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Domestication of *Brassica oleracea* L.

LORENZO MAGGIONI

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Lorenzo Maggioni
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Plant Breeding
Alnarp

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Cover: *Brassica rupestris* Raf. at the Norman Castle of Monte Consolino, Calabria, Italy, July 2007.
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Abstract

Various theories exist about the progenitors of *B. oleracea* L. (cole crops) and the area of its domestication. Relatively recent molecular data have pointed at the wild species growing on the European Atlantic cliffs as the closest relatives to all the cultivated types.

Compatibly with all the existing observations, an alternative hypothesis is formulated here, namely that the domestication of *B. oleracea* occurred in a Mediterranean location, while the crop reached the Atlantic coast only at a later date, with the movement of people and cultures. The wild Atlantic populations are supposed to be re-naturalized escapes from the fields. If this theory could be confirmed, it would be plausible for all the cole crops to show the highest affinity with the Atlantic populations, since these belonged to the same genetic pool that underwent a domestication bottleneck.

Our experimental work revolved around attempts to test the above-mentioned hypothesis through indirect means. Linguistic, literary and historical aspects of the use of cole crops indicate a deep-rooted knowledge and use of cole crops as well as of their wild relatives by the ancient Greek and Roman civilizations. Moreover, words used to name the coles throughout Europe largely if not exclusively derive from Greek or Latin roots. Analysis of the genetic diversity of leafy kales from around Europe did not find a geographic pattern and a directionality. Agro-ecosystems mimicking a situation of initial domestication, with cole crops and one of their wild relatives (*B. rupestris* Raf.) growing in close proximity to one another were analysed. Patterns of gene flow have been identified and domestication scenarios have been described.

Evidence of populations of coles escaping from the field and becoming indistinguishable from truly wild populations was obtained with molecular marker studies. Finally, attention was given to the legal aspects of collecting wild specimens of genetic resources in Europe, with an analysis and discussion about this issue.

This work analyses strengths and weaknesses of the existing theories of domestication of *B. oleracea* and supports an alternative and original hypothesis that will deserve to be further tested in future before a conclusive statement can be made on the origin of the coles.

Keywords: Brassica oleracea L., domestication, genetic resources, molecular markers, gene flow, collecting missions, linguistic studies, ancient ethnobotany

Author's address: Lorenzo Maggioni, Bioversity International,
Via dei Tre Denari 472/a, 00057 Maccarese (Fiumicino), Roma, Italy
E-mail: l.maggioni@cgiar.org

Dedication

To Cecília

Veulettes-sur-Mer, July 2012

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List of Publications

This Thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Maggioni, L., Bothmer, R. von, Poulsen, G. & Branca, F. (2010). Origin and domestication of cole crops (*Brassica oleracea* L.): linguistic and literary considerations. *Economic Botany*, vol. 64 (2), pp. 109–123.

II Christensen, S., Bothmer, R. von, Poulsen, G., Maggioni, L., Phillip, M., Andersen, B.A. & Bagger Jørgensen, R. (2011). AFLP analysis of genetic diversity in leafy kale (*Brassica oleracea* L. convar. *acephala* (DC.) Alef.) landraces, cultivars and wild populations in Europe. *Genetic Resources and Crop Evolution*, vol. 58 (5), pp. 657–666.

III Maggioni, L., Bagger Jørgensen, R., Bothmer, R. von, Poulsen, G. & Branca, F. (2013). Signs of inter-crossing between leafy kale landraces and *Brassica rupestris* in south Italy. In: Branca, F. & Tribulato, A. (eds) *Proceedings of the VI International Symposium on Brassicas and XVIII Crucifer Genetics Workshop. Acta Horticulturae* 1005, pp. 165-172.

IV Maggioni, L., Bothmer, R. von, Poulsen, G., Branca, F. & Bagger Jørgensen, R. (2014). Genetic diversity and population structure of leafy kale and *Brassica rupestris* Raf. in south Italy. *Hereditas*, vol. 158, pp. 145–158.

V Maggioni, L., López Noriega, I., Lapeña, I., Holubec, V. & Engels, J. (2015). Collecting plant genetic resources in Europe: a survey of legal requirements and practical experiences. In: Coolsaet, B., Batur, F., Broggiato, A., Pitseys, J. & Dedeurwaerdere, T. (eds) *Implementing the*

Nagoya Protocol. Comparing Access and Benefit-sharing Regimes in Europe. Leiden; Boston: Brill | Nijhoff, pp. 327-362.

VI Maggioni, L., Bothmer, R. von, Poulsen, G. & Lipman, E. Domestication, diversity and use of cole crops (*Brassica oleracea* L.), based on ancient Greek and Latin texts (submitted manuscript).

VII Poulsen, G., Maggioni, L., Smolka, A., Bagger Jørgensen, R. & Bothmer, R. von. Use of molecular markers for taxonomical identification of *Brassica* (manuscript).

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The contribution of Lorenzo Maggioni to the papers included in this Thesis was as follows:

I Researched and drafted manuscript and final paper collated with editorial input from co-authors.

II Participated in the design of the research, procurement of material and editorial inputs.

III Researched and drafted manuscript and final paper collated with editorial input from co-authors.

IV Researched and drafted manuscript and final paper collated with editorial input from co-authors.

V Provided idea of hypothesis, researched and contributed to the writing of the paper.

VI Researched and drafted manuscript and final paper collated with editorial input from co-authors.

VII Participated in the design of the research, procurement of material and contributed to the writing of the paper.

Abbreviations

ABCD	Archaeobotanical Computer Database
ABS	Access and benefit sharing
ACK	Ancestral crucifer karyotype
AEGIS	A European Genebank Integrated System
AEGRO	An Integrated European In Situ Management Work Plan: Implementing Genetic Reserves and On Farm Concepts *(EU-funded project)*
AFLP	Amplified fragment length polymorphism
BAC	Bacterial artificial chromosome
BCE	Before the Common Era
BP	Before the Present
CCP	Comparative chromosome painting
cDNA	Complementary DNA
CE	the Common Era
CGN	Centre for Genetic Resources, the Netherlands
CMS	Cytoplasmic male sterility
cpDNA	Chloroplast DNA
DNA	Deoxyribonucleic acid
EC	European Commission
ECPGR	European Cooperative Programme for Plant Genetic Resources
EST	Expressed sequence tag
EU	European Union
EURISCO	European Plant Genetic Resources Search Catalogue
FAO	Food and Agriculture Organization of the United Nations
FISH	Fluorescent *in situ* hybridization
GRIN	Germplasm Resources Information Network
IBPGR	International Board for Plant Genetic Resources *(now Bioversity International)*
ISSR	Inter simple sequence repeat

ITS	Internal transcribed spacer
LDL	Low-density lipoprotein
Mya	Million years ago
OECD	Organisation for Economic Co-operation and Development
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PGRFA	Plant Genetic Resources for Food and Agriculture
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SEM	Scanning Electron Microscope
SINE	Short interspersed nuclear elements
SMTA	Standard Material Transfer Agreement
SNP	Simple nucleotide polymorphism
SSR	Simple sequence repeat
UPM	Universidad Politécnica de Madrid, Spain
USDA	United States Department of Agriculture
WIEWS	World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture

1 Introduction

1.1 *Brassica oleracea* L. and its relatives

1.1.1 Economic importance

The plant family Brassicaceae (or Cruciferae) includes some of the world's most economically important crops, especially members of the genera *Brassica* L. (coles, mustards, oilseed rapes, turnips, etc.), *Raphanus* L. (radish), *Armoracia* G. Gaertn. & al. (horseradish), *Lepidium* Fabr. (garden cress), *Nasturtium* W. T. Aiton (watercress), *Eutrema* R. Br. (wasabi) and *Eruca* Mill. (rocket) (Al-Shehbaz *et al.* 2006). These cultivated species offer a large variety of leaf and root vegetables, oilseed and condiment crops.

The *Brassica* vegetables are a dietary staple in many parts of the world, while the *Brassic*a oilseed crops provide 14% of the world's edible vegetable oil and the third most important source of edible oil after soybean and palm (OECD, 2012). Among the vegetables, the cultivated varieties of the species *Brassica oleracea* L. are often referred to as 'cole crops', comprising broccoli, Brussels sprouts, cabbage, cauliflower, kales and kohlrabi, but not the *Brassica rapa* L. vegetables (Haynes *et al*., 2009; OECD, 2012).

Cole crops are grown worldwide, with the exception of some tropical areas. Among these, cabbage is most widely produced, followed by cauliflower and broccoli. Cabbage is more important in northern and eastern European countries, while cauliflower is more important in southern Europe, USA and Mexico. Other crops are very important on a more local basis, such as Portuguese kales (tronchuda) in Portugal and Spain and a great array of leafy kales are common in European home gardens, with local variations.

The FAO estimates (FAOSTAT, 2015) of the world commercial production for human consumption of *B. oleracea* are aggregated in two groups: 'cabbages and other brassicas' and 'cauliflowers and broccoli'. In 2013, total yield estimates for these two items combined amounted to over 93 million tonnes (respectively 71.4 and 22.3) from about 3.7 million hectares (respectively 2.44 and 1.25). Asia held the highest production share, with 74.5% of cabbages and other brassicas (compared to 16.2% in Europe) and with 82.7% of cauliflowers and broccoli (10.3% in Europe). The annual growth rates between 2009 and 2013 were globally positive for both groups of crops (see Table 1, with world and EU trends compared). These figures do not include production of kitchen gardens or small family gardens.

Table 1. *Annual growth rates between 2009 and 2013 (FAOSTAT, 2015)*

Crops	Area harvested		Production		Yield	
	EU	World	EU	World	EU	World
Cabbages and other brassicas	-1.38%	1.47%	-1.54%	2.10%	-0.17%	0.62%
Cauliflowers and broccoli	0.63%	2.57%	1.12%	3.10%	0.49%	0.51%

1.1.2 Taxonomy of *Brassica oleracea* L.

Brassica oleracea L. is classified as follows:

- Order Brassicales (= Cruciales)
  - Family Brassicaceae (= Cruciferae)
    - Tribe Brassiceae
      - Subtribe Brassicinae
        - Genus *Brassica*
          - Section *Brassica*
            - Species *Brassica oleracea* L.

Systematic studies within the family Brassicaceae and especially regarding the genus *Brassica* have historically been challenging and generated a confused nomenclature with a large number of synonyms. Important taxonomic treatments of the cultivated brassicas were made, among others, by Schulz (1919, 1936), Bailey (1922; 1930), Helm (1963), Nieuwhof (1969), Diederichsen (2001) and Gladis and Hammer (2001; 2003). A detailed review of the history of the taxonomy of the genus *Brassica* until their time was given by Prakash and Inata (1980).

The underlining reasons for these difficulties are partially related to ancient polyploidizations occurring in the family, which confers the ability of several taxa to intercross and generate stable hybrids, in some cases even after millions of years of divergence. Additionally, convergence of traits is so predominant in the family that virtually every morphological character arises with similar states in different lineages without an immediate common ancestor.

Family Brassicaceae

The family has recently undergone molecular systematic studies and the substantial achievements made are kept up to date on the online BrassiBase project database (Koch *et al*., 2012; Kiefer *et al*., 2014). The resolution of many remaining problems, especially regarding the delimitation of tribes and genera, is still in progress (Al-Shehbaz, 2012).

As currently delimited, the Brassicaceae comprises 49 tribes, 321 genera and 3660 species (Al-Shehbaz, 2012). The species of this family have flowers with 4 sepals, 4 equal cruciform petals, 6 stamens (4 long, 2 short), and an ovary with two parietal placentae. The fruit is called siliqua and is a capsule with a false septum (so called because it does not represent the wall between carpels; Hedge, 1976).

Tribe Brassiceae

The tribe includes most of the cruciferous crops. It was considered one of the most distinctive and the most natural of all tribes of the Brassicaceae (Al-Shehbaz, 1985). Tribal members are morphologically characterized by having conduplicate cotyledons (i.e. the cotyledons longitudinally folded around the radicle in the seed) and/or transversely segmented fruits that have seeds or rudimentary ovules in both segments (heteroarthrocarpic) and, if present, only simple (not stellate, bifid or branched) trichomes or hairs (Gómez-Campo, 1980; Warwick and Hall, 2009). This tribe has been confirmed to be monophyletic on both morphological and molecular evidence (references in Arias and Pires, 2012) and it currently includes 47 genera and 227 species (Al-Shehbaz, 2012). Apart from all the brassica crops included under the genus *Brassica*, this tribe also includes other important genera, such as *Diplotaxis*, *Eruca*, *Raphanus* and *Sinapis* (sources of edible leaves, roots and condiments).

Subtribe Brassicinae

The subtribe was recognized in the comprehensive taxonomic treatments made by Schulz (1919; 1936) as one of seven subtribes (Brassicinae, Cakiline, Moricandiinae, Raphaninae, Savignyinae, Vellinae and Zillinae). Gómez-Campo (1980) then proposed reducing to six subtribes by including the Savignyinae in the Vellinae. Brassicinae and Moricandiinae are characterized by elongated, siliquose fruits, whereas the other subtribes generally have reduced, shortened fruits. Together with *Brassica*, other economically important genera such as *Diplotaxis*, *Eruca* and *Sinapis* are traditionally included among the Brassicinae, and *Raphanus* among the Raphaninae. The morphological distinctness of subtribes Brassicinae, Moricandiinae and

Raphaninae was considered not well substantiated by Al-Shehbaz (1985) and Warwick and Black (1994). Gómez-Campo (1999) confirmed that the division of the tribe Brassiceae into subtribes had been an unresolved issue for long time. The difficulty has been to establish adequate criteria and the results of molecular analysis should better guide the splitting of the tribe into homogeneous groups.

Genus Brassica

A description of the genus *Brassica*, taken from *Flora Europaea* (Heywood, 1964) is the following:

> Herbs or small shrubs. Leaves entire to pinnatipartite. Sepals erect or patent, the inner larger than the outer. Petals yellow or white, clawed. Lateral nectaries prismatic, intense green. Ovary sometimes with a short gynophore. Fruit a siliqua with a *long* or short beak; valves convex, with a prominent median vein. Seeds in 1-2 rows in each loculus, globose or rarely ovoid

The BrassiBase records 38 species within the genus *Brassica*. Among these we find the domesticated brassica crops (*B. carinata*, *B. juncea*, *B. oleracea*, *B. rapa* and *B. napus*), semi-domesticated or relic crops (*B. nigra* and *B. tournefortii*), wild species occasionally harvested for food or brought into cultivation (*B. fruticulosa* and *B. cretica*) and other wild species.

Recent molecular studies with a few nuclear and chloroplast markers revealed that *Brassica,* as well as *Diplotaxis, Erucastrum* and *Sinapis* are polyphyletic genera (Warwick and Sauder, 2005; Warwick and Hall, 2009; Hall *et al*., 2011). Therefore, it is expected that future detailed studies with more markers, together with a critical evaluation of all morphological characters will lead to nomenclatural re-adjustments and re-organization of the generic circumscription, in order to recognize monophyletic genera that truly represent the phylogenetic relationships within the tribe. However, certainly all crops of the genus *Brassica* will remain in this genus (Al-Shehbaz, 2012).

Section Brassica

The genus *Brassica* is divided in three sections: (1) sect. *Brassica*, (2) sect. *Brassicaria*, (3) sect. *Melanosinapis* (Diederichsen, 2001). Section *Brassica* has been subject to different delimitations by different authors. Stork *et al*. (1980) and Snogerup *et al*. (1990) defined this section as including only the species with x=9 genome, characteristic of *B. oleracea* (i.e. the C-genome). Such delimitation corresponds to the cytodeme concept of Harberd (1976; see below, chapter 1.1.4). A cytodeme is composed of all the species sharing the same chromosome complement and that can be easily crossed to give a hybrid which is neither weak in vigour, nor of low fertility. This same concept is conveniently maintained in Diederichsen (2001), but only by creating an informal headline '*B. oleracea* group'. Conversely, the formal section *Brassica,* reflecting the definition of section *Brassicotypus* made by Schulz (1936), is based on morphological characters, and includes also other major informal brassica crop groups (*B. rapa*, *B. juncea*, *B. napus*, *B. carinata* and *B. tournefortii* groups).

Species Brassica oleracea *L.*

The species name was validly published for the first time by Linnaeus in his *Species Plantarum* (1753, p.667). At this occasion Linnaeus used the following diagnostic phrase name: *radice caulescente tereti carnosa*, referring to the round, fleshy root/stalk of the cabbage. At the same time, he indicated as a synonym the variety name *sylvestris*, referred to the phrase name *Brassica maritima arborea seu procerior ramosa,* encountered in Morison's *Historia Plantarum* of 1680 as well as assigned by Ray to the "Wild Colewort" in his *Historia Plantarum* of 1686. This indicates that Linnaeus identified the cultivated forms as the same species as the wild kales growing on the coastal cliffs of England (Oost *et al*., 1989).

Infraspecific taxonomy and nomenclature

In his *Species Plantarum*, Linnaeus also assigned ten varietal names to different cultivated forms of *Brassica oleracea*, including cabbages, cauliflowers, various leafy kales and kohlrabi (as well as, by mistake, to a form of *B. napus* that he called *napobrassica*).

The large variety of forms of cultivated *B. oleracea* has been the object of several attempts of systematization. Among the most comprehensive are those of Schulz (1919; 1936), Bailey (1922; 1930), and Helm (1963). A good summary of the nomenclatural history of *B. oleracea* was given by Prakash and Hinata (1980). More recent systematization treatments were made by Diederichsen (2001) and Gladis and Hammer (2001; 2003), where all existing synonyms can be found.

The various nomenclatural and taxonomic treatments of the cultivated forms were similar, but differentiating either in the names assigned to the various clusters of crops and/or in the use of different ranking categories (convariety, variety, form) and the number of clusters. In the work made by Gladis and Hammer (2001) a different approach is proposed, since all the cultivated and wild taxa belonging to the same C-genome are grouped under the species *B. oleracea*. The cultivated forms are first ranked at the subspecific level and then divided in convarieties and varieties. The rationale behind this treatment is that several taxa that are part of the *Brassica oleracea* group (Section *Brassica sensu* Stork) are equally related to the cultivated forms as the wild Atlantic *Brassica* identified by Linnaeus as *Brassica oleracea*. Therefore, Gladis and Hammer (2001) identified eight wild, one intermediate and one cultivated subspecies under *Brassica oleracea*.

The proposed change was not widely adopted in subsequent literature. For example, both the OECD (2012) consensus document and the GRIN (Germplasm Resources Information Network) taxonomy managed by the United States Department of Agriculture treat only the wild Atlantic brassicas as varieties of *B. oleracea* at the same level of the cultivated varieties (USDA, 2015). As an example of the different approach, a comparison of the nomenclature assigned respectively by GRIN and by Gladis and Hammer to cauliflower is the following:

GRIN: *Brassica oleracea* var. *botrytis*
Gladis and Hammer (2003): *Brassica oleracea* subsp. *capitata* convar. *botrytis*

The proposal by Gladis and Hammer deserves attention, since it responds to the recommendation made by Harlan and de Wet (1971) that crop species and their close wild relatives should be treated as different subspecies within one species. Therefore, they include all the cultivated types of *B. oleracea* under the new subspecies name *capitata*, while the wild Atlantic species maintains the subspecific name *oleracea* and other Mediterranean wild species are downgraded from the species level to subspecies of *B. oleracea*. Also the decision to treat all the C-genome wild brassicas as members of the same species has some merit, since several of these taxa can intercross with the cultivated forms (see below). However, there are also drawbacks with this interpretation. The traditional nomenclature of the cole crops is rather deep-rooted among agronomists and horticulturalists who are reluctant to adopt new long name combinations. Moreover, interfertility among the wild Mediterranean taxa and the cultivated *B. oleracea* does not have the same rate of success for all the taxa. Also molecular phylogenetic studies place the various taxa in close but different clusters. Finally, the derivation of the cultivated taxa from the wild Atlantic species is questioned by this Thesis. In summary, it is perhaps premature to consider all the C-genome wild taxa as part of the same species *B. oleracea*. More comprehensive molecular studies should help clarifying the taxonomic status of these taxa.

The most important crops of the *Brassica oleracea* group are listed in Table 2, according to the GRIN nomenclature:

Table 2. *Nomenclature of cultivated* Brassica oleracea *L. according to GRIN*

B. oleracea L.	Common synonym	Common names
var. *alboglabra* (L. H. Bailey) Musil	*Brassica alboglabra* L. H. Bailey	Chinese Kale, Kailan
var. *botrytis* L.		Cauliflower
var. *capitata* L.		Red/White/Shetland cabbage
var. *costata* DC.		Portuguese kale; tronchuda kale
var. *gemmifera* DC.		Brussels sprouts
var. *gongylodes* L.	*Brassica caulorapa* (DC.) Pasq.	Kohlrabi
var. *italica* Plenck		Broccoli
var. *medullosa* Thell.		Marrow-stem kale
var. *palmifolia* DC.		Palm kale; Jersey kale
var. *ramosa* DC.	subsp. *fruticosa* Metzg.	Thousand-head kale; branching bush kale
var. *sabauda* L.		Savoy cabbage
var. *sabellica* L.		Curly kale
var. *viridis* L	var. *acephala* DC.	Kale, Collard

1.1.3 The other *Brassica* crops

Together with *B. oleracea*, five other closely related brassica crops are of primary economic importance: *B. nigra* (L.) Koch (black mustard); *B. rapa* L. (turnip); *B. carinata* A. Braun. (Abyssinia mustard); *B. juncea* (L.) Czern. (brown mustard) and *B. napus* L. (oilseed rape).

The relationship between these six cultivated *Brassica* crops was clarified by the cytogenetic analysis and crossing experiments carried out between 1922 and 1937 by Karpechenko, Morinaga and his student Nagaharu U (Prakash and Inata, 1980 and references therein). The genetic relationships are elegantly explained by the so-called ‘Triangle of U’ (1935), shown in Figure 1.

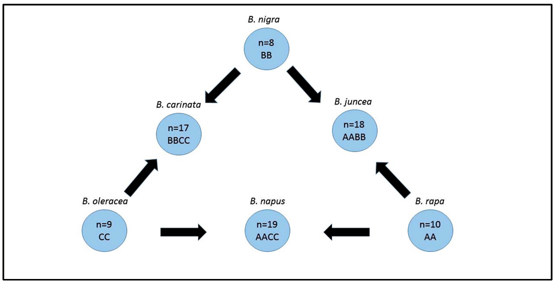

Fig. 1. The Triangle of U representing the genomic relationships among *Brassica* species.

By intercrossing with each other, the three basic diploid species *B. nigra* (n=8, genome code BB), *B. oleracea* (n=9, CC) and *B. rapa* (n=10, AA) have generated three amphidiploid taxa, *B. carinata* (n=17, BBCC), *B. juncea* (n=18, AABB) and *B. napus* (n=19, AACC). Subsequent studies have confirmed these genomic relationships through re-synthesis of the three amphidiploids from their parents as well as with studies on phenolic compounds, protein patterns, isozymes, nuclear and chloroplast DNA and fluorescent *in situ* hybridization (OECD, 2012 and references therein).

1.1.4 The *Brassica oleracea* group (C-genome)

Knowledge of the relationship between a crop and its relatives allows a better understanding of its origin and evolution, as well as defining the boundaries of the genepools in the sense of Harlan and de Wet (1971). Members of the primary and secondary genepools can more easily intercross and are therefore the target taxa for genetic resources conservation and use. Knowledge of the potential for hybridization is important not only for breeding purposes, but also to better manage the seed production and to monitor the non-intended gene flow and the ecosystem dynamics.

A successful approach to identify biological relationships among taxa was utilized by Harberd (1976), with the intention to offer a guidance to the breeders on which wild species are sufficiently related to the crop plants to be of potential value. He included all the different taxa sharing a common chromosome complement, independently from their genetic or ecological variability and taxonomic status, into a single cytodeme, characterized by a common basic chromosome number and by high interfertility within the group. He thought the cytodeme to be the most important unit of evolution, since members of each cytodeme must be sharing the same lineage by descent. The concept of '*Brassica* coenospecies' was also defined by Harberd (1976), encompassing those species and genera, which sufficiently relate to the six brassica crops to be capable of experimental hybridization with them. On this basis, Warwick *et al*. (2009) have listed 45 diploid and 6 amphidiploid cytodemes composing the *Brassica* coenospecies. Every basic chromosome number (x) between 7 and 14 is represented among the diploid species, with a mode at 9 and 10. Apart from *Brassica*, other genera are represented in the coenospecies, such as *Coyncia*, *Diplotaxis*, *Enarthrocarpus*, *Eruca*, *Erucastrum*, *Hirschfeldia*, *Moricandia*, *Raphanus*, *Sinapis*, *Sinapidendron and Trachystoma.*

The '*Brassica oleracea* group' or 'cytodeme' or '*Brassica* section *Brassica*' is therefore not a formal taxonomic unit although Gladis and Hammer (2001) have proposed to treat all the wild species within this cytodeme as subspecies of *B. oleracea*. This group is a homogeneous entity of taxa, which are highly interfertile (to various degrees) and share the same chromosome genome C of *B. oleracea*. By this definition, this group also corresponds to the *B. oleracea* primary genepool *sensu* Harlan and de Wet (1971).

General description

The wild species of the *Brassica oleracea* group are described by Snogerup *et al*. (1990) as follows:

> Perennials, as adults with a woody stem and usually also woody branches (…). Leaves in some species semicoriaceous, in others of herbaceous structure but rather thick, hairy or glabrous. Flowers usually protrandrous, with stigma ripening one to a few days after the opening of anthers. Sepals erect to erecto-patent during anthesis (…). Petals large, white to light yellow, usually patent at anthesis (…). Fruit a siliqua, usually with the seeds in a one-row arrangement in each loculus and often alternating between the loculi, only in *B. macrocarpa* and *B. hilarionis* in two intersected rows in each loculus. Seeds always spherical or almost so, 1.5-3.5 mm in diameter, with a more or less conspicuous superficial network.

These taxa are often chasmophytic, i.e. specialized to grow in the crevices of rocks, usually found on more or less vertical cliffs by the coast, commonly on limestone substrates. However, growth on gentle rocky slopes, macchia or ruderal substrates is also possible, including rocky areas quite distant from the coast. The restriction to a cliff habitat is mainly dependent on the presence of grazing animals, as well as on competing vegetation. Populations can be found from the sea level up to high elevations, the highest recorded by the author at ca. 1400 m for *B. incana* in the Sorrento Peninsula, Italy.

Population size can be variable between few individuals up to very large populations with several thousands of individuals. Populations are very often well delimited and contained within isolated locations, but there are also cases of large groups of populations occupying extended cliff systems, with the possibility to exchange genes across long distances. Sympatry normally does not occur, although this aspect would require a more thorough investigation, also to explain hypothesized cases of hybridization events (see below). The life span of individuals is usually 2 to 6 years. The time of flowering is variable, with *B. cretica* beginning to flower in the first or second year, while wild *B. oleracea* remains in a vegetative state for several years and requires vernalization. Only a fraction of each population flowers each year, with an average of 50% of the plants. All the taxa present a high degree of self-sterility, which is however never complete. The geographic distribution according to Snogerup *et al.* (1990) is reproduced in Figure 2.

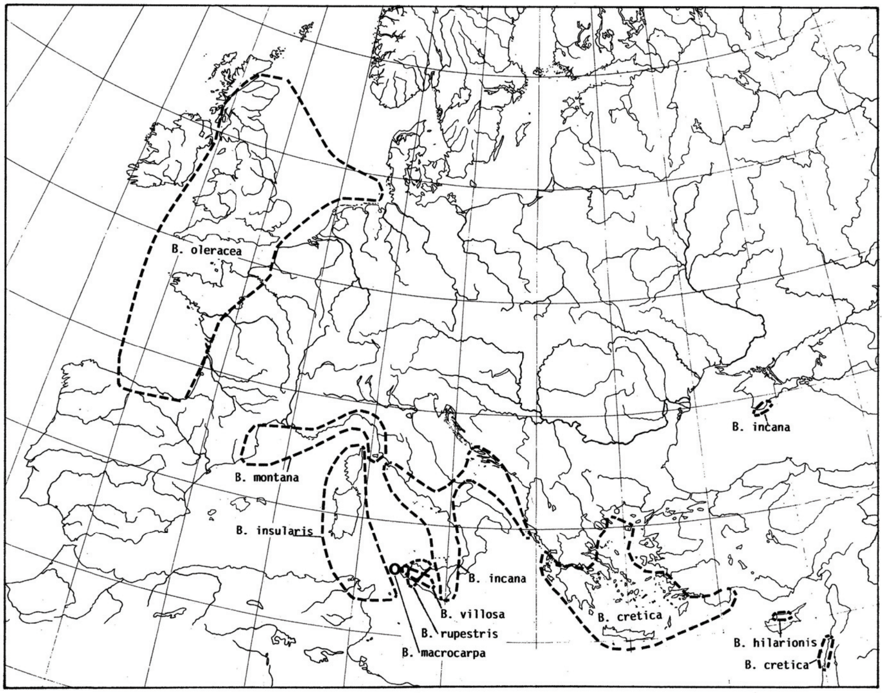

Fig. 2. Distribution of the species of the *B. oleracea* group according to Snogerup *et al.* (1990), reproduced with permission of the Botanischer Garten und Botanisches Museum Berlin.

Taxonomic treatment

A variable number of wild taxa have been described within the *Brassica oleracea* group and alternative ranking of species, subspecies or varieties have been assigned to them. It is beyond the scope of this Thesis to reconstruct and comment the history of the proposed taxonomic arrangements and the related babel of existing synonyms. Full taxonomic treatments of the group were published for example by Schulz (1919), Onno (1933), Snogerup *et al.* (1990), Heywood and Akeroyd (1993), Gustafsson and Lannér-Herrera (1997), Diederiechsen (2001) and Gladis and Hammer (2001; 2003). Taxonomic keys are available in Snogerup *et al.* (1990), Heywood and Akeroyd (1993), Gladis and Hammer (2003) and Brullo *et al.* (2013).

The difficulty to find agreements on the number and ranking of taxa of this group are related to the fact that morphological characters are subject to a large variability that is often influenced by environmental factors. Isolation of several populations easily leads to founder effects, genetic drift and the creation of differentiated populations, which are sometimes split into new species or subspecies. At the same time, the continuing ability of the various taxa to intercross and generate more or less fertile hybrids leads others to lump these same taxa, even up to the extreme of recognizing only one species.

Morphological traits that are not fully reliable for their discriminating ability have been studied by Snogerup *et al*. (1990). These authors noticed a large variation in leaf shapes and in sizes and shapes of the siliqua, which vary considerably among individuals and populations, as well as in cultivation experiments, due to differences in nutrition, pollination, etc.

A numerical taxonomy study carried out on Sicilian taxa by Maggioni (1996) also confirmed that quantitative characters related to the sizes of leaves and siliqua were not useful to discriminate the various species, due to a large intraspecific variability.

An additional challenge to the identification of taxa is the reported event of crosses among wild species. For example, Tatout *et al*. (1999) suggested that hybridization between *B. incana* and *B. villosa* generated *B. drepanensis* in western Sicily, although they did not elaborate on the current absence of *B. incana* from west Sicily. On the basis of morphological traits, Maggioni (1996) hypothesized a hybridization between *B. incana* and *B. rupestris* in northeast Sicily.

Hybridizations between wild species and cultivated *B. oleracea* are frequently reported and a specific taxonomic nomenclature for these 'intermediate races' has been proposed by Gladis and Hammer (2003), that is *Brassica oleracea* subsp. *capitatoides* Gl. & Hm. Specific cases of gene flow between cultivated and wild species have also been discussed in Paper III and Paper VII.

Finally, escapes from the field and re-naturalization of *B. oleracea* is another frequent phenomenon, which makes the taxonomic identification of given populations even more difficult. Examples are listed by Snogerup *et al*. (1990) and further discussed below and in Paper II and Paper VII.

The following list of 11 accepted wild species of the *Brassica oleracea* group, including three subspecies of *B. cretica*, is taken from the GRIN taxonomy for plants (USDA, 2015). Here, the scientific names are verified, in accordance with the international rules of botanical nomenclature, by taxonomists of the USDA National Germplasm Resources Laboratory, using available taxonomic literature and in consultation with taxonomic specialists:

1. *Brassica bourgeaui* (Webb ex Christ) Kuntze

Endemic to the Canary Islands. Only known from a few populations and considered to be at risk of introgression from colonies of *B. oleracea* escaped from the fields (Marrero, 1989). According to Borgen *et al*. (1979), the morphological differences between *B. bourgeaui* and *B. oleracea* are so slight that the taxonomic rank of *B. bourgeaui* as a separate species can be questioned. The status of this taxon is further discussed in Paper VII.

2. *Brassica cretica* Lam.

This species has sometimes been cultivated in Greece. Former cultivation is also supposed for Lebanon (Diederichsen, 2001). Flowers white (suspb. *cretica*) or different shades of yellow. Three subspecies are known, distinguished by the leaf shape and flower colour:

a) *Brassica cretica* Lam. subsp. *cretica*
Kriti, north Peloponnisos, central to south Lebanon (probably introduced).
b) *Brassica cretica* Lam. subsp. *aegaea* (Heldr. & Halácsy) Snogerup *et al*.
South Greece, south-west Turkey and Israel (Mt Carmel) (probably introduced).
c) *Brassica cretica* Lam. subsp. *laconica* M. A. Gust. & Snogerup
South Peloponnisos.

3. *Brassica drepanensis* (Caruel) Damanti

A few populations in the area of Trapani (Sicily), with a characteristic shape of the pod (smaller fruit, with slender beak). It has a doubtful status and is included as a subspecies of *B. villosa* in the Italian checklist of the vascular flora (Conti *et al*., 2005). Recently, molecular data supported its status of species (Tatout *et al*., 1999).

4. *Brassica hilarionis* Post

Endemic to the Kyrenia range in northern Cyprus. Flowers white or purplish. Leaves with lamina generally undivided and short- or non-petiolated. Broader siliqua.

5. *Brassica incana* Ten.

Thyrrenian coastal or sometimes internal areas of central and south Italy, east Sicily, Tremiti Islands, some coastal localities in Croatia and Ionian islands in northwest Greece. It is also present in south Crimea, probably as an introduction. Local variants in the Croatian islands have been attributed the status of species or varieties, based on fruit characters, such as *B. botteri* Vis., *B. cazzae* Ginzberger &Teyber and *B. mollis* Vis. (Maggioni and Eastwood, 1997). Hairy leaves, ususally with a semiamplexicaul wing at the base of petiole, flowers yellow.

6. *Brassica insularis* Moris

Present in Sardinia, Corsica, Tunisia, Algeria and on the Island of Pantelleria, south of Sicily. Isolated populations in Corsica were treated as four separate varieties owing to their large variation (Widler and Bocquet, 1979). Usually glabrous leaves and flowers white. *B. atlantica* from North Africa is generally considered a synonym of *B. insularis*, although Véla and Pavon (2012) still distinguish two populations from northeast Tunisia as *B. atlantica* (Coss.) O. E. Schulz.

7. *Brassica macrocarpa* Guss.

Endemism of the Egadi Islands, west of Sicily. Only present on the Islands of Favignana and Marettimo (Maggioni and Eastwood, 1997). Siliqua wide, with thick valves, stout and conical beak, flowers yellow, leaves glabrous and petiolate. Experimental fields have been grown on the Island of Levanzo and the artificial reintroduction on this island is being considered.[1]

8. *Brassica montana* Pourret

Formerly known as *B. oleracea* subsp. *robertiana*. Distributed along the coasts of the northern Mediterranean Sea from northeast Spain to northwest Italy. Also occurring in inland locations (such as the Apuan Alps, Tuscany) with a localized presence on the northern Apennines and along the Thyrrenian coast (Conti *et al.*, 2005). Leaves glabrous, flowers light yellow. A population on Monte Conero near Ancona on Italy's Adriatic coast (Onno, 1933; Biondi *et al.*, 2002) has been considered an escaped population of cultivated *B. oleracea* by Snogerup *et al.* (1990). Two populations, from Monte Conero and Ponza Island have been investigated in Paper VII.

9. *Brassica oleracea* L. var. *oleracea*

Also known as *Brassica oleracea* L. subsp. *oleracea*. Distributed along the European Atlantic coasts in northern Spain, France, United Kingdom and Helgoland Island (Germany). At least one population was formerly known from Ireland (Snogerup *et al.*, 1990). Some morphological traits (such as the greyish surface of the leaves) are very similar to those of the cultivated *B. oleracea*. Various authors have regarded some of these populations as introductions (Grenier and Godron, 1848; Watson, 1870; Mitchell, 1976). Frequent introgression from cultivated taxa has also been suggested (Diederichsen, 2001).

10. *Brassica rupestris* Raf.

Sicily and Calabria. Usually a conspicuous main stem branching only apically to form the inflorescence. Leaves with long petiole, hairs with bulbose

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[1] Ferdinando Branca, personal communication

base, flowers light yellow. The Italian checklist of the vascular flora distinguishes two subspecies, *hispida* and *rupestris* (Conti *et al*., 2005).

11. *Brassica villosa* Biv.

Endemic of Sicily (only central and west part). Leaves hairy, without wings at the base of the petiole. Flowers light yellow. The Italian checklist of the vascular flora distinguishes five subspecies: *bivoniana*, *brevisiliqua*, *drepanensis*, *tinei* and *villosa* (Conti *et al*., 2005).

Three additional taxa should be mentioned, that were recently proposed by Italian botanists, but are not considered by the GRIN taxonomy and therefore they are either not yet accepted or not included as synonyms of the above names. These three species were described on the basis of morphological traits and were not yet included in crossing experiments or molecular studies to determine their genetic relationship with the other taxa. They are the following:

➢ *Brassica raimondoi* Sciandr., C. Brullo, Brullo, Giusso, Miniss. et Salmeri

This taxon was described only from the cliffs of Castelmola (Messina, Italy), being sympatric to *B. incana*, from which it is distinguished mainly for the white flower colour and the seed coat microsculpture. Chromosome number 2n=18 (Sciandrello *et al*., 2013). About this same site, Snogerup *et al*. (1990) reported of risks of introgression into *B. incana* from white-flowered cultivated forms. The same authors also reported of the existence of white-flowered *B. incana* in a nearby location in East Sicily.

➢ *Brassica thyrrena* Giotta, Piccitto et Arrigoni

Endemic of central-east Sardinia, where at least six populations were identified. It differentiates from *B. insularis* mainly by its yellow-coloured flowers and earlier phenology. Chromosome number 2n=18 (Giotta *et al*., 2002).

➢ *Brassica trichocarpa* C. Brullo, Brullo, Giusso & Ilardi

Endemic of an exclusive location near Mt. Cuccio, close to Palermo, Sicily. Despite the hairy habit, similar to *B. villosa*, which was typified from a nearby location, this new taxon shows very distinctive characters with its pubescent ovary and very short, wide and hairy fruit. Chromosome count is however not confirmed (Brullo *et al*., 2013).

Finally, *Brassica balearica* Pers. had been included in the *B. oleracea* group by Onno (1933). The chromosome number of this taxon, endemic to the Island of Mallorca, Spain, has been erroneously reported as 2n=18 or 2n=36, while the effective number is 2n=32 (Dahlgren *et al*. 1971; Snogerup and Persson, 1983). It has been shown through hybridization experiments

(Snogerup and Persson, 1983) and confirmed by molecular means (Tonosaki and Nishio, 2010), that *B. balerica* contains a *Brassica* C-genome and that it is a probable polyploid resulting from the cross between a C-genome progenitor with 2n=18 and an unknown parent with 2n=14. Following the successful hybridization experiment with *B. insularis* (Snogerup and Persson, 1983), *B. balearica* is considered a member of the tertiary genepool of *B. oleracea* and therefore of potential value in cole breeding.

1.1.5 Interspecifc and intergeneric hybridization

Interspecific crosses within the genus *Brassica* as well as intergeneric crosses have been successfully attempted since the 19th century (Prakash and Inata, 1980). As reported above, several experiments were initially made with the intention to understand the genomic relationships of the cultivated crops and subsequently they were inspired by the possibility to transfer useful traits from distant species.

Several incompatibility barriers can be encountered during these experiments, which often can be overcome in various ways, ranging from the appropriate choice of the female parent, the use of chemicals, repeated or mixed pollinations, style excisions or grafting, and more recently *in vitro* techniques such as *in vitro* fertilization, embryo rescue and protoplast fusion (Prakash and Inata, 1980; Hu, Li and Mei, 2009).

A summary of the literature compiled on interspecific and intergeneric cytodeme hybridization between members of the tribe Brassiceae is available from Warwick *et al*. (2009). The production and maintenance of progenies from F_1 hybrids between brassica crops and distant taxa was reviewed by Kaneko *et al*. (2009).

Brassica oleracea hybridizes with difficulty with *Brassica rapa*, but this cross can be facilitated by *in vitro* techniques like embryo rescue. This must have happened naturally anyway, possibly more than once, since *Brassica napus* is the result of this cross (McNaughton, 1995). The hybridization between *B. oleracea* and *B. napus* is also extremely difficult, while the cross between *B. napus* and *B. rapa* is not very difficult to make (Yarnell, 1956).

Considering the primary genepool of *Brassica oleracea* itself, as indicated above, all the species belonging to it are interfertile. The results of crossing experiments performed to determine the potential gene transfer from one to another species of this group were published by Gustafsson (1982), with the following findings:

> 1) breeding barriers, resulting in reduced hybrid fertility, exist between some of the wild species and cultivars but not between others.

2) male fertility and seed setting is variable in both F_1 and F_2, but in most combinations sufficient to allow the production of further hybrid and backcross generations.

3) in most cases genes or gene blocks, which are valuable from an agricultural point of view, can be transferred from wild species to different cultivated forms.

Crossing experiments between cultivated or wild *B. oleracea* and six wild species of the *B. oleracea* group were carried out by Kianian and Quiros (1992). High fertility hybrids were produced by *B. bourgeaui*, *B. cretica*, *B. montana* and wild *B. oleracea*. Hybrids with reduced fertility values were obtained from crosses with *B. incana*, *B. insularis* and *B. rupestris*.

More recently, Bothmer *et al.* (1995) reported the results of an extensive research to define in detail the relative fertility of the species belonging to the section *Brassica*. The crossing programme included 10 wild taxa and 23 accessions representing 6 major cultivated forms and landraces of the *B. oleracea* group. They showed that all the cultivated forms were closely related, with very weak crossing barriers among them. Moreover, the wild forms of *B. oleracea* were significantly more interfertile with the cultivated forms than with any of the other wild species. Fertility in crosses between wild relatives and cultivated forms ranged between 52-79% in F_1 and 56-84% in F_2, with the lowest values obtained by crosses involving *B. macrocarpa*, *B. montana* and *B. rupestris*; the highest values were obtained in the crosses involving *B. cretica*. Some discrepancies between the experiments of Kianian and Quiros (1992) and those of Bothmer *et al.* (1995) relate to the very different fertility rates recorded for *B. montana* and *B. insularis*. These differences might be attributed to the use of materials from different sources and geographic locations, as well as to the different environmental conditions under which the crosses were performed.

1.1.6 Phylogenesis

Studies based on cytological analysis, molecular markers and sequence data provide the basis to understand the evolution of the family and of its lineages. A better knowledge of the phylogeny and genetic relationships of the brassica crops and their wild relatives can explain, *inter alia*, the evolution of the chromosome numbers and the origin of physiological traits, and indicate the most important wild germplasm that could be used for crop improvement.

Classical taxonomy of Brassicaceae has relied on characters that exhibit substantial homoplasy, especially the fruit traits. An explosion of fruit morphologies, likely controlled by few genes, occurred independently of other morphological aspects and therefore led to inadequate taxonomy, for example obscuring the true relationships within the tribe Brassiceae (Al-Shehbaz, 2006).

Given the results of molecular phylogenesis, the *Brassica*'s genepool base may actually be larger than formerly thought on the basis of traditional taxonomy. Previously unsuspected closely related genera may thus be relevant sources of traits for use in crop improvement (Warwick and Hall, 2009).

Brassicaceae and Brassiceae phylogeny

Early studies by Röbbelen (1960) on the three diploid crop brassicas established that only six of their chromosomes are unique, while the others are homologues. This discovery led to the hypothesis of a now-extinct ancestor with x=6 chromosomes from which today's species were derived.

Clarification of the cytological history and phylogenesis within the family Brassicaceae was then spurred by the studies made on *Arabidopsis*, which became a model organism for cytogenetic studies in the 1990s and for comparative genomic studies after the sequencing of its genome in the year 2000.

As a result of comparative genetic mapping, comparative chromosome painting (CCP) (i.e. fluorescent *in situ* hybridization or FISH) and sequencing, the following evolutionary events were reconstructed (partly reviewed in Lysak, 2009):

- An ancestral crucifer karyotype (ACK) with n=8 chromosomes can be hypothesized, representing a fictious extinct species *Crucifera ancestralis*, from which the *Arabidopsis* karyotype derived as a result of 3 chromosome fusions.
- A set of up to 24 conserved genomic blocks (named A to X) can be identified as belonging to the ACK. These were subsequently reshuffled through various rearrangements within the Brassicaceae (for example, 21 genomic blocks of *Arabidopsis* can be assembled to create the extant genome of *B. napus*).
- The whole family Brassicaceae underwent three rounds of whole-genome duplication. These events could only be detected through sequencing and they were estimated to have occurred before the *Arabidopsis-Brassica* split, i.e. not earlier than 24 Mya and possibly about 40 Mya (paleo-polyploidization events; Franzke *et al*., 2011). The duplication at this time of a proto-karyotype of n=4 chromosomes is hypothesized of having played a pivotal role in the ecological diversification and dramatic increase of species number of Brassicaceae. The Irano-Turanian region, which hosts the highest diversity of Brassicaceae and particularly Turkey with its peak of 560 species, is believed to be the cradle of the family.
- Three major lineages (I, II and III) are distinguished within the family Brassicaceae. The tribe Brassiceae is placed within the lineage II, together

with the tribes Thelypodieae, Sisymbrieae and Isatideae (Franzke *et al*., 2011).

- A number of *Brassica* and other Brassiceae genomes underwent triplication through a hexapolyploidization event, since three copies of ancestral genomic blocks can be observed cytogenetically with CCP analysis. This event was dated as occurring between 8 and 17 Mya (meso-polyploidization event).
- Rearrangements of the triplicated blocks, including chromosome duplications, deletions, translocations, transpositions of elements, deletions and fusions, have led to the present variation of chromosome numbers within the Brassiceae (Lysak *et al*., 2005; 2007; Lysak, 2009; Warwick and Hall, 2009).
- Apart from the above-mentioned tribe-specific cytogenetic data, the tribe Brassiceae is confirmed to be monophyletic also on the basis of cpDNA markers, as well as on a morphological basis, as indicated in the previous chapter 1.1.2.
- Several molecular studies based on restriction site analysis of chloroplast and nuclear DNA markers (references in Warwick and Hall, 2009) have shown little support for the subtribes Brassicinae, Moricandiinae and Raphaninae. Former members of these subtribes are split across the Rapa/Oleracea and Nigra lineages, also called *Brassica* and *Sinapis* lineages and including all the brassica crops. Within their lineage, *Brassica rapa* (A-genome) and the *B. oleracea* group of species (C-genome) resulted the most closely related to each other. The most important genera in the Brassiceae, *Brassica*, *Diplotaxis*, *Erucastrum* and *Sinapis* all showed a polyphyletic nature, based on cpDNA markers, ITS, *mat*K and *phy*A sequences (references in Warwick and Hall, 2009).
- The most recent formation of allopolyploids, such as those represented in the triangle of U (neo-polyploydization events), have recently been re-confirmed through the identification of the A-, B- and C-genomes through fluorescent *in situ* hybridization (Snowdon *et al*., 1997).
- The latest studies by Arias and Pires (2012), based on data from four rapidly evolving non-coding chloroplast regions, indicated a subdivision of the overall tribe Brassiceae into eight lineages, called by these authors Cakile, Crambe, Henophyton, Nigra, Oleracea, Savignya, Vella and Zilla. The relationships between these lineages were fully resolved, with high bootstrap values. The Oleracea lineage includes the A- and C-genome species, as well as *B. napus* (A- and C-genomes), *B. juncea* (A- and B-genomes) and other *Brassica*, *Diplotaxis*, *Eruca*, *Raphanus* and other species. The Nigra lineage includes *Brassica nigra* (B-genome), *B. carinata*

(B- and C-genomes), *B. fruticulosa*, *B. tournefortii* and other *Brassica* species, as well as *Sinapis* and other genera. Although very strongly supported, the phylogenetic tree presented by these authors still requires to be tested with nuclear genes, since incongruence of chloroplast-based with nuclear-based topologies were shown to occur (Hall *et al.*, 2011). The incongruence is likely due to the fact that chloroplasts only trace the maternal lineage, while several past hybridization and polyploidization events have occurred within the family.

- Further studies by Arias *et al.* (2014), using a combination of molecular phylogenetics, diversification analysis and historical biogeography, found the evidence that the tribe Brassiceae originated in the area at the intersection between the Arabian Peninsula and North Africa (Saharo-Sindian region), about 24 Mya. The Nigra lineage then diverged from the Oleracea/Rapa lineage about 20 Mya and the former continued to differentiate in the southwestern part of the Mediterranean region, while the latter diversified in the Saharo-Sindian region and later colonized the European Mediterranean through the east. A 'core Oleracea' clade, containing *B. rapa* and the *B. oleracea* group of species originated in the northeastern Mediterranen ca. 6.5 Mya, possibly at the time of the Messinian Salinity Crisis. At this time, the Mediterranean went through cycles of partial desiccation lasting several hundred thousand years, reducing its extension, while salt deposits were accumulating and new drier and open environments were being created. According to these authors, *B. oleracea* and its C-genome wild relatives diversified in the northeast Mediterranean region about 1.5 Mya and then spread through the rest of Europe. At about the same time (ca. 2 Mya), *B. rapa* ancestors dispersed throughout the Irano-Turanian region reaching Central Asia. According to this scenario, after 20 Mya of evolutionary divergence, *B. rapa* and *B. oleracea* maintained the ability to hybridize with *B. nigra* and generate the respective allopolyploid *B. juncea* and *B. carinata* at the crossroads of their distribution area or in cultivated environments.

Genetic relationships between the species of the B. oleracea *group*

In her doctoral Thesis, Lannér (1997) summarized the existing knowledge of the genetic relationships within the *B. oleracea* cytodeme. On the basis of crossing experiments (Kianian and Quiros, 1992), high fertility values were detected within three groups of species (or karyotypic types), namely: 1) *B. bourgeaui*, *B. cretica*, *B. montana* and *B. oleracea*; 2) *B. incana* and *B. insularis*; 3) *B. rupestris*. High fertility was also registered by Snogerup (1993) in crosses between *B. rupestris* and *B. macrocarpa* (89%), but not in crosses between *B. incana* and other species (*B. macrocarpa*, *B. rupestris* and *B. villosa*) (24-64%). Low fertility was shown in all crosses with *B. cretica*.

Various molecular methods were applied by different authors to various sets of species (Song *et al*., 1990; Warwick and Black, 1993; Lannér, 1997). These studies are not immediately comparable among one another, since the markers used (nuclear and chloroplast RFLPs, random genomic cDNA probes and a variable intergenic region of the chloroplast genome) and the sets of compared species always differed. Overall, the results do not allow a clearcut understanding of the genetic relationships. The only somewhat consistent pattern emerging from the above studies was the close association between the Sicilian *B. rupestris* and *B. villosa*.

A clearer pattern was provided by the studies of Mei *et al*. (2010), who carried out a robust analysis of 10 wild taxa and 7 brassica crops of the *B. oleracea* cytodeme, with 301 polymorphic bands from 11 AFLP primers, combined with 518 polymorphic bands from 108 SSR primers. The results indicate that the Sicilian wild taxa *B. macrocarpa*, *B. rupestris*, *B. insularis* and *B. villosa* cluster together and are more distantly related to the cultivated crops than other C-genome wild taxa. These findings were in line with previous studies made by Lannér *et al*. (1997), Lázaro and Aguinagalde (1998b), Lannér (1998), Tatout *et al*. (1999) and Geraci *et al*. (2001).

A fully resolved phylogeny of the tribe Brassiceae was obtained by Arias and Pires (2012), using sequence data from 4 chloroplast non-coding regions. Also in this case, a well-supported clade separates the Sicilian wild taxa *B. macrocarpa*, *B. rupestris*, and *B. villosa* (position of *B. insularis* not shown in the paper) from the remaining C-genome taxa, including *B. oleracea* crops.

1.1.7 Genetic resources

Genetic resources provide the diversity that is the basis for crop improvement. It is critical that these resources be properly conserved and made accessible for use in breeding and research. Genetic resources can be defined using the genepool concept of Harlan and de Wet (1971). In the case of *Brassica oleracea*, the primary genepool, including all the taxa that easily give fertile hybrids, can be represented by the C-genome taxa of the *B. oleracea* group. The secondary genepool, represented by the taxa that are potentially capable of experimental hybridization with *B. oleracea*, is extended to the so-called *Brassica* coenospecies (see above).

Genetic resources are conserved as *ex situ* seed samples in genebanks and are also present as *in situ* populations (in natural environments or in the farmer’s fields). Molecular resources are also increasingly useful, including expressed sequence tags (ESTs), full length cDNA clones of many genes, bacterial artificial chromosome (BAC) libraries, etc.

A review of the status of genetic resources of cruciferous crops was published by IBPGR (1981). At this time, priorities for collecting specific geographic areas with evident gaps in the collections were outlined. In the following years, several international (Meer, 1984) and national collecting missions (Gass *et al*., 1994; Maggioni *et al*., 1997) were organized, at least partially filling the gaps. *Brassica* genetic resources were again globally reviewed in recent years by Boukema and Hintum (1999) and by Maggioni (2004) for the European region. More recently, an overview of the availability of wild (2n=18) *Brassica* was provided by Maggioni *et al*. (2013), while the molecular resources were reviewed by Knee *et al.* (2011).

Among the online databases offering information on the *ex situ* collections, FAO WIEWS (2015) offers metadata at the global level, accounting for over 19 000 *ex situ* accessions of *B. oleracea*. According to this catalogue, the largest national collections are in the UK (24%), USA (10%), China (9%), France (8%) and Germany (7.5%). The European Plant Genetic Resources Search Catalogue EURISCO (2015) offers information with detailed accession level, accounting for over 11 500 *B. oleracea* accessions conserved in European genebanks. According to this catalogue, the largest European collections are in the UK (35%), Russian Federation (13%), Germany (13%) and Spain (7.5%). The ECPGR *Brassica* Database (http://documents.plant.wur.nl/cgn/pgr/brasedb/), managed by the Centre for Genetic Resources, the Netherlands (CGN), includes data from 65 European institutions and lists ca. 11 400 *Brassica oleracea* accessions. This database is also linked to evaluation data on disease, pest and abiotic stress resistance generated during EC-funded projects.

Independently from discrepancies between catalogues, which are related to different data flow mechanisms, it is evident that the *Brassica* collections are dispersed across several genebanks. The numbers of accessions, the coverage of crop types and of geographic origins indicate the presence of a large amount of genetic diversity currently conserved. However, from the information at hand, it is difficult to evaluate the actual availability and quality of the material, as well as to trace duplications and possible remaining gaps. Inevitably, the quality of conservation and the modality of access to the various collections are expected to be variable. In any case, the '*Brassica* complex' (including *Brassica* and other genera) is part of the list of crops in Annex I of the legally-binding FAO International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2002). This list establishes the Multilateral System, whereby all Annex I crops' accessions in the public domain and under the control of the governments that are parties to the Treaty, should be exchanged according to standard terms and conditions for access and benefit-sharing. In Europe, the initiative of the European Cooperative Programme for Plant Genetic Resources (ECPGR) to establish A European Genebank Integrated System (AEGIS) not only aims at harmonizing the procedures of access to germplasm of all crops, in line with the principles of the Treaty, but also intends to guarantee a high-quality long-term conservation under the shared responsibility of the AEGIS member countries (ECPGR, 2009). Presently, 462 *Brassica oleracea* accessions conserved in the Netherlands are formally included in the decentralized European Collection under the AEGIS framework.

Wild species belonging to the C-genome have been the object of several IBPGR-sponsored collecting missions between 1982 and 1988, collecting over 230 samples of both Mediterranean and Atlantic taxa (IBPGR, 1993). These initiatives, together with a number of national collecting missions, specifically in Italy and Spain (Hammer *et al*., 1987 and references therein; Gómez-Campo *et al*., 2005; 2008) have secured a fairly good representation of the genetic diversity of the wild species in genebanks and built up a good knowledge base of the distribution of *in situ* localities, size of populations, extent of variability and existing threats (Snogerup *et al*., 1990). Specialized collections of wild species were established at the Crucifer Seed Bank of the Universidad Politécnica de Madrid (UPM), Spain and at the University of Tohoku, Japan (Gómez-Campo, 2009).

As a matter of fact, the existing collections still present gaps that should be filled in (low or absent representation of sites from the Adriatic coasts of Albania, Croatia and Italy, the Aegean coasts of Greece and Turkey, northern Cyprus, Algeria and Tunisia). Unfortunately, also material already under conservation is often not available due to low seed quantities. Regeneration and multiplication is expensive, since it requires the management of special pollinators and the adoption of isolation measures to avoid contamination across different accessions. The Mediterranean species are easy to reproduce under the environmental conditions of their origin, but may fail to go to flower or set seeds in more northern conditions.

Numerous are therefore the reasons to maintain *in situ* populations, not only to guarantee the continued survival of natural evolutionary processes, but also to complement *ex situ* conservation with alternative sources of material to be used for research and breeding. The importance of facilitated access to *in situ* populations and the existing constraints are dealt with in Paper V.

The establishment of *in situ* genetic reserves of wild populations has been recommended on numerous occasions by the ECPGR *Brassica* Working Group

(Gómez-Campo and Gustafsson, 1995; Gustafsson, 1995; Maggioni *et al*., 1997). Sicily has often been pointed out as a priority area due to the high diversity that can be found in this island where at least seven species are present (Raimondo, 1997; Branca *et al*., 2012). Other priorities were also identified in a case study on *Brassica* carried out by the EU-funded project AEGRO (Kristiansen and Bjørn, 2012). A recently approved ECPGR concept for the conservation of crop wild relatives (Maxted *et al*., 2015) promises to set the scene for the effective establishment in Europe of a network of genetic reserves and the wild brassicas are expected to play a prominent role as unique European target species for priority reserves.

Local forms of cole crops are still extensively grown in home gardens all over Europe and beyond, as testified by several papers pointing out at the rich diversity still present on farm (Dias, 1995; Cartea *et al*., 2002; Laghetti *et al*., 2005; Balkaya and Yanmaz, 2007; Padilla *et al*., 2007; Farnham *et al*., 2008; Matotan and Samobor, 2008; Vilar *et al*., 2008; Scholten *et al*., 2008; Kahn *et al*., 2010; Branca *et al*., 2013; Papers II and IV). A comprehensive inventory of on-farm diversity is not available. This is a difficult task to achieve considering the effort that would be required to keep such a database up to date in view of the variable presence of material in on-farm conditions. However, national or local inventories of landraces have been prepared with different approaches by various countries and local administrations (Veteläinen *et al*., 2009). A compilation of existing inventories has recently been promoted by the EC-funded Project PGR Secure (www.pgrsecure.org).

Among the genetic resources, an increasingly important section is composed of special genetic stocks, such as diversity sets and mapping populations, as well as clone libraries. A special set of resources is also represented by genomic and phenomic information. A web site collating and exchanging open source information related to *Brassica* genomics and genetics, including registries of reference datasets and nomenclature standards, a comprehensive compilation of ongoing public domain genome sequencing and searchable access to databases of genetic tools and stock material, is *brassica.info* (www.brassica.info), which is hosted and maintained by the Australian Southern Cross University on behalf of the Multinational Brassica Genome Project.

1.1.8 Breeding prospects

The large plasticity of *B. oleracea* has given rise to an impressive differentiation of crop types in Europe. Within each crop, a great variability of morphologies, colours, productive periods and environmental adaptabilities has been documented (Helm, 1963; Branca, 2011; Branca *et al.* 2013).

Heterogeneity of populations is dependent from the crop's reproductive system, with prevalence of self-incompatible outcrossing types. During the 19th and 20th centuries, mass and recurrent selection were applied to achieve a greater uniformity and higher productivity, in parallel with the development of large-scale production for the market. The introduction of F_1 breeding programmes started as early as during the 1950s, with the aim of exploiting heterosis and obtaining harvest uniformity for industrial purposes.

Inbred lines can be obtained by overcoming the self-incompatibility barriers, either with hand self-pollination prior to anthesis, when the self-incompatibility system is not yet functional, or with chemically inducing methods (NaCl or CO_2). More recently, the use of double haploids obtained through microspore culture was widely used to create parent inbred lines. For the production of hybrid seed, the sporophytic self-incompatibility mechanism has been put to use, although sometimes this incompatibility may be disrupted by high temperature, high humidity or other environmental conditions. The transfer of the Ogura cytoplasm from radish (*Raphanus sativus*) to *B. oleracea* via protoplast fusion (Pelletier *et al.*, 1988) offered a valuable source of cytoplasmic male sterility (CMS). This CMS has been routinely transferred to several lines, to be used as parents for hybrid production. Another source of CMS has also been transferred from *B. rapa* to *B. oleracea*, also with protoplast fusion (Cardi and Earle, 1997).

Genetic improvement of the cole crops in the last decades mainly concerned cabbages, cauliflower, broccoli and Brussels sprouts. The breeding goals resulted in vigorous, high yielding and uniform F_1 cultivars. Biotic and abiotic stress resistance have been an important breeding objective, which will continue to be important, particularly for organic and low-input farming systems, but also for conventional agriculture in view of climate change adaptation and sustainable cultivation. Additionally, appearance (colour and shape), taste and nutritional qualities have become increasingly important to address the consumer's preferences. Suitable traits for high commercial quality, such as size and shape, colour persistence, firmness and bruise resistance have been pursued.

In the case of cabbage, high head weights are demanded for industrial processing, but also small heads and low leaf size for dense planting are needed. Market demands and preferences can vary in different countries, with compact head desired in central Europe for industrial processing, but flat or pointed cabbages preferred elsewhere. Harvest date and winter hardiness are important traits, as well as resistance against bolting and splitting of the heads. Good standing ability is important for plant health and for mechanical harvesting (Becker *et al.*, 1999).

Major breeding achievements of cauliflower and broccoli have been the modification of the harvest index, with an increased reproductive portion. Size uniformity is requested by the freezing industry. The cauliflower curd colour is subject to consumer preferences and work has been dedicated to obtain purely white or creamy white heads, but also to develop more colourful green, purple and orange types, with improved nutritional quality derived from anti-oxidant antocyanins and carotenoids (Acciarri, 2004). Reduction of glucosinolate content has been pursued to obtain a smoother taste, although these cultivars resulted more sensitive to insect damage (Branca, 2011).

A source of resistance to Fusarium yellows (*Fusarium oxysporum*) has been successfully used in cabbage cultivars in North America for more than 70 years. This condition identified in 'Winsconsin Ballhead' and designated 'Type A', is an example of single dominant long-lasting resistance (Quiros and Farnham, 2011).

Sources of resistance were identified against clubroot (*Plasmodiophora brassicae*). However, only few have been effectively used in cultivar development, since this destructive disease maintains a large variation. Similarly, breeding lines have been developed with resistance to black rot *(Xanthomonas campestris*), possibly the most serious disease of crucifers worldwide, but the inheritance of resistance is unstable, since it is influenced by the pathogen's isolates (Quiros and Farnham, 2011). Downy mildew (*Hyaloperonospora parasitica*) can be very destructive to young seedlings. Research has identified both monogenic and multigenic sources of resistance and associated molecular markers have been localized on the *B. oleracea* genetic map (Quiros and Farnham, 2011). Resistance to Verticillium wilt (*Verticillium longisporum*) has been identified in *B. incana* (Happstadius *et al*., 2003). No specific resistance genes are known that can prevent damage by insects. Those that have been studied are quantitative in nature and with low heritability. A relative resistance to several insect pests has been associated to a glossy leaf phenotype (Quiros and Farnham, 2011). Trichome-based resistance against flea beetles was studied in *B. villosa* (Palaniswamy and Bodnaryk, 1981) and resistance to the cabbage aphid *Brevicoryne brassicae* was detected in *B. fruticulosa* (Pink *et al*., 2003). Resistance to white fly (*Aleyrodes proletella*) that is expressed by plants at the early age of 6 weeks was found for the first time in *B. villosa*, *B. incana* and *B. montana* accessions by Pelgrom *et al.* (2015). In *B. incana*, the presence of trichomes is likely to be responsible for the observed resistance (Vosman *et al*., 2015).

The opportunity to breed for higher content of potentially beneficial (antioxidant and anti-cancer) glucosinolates has been used with the development of the 'Beneforte' broccoli, resulted from a cross with *B. villosa* from Sicily (Mithen, 2014). Recent evidence from human studies indicated that consumption of high glucoraphanin broccoli significantly reduce plasma LDL-cholesterol (Armah, 2015).

The rapidly growing knowledge of the *Brassica oleracea* genome offers today an assembled sequence covering about 75% of the estimated genome size (Parkin *et al*., 2014), and the availability of thousands of markers and of highly saturated genetic maps (http://www.brassica.info/resources). These tools will undoubtedly help the future breeding efforts. Marker-assisted selection and genetic transformation (Poulsen, 1996) will also provide increasing precision and extended options to aid conventional breeding.

1.2 Domestication and origin of crops

1.2.1 Definition of domestication and origin of crops

The first chapter of the *Origin of species* was dedicated by Charles Darwin (1859) to the study of variation under domestication. The modifications of plants and animals under man's control served Darwin well to point out the strong and visible effects of artificial selection. As specified by Harlan (1975), to domesticate means to bring into the households. With reference to plants, it is important to distinguish cultivation from domestication, since these terms have sometimes been used as synonyms or have been attributed different meanings, as reviewed by Ladizinski (1998). Still today, the concept of domestication may be approached in different ways. For example, an anthropological view holds domestication as a sustained multigenerational, mutualistic relationship between two organisms (Zeder, 2015). Such definition emphasizes the coevolving mutualism between the manager and the managed resource, but it seems to fall short of addressing the key effects of such relationship, which are the hereditary changes. In this Thesis, 'cultivation' refers to all the activities related to growing plants in a more or less manipulated context, while 'domestication' is an evolutionary process during which genetic shifts or alterations occur. As clearly pointed out by Harlan (1975), as a result of such process, a fully domesticated plant or animal is completely dependent upon man for survival, but different degrees of differentiation or intermediate conditions may exist between the wild and the fully domesticated condition. It is possible to cultivate wild plants, although continued cultivation will tend to increasing adaptation to a cultivated environment and decreasing adaptation to the original habitat. Therefore, not all the cultivated plants are necessarily fully domesticated.

A certain degree of ambiguity also surrounds the word 'crop', as indicated by Harlan (1975) who titled a chapter of his book with the question "What is a crop?”. In general terms, any type of harvest, either from the wild or from a cultivated field may be called a ‘crop’, independently from the level of domestication of the species involved. In more restrictive terms, crops are considered those cultivated plants that have developed domesticated forms. In this sense, the search for the ‘origin of crops’ is related to understanding when, where and how a certain plant has acquired those traits that distinguish it from its wild progenitor and that can only be maintained with human intervention, since they would be disadvantageous and selected against in a natural context.

The origin of crops through domestication is therefore an important theme, that is part of the wider proposition of understanding the origin of agriculture, together with the revolution in human societies that it has brought about since its establishment about 10 000 years ago. The presence in the archaeological records of plant remains showing evidence of domestication is essential to orientate scholars in place, space and time regarding the introduction and development of agriculture. Groups of specific traits recurring in the cultivated plants supported the concept of a ‘domestication syndrome’ (Hawkes, 1983; Hammer, 1984), comprehending traits that differentiate the crop from its wild progenitor. In some cases these traits make the crop unable to survive in the wild, and in all cases they make them more suitable to use by farmers. Non-shattering ears of cereals, suppressed distribution mechanisms (indehiscence) and increased size of grains or edible parts can be recognized in archaeological records and testify the domestication process. Loss of seed dormancy, reduced branching to a single central stem, uniform flowering and seed maturation, increased selfing behaviour, vegetative reproduction replacing sexual reproduction and changes in secondary metabolites, such as the loss of bitter or toxic compounds, are on the other hand not easy to identify in the archaeological remains.

A useful distinction about phenotypic changes during domestication is between pure ‘domestication traits’ and other ‘improvement traits’ (Olsen and Wendel, 2013). While the former are fixed during the initial process of domestication and are common to the entire crop species, the latter are variable among cultivars of the crop and relate to adaptations to specific improvement needs (morphology or pigmentation of edible parts, adaptation to different climates, nutritional composition, etc.).

The genetic architecture and molecular basis of the changes brought about by domestication and crop improvement is being increasingly understood. Generally, selection for domestication traits drives the crop through a bottleneck of genetic drift, decreasing its diversity compared to the wild progenitor. In this case, specifically targeted genomic regions can present a molecular signal of selection, with very low levels of genetic diversity that indicate domestication-related traits. Thanks to a combination of molecular technologies and improved experimental design, including nested association mapping, genome-wide association studies, population genetic screens and candidate gene approach, an increasing number of domestication and crop improvement related genes has been identified (Olsen and Wendel, 2013). The resulting picture shows that not only loss-of-function mutations, but also selection on regulatory genes and on transcriptional regulators are involved in the domestication process. Actually, genome-scale systems biology is starting to show that a very large number of genomic regions is involved into a 'large-scale rewiring' of the transcriptome in response to domestication and therefore this field of research is also offering new insights for understanding evolutionary processes as a whole (Olsen and Wendel, 2013).

The search for the actual location(s) and specific wild population(s) from which a crop has derived (the origin of a crop) offers the opportunity to understand the distribution of the genetic diversity of a given crop's genepool. This knowledge has implications for a better conservation and use of the genetic resources. The identification of the correct progenitor wild species will indicate which taxa are closer to the given crop and can be used for introgression of useful traits, as well as which efforts should be made for their preservation. Moreover, the identification of a specific progenitor population or geographic area where the crop has evolved can hint to the opportunity to look for additional diversity in more distant geographic areas. Understanding the genetic changes following domestication can also better guide the strategies for crop improvement (Gepts, 2004).

1.2.2 Methodologies to discover the origin of crops

Precursor works on the origin of crops, exemplified by the linguistic comparisons of de Candolle (1885) and by the studies of variation and distribution of crops by Vavilov (1926), are being updated today by a wealth of data derived from many disciplines, including archaeology, cytogenetics, molecular genetics, chemistry, anthropology, linguistics and eco-geographic modelling. As reviewed by Zohary *et al.* (2012), the main sources of information are plant remains from archaeological excavations, the evidence derived from the living plants and other pertinent sources. The main questions to be answered are listed by Zohary *et al.* (2012) as follows:

(i) When and where do we find the earliest signs of domesticated crops?
(ii) How and when did the crops spread to attain their present distributions?
(iii) What were the early cultigens like?

(iv) What were the main changes in the crops once they were introduced into cultivation?

(v) Where and when did these changes take place?

Partial answers to these questions may be derived from the identification of plant remains in the ‘fossil records’. These may consist of charred (carbonized) plant material that were exposed to fire under low oxygen condition and therefore preserved their morphological or anatomical features.

Grains or other plant material may leave imprints on pottery, daub or bricks. Desiccation under extreme dryness allows perfect preservation of grains, fruits, parts of vegetables, leaves and flowers, such as in Egyptian tombs, sites on the shores of the Red Sea and caves in the Dead Sea basin. Waterlogged preservation in anaerobic conditions is the case of lake-shore dwellings, bottoms of old wells as well as the stomach content of corpses retrieved from bogs. Phytoliths in food residues (siliceous microscopic structures found in some plant tissues and persisting after the decay of the plant), human feces (coprolites) and organic residues in ancient vessels are other potential sources of information.

Evidence from the living plants involves the search for the wild progenitors, i.e. the nearest relatives of the domesticated crop. These can be identified by classical taxonomic studies (comparing the morphology and anatomy of the various species) or by comparisons based on cytogenetic analysis or on genetic affinities determined by analysing DNA, proteins or other metabolites.

The distribution and ecology of the wild progenitors, when known, can narrow down the geographic area of the original domestication, under the caveat that the current distribution has not changed since the time of introduction into cultivation.

Other pertinent sources of information include historical information (representation of plants in art, documentations in inscriptions or literature), linguistic comparisons of the names of plants in various languages and other circumstantial evidence, based on geological, climatic, anthropological or other evidence.

1.2.3 Status of knowledge of crop domestication in Europe

Based on an increasingly rich set of molecular data and archaeological findings, it is known today that agriculture and the domestication of plants and animals started independently in at least eleven regions of the world, following the end of the last Ice Age between 12 000 and 11 000 years ago (Larson *et al.* 2014). Climate change and human population pressure are considered the most important driving factors of the switch from hunting and gathering to the adoption of agriculture, but also alternative cultural factors, independent from the scarcity of resources, have been proposed and the debate on different possible explanations is still showing tension among different scholars (Gremillon *et al*., 2014; Larson *et al*., 2014; Zeder, 2015).

The most important centre of domestication for the beginning of agriculture in Europe is the Fertile Crescent. Other centres are located in tropical or subtropical regions of Africa, Asia and America, with the exception of eastern North America and Central Asia (Gepts *et al*., 2012).

The earliest signs of definite plant domestication are dated ca. 10 500–10 100 calibrated years BP, appearing in a series of farming villages of the Fertile Crescent. The data refer to cereals (emmer, barley and einkorn wheat) showing spikelet forks with rough disarticulation scars. A group of annual legumes (lentil, pea, chickpea and bitter vetch) were domesticated at about the same time and accompany the first cereals in the archaeological records. This group of crops, together with flax, constituted a ‘Neolithic crop assemblage’ that rather rapidly was adopted, spreading both west (to Europe) and east (to Central Asia and the Indian subcontinent). By the end of the 8th millennium BP, these crops, in various combinations, were grown all over Europe, in Egypt and Central Asia (Zohary *et al*., 2012).

The geographic distribution of the identified wild progenitors of the above-mentioned ‘founder crops’ generally corresponds today with the area of the earliest archaeological findings, centred in the Fertile Crescent. For example, wild emmer wheat and wild chickpea are endemic to the Fertile Crescent and therefore domestication can only have occurred in this restricted area (Zohary *et al*., 2012). Other crops were brought into domestication in Europe, outside of the Fertile Crescent, as testified for example by the case of poppy (*Papaver somniferum*), with a central-western Mediterranean distribution of the wild progenitor and a well-documented archaeological series of remains from western Europe (Zohary *et al*., 2012).

It is not easy to distinguish wild from domesticated remains of fruit trees in the archaeological records, therefore domestication of fruit crops is known on the basis of circumstantial evidence, such as the presence of fruit remains or artefacts in areas where the wild forms do not occur. Indications exist that olive, grapevine, fig, date palm and pomegranate formed a first wave of domesticated fruit trees in West Asia and Europe, which were introduced into orchards after the domestication of grain and pulses, certainly in the fifth millennium BP, if not earlier. Cultivation of these fruits depended on a fully settled way of life and the invention of vegetative propagation. A second wave of fruit crops entered into cultivation much later, with evident signs of domestication only in the third millennium BP. These crops, including apple, pear, plum, sweet cherry, carob and pistachio, required the mastering of the grafting technology, which is documented in texts of ancient Greek literature (Zohary *et al*., 2012).

Vegetables are the least-known group of domesticated plants, owing to the perishable nature of the anatomical parts that are used for food. Only exceptional findings come from particularly favourable conservation environments such as the dry conditions of the Egyptian tombs or Judean desert caves. In these contexts, dating back to the second and first millennia BCE, garlic, leek, onion, lettuce, melon and watermelon remains could be recovered and identified. Alternative sources of knowledge are the Mesopotamian Bronze Age literary sources and drawings and descriptions found in Egyptian tombs (Zohary *et al*., 2012). A list of plants grown in the garden of Merodach-Baladan in Babylonia (ca. 2720 BP) enumerates for the first time beet, turnip and cress (Körber-Grohne, 1995). By classical times, Jewish, Greek and Roman sources indicate that the number of cultivated vegetables had largely increased, including also coles, celery, carrot, parsnip, asparagus and many others.

Among the recent vegetables domestication works, a significant example was the study of the genetic structure and domestication of carrot with 4000 SNPs developed from sequencing data of the carrot transcriptome (Iorizzo *et al*., 2013). Analysis of a sample of 84 genotypes of widely dispersed wild and domesticated carrot accessions revealed that wild carrots from central Asia were genetically most similar to cultivated types and therefore an origin of domesticated carrot in Central Asia was suggested. Also an absence of genetic bottleneck was noted, similarly to results obtained on einkorn wheat (Kilian *et al*., 2007), pepino (Blanca *et al*., 2007) and chicory (Cutsem *et al*., 2003), and differently from domestication bottlenecks observed in soybean (Hyten *et al*., 2006), rice (Londo *et al*., 2006), barley (Morrel and Clegg, 2007), maize (Tenaillon *et al*., 2004) and wheat (Haudry *et al*., 2007). The lack of genetic diversity reduction was interpreted in carrot to be linked to the outcrossing behaviour of this crop, the extensive use of an open-pollinated breeding approach before the development of hybrid cultivars in the 1950s, and the existence of a bidirectional gene flow between wild and cultivated carrot (Iorizzo *et al*., 2013).

Several items related to domestication research remain controversial. For example, fixation of domestication traits has been suggested to undergo a rapid evolution of a few centuries in cereals (Hillman and Davies, 1992; Abbo *et al*., 2010; Haldorsen *et al*., 2011) or alternatively to require a protracted evolutionary process lasting various millennia (Tanno and Willcox, 2006; Allaby *et al*., 2008; Brown *et al*., 2009). Another controversy relates to the question whether domestication took place in a rather restricted core area where domesticated varieties originated from a single or relatively few events and then were diffused outside of their area of origin, or whether the domestication of each crop repeated itself several times in different and independent geographic contexts. The former view is supported for example by Abbo *et al*. (2010) in the case of the Old World founder crops, based on archaeological data and eco-geographic and genetic studies, all pointing at southeastern Turkey as the area of origin of domesticated einkorn (Heun *et al*., 1997), emmer wheat (Özkan *et al*., 2002; 2005), lentil (Ladizinski, 1999) and chickpea (Ladizinski, 1995). The alternative vision suggests a dispersed group of parallel processes of plant domestication proceeding at different pace in multiple locations both within southwest Asia and in other regions (Fuller *et al*., 2011 and references therein).

Among the conclusions of a review of patterns and processes in crop domestication of 203 global food crops (Meyer *et al*., 2012), multiple origins were recorded to be proposed for only a small subset of the dataset (19%). The most common registered domestication syndrome traits were the changes in secondary metabolites affecting flavour, pigments and toxicity. Major gaps were found in the literature for many crops species, particularly with regard to ancestors, regions of origin and domestication dates.

Challenges in domestication research will be addressed by filling gaps in geographical and genomic maps, in order to better understand the relationship between domesticates and their wild ancestors. To this end, increasing sampling and methodological developments, such as geospatial modelling (Etten and Hijmans, 2010) will be useful. Bottlenecks leading to extinction and introgression that can lead to conflicting interpretations of genetic data, may be reduced in future with increasing analysis of ancient DNA to compare modern populations with those of the past (Jaenicke-Després *et al*., 2003; Kistler and Shapiro, 2011; Larson *et al*., 2014).

1.2.4 Studies on the domestication of *Brassica* crops

A number of studies to understand the origin of some of the *Brassica* crops of the U triangle (*B. rapa*, *B. napus* and *B. juncea*) have been concluded very recently.

A study to investigate the genetic structure and centre of origin of *Brassica rapa* was carried out by Guo *et al*. (2014), based on 51 SSRs detecting 715 alleles at polymorphic loci in 173 *B. rapa* accessions with a worldwide geographic distribution and including wild types. It was possible to distinguish this collection into three geographic groups. The wild types belonged to the Old World group (Europe, West Asia and North Africa) and this observation confirmed the Old World origin of *B. rapa*. A second group with high levels of genetic diversity, representing East Asia accessions was considered the effect of a secondary centre of diversification, while the third group, with low levels of genetic diversity, included migrants into East, South and Central Asia along the trade routes between east and west in Neolithic times.

The origin of *B. napus* was investigated by Allender and King (2010) with a combination of chloroplast and nuclear markers. Six chloroplast SSRs and AFLPs detecting 102 bands were applied to a sample of 198 accessions representing 6 *Brassica* species. The study concluded that it is very unlikely that *B. oleracea* or any other C-genome species acted as the maternal progenitor of most of the *B. napus* accessions. On the other hand, 'spring broccoli raab', a variety of *B. rapa* from southern Italy, was suggested to be the closest extant relative of the maternal ancestor of *B. napus*. As the existence of truly wild *B. napus* populations has never been documented, it is reasonable to speculate that the initial hybridization between *B. rapa* and *B. oleracea*, giving rise to the amphidiploid *B. napus* must have occurred in a cultivated context where the two crops were grown together. At the same time, multiple hybridization events are indicated by the presence of three different cytoplasms among the *B. napus* samples tested, although some of these may be the result of post-speciation introgression or deliberate interspecific crossings. Reduced diversity present in *B. napus*, as measured by the nuclear markers, is supportive of the relatively recent origin of this species.

Comparison of the orthologous genes between the A and C subgenomes in *B. napus* and the respective progenitor genomes suggested a divergence 7500 to 12 500 years ago and a formation of *B. napus* after this date (Chalhoub *et al*., 2014).

Kaur *et al*. (2014) suggested a polyphyletic origin of *B. juncea*, with *B. nigra* and *B. rapa* participating as cytoplasm donor parents in several independent hybridization events. This result was obtained by analysing 246 accessions of *B. juncea*, *B. rapa* and *B. nigra* using 9 chloroplast and 8 nuclear SSR markers, which were developed using sequence information of candidate genes related to flowering and seed dispersal. This work also confirmed the existence of two groups in *B. juncea*, one comprising East European and some Indian accessions and another one comprising accessions from India and China. The region of origin of *B. juncea* was suggested by Chen *et al*. (2013) to be in the Middle East and neighbouring regions where the distribution area of the progenitors overlapped. However, according to Kaur *et al*. (2014), the ancestral *B. rapa* genotypes that participated in original hybridization events might be extinct.

1.3 Domestication of *Brassica oleracea* L.

1.3.1 Status of knowledge and existing theories

Archaeological evidence

Very limited evidence about the prehistoric use of *B. oleracea* can be derived from archaeobotanical literature. This crop is mainly used for the leaves and other fleshy parts that are highly perishable and are usually not included among carbonized archaeological remains.

Brassica seeds are occasionally found in prehistoric archaeological excavations. However, reliable evidence of the use of *B. oleracea* in Neolithic or Bronze Age sites is currently missing, although records for *B. rapa* and *B. nigra* are better documented (Schlichterle, 1981; Körber-Grohne, 1995). Neuweiler (1925) described an imprint of a leaf and one broken seed of *B. oleracea* that were found together with three dozens of *B. rapa* seeds among the sediments inside a late Bronze Age terra-cotta vase at the pile-dwelling site of Alpenquai, by the lake of Zurich, Switzerland. The author believed that the leaf could be attributed to var. *capitata* (head cabbage), on the basis of the leaf veins. However, both Schlichterle (1981) and Körber-Grohne (1995) considered the determination of the cabbage leaf made by Neuweiler rather questionable and the possibility was advanced that the seeds could be the result of a secondary deposit inside the vase.

The systematic presence of wild *B. rapa* seed is documented from several Neolithic dwelling sites near lakes and marshes on the northern side of the Alps, in Switzerland, or in the French Jura. Since these seeds are usually found dispersed among other plant remains in a disorderly way, they have been interpreted as occasional weeds, but at some point these seeds may have been conscientiously collected and appreciated for their oil content as integration to the diet. This hypothesis was made by Schlichterle in 1981, following the finding of a clump of carbonized seeds of oil-rich species such as wild *B. rapa* and *Descurainia sophia* near the lake of Constance (Körber-Grohne, 1995).

The difficulties in the identification of archaeobotanical *Brassica* records are well described by Tomlinson and Hall (1996), who pointed out that there are no significant differences in seed size and shape which are appropriate for the identification of *Brassica* fossil material, while the only distinguishing characters are the reticulate patterns on the seed surface (testa) (Berggren, 1960; 1981). Therefore, only very well preserved seeds can be identified with certainty. A survey of the Archaeobotanical Computer Database (ABCD) indicates that this is a very rare occurrence. The ABCD is an electronic compilation of information about plant remains from archaeological deposits throughout the British Isles (Tomlinson and Hall, 1996). There is no certain record for *B. oleracea* in this database, but only a few uncertain identifications, the oldest being related to 4 seeds found in a sewer deposit apparently serving the bath house of the legionary fortress at a late Roman site by Church Street, York, England (Greig, 1976). *Brassica* sp. samples were on the other hand found in prehistoric times in various sites from England, Ireland and Scotland. The earliest records were dating back to the Neolithic period (5 waterlogged *Brassica* sp. seeds found in a turf layer of a burial cairn in Meath County, Ireland, dated 2650–2230 BCE) (Groenman-van Waateringe and Pals, 1984; Monk and Williams, 1984) or even to the earlier middle Neolithic. To this last period were related 62 waterlogged *Brassica* sp. seeds documented by Greig (1991) from a channel sediment by a riverside settlement at Runnymede, Berkshire, England.

The online database of Dr Helmut Kroll on *Literature on archaeological remains of cultivated plants 1981-2004* (http://www.archaeobotany.de) is another precious source of information. However, no certain evidence could be found of *B. oleracea* archaeological remains before Roman times and even these cannot usually be definitely identified at the species level in the case of *Brassica*. For example, desiccated and charred *Brassica* seeds were found in the midden deposits (accumulations of domestic refuse) in the excavations of the Roman Egyptian fort of Mons Claudianus. Here, on the basis of morphological analysis (prominence of the reticulum), Veen (2001) recognized several seeds resembling *B. oleracea*, one that may represent turnip and several other intermediate forms between *B. rapa* and *B. napus* and hypothesized that cabbage species must have been cultivated around the fort, as a precious source of Vitamin C. However, only black mustard (*B. nigra*) could definitely be identified at the species level.

An alternative approach to the difficult determination of leafy vegetable constituents of the diets of early societies was proposed by Evershed *et al.* (1991). Through gas chromatography and mass spectrometry analysis, these authors have been able to detect *Brassica* leaf wax components associated with potsherds from a late Saxon/Medieval (9th-13th centuries) settlement in Northamptonshire, UK. The findings provided the first evidence for the utilization of vegetables of this genus on the site. The study suggested that the pots were used for cooking cabbage, however a precise determination of the types in cultivation at the time was not feasible and *B. rapa* (turnip) could not be excluded either.

Perspectives for more reliable identification of archaeological *Brassica* seeds at the species level may come in future from analysis through Scanning Electron Microscope (SEM). In his unpublished Thesis, Fraser (1981) studied *Brassica* using SEM.

Domestication traits

Polymorphism within cultivated *B. oleracea* is considered one of the most striking examples of crop variability triggered by human selection (Helm, 1963). Each crop type is distinguished by domestication traits that are not commonly found among the wild populations. Cabbages form a head that is made of leaves surrounding the terminal bud. Similar smaller heading structures develop at the leaf axils of Brussels sprouts. The stem is entirely swollen along its length in the marrow-stem kale, while it is short and thickened in a bulb-like shape in the case of kohlrabi. Broccoli and cauliflowers are characterized by an arrested switch from vegetative growth to flowering (Smith and King, 2000). The typical curd of cauliflowers consists in proliferating, arrested inflorescence and floral meristems, while Calabrese broccoli are characterized by a fused inflorescence of several arrested floral spikes with proliferation of almost fully developed floral buds (Carr and Irish, 1997). A vast range of different leafy kales exist, either with single or branched, short or tall stem, producing fleshy leaves with different shapes (collards, curly kale, Galega kale, palm kale, Scottish kale, tronchuda, etc.).

It is not so easy to define which domestication traits are common to all of the above-mentioned types, as opposed to the wild species. Thompson (1976) suggested that selection, at early stage in domestication, must have been for less bitter-tasting plants, while other early selection criteria were less fibrous, thicker stems and more succulent storage organs. Snogerup *et al*. (1990) found that the cultivated *B. oleracea* forms have a special glaucous leaf surface, which they have in common with the wild *B. oleracea*, but not with the Mediterranean species. The structure of the epicuticular wax layer of *B. oleracea* (and *B. bourgeaui*) was indeed confirmed to be different from that of the other 2n=18 species (Gómez-Campo *et al*., 1999). According to Dixon (2007), cultivated brassicas have broadly expanded thin leaves, well supplied with chlorophyll, that are better suited for absorbing and utilizing solar radiation and ample supplies of water and fertilizers. Conversely, wild plants have thick leaves containing less chlorophyll and many more cell wall components, to increase the efficiency of water conservation in dry, rocky habitats.

Earliest signs of cultivation of the crop types of B. oleracea

Leafy kales are considered the earliest cultivated brassicas, originally used for human consumption or to feed livestock. These are the first types documented in ancient Greek and Roman literature (see Papers I and VI). An early written reference to the leafy kales grown in north-west Iberia dates back to a timetable of the Iberian farm activities of 961 CE. As reported by Dozy (1961, in Dias, 1995), Galega kale types or berzas were among the vegetables included in this document. The earliest Medieval drawings of leafy kales are contained in handbooks known as *Tacuina sanitatis*, illustrated in late 14th century Lombardy. These manuals were principally based on the *Taqwim al ˉsihha* [Maintenance of Health], an 11th-century Arab medical treatise by Ibn Butlan of Baghdad. Kales are shown growing in enclosed gardens, plants are harvested and baskets are filled with leaves (Daunay *et al.*, 2009). A note written by Sturtevant in the late 1800s (Hedrick, 1919) reports that Ray journeyed into Scotland in 1661 and said that people used “much pottage made of coal-wort which they call keal”. Sturtevant commented that this was probably the form of cabbage known to the ancients.

Several crop types are mentioned by Pliny the Elder in the I century CE. The descriptions do not allow a safe identification, but certainly he speaks of heading types, since *Cumanum* has a wide head (*capite patulum*), *Lacuturnense* also has wide heads (*capite praegrandes*) and some of them are folded into a globe (*in orbem conlecti*), others are long and sinewy (*in latitudinem torosi*). The description of *Aricinum* reminds of broccoli sprouts, since almost under every leaf peculiar shoots are thrown out (*sub omnibus paene foliis fruticat cauliculis peculiaribus*). Marrow-stem kale seems to be described by the traits of *Pompeianum*, which is thin at the root, increasing in thickness as it rises among the leaves (*caule ab radice tenui, intra folia crassescit*).

An early indication of what could have been kohlrabi derives from a manuscript dated between 770 and 800 CE. This document is known as *Capitulare de villis* and contains the instructions established by a Franc sovereign (possibly Charlemagne) to regulate the use and the management of his domains (Bloch, 1923). Horticultural gardens are here (article LXX) recommended to be cultivated with, among others, *caulos* and *ravacaulos* (University of Leicester, 2008). The last mentioned implies a kale with a turnip-like outgrowth, possibly kohlrabi (Toxopeus, 1974). Kohlrabi was depicted by Renaissance herbalists, for example by Fuchs (*caulorapum vel rapicaulis*), painted between 1542 and 1565.

The library of the Abbey of St. Gall, Switzerland, preserves a unique document of the early 9th century, known as the Plan of St. Gall, which is dedicated to Gozbertus, the abbot of St. Gall from 816-36 (Horn and Born, 1979). The Plan was a copy of an original blueprint of an ideal monastery created at two Carolingian reform synods held at Aachen in 816 and 817. The cloister garden was supposed to host a number of vegetables, most of them corresponding to the recommendations of *Capitulare de villis*, and *caulas* were also included (Berschin, 2002), but it is not possible to know whether these were heading or leafy types. According to Olivier de Serres, writer of a text book of French agriculture *Théâtre d'Agriculture* published in 1600, white cabbages came from the north and the art of making them head was not known at the time of Charlemagne (quoted by Sturtevant, 1887). According to Gates (1953), head cabbage appears in the texts by Hildegard von Bingen (1098-1179). Hildegard also recognized red cabbage. She was a remarkable woman who wrote about theology, medicine and botany. Reference to seemingly cabbage type of coles can also be found in the Spanish Arabic treatise on agriculture *Kitab-al-falaha* [Book of agriculture], written in the 12th century by Al Awam (Cubero, 2002, chapter XXIII). Here, these coles are classified as 'Christian vegetables' (*bakalato-l-ansar*), as opposed to the cauliflower, which is called 'Syrian cole' (see below). Albertus Magnus (1193?–1280), also known as Albert of Cologne, was a Dominican friar, philosopher and theologian, who displayed an encyclopedic knowledge of topics including botany, geography, zoology, etc. He seems to refer to a headed cabbage when he mentions *caputium* in his work *De vegetabilibus et plantis* (lib. VII c. 90), written during the 1250s, but there is no description (Sturtevant, 1887). As noted by Sturtevant (1887), the word *cabaches* and *caboches* used in England in the 14th century indicates that cabbages were known by then and distinguished from other coles (*coleworts*). In *The Forme of Cury* (Project Gutenberg, 2013), a roll of ancient English cookery, compiled about 1390 by the Master-Cooks of King Richard II, a recipe of *caboches in potage* in Middle English can be found:

> Take Caboches and quarter hem and seeth hem in gode broth with Oynouns y mynced and the whyte of Lekes y slyt and corue smale and do þer to safroun an salt and force it with powdour douce

According to Sturtevant (1887), the first unmistakable descriptions of cabbage were recorded in 1536 by the botanist Ruellius in his *De natura stirpium*, p.477. He calls them *capucos coles* or *cabutos* and describes the head as globular and often very large, reaching 45 cm in diameter. Ruellius also describes a loose-headed form called *romanos*. The interpretation of Sturtevant (1887), considering the difficulty of heading cabbages in the warm climates, is that Roman varieties were not solid-heading types, perhaps corresponding to the Savoy cabbage. Cabbages are common in Renaissance paintings and herbals (Toxopeus, 1979). For example, Dodoens (1554) described white cabbage, Savoy cabbage and red cabbage (Zeven and Brandeburg, 1986).

Regarding cauliflower, the earliest reference that can be clearly attributed to this type is found in the 12th century Spanish Arabic treatise by Al Awam (Cubero, 2002). Here the word *qunnabit*, current Arab word for 'cauliflower', is used. Two kinds are described, also named ‘Syrian coles’: one is closed and huddled together, the other is open, with a head that splits in various branches. This seems to be a distinction between cauliflower and broccoli. Al Awam also quotes three types that are referred by the *Nabatean Agriculture*, an Arab book translated in the 10th century from Syrian materials dating back to the 3rd century CE. The three types are large (bright yellow), medium (light yellow whitish) and small (white-yellow). The first illustrations of cauliflowers appear in the herbals and paintings of the 16th century. In a collection of reproductions of paintings by Dutch artists of the 16th and 17th centuries, Zeven and Brandeburg (1986) have recognized 16 paintings with cauliflowers, mostly of a normal size and well closed curd, some with loose curd and others with stems ca. 25-30 cm long. Illustrations by German herbalists Öellinger and Fuchs are among the earliest. The illustration with no name in a manuscript completed before 1553 by Öellinger shows a small white head in the middle of several fleshy leaves at the top of a high stalk, unlike any known modern type. The illustration by Fuchs (ca. 1542-1565), named *Brassica capitata cypria*, shows a loose white head, seemingly an intermediate form between broccoli and cauliflower. The illustration by the Dutch Dodoens (1559), named *Brassica Pompeiana aut Cypria*, gives a small curd surrounded by several leaves on top a short stalk. Probably based on the above-mentioned sources, various authors reported that cauliflowers might have originated in the east Mediterranean (Cyprus or Syria) and tradition was that Genoese imported seed around 1490 from the Levant to Venice and Vienna from where it spread to northern Europe (Miller, 1807 in Giles, 1941; Jensma, 1957; Hyams, 1971). According to Boswell (1949), cauliflower was referred to as ‘Cyprus coleworts’ and Cyprus was mentioned as the source of seed for planting in England.

Tender sprouts were in high demand in Roman times, starting around the first century BCE. They were called *cymae* and possibly from these forms other types of broccoli (either sprouting or heading) evolved (see Paper VI). However, apart from the above-mentioned description by Al Awam, there is no specific account or figure of broccoli made by early botanists, possibly because they were confused with the cauliflower, as shown by the quoted illustration by Fuchs. An illustration of what could be considered a sprouting broccoli is given by Dalechamp (1615). It is here called *Brassica asparagoides* or *Chou d’Asperge de Dalechamp*. Several small short fleshy and leafy floral shoots, said to be similar to asparagus shoots, are produced. According to Thompson (1976), the sprouting broccoli was not mentioned until 1660 and was referred to as ‘sprout colli-flower’ or ‘Italian asparagus’ in Miller’s Gardener’s Dictionary of 1724. The molecular steps of the switch from broccoli to cauliflower have been proposed by Smith and King (2000) after a genetic and molecular study of the genes *BoCAL-a* and *BoAP1*. These authors have consequently proposed that the cauliflower curd arose in southern Italy from a heading Calabrese broccoli through an intermediate Sicilian purple type. Italy is indeed a centre of diversity for both broccoli and cauliflowers, which are present in myriad of conformations and regional types and believed to be the source of the material subsequently spreading to the North of Europe (Sturtevant, 1887; Giles, 1941; Crisp, 1982; Gray, 1982; Massie *et al*., 1996).

Brussels sprouts were not illustrated during the 16th and 17th centuries, with the possible exception of a drawing by Dalechamps in his 1587 herbal, which is called *Brassica capitata polycephala*. This illustration however seems to be a many-headed cabbage and its affinity with Brussels sprouts is put into doubt (Hodgkin, 1995). In a lecture given in 1818, van Mons of Louvain University, reported market regulations of some Belgian towns mentioning *spruyten* as early as 1213 (Hyams, 1971). *Sprocq* served at a wedding party were mentioned by Charles the Bold, Duke of Burgundy, in 1472 (Hyams, 1971). Less uncertain records of Brussels sprouts are only available in the 18th century from Belgium and 19th century from France (Thompson, 1976).

Identification of the wild relative

In his taxonomic arrangement, Linnaeus considered all the cultivated forms of *B. oleracea* to belong to the same species growing on the cliffs of Dover, implying that they had all derived from the wild species growing on the coasts of England. He accepted the variety name *sylvestris* as a synonym for the wild types. Augustin de Candolle (1824), speaking of the wild cabbage (*Brassica oleracea sylvestris*), described its distribution in France (Normandy), Britain (Yorkshire, Wales, Cornwall and especially about Dover), as well as in Greece, as mentioned by Dioscorides and by Sibthorp, author of *Florae Graecae Prodromus*. By comparing the morphologies of the wild and cultivated coles, de Candolle (1824) was convinced that the wild plants could 'degenerate' to the various cultivated forms, since "the leaves shooting from the summit of the sterile branches form a kind of rose, giving to the wild plant the intermediate aspect between the two grand races, the Round-headed Cabbage, and the Cavalier or Tall Cabbage". Moreover, "the leaves of the wild cabbage are in every respect like those of the Garden Cabbage, fleshy, glabrous, and of a bluish green". Regarding the inflorescence, he gave credence to "the possibility of increasing the natural disposition of the panicle to form a corymb, and this determines the character of the Cauliflower".

Alphonse de Candolle (1883; 1885) described a wider geographic distribution of the wild *B. oleracea*, that could be found on the rocks by the sea-shore in the Isle of Laland [Lolland], Denmark, the Island of Helgoland, the south of England and Ireland, the Channel Isles Jersey and Guernesey, Normandy, Charente Inférieure and the islands off its coast. It was also present on the north coast of the Mediterranean, near Nice, Genoa, and Lucca. Therefore, Alphonse de Candolle included under the same species also what is currently considered a different species, *B. montana*. On the other hand, he excluded, as not confirmed, the presence of wild cabbage in Greece, but he distinguished *B. cretica* (and *B. balearica*) as separate species growing in Mediterranean islands. He made an interesting consideration about the geographic distribution of the wild cabbage as he knew it:

> Its distribution into somewhat isolated places, and in two different regions of Europe, suggests the suspicion either that plants apparently indigenous may in several cases be the result of self-sowing from cultivation, or that the species was formerly common, and is tending to disappear. Its presence in the western islands of Europe favours the latter hypothesis, but its absence in the islands of the Mediterranean is opposed to it.

The considerations made by Sturtevant in the late 19th century and edited by Hedrick (1919) are worth reporting entirely since they set the base for subsequent elaboration regarding the identification of the ancestors of cole crops:

> At Dover, England, wild cabbage varies considerably in its foliage and general appearance and in its wild state is used as a culinary vegetable and is of excellent flavor. This wild cabbage is undoubtedly the original of our cultivated varieties, as experiments at the garden of the Royal Agricultural College and at Cirencester resulted in the production of sorts of broccoli, cabbages and greens from wild plants gathered from rocks overhanging the sea in Wales. Lindley groups the leading variations as follows: if the race is vigorous, long jointed and has little tendency to turn its leaves inwards, it forms what are called open cabbages (the kales); if the growth is stunted, the joints short and the leaves inclined to turn inwards, it becomes the heart cabbages; if both these tendencies give way to a preternatural formation of flowers, the cauliflowers are the result. If the stems swell out into a globular form, we have the turnip-rooted cabbages.
>
> Other species of *Brassica*, very nearly allied to *B. oleracea* Linn., such as *B. balearica* Richl., *B. insularis* Moris, and *B. cretica* Lam., belong to the Mediterranean flora and some botanists suggest that some of these species, likewise introduced into the gardens and established as cultivated plants, may have mixed with each other and thus have assisted in, giving rise to some of the many races cultivated at the present day.

Also other authors (Henslow, 1908; Giles, 1941) write about the experiment made at the Royal Agricultural College, Cirencester, which was considered a proof of the origin of many cultivated varieties. The experiment was carried out by Prof. Buckman, starting from seed from a wild population collected in Llandudno, Wales. Some of the raised varieties had “short petioles and the close-hearting condition of cabbages, both green and red”. “Others, with longer petioles and lyrate leaves, seem to take on the looser method of growth of kales” (Henslow, 1908). Giles (1941) found that the seed catalogue of Messrs. Suttons of 1864 was offering ‘Buckman’s New Hardy Sprouting’. This broccoli was said to have been obtained after several years sowing, selecting and resowing. Good heads of white broccoli were produced, some of them sprouting, but the majority close-headed. In a letter dated 6 November 1863, Prof. Buckman said that it was “derived from the wild cabbage without hybridization”.

A similar experiment is reported by Gates (1953), indicating that Hegi (1919) cited H. Hoffmann (without a reference), for the statement that he grew seeds of a wild population from Helgoland and obtained different varieties like the cultivated sorts. However, in this case the conclusion of Hegi was that the plants from Helgoland were naturalized, not wild, as they were unknown in the 16th century list of its plants.

A ‘reverse proof’ of the origin of cole crops from the wild *B. oleracea* is found by Gates (1953) in the experiments of Kristofferson (1924), who crossed cabbage and kale on a large scale. The hybrids were showing huge rosettes, quite similar to those that can be obtained from wild seed. Gates (1953) interpreted this as a case of reversion to the wild ancestor of cabbage and kale. Similar experiments made by Sutton (1908) with crossings between cabbages, kohlrabi, thousand-headed kale and other forms always gave rise to F_1 that were unlike either parent and represented an approximation of the ancestral condition (Gates, 1953).

In his account of the wild *B. oleracea*, Bailey (1930) remarked about its affinity with the cultivated coles and that he had followed “the general assumption that the kales, heading cabbages and cauliflowers are derivatives of *B. oleracea* of the cliffs and shores of Europe”. He had often grown the wild plants from seed and had “not observed variations suggesting the highly modified domesticated races”, but these races were “apparently fairly within the range of possibilities of the wild species”. However, he invited to critically investigate all the related native species and the diverse regional developments of the domesticated classes, since “there may be surprises in the phylogeny”.

A more complete perspective of the geographic distribution of the wild relatives of *B. oleracea* across the Mediterranean became evident after the works of Schulz (1919; 1936), Onno (1933) and especially Snogerup *et al.* (1990), which is still the most accurate, even though an update would be necessary to account for a few gaps and new discoveries made by subsequent explorations (see Figure 2, p.22).

A wider knowledge of the distribution of wild kales, coupled with the generalized perception that at least some of the crop types (broccoli and cauliflowers) originated in the Mediterranean area, led various authors to suggest the involvement of Mediterranean species in the origin of cole crops. Hegi (1919) supposed var. *sylvestris* from Italy (i.e. *B. montana*) to be the progenitor of various types. Netroufal (1927) hypothesized *B. montana* to be the ancestor of cabbages and kales and *B. rupestris* to be that of kohlrabi. Schiemann (1932) presumed that different Mediterranean wild species should be the sources of the cole crops. Schulz (1936) proposed *B. cretica* as the probable ancestor of the cauliflowers. Giles (1941) stated that all the types of broccoli and cauliflowers had their origin in the wild *B. oleracea*. At the same time, in a somehow contradictory way, he conceded that the origin of the cauliflower was supposed to be Cyprus or some other part of the Mediterranean, as well as that it was generally accepted that the home of broccoli was Italy. Based on the above-mentioned notion of ‘degeneration’ of cole crops in crossing experiments, the geographic distribution of wild relatives, historical considerations, and his own observations of the effects of cultivation on wild *B. oleracea* (“it shows enormous increase in size”), Gates (1953) concluded that cabbage, Brussels sprout and kale could have been derived from *B. oleracea* from western Europe, while cauliflower and broccoli probably derived from *B. cretica* and kohlrabi may have derived from one of the middle Mediterranean species. Lizgunova (1959) proposed a multiple origin from different wild forms. Helms (1963) suggested a triple origin, but did not indicate the probable wild ancestors. Toxopeus (1974) stated that the *B. oleracea* crops were native only to western Europe, although he provided at the same time historical evidence that could have been differently interpreted.

A thorough review of the existing evidence of the possible relations between the wild forms of the *Brassica oleracea* group and the cultivated ones was made by Snogerup in 1980. This was based on a research programme carried out at the University of Lund, Sweden, aimed at an experimental revision of the wild 2n=18 brassicas, involving extensive crossing and cultivation experiments as well as direct exploration and collecting of wild populations. Results of crossing experiments, at that time still preliminary, confirmed indication that all crossings give at least semi-fertile progeny. These results could not give absolute evidence of the ancestry of the cole crops, but were not in contradiction with the assumptions of previous authors, such as

Schulz (1936), that *B. cretica* could have been involved in the origin of cauliflower and broccoli. At the same time, it was clear that, as soon as different cultivars or species of the group occurred to grow side by side, crossing would occur. Therefore, the history of present-day cultivars may be clouded by events of hybridization and introgression from other cultivated or wild forms and it may be very difficult or impossible to be traced back. Based on comparison of morphologies, Snogerup (1980) suggested a multiple origin of the cultivated coles. In particular, the west European *B. oleracea* was proposed as the progenitor of cabbages and Brussels sprouts, owing to the greyish surface texture of the leaves that they share with this wild species. Stem kales were found to have a strong dominating main stem in combination with hairs, at least on the seedlings, in common with *B. incana*, *B. rupestris* and other Sicilian species that he grouped in a so-called *rupestris-incana* complex. Branching bush kales were found to have a branching, shrubby habit and fleshy leaves in common with *B. cretica*. The origin of broccoli and cauliflowers remained undecided, since these have a leaf surface reminiscent of the cabbages and therefore it is hard to reconcile historical, geographical and morphological evidence.

A multiphyletic origin of the cultivated forms of *B. oleracea* from a number of wild species was also proposed by Mithen *et al*. (1987) with a different approach, comparing the glucosinolate profile of the different taxa. The high level of glucoiberin within *B. rupestris*, as opposed to other wild species, was found to be comparable to the amounts within cabbage and Savoy cabbage. Similarly, sinigrin, also common in many *B. oleracea* cultivars, was only detected in Greek and Tunisian wild populations. The authors thought that these results supported the thesis that cultivated *B. oleracea* did not derive from the wild populations of western Europe. However, out of two tested populations of wild *B. oleracea* from the UK, one from Cornwall was found to also contain sinigrin and was interpreted to be an escape from cultivation. The other one from Glamorgan, without sinigrin, was believed to be truly wild. A different scenario was suggested by a chemosystematic survey on 12 wild n=9 taxa, finding the same flavonoid pattern (total absence of isorhamnetin) in cabbage and in every studied wild population of *B. montana*, *B. oleracea* and *B. bourgeaui* (Aguinagalde *et al*., 1992). The authors noted the geographic correlation of these species and associated the data obtained with the hypothesis of a domestication based exclusively on the Atlantic plants.

Results of extensive crossing experiments between cultivated and wild *B. oleracea* cytodeme species were completed in the 1990s. Kianian and Quiros (1992) included *B. alboglabra*, *B. bourgeaui*, *B. cretica* subsp. *cretica*, *B. montana* and *B. oleracea* within the same karyotype group, based on their ability to form fully fertile hybrids. *B. incana*, *B. insularis* and *B. rupestris* were found to produce only semi-fertile hybrids with other species, except full fertility was noted in *B. incana* × *insularis* hybrids. By contrast, Bothmer *et al.* (1995) noted high fertility of hybrids between 23 accessions representing the major cultivated forms of *B. oleracea* and 10 wild taxa (*B. cretica* subsp. *aegaea, B. cretica* subsp. *cretica*, *B. insularis*, *B. rupestris*, *B. villosa*, *B. incana*, *B. macrocarpa*, *B. montana*, *B. oleracea* and *B. bourgeaui*). The levels of pollen fertility were especially high in crosses involving wild *B. oleracea* and *B. bourgeaui* (respectively 87% and 93%) in F_1, while crosses with the other species ranged between 52-79% in F_1 and 56-84% in F_2. Crosses with lower fertility tended to be those involving *B. macrocarpa*, *B. montana* and *B. rupestris*. After wild *B. oleracea* and *B. bourgeaui*, the highest values were obtained with *B. cretica*. The conclusions confirmed that no fertility barriers exist within the *B. oleracea* cytodeme and all members belong to either the primary or secondary genepool. Data from crossing experiments did not give sufficient information to reconstruct the differentiation steps that occurred during the domestication of *B. oleracea*. However, strong indications were given that the close affinity between the wild *B. oleracea* and the cultivated coles may depend on an ancient domestication from this taxon. Even if different wild forms had been brought under cultivation, those of *oleracea* origin had a major role.

In the late 20th century, the use of molecular markers to investigate relationships between cole crops and their wild relatives started to bring new evidence for consideration and several studies have been published during the last 25 years. A research by Hosaka *et al.* (1990) revealed the presence in a kale variety of DNA fragments characteristic of *B. incana* and *B. insularis*. These data did not help to identify a specific wild relative, but indicated the common occurrence of introgression events and the affinity of various wild species with the cultivated crops.

A study by Song *et al.* (1990), based on RFLPs obtained with random nuclear sequences and one seed protein gene used as probes, analysed nine cultivated types of *B. oleracea* and thirteen n=9 wild brassicas. All of the cultivated types (including broccoli, cabbage, thousand-headed kale, Portuguese tree kale, Chinese kale, kohlrabi, borecole and cauliflower) clustered together and eleven wild accessions formed a separate cluster. Wild *B. oleracea* and *B. alboglabra* occupied an intermediate position, but genetically closer to the cultivated group, indicating that these two taxa were the closest ancestors of the cultivated types. This work presents some imprecisions in the choice or identification of the materials: Chinese kale and *B. alboglabra* are expected to be the same type of plant, but in this work they were used either as a cultivated or a wild sample (and they clustered in separate groups); accession 1952 from UPM (*B. cretica* subsp. *atlantica*) is an ambiguous accession name, more likely to be considered *B. insularis*, according to data available from the ECPGR *Brassica* Database. In any case, a strong argument was given by Song *et al*. (1990) in support of a monophyletic origin of the cole crops and of an ancestor more similar to the Atlantic *B. oleracea* than to any Mediterranean wild species.

Different chloroplast and nuclear markers have been used, ranging from isozymes, RFLPs, RAPDs, AFLPs, etc. As described by Lannér (1997), such type of studies often give contrasting results, since different markers have different discriminatory potential, depending on whether they match conserved sequences or more variable regions of the genome and also depending on the level of genetic organization at which the taxa are compared, such as interspecific or intraspecific. Moreover, materials of different origins are often used in the investigations and their identity is sometimes uncertain, owing to different taxonomic interpretations of the original collector or the providing genebank.

Although a conclusive scheme of the relationships among the studied taxa has therefore not been confirmed, some recurrent patterns can be traced:

- Wild *B. oleracea* and *B. bourgeaui* always show the highest affinity with the cultivated crops (Warwick and Black, 1993 based on cpDNA restriction site polymorphism; Tatout *et al*., 1999, based on 21 short interspersed nuclear elements (SINE); Allender *et al*., 2007, based on 6 chloroplast SSR markers; Mei *et al*., 2010, based on 301 AFLP and 518 SSR polymorphic bands; Zamani-Nour *et al*., 2013, based on 40 cpSSR primers).
- In several occasions, *B. incana* appears to be a taxon that is genetically close to *B. oleracea* (Lázaro and Aguinagalde, 1998a, based on 5 enzyme systems; 1998b, based on RAPDs; Tatout *et al*., 1999; Allender *et al*., 2007; Mei *et al*., 2010; Zamani-Nour *et al*., 2013).
- The Sicilian species, *B. rupestris*, *B. macrocarpa* and *B. villosa* generally cluster together and seem to be more distant from *B. oleracea* than other species (Warwick and Black, 1993; Lázaro and Aguinagalde, 1998b; Lannér *et al*., 1997, based on random genomic and cDNA RFLPs; Tatout *et al*., 1999; Geraci *et al*., 2004, based on 5 enzyme systems; Allender *et al*., 2007; Mei *et al*., 2010; Zamani-Nour *et al*., 2010).
- *B. cretica*, *B. hilarionis*, *B. insularis* and *B. montana* position themselves in variable clusters in the different analyses and easy generalizations are not possible.

Regarding *B. oleracea*, it should be noted that Allender *et al*. (2007) found a large uniformity of its chloroplast genome, since the vast majority of 105 samples of cultivated and wild material exhibited the same haplotype, contrasting with a wealth of diversity of haplotypes among the Mediterranean populations. The same chloroplast haplotype was also found in 10 out of 11 *B. incana* accessions. Also Panda *et al*. (2003), using 12 amplified chloroplast fragments cleaved by restriction enzymes in 36 combinations, had not found variation in chloroplast PCR-RFLP profiles between one cultivated accession and nine wild *B. oleracea* samples from France, Spain and the UK.

In summary, strong molecular genetics evidence, together with fertility tests, point at the wild *B. oleracea* from the Atlantic coasts of Europe as the closest relative of all the cultivated crops. Introgressions from other Mediterranean wild species into cultivated crops have also been documented. It remains to be explained how this scenario can be reconciled with the historical tradition that at least some type of crops, such as broccoli and cauliflowers, had a Mediterranean origin. The glucosinolate profile pattern that convinced Mithen *et al*. (1987) to suggest a polyphyletic hypothesis is also in contrast with the single derivation of all cole crops from the wild Atlantic populations.

Theories of domestication

As described in the previous section, before 1990, i.e. before the first relevant results of molecular genetics, theories of domestication of the cole crops were sharing a general polyphyletic view that *B. oleracea* var. *oleracea* was one of the ancestors and that other Mediterranean species had likely been involved in the origin of some of the cole crops (Thompson, 1976; Snogerup, 1980). After 1990, theories of domestication were formulated from the starting basis of a monophyletic origin, and with the intention to reconcile with it the history of the crops. These attempts are summarized below.

Song *et al.* (1990), who were the first to substantiate the monophyletic origin with molecular data, suggested that the earliest cultivated cole crop, originating from a single ancestor similar to wild *B. oleracea*, was a leafy kale, from which different types of kales evolved along the Atlantic and Mediterranean coasts from Wales to Greece. Specialized forms derived from local kales then adapted to different areas, possibly broccoli and cauliflowers appearing in Italy, Portuguese tronchuda cabbage in Portugal, etc. Regarding the time and location of the first domestication events, this proposal remains vague, also considering the unknown geographic distribution of the proposed hypothetical ancestor.

Following the logic of Song *et al*. (1990) and of Snogerup *et al*. (1990), Hodgkin (1995) further elaborated that it would seem unlikely that wild

B. oleracea was ever distributed in the Mediterranean region. Therefore, he concluded that the evidence indicates that early crop types originated on the Atlantic coasts and from there they were brought to the Mediterranean where selection for many other different forms occurred. Intercrossing with Mediterranean wild species was likely to take place and bring in additional specific traits, not present in *B. oleracea*.

Gómez-Campo and Prakash (1999) described the hypothesis of cultivation of primitive forms (kales) by the Celts, with a subsequent movement of these forms to the east Mediterranean region (by the end of the second millennium BCE) where domestication would have been completed with an explosive diversification of the cultivated forms. These authors mentioned possible occasions of contact and exchange between Celts and Mediterranean populations during the Celtic invasions of southeastern Europe (VI-IV centuries BCE). An earlier contact (around the IX century BCE) might have occurred through the 'tin route', linking the British Islands with the east Mediterranean, since Phoenicians used to travel to the Atlantic harbour of Gades (Cádiz) to buy the tin that was extracted from the Cornwall mines.

The idea of attributing the first steps of domestication of the cole crops to the Celts, as reported by Gómez-Campo and Prakash (1999), was probably suggested by the linguistic commentary of de Candolle (1885). This author mentioned different words used by Celtic languages (such as *kap*, *cal* and *bresych*) to indicate the coles. The diversity of words used in European languages was for de Candolle an indicator pointing at a European as opposed to Asian origin of the coles. The linguistic treatment of de Candolle is commented in Paper I.

Dias (1995) reported that the Portuguese tronchuda cabbage is also known as *couve penca* and that *penca* is an expression of pre-Roman and probably Celtic origin. Additionally, he mentioned the possibility that primitive coles might have been introduced to Portugal by the Celts or that the invading Celts found them already domesticated by Ligurians and Iberians.

Smith and King (2000) and King (2003) discussed the domestication of cauliflower. While admitting that its history was incompletely known, they gave credence to the arrival to Italy of precursor material from the east (Aegean area), based on historical accounts. The genetic model proposed by them brought complementary evidence to suggest that the cauliflower arose in southern Italy from a heading broccoli via an intermediary Sicilian crop type.

Allender *et al*. (2007) wondered whether the low level of chloroplast genetic diversity that they found in *B. oleracea* (wild and crops) could be at least partially explained by the process of domestication. They remarked the presence of still scant reliable biological or historical evidence of the nature and location of initial domestication. On the other hand, the conclusion made by Mitchell (1976) that most of the wild populations around the British coast originated as escapes from cultivation could explain the lack of chloroplast diversity in the UK populations tested in their study.

1.3.2 Formulation of a working hypothesis

The above sections have shown that current theories of domestication of *Brassica oleracea* do not give a conclusive answer to the location and timing of initial domestication as well as to which wild relative was involved. The wild *Brassica oleracea* is with no doubt genetically very close to the cole crops, but its limited distribution along the Atlantic coasts and the monophyletic pattern revealed by molecular data compel to speculate about an introduction of domesticated coles from the west to the east, which seems at least in part contradicting historical evidence.

An alternative hypothesis was inspired by the observations made by several authors (details in the Discussion) that *B. oleracea* tends to escape from cultivation and re-naturalize. What if all the wild *B. oleracea* populations of the Atlantic coast were not representing a natural species, but were all mere escapes from cultivation? According to such a scenario, initial domestication could have taken place somewhere in the Mediterranean area, where truly wild $2n=18$ species thrive. Cultivation of various forms of coles was part of a deep-rooted tradition, both for Greek and Roman civilizations. If not earlier, possibly as a consequence of the Roman expansion and colonization of north-west Europe, cole crops were introduced into this area. Escapes from cultivation then occurred and the plants found a suitable habitat for stabilized naturalization, starting from the coastal cliffs near villages. If this scenario is correct, the genetic distance of the Atlantic populations from all the other cultivated coles would be expected to be closer than that of the other wild relatives, since both cultivated crops and Atlantic populations would have shared the same domestication bottleneck. Equally plausible would be for the Atlantic populations to share the same chloroplastic haplotype with all the cultivated crops, with little variation. The proposed design is also in line with the historical evidence.

Research carried out as part of this Thesis served to test the above-mentioned aspects of a theory on the domestication of *B. oleracea*. After a description in the next section of the results obtained, the Discussion will analyse whether and to what extent this theory has gained support after our studies, what are the remaining elements of criticism and which experiments could be conceived to validate or falsify this hypothesis.

2 Methodology and results

Various studies have been carried out, that in different ways bring insights on the process of domestication of *B. oleracea*.

Considering the absence of archaeological remains, it is however possible to try to derive the origin of the crop by following linguistic signals and ancient literary records.

We found traditional agro-ecosystems that are likely to mimic primeval conditions when the first domesticated kales were grown in close proximity of the wild progenitors. Genetic analysis of crops and wild species in these environments, including diversity and gene flow, offer information on possible patterns of domestication, as well as suggestions on the correct management of genetic resources.

Analysis of the diversity of leafy kales across Europe was carried out in the hope of revealing a gradient of geographically structured diversity among the most primitive domesticated forms currently grown in Europe.

Molecular markers were used to investigate the taxonomic relatedness among populations of dubious identity, including possible escapes from the field. These latter studies were planned, among other reasons, also in the hope of better understanding the ability of the cole crops to re-naturalize and the possibility to distinguish escapes from cultivation from truly wild populations.

A study was completed on the legal aspects of collecting in Europe. Collection of adequate material was an essential element of the entire body of this research, enabling to study diversity and relationships based on offspring from individual plants. Simply receiving material from genebanks would not have been sufficient. Our collecting experience is described in the context of a wider framework, which includes challenges of legal and social nature.

Additional lines of research have been initiated, specifically an investigation of the wild Atlantic populations of France and Spain, aiming at verifying their genetic structure and diversity. The assumption is that if these populations are ancient escapes from the fields, they might show very different patterns from the wild populations of the Mediterranean.

We considered the hypothesis of an existing correlation between the locations of Atlantic populations and the sites of ancient Roman villages. The rationale of a study to verify this hypothesis will be described below.

2.1 Linguistic and historic approaches

A survey of ancient literary sources and linguistic considerations is the subject of Papers I and VI. Ancient words have been looked up that could be referred to the coles in ancient languages. Coles are not mentioned in the Bible, they are not included among the vegetables of the Babylonian gardens and of the Assyrian feasts and they are not identifiable in Egyptian lists of vegetables. A Sanskrit word for coles has not been found in the Hindu Upanishads.

An extensive search of any reference made to the coles by the ancient Greek and Latin literatures was based on *Thesauri* (catalogues of all the forms of the words used in a language and their location in ancient texts, as well as notes on the history of the words) and *Lexica* (vocabularies including all the words and expressions of a given language and their meaning).

The most ancient works of Greek literature, the Homeric poems, contain about 50 botanical names, but these do not include the coles. The oldest written accounts that can be safely referred to cole crops is the use of the Greek words *krambē* (starting from the VI century BCE) and *rhaphanos* (from the V century BCE), respectively in the Doric and Attic dialects. Contemporary philologists have explained that these words were applied to the same plant. The word *kaulos*, meaning ‘stem’, acquired the meaning of the entire cole plant as of the IV century BCE. In the Latin world, the most ancient word for cole was *brassica* (first attestation in a comedy by Plautus in the III century BCE), but also *caulis* was used, initially with the same meaning of the Greek word for ‘stem’, and subsequently, as of the I century BCE, meaning the entire plant, like in Greece. Another possible link between the Greek and Latin languages is the word *braskē*, only mentioned by Hesychius as a local name used in the ancient Greek colonies of south Italy, having replaced the use of the original word *krambē*. Therefore, a possible succession from *krambē* to *braskē* and then to *brassica* can be hypothesized. The word *raphanus* in Latin was not applied to the coles like in Greece, but had the meaning of ‘radish’ (*rhaphanis* in Attic Greek). The word *crambe* is rarely used in Latin, with reference to Greek quotations of the word *krambē*. Other relevant words were used in Latin: *holus, cyma* and *caput. Holus* (or *olus*), was initially used to designate all the green vegetables, but later was often referred to specifically mean the cole crops. The current binomial scientific name *Brassica oleracea* still conserves the adjective *oleracea* from '*olus*, *-eris*'. *Cyma* (from the Greek *kyma,* with the same meaning), indicated young sprouts or tender inflorescence tops, which were especially appreciated at least by the time of Pliny (I century CE) and onward. Pliny utilized the word *caput* (head) to describe the products of the types of coles in use at his time. The medieval word *caputium* (from *caput*) was later adopted to designate the headed cabbages (*Brassica oleracea* var. *capitata*).

The linguistic analysis indicates that several words were used by Greek and Roman authors referring to the coles. The earliest Greek records date back to the VI (*krambē*) and V (*rhaphanos*) centuries BCE, while *brassica* appears in the earliest surviving intact works of Latin literature (III century BCE). The words *kaulos* and *caulis* ('stem') are used to mean the entire cole plant, since the IV and I centuries BCE, respectively in Greece and in Italy. This rhetorical figure of speech is termed synecdoche, whereby a part of something is used for the whole. Evidently, the upright stems of the leafy kales were initially the most significant traits of the coles in use at the time and it came natural to call these plants 'stems'. The word *caulis* took over and became predominant after the I century CE, replacing almost entirely the word *brassica*. *Caulis* remained in use, with little variation, in many European languages, even after the plant started to differentiate into many forms, where the stem was no longer always the most evident anatomical part. Hence, we have the words *cal* in Irish, *cavolo* in Italian, *chou* in French, *col* in Spanish, *cole*, *collard* and *kale* in English, *couve* in Portuguese, *Kål* in Scandinavian languages, *kaali* in Finnish, *kaol* and *kol* in Breton and *Kohl* in German. On the other hand, *brassica* is only attested for Italian and Sicilian and was passed, according to Ernout and Meillet (1932), to the Irish *braissech*, the Welsh *bresych* and the Serbian *bróvska*. Another group of words currently in use in Europe also derive from a Latin word (*caput*) and clearly refer to the ability of the coles to form heads: *cabbage* in English, *cabus* in French, *kapsas* in Estonian, *Kappes* in German, *kaposzta* in Hungarian, *kapusta* in Slovak and *kupus* in Croatian.

De Candolle (1885) made the point that he could identify four or five distinct roots in Europe for words meaning 'coles', while the terms used in Asiatic languages were rare and modern. His conclusion that the coles were of European origin can be accepted. However, all the 'Celtic' roots quoted by de Candolle actually seem to derive from Greek (*krambai* and *kaulos*) or Latin (*brassica*, *caput*, *caulis*) origins. The old Irish *braissech* and the Welsh *bresych* are sometimes considered the source of the Latin *brassica*, but the reverse is also equally possible (Hervé, 2003). On the other hand, the origin of *brassica* may be tracked down to the word *braskē*, used in the ancient Greek colonies of south Italy, if Hesychius, Greek grammarian of the IV century CE, is correct.

From the analysis of literary sources it was possible to trace the proof of an already well consolidated tradition of knowledge and use of coles, dating at least to the VI–V centuries BCE in Greece. For the ancient Greeks, coles were a leafy vegetable to be boiled and dressed with olive oil. Only minimal diversity was known (either smooth or curly leaves) and the wild plant was still considered an option to bring on the table, even if more bitter or pungent. First descriptions of different cultivated varieties date back to the IV century BCE with Theophrastus. He clearly indicated that both wild and domesticated types were used. The coles were familiar enough as to be frequently mentioned in verbal expressions of comic exclamatory tone (By the *krambē*!). Great emphasis was given to their medicinal properties. Among these, the alleged antidotal effect against drunkenness was popular knowledge, while physicians compiled systematic handbooks to record properties and preparations against all sorts of diseases. From Latin literature we also derive the impression that coles were vegetables of widespread use, present in all vegetable gardens and extremely familiar in all spheres of life. The virtual absence of morphological descriptions offered by the Greeks and the earliest Latin scholars is eventually replaced in the I century CE by the description of at least 12 different types of cultivated forms, geographically distributed in central and south Italy, with the majority from locations in Campania and Calabria, where several ancient Greek colonies were settled. For the first time, the literary descriptions allow us to infer that this crop was no longer just a leafy vegetable, but that also heading types had appeared. More or less at the same time it becomes apparent that a product in high demand is not just the leaves, but rather the tender sprouts (*cymae* or *coliculi*), growing from the leaf axils and re-shooting after being cut back. No clear reference to this use had been made earlier in the Roman or Greek world. The value of the sprouting inflorescences of either *B. rapa* or leafy kale *B. oleracea* is well known in present-day Italy (where they are still called *cime* or *broccoletti*). Interest in this part of the plant might have exercised a selection pressure eventually leading to the development of broccoli and then cauliflowers.

The use of wild plants also seems to fade away with time, in the face of the abundance of new varied and productive types. These are described by Pliny not without a note of dismay for the modern ‘monstrosities’ reaching the tables of his contemporaries (I century CE).

The overall picture emerging from this study stands out against the lack of equivalent historical records pertaining to *B. oleracea* from any other geographical area falling outside of the remit of the ancient Greek and Roman civilizations. It is therefore in this area, and for temporal reasons most likely the Aegean, that the domestication of *B. oleracea* can most logically have occurred.

2.2 Studies within a model agro-ecosystem in south Italy

A few agro-ecosystems were identified in Calabria and Sicily (south Italy), where leafy kale is grown for self-consumption in home gardens, sometimes very near to populations of the wild relative *B. rupestris* Raf., which is endemic to Calabria and Sicily. This type of scenario is supposed to be very similar to what must have happened at the time of domestication, with the contemporary presence of wild brassicas and of the first leafy kale domesticates. The results of the studies made in this context are described in Papers III and IV.

Our aim was to compare, with the use of AFLP markers, the genetic diversity and population structure of collected cultivated and wild material as a whole, of Calabrian versus Sicilian populations and of the various populations collected within each local ecosystem. We searched for signs of gene flow and wished to investigate whether it is possible to use molecular means to distinguish wild from cultivated populations and leafy kales grown in different home gardens from each other.

Answers to the above questions were expected to shed light on the relevance of the presence of wild plants near home gardens and their influence on the diversity of cultivated plants, including aspects related to the processes involved in the domestication of the species. Indications could also be sought on the best strategy for sampling (*ex situ* conservation) and on the most appropriate *in situ* conservation strategy.

For the first time, molecular analysis was applied to C-genome wild brassica populations and to cultivated kale growing in the same area, based on samples collected at the same time. The choice of AFLP markers (binary dominant markers in diploid individuals) was made considering that they offer several polymorphic loci dispersed over the genome, unsurpassed by most other marker systems, therefore they are often indicated as very suitable for estimating and monitoring genetic diversity and to give robust and repeatable results.

As a measure of internal genetic diversity for diploids, it was possible to use the expected heterozigosity (biased or unbiased), also called Nei's genetic diversity, $He= 2pq$, which was calculated by the software GenAlEx for binary diploid data. A second measure that was used for dominant markers was the Shannon Index $I = -1^* (p * \text{Ln} (p) + q * \text{Ln}(q))$. Results showed that local leafy kales had higher variability than wild populations. This pattern was consistently shown both by Nei's genetic diversity and Shannon Index. Individual populations with peaks of diversity could also be identified (i.e. He was ranging between 0.12 and 0.26 within the wild populations and between 0.10 and 0.36 within the leafy kales). Sicilian material did not differentiate from Calabrian material. Wild populations remained distinct from cultivated material. Additionally, most wild populations were distinctively isolated from each other. On the other hand, it was not possible to molecularly distinguish even geographically distant leafy kale populations from each other or from different *B. oleracea* crops.

Some of our results were unexpected and required explanations or interpretations. In fact, the total diversity of both Calabrian ($H_T = 0.36$) and Sicilian ($H_T = 0.37$) kales (respectively 6 and 16 accessions) was higher than the total diversity ($H_T = 0.32$) of 17 European leafy kale accessions measured in Paper II, where the most diverse accession had $H = 0.27$. This may be an indication that the kales collected by us in south Italy represent an important value in terms of genetic diversity. It should also be kept in mind that other *B. oleracea* varieties (broccoli, cauliflower and Savoy cabbage) are also grown in the same area in south Italy, including both local self-reproduced populations and modern cultivars, and these might have contributed to increased diversity through exchange of genes with leafy kales. The same explanation can also justify that cultivated kales held higher levels of total and average diversity compared to *B. rupestris*. The lower level of diversity shown by the wild populations can also be understood, if we consider that these populations are often confined to limited areas and therefore may likely be subject to genetic drift effects.

Absence of private bands in the wild samples, compared to leafy kales, was also contrasting with the clear qualitative difference between cultivated and wild material, as shown by principal coordinate analysis (PCoA). It should therefore be accepted that the pattern revealed by AFLPs here gives a general indication of the level of diversity, without the possibility to capture all the morphological, biochemical or other functional differences.

It was possible to detect signs of intercrossing between leafy kales and *B. rupestris*, with gene flow in both directions. These were revealed by spatial visualization of genetic distances and by patterns detected with softwares AFLPOP and NewHybrids. Specific individuals were identified, carrying molecular bands uncommon in the wild populations, but frequent in home garden material. A hybrid population was also detected, resulting from crossings between wild and cultivated plants and subsequent backcrossing to both source populations.

Regarding the issue of the domestication of *B. oleracea*, our data indicated a clear molecular distinction between *B. rupestris* and both the cultivated local kales and other *B. oleracea* crop types. The genetic diversity of the Calabrian and Sicilian kales was larger than that shown by *B. rupestris*, therefore we tended to believe that *B. rupestris* is not the most likely candidate as a progenitor of the *B. oleracea* crops. This conclusion is in line with the findings from Mei *et al*. (2010) that *B. rupestris* and other Sicilian species showed such a high comparable genetic distance from *B. oleracea* (based on AFLP and SSR), that there is a low possibility that current *B. oleracea* crops originated in Sicily.

Even though the locations that we studied in south Italy may not correspond to the original sites of domestication of *B. oleracea*, we wished to highlight that the agro-ecosystems under study represented a model scenario for the conditions that could have led to domestication of leafy kales in antiquity, albeit in a different location and starting from a different wild species. In fact, in our model study we found that the wild plants were growing around or inside the villages where farmers tended their home gardens. The wild species is still consumed by humans on special occasions. Some people like their pungent taste, although generally the ‘sweeter’ taste of the domesticated leafy kale is preferred. This trait has possibly been the first criterion of selection towards domestication. The observed agro-ecological and ethnobotanical elements that might have had an influence at the time of domestication were the following: 1) occasional use of the wild relative for human consumption; 2) co-existence of cultivated (domesticated) and wild plants in close proximity; 3) conscious selection made by farmers who eliminate ‘off-type’ plants bearing wild traits from their fields; 4) ongoing inter-crossing between wild and cultivated plants with possible bidirectional introgression. We have consistently registered the above elements both in Calabria and in Sicily and we assume that the same scenario can be found in several other locations in the Mediterranean area and that it can be representative of the original steps leading to domestication of *B. oleracea*.

2.3 Patterns of diversity of kales in Europe and taxonomy issues

A comparison of the genetic diversity and population structure of twelve leafy kale landraces from several parts of Europe was carried out with AFLP markers in the study described in Paper II. Wild *B. oleracea* from the UK and north Spain and two leafy kale cultivars were also included in the study.

The genetic diversity of the landraces showed to be variable, with Nei’s values between 0.12 and 0.27. A higher genetic diversity was found for samples originating from areas were landraces are widely grown, such as in Portugal and Turkey. Cross-pollination between fields and/or exchange of seeds among farmers were given as possible explanations for higher levels of diversity. Wild samples showed intermediate levels of genetic diversity, within the same range of the landraces. Study of the population structure revealed that the largest component of the total genetic variation (62%) was found within accessions and most of the samples, with a few exceptions, grouped in one cluster in a PCoA. It was therefore not possible to clearly distinguish the landraces from the wild populations, which were separated by only 4% divergence. A correlation between genetic and geographical distance was not found. Despite the relative similarity between some of the accessions, most of them were clearly genetically identifiable from each other (38% of the variation in the data set was explained by a statistically significant differentiation among accessions). This indicates that the accessions have been separated from each other long enough for selection and genetic drift to have taken place.

A specific test was made on a wild-growing population in Rødvig, Denmark, that was included in the study to determine if the population was truly wild or an escape from cultivation. Based on PCoA and cluster analysis, this accession was found to have a close genetic relationship with 'Høj Amager', a Danish curly kale cultivar. Therefore, it seemed most likely not to consider the population at Rødvig as truly wild, but naturalized from a cultivated origin.

Regarding domestication of *B. oleracea*, this study did not give indications on the centre of origin, since no geographical trends in the genetic variation of accessions could be found.

Distinguishing whether a population is truly wild or escaped from cultivation, and even the taxonomic identification of certain populations represent a challenge. Paper VII tried to address this problem with molecular analysis of a number of ambiguous cases, based on inter simple sequence repeat (ISSR) dominant markers. This technique does not require sequence knowledge. Based on randomly extended repeat sequence primers, sequences between two SSRs are PCR-amplified, detecting a higher level of polymorphism than is detected with RFLP or RAPD analyses. Genetic diversity and population structure were measured using GenAlex software, while population assignment tests were made with AFLPOP software.

Studies made to determine the status of potentially escaped populations did not give clear-cut indications. For example, the *B. oleracea* population growing on the Island of Helgoland in the North Sea was not clearly distinguishable from a Danish cultivar, an Italian landrace and a wild population from the UK.

The assignment test indicated a high likelihood of hybridization between landraces and wild populations. Obviously the Helgoland population cannot be a hybrid between the populations used as test parents, since these are geographically too distant, but the results show that its genomic constitution is compatible with that of a hypothetical hybrid between an Italian leafy kale and a wild *B. oleracea* from the English coast. This result is showing once more that C-genome *B. oleracea* populations are sharing the same genepool. The reason why the geographically isolated Helgoland's population is not showing genetic isolation cannot be reconstructed in detail with the methodology used. However, a history of escaping from cultivation and/or hybridization with cultivated kales seems the most likely scenario.

Also the putatively wild population of Rødvig was tested again with ISSR and compared with the 'Høj Amager' curly kale. In this case, the close similarity between these two populations could not be confirmed, while the Rødvig population showed more similarity with wild *B. oleracea* from UK and France. Since the reference samples and molecular markers used in Papers II and VII differed, the results obtained were a confirmation that comparisons across experiments are not reliable whenever the variables are not perfectly stable. The case of Rødvig was subsequently enlightened by a first-hand witness, informing us that he observed that cabbages dumped over the cliff in that locations were rooting and sprouting in 1997.[2] We have therefore a modern testimony of a naturalized cabbage losing its crop traits in the wild and showing a genetic pattern that is compatible with that of wild *B. oleracea* populations of the Atlantic coasts.

The analysed case of a population growing wild on the coastal cliffs of Monte Conero, near Ancona, Italy, also gave indication of a complex situation, with assignment tests pointing at a cultivated origin. This population is locally considered to belong to the species *B. montana*, morphologically characterized by the absence of hairs on the leaves, but the genetic pattern shown by our study is more similar to hairy *B. incana* from central Italy than to other populations of *B. montana* growing further west. Following our studies, a hybrid origin is hypothesized, although no populations of *B. incana* are known to exist in this area. Similar ambiguities were revealed by the study of a *B. montana* population from Ponza Island in the Thyrreanian Sea. Also in this case, even though morphologically glabrous, genetic identity and assignment tests of the Ponza sample rather suggest similarity with hairy *B. incana* or the status of hybrid with cultivated material.

Studies carried out with ISSR did not provide conclusive statements regarding the taxonomic status and the wild vs. escaped status of the accessions

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[2] Niels Jacobsen, personal communication

analysed. However, indications from these molecular markers were clear enough to increase the suspicion that many ‘wild’ populations might be escapes from cultivation and/or the result of complex hybridizations. It also became clear that taxonomic identification cannot solely rely on morphological characters, which should be applied with caution for C-genome species, and that a species such as *B. montana* needs a careful revision, especially at the margins of its geographical distribution.

2.4 Collecting missions: legal and social aspects

Research carried out for this Thesis required the organization of collecting missions to procure fresh seed samples of the appropriate quality and composition to carry out the molecular analysis. In some cases it has been sufficient to request samples from genebanks. This approach has been efficient and allowed us to quickly test material originating from several distant parts of Europe without much effort. However, in other cases, our research could have not been accomplished without direct survey of the territory, collecting of samples in sufficient quantities and reflecting the existing population structure. For population genetic studies, we aimed at collecting seed from as many as 30 mother plants per each population and we kept the progeny of each individual separate. In this way, we could measure the genetic diversity within and among populations. It would not have been possible to solely rely on genebank samples for this purpose. Additionally, our field surveys allowed us to identify peculiar agro-ecosystems in Calabria and Sicily, consisting of several neighbouring home gardens positioned at variable distances from wild populations. Field observations made us recognize situations of potential hybridization between cultivated and wild forms and at least in one case in Caltavuturo, Sicily, we harvested seed from a patch of plants looking like hybrids, positioned at the margin of a wild population and very close to a home garden. Dialogue with farmers and locals in south Italy allowed us to better understand the cultivation practices and learn that wild species were still occasionally used and valued for their pungency, although milder domesticated species were mostly preferred. Hybridization occurrences in home gardens were clearly well known and off-types easily recognized and eliminated by farmers. We were told of food traditions and medicinal beliefs showing a close parallel with recipes and narrations found in the ancient Greek and Latin literature. For example, we heard of the antidotal effect of pieces of raw brassica stems against drunkenness. This information was repeated by the local policeman in Pazzano, Calabria, with similar confidence but more lively words than we read in a book by Theophrastus.

Harvesting of seed from nature or from farm fields, transfer of genetic resources across countries and their use for research, breeding and education are subject to an international regulatory framework – Convention on Biological Diversity (United Nations, 1992), International Treaty on PGRFA (FAO, 2002) and Nagoya Protocol (United Nations, 2010) – and to national and local legislation and procedures. Our experience in requesting permission from appropriate authorities for collecting wild *B. oleracea* on the coasts of north Spain and west France has been the occasion to analyse the issue in a wider context and from the perspective of the plant collector. Paper V analyses the legal framework of collecting PGRFA in Europe, as well as used practices and procedures. This Paper was built out of a questionnaire sent to genebank curators all over Europe, with the aim to understand the different approaches of each country and the impact of the existing regulatory framework on collecting activities. An analysis was then made of existing and evolving laws and regulations, of their perceived and effective impact, and of the opportunities to create a more transparent and effective framework in Europe. A number of difficulties were highlighted, resulting from the combination of international rules on access and benefit-sharing with pre-existing national laws and administrative procedures that both add complexity to and influence the way international conventions are implemented. It was found that the main difficulty for a plant collector lies in finding the appropriate entry point in each country and the correct procedure to be followed.

This Paper concludes with a few recommendations directed to European policy-makers and administrators. These recommendations call for the establishment of fixed and easy procedures and for the regular adoption of the Standard Material Transfer Agreement (SMTA) designed to facilitate and regulate the access to material within the Multilateral System. Regular use of the SMTA for transfer of *in situ* material would allow providers, collectors and further users in Europe to certify compliance with national access and benefit-sharing requirements under the Nagoya Protocol and the EU Regulation No. 511/2014. Plant collecting missions would greatly benefit if European countries adopted harmonized approaches to monitor plant collecting. This opportunity has not been taken with the launching of the above-mentioned EU Regulation on the implementation of the Nagoya Protocol. At the same time, pressure on collectors has increased to provide clear evidence of compliance with national access and benefit-sharing (ABS) rules or with the legality of collecting activities in the absence of such rules.

2.5 Ongoing research

Additional ongoing experiments are expected to add information in support of a theory of domestication. In particular, collecting missions have been carried out in 2011 and 2012, respectively to the Atlantic coasts of north Spain and of north and west France. A total of 19 accessions of wild *Brassica oleracea* were collected in Spain (6 from the Basque Country, 4 from Cantabria and 9 from Asturias). Additionally, 3 samples of cultivated *B. oleracea* leafy kales were obtained from local home gardens in Asturias. In France, 34 populations were collected (1 from Picardie, 30 from Haute-Normandie, 2 from Basse-Normandie and 1 from Charente-Maritime). Molecular analysis with AFLP markers will be carried out on these populations. Previous studies were carried out on Atlantic populations of *B. oleracea*. Lannér-Herrera *et al.* (1996) studied populations from the UK, France and Spain with isozymes and found high genetic diversity, significant differentiation among populations, but no correlation between genetic and geographical distances. At the local level, Raybould *et al*. (1999) found significant differentiation among populations on the Dorset coast and evidence of isolation by distance at both isozyme and miscrosatellite loci. It could be inferred that complete genetic isolation was however prevented by low but significant levels of gene flow between Dorset populations. Watson-Jones *et al*. (2006) analysed with AFLPs the genetic diversity of eight British populations and found high levels of genetic variation (Nei values ranging between 0.18-0.33), no significant relationship between genetic and geographic distance and significant differentiation between populations (F_{ST}=0.22). As previously mentioned, Allender *et al*. (2007) found almost no variation in chloroplast haplotypes of British wild *B. oleracea* populations. Populations from north Spain were studied by Sánchez-Yélamo (2014) with isozymes and in this case it was possible to show high polymorphism levels, significant differentiation among populations and a reasonable clustering of geographically homogeneous groups of populations.

Our study will complement the previous studies by analysing for the first time French and Spanish populations with AFLP markers, thereby allowing a comparison with the British analysis that was carried out with the same markers by Watson-Jones *et al*. (2006). From the resulting population structure, we hope to be able to distinguish a pattern that can bring insights on the relationship among the studied populations, also in comparison with the pattern shown by cultivated local kales that will be included in the experiment. A future comparison between the structure of the Atlantic populations and the structure of wild Mediterranean populations should be able to offer further indications on the differences or similarities among these species.

A very close association between the sites of wild *B. oleracea* and towns and villages has been documented in detail by Mitchell (1976) for the British populations. Based on his findings, he concluded that most extant populations of the British Isles seemed to have originated from cultivation and that it seemed likely that Romans or Saxons had brought the coles to Britain.

During our collecting missions to the Atlantic coasts of France and Spain, we also noticed a close relationship between wild populations and inhabited places. An accurate study of these areas for a possible correlation between brassica sites and ancient Roman villages or modern towns is a possible line of research that could bring additional circumstantial evidence to the hypothesis of naturalization of the Atlantic populations. The role of birds, particularly seagulls, in the dispersal of seeds (Gillham, 1970) is a possible complicating factor that would also deserve further investigation.

3 Discussion

The studies carried out as part of our research have deepened our knowledge of the ancient literature and of linguistic aspects related to the domestication of *B. oleracea*. Our literature survey is the most complete on this subject and the mass of collected information brought new arguments to the domestication hypothesis. Molecular studies have revealed patterns of diversity and structure of specific populations that contributed to paint new elements of the relationships among C-genome species. Studies on wild populations, sometimes suspected to be escapes from cultivation, have generated relevant results regarding the domestication theory.

3.1 Literary, linguistic and historical evidence

No evidence has been found of the use of a word referring to the ‘coles’ by ancient Fertile Crescent or Egyptian civilizations. The most ancient words with this function were found in ancient Greek literature (VI-V centuries BCE). Several words were referred to the ‘coles’, with dialectal variations, *krambē* (Doric), *rhaphanos* (Attic), *braskē* (Greek colonies in south Italy), *kaulos* (initially meaning just ‘stem’ and then the entire plant) and *kyma* (young shoots or inflorescence tops). A similar variety of words was used by the ancient Romans, starting with *brassica* (III century BCE), then *caulis* (also in this case only referred to ‘stem’ until the I century BCE and then becoming the predominant word to refer to the entire plant). *Crambe* and *cyma* were also used in Latin with the same meaning as in the Greek language. The Latin word *caput* (head) takes the meaning of the entire cole plant only in Medieval times, but already Pliny was talking about *capita* (heads) as products of the coles in the I century CE. Current European languages invariably refer to coles with words deriving from either one or the other of the ancient Greek and Latin words. Richness of terms used in ancient Greek and Latin languages point at

Greece and central and south Italy as the areas where the cole crops (and the accompanying words to define them) should most likely have originated.

Several etymological hypotheses about the word *brassica* have been proposed and often uncritically reported in articles discussing about domestication of the coles, for example Dixon (2007) and Prakash *et al*. (2012). However, we found that the only hypothesis that is given some credit by lexicographers (Facciolati *et al*., 1864) refers to the word *braskē*, used instead of *krambē* by the Greek colonists living in *Magna Graecia* (south Italy). Regarding the claim that the word *brassica* derived from the Celtic words *bresych* or *braissech*, we have found that etymologists rather suggest the opposite, i.e. derivation of the Celtic words from the Latin *brassica* (Ernout and Meillet, 1932). Recently, it has been suggested that the Scottish word *praiseach,* with multiple meanings of brassica, pottage, brass, slut, etc., and corresponding to the Irish *braissech,* has the same root as the Scottish *prais* (pot), which is also the same root as the Greek word *brazein* (to boil) (Regueiro Castro, 2014). According to this line of thoughts, the Celtic words would have been created independently for a crop that used to be boiled as a soup in a pot. Similarly independent would have been the adoption by the Greeks of different words to identify the coles (*krambē* or *rhaphanos*) and the act of boiling them (*hepsein*, and not *brazein* was used) as exemplified in a fragment of the V century BCE by Crates of Athens:

> *tōn rhaphanōn hepsein chrē* [coles are usually boiled]

Should more attention be given by professional linguists to the origin of the word *brassica*, further light might be shed on its etymology and the most likely sequence of steps of its introduction among different cultures. In any case, it can be pointed out that no word similar to *brassica* was used in Greece, except for *braskē*, as mentioned above. Therefore, the hypothesis that Celts might have passed the word (and the crop) to the ancient Romans does not hold regarding the Greeks, who were independently using their own words to identify the coles. On the opposite, it is known that the Greeks passed their words (*krambē, kaulos, kyma* and possibly *braskē*) to Romans (*crambe*, *caulis*, *cyma* and *brassica*). The word *rhaphanos* (coles for the Greeks from Athens), was also used by the Latins for a different crop (*raphanus* = radish).

Ancient Greek literature gives indications that the coles were an extremely familiar crop in all spheres of life, which is already evident from VI and V centuries BCE fragments of comedy poets. It was a leafy vegetable to be boiled and dressed with olive oil. It was familiar enough as to be frequently mentioned in verbal expressions of comic exclamatory tone (By the *krambē*!). Great emphasis was given to its medicinal properties. Among these, the alleged antidotal effect against drunkenness was popular knowledge, while physicians compiled systematic handbooks to record properties and preparations against all sorts of diseases. The only descriptions of the type of crop in use are referring to the leaves (either smooth or curly). Therefore, it can be interpreted that only leafy kales were known by scholars such as Theophrastus (IV century BCE) and Dioscorides (I century CE). The relationship between the wild relative and the cultivated plant was rather clear to Theophrastus, since he thought that the cultivated kinds existed also in a wild form and that the distinction between them was simply due to cultivation, i.e. depending whether the plants received human attention or not. Theophrastus also acknowledged the pungent and stronger taste as one of the main differences between the wild and the cultivated kind of coles.

Linguistic considerations, attesting of an independent origin of the words used in Greece for the coles, together with abundant presence of the coles in everyday's ancient Greek life, seem to indicate a probable local domestication, while it is very unlikely that the tradition to grow coles could be received from the Celts or others. It should also be noted that the first interactions between Celts and Greeks in southeast Europe cannot be traced back earlier than the IV century BCE, with possibly the first contact occurring in 335 BCE, when the Celts sent representatives to pay homage to Alexander the Great while he was engaged in wars against the Thracians (Wikipedia, 2015).

Based on the ancient Greek linguistic, literary and historic elements described above, it would be very difficult to explain a monophyletic origin of the coles from a wild relative exclusively growing on the Atlantic coasts of Europe.

Coles are mentioned since the earliest entire works of Latin literature that have reached us, written by Plautus and Cato in the II century BCE and the familiarity of ancient Romans with this crop is evidently expressed there as well as in later works. Reference was made by Cato and others to the Greek knowledge and uses of the cole, with special reference to its medicinal properties. Gradual replacement of the word *brassica* with *caulis,* which is almost complete after the I century CE, indicates that the 'stem' had been the outstanding anatomical trait characterizing these plants and indeed, no description of something different from a leafy kale can be found until the I century CE. However, by this time, Pliny describes various kinds of coles that are valued for different anatomical parts. The head (*caput*) of *Tritianum* could reach 30 cm, *Cumanum* had a wide open head (*capite patulum*), *Aricinum* was valued for its small shoots (*cauliculis*) thrown out from the leaf axils, *Pompeianum* had a stock increasingly thick as it rose among the leaves (*caule intra folia crassescit*), *Lacuturnenses* had a very large head (*capite* *praegrandes*) and had only lately come into fashion. Most types were named after localities from central and south Italy, as if they were local landraces. Pliny was amazed by the new 'monstrosities' that were not accessible to the poor people (*monstro pauperibus interdicta*), such as coles so big that the poor's man table was not large enough to hold them (*caule in tantum saginato, ut pauperis mensa non capiat*). Also *cymae* (cole sprouts or broccoli) were much valued and at the time of Diocletian the maximum price of a bunch of best quality *cymae* should not exceed 4 *denarii*.

An explosion of variety of forms is documented for Italy by the Latin literature, starting with the I century CE. As no contemporary illustrations are available, it cannot be clarified whether the 'heads' of which Pliny was talking about were cabbages or cauliflowers. The Italian localities quoted by Pliny, giving names to the various types, mostly correspond to an area still bearing high diversity of broccoli and cauliflower. The Latin words *caulis* and *caput* have passed their roots to modern words that in most European languages mean 'cole' (Kohl, cabbage, etc.). Although it is never mentioned in Latin literature that cole types were imported from foreign areas, it cannot be excluded that some types (such as cabbages) were developed in more northern areas. It is noticeable that the earliest Medieval descriptions that can be attributed to cabbages (var. *capitata*) are from the German Hidegard von Bingen and Albertus Magnus (11th century), while the earliest Medieval descriptions of cauliflower and broccoli are found in a Spanish Arabic treatise of the 13th century in which these crops are called 'Syrian coles', as opposed to other coles that are named 'Christian vegetables'. Later illustrations of cauliflowers (Fuchs and Dodoens in the 16th century) are described as *Brassica Cypria* or *Pompeiana*, pointing at Italian or Aegean origin.

In summary, with a systematic compilation and analysis of available references from the ancient Greek and Latin literature, our studies have reinforced the evidence that linguistic, historical and literary information is not compatible with a monophyletic origin of the *B. oleracea* crops, if such a single origin is attributed to the Atlantic *B. oleracea* var. *oleracea*.

3.2 The dubious wild status of Atlantic *B. oleracea*

Since 1990, molecular data have consistently pointed at *B. oleracea* var. *oleracea* (and *B. bourgeaui*) as the closest progenitors of all the cole crops. This pattern was confirmed using nuclear and chloroplast markers, based on RFLP, SSR, SINE and AFLP. Also crossing experiments published in the 1990s confirmed these Atlantic taxa to be closer to the cole crops than any other wild species. As indicated in the Introduction, historical data would reconcile with genetic data if all the wild Atlantic populations were escapes from cultivation.

In his study on populations of *Brassica oleracea* subsp. *oleracea* of the British Isles, Mitchell (1976) reports contrasting opinions of botanists of the 19th and 20th centuries, some of them regarding the status of a number of British populations as probably adventive. For example, the conclusions of Watson (1870) were:

> Denizen? …Coast cliffs, native? Inland only as an alien…Wild on the western coasts of France? – N.B. Exceedingly difficult to trace the native habitat of this plant

Personal visits of the British populations and historical investigation made by Mitchell (1976) led him to suggest that the large majority could be considered adventive, especially considering their strict association with towns and villages. The ephemeral nature of many populations that have gone extinct was also considered notable and the short-living populations were presumed to have been introduced. Other existing populations could be pointed out as almost certain introductions since plants extended up from back gardens or grew particularly around old allotments which had been cultivated for centuries. The population from Dover (East Kent) was considered unique in the sense that records of its existence in a wild status could be traced back as far as 1551 and therefore it holds the strongest claim to be truly wild. However, Dover had extensive garrisons for centuries and was one of the main areas of Saxon settlement during the early post-Roman period (Mitchell, 1976).

Watson-Jones *et al*. (2006) listed the Atlantic populations of *B. oleracea* as being introduced and subsequently naturalized, although they noted difficulties in distinguishing native from alien populations along the sea coast and noted as well that Preston *et al*. (2002) had listed all coastal populations in England and Wales as native.

Allender *et al*. (2007) suggested that lack of chloroplast diversity in tested UK wild accessions would be explained if they were escapes from cultivation.

Contrasting opinions about the native status of the French populations were also expressed in the past and the adventive status was suggested by Grenier and Godron (1848).

The wild status of the population from Helgoland, Germany, has been repeatedly disputed (Körber-Grohne, 1995). According to Hegi (1919), it was naturalized, since absent from a 16th-century list of plants. It was later documented in the *Flora Danica* (Hornemann, 1830-1834, p. 5 and Table MMLVI). Our studies have focused on this population and suggested as most likely either a direct introduction from cultivation or a hybridization with cultivated kales.

Our study of the Danish population of Rødvig showed that molecular data can give contrasting results, but also that the choice of reference material is critical. Since we compared the Rødvig population with a Danish curly kale on one side and with 'wild' UK or French populations on the other side, we may have missed the comparison with a truly wild population, especially if truly wild *B. oleracea* populations do not exist.

The case of the Rødvig population is exemplary, since we learned from a local witness that it was undoubtedly an introduction from a cabbage dump. This population is at first sight indistinguishable from any other 'wild' Atlantic population. The main domestication traits (in this case we know that it was derived from a cabbage) are no longer maintained. This situation corresponds to descriptions that can be found in literature (Mitchell, 1976) about the reversion of populations escaped from cultivation that become morphologically indistinguishable from 'native' plants within a few years. Mitchell also quoted an observation from Syme (1863):

> Red cabbage of neglected gardens at the sea-side pass back in a few generations to the condition of the wild cabbage

While it is plausible that hybrids between different cole crops may lose the phenotype determined by homozygote recessive traits of one of the parents, it is not immediately clear why cabbage plants abandoned on a cliff should not continue to generate cabbages in their progeny, but rather revert to the wild leafy type. One possible explanation is that under unfavourable conditions of water and nutrient stress, selection is quickly acting in favour of 'wild' types that are better adapted to survive in the natural environment, especially if there is a large heterozygosity available for selection. This hypothesis might be tested with appropriate experiments of artificial naturalization and then reintroduction into cultivation at different post-naturalization times. Whatever might be the mechanism behind the loss of certain domestication traits in naturalized populations, the occurrence of such 'reversion to the wild' may explain many situations of dubious wild status of British (and other Atlantic) populations. It can also explain those cases of naturalized *Brassica oleracea* populations growing wild in North America, where it has become established on sea-facing cliffs in California (Al-Shehbaz, 2013) and New Zealand (Tsunoda, 1980; Rakow, 2004).

The other Atlantic species, *B. bourgeaui* from the Canary Islands, is mainly distinguished from *B. oleracea* based on leaf morphology and its taxonomic status was considered dubious by Borgen *et al*. (1979) and by Snogerup

(1980). Snogerup *et al.* (1990) thought that it deserved the status of species, although it seems that they based their analysis only on cuttings from one plant belonging to a population of two plants discovered by Borgen and Elven (1979) in La Palma. Populations of *B. oleracea* that escaped from cultivation are common in the Canary Islands (Marrero, 1989) and cannot be distinguished from wild *B. oleracea*, which is listed for these islands by Eriksson *et al.* (1979). As said above, *B. bourgeaui* is genetically very close to the cole crops and this is not surprising, if we assume that it has evolved directly from populations escaped from the fields. Our own molecular analysis of an accession from the Canary Islands passed to us under the name *B. bourgeaui* and that was growing wild on the Island of Hierro, indicated its higher affinity with cultivated coles than with other wild North Atlantic populations, thus confirming the occurrence of escapes from cultivation in the Canary Islands.

In summary, we have made field observations, generated molecular data and collected bibliographic accounts that are all compatible and actually reinforcing the theory that considers the wild Atlantic populations to be all introductions by humans.

3.3 Challenges to the theory of a Mediterranean domestication

A concept in opposition to our theory was presented by Gómez-Campo and Prakash (1999), who said that all the Mediterranean kales should be considered mere escapes from early cultivations. This statement does not seem to be justified for a number of reasons:

- There is a high diversity of species in the Mediterranean and some of them (the Sicilian *B. rupestris*, *B. macrocarpa* and *B. villosa*) (Mei *et al.*, 2010 and Paper IV) show both higher genetic distance from *B. oleracea,* and lower fertility upon hybridization with it, than would be expected from escapes from cultivation.
- The high Mediterranean diversity of chloroplast haplotypes is contrasting with the near uniformity of *B. oleracea* (Allender *et al.*, 2007). It would be difficult to believe that sustained differentiation took place in the Mediterranean in a short time period after domestication, while no differentiation would have taken place in the original truly wild Atlantic progenitor.
- Chloroplast phylogeny studies (Arias *et al.*, 2014) support a Mediterranean origin for ancestors of *B. oleracea*.
- Historical, linguistic and literary evidence is not compatible with an introduction of the cole crops to Greece from the west.

Another objection to the hypothesis that all the *B. oleracea* subsp. *oleracea* populations of the European Atlantic coasts could be introductions was formulated by Snogerup *et al*. (1990). These authors considered highly improbable that all the cole crop types could have originated from Mediterranean species, since the cultivated forms have a glaucous leaf surface in common with wild *B. oleracea*, while this trait is not present in any of the Mediterranean species. Most importantly, they did not consider possible that a crop of Mediterranean origin could have naturalized repeatedly and successfully in northwest Europe but not in its area of origin.

In reply to this well-argued criticism it can be noted that a number of cases of naturalization from cultivation (introduction by man) in the Mediterranean area have actually been listed by Snogerup *et al*. (1990) themselves. A few populations of *B. cretica* are occurring locally far outside their distribution area, at Mount Carmel, Israel and from central to south Lebanon. One population of *B. incana* in Crimea is also outside of the distribution area of this species. These cases have been interpreted by Snogerup *et al*. (1990) as introductions due to ancient use of these species for human consumption. In support of this interpretation, these authors reported their knowledge of cultivation of *B. cretica* in the Island of Samos until 1962. The Crimea population was cultivated in Lund and found to be more interfertile with cultivated *B. oleracea* than with other *B. incana* material. Therefore, it was thought to be a probable introduction resulting from former introgression. A deeper investigation of these populations, their history and the genetic status and extent of use of *B. cretica* for direct consumption in the Aegean area seems to be necessary before elaborating further interpretations.

Another possible introduction refers to the location of Monte Conero, south of Ancona (Italy). Snogerup *et al*. (1990) regarded this population as escaped *B. oleracea*. However, local botanists assigned it to *B. montana* (Biondi *et al*., 2002). Our molecular investigation on this population (Paper VII) seemed to suggest that it is indeed more likely an escape from cultivation than *B. montana*. This example confirms that *B. oleracea* may indeed naturalize in a Mediterranean context and then become indistinguishable from a wild species. Figures 3 and 4 show the similarity between the probably escaped *B. oleracea* of Monte Conero and a wild *Brassica oleracea* from Torimbia (northern Spain).

Figure 3. Monte Conero, May 2005.

Figure 4. Torimbia, Asturias, July 2011.

The case of Monte Conero is not isolated. Another population that is probably an escape from cultivation was observed in 2014 in San Marino, at a location where it was identified as *B. oleracea* subsp. *robertiana* (= *B. montana*) by Biondi and Vagge (2004). A comparison between the San Marino population and a wild *B. oleracea* from north Spain is shown in Figures 5 and 6.

Figure 5. Getaria, Basque Country, July 2011.

Figure 6. Borgo Maggiore, San Marino, August 2014.

The glaucous and waxy leaf surface, typical of cultivated crops, is shown by the leaves of plants from the San Marino station in Figure 7.

Figure 7. Glaucous leaves of the San Marino population.

Ambiguities in determination of *B. montana* were highlighted in Paper VII, indicating that the diagnostic traits currently used for this species may be insufficient to always distinguish it from other species or from escapes.

Escapes from cultivation of *B. oleracea* are frequently noted in Italy, generally giving rise to ephemeral groups of individuals, easily subject to grazing or removed by human activities along the roads or in open spaces. Also Snogerup *et al.* (1990) noted the threat of introgression from cultivations and from escapes found in close proximity of local, wild populations. They also noted that hybridization may occur in these situations, such as in the case reported by them of 38 healthy hybrid plants noted in 1984 at 250 m from a *B. incana* population in Casamicciola, Island of Ischia.

We also confirmed the presence of hybrid plants growing between a leafy kale garden and a *B. rupestris* population in Caltavuturo, Sicily, as well as common occurrence of reciprocal introgression between cultivated and wild plants (Paper III). In such situations, escapes from cultivation are most likely 'absorbed' by existing populations and this may be the reason why naturalized populations in a Mediterranean context often do not look like the Atlantic *B. oleracea* subsp. *oleracea*. In those cases where they escape and colonize habitats that are free of local species, they may be classified as *B. montana*, such as in the Monte Conero and San Marino examples. It should also be noted that the distinctive traits between C-genome species are not always clear cut, while intraspecific variability is very large. An interesting example of such variability was found by Snogerup *et al.* (1990) for the leaf shape of a *B. rupestris* population of Rocca Busambra, Sicily. This population is far from other populations and no cultivation of *B. oleracea* was observed in the surrounding, therefore introgression was excluded. However, all plants showed a glaucous and waxy leaf surface, which is therefore not an exclusive trait of the Atlantic populations, but is within the remit of other species as well.

A final observation is that there are no clearly documented occurrences of two species sharing the same habitat. Even in mainland Sicily, where at least five species (*B. drepanensis*, *B. incana*, *B. rupestris*, *B. trichocarpa* and *B. villosa*) broadly share the same territory, each cliff complex remains monospecific. Genetic drift due to isolation and sometimes introgression from cultivated crops result into complex situations, making taxonomic identification uneasy.

In summary, it can be assumed that when *B. oleracea* naturalizes in a Mediterranean context, it is either disguised by already existing populations of other species occupying the suitable habitat, or it takes a habit that may not be recognized as *B. oleracea* var. *oleracea* and it is sometimes taxonomically classified as *B. montana*.

4 Conclusion

Our hypothesis that coles were initially domesticated in a Mediterranean context, as opposed to an Atlantic context, has gained support from historical and linguistic studies. Additionally, analysis of the existing bibliography, field observations and molecular data from our studies have added enough elements to make our starting hypothesis plausible and defendable.

However, in order to confirm our theory, it will be necessary to resolve at least two issues: 1) to prove without doubts that all the wild Atlantic kales are in fact introductions from cultivated fields; 2) to identify the nearest Mediterranean progenitor of the cole crops.

Alternatively, our theory could be falsified if archaeological or literature findings would be able to prove the existence of cole crops in Atlantic contexts before any contact occurred between northwestern populations and Roman or Greek or Phoenician traders. It would also be falsified if the Atlantic populations of *B. oleracea* could be proved to be truly wild.

A number of experiments or investigations can be conceived to clarify the status of the Atlantic populations (either wild or adventive):

- verify the existence of a correlation between ancient Roman settlements and the position of the wild populations;
- survey the genetic pattern, behaviour and origin of *B. oleracea* populations growing wild in other continents (indications exist for California and New Zealand), where it is safe to state that the plants have escaped from cultivation; verify their genetic diversity and population structure with various molecular markers, looking for differences from, and/or similarities with the European Atlantic populations;
- verify experimentally whether few generations are sufficient for various *B. oleracea* crop types to escape from cultivation and to become

habitually similar to wild populations in a natural habitat; verify the loss of domestication traits;

- carry out paleobotanical studies about the presence of *B. oleracea* growing on the Atlantic side of Europe before the start of crop domestication.

The above-mentioned lines of investigation could probably only build up additional circumstantial evidence on the validity of our theory and not lead to a final proof.

The identification of the nearest progenitor of the cole crops, assuming that it cannot (or not only) be the Atlantic *B. oleracea,* is still not verified, based on the existing bibliography and our own studies and observations.

We tended to exclude *B. rupestris*, which was the focus species in our model agro-ecosystem studies. This is in line with the findings of Mei *et al*. (2010) and others, that the Sicilian species are genetically more distant from *B. oleracea* than other species. The impression deriving from ancient Greek literature is that the Greeks independently domesticated leafy kales and this would point at *B. cretica* as a strong candidate. Indeed, *B. cretica* (after *B. oleracea* and *B. bourgeaui*) showed the highest values of pollen fertility (79% in F_1 hybrids) following crossings with cultivated forms (Bothmer *et al*., 1995).

B. cretica showed a large variability of haplotypes in the study made by Allender *et al*. (2007), including the occurrence (1 plant out of 9 tested) of the same haplotype that is prevalent in *B. oleracea*. In this same study, the other species prevalently showing the same haplotype as *B. oleracea* was *B. incana* (10 plants out of 11 tested). In crossing experiments with cultivated forms, *B. incana* also gave good results, with a pollen fertility of 78% in F_1 hybrids, although in the experiments of Kianian and Quiros (1992) *B. incana* only produced semi-fertile hybrids with *B. oleracea*.

Based on the above observations, *B. cretica* and *B. incana* are probably the best candidate species to be further investigated. Each of these two species has a rather wide distribution area (Aegean in the case of *B. cretica*, Adriatic and Thyrreanian in the case of *B. incana*). Ideally the entire distribution area of the two species should be sampled and analysed and not only a limited number of accessions, as it is usually done. Morphological variation in about 20 populations of *B. cretica* was studied by Snogerup *et al*. (1990), who found considerable variation in most of the characters studied with the largest part of variation observed among populations. A comparable study of the variation of *B. incana* across its distribution range is not available, also considering that Adriatic populations are not represented in genebank collections.

B. montana could be a third candidate for further investigation, but the available data are somehow contradictory. In fact, this species was proposed in the past as a possible ancestor of some cole types. Originally, it was also considered by de Candolle (1885) and by Hegi (1919) to belong to the same *sylvestris* variety as the wild *B. oleracea*, considering the morphological similarity among the two species and their relative geographic proximity. The flavonoid pattern of *B. montana* was shown to be similar to that of *B. oleracea* by Aguinagalde *et al*. (1992), while Kianian and Quiros (1992) included *B. montana* in the same karyotype group as *B. oleracea*, based on its ability to form fully fertile hybrids. However, contrasting results were shown by various molecular analysis, where *B. montana* does not always stand out as the closest relative of *B. oleracea*. Moreover, Bothmer *et al*. (1995) positioned *B. montana* among the species with lower fertility rates following experimental crosses with *B. oleracea*. Finally, *B. montana* did not share any haplotype with *B. oleracea* in Allender's experiments (Allender *et al.*, 2007).

Understanding the phylogenetic position of *B. montana* could be important for the clarification of the wild status of *B. oleracea*. Assuming it is correct that *B. oleracea* varieties and their wild relatives diversified in the northeastern Mediterranean and later spread through the rest of Europe (Arias and Pires, 2014), it would be logical if *B. montana,* which is geographically positioned between the distribution area of *B. oleracea* and all the other Mediterranean species, would be the penultimate species in a sequence of differentiation from the east to the west. Should *B. montana* be confirmed to be more distant from *B. oleracea* than other species, the wild nature of the Atlantic *B. oleracea* could be disputed also from this different angle. Incidentally, the taxonomic boundaries of the species *B. montana* should be defined with better precision, considering the uncertainties evidenced by our Paper VII.

A conclusive experiment that might definitively resolve the genetic relationships among the C-genome species and position them in a correct phylogenetic sequence has not yet been completed, although many attempts were made, with contrasting results, as described in the Introduction. A large number of molecular markers (301 polymorphic bands from 11 primer combinations of AFLP and 518 polymorphic bands from 108 primer combinations of SSR) were used by Mei *et al*. (2010) and were sufficient to clearly separate a group of species (*B. insularis*, *B. rupestris*, *B. villosa* and *B. macrocarpa*) as more distant than others from *B. oleracea.* However, the relationships between the other species were not fully resolved. The use of both a large number of markers and a large number of samples representing the entire distribution area of each species might give clearer results. Part of the difficulty may be due to the close genetic distance between the remaining

C-genome species, coupled with the continuing possible intercrossing between wild populations and cultivated crops in the last two thousand years, which makes it difficult to reconstruct the phylogenetic thread.

Future studies of genome evolution, based on comparison of genome sequences, may contribute to resolving pending questions. The recent comparison between the entire sequences of a *B. rapa* crop and *B. oleracea* var. *capitata* (cabbage) allowed to estimate the divergence between these species at ca. 4.6 Mya, based on synonymous substitutions (Ks) rates of genes (Liu *et al*., 2014). Other revealing comparisons consist in the analysis of transposable elements insertion times, differentiation of paralogous and orthologous gene sequences and of their expression. Undoubtedly, future comparisons of entire genomic sequences of the wild species and of the various *oleracea* crops will clarify phylogenetic steps, genetic relationships and the domestication of *B. oleracea*.

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Various theories exist about the progenitors of *Brassica oleracea* L. (cole crops) and the area of its domestication. This work formulates an alternative hypothesis, namely that the domestication of *B. oleracea* occurred in a Mediterranean location, while at a later date the crop reached northwest Europe, where it returned to the wild on the Atlantic cliffs. Linguistic, literary and historical aspects, as well as genetic studies of model agro-ecosystems and the investigation of cole populations escaped from the fields bring support to our hypothesis through indirect means.

Lorenzo Maggioni received his MSc degree from the School of Biological Sciences, University of Birmingham, UK and his undergraduate degree in Agricultural Sciences from Università Cattolica del Sacro Cuore, Piacenza, Italy.

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