Diversity in natural populations of wild cabbage *(Brassica oleracea* L.*)*

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Abstract

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Brassica oleracea L. belongs to the *B. oleracea* cytodeme with $2n = 18$. Biosystematic studies show that the cytodeme is composed of distinct species or groups of regionally distributed species. Wild populations of *B. oleracea* are distributed along the Atlantic coasts of Spain, France, and Great Britain, where they usually grow in steep parts of maritime cliffs consisting of limestone or chalk. The size of the populations varies from one locality to another and a considerable intrapopulational variation is present in most of the populations regardless of size. Isozyme analysis indicates that even adjacent populations, geographically separated by a few kilometres only, may show quite different isozyme patterns. Thus, in most parts of the distribution area the species is represented by spatially well isolated populations and local adaptation has often given rise to morphologically distinct populations. The gene flow between adjacent populations seems to be small.

The wild Mediterranean *Brassica* species with the chromosome number $2n = 18$, form a polymorphic aggregate of species belonging to section *Brassica*. It consists on one hand of crop plants, as a result of domestication of *B. oleracea* in particular, and on the other of numerous wild species, which are perennial and inhabit maritime biotopes, mostly coastal cliffs or rocky islets. Biosystematic studies show that the complex is composed of distinct species or groups of regionally distributed species (Gustafsson 1979, 1982, Gustafsson & al. 1983, Snogerup & al. 1990). *B. olereacea* differs from all the other species in the cytodeme by its Atlantic distribution and the glabrous, waxy leaves with a greyish-blue surface.

The species *Brassica oleracea*

The Linnean species *B. oleracea* comprises crop plants as well as wild plants. Ancient literature reveals that the domestication process started very early (Toxopeus 1979).

Theophrastos (370-285 B.C.) makes clear that several edible coles were cultivated in Greece representing not only primitive forms similar to the wild species of Greece, but also more domesticated forms similar to stem kales and types with curly leaves. Most authors believe that the cultivars have a monophyletic origin, that is originate from wild, western European *B. oleracea.* It is true that several cultivars show morphological traits which are also observed in *B. oleracea,* but on the other hand many cultivars show morphological characteristics which are found in other wild species. The cultivars are probably of polyphyletic origin, but back crossing to wild and cultivated *B. oleracea,* which has occurred frequently during centuries, makes it difficult to trace the origin of the parental species.

Habitat and population structure

The populations of wild *B. oleracea* are distributed along the Atlantic coasts of northern Spain, western and northern France, the British Isles, and Helgoland. They grow in steep parts of maritime cliffs consisting of limestone or chalk, but in situations protected from grazing, plants can be found below the cliffs in scree and among shrubs. In France and Great Britain some populations have colonized steep, grassy slopes. Adjacent populations are isolated from each other by unsuitable habitats. Usually, a single population is restricted in size and distribution and the number of plants comprising a population may vary from one location to another. During a collecting mission in 1988 the sizes of 44 populations of *B. oleracea* were estimated as to the total number of individuals (Table 1). The Spanish populations tend to be small in number, mostly due to restricted areas of suitable cliffs. The situation for the French populations is quite the opposite, many of the populations are large and distributed over fairly large distances. In Britain, population size covers the entire range from very small up to very large.

Table 1. Size of 44 populations of wild *Brassica oleracea,* all estimated in August 1988. N indicates the number of populations.

Origin	Population size (number of individuals) 1 - 50 - 100 - 500 - 1000 - 5000 - 10000 >10000							N
Spain	4	4	1	0	1	1	0	11
France	0	0	1	3	2	4	4	14
Britain	1	3	2	2	6	2	3	19
Total	5	7	4	5	9	7	7	44
Category	small			medium-sized			large	
%	27			41			32	

Diversity in populations of wild *B. oleracea*

Investigations in the field indicate that the morphological variation within the *B. oleracea* group is considerable and that even small populations may maintain a fairly large intrapopulational variation. Moreover, individual plants of small populations did not seem

to suffer from inbreeding by means of reduced vegetative growth, flowering or reduced seed set. The species *B. oleracea,* which shows a large range of variation in population size, was selected as a model for further studies of the mode of variation within and between wild populations of various size.

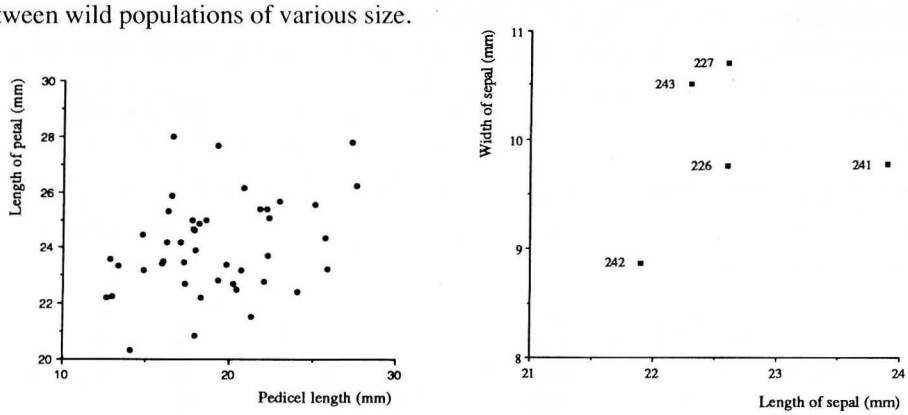

Fig. 1. Morphological variation in wild populations of *B. oleracea.* The measurements have been carried out on 50-60 plants grown in a heated greenhouse. To the left: mean values for length of petal and length of pedicel of 44 wild populations of *B. oleracea.* To the right: sepal size in five British populations. Number 226 and 227 represent two adjacent populations from Folkstone in Kent, and the other, adjacent populations from Wales. Numbers 241 and 242 originate from Llandudno and 243 from Llandulas.

Morphological variation

Comparative cultivation experiments were conducted with 44 wild populations. Each population was represented by 50-60 plants grown in a heated greenhouse with 16 h of light. In total, eleven characters were analysed and the variation in two generative characters is shown in Fig. 1 and Table 2. Statistical analysis indicates that there are significant differences between populations in all morphological characters. The pattern of variation can be summarized as follows: the largest part of the variation exists among populations; even adjacent populations may differ significantly in many characters; small populations with less than 100 plants may also exhibit a large variation; and component analysis indicates that no regional differentiation is obvious.

Male sterility

In the cultivation experiments, the frequency of male sterile plants was investigated. Male sterile plants with rudimentary anthers were found in populations of various size, but the frequency seems to be higher in small than in intermediate and large populations. In ten out of eleven Spanish populations male sterile plants were found, with the highest frequencies in populations Es 204 and 202 with 40 and 32 %. In material from France and Great Britain male sterile plants are observed in 9 out of 14 and 6 out of 19 populations. The highest frequencies were 10.9 and 7.8 % in British populations and 7.5 % in French.

Probably, female sterility is rare, as the seed set of male sterile plants after open pollination was as high as in 'normal' plants.

Table 2. Length of sepal in twelve populations of wild *B. oleracea* in relation to size (number of individuals). The results are based upon 50 to 60 plants of each population.

Population		Size	Sepal length mean +/- 2 sd
Es	210	75	13.8 +/- 3.2
Es	209	80	13.5 +/- 2.5
GB	226	400	11.0 +/- 3.0
GB	236	500	12.7 +/- 3.2
Fr	211	550	12.4 +/- 3.0
GB	227	900	11.2 +/- 2.9
GB	243	2500	10.9 +/- 2.4
GB	242	4500	11.5 +/- 2.5
Es	200	5000	12.0 +/- 2.8
Fr	224	8000	13.7 +/- 3.2
GB	225	10000	13.3 +/- 3.0
GB	241	10000	12.0 +/- 2.9

Enzyme electrophoresis

Isoenzyme polymorphism was investigated in twelve populations, three originating from Spain, two from France and seven from the British Isles. The standard procedures have been used (Lannér-Herrera & al. 1996) and the enzymes PGI (1 locus), Dia (1 locus), PGD (1 locus) and PGM (2 loci) have been investigated. Some results are summarized in Table 3 and Fig. 2 and 3.

A considerable intrapopulational variation is present in most of the populations regardless of size. However, the populations Fr 211 and GB 227 deviate from the others by having low values, 0.10 and 0.17 respectively. Fr 211 is the southernmost of the French populations and is effectively isolated from all others. The cliff area at this site, suitable for colonization, is limited and probably the population has been founded by a few individuals. This founder effect may explain the remarkably small variation. The reason for the narrow variation in GB 227 is not known.

The gene diversity in subdivided populations, GST, was determined over all populations, among populations within national regions and between regions (Fig. 2). The results show that the interpopulational variation is considerable in Great Britain and France, but rather small between Spanish populations.

Even in the adjacent populations from Wales, in Fig. 3 represented by GB 241, 242, and 243, which are geographically separated by a few kilometres only, show quite different isozyme patterns. For instance, in locus PGM 2 (Fig. 3) the allele b is dominating in population GB 242, while c is the most common allele in populations GB 241 and GB

243. Similar results have been obtained in two adjacent populations from Folkstone in Kent (GB 226 and 227).

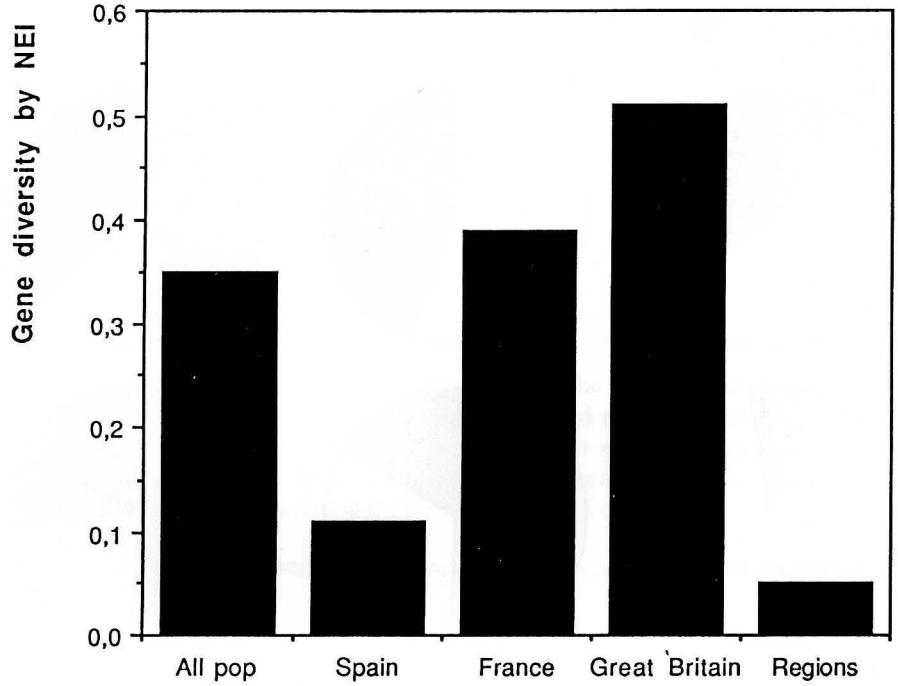

Fig. 2. Summary of gene diversity in subdivided populations, GST. The results are based upon isozyme variation at 5 loci.

Table 3. Isoenzyme variation (gene diversity by NEI) within twelve populations of wild *B. oleracea* of various size. The index varies between 0 and 1, where 0 means a completely homogeneous population and 1 a completely heterogenous one.

Population		Size	Index
Es	210	75	0.41
Es	209	80	0.42
GB	226	400	0.30
GB	236	500	0.37
Fr	211	550	0.10
GB	227	900	0.17
GB	243	2500	0.38
GB	242	4500	0.38
Es	200	5000	0.42
Fr	224	8000	0.42
GB	225	10000	0.42
GB	241	10000	0.50

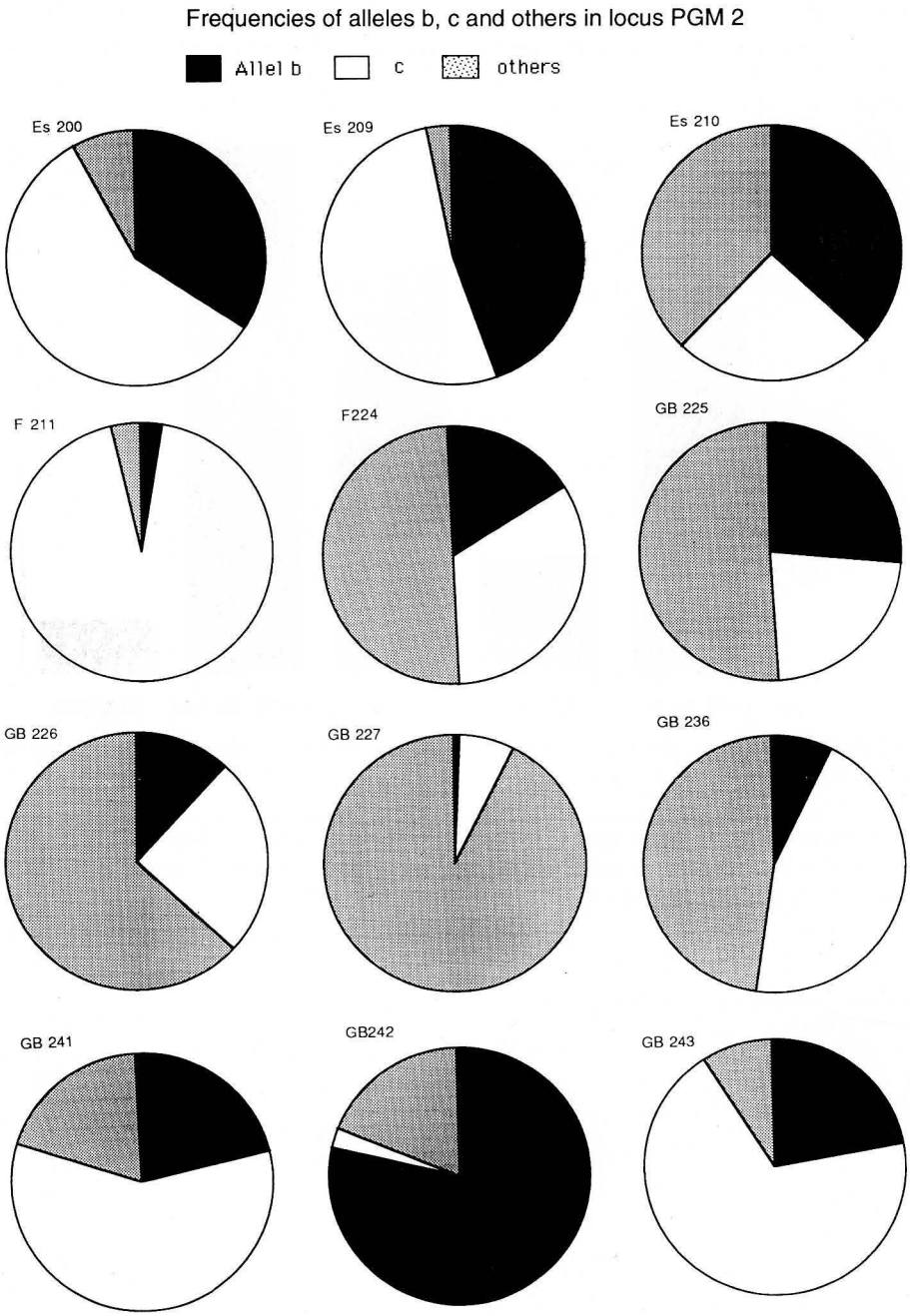

Fig. 3. Allele frequencies in locus PGM-2 in twelve populations of wild *B. oleracea.*

Table 4. Mean frequency (%) of homozygous loci in populations of various size. The results are based on isozyme variation. The figures are calulated as follows: percentage of plants homozygous for any allele in locus GPI + the frequency of homozygotes in DIA + the frequency in any other loci divided with the number of loci analysed.

Population		Size	Homozygosity	Population		Size	Homozygosity
Small populations				Medium - large populations			
GB	233	10	62.0	GB	235	2 000	54.5
Es	210	75	67.6	GB	243	2 500	84.2
Es	209	80	61.9	Fr	218	3 000	60.0
GB	226	400	64.9	Fr	212	4 000	60.3
GB	236	450	78.0	GB	242	4 500	71.0
Fr	211	550	89.5	Es	200	5 000	60.1
GB	227	900	86.1	Fr	224	8 000	60.8
				GB	228	9 000	58.7
Average: small			72.9	GB	225	11 000	57.4
medium - large			62.2	GB	241	12 000	55.1

The frequency of homozygous genotypes has been calculated and the results are summarized in Table 4. On average small populations have high frequencies, between 62 and 90 %, with the largest frequencies in the French population Fr 211 (89.5 %) and the British GB 227 (86.1 %).The corresponding figures for populations larger than 2 000 plants are less, on average 62.2 %, with extraordinarily high frequencies in the British populations GB 243 (84.2 %) and GB 242 (71 %).

Crossing experiments

In order to study reproductive isolation, all the wild species were crossed with each other and with cultivated forms of *B. oleracea.* All combinations were compatible, but most crosses show some reduced fertility in the hybrid offspring. By far, the most viable and fertile hybrids were found in crossing combinations between wild populations of *B. oleracea* and cultivated forms. Even in hybrids with reduced male fertility the seed set was high enough to secure further offspring. These crossing experiments indicate that all cultivated forms including the Asiatic *B. alboglabra* and the wild populations belong to the same biological species, *B. oleracea* (Bothmer & al. 1995).

Differentiation pattern

In wild *B. oleracea* there is a remarkably high variation in small populations, although the frequency of homozygous loci is higher in small than in intermediate and large populations. Significant differences exist between populations in most morphological traits as well as in distribution of alleles. Even, geographically closely adjacent populations may

differ considerably from each other and isozyme analysis, in particular, indicates that gene flow between populations is highly restricted.

Male sterility is most pronounced in small populations and the highest frequencies are observed in Spanish populations. A certain degree of male sterilility in small populations favours outcrossing, which decreases inbreeding depression, but increases the number of new recombinants.

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