BOTANICAL FILES ON LETTUCE (*LACTUCA SATIVA*)

On the chance for gene flow between wild and cultivated Lettuce (*Lactuca sativa* L. including *L. serriola* L., Compositae) and the generalized implications for risk-assessments on genetically modified plants

by

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Summary

In Botanical Files, a study of the real chances for gene flow from cultivated plants to the wild a system of dispersal codes (D_{pdf}) was introduced (see text box D_{pdf}).[37] They are indications of already occurring gene flow from cultivated plants to the wild flora, as can be deduced from herbarium collections and floristic archives. These codes apply to the Netherlands only.

One of the crops of which the real chances for gene flow could not be determined, because of uncertainties regarding the relationship between the cultivated plant and its wild relatives, is Lettuce (*Lactuca sativa* L.). Its relationship with the wild *L. serriola* L. is accepted to be very close, but the species are considered to be distinguishable. In a field trial, using 350 specimens from 67 genetically different populations, the distinction between the two species proved to depend largely on character states usually connected to domestication, like absence or presence of prickles, retention of achenes, leaf texture and colour.

The consequences are that both wild and cultivated lettuce must be considered to belong to the same species. The finding of some 'domesticated' character states in 'wild' lettuces indicates an already ongoing gene flow between cultivated lettuce and the wild flora, and the D_{pdf}-code is adapted accordingly, indicating a **substantial chance for gene flow** from cultivated lettuce to its wild relative in the Netherlands.

If the scope of Dispersal codes as in Botanical Files is extended to Europe, European D_{pdf}-codes are needed. In this report a model is proposed using a plant geographical division of Europe into six vegetational regions. For each species six D_{pdf}-codes, summarizing the chances for gene flow to each of the regions, should be developed. For the major part of the species this can be done using the information already present in national herbarium collections.

1. Introduction

This report, *Botanical Files on Lettuce*, is a continuation of the report *Botanical Files* which presented the possibilities for gene flow from cultivated plants to the wild flora of the Netherlands.[37] *Botanical Files* was prepared for the Ministry of Housing, Spatial Planning and Environment, and can be used as a tool for the evaluation of risk assessments for Genetically Modified Organisms (GMO's).

In the previous report 42 crop species were studied, to decide which (floristic) factors should be considered when assessing the possibility for gene flow from a certain cultivated plant to the wild flora. This question had become an urgent one in the public discussion on the introduction and use of GMO's.

Gene flow

There are two ways in which a cultivated plant may cause gene flow to the wild flora: by hybridizing with wild relatives and by running wild itself. So the chances for gene flow depend both on the degree of relationship of the cultivated plant with its closest wild relative, and on the fitness of cultivated plants in a more or less natural, i.e., non-cultivated environment.

It must be stated explicitly, that the nature of the inserts in GMO's was not included in the study, nor the effect of the inserts on the organisms themselves. The study was confined to the already occurring gene flow, which can be inferred from the distribution of plants present in the wild in the Netherlands now and in the past, and from their relationships with cultivated plants (see text box Gene flow).

During the study for Botanical Files it became clear, that taxonomic relationships of cultivated plants and their relatives in the wild, must be fully understood before the real chances for gene flow can be determined. Of most of the cultivated plants in the study, their relationship to the species of the same genus belonging to the wild flora of the Netherlands could be determined without doubt. Of some species however, the exact relationship with wild relatives could not be ascertained because of lacking or incomplete information. For these species further research was recommended.

One of the crops suited for this purpose is *Lactuca sativa* L., Garden lettuce, cultivated on a small but wide-spread scale in the Netherlands, both indoors as a greenhouse vegetable, and outdoors. It is considered to be closely related to Prickly Lettuce (*Lactuca serriola* L.), which is a component of the wild flora of the Netherlands.

Prickly lettuce is generally found on sunny, more or less dry, rich, usually calciferous and/or stony places, and is associated with soil disturbance. It was rare in the Netherlands until the first half of this century, but since the 1960's it has suddenly become invasive in the Western part of the Netherlands.[12 13 23 41] The same phenomenon was recently recorded in Warwickshire, Britain.[5] On the other hand, Garden lettuce is assumed to be only occasionally found in the wild, and then usually in unnatural habitats, like rubbish dumps, waysides, old allotments, etc.[22] Both species are considered to be predominantly self-fertilizing.

Prickly lettuce (Fig. 4, p. 21) is usually considered to have bluish green leaves with a row of prickles on the midrib, and with cauline leaves usually twisted to the vertical plane. The capitula are grouped to a rich pyramidal or plume-like inflorescence, and have reflexed involucral leaves. Garden lettuce (Fig. 5, p. 21) is described as having often light-coloured, glabrous leaves, forming large heads, and with cauline leaves not twisted to the vertical plane. The capitula are grouped to a flat-topped, umbel-like inflorescence, and have erect involucral leaves.

Because this is the commonly held opinion of Garden lettuce, a wild growing lettuce with yellowish green leaves and no prickles will be considered to be an escape, whereas an observation of a vegetative or poorly flowering specimen of a more or less prickly, bluish-green cultivar will be recorded as wild. Because taxonomists on wild plants are usually not familiar with the variation within cultivated plants, escapes resembling wild plants can be overlooked. This implies that the recorded number of escaped cultivated lettuce in the Netherlands is low, probably lower than it actually is.

This has implications for the assessment of gene flow from cultivated lettuce to the wild flora. If the actual escapes are not noticed as such, they will be recorded as wild, and therefore as natural. Without this knowledge, the information in literature and the herbarium would yield the following D_{pdf}-code:

D_p = 3 for a wild relative of a different species
D_d = 2 for occasional escapes, but no lasting populations
D_f = 2 for an Hour Block Frequency of 6 for Prickly lettuce

Summary D_{pdf}-codes

In the report Botanical Files[37] a code was developed to summarize the information on chances for the already occurring gene flow from cultivated plants to the wild flora of the Netherlands: D_{pdf}. It is a three-digit code, combining codes for Dispersal by Pollen (D_p), Dispersal by Diaspores (D_d) and a Frequency of Distribution of the closest wild relative (D_f). The higher the code for a particular crop, the higher the chances for gene flow from that crop.

Dispersal by Pollen (D_p) is related to the taxonomic relationship of the species involved. This dispersal will only have effect if pollen lands on a receptive stigma of a wild relative. Then hybridization may occur, resulting in a new combination between wild and cultivated plants.

0: No wild species from the same genus native to the Netherlands
1: Wild species of the same genus native to the Netherlands; hybrids impossible
2: Hybrids possible, spontaneous hybrids not found in the wild
3: Spontaneous hybrids with different species found in the wild
4: Crosses with same species in the wild possible but undetectable
5: Crosses with both the same and another species in the wild possible

Dispersal by Diaspores (D_d) is related to the dispersal capacity of the diaspores of the cultivated plant, and to the fitness of individuals resulting from these diaspores, which might lead to populations in the wild.

0: No records of cultivated plant outside cultivation
1: Records of cultivated plants outside cultivation, no viable offspring in the wild
2: Records of cultivated plants outside cultivation, some viable offspring in the wild
3: Records of cultivated plants outside cultivation, new populations are founded
4: Escapability unknown because the same species is native to the Netherlands

To the two qualitative dispersal codes, a quantitative code was added, the *Frequency of Distribution* (D_f), showing the distribution of the cultivated plant in the wild.

0: The cultivated species is not native to the Netherlands and is not found in the wild
1: The nearest wild relative is extremely rare to rare (Hour-block Frequency Class of 1–6); cultivated species is not found outside cultivation
2: The nearest wild relative is less rare to rather common (Hour-block Frequency Class of 6 or 7)
3: The nearest wild relative is very common (Hour-block Frequency Class of 8 or 9)

This is a score implying a minimal chance for gene flow by Garden lettuce to the flora of the Netherlands (see text box D_{pdf}-codes).[37]

If the information in the literature on experimental data, and on the taxonomy of cultivated plants is included, it becomes clear that the information in this case is not sufficient to correctly assess the real chance for gene flow from cultivated lettuce.

All authors on the taxonomy and genetics of lettuce agree, that Garden lettuce and Prickly lettuce are species with a very high degree of variability. Use of only herbarium material of wild specimens in this study would have had the disadvantage, that not all variation between the populations might have been covered. It was therefore necessary to get more information than could be assembled from the herbarium material, which is usually a sample of the variation that is found in the wild.

This was the reason for a field trial with 67 populations of *L. sativa*, *L. serriola* and *L. virosa*, grown under the same conditions and scored on different characters in comparable stages (Fig. 6, p. 21). The cultivated lettuce were of undoubted and well-recorded origin, and most of the *L. serriola* and *L. virosa* were of truly wild origin within Europe, the Mediterranean area and China. The other 'wild' lettuces were obtained from botanical gardens and one accession is thought to be of hybrid origin. These specimens formed the basis for a research into the taxonomic validity of the characters used to separate the species *L. sativa* and *L. serriola*.

This new study aims to answer the questions about the relationship between *L. sativa* and *L. serriola*, and the implications of this relationship for the assessment of gene flow from cultivated lettuce to the wild flora. It reveals that the original D_{pdf}-code for *L. sativa* cannot be upheld against the new information which has been derived from the cultivation experiments performed. In fact, the expected chance for gene flow from *L. sativa* to the wild flora is much greater than previously thought.

Another reason for the study of the relationship between wild and cultivated lettuce is the feasibility of D_{pdf}-codes for Europe. It must be stressed, that the dispersal codes of Botanical Files apply to the flora of the Netherlands only, which is only a very small part of Europe. If the scope would be extended to Europe, more information should be considered. This project is an attempt to list the extra factors needed to assess the consequences of a genetically engineered crop for the flora of Western Europe. In order to understand the implications of this enlargement of the scale, we studied the probable gene flow between wild and cultivated lettuce in Western Europe as a case study for a 'European D_{pdf}-code' in Chapter 7.

2. Historical part: *Lactuca sativa* L. and *L. serriola* L. in Western Europe

Introduction

The name Lettuce is linked to the genus *Lactuca* since Linnaeus' time. Before that, other species of the Compositae were often also depicted as 'Lettuce', because of similar use and appearance.[26] Since then the taxonomy of the genus and its close allies has been revised several times, 'oscillating between splitting and lumping'.[11]

Especially the question of the relationship between cultivated lettuce and its nearest relatives in the wild has been the subject of study by many authors. This chapter summarizes the treatment of this relationship, the genetics of the group and the different hypotheses and arguments for the ancestry of cultivated lettuce.

Terminology

One of the problems related to the question of the relationship between wild and cultivated Lettuce is one of terminology. This becomes evident when combining information from two usually unrelated fields, both dealing with plant species and their relationships: the field of cultivated plant taxonomy and that of the study of the wild flora.

The two fields both deal with plants, and with their names, but they differ in dealing with the concept of plant species. Generally botanical names have the same usage, but when communication is lacking on a scientific level, for instance between taxonomists specialized in cultivated plants and others specialized in the wild flora, differences can occur. This can result in the situation that a certain plant is known to both groups under different names. Unfortunately this is in contrast to the idea that scientific names refer to only one, clearly defined taxon all over the world.

This difference in attitude may have implications for the assessment of the chances for gene flow from a genetically engineered crop to the wild flora. If it is not certain to what species the relatives of the cultivated plant can be assigned, the implications of genetically engineered plants may be misjudged. Overestimation of the chances for gene flow could lead to unnecessary research into controlling measures to prevent a harmless plant from spreading, and underestimation could lead to uncontrolled gene flow to the environment. Either situation is undesirable.

This problem is also present in the situation of Lettuce. In both fields the vernacular name 'Lettuce' is known, and it is linked to a botanical name: the genus *Lactuca*, or part of it. But there is a difference in the use of the names, which can cause confusion when both fields are combined.

In the field of cultivated plants, 'Lettuce' usually refers to the very variable species *Lactuca sativa* that is cultivated throughout the temperate regions. It consists of several thousands of cultivars, of which habits and relationships are accurately known. Each year new cultivars are introduced, originating from crossing or selection, with new resistances, habits or yields. In this field wild species of Lettuce are also recognized, but they are of potential breeding interest (usually resistance to pathogens) only.[8 25 29]

In the botanical field 'Lettuce' is the vernacular name of a genus, *Lactuca* L. confined to mainly the temperate and warm regions of the Northern hemisphere.[11 34]

The genus Lactuca L.

The genus *Lactuca* L. consists of about 100 species, of which 17 are found in Europe. These species are usually classified into four sections, listed below.[11 36] The numbers in brackets refer to the number of species belonging to that section, and which are considered to be native or regularly found in Europe.[11]

Sect. *Phaenixopus* (Cass.) Benth. in Benth. & Hook. f.	(3)
Sect. *Mulgedium* (Cass.) C.B. Clarke	(2)
Sect. *Lactucopsis* (Schultz-Bip. ex Vis. & Panb.) Rouy	(3)
Sect. *Lactuca* – subsection *Lactuca*	(6)
– subsection *Cyanicae* DC.	(3)

Of this genus three species are considered to be part of the flora of the Netherlands:

Least lettuce (*L. saligna* L.)
Blue lettuce (*L. tatarica* (L.) C.A. Meyer)
Prickly lettuce (*L. serriola* L.)

There are two other species, not considered to be native, that are found occasionally in the Netherlands. One is described in the Flora of the Netherlands as a cultivated plant, sometimes running wild: Garden lettuce (*L. sativa* L.) and the other is Great lettuce (*L. virosa* L.), recorded as a neophyte in the Dutch flora, which is probably establishing.[22] The English vernacular names were taken from the Flora of the British Isles.[34]

Of these five species of *Lactuca* in the Netherlands, we included three in the present study: *L. serriola* and *L. sativa* to study their mutual relationships, and *L. virosa* because it is a closely related, yet distinguishable species. They are distinguished in taxonomical literature on several morphological characters. A summary of the opinions in literature on the delimitations of the species is given in Appendix 1.[3 10 11 22]

Genetics

The species in the study belong to the section *Lactuca* subsection *Lactuca* which is reproductively isolated very well from the other sections within the genus.[11 31] Within the subsection the relationships have been studied by many researchers, and much is published about the respective inter-breeding possibilities and the genetics of the different species within *Lactuca*.

In the past various crossing experiments with *L. serriola*, *L. sativa* and *L. virosa*, among others, have been carried out. These experiments have shown that the great majority of *L. sativa* × *L. serriola* and reciprocal are highly successful. The different hybrids often yield a 100% fertile, normal looking offspring, regardless of which species was used as the maternal parent. Usually the characteristics of *L. serriola* were reported to predominate in the general habit of the plants.[36]

Of the many other interspecific crossing experiments within the genus *Lactuca* we mention those involving *L. virosa* with both species. *Lactuca sativa* × *L. virosa* and reciprocal yield at best sterile hybrids, or hybrids dying in the rosette stage. *L. virosa* × *L. serriola* and reciprocal crosses produce usually sterile hybrids, or sometimes hybrids with a very limited fertility.[36 38]

Cyto-genetical studies[20 21 42] analyzed the meiosis of the different species, and of crosses between them, within the genus *Lactuca*. The chromosome behaviour of *L. sativa* was found to be stable, and the chromosomes of *L. serriola* to be very similar to those of *L. sativa*. The chromosome configurations of *L. sativa* × *L. serriola* and reciprocal crosses showed no aberations. The number of irregularities was even lower than in the pure lines or in the crosses between the different populations within the species.[20]

The chromosome configurations of *L. virosa* × *L. sativa* as well as of *L. virosa* × *L. serriola* and reciprocal crosses show univalents, lagging chromosomes and inversion bridges, indicating structural chromosomal differences. These results imply a much less close relationship of *L. virosa* with both *L. sativa* and *L. serriola* than of *L. sativa* with *L. serriola*.[20]

These results were confirmed recently by chromosome banding research. The patterns obtained with C- and N-banding and $AgNO_3$ staining have shown that the chromosomes of *L. sativa* and *L. serriola* share a virtually identical morphology. The chromosomes of *L. virosa* are quite distinct from both of them.[18]

Ancestors

There are three main hypotheses about the ancestry of cultivated lettuce:

1. Lettuce arose from wild forms of *L. sativa*. Arguments are that some character states of cultivated lettuce, like a flat-topped inflorescence or an ovate leaf-shape, are not found in *L. serriola*, while prickles would be absolutely absent in *L. sativa*. Furthermore, some authors claim to have observed wild forms of *L. sativa*.[6]
2. Lettuce originated directly from *L. serriola* without hybridization with any other species. Arguments in favour are that there are no reliable records of a true wild form of *L. sativa*, and that the variation of cultivated lettuce can be fully explained by the variability of *L. serriola*, without assuming additions from other *Lactuca* species.[7 9 31]
3. Lettuce originated from a hybrid origin of species of the subsection *Lactuca*: *Lactuca virosa*, *L. serriola* and/or *L. saligna*. There are three possibilities within this hypothesis: one hybrid population could have been the progenitor of both *L. sativa* and *L. serriola*, one developing by active human selection and the other by adapting to the anthropogenic environment; *L. sativa* may be the derivative of progenitors of *L. serriola* and a third species; or, conversely, *L. serriola* may be the weedy derivative from progenitors of *L. sativa* and a third species.[6 17 38] Some character states of *L. sativa*, like pointed leaf shape and spotted anthocyanin in leaves are not found in *L. serriola*, but they are present in the other two species, implying that these species contributed the character states to cultivated lettuce.[19]

The second hypothesis is generally considered to be plausible, although the third hypothesis is not discarded by all. Recent studies of Kesseli, Ochoa and Michelmore have contributed to the discussion about the ancestry.[17] They showed that contrary to what was mentioned about the absence of pointed leaf shape and anthocyanin in *L. serriola*[19], these character states are present in some populations of the species (see Fig. 7, p. 22), and that the absence in Lindqvist's collection is based on a limited sample of populations. Therefore the hypothesis that *L. saligna* and/or *L. virosa* contributed to the origin through hybridization is discounted. The possibility of another, third species, extant or extinct, contributing to cultivated lettuce could not be ruled out, but was rendered implausible.[17]

In short, most authors on the origin of cultivated lettuce agree that *L. serriola* and *L. sativa* have at least one ancestor in common, but the exact identity of that ancestor, and the relationship of both species to it and to each other is not certain. It is however important to correctly assess this relationship, because of its implications for the assessment of the occurring gene flow from cultivated lettuce to the wild.

Characters

Prickly lettuce and Garden lettuce are traditionally distinguished from each other, on several characters. Most of them are considered to be more or less stable, others are overlapping to some extent.

Prickly lettuce is recorded to have bluish green leaves with a row of prickles on the midrib beneath, and the stem is usually prickly in the basal part. Latex is abundant in all parts, whitish and bitter. The capitula are grouped to a rich pyramidal or plume-like inflorescence, and have reflexed involucral leaves after fruit set. The achenes are grey to brownish.

Garden lettuce is described as having often light-coloured, glabrous leaves, forming large heads, and a glabrous stem. Latex is abundant in all parts, white and not bitter. The capitula are grouped to a flat-topped, umbel-like inflorescence, and have erect involucral leaves. The achenes are white, amber or greyish brown.[11 12 13 22]

The character state 'cauline leaves twisted to one vertical plane' is usually attributed to wild growing specimens of Prickly lettuce only (Fig. 8, p. 22). It is known as Compassing, because of the orientation of the leaves in the North-South plane, with the leaves facing East and West. Prickly lettuce is mainly growing on sunny, open places, and it is assumed that the twisting prevents the hot mid-day sun from burning the delicate chlorophyll in the leaves. Compassing is usually found in specimens growing in full sun, otherwise it is far less obvious.[39]

The aforementioned characters seem sufficient to warrant a clear distinction between the two species. However, their distinguishing quality should be evaluated in conjunction with their origin.

In the literature on cultivated plants several papers can be found on 'domestication characters'. These characters are related to the use of plants by man, and the subsequent change of the genetical structure of the plants during the process of domestication. The change from a wild organism to a domesticate is generated by the continuous, and especially in later periods conscious, selection of those organisms that are best adapted to the anthropogenic environment.[14 43]

Most of these domestication characters are related to the behaviour of the plants in cultivation, to harvesting of the desired organs, and to consumption or other uses. Some characters generally linked to the cultivation of herbaceous plants are listed below[14 33 44]:

a. rapid growth, frequently leading to an extraproportional increase of desired organs;
b. a short life-cycle, for quick harvesting and the prevention of pest development;
c. absence of herbivore deterrents like poisons or prickles, preventing harvest and/or consumption;
d. absence or disabling of dispersal mechanisms to prevent the plants from spreading, especially when the dispersing organs are to be harvested, and generally for gathering the basis for the next growth season;
e. better consumption qualities, or qualities for further use of the plant or its parts.

Translated to the situation of Leaf (Butterhead, Cos, Crisp, Cutting and Latin), Stalk and Oilseed lettuces, which are cultivated for their leaves, stems and seeds, respectively, the domestication character states for lettuce can be listed as follows:

a. rapid formation of a big, harvestable head or thick stem;
b. annual plants instead of biennials (wild lettuce); absence of dormancy in the seeds ensures growth directly after sowing;
c. no or few prickles on the midribs of the leaves and no prickles on the stems of Leaf and Stalk lettuces, less than wild lettuce on those of the Oilseed lettuces;

d. erect involucral leaves, retention of the achenes after fruit set, especially for Oilseed lettuces;
e. tender, light green to dark red leaves in Butterhead and Cutting lettuces; crisp leaves in Crisp lettuces; thick, succulent stems in Stalk lettuces (with inedible leaves); very many capitula with many seeds in Oilseed lettuce.

When this list is held against the traditional distinction of wild and cultivated lettuces as found in literature, most of the distinguishing character states prove to be linked to domestication. Then only a few character states that are not considered to be under the influence of conscious human selection remain:

- the shape of the inflorescence is supposed to be pyramidal in wild lettuce and corymbose in cultivated lettuce
- the colour of the achenes, grey to brownish in wild lettuce, and white, amber or greyish brown in cultivated lettuce
- compassing of the leaves in wild lettuce only

Some of the characters can be found to be overlapping in a herbarium study. Brown achenes of cultivated lettuce cannot be distinguished from achenes of wild lettuce. Furthermore, poor specimens of escaped cultivated lettuce show inflorescences with a more plume-like than corymbose habit, which suggests an influence of the environment. The third character, whether the leaves were compassing or not, could not be determined in herbarium material. These characters were studied in the field trial that is described in Chapter 4.

3. Domestication of Lettuce

Introduction

Lettuce is a very 'old' vegetable, it was taken into cultivation and domesticated long before Roman times. It is widely assumed that the domestication of lettuce took place in South-West Asia, in the region between Egypt and Iran. The highest number of related species is found between the Tigris and Euphrates rivers, which suggests this region to be the centre of origin for cultivated lettuce.[44] The hypotheses that are posed to the question which of these species contributed to the ancestry of Lettuce were discussed in the previous chapter, under *Ancestors*.

Early domestication

Lettuce has been cultivated for a long time, as can be deduced from archaeological evidence. The first indications for the existence of a lactiferous vegetable can be found in hieroglyphs and wall paintings of the Fourth Kingdom (± 2500 B.C.) in Egypt. Regularly occurring depictions of elongated cob-like structures with bound, pointed leaves from that time can be interpreted as depicting simple forms of lettuce (Fig. 1). Other interpretations of these paintings, like cypress or artichoke, are less likely because those species were not known in Egypt at that time.[16]

The use of Lettuce in the early times is not clear. As mentioned before, there are hieroglyphs of 2500 B.C. referring to a latex-containing vegetable. On the wall paintings of a later time, leaf marks on the bases of the lettuce plants suggest that the

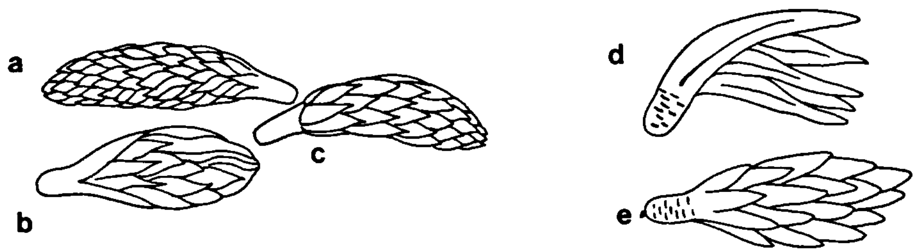

Fig. 1. Depictions of lettuce on wall paintings and tombstones in Egypt.[16]
a, b, c. Fourth Kingdom. – d, e. Middle Kingdom.

plants have been used as a gathering or cutting lettuce (Fig. 1d, e). Apparently Lettuce also had a sacral meaning, indicated by the depiction of lettuce plants on altars of the god Min (Amon), the god of fertility.[26] This link to fertility might be traced back to the abundance of milky latex, which is a common feature of the genus *Lactuca*.

Another possible function of Lettuce concerns the use of the fruits. The full-grown seeds contain up to 35% oil, of which a considerable percentage non-saturated fatty acids[15], so use as an oilseed crop for human consumption is suggested. However, the Ancient Egyptians had many other oil-bearing crops with larger, easier harvested seeds, so it will not have been a major crop.[16,26] In the seed banks of the CGN in Wageningen several populations of Oilseed lettuce from Egypt can be found, named either *Lactuca sativa* or *L. serriola*, and sometimes with intermediate habits. Often the plants remind of a *L. serriola* without prickles.[4]

Further development of the different groups of cultivated lettuce

Cos and Cutting lettuce

From Egypt the vegetable lettuce spread throughout the Mediterranean region in the first centuries B.C., as can be deduced from different Greek and Roman sources. The original plants were much like a loose Cos lettuce, and they were often earthed up. Although the Romans must have taken this lettuce with them to Northern Europe, there are no records of lettuce cultivation persisting after their retreat.[26] These lettuces were presumably the ancestors of the numerous forms of heading lettuces (see Butterhead and Crisp lettuce) and Cutting lettuces of Western Europe (Fig. 9, p. 22).

Oilseed lettuce

Oilseed lettuce, that is assumed to have been developed in Egypt around the same time as the vegetable Cos lettuces, can still be found in that region, and seems more or less unaltered (Fig 9, p. 22).

Latin lettuce

A poorly heading type is the group of Latin lettuces which have usually thick, leathery greyish green leaves. The origin is not very clear, but it must have been developed in the Mediterranean region, where it is still grown and consumed (Fig. 9, p. 22).

Heading lettuces: Butterhead and Crisp lettuce

The first indications of lettuce cultivation in North-West Europe date from descriptions in late fifteenth century herbals. Lettuce was supposed to have several uses: it was used as a vegetable, and as a medicinal plant for its thirst-quenching quality and its stimulating effect on the digestive system. The engravings show loose Cos lettuces and primitive heading lettuces.[26] The latter have led to the development of the modern Butterhead lettuces in Europe through selection on soft, tender leaves (Fig. 11, p. 23, for a red variety). Introduction of a Batavia-type heading lettuce to Northern America soon after its discovery, resulted in the development of the Crisp or Iceberg lettuce (Fig. 10, p. 23), which became dominant in the beginning of this century.[31]

Stalk lettuce

The introduction of lettuce plants to China presumably has taken place between 600 and 900 A.D. The plants developed from a loose-leafed vegetable to the so-called Asparagus lettuce or Stalk lettuce, of which the extremely thickened stems are consumed (Fig. 12, p. 23). This vegetable was introduced in Northern Europe in the nineteenth century, but has not become very popular.

4. Field trial

Introduction

In Chapter 2 the characters traditionally used to distinguish wild and cultivated lettuce were compared with characters linked to domestication. Most of these characters proved to be strongly influenced by human selection.[14 33 43] The rest of the distinguishing characters were either found to be overlapping (brown achenes are found in both wild and cultivated lettuce) or influenced by the environment, like the shape of the inflorescence (see also Chapter 2).

This was the reason for a field trial with wild and cultivated lettuces grown together. This enabled a comparison of the genetical diversity with the exclusion of environmentally influenced variation. With this field trial standardized information became available from all developmental stages of both wild and cultivated lettuce plants. It was possible to follow the development of some morphological distinguishing characters, like leaf position along the stem or shape of the inflorescence, as well as the distribution of prickles, over a period of time from the same plant.

To this must be added that in herbarium collections vegetative stages of wild lettuce usually are poorly known, as are flowering and fruiting stages of cultivated lettuce. This field trial enabled a full comparison of all stages of all groups. In all, 67 genetically different populations of wild and cultivated lettuces (including wild *L. virosa*) have been investigated using both vegetative and generative characters of 350 living plants.

Apart from this, the field trial was also useful for the identification of the different phenetic groups within cultivated lettuce. For instance a head of a Crisp lettuce can lose most of its characteristic shape when dried for herbarium purposes. The distribution of character states within and among the populations as well as within and among the species could be investigated using the information obtained in the field trial. This survey of character states on many different specimens within the species added invaluable information to the research on herbarium material.

The trial was held from April to July 1993, on the fields of the Centre for Plant Breeding and Reproduction Research in Wageningen (CPRO/DLO), with the assistance of the experts on vegetables of the department for Registration and Plant Breeders' Rights (RKO) and of the Centre for Genetic Resources, the Netherlands (CGN), both part of CPRO/DLO. The fields were maintained by the garden staff of CPRO/DLO.

As in the field trial the emphasis was on the variation within and between the different groups, it was important to eliminate environmental factors. All populations of Lettuce in the CGN and RKO seed banks are regularly tested for homogeneity, so the variation within the populations was expected to be minimal. The environmental effects were minimized by using a randomized block design of 67 populations in 3 replications, by which they were spread evenly over all beds.

Tabel 1. Number of populations of *Lactuca* species in the field trial, per phenetic group. The names are the botanical names under which the accessions were received from the seed banks.
RKO: Registration and Plant Breeders' Rights
CGN: Centre for Genetic Resources, The Netherlands

L. virosa CGN		**11**
L. serriola CGN		
Of wild origin	19	
Cos lettuce	1	
Oilseed lettuce	1	
Total *L. serriola*		**21**
L. sativa CGN		
Of 'wild' origin	3	
Crisp lettuce	1	
Latin lettuce	1	
Cos lettuce	3	
Cutting lettuce	1	
Stalk lettuce	2	
Oilseed lettuce	4	
Total *L. sativa* CGN		**15**
L. sativa RKO		
Butterhead lettuce	2	
Crisp lettuce	1	
Latin lettuce	5	
Cos lettuce	3	
Cutting lettuce	9	
Total *L. sativa* RKO		**20**
Total number of populations		**67**

Material

For this field trial 67 populations of *Lactuca sativa*, *L. serriola* and *L. virosa* were used from the collections of RKO and CGN. The wild populations were selected for variation in morphology, but also on their countries of origin. Because the Mediterranean region is supposed to be the centre of origin of cultivated lettuce (see Chapter 3, Domestication), a higher number of populations of wild *L. serriola* and of primitive cultivated *L. sativa* from Israel, Egypt and Turkey was included. A selection of 11 populations of the rather rare, but closely related *L. virosa*, all from wild origin, was added to serve as a reference species. The selection of the wild lettuce populations was made in close cooperation with the experts of CGN.

The populations of the very variable group of cultivated lettuce were selected from the seed banks of RKO, in cooperation with the vegetable experts of CPRO/DLO. The selection criteria were mainly leaf shape, seed colour, and overall morphological distinctness. The populations were chosen to represent the variation within the different phenotypic groups as distinguished by the CGN Lettuce collection.[28 30]

Table 1 summarizes the numbers of populations per group of the lettuces in the field trial. See Appendix 3 for a more detailed account of the accession numbers of each of the populations and their origins. For some accessions, alternatives are suggested for the phenetical group names under which the populations were received.

Methods

The seeds of the populations of Garden lettuce (*Lactuca sativa)* were treated according to the standard practice of the lettuce cultivar trials of the RKO: they were sown on March 15, on flat trays with normal potting soil, and pricked out to fresh plant trays after a week. After one month in a cool greenhouse, the young plants were planted out on the field on April 22, with the aid of the CPRO field staff.

The wild lettuces used in this trial, *L. serriola* and *L. virosa*, are annual to biennial herbs; if biennial, they usually flower in their second year. To induce flowering in the first year, the seeds of the wild populations therefore had to be vernalized, i.e. treated with a cold period just after the emergence of the rootlet, to simulate a winter period (see text box). After the vernalization treatment, on March 30, the young, etiolated plants were pricked out to plant trays, and kept in the greenhouse until they were strong enough to be planted outside. On April 28 they were planted out on the field, a week later than the Garden lettuces.

Vernalization

The seeds were sown on wet filter paper in Petri dishes, kept at 10°C for two days to remove dormancy, and then placed at room temperature for germination. Most seeds germinated within one day, some in two. One week later the seedlings, with rootlets of 2–20 mm, were transferred to fresh wet filter paper in new Petri dishes, and placed in a refrigerator. They were kept at 1°C in the dark for c. six weeks. Every two weeks the seedlings were checked for fungus, the infected plants were removed before they could infest the whole population.

Observations

During the field trial the morphological characters were observed on two plants of each field (Fig. 13, p. 24). The scored plants were always the third and sixth of the row. If one of the plants died or failed to flower, the fourth, respectively fifth, were used as a replacement. In some populations only one or two plants remained, so that they were used, irrespective of their place in the row. The character states were scored in a number of series, according to the stage of development. The vegetative characters could be scored in two sessions in May, but as flowering was not simultaneous, the generative characters had to be scored over a longer period of time from May to July. See Appendix 2 for the characters scored during the respective stages.

All observations were recorded using a FieldWorker 60 field terminal with a Micronic programme. The data were transferred to the VAX computers of the CPRO/DLO directly after each series of observations, and prepared to be combined and ordered in a spreadsheet using Lotus and QuattroPro. The matrix with all data was analyzed using SPSS/pc with the HOMALS, PRINCALS and CLUSTER modules for homology analysis, principal component analysis, and cluster analysis, respectively. The results are presented in the next chapter.

The matrix with the basic data from the field trial observations on the 350 plants is stored in Lotus-format in the VAX-computer at CPRO/DLO. More passport information of the accession numbers of the wild and cultivated lettuces can be obtained at the databanks of CGN and RKO (see also Appendix 3).

Herbarium material

During the field trial three plants per population were collected for herbarium preservation:

- a young plant of which the central axis had started to stretch;
- a plant with a young inflorescence;
- a branch or top of a full grown inflorescence, preferably with ripe achenes.

The last stage could not be collected from all populations, because not all plants reached maturity during the time of the field trial. The plants were dried with the kind cooperation of the staff of the Herbarium Vadense of the department of Plant Taxonomy and Geography, Agricultural University Wageningen. They were mounted and labelled by Mrs. Sabatino and Mrs. Uenk, staff members at CPRO-DLO. The specimens are kept at the Rijksherbarium in Leiden as SAB & UEN 111 to 113, 122 to 124, 128, 130 to 133, 135 to 137, 140 and 141, 143 to 182, 189 to 202, 210 to 237 and 243 to 338 for future reference.

Photography and microscopy

Of some of the plants fresh leaves and tips of young stems were harvested and examined using light microscopy. Especially the prickles on the midrib, on the secondary nerves and on the stems were examined and if possible dissected from the inner layers, to examine which layers contributed to the prickles. The preparations have been photographed.

During the field trial the different habits of cultivated and wild lettuces were photographed for further reference, as well as the character states that were scored in the respective stages. Two complete sets of the slides, photos and corresponding documentation are kept at the Rijksherbarium and CPRO/DLO, respectively.

5. Results

Introduction

The observations on the 350 live lettuce plants in the field trial were assembled to one spreadsheet, and the data were processed in several ways, explained below. Combined with the information obtained from herbarium material these data provided a basis for the reassessment of the taxonomical validity of the traditionally used characters for the distinction between wild and cultivated lettuce.

Results herbarium study

The importance of a good herbarium collection has already been stressed in the previous report.[37] Therefore the many *Lactuca serriola*, *L. sativa* and *L. virosa* collections of the Rijksherbarium were the basis for the study of the distribution and delimitation of the species that were subject of our research.

In the Rijksherbarium several collections of wild lettuces from the Netherlands, Europe and the Eastern part of the Mediterranean are kept, and most specimens were clearly wild lettuces. However, there were wild lettuces that showed some of the character states usually attributed to cultivated lettuce: a not clearly pyramidal inflorescence, or absence of prickles. Sometimes it was not clear whether they were escapes from cultivation or resulted from introgression from cultivated lettuce.

The few herbarium specimens from the wild identified as *L. sativa* labels, were more or less light yellow, and mostly glabrous or with some, small prickles. Some smaller specimens had small inflorescences with few branches and few capitula, which were plume-like instead of corymbose.

Results field trial

The first treatment of data obtained on the 350 individual plants was an inventorial procedure using the statistical program SPSS/pc. With Principal Component Analysis the data were plotted, and three or four groups, with no absolute demarcations, could be discerned. One group consisted of all *L. virosa*, one of wild *L. serriola*, and one of cultivated lettuce, including accessions named *L. serriola* that should be attributed to the phenetical group of Oilseed lettuces. The results are presented in Figure 2.

The Principal Components were fruit characters, prickliness, position of the involucrum, and shape of inflorescence. Some of these are domestication characters, as was shown in Chapter 2, the fruit characters were mainly used to separate *L. virosa* from the other taxa.

Clustering of the same data, using Within Group Averages as a clustering criterium, showed more or less the same three clusters. Some wild lettuces, that later could be attributed to the Oilseed lettuces, were found in the cultivated lettuce cluster. We used extra weight on the fruit characters to compensate for the number of characters scored on vegetative organs, which was higher than the number of characters on the generative organs.

The shape and colour of the achenes are some of the characters used to separate *L. virosa* from other lettuces.[27] These characters, however, can not be used for distinction within the *serriola-sativa* complex. The shape of the achenes is constant for

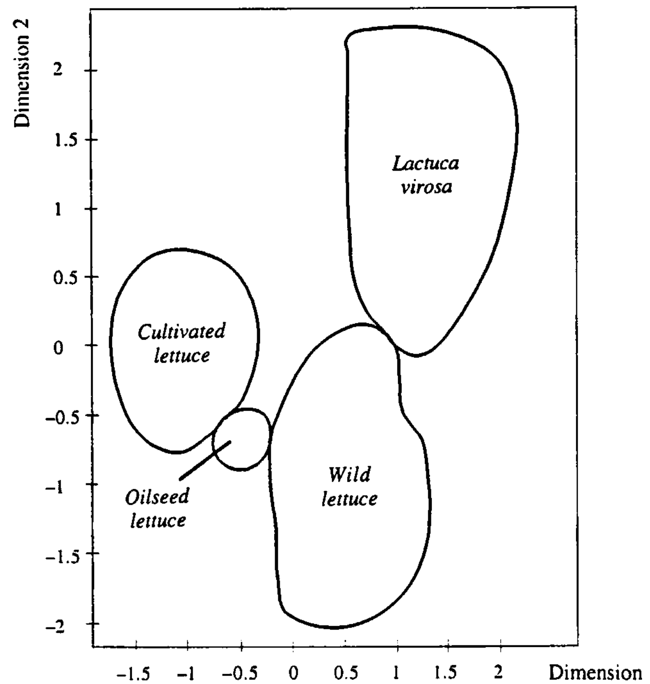

Fig. 2. Plotting of wild and cultivated lettuce using Principal Component Analysis on 20 morphological characters.

all accessions, while the colour is brown in the wild accessions, and white or brown in the cultivated group. Brown achenes from cultivated lettuce are not distinguishable from those of wild lettuce.

Finally the groups were separated in a more traditionally morphological way, by sorting the individual plants on their character states, regardless of the previously assigned botanical name. Each group resulting from one division was sorted on other character states until more or less homogeneous groups consisting of only *L. virosa*, wild lettuce or cultivated lettuce remained. The division using the least steps, and giving a combination of the least number of groups is shown in Figure 3.

As could be expected, the *L. virosa* plants distinguished easiest on the achene character states 'purplish-black seed coats' and 'broad wings'. The other plants all had 'white or brown seed coats' and 'narrow wings'. The best division within the *serriola-sativa* group was obtained using the following characters: prickles on the stem and the midrib of the cauline leaves, the position of the involucral leaves after fruit set and the shape of the inflorescence. These characters enabled a division with the least steps to reach a satisfactory number of homogeneous groups.

Apart from using these domestication characters, a logical division of the *serriola-sativa* complex could not be made. It implies that *L. serriola* and *L. sativa* are not only very closely related, but that their distinction is mainly based on domestication characters, as mentioned in Chapter 2.

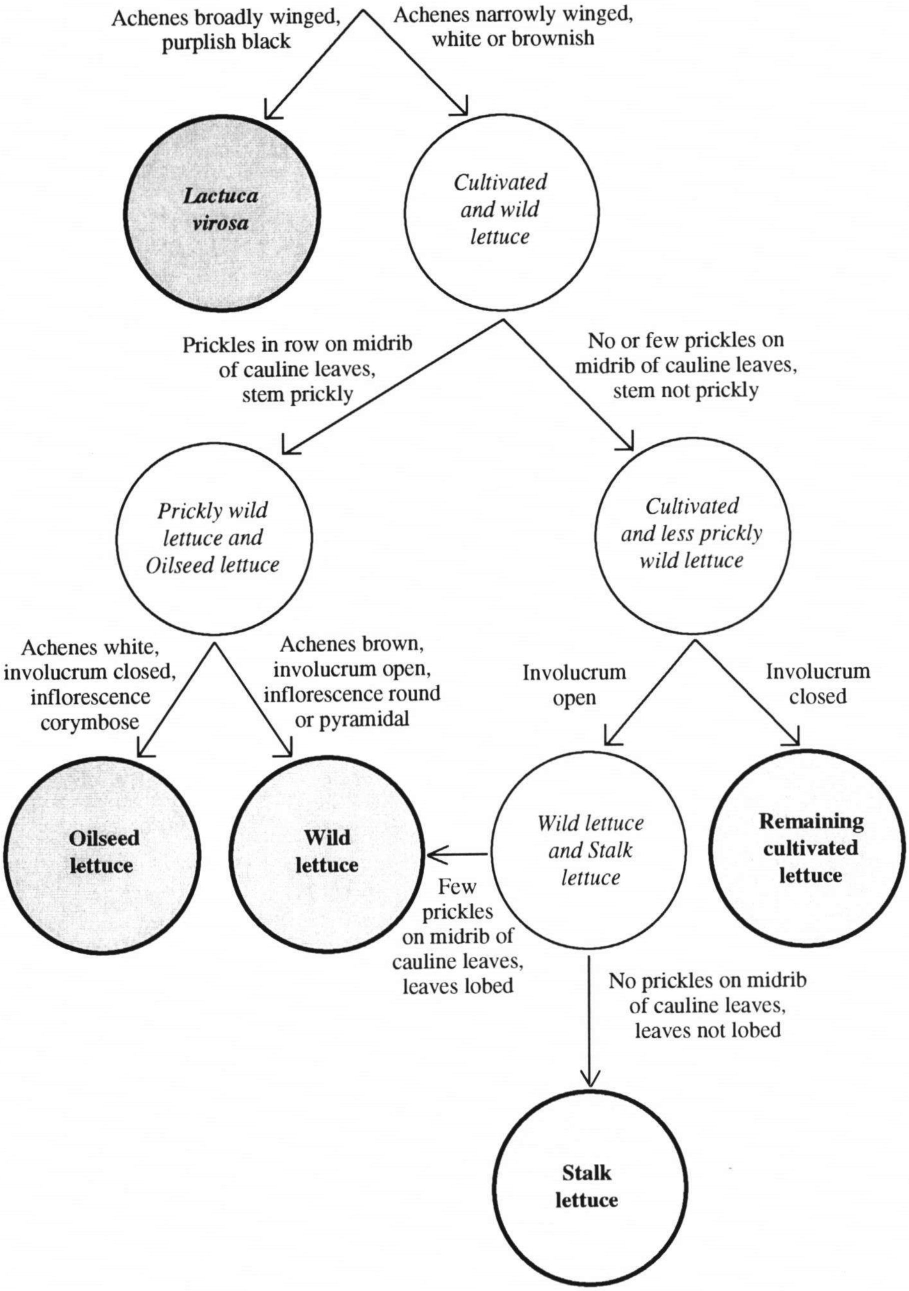

Fig. 3. Subdivision of the wild and cultivated lettuces in the field trial into more or less homogeneous groups, on the characters mentioned at the arrows. The arrows only indicate the groups resulting from a single division on the character states; they do not present any supposed order of development of cultivated lettuce or phylogenetic relationships.

Legends to Figures 4–15

Fig. 4. General habit of Prickly lettuce, grown in an experimental field in Wageningen, The Netherlands.

Fig. 5. Garden lettuce grown, in an experimental field in Wageningen, The Netherlands.

Fig. 6. Part of experimental field with some of the 67 populations of wild and cultivated lettuce.

Fig. 7. Pointed leaf shape and anthocyanin in Prickly lettuce (CGN 910401).

Fig. 8. Cauline leaves of Prickly lettuce in full sun, twisted to the vertical plane: 'Compassing'.

Fig. 9. From left to right: Latin lettuce, Oilseed lettuce, wild lettuce, and Cutting lettuce and two wild lettuces in an experimental field in Wageningen.

Fig. 10. Young plants of Crisp lettuce (Van Sal, RKO 87454).

Fig. 11. Flowering red Butterhead lettuce (Rougette du Midi, RKO 87447).

Fig. 12. Stalk lettuce (CGN 10892).

Fig. 13. Observations in the field.

Fig. 14. Change of colour from yellowish to bluish green in flowering stems of cultivated lettuce (CGN 4733). Note twisting of the cauline leaves.

Fig. 15. Bluish green cauline leaves in flowering stems of cultivated lettuce (RKO 90207).


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6. Discussion and conclusions

From the results of the morphological study of 67 living populations in the field trial with wild and cultivated lettuces from different parts of Europe, Russia, Turkey, Egypt, Afghanistan, Iran and China (see Appendix 3) the following can be concluded. Cultivated lettuce can only be distinguished from its wild relative Prickly lettuce by the relative absence of prickles on the midrib of the cauline leaves, the absence of prickles on the main stem, and retention of the achenes after ripening. These characters are all considered to be strongly influenced by centuries of domestication processes (see Chapter 2).[14 33]

The other morphological characters, traditionally used to separate wild and cultivated lettuce, all show overlap to a smaller or larger extent between the groups. On those characters, compassing of the leaves, shape of the inflorescence, and colour of the achenes no clear demarcation can be made between wild and cultivated lettuce (see also Appendix 4). They are treated separately below.

The character state 'cauline leaves twisted to one vertical plane', surprisingly was found in all lettuces in the trial. In all specimens at least some leaves could be found with one margin clearly higher than the other. Even the plants of *L. virosa* showed a slight to marked twisting in their cauline leaves. The phenomenon may be more conspicuous in Prickly lettuce, but it is present in all (see also Fig. 14, p. 24).

The shape of the inflorescence is not as stable as is assumed in literature.[11 22] In all groups of wild and cultivated lettuce rounded panicles were observed, that could not be assigned to either a pyramidal or corymbose shape. This character shows so much overlap across the groups that it has no distinguishable value.

The colour of the achenes was already mentioned, the brown achenes of wild lettuces being not distinguishable from brown achenes of cultivated lettuce. Both the colour and the short, bristle-like appendages on the apical part of the achene show a complete overlap with those found in cultivated lettuce.

The character Leaf colour was mentioned in Chapter 2: Cultivated lettuce is supposed to have bright yellowish green leaves, as opposed to the bluish green leaves of Prickly lettuce.[22] Apart from the red-leafed varieties, which obviously have no yellowish leaves, there were several types of cultivated lettuce showing a slight to marked change of colour from yellowish green below to bluish green along the stem during the flowering stage (Figs. 14 & 15, p. 24).

On the basis of these results – the lack of distinguishing morphological characters – strong doubt must be set against the current opinion that *L. sativa* and *L. serriola* belong to separate taxonomical species. There are indeed distinct morphological differences that can be found separating wild and cultivated lettuce[11] but most of these differences must be supposed to originate from human intervention.[4 43]

Apart from that, crossing experiments between several combinations of wild and cultivated lettuces have shown, that the two taxa are fully interfertile, and that their offspring is usually 100% fertile.[36 38] In cyto-genetical research the chromosome morphology of the two taxa proved to be virtually identical, and the meiosis of the crosses to be regular.[18 20 42] These facts in themselves justify, irrespective of attitude on species concept, that the very interfertile groups of wild and cultivated lettuce belong to one and the same species.

Thus, morphological and experimental observations do not allow a distinction on a specific level for these two taxa. Domestication characters are under human influence, and they have usually developed through selection for traits desired by man that are unfit for survival under wild circumstances. Such characters alone do not permit a specific rank for the cultivated group.

It is therefore concluded that both Garden and Prickly lettuce belong to one, very variable species, *L. sativa* L., within which a group of wild lettuce can be distinguished on its prickliness, brown achenes and an open involucrum after fruit set. The rest within the species developed through human selection and consequent breeding.

The group of cultivated lettuce can morphologically be divided into three more or less distinct groups: first a group of which the seeds are harvested, of which some populations have prickles and others have not. Often the habits of the plants remind of a less prickly or glabrous wild lettuce.[4] Secondly a group of which only the stems are consumed. Their leaves are morphologically similar to those of the wild lettuces, and they are equally inedible. And thirdly a large and diverse group of which the leaves are consumed, which shows all domestication characters connected with harvest and consumption of the fresh leaves.

The fact that these groups segregate so clearly, suggests that they may have genetically somewhat different gene pools in their ancestry. The populations that were selected for the different uses may not have represented the full genetic diversity within the species: The Stalk lettuces were developed in Asia, far from their centre of origin, and probably from a limited sample of their ancestral population. The same goes for the group of the Oilseed lettuces. Because of their use as an oil crop only, this group shares a relatively high number of character states with wild lettuce.

The implication of this conclusion for the chances for gene flow from cultivation to the wild population of Prickly lettuce is that gene flow must be considered to be possible, and probably already occurring, as is the case with all crop species with a wild relative belonging to the same species that is native to the Netherlands. The preliminary D_{pdf}-code of 3.4.9 for Lettuce must then be revised to **4.4.2.** in the model system of Botanical Files[38], but see Chapter 7. This code implies a **substantial chance for gene flow** from cultivated lettuce to its wild relative in the Netherlands.

In this study we have not been able to prove that the rapid dispersal of Prickly lettuce in the Netherlands would indeed be connected to occurring gene flow from cultivated lettuce. This would require further, molecular-genetical research. For risk-assessment research concerning the introduction of genetically engineered organisms it would be important to know, whether the wild flora has already had any substantiated genetical influences from a crop that is known as a self-fertilizer.

7. Towards European Botanical Files

Introduction

The Dispersal code system as developed in the previous report was explicitly meant as a code for the Netherlands. If the scope is extended to Europe, a number of other factors must be taken into account. The present study serves as an inventorial study to see which of these factors are important for the assessment of the real chances for gene flow from cultivated plants to the flora of Europe.

A European Dispersal code system must account for differences in chances for gene flow in the different parts of Europe. The difficulty in this, is the diversity found within the area. In our opinion, a single code summarizing the chances for the Western European as well as the Mediterranean region would not be feasible. It would be better to have a model with a number of codes: one code per region for each species. An important question is whether there is enough information available (to be extracted from herbaria and botanical literature) to construct D_{pdf}-codes for Europe which are comparable to the ones presented in *Botanical Files*.

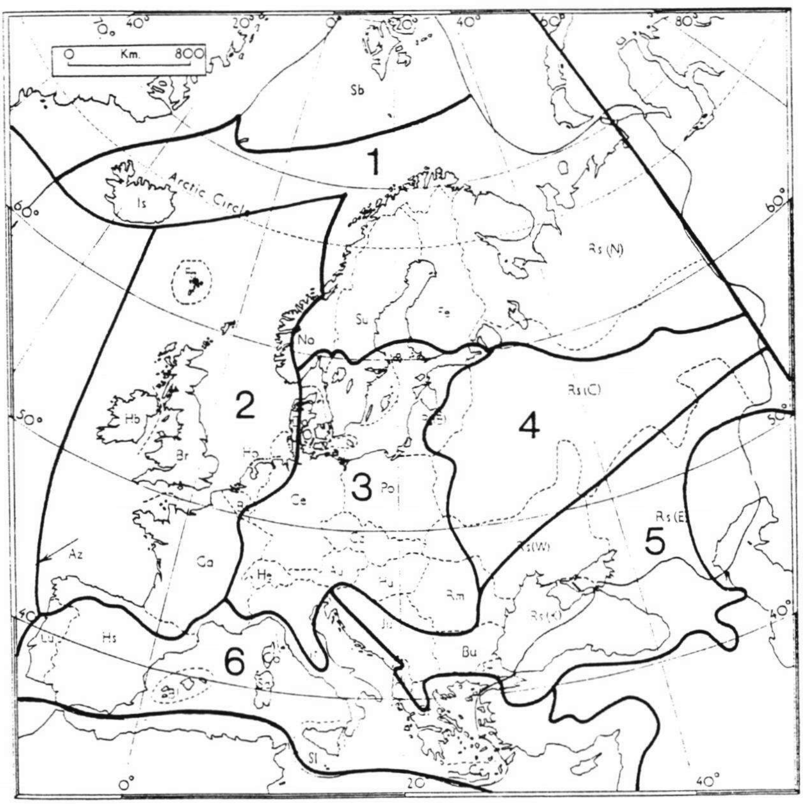

Fig. 16. Plant geographical regions of Europe.[32]

1: North Europe
2: West Europe
3: Central Europe
4: East Europe
5: South-East Europe
6: South Europe

European plant geographical regions

In order to establish regional D_{pdf}-codes, Europe should at first be divided in a way that is meaningful for the distribution of plants in the area. Unfortunately, state borders have no biological importance at all, so they must be left out of consideration. The best way is to use a classification into plant geographical regions, because they reflect the historical response of numerous different plant species to important environmental factors like climate.

An important source for European D_{pdf}-codes is the paper of Schaminée et al.[32] In this study Europe was divided into 6 plant geographical regions, in accordance with the plant geographical classifications of Walter & Straka, Meusel & Jäger and Takhtajan.[24 35 40] See Figure 16. Mountainous areas have not been taken into account, but in those regions the real chances for gene flow from cultivation to the wild are expected to be low for all plant species.

The importance of the contribution of Schaminée et al. to the plant geography of Europe is the quantitative approach. For each region the coverage of a plant species is given in a 5 digit scale. In the majority of cases, this regional coverage can be used as a measure for chances for dispersal: if the wild relatives are common, pollen dispersal will be likely to occur, and the conditions for dispersal by seed are more favourable than in regions where the wild relatives are already scarce themselves.

Towards European D_{pdf}-codes

The principle of a D_{pdf}-code has been accepted for risk assessment studies in Switzerland.[2] The codes were improved by some slight alterations giving each dispersal factor the same scale from 0 to 5, with 9 as an indication of unknown chances because of lacking information. If this improved system is applied to the European situation, the codes for a certain European region would be as presented in the text boxes on the next pages. The implications per dispersal code are presented at page 000.

Regional D_p-codes

The chances for hybridization (D_p-code, see text box European codes for D_p) depend on the regional floristic composition: on the availability of compatible wild relatives in the direct surroundings. For nearly all European regions the D_p-code can be established accurately enough for our purposes. The occurrence of hybrids in the wild is known reasonably well throughout Europe.

Regional D_d-codes

The climate is not constant throughout Europe. A cultivated plant with a low D_d-code because of a low fitness to the Atlantic climate, might be suited to a Mediterranean or Centreuropean climate. This will affect its possibilities for escape from cultivation in that region, and therefore the chance for gene flow to the wild. This should result in that species receiving a higher D_d-code for that region (see text box European codes for D_d).

European codes for D_p (Dispersal of Pollen)

0: No chance for D_p to the wild, even when the cultivated plants come into flower. In this region of Europe there are no wild species of the same genus as the cultivated plant.

1: No chance for D_p to the wild, even when the cultivated plants come into flower. Crossing experiments have shown that wild species of the same genus in this region of Europe are not compatible with the cultivated plant.

2: Chance for D_p to the wild is very small, but gene flow under special local conditions to be considered possible. There are no records of spontaneously formed hybrids of the cultivated plant with wild species of the same genus in this region of Europe. However, hybridization is possible under experimental conditions without artificial help.

3: Chance for D_p to the wild is low, but under favourable circumstances considerable. There are records of spontaneously formed hybrids of the cultivated plant with wild species of the same genus in this region of Europe. Local situations have to be studied carefully in risk assessments of field experiments.

4: Chance for D_p to the wild is real. Hybrids of the cultivated plant with wild species of the same genus in this region of Europe occur fairly often.

5: Chance for D_p to the wild is very real. Hybrids of the cultivated plant with wild relatives of the same genus in this region of Europe occur very often, they are fertile and backcross frequently.

9: Data too scanty or lacking at all, no evaluation possible.

European codes for D_d (Dispersal by Diaspores)

0: No chance for D_d to the wild. Seeds are sterile or otherwise deficient, they have lost their reproductive function.

1: Chance for D_d to the wild very low. Escape occurs only occasionally and under very favourable conditions, plants usually survive only for one season.

2: Chance for D_d to the wild low, but under favourable conditions considerable. Some escapes are found locally. Further population dynamic research necessary. For risk assessment the position of the plants in the regional ecosystem can be of importance.

3: Chance for D_d to the wild small but real. Fruiting of the cultivated plant is essentially undesirable. Further population dynamic research necessary. For risk assessment the position of the plants in the regional ecosystem can be of importance.

4: Chance for D_d to the wild real. Fruiting of the cultivated plant occurs normally, and escapes are found in several localities.

5: Chance for D_d to the wild very real. Fruiting of the cultivated plant occurs very frequently and abundantly, and escapes are found widely, or are undetectable because the same species is native to this region of Europe.

9: Data too scanty or lacking at all, no evaluation possible.

Regional D_f-codes

The distribution of wild plants in Europe is mostly not known on such a small scale as that of hour-squares (5 x 5 km^2) in the Netherlands. This affects the D_f-code. In general, the floristic knowledge of different regions within Europe is sufficient to maintain a kind of frequency-code.

It should be noted, however, that in large parts of Europe most of the floristic knowledge covers areas with a (half-)natural vegetation rather than unnatural vegetations. This means that the chances for gene flow from cultivation to the wild flora can be predicted fairly well for (half-)natural vegetations, but not so for urban areas or areas with a high degree of agricultural activity.

European codes for D_f (Frequency of Distribution)

0: No plants of this species or of a wild relative are found in the wild.

1: Plants of this species or of wild relatives are very rare in the wild, and have their stable place in the regional ecosystem. Chances for hybridizing or blending with the wild population are negligible, if location to grow transgenic plants is chosen ap propriately.

2: Plants of this species or of wild relatives are very rare, but occur sporadically. Distribution is difficult to predict and essentially uncontrollable. Chances for hybridizing or blending with wild populations are low, but unpredictable.

3: Plants of this species or of wild relatives are not very common in the wild, and have their stable place in the regional ecosystem. Chances for hybridizing or blending with the wild population are small.

4: Plants of this species or of wild relatives are not frequent, but well distributed over the whole region. Chances for hybridizing or blending with the wild population are considerable under favourable conditions.

5: Plants of this species or of wild relatives are common, and well distributed over the whole region. Chances for hybridizing or blending with the wild population are considerable and cannot be prevented.

9: Data too scanty or lacking at all, no evaluation possible.

Summary of the chances for gene flow per region

The combined scores for the D_p-, D_d- and D_f-codes for a certain cultivated plant can be summarized to a European D_{pdf}-code, indicating the expected gene flow from that plant species to the wild flora of a certain region of Europe:

No	If D_p or D_d or D_f is 0 or 1, provided the others do not exceed 2.
Minimal/local	If D_p or D_d or D_f are not below 2 and the others do not exceed 3.
Substantial	If D_p or D_d or D_f are not below 3 and the others do not exceed 4.
Serious	If D_p or D_d or D_f are not below 4 and one of those is in position 5.
Unknown	If D_p or D_d or D_f is in position 9, further research necessary.

An example: European D_{pdf}-codes for Lettuce (Lactuca sativa)

The results of our new view on the relationship between wild and cultivated lettuces taken into account, the following facts are of importance for the European D_{pdf}-code for Lettuce (*Lactuca sativa*):

1. The distribution and ecology of wild Lettuce (under the scientific name *Lactuca serriola*) as summarized in Flora Europaea which reads as follows: 'Roadsides, waste places and sand-dunes. Much of Europe, but only as an alien in the north.' [10]
2. The coverage-score in Europe: +34444, meaning 'rare' in the North, 'slightly less common' in the Western part and 'very common' in the rest of Europe.[32]
3. The notion that '*Lactuca serriola*' is the closest wild relative, and that hybridization with other *Lactuca*-species has not been recorded from anywhere in Europe.
4. The notion that self-fertilization is the rule for both wild and cultivated lettuce all over Europe.
5. The fact that cultivated Lettuce is usually prevented from flowering as it is a vegetable; Oilseed lettuce, however, is especially cultivated for the seeds, but is grown mainly in Egypt and Afghanistan. The flowering of lettuce in home gardens and allotments is not recorded but expected to happen on a local scale.

The improved D_{pdf}-codes[2] (see also text boxes), would be as follows for the European regions 3, 4, 5 and 6 (Central, East, South-East, and South Europe):

$D_p = 3$	(self-fertilization is the rule in Lettuce);
$D_d = 3$	(fruit setting usually does not occur in cultivation – under the assumption that Oilseed lettuce is not grown as a crop in South Europe; otherwise D_d would be probably 4);
$D_f = 5$	(plants of this species and their wild relatives are common and well distributed over the whole region, chances for hybridizing or blending with wild populations must be expected and cannot be prevented in field experiments).

For region 2 (West Europe) too, the code would be 3.3.5, because Lettuce is still common enough (and expanding!) in that region.

For region 1 (North Europe) however, the code would be 3.1.2 (no gene flow is expected).

If the present study would not have been performed, the code would have been as follows:

$D_p = 2$ (no records of spontaneously formed hybrids, but hybridization is possible under experimental conditions);

$D_d = 3$ (fruit setting usually does not occur in cultivation – under the assumption that Oilseed lettuce is not grown as a crop in South Europe; otherwise D_d would be probably 4);

$D_f = 2$ (plants of this species occur only sporadically in the wild, chances for hybridizing are scanty; gene flow may be expected, but in most cases on a very local scale only).

Discussion and conclusions

It is possible to establish dispersal codes for 6 plant geographical regions of Europe. For this purpose, reliable information is needed on the following subjects:

a. hybridization, experimentally and/or spontaneously;
b. distribution density of the wild relatives;
c. fitness of the offspring from hybridization;
d. the extent to which the cultivated plant is self-fertilizing or cross-fertilizing (and whether it is wind or insect pollinated);
e. the dispersal possibilities from culture to the wild;
f. the fitness of the diaspores formed in the wild;
g. the amount of diaspores normally produced;
h. the rareness of the wild relatives;
i. the 'nature value' of the wild relatives.

The *Lactuca* study has made it clear that the taxonomic relationships between cultivated plant and wild plant must be sufficiently understood – otherwise, the chances for gene flow will be (much) underestimated. Furthermore it is clear that further research on the actual population dynamics of many species with a high chance for gene flow to the environment is desirable to get more information on a detailed level for those species with a relatively high D_{pdf}-code.

The amount of relevant information on the subjects a–i that is already available for inclusion into a Europe-oriented database is probably large enough to produce a kind of European Botanical Files within a few years. The (eventual) presence of such files is a prerequisite for a first estimate of the chances for gene flow from cultivated plants to the environment, leading to well-informed prioritizing of research programmes on the possible gene flow from GMO's to the wild flora.

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Appendix 1.

Comparison of the delimitations of *Lactuca sativa*, *L. serriola* and *L. virosa* according to three different authors.

F.76: V. Feráková, 1976.[10]
F.77: V. Feráková, 1977.[11]
Boom: T. van den Boom, 1986.[3]
Meij.: R. van der Meijden, 1990.[22]
All: all aforementioned authors, or sometimes all other authors.

	Lactuca sativa	*Lactuca serriola*	*Lactuca virosa*
F.	up to 1.20 m	up to 2.00 m	up to 2.00 m
Boom	(0.20–)0.40–1 m	(0.15–)0.35–2 m	0.40–1.40 m
Meij.	0.60–1 m	0.60–1.20 m	0.50–1.50 m
All	stem glabrous	glabrous or prickly, mainly in basal part	glabrous, or basal part prickly
Radical leaves			
All	undivided or runcinate-pinnatifid	pinnate-lobed to pinnatisect, sometimes non-lobed	non-lobed or pinnatisect
F.	–	spinulose on midrib beneath	spinulose on midrib beneath
Boom	midrib glabrous or with some prickles beneath	midrib glabrous or prickly beneath	midrib with short prickles beneath
	–	–	also lateral nerves
Meij.	glabrous beneath	midrib beneath with often more than 2 mm long prickles	midrib beneath often with few, often less than 1 mm long prickles
Cauline leaves			
All	usually undivided	less lobed to non-lobed	non-lobed or pinnatisect
Meij.	yellowish-green	bluish green	–
All	midrib glabrous or rarely with a few prickles	midrib glabrous or prickly beneath	midrib glabrous or prickly beneath
Meij.	glabrous beneath	midrib beneath with often more than 2 mm long prickles	midrib beneath often with few, often less than 1 mm long prickles
F.77	–	spinous-ciliate on lateral nerves beneath	sometimes spinous-ciliate on lateral nerves beneath
Boom	–	lateral nerves sometimes prickly	lateral nerves prickly
All	not held vertically	in full sun held vertically	usually not twisted
Bracts			
Boom	with big amplexicaul auricles	with circular, elliptic or oblong auricles	with circular amplexicaul auricles, sometimes nearly acute in the lower bracts

Inflorescence			
F.	dense corymbose panicle	pyramidal or spike-like panicle	long, pyramidal panicle
Boom	large corymbose panicle sometimes nearly pyramidal side branches erect	large, pyramidal panicle to nearly corymbose or thin plume-like	large open panicle, cylindrical to pyramidal
Flower heads			
F.	7–15(–35) florets	7–15(–35) florets	c. 15 florets
Boom	10–16 florets	12–22 florets	15–23 florets
Boom	involucrum without anthocyanin	involucrum sometimes tinged or spotted violet	involucrum sometimes with violet spots and/or apex
All	Involucrum not reflexed when ripe	reflexed when ripe	reflexed when ripe
F.76	Ligulas pale yellow, often violet-streaked	pale yellow	pale yellow
F.77	ligulas yellow	pale yellow, often violet tinged	pale yellow
Achenes			
F.	6–8 mm	6–8 mm	6–10 mm
Boom	3–5 × (0.7–)1.1–1.9 mm	2.8–5 × 0.8–1.4 mm	3.7–6.0 × 1.0–2.0 mm
Meij.	3.5–4(–5) × 1–1.5 mm	c. 3 × 1 mm	4–5 × 1.5–2 mm
All	narrowly or broadly obovate	obovate or elliptical	broadly elliptical or obovate
All	whitish, or grey to middle brown with whitish-light brown ribs, or olive green or middle to dark brown with dark spots	light to dark brown or grey, sometime olive green, usually with dark spots	dark brown to purplish black
F.	often finely muricate at apex	short fine bristles towards apex	rugose but not spiculate or setose
Boom	muricate, with small colourless appendages at apex	muricate, with clear colourless appendages at apex	muricate, and sometimes with a few dark prickles at apex
Meij.	glabrous or finely muricate at apex	finely muricate at apex	glabrous
F.76	5–9-ribbed	5–9-ribbed	5-ribbed
Boom	6–10 ribs	4–9 ribs	4–8 ribs
All	2 narrow wings	2 narrow wings	2 (3) broad wings

Appendix 2. List of characters scored during the field trial.

Observations before planting, February 12, 1993.

1. Form of the achene
   - 1: flat, with broad wings
   - 2: flat, with small wings
2. Colour of the seed coat of the achene
   - 1: white
   - 2: amber
   - 3: brown
   - 4: black

Observations in the four leaf stadium. May 6–7, and 12–13

Young rosette leaves

3. Prickles on the midrib
   - 1: no prickles
   - 2: scattered prickles
   - 3: prickles over the length of the midrib
4. Prickles on secondary veins
   - 1: no prickles
   - 2: few scattered prickles
   - 3: rows of prickles
5. Form of leaf
   - 1: round
   - 2: irregularly lobed

Observations in the vegetative period. May 24 and 28, June 1, 4 and 11, 1993

Full grown rosette leaves

6. Prickles on the midrib
   - 1: no prickles
   - 2: scattered prickles
   - 3: prickles over the length of the midrib
7. Prickles secondary veins
   - 1: no prickles
   - 2: scattered prickles
   - 3: rows of prickles
8. Lobes
   - 1: not lobed
   - 2: lobed, incision to 1/3 from the outer margin
   - 3: lobed, incision to 2/3 from the outer margin
   - 4: lobed, incision irregular
9. Number of lobes
   - 9: lobes irregular

Observations in the generative period, June and July 1993

Stem leaves, on knee height (50–60 cm above ground)

10. Prickles midrib
    - 1: no prickles
    - 2: scattered prickles
    - 3: a short row, not over the entire length of the midrib
    - 4: prickles over the length of the midrib
11. Prickles secondary veins
    - 1: no prickles
    - 2: scattered prickles
    - 3: secondary veins clearly prickled
    - 4: secondary and tertiaire veins clearly prickled
12. Lobes
    - 1: not lobed
    - 2: lobed, incision to 1/3 from the outer margin
    - 3: lobed, incision to 2/3 from the outer margin
    - 4: lobed, incision more than 2/3 from the outer margin
13. Number of lobes
14. Presence of prickles on the stem
    - 1: no prickles
    - 2: some prickles under leaf attachment
    - 3: scattered prickles
    - 4: densely prickled
15. Colour of stem prickles
    - 1: white
    - 2: white, base with anthocyanin

Observations on the inflorescence

16. Involucral leaves after fruitset
    - 1: closed
    - 2: open
17. Presence of anthocyanin
    - 1: no
    - 2: yes
18. General outline synflorescence
    - 1: Flat, no distinct central axis
    - 2: More or less rounded, side branches lower than central branches
    - 3: Pyramidal, with a clear central axis

Appendix 3. Accession numbers of *Lactuca sativa*, *L. virosa* and *L. serriola*, with cultivar (group) name, origin and annotations.

The botanical names are the names under which the accessions were received by the seed banks. Some alternatives are suggested in the last column.

RKO: Department of Registration and Plant Breeders' Rights, CPRO/DLO, P.O. Box 16, 6700 AA Wageningen, The Netherlands

CGN: Centre for Genetic Resources Wageningen, CPRO/DLO, P.O. Box 16, 6700 AA Wageningen, The Netherlands

1. *Lactuca sativa* L.

1a. CGN accessions

Number	Cultivar name	Source/Origin	Remarks
Of 'wild' origin			
04897			Oilseed?
04898		Turkey	Oilseed?
05085			Oilseed?
Crisp lettuce			
13386		Netherlands landvariety	
Latin lettuce			
05999		Gradina Botanica a Universitatii din Cluy-Napoca, Rumania	
Cos lettuce			
05348	Balady	University of Assiut, Egypt	
04589	Gobekli Marul	University of Ege, Bornova Izmir, Turkey	
04733	Kahu	Iran, through Beltsville Agricultural Research Centre, Beltsville, Maryland, USA	
Cutting lettuce			
05815		Jardim e Museu Agricola do Ultramar, Lisbon Belem, Portugal	
Stalk lettuce			
10932	Cabbage lettuce	Forschungs Institut Gemüsebau, Olomouc, Czechia	
11387	Tianjin Big Stem	China	
Oilseed lettuce			
04786		Afghanistan	Cos?
05115	Balady	University of Assiut, Egypt	
05342		IBPGR Egypt Expedition, Giza, Egypt	
09356		IBPGR Egypt Expedition, Giza, Egypt	Cos?

1b. RKO accessions

Butterhead lettuce

91283	Gotte Jaune d'Or
87447	Rougette du Midi à Graine Noire

Crisp lettuce

92180	Chou de Naples

Latin lettuce

88380	Deer tongue
89434	Gallega d'hiver
91232	Madrilene
89431	Sucrine
91239	Sudia

Cos lettuce

91162	Hector
87324	Kasseler Strünkchen
88181	Little Leprechaun

Cutting lettuce

89428	À Couper à Feuille de Chène blonde à Graine Noire
89276	American Gathering
85432	Australischer Gelber
89235	Black Seeded Simpson
87323	Hohlblättriger Butter
90207	Monet
92331	Red Salad Bowl
88102	Ruby
90275	Waldmann's Dark Green

2. *Lactuca serriola* L.

CGN accessions

04667	Botanische Tuinen van de gemeente Rotterdam, The Netherlands	
05800	Jardim Botanico da Universidade de Coimbra, Portugal	
05939	Agricultural Research Organisation, The Volcani Centre, Bet Dagan, Israel	
10892	China	Stalk?
10938	Bulgaria	
11323	Denmark	Stalk?
11408	Afghanistan	wild × cult.?
900030	Armenia	
900036	Armenia	
900037	Armenia	
900052	Daghestan, Russia	
900057	Daghestan, Russia	
910401	Turkey, Hatay	
910402	Turkey, Hatay	
910403	Turkey, Antalya	
910409	Turkey, Afyon	
910411	Turkey, Izmir	
910412	Turkey, Eskesehir	
910414	Turkey, Balikesir	

(*Lactuca serriola* L. continued))

Cos lettuce

04777	Egypt	Oilseed?

Oilseed lettuce

04769	Egypt

3. *Lactuca virosa* L.

CGN accessions

05793	Botanical Garden of the University, Szeged, Hungary
05941	Agricultural Research Organisation, The Volcani Centre, Bet Dagan, Israel
09316	Great Britain, through California University Agricultural Research Station, Davis California, USA
09365	Iran, through Prof. T.H. Hewer Vine, Bristol, United Kingdom
13302	Zentral Institut für Genetik und Kulturpflanzenforschung der A.D.W. Gatersleben, Germany
13339	Spain, through Centre for Genetic Resources, Wageningen, The Netherlands
13362	France, through Centre for Genetic Resources, Wageningen, The Netherlands
14289	Spain, through Centre for Genetic Resources, Wageningen, The Netherlands
900045	Russia, Daghestan
900048	Russia, Daghestan
900049	Russia, Daghestan

Appendix 4. Scores for a set of characters used in the field trial

A cross indicates presence of that character state in at least one individual of that population. More than one character state can be present in one population. The registration numbers are the same as in Appendix 3, Accession numbers.

Seed:

Shape of the achene*
- 1: flat, with broad wings
- 2: flat, with small wings

Colour of the seed coat of the achene**
- 1: white
- 2: brown
- 3: black

Radical leaves:
- 0: no rosette leaf present
- 1: no prickles
- 2: scattered prickles
- 3: a row of prickles over the entire length

Cauline leaf midrib:
- 1: no prickles
- 2: scattered prickles
- 3: a short row, not over the entire length of the midrib
- 4: prickles over the length of the midrib

Cauline leaf secondary veins
- 1: no prickles
- 2: scattered prickles
- 3: secondary veins clearly prickled
- 4: secondary and tertiaire veins clearly prickled

Stem prickles
- 1: no prickles
- 2: some prickles under leaf attachment
- 3: scattered prickles
- 4: densely prickled

Involucrum***
- 1: closed after fruit set
- 2: open after fruit set

General shape inflorescence
- 1: flat, no distinct central axis
- 2: more or less rounded, side branches lower than central branches
- 3: pyramidal, with a clear central axis

*) In Tabel next pages: s
**) In Tabel next pages: c
***) In Tabel next pages: Invol

Registr.	Achene		Radical leaves				Cauline leaves								Stem				Invol		Infloresc		
number	s	c	midrib				midrib				secondary				prickles				open		shape		
			0	1	2	3	1	2	3	4	1	2	3	4	1	2	3	4	1	2	1	2	3
Wild lettuce																							
04667	2	2			x					x	x	x	x			x				x		x	
05800	2	2				x				x		x	x				x			x			x
05939	2	2	x							x	x	x				x	x			x		x	x
10938	2	2				x				x		x				x				x		x	x
900030	2	2			x					x			x			x							x
900036	2	2			x			x		x	x		x	x	x		x			x		x	x
900037	2	2			x	x				x	x	x			x	x				x		x	x
900052	2	2				x				x		x	x				x						x
900057	2	2			x	x				x	x	x				x	x			x		x	x
910401	2	2			x	x				x	x	x	x			x	x			x		x	x
910402	2	2				x				x			x			x	x			x			x
910403	2	2				x				x			x			x	x	x		x		x	x
910409	2	2				x				x				x				x		x			x
910411	2	2				x				x			x			x	x			x		x	x
910412	2	2				x				x		x	x	x		x	x	x		x		x	x
910414	2	2			x					x	x	x	x			x	x			x		x	x

Registr.	Achene		Radical leaves				Cauline leaves								Stem				Invol		Infloresc		
number	s	c	midrib				midrib				secondary				prickles				open		shape		
			0	1	2	3	1	2	3	4	1	2	3	4	1	2	3	4	1	2	1	2	3
Wild x cultivated ?																							
11408	2	1			x	x	x			x	x		x		x	x			x	x	x	x	
11408	2	2			x		x			x	x				x				x		x		x
Oil-seed lettuce of "wild" origin																							
04897	2	1	x							x	x						x		x		x		
04898	2	1	x							x	x	x				x			x		x		
05085	2	1	x							x	x					x			x		x		
Butterhead lettuce																							
87447	2	2		x	x		x				x				x				x		x		
91283	2	1		x	x		x				x				x				x		x		
Cos lettuce																							
04589	2	1		x	x		x				x				x				x		x	x	
04733	2	2			x		x	x		x	x				x				x		x		
04786	2	1			x				x			x							x			x	
05348	2	1			x		x				x				x				x		x	x	
09356	2	1			x		x				x				x				x		x	x	
87324	2	1			x			x			x				x				x		x	x	

Registr.	Achene		Radical leaves				Cauline leaves								Stem				Invol		Infloresc		
number	s	c	midrib				midrib				secondary				prickles				open		shape		
			0	1	2	3	1	2	3	4	1	2	3	4	1	2	3	4	1	2	1	2	3
Cos lettuce, continued																							
88181	2	2		x	x		x				x										x	x	
91162	2	1		x	x		x				x				x				x		x	x	
Cutting lettuce																							
05815	2	2			x		x				x				x				x			x	
85432	2	1		x	x		x	x			x								x		x	x	
87323	2	1		x			x				x				x				x		x	x	
88102	2	1			x		x	x			x				x						x	x	
89235	2	2			x			x	x	x	x								x		x		
89276	2	1		x	x		x	x			x								x		x	x	
89428	2	2		x	x		x				x				x				x		x		
90207	2	2		x			x				x				x				x		x		
90275	2	2		x			x				x								x		x		
92331	2	2			x			x	x		x					x					x		
Crisp lettuce																							
13386	2	1			x			x	x		x											x	
92180	2	1			x														x			x	

Registr.	Achene		Radical leaves				Cauline leaves								Stem				Invol		Infloresc		
number	s	c	midrib				midrib				secondary				prickles				open		shape		
			0	1	2	3	1	2	3	4	1	2	3	4	1	2	3	4	1	2	1	2	3
Stalk lettuce																							
10892	2	2			x		x				x				x				x		x		
10932	2	1		x	x		x				x				x				x		x		
11323	2	2			x		x				x				x				x		x		
11387	2	2			x	x	x				x										x		
Latin lettuce																							
05999	2	1			x			x	x		x				x				x		x	x	
88380	2	2		x	x										x				x		x		
89431	2	1		x											x						x		
89434	2	2			x		x				x				x				x		x		
91232	2	2			x										x				x		x		
91239	2	2			x														x		x		
Oilseed lettuce																							
04769	2	2	x				x				x				x				x		x	x	
04777	2	2	x				x				x				x				x		x	x	
05115	2	2	x				x				x				x				x		x		
05342	2	2	x				x				x				x				x		x		

Registr.	Achene		Radical leaves				Cauline leaves								Stem				Invol		Infloresc		
number	s	c	midrib				midrib				secondary				prickles				open		shape		
			0	1	2	3	1	2	3	4	1	2	3	4	1	2	3	4	1	2	1	2	3
Lactuca virosa L.																							
05793	1	3				x				x			x				x	x		x			x
05941	1	3			x	x	x				x				x					x			x
09316	1	3				x			x	x	x	x					x	x		x			x
09365	1	3			x	x			x	x	x	x				x	x			x			x
13302	1	3				x				x			x					x		x			x
13339	1	3				x				x		x	x				x	x		x			x
13362	1	3				x				x			x					x		x			x
14289	1	3				x				x			x					x		x			x
900045	1	3			x	x			x	x	x		x		x	x				x			x
900048	1	3			x				x	x	x	x				x				x			x
900048	1	3			x				x		x					x				x			x
900049	1	3				x		x	x	x	x					x				x			x

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F.T. de Vries, R. van der Meijden & W.A. Brandenburg: Botanical Files. A study of the real chances for spontaneous gene flow from cultivated plants to the wild flora of the Netherlands. 100 pp. — Dfl. 20.00.

Summary

Separate 'Botanical files' have been made for 42 species of cultivated plants. Each file gives information about the cultivated plant itself (use, origin, etc.), its wild relatives in the Netherlands, a report on actual hybridization and/or crossing (indicating gene flow by pollen), and observations on escapes from the field to nature (indicating gene flow by diaspores); the information is summarized to a conclusion and a numerical code, indicating the possible ecological effects of the cultivated plant on the wild flora of the Netherlands. This study was especially undertaken for questions regarding biosafety research on Genetically Modified Organisms (GMO's). The sources are the herbarium collections of the State Herbarium at Leiden, floristic archives and botanical literature, as well as expert judgment on the flora of the Netherlands and crop plants. An important consideration is that the *absence* of certain hybrids in the State Herbarium can be interpreted as a decisive indication that such a hybrid does not occur in the wild in the Netherlands. The botanical files show that in *c. 50%* of the cases no gene flow is expected; in *c. 15%* of the cases small, often local-scale effects are expected; in *c. 25%* of the cases considerable gene flow to the wild is expected; in *c. 10%* of the cases further research should be done before a definite conclusion can be drawn (most of the cases need further taxonomic research).