HISTORICAL AND MOLECULAR BIOGEOGRAPHY OF PRICKLY LETTUCE (*L. SERRIOLA* L.) AND EVALUATION OF HYBRIDISATION WITH THE CROP (*L. SATIVA* L.)

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PhD Thesis
Université de Neuchâtel - Faculté des Sciences - Laboratoire de Botanique évolutive
Neuchâtel - Suisse
soutenue le 24 mai 2006

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IMPRIMATUR POUR LA THESE

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General summary

Prickly lettuce *(L. serriola)* distribution has expanded throughout Europe during the past centuries. In the present thesis, we document its spread in time and space by the investigation of historical material. We show that the colonisation of Northern Europe occurred during the end of 18th century and the first decades of the 19th century. Most of Europe was colonised by the beginning of the 20th century. The colonisation is recent and several factors that could explain this massive spread are discussed, in particular the influence of environmental and socio-economic changes on the distribution of the species. This includes different factors as climate warming, globalisation of trade and travel, development of transportation networks and the increased availability of disturbed and ruderal habitats. Its expansion has also been documented using several molecular tools (nuclear microsatellites and chloroplast PCR-RFLPs). The genetic diversity of present wild populations collected all over Europe was analysed. The results were in accordance with the life history strategy of the species. We found a very strong population structure and most of the genetic variation was distributed between populations. A strong pattern of isolation by distance was found, mainly caused by low pollen flow between populations and confirmed by a very high overall inbreeding coefficient and very low amount of observed heterozygotes. No clear grouping patterns could be highlighted at the continental scale. The higher genetic diversity was found in South of France, northern Italy and eastern Europe, while less was present in Great Britain, north Scandinavia and Iberian Peninsula. Kriging of Gene Diversity showed a tendency of decrease westwards and northwards from Eastern Europe. Finally, we studied the hybridisation between prickly lettuce (*L. serriola* L.) and crop lettuce empirically, by planting the crop in the middle of two fields and wild plants at different distances from it, in two locations in Switzerland during the summer 2003 and 2004. These experiments were carried out in order to assess the risk of (trans)gene escape through pollen flow. Despite flowering mechanisms ensuring self-fertilisation in both species, hybridisation has been found to be the rule rather than the exception at close distances.

Context
This PhD thesis has been carried out in the framework of the EU-funded project "Analysis of gene flow from crop to wild forms in lettuce and chicory and its population-ecological consequences in the context of GM-crop biosafety" (ANGEL), coordinated by Dr. C. van de Wiel, from Wageningen (Quality of Life and Management of Living Resources - QLK-CT-2001-01657).

Key words: hybridisation, hybrid, risk assessment, pollen flow, global change, climate change, bioclimatic niche, disturbance, biogeography, SSR, PCR-RFLP, RAPD, population genetic, *Lactuca serriola*, *Lactuca sativa.*

Thesis outline

We contributed to ANGEL project with three different approaches: 1) we studied historical aspects of the expansion of prickly lettuce in Europe by the analysis of historical material (herbaria and literature); 2) The genetics of present wild populations was analysed using two types of molecular markers, nuclear microsatellites and chloroplast PCR-RFLPs; 3) we quantified the hybridisation rate between crop lettuce and its wild relative with a field experiment.

1) The historical approach aimed to reconstruct the expansion of *L. serriola* through the North, and to understand the main past and recent factors that facilitated its spread.

Floristic and herbaria data are of prime importance to trace historical colonisation events, which are the basis for the management of modern environmental issues: the influence of environmental changes on species, invasions or biodiversity changes among others. Botanists are very attentive to the floristic composition of their region and usually have the tendency to look for rarities. Generally speaking, they often observe a "newcomer" very quickly, and collect it preferentially, while they are more lax toward common plants. Thus, herbaria can inform us quite precisely about the date of arrival of a taxon in a region.
A large dataset was built up through literature search as well as floristic and herbarium surveys. For all herbarium and literature data, we searched for the geographical coordinates. The occurrences were represented by GIS software (Geographical Information System) in order to display the evolution in time and space of *L. serriola* spread in Europe. Many factors, possibly responsible of this expansion, will be exposed and discussed in chapter 1.

2) By analysing *L. serriola* with molecular markers, we aimed to characterise the genetic diversity of the species on a spatial scale, and to provide information on overall polymorphism and on out- or inbreeding in European populations. The central question behind this study, was to understand the process of colonisation of Europe by prickly lettuce from its area of origin (Eastern Europe and Mediterranean region) by identifying how the genetic diversity is distributed within the species.

The study presented in chapter 2 also provides insights for a better understanding of the biology and the life history strategy of the species.
In addition, it has been widely recognized that the genetic diversity of crop species and their wild relatives should be conserved as a resource for future programmes of genetic improvement (e.g. Lebeda 2002) and that the genetic base of crops should be broadened by use of wild germplasm resources. Yet, *L. serriola* is already used for breeding purposes. In this perspective, a better understanding of its intra and inter-population genetic diversity, as well as of its distribution across Europe is essential, as it can favour a more rational and efficient effort in sampling and screening wild populations for gene bank collections and future programmes of genetic improvement.

3) The field experiment carried out in 2003 and repeated in 2004 aimed to quantify, in natural field conditions, the hybridisation rate as a function of the distance between *L. sativa* and its wild relative *L. serriola.*

This kind of study is indeed essential in the context of GE lettuce cultivation. It could help to assess the potential risk of (trans-)genes escape into wild populations through pollen flow, and represents a first step toward the understanding of the mechanisms of introgression. It has been widely demonstrated that the occurrence of hybridisation in nature depends on the relatedness of the species concerned and on local environmental factors that can affect it. As it is further explained in chapter 3, *L. sativa* and *L. serriola* are very closely related and do hybridise at a significant rate. Due to the large number of seeds produced by single plants, the number of hybrids produced can be large as well. Moreover, hybrids are completely fertile and self-compatible. This can facilitate (trans-)genes introgression into wild populations, as continual hybridisation is not necessary. Even though we did not use transgenic varieties to address the question of crop-to-wild gene flow, our results have clear implications for the risk assessment of cultivation of transgenic lettuce.

Introduction

The genus ***Lactuca***, prickly lettuce and its related crop

The genus ***Lactuca***

The taxonomical position of the genus *Lactuca* is unclear and divergent opinions persist. Feràkova (1977), de Vries & Jarvis (1987), Prince & Carter (1977) made an historical survey of taxonomical studies. The genus *Lactuca* belongs to the Asteraceae family, subfamily Cichorioideae, tribe Lactuceae Cass. (Cichorieae, according to Stebbins (Stebbins 1953)), subtribe Lactucinae Dumort (Bremer et al. 1994). *Lactuca serriola* and *L. sativa* belong to the *Lactuca* section and subsection and to the *serriola* group. More recently, changes in *Lactuca* ssp. classification, based on molecular data, have been proposed (Koopman et al. 1998, Koopman 1999). The genus *Lactuca* L. consists of about 100 species, of which, 17 are found in Europe (Frietema de Vries et al. 1994); only 16 according to Lebeda (Lebeda et al. 2004). Mejias (1993) consider it as one of the most evolved and successful taxa within Asteraceae. Recently, several studies have demonstrated the close relatedness of all related *Lactuca* species, using morphological traits (de Vries & van Raamsdonk 1994), chromosome banding patterns (Koopman et al. 1993), isozymes (Kesseli & Michelmore 1986), RFLPs (Kesseli et al. 1991), RAPDs (Wycott and Fort 1994) and microsatellites (van de Wiel et al. 1998).The genus is distributed in temperate and warm regions of the northern hemisphere. Its most important centre of diversification is the Mediterranean region, where about 20 species can be found (Feràkovà 1977). The more common European species are *L. serriola*, *L. saligna* and *L. virosa* (Lebeda et al. 2004).

Prickly lettuce, ***Lactuca serriola*** L.

L. serriola is native to southern Europe, northern Africa and western Asia, but it has been widely introduced elsewhere (Carter et al. 1985; Zohary 1991)。It is the most variable and widely distributed species of the genus (Lebeda et al. 2004). In the putative regions of origin (Near East), it occurs in primary habitats such as rocky gullies or open rocky slopes, whereas in other distribution areas it grows mainly in synanthropic disturbed areas (Lebeda et al. 2004) such as waste places, embankments, along ditches and roads, field margins, fallows and cultivated fields (Feràkovà 1977; Zohary 1991; Lebeda et al. 2001)(Fig.1). *Lactuca serriola* is a large spring or winter annual therophyte that reproduces only by seeds. Seedlings develop into a basal rosette with a long taproot. It grows mostly on sunny exposure and is a pioneer of open habitats (Lebeda et al. 2004). It flowers from July until September and seeds are shed from August until late September. The temperature and the photoperiod are probably two determinant factors controlling the blooming and seed production and quality (Rappaport et al. 1956). The flowering stem is erect, 0.5 to 1.8 m high, spiny or not near the base, branched near the top at the beginning of anthesis and then branching at the aisselle of leaves and/or from the rosette when growing conditions are optimal. Cauline green waxy leaves are alternate, sessile and clasp the stem. Leaves are naturally oriented vertically with the lamina perpendicular to the east and the west. Leaf compassing reduces water loss without affecting the carbon gain, resulting in higher reproductive output compared to a horizontal leaf orientation (Werk et al. 1986). A row of spine is present along the midrib on the underside of the leaf (Fig.2). The plants contain white bitter latex.

Figure 1. L. serriola *in a dump site*

Figure 2. *Spines on the underside of the leave*

Figure 3a. *Capitula of* L. serriola

Figure 3b. *Capitula of* L. serriola *with open florets*

The numerous flower heads (capitula) are small and grouped into rich pyramidal or plume-like inflorescences (Frietema de Vries et al. 1994), composed of 10 to 30 pale yellow ligulate flowers (Weaver et al. 2003) (Fig.3a and b). The seeds are greyish to brown, 3 to 4 mm long, with short fine bristles near the apex, ending in a beak with an attached pappus, 4 to 5 mm long. When the seeds are ripe, the involucral bracts reflex and the seeds are wind-dispersed (Fig.4). The leaf shape variability of *L. serriola* brought botanists to describe two main forms: *L. serriola* f. *serriola* with pinnate-lobed cauline leaves and *L. serriola* f. *integrifolia* with entire cauline leaves (Fig.5 and 6). Both forms have a row of spine along the midrib on the underside of the leaf. The *integrifolia* form is also mentioned under synonyms *L. serriola* var *integrata* Gren. Et Godr., *L. dubia* Jord., and *L. integrata* A. Nels (Feràkovà 1977). It is found throughout the *L. serriola* distribution area, but with different frequencies of occurrence (Lebeda et al. 2004). In 1785, Allioni described *L. angustana* in Val d'Aosta (Italy) (*in* Dal Vesco et al. 1987/88). This species has entire or pinnate leaves without spines (Fig. 5 and 6) and has been integrated in the *L. serriola* f. *integrifolia* group. However, this classification should be revised since both leaf shapes can be found within *L. augustana* species (Fig.7). *L. serriola* and *L .augustana* can be found in sympatry.

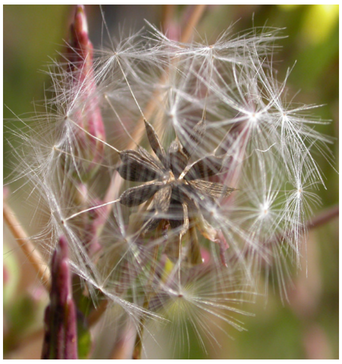

Figure 4. *Wind-dispersed seeds*

Cultivated lettuce, ***L. sativa*** L.

L. sativa is the only species of the genus with an economic importance and a worldwide distribution (Lebeda et al. 1999). The domestication of lettuce has not been completely resolved yet, but most likely, it occurred in southwest Asia (Zohary 1991), probably between Egypt and Iran (Hintum et al. 1999). Cultivated lettuce appeared circa 2500 BC on Egyptians wall paintings and relieves on tomb walls (De Vries 1997) (Fig.8). *L. serriola* is one of the direct ancestors of lettuce, as shown in studies with molecular markers (Kesseli et al. 1991; Hill et al. 1996). The genetic transformation of *L. sativa* is possible since many years (Mallory-Smith et al. 1990; Curtis et al. 1999). It has already been engineered for many traits like herbicide resistance (Nagata et al. 2000; Lebeda 2002, Mohapatra et al. 1999, McCabe et al. 1999), pathogen resistance (Okubara et al. 1997), virus resistance (Dinant et al. 1997), frost tolerance (Pileggi et al. 2001) iron accumulation (Goto et al. 2000) and other agronomic traits (Curtis et al. 1999).

Figure 5. *Pinnate leaves*

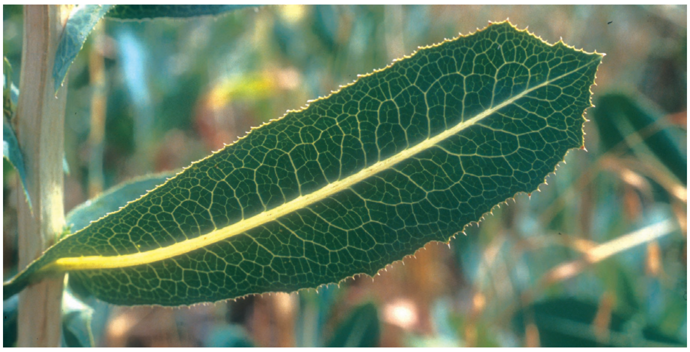

Figure 6. *Entire leaves*

L. sativa – L. serriola ecocomplex

L. sativa and *L. serriola* are extremely closely related and are often considered as conspecific (Whitaker 1939; Lindqvist 1960 a; De Vries 1990) or ecospecies (De Vries et al. 1994). The two species are diploid, and their chromosome length and genetic background are equivalent (Lindqvist 1960 a; Koopman et al. 2001, Thompson et al. 1941, Lindqvist 1960 a). No pre- or postzygotic barriers preventing hybridisation between the two species exist (Lindqvist 1960 a; De Vries et al. 1994), and hybrids are obtained easily in both directions (Thompson et al. 1941; De Vries 1990, Lindqvist 1960 a). Frietema de Vries et al. (1994) consider the two taxa as one single species, differing only in characters connected to domestication like rapid growth,

Figure 7. *The two leaf shapes found together within* L. augustana *species*

Photo. Nicola Schoenenberger

Figure 8. *Egyptian wall paintings representing cos letuce*

short life-cycle, quick harvesting and prevention of pest development, absence of dispersal mechanisms preventing the spread of seeds (erect involucral leaves), absence of dormancy and no or few prickles on the leaves and none on the stem. The involucre character has been found to provide a very reliable mean to separate *L. sativa* (erect involucre) and *L. serriola* (reflexed involucre) (Frietema de Vries et al. 1994, Fig. 9 and 10). Hintum and Boutema (1999) and Hintum et al. (1999) stressed that other species like *L. altaica, L. augustana, L. scarioloides, L. dregeana, L. aculeata* appear to be fully sexually compatible with the *L. sativa - L. serriola* group and can even be considered synonyms of *L . serriola*. Finally, the Dpdf code (Dispersal by pollen, diaspore and frequency of dispersion) established by De Vries (1994) for *L. serriola* in Europe is 3.4.5, which indicate that gene flow between *L. sativa* and *L. serriola* is expected.

Figure 9. *Reflexed involucre*

Figure 10. *Erect involucre*

Floral mechanism and pollination

The floral mechanisms of *Lactuca serriola* and *L. sativa* ensure a high degree of self-fertilization, and all the species of the serriola group are self-fertilizers (Mejias 1993). The flowers possess an incomplete protandry (Mejias 1994): while the flowers heads are open, the pistil grows through the tube formed by the five anthers. The style emerges through the anther tube and branches when it is about 2 mm above the tube (Fig.11 and 12). These two branches curl back upon themselves and usually make contact with pollen grains on the sides of the style, which result in a self-pollination process. The flowers heads are open only during the morning, in function of the temperature. On warm bright days, the heads open early in the mornings and remains open few hours, even less. On cold days, flowers remain open longer, while on rainy

Figure 11. *Emergence of the styles from the antherinal tube*

Figure 12. *Growth of the style*

days flowers do not open. After a short time the petals close and don't open again; but new flower heads open each days (Lindqvist 1960 a).

Since the pollen is not windblown, pollinators are essential for the effective transfer of pollen to the stigma, within and likely between flower heads. The temperature influences positively in-vivo pollen germination in cultivated lettuce (Eenink 1983).

Hybrid description

Morphologically, prickly X crop lettuce hybrids can be easily distinguished from wild parents only at the rosette stage, when they look like the paternal cultivar (Fig.13). On the opposite, hybrids are not easily recognisable as they grow, since their morphology gets closer to that of the wild parent: The leaves compass, the plant gets spiny and finally the adults scatter their seeds. The F1 hybrids combine the adaptations to wild habitats of the wild parent (Fig.14) but possess some traits related to the domestication of the cultivated parent.

Figure 14. *A spontaneous F2 hybrid growing in a typical ruderal habitat as do its wild parent*

Figure 13. *Comparison of a wild plant (right) a cultivated plant (left) and their hybrid (centre) at the rosette stage. The hybrid look like its cultivated parent*

More than twenty years after the introduction of genetically engineered crops in agriculture, the public controversy on transgenic crops is still extremely present, especially in Europe. After the end of the ban of transgenic varieties in European countries from 1999 to 2004, these latter have adopted a new directive (2001-2018 CE), which strongly takes into account the precautionary principle, but allows cultivation through an authorisation process. For example, in 2005, Germany, Spain, France, Portugal, Czech Republic and Romania grew GM crops for forage.

Because of the cultivation and commercialisation of GM crops, the need to establish national regulations and strategies that should enable coexistence of all agricultural production systems has emerged. In addition, the public pressure for the application of the precautionary principle has raised the need of scientific studies aimed to assess potential risks for natural and agro-ecosystems caused by the cultivation of genetic engineered organisms (GEOs).

Nowadays, in order to legitimate its decisions, the European commission accompany its recommendations on coexistence with highly supported financially research projects. However, while surveys clearly indicate that a majority of European citizen are against a GM alimentation, scientific studies aimed to assess the potential local environmental and social benefits of GEO cultivation are, to my knowledge, still lacking. This is hardly comprehensible, since these kinds of studies would probably be more efficient to convince the population to adopt such a technology.

In our perspective, one of the main concerns relative to the cultivation of GM crops is the transgenes escape into wild populations in agricultural areas. Expression of transgenes in wild and/or weedy introgressants could lead to increased performances and/or weediness. The negative impacts could be both, economic if super-weeds emerge and/or environmental if new highly competitive organisms are dispersed in natural and agro- ecosystems.

These risks are likely to be higher when wild plants already show weedy tendencies (Snow et al. 1997). Yet, prickly lettuce occurs as a weed in Canada and in Argentina in a variety of crops where no-till or conservation tillage systems are used (Weaver et al. 2003). In Southern-Australia, *L. serriola* infested cropland and wheat fields for the first time in 1994. Therefore, it is conceivable that it could become a more aggressive weed by the introgression of advantageous traits. It should also be noted that the introgression of herbicide tolerance genes is not necessary for the emergence of resistant strains of wild and weedy species; sulfonylurea-resistant prickly lettuce has indeed already been dicovered in Idaho (US) in 1987.

In Europe, where prickly lettuce expanded tremendously during the past few centuries, it does not seem to pose problems in agricultural areas, although it has already been described as an invasive weed occupying ruderal places (Lebeda et al. 2004).

Hereafter, the main ideas, results and discussion of the three different approaches of my thesis are exposed and briefly summarized. The order is the same of the three chapters that follow this introduction. Each chapter has been or will be submitted for publication in peer reviewed scientific journals.

Summary of the chapters

Chapter 1

Prickly lettuce (*L. serriola* L.) has benn expanding throughout Europe during the past 250 years. We documented its distribution shift through Europe by the investigation of 1785 herbarium sheets from 24 herbaria including 15 European countries. More precise analyses were carried out for Germany, Austria and Great Britain by investigating 976 literature occurrences. Floristic and herbaria data were consistent: the colonisation of Northern Europe occurred during the end of 18th century and the first decades of the 19th century. At the beginning of the 20th century, the species had colonized most of Europe at the exception of the Northern part of the United Kingdom and Northern Scandinavia. Presently, the Northern limit of the distribution area is located near the latitude 66 º N in through Sweden and near 56 º N in Scotland.

Subsequently, we tried to understand the past and the more recent factors, which allowed this spread. The recent concerns about the impact of climate change and its influence on the dynamics of plant communities and species (e.g. range expansion or contraction) brought us to the hypothesis that global warming could have contributed to the expansion of *L. serriola* to the North. In order to test this hypothesis, we built predictive models for the bioclimatic niche of the species and showed that the climate warming in Europe increased the number of sites suitable for the species. *L. serriola* responded to climatic changes by migration to maintain its distribution range in equilibrium with its climatic niche. However, since climate change is a relatively recent phenomenon it couldn't trigger the distribution shift of *L. serriola* towards Northern Europe in the past centuries. Other extrinsic factors as the changing patterns of land use by humans, the development of transportation networks and the globalisation of trade have lead to an increased availability of disturbed and ruderal habitats, favourable to *L. serriola*. Intrinsic factors, like the reproductive and dispersal strategies of *L. serriola*, combined with the extrinsic factors presented above, probably favoured the dispersion of the species over large distances.

Chapter 2

In order to characterise genetically this recent expansion, we carried out a study using molecular markers on 2622 individuals from 101 wild populations collected along a North-South and East-West transect across Europe. Microsatellites (SSR) and chloroplastic (PCR-RFLPs) markers were used to detect polymorphism within the species.

The genetic differentiation between populations was very high (G'st =0.880). Most of the genetic variation was indeed found between populations, but only little within (Hs = 0.35). We found a very strong pattern of isolation by distance, which is probably due to non-adaptive factors, as limited dispersal of pollen. This seems to be confirmed by the low amount of heterozygous individuals (Ho = 0.014) and the very high overall inbreeding coefficient (Gis = 0.96), which indicate a high selfing rate and an extremely low level of gene flow between individuals and populations. Despite the high overall Gst and the strong pattern of isolation by distance, only a very weak correlation between the distribution of genetic diversity and the geographic distribution could be identified at the continental scale. However, this does not mean that Gene Diversity is equally distributed over Europe. Kriging of Gene Diversity showed that most of the diversity is found in south-eastern populations and in central Europe, while it decreases northwards and eastwards from these regions. Finally, some polymorphism observed with highly conserved chloroplastic PCR-RFLPs markers seem to indicate that several founder events occurred in some populations.

The genetic data were compatible with the biology of the species and very well illustrated the life history of the species, which grows mainly in disturbed areas and whose spread is closely related to human activities, mainly to transportation. *L. serriola* seeds may rapidly disperse on both short and long distances. Empty patches may be colonised by few seeds; the plants that grow from those seeds produce a large amount of self pollinated and genetically very homogeneous seeds (offspring) that further fill the habitat. These results support the historical description presented in chapter 1.

Chapter 3

In order to assess the risk of gene transfer from GM or conventional crops to wild relative, quantifying hybridisation through pollen flow is a key issue. Moreover, gene flow between conventional and well adapted lettuce cultivars and prickly lettuce has been proposed as a hypothesis to explain the recent massive spread of the wild species in Europe, but no study has been previously performed in order to determine how easily the two species can hybridize. Yet, this is the first step toward the understanding of the mechanisms of introgression. In the present study, pollen flow from cultivated lettuce (*Lactuca sativa* L.) to its wild relative *L. serriola* was investigated by experiments carried out in natural field conditions. During the summer 2003 and 2004, an experiment was carried out in two locations in North-Switzerland. In each field trial, 216 plants of *L. serriola* were planted in 12 rows of 18 plants at 0, 1, 5, 15, 25, 40 m North and South of a 25 x 1 m plot composed of 400 crop plants lettuce (*L. sativa*). All the seeds of the wild species were collected, and the progeny was screened both, morphologically and with RAPD markers, in order to detect natural hybridisation events. Mean hybridisation rates (MHR) and incidence of hybridisation (IH, percentage of wild plants that produced at least one hybrid) decreased with the distance, but at unequal rates. MHR was consistent with out crossing-rates in crop lettuce cultivars found in the literature.

Hybridisation events occurred up to the maximal distance tested (40 m) and individual hybridisation rates varied between 0 to 26%. The incidence of hybridisation was higher than 80 % at 0 m and 1 m. It ranged from 32 % to 62 % at 5 m and from 5 to 21 % at 15 m. The incidence of hybridisation was higher at 25 meters than at 15 m with ranges from 12 to 22 %. At 40 m, 4 to 5 % of the wild plants produced hybrids.

In sympatric crop-wild populations, pollen flow from cultivated lettuce to its wild relative has to be seen as the rule rather than the exception, at least at close distances. Moreover, despite the floral mechanisms ensuring self-fertilisation in both species, incidence of hybridisation can be relatively high even at long distances. Common assumption that autogamy, at least in lettuce, will hamper crop (trans-)gene transfer to wild species should probably be revised.

Conclusion

We showed that prickly lettuce has spread rapidly in the last century and has now colonised most Europe. Environmental and socio-economic changes are probably responsible for this expansion. Molecular data confirm the high mobility and the biology of the species. *L. serriola* is still in a dynamic of colonisation and gets more and more abundant in disturbed areas. Our findings also clearly indicate a non negligible potential for crop-wild lettuce hybridisation in sympatric populations. Even low hybridisation rates could lead to the formation of a non negligible number of self fertile hybrids. The hybrid fertility could facilitate introgression of crop genes into the wild species, if the introgressed traits are not deleterious. Moreover, genotypes having an environmental advantageous trait would probably expand rapidly, since selfing would ensure the heredity of this trait in the progeny.

In conclusion, although the species is actually a weed in the sense of Baker (1974), *i.e.* a synanthropic plant species that poses no problems, if transgenic varieties were cultivated and if the inserted traits could confer a selective advantage to the wild species in agricultural areas, the trait is expected to be introgressed rapidly. These traits could become the predominant alleles, since lettuce is a self fertilizing plant. Subsequent selfed and/or introgressed generation could become invasive even in agricultural areas where the species is not a problem yet.

Perspectives

The quantification of the out-crossing rate of *L. serriola* and of hybrids is still unanswered. This remains of prime importance for introgression studies; even though self fertilisation prevails.

In 2004, in Val d'Aosta (Italy), I found a spontaneous hybrid growing in a wall close to a private garden were cultivated lettuce was grown (Fig.15). This indicates that hybridisation between the two species is an ongoing process. However, data on the dynamic of hybrids populations in natural conditions are lacking. In order to satisfy my curiosity, in 2004, I planted a hybrid in a dump colonised by a wild population of *L. serriola.* The single F1 hybrid produced viable offspring and its progeny persisted until present time. These observations were made only on the base of morphological observations. However, F2 and subsequent generations segregate for morphological traits of the cultivated parent, as they are often recessive (the segregation is mendelian). Thus, in a F2 generation, the hybrid origin of only ¼ of the selfed F1 progeny was recognisable morphologically. Consequently, morphology based observations would probably underestimate the real hybrid population size. Such observations represent the basis for demographic predictive models as that developed by Hooftman et al. (2005). Therefore, experiments that would assess the real population turnover under natural conditions could represent a perspective of further studies.

We observed the results of a sexual reproduction through pollen flow, but questions related to pollen germination and pollen performances remain unanswered. Further experiments are also needed to assess the hazard components, i.e. the consequences of transgene expression and /or introgression into the wild relative.

As exposed briefly in the introduction, a major factor for the acceptance of GM organisms by people, is to demonstrate scientifically their utility for the society and the environment. Thus, studies carried out in this perspective are as important as risk assessment studies, and should even be done before these latter. They represents, in my sense, key perspectives for future works.

Figure 15. *Spontaneous wild hybrid growing in a wall close to a private garden*

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Aknowledgements

Merci !

François et Roberto pour m’avoir donné l’occasion de vivre cette expérience ANGELesque, pour la collaboration, les conseils et l’aide apportée lors de la préparation et de la relecture des travaux.

Philippe pour m’avoir accueilli dans ton laboratoire. Tu as su me transmettre ta passion au travers de tes qualités humaines. Tu m’as appris que la Nature enseigne à qui sait regarder. Je garderai bien dans mon cœur ces escapades floristiques de bonheur.

Au jury de ma thèse ; Hans den Nijs, Gregor Kozlowski et Peter Stamp

A Smart, Phils et Nic Belmonte pour votre amitié, pour les aventures floristiques et toutes les autres !

Xavier Morin pour l’accueil à Montpellier et pour les petits foots entre un modèle climatique et l’autre...

A la « 61 » puis « 93 » puis « rondin » connexion pour ces 8 années passées en votre compagnie. Elles se passent de commentaires, vous pensez bien ! Sans vous je n’y serais jamais arrivé !!!

Chip de m’avoir accompagné te ton amour et de ta tendresse durant toutes ces années.

A toute ma famille qui à tout fait pour rendre possible cette aventure. Merci Mà, Pà et Nadia !

CHAPTER 1

HISTORICAL BIOGEOGRAPHY OF *LACTUCA SERRIOLA* L. IN EUROPE

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Abstract

Lactuca serriola is a ruderal plant originating from southern Europe, northern Africa and western Asia. In the present study, we investigated the European northern shift of *L. serriola* during the last 250 years. For that purpose, we investigated 1785 herbarium sheets from 24 herbaria including 15 European countries. Detailed analysis was carried out for Austria, Germany and Great Britain through the investigations of 976 literature occurrences.

By modelling the distribution of the species bioclimatic niche, we showed that the climate warming in Europe increased the number of sites suitable for the species. *L. serriola* responded to climatic changes by migration, maintaining its distribution range in equilibrium with its climatic niche. Non climatic influences of global change, like the globalization of trade and industrialization have probably contributed to its rapid spread. Moreover, the influence of crop to wild hybridization as possible cause of an increased colonization aptitude is discussed. Our work highlights the importance of historical, floristic and herbarium data for the understanding of the expansion of a species. Data witnessing the past events are the basis for the management of modern environmental problematic such as the species reaction to environmental change.

Keywords : *L. serriola*, distribution shift, climate change, gene flow, global trade, disturbance.

Introduction

Lactuca serriola L. or prickly lettuce (Asteraceae) is a large spring or winter annual herb native from summer-dry Mediterranean climate (Gallardo et al. 1996). Its original distribution is southern Europe, northern Africa and western Asia**,** but it has been widely introduced in other regions (Carter et al. 1985; Zohary 1991). The species has now a worldwide synanthropic distribution and belongs to the Mediterranean group of ruderal plants that enlarged their distribution area during the last few centuries (Landolt 2001)**.**

Global change, which components are linked to global industrialisation and global trade, is a concept that brings together many environmental changes and subsequent ecological consequences. It is an emergent phenomenon and will radically reshuffle the places where species grow (Sala et al. 2000). It includes invasion of alien species in natural environments, biodiversity changes, climate changes, increased nitrogen deposition and changing patterns of land use like destruction and fragmentation of habitats (Dukes et al. 1999).

Recently, concern about the impact of the current climate change on environment and organisms has greatly increased. Distributional latitudinal shifts have already been documented for many kinds of organisms (Parmesan 1996; Perry et al. 2005; Huntley et al. 1995; Walther et al. 2002; Root et al. 2003; Walther et al. 2005); it is now demonstrated that climate warming can affect the dynamics of plant communities and influence species' range expansion and contraction (Davis et al. 2001; Parmesan et al. 2003). However, correlation between climate changes and single species distribution range shifts are mostly investigated on small geographical areas (Pounds et al. 1999; Johnstone et al. 2003; Grabherr 1994); (Kennedy 1995; Sturm et al. 2001; but see Walther et al. 2005)

Annual precipitations and temperatures increased at the medium and high latitudes of the Northern hemisphere and a global warming of 1.4 to 5.8°C, depending on the climate change scenario considered, is expected over the next century (Dukes et al. 1999, IPCC 2001). Since the end of the 19th century, the Earth global temperature increased on average by 0.6 °C, and by even more if only the continents are considered (i.e. without oceans). From 1946 to 1975 temperature decreased in the Mediterranean region, Central Europe and Great-Britain, while it continued to increase in Scandinavia and the rest of Europe. From 1976 until nowadays, it increased faster all over Europe, the nineties being the warmest decade of the 20th century (IPCC 2001).

Improving our understanding of how climate and dispersal dynamics interact to drive migration rate is an important component for predicting future ecosystem response to global change. In this context, (Prince et al. 1985) showed that the geographical distribution limit of *L. serriola* in Great Britain corresponded to climatic variables which relate to the warmth and dryness of the summer; they would exert a dynamic control over its distribution range (Prince et al. 1985) and would be implicated in the control of its altitudinal limit. The temperature and the photoperiod are probably two determinant factors controlling the blooming (Prince et al. 1978). The seed germination is affected by climatic variables as rainfall and temperature; for instance low temperature might slow it down (Carter et al. 1985).

Seed production and germination are crucial factors of colonization success for *L. serriola.* Using a population dynamics epidemic model, Carter & Prince (1981) showed by simulations that a little change in the number of seeds produced is sufficient to change the balance of colonization to extinction rates, and therefore to explain a sharp biogeographic range limit. We thus hypothesize here that climate, through its influence on *L. serriola* seed set production, has contributed to variations of its geographic range over past periods.

The spread of *L. serriola* is very closely related to human activities, mainly with the increased of transports (Lebeda et al. 2001) and of suitable habitats. It grows in disturbed areas (Lebeda et al. 2004) such as waste places, embankments, along ditches and roads, field margins and fallow (Feràkovà 1977; Zohary 1991; Lebeda et al. 2001). The changing pattern of landuse and the development of transport network, which are other components of global change, have lead to an increase availability and a better connexion of disturbed and ruderal habitats favourable to *L. serriola*. Indeed, the species is considered as a “r” strategist (Mejias 1993; Frietema de Vries et al. 1994; Mejias 1994) as its evolution has tended towards a short life cycle, strong self-fertilization ability, quick germination and yellow flowers (Lebeda et al. 2001). *L. serriola* is described as an invasive weed occupying ruderal places (Lebeda et al. 2004). However, the growing field of invasion ecology has seen a multiplication of terms to describe various phenomena. There exists much confusion concerning the terms “invasive”, “naturalized”, “alien”, etc. (Kowarik 2003, Richardson et al. 2000). An invasive species is defined as an alien (non indigenous, non native, introduced, exotic) species which becomes established in natural or semi-natural ecosystems and acts as an agent of change. Its establishment and spread threaten ecosystems, habitats or species with economic or environmental harm (Weber 2000). It is important to differentiate this latter concept from the weed concept well defined by Baker (1974): “a plant is a weed if, in any specified geographical area, its populations grow entirely and predominantly in situations markedly disturbed by man”.

Taking a genetic rather than an environmental point of view, hybridization of wild plants with their crop relatives could additionally drive genetic changes in wild populations that could possibly lead to the apparition of new adaptive characters. These could lead to increase the invasiveness and/or the weediness of the introgressant (Snow et al. 1997), and consequently widen the overall ecological tolerance of the species.

Assessing the crop-to-wild hybridisation rate requires specific experiment, which is currently the topic of a separate study. We focus here on describing the spread of *L. serriola* in Europe and assessing the influence of climate on species’ distribution. For that purpose, we compile historical data from natural history collections and relate these to past and present climate (e.g. Walther et al. 2005).

Due to the long history of floristic studies in Europe, data could be assembled for *L. serriola* in Central and Northern Europe. Yet, no study has documented in time and space the spread and test the hypothesis of a climatically induced distribution shift of *L. serriola* during the last centuries in Europe. The results are discussed in the context of climate change, but considering also the increase of human disturbance and gene introgression.

Material and methods

Species occurrence data

Data on the geographic distribution of *Lactuca serriola* were assembled through literature search as well as floristic and herbarium surveys. Floristic investigation of the literature of 25 European countries resulted in the gathering of 1365 *L. serriola* occurrence records. The survey of the literature data focused mainly on Germany, Austria and Great Britain (71 % of the dataset, 975 occurrences). In addition, 24 herbaria from 15 European countries were also screened, resulting in 1785 *L. serriola* herbarium sheets. The herbarium data were more equally distributed amongst all European countries.

For all herbarium and literature data, we searched for the geographical coordinates of *L. serriola* localities. Six categories of precision were used: (i) exact coordinates, (ii) precision to 50 km, (iii) precision to 100 km, (iv) precision to 300 km, (v) precision to 500 km, and (vi) precision to more than 500 km. On maps, only locations with coordinates up to 100 km precision for the herbarium and up to 300 km for the literature were considered. The occurrences of *L. serriola* were cumulated on the maps.

The indication about abundance (if present in the literature) has been also defined in six categories: ND – no data at all; A – absent, P – present without abundance indication; R – very rare to scarce; S – scattered; and F – common to frequent. Herbaria sheets gave us no information about abundance, therefore only the “P” abundance category has been used.

Distribution data for **L. serriola**

Maps of the *L. serriola* spread in Central and Northern Europe using all herbarium data were prepared based on the occurrence data collected. For this, Europe was divided in grid squares of 50 km x 50 km. We considered a square as colonised if it contained at least one occurrence. The southern limit of the study area is defined by the Pyrenees, Southern France and Northern Italy, while it is limited Eastward by the oriental boarders of Austria, Czech Republic, Germany and Scandinavia. The occurrences of *L. serriola* were cumulated on the maps. Six time periods were defined. The first time period was 1765-1820, followed by four 30-year periods: 1821-1850, 1851-1880, 1881-1910, 1911-1940, 1941-1970. The last period spans 1971-2000.

As mentioned before, we had sufficient occurrences (more than 50 *L. serriola* literature locality indications) and well distributed over the time periods for three countries: Germany, Great Britain and Austria. To follow the spread of *L. serriola* in these countries, we have additionally divided the data according to the administrative units (shires for Great Britain and “Bundesländer” for Germany and Austria), abundance and the six time periods. The first time interval was between 1632 and 1800, followed by five 40-year periods: 1801-1840, 1841-1880, 1881-1920, 1921-1960 and 1961-2000. The occurrences of *L. serriola* were cumulated in the maps (except for category A – absent). They include both the herbarium data (placed into category P – present, without abundance indication) and the floristic data. The time periods were different than those used to build herbarium maps in order to equilibrate the data between categories.

For the test of climate-induced shift in distribution, we only split the species dataset into two periods: a first time period comprising years 1901 to 1920 and a second one comprising years 1981 to 2000. The species dataset for period 1901 to 1920 includes all 0.5° x 0.5° cell known to have been once occupied by the species before 1920, whereas dataset for period 1981 to 2000 include all cells known to have been once occupied until present days. By this mean, we assume that once the species has colonized a cell, it remains there unless the climate becomes unsuitable.

Climate data

We used the CRU TS 2.0 data set (from the Climatic Research Unit, University of East Anglia, United Kingdom), which provides monthly means of minimum temperature as well as precipitation from 1901 to 2000 for a 0.5° x 0.5° grid resolution (55 km x 55 km). These data have been obtained by interpolation of observed climatic data of more than 20,000 weather stations all over the world (New et al. 2000). We used a grid of points for Europe, which comprises 8566 points.

For the same two time slices considered for species data, we calculated a set of eight climatic predictors that could potentially have an influence on blooming and seed production as warmth and dryness of the summer and seed germination as rainfall and temperature. These predictors comprised the mean sum of precipitation during winter (December to February), spring (March to May), summer (June to August) and autumn (September to November), the mean temperature of spring and summer, the mean number of months with minimum temperature superior of 10°C and finally the mean number of months without frost.

Statistical modelling

A species distribution model was fitted to 1901-1920 species and climatic data. All grid squares occupied by at least one occurrence of the species were considered as presences, all others as absences. Generalised additive model (GAM; (Hastie et al. 1987; Yee et al. 1991) see (Guisan et al. 2002) was used to fit the model on a random sample (70%) of the initial data. The accuracy of the model was assessed using the area under the curve (AUC) of a receiver operating characteristic (ROC) curve on the 30% remaining data (test set for independent evaluation; (Pearce et al. 2000; Guisan et al. 2002).

The model calibrated for 1901-1920 was then used to generate a projection of climatically suitable habitats under 1981-2000 climatic conditions. The AUC measure was calculated to assess the adequation between the predictions of the 1981-2000 model and the species data for the same period. An AUC value was also calculated for 1901-1920 species data to check if the adequation between the projected model 1981-2000 and species data for the same period is higher than for period 1901-1920. An AUC value of 1 means perfect agreement, an AUC of 0.5 means that predictions are not significantly different than random, and values between 0 and 0.5 means predictions worst than random. The following interpretation scale is usually used for ranking model predictions based on AUC (Swets 1988): > 0.9 good, 0.7-0.9 useful, and < 0.7 poor.

Results

Colonization of Europe from herbarium data

The changing distribution patterns are shown in Figures 1(a to g). The maps clearly show a spread of the species northwards since the beginning of the 19th century. Moreover, the results indicate that colonization in Great Britain and Scandinavia occurred from the South-East to the North-West. Today, the Northern boundary of the distribution area runs near the latitude 66 ° N through Sweden and then near 56 ° N through Scotland in Great Britain.

First occurrence of *L. serriola* in Europe according to the herbarium survey is for Belgium in 1765. Until 1820 few records of the species are available: *L. serriola* is present in Belgium in Southern-France and Germany. In Switzerland prickly lettuce has already colonised the country and is found north and south of the Alps. Interestingly, in 1802 it was already found at high altitudes (Zermatt, 1620m) (Fig.1a).

In South-East England (Northfleet) the species was collected for the first time in 1830 and in Sweden (Lund) in 1828 (Fig. 1b). Therefore, the first steps of the colonization of Great-Britain and Scandinavia took place already at the beginning of the 19th century.

In 1850, *L. serriola* was widespread in Central Europe (France, Belgium and Germany) (Fig.1B). Since 1851 to 1880, the number of records increased for Central Europe. The spread northwards continued: prickly lettuce was recorded on the north-eastern coast of England **(**Hartlepool, Cleveland**)**. In Sweden, the species progressed from the coast of Baltic Sea to Ostergötland (Fig. 1c) and colonised regions below 100 meters. The thirty next years (1880 to 1910) are characterised by a westwards spread; *L. serriola* progressed to Norwegian border (herbarium record (HR) in 1903 in Langbro, Sweden) and settled in the centre of Norway in 1906 (Bratsberg). It colonised Wales, Cornwallshire and Devonshire in Great Britain (Fig. 1d) staying below 100 meters and avoiding the Cambrian Mountains and the centre of the island.

The first herbarium record in Denmark (Copenhagen) is dated from 1881 after Swedish colonization. The colonization of The Netherlands started at the very beginning of the 20th century with a first herbarium sheet dated from 1904.

Then, until 1940, prickly lettuce colonised the centre of Great Britain, the north-western coast of Wales and England and migrated above 100 meters in Cirencester. In Sweden, *L. serriola* progressed northwards as shown in figure 1e, colonizing altitudes above 100 meters. From 1941 to the end of the century, the species progressed of 700 km northwards along the Swedish coast (Fig. 1f and 1g).

Sometimes, indications on herbaria sheets inform on the dynamics of populations and the circumstances in which *L. serriola* arrived. We could read on a herbaria sheet dated from 1874 of Besançon (France): "abundant since the construction of the railway". In a herbaria sheet collected in Somerton (GB) in 1915, *L. serriola* was recorded as “ballast alien”; in a sheet collected in Penryndendreath (GB) from 1922, it was recorded as “adventive by railway “. In 1936, in a sheet from Colchester: “not noticed for some years, abundant for this year”.

Colonization of Austria, Germany and Great-Britain from floristic and herbarium data

Since both type of data (floristic and herbarium) are used together, records coming from our herbarium survey will be annotated as HR.

Austria is of particular interest because of its orographic relief (Figure 2, a to f). Until 1840, only 2 items are available (Fig. 2b). One is the absence of *L. serriola* in the “*Enumeratio Stirpium Plantarum quae sponte crescunt in agro Vindobonensi*” of Jacquin in 1762 (Jacquin 1762) (Fig. 2a) and the other is an HR of 1819 in Clausen (Tirol) (Fig. 2b). The mountainous region of the centre has been colonised later. “Die Flora von Bad Aussee” in 1956 indicates the absence of prickly lettuce, while nowadays, it is mentioned as rare (Figure. 2f) (Rechinger 1956).

Germany has been rapidly colonised (Figure 3, a to f). The species was mentioned for the first time in the Flora Halen in 1761 (Leyser 1761). The first HR was in 1819 in Plauen-Dresden. In 1800, *L. serriola* was everywhere except in Saarland (south-west Germany), probably due to the lack of data and not to real absence. Since 1800 its abundance increases from the South to the North. At the beginning of the 20th century, Germany was almost totally colonised.

Great Britain is presented in Figure 4 (a to f). The oldest mention of *L. serriola* is a record of 1632 in the Flora of Middlesex in 1889 (Trimen 1869). However, it might be due to a misidentification. According to Oswald (Oswald 2000) “the confusion occasioned by the failure of most British Flora of the nineteenth century to recognise that *L. serriola* can have simple leaves has led to some uncertainties about the past status of this species and *L. virosa* in Britain”. Prince and Carter (1977) stated that the general practice was to call unlobed-leaved plants *L. virosa*. They concluded that most pre-1930 records could as easily refer to *L. serriola* as to *L. virosa*.

The expansion of *L. serriola* started from the south-east. In 1785, *L. serriola* is mentioned in the Flora Cantabrigensis (Relhan 1785) in the Isle of Ely (Fig. 4a). Until 1880, it remains confined in the South-East, spreading from the initial record location to reach Hartlepool (Cleveland) in 1866 (HR) (Fig. 4b).

At the end of the 19th century, the number of records increased in the eastern part and the north-eastern part of England was colonised (Fig. 4c). In 1889, a first HR for Wales (Bangor,Wrexham) is registered. In the Flora of Glamorgan (Wade 1994), *L. serriola* is mentioned in Porthcowl in 1897. Then, in 1902 and 1907, prickly lettuce was recorded in the Pembrokeshire and in Cardiff respectively. In 1901, *L. serriola* is mentioned as casual in the

Flora of Cornwall (Davey 1909) and in 1908 HR in Par is registered. It has been collected along a railway bank near Newton Abbot (Devonshire) in 1909 (Fig. 4c).

At the beginning of the 20th century, prickly lettuce expanded to the western part (Fig. 4d). An intriguing fact is that at this time, *L. serriola* was mentioned as absent from the central part in the flora of Wiltshire (Preston 1888), of Bournemouth (Linton 1919) and of Warwickshire (Bagnall 1891). Thereafter, it colonized all the southern and central parts of England and finally spread in Scotland, where the first record in the Flora of Angus is mentioned in 1967 (Ingram 1981) (Fig. 4e and 4f).

Since 1950, *L. serriola* increased its abundance in the areas colonised, as in the Warwickshire, where prickly lettuce has been recorded for the first time in 1959 ((Bowra 1992)). For an example of populations explosions, see (Bowra 1992). A similar situation of expansion is described for Netherlands (Hooftman, in press) and for Switzerland (Cottet et al. 1891; Jaquet 1930; Landolt 2001; Purro et al. 2003) and is probably occurring in many other countries.

Modelling climate-induced shift

The 1901-1920 model retained all initial environmental predictors, each of these proving statistically significant to explain the distribution of the species. The climatic conditions that were found to favour *L. serriola* presence in Europe were : temperature in Spring >5°, temperature in Summer <15°, 1 to 3 month of temperature >10°, less than 4 months of frost , less than 300mm of rain in winter, more than 250mm of rain in Spring, less than 550mm rain in summer and less than 350 mm rain in autumn (fig 5).

The model fit was rather high, with 65% of the deviance explained. The AUC evaluation on independent data was 0.80, corresponding to a fair agreement between predicted and observed data (Table 1). The species is predicted to have a high probability of presence in all central Europe except the Mediterranean coast, the Aquitaine Atlantic coast, a large part of Scotland as well as Scandinavia. Note however that the southern Swedish coast of the Baltic Sea have intermediate values of probability (fig. 6a).

Model projections for 1981-2000

Figure 6b illustrates the projection of the 1901-1920 model into the 1981-2000 climate. The AUC values between observed and projected data for this period were 0.79 (Table 1). This value is only slightly lower than the AUC for the period 1901-1920, indicating that the species has tracked its climatic niche through the last century. Note also that the agreement between the observed data for timeslice 1901-1920 and the values projected for 1981-2000 is lower (AUC= 0.74), which confirms this hypothesis.

The main differences in the predicted, respectively projected, spatial pattern of probability of presence indicates a north-eastern shift of distribution of the species. In particular, Denmark and the southern Swedish coast of the Baltic Sea have become much more suitable for the species at the end of the century, as well as the eastern part of England. Oppositely, western France and England are predicted not to be any more suitable for the species.

Table 1. Model evaluation: AUC calibration and evaluation values, sensitivity (percentage of presence predicted as presence by the model), specificity (percentage of absence predicted as absence by the model) and model fit (percentage of explained variance).

	AUC Calibration dataset	AUC Evaluation dataset	sensitivity	specificity	model fit (D^2)
Prediction 1901 - 1920	0.837	0.802	72.967	74.000	0.65
Projection 1981 – 2000	-	0.789	70.117	70.0831	

Discussion

Using natural history collection data to quantify range change

For this study, we assumed: (1) that the effect of climate change must be already visible for this species, and in particular that it caused its distribution range to shift (overall northward); and (2) that the data collected were appropriate to evidence such a range shift.

The use of herbarium specimens suffers from several biases including problems of identification of the plant in the field, accessibility of sampling site and variability of the sampling effort over a long time period. Some groups of species (e.g. aliens or garden escape) were recorded less consistently (Delisle et al. 2003), whereas other were more frequently recorded (rare, endangered or conspicuous species). Furthermore, the spread of invasive species is usually a very rapid phenomenon; therefore time scale of invasion might not be represented correctly by herbarium records.

Yet, our floristic and herbarium data revealed consistent trends: the colonization of Northern Europe by *L. serriola* happened during the end of 18th century and the first decades of the 19th century. During the same time, the species increased its abundance in the countries of Central Europe. At the beginning of the 20th century, the species had colonized most of Europe. Locally, westwards migration of the species was observed from Sweden to Norway, from South England to Wales, from Wales to Ireland (first record in 1996) (Preston et al. 2002) and from the east of Austria to the west. Our data set does not allow any inference on colonization of the Iberian Peninsula either from France or from North Africa. In Scandinavia, although the first steps of colonization took place early from the south, the species is still not widespread yet.

The colonization of lower altitudes and flat countries took place more rapidly than of mountainous regions. Mountains are significant obstacles for plant migration, making transportation from one valley to the next more likely to take place by human. In southern Europe, *L. serriola* has been observed generally at higher altitude than in northern areas, although it has been collected at 680 meters in Bratsberg in Norway. *L. serriola* is now abundant in Central Europe (Lebeda et al. 2001) and in England (Bowra 1992) and is still in a dynamics of colonization. Hooftman et al. (in press) showed for instance that *L. serriola* is spreading rapidly through The Netherlands.

In the city of Zürich (Switzerland), the first plants were discovered in 1887. Today the species grows in 90% of the inventoried mapping units of the city (Landolt 2001). The same phenomenon was observed in Fribourg (Switzerland): in 1891, the species was still absent, in 1930, only one small population was mentioned, and today the species was inventoried in 50% of the mapping units (Cottet et al. 1891; Jaquet 1930; Purro et al. 2003). In Switzerland, the species was recorded as rare before 1900 but had totally colonised the country by the end of the 20th century.

Today, the northern boundary of the distribution area runs near 66 ° North through Sweden and then near 56 ° North through Scotland.

Different factors of global planetary change - including climate change, globalisation and increase of trade and travel, increase of disturbance through the development of trade routes and urban areas - are responsible for the spread of *L. serriola* (Fig. 7). They had direct consequences on the modification of ecosystems and acted directly on population and metapopulation dynamics of *L. serriola.* Hybridization with the crop could also have contributed to the expansion of *L. serriola* towards the North, by introgression of adaptative traits.

It is difficult to assess the relative importance of each factor as they interact. Hereafter, we discuss how each of them might have influenced the distribution of prickly lettuce.

Invasiveness of L. serriola in Europe

Until recently, alien species invading agricultural systems and/or colonizing natural areas have become a subject of concern for international organisations as well as scientific community dealing with biodiversity conservation and weed control. In Europe, *L. serriola* colonises man-made habitats and poses no problems in natural and agricultural ecosystems yet. *L. serriola* possesses some life history traits typical of invaders. However, at the present time, even if it may grow in populations of many thousands of individuals, we think that the term invasive does not describe correctly the status of the species in Europe, while Baker’s weed definition would be more accurate (Baker 1974).

In Canada and in Argentina, it occurs as a weed in a variety of crops where no-till or conservation tillage systems are used (Weaver et al. 2003). Moreover, in the United States as well as in Australia, herbicide-resistant populations have already been found (http://www.weedscience.org/in.asp). In Europe no such case has been described yet.

Climate change

The only investigations that studied the relation between *L. serriola* distribution and climate were carried out in Great Britain. In a transplant experiment, no physiological changes to particular climatic factors could be highlighted for the species beyond its distributional limit (Prince et al. 1985). The authors stressed that even if physiological responses were undetectable or slightly reduce the performance of the plant at the individual level, the secondary effect induced on populations or the tertiary effects on the dynamics of metapopulations could be high. Thus, climatic factors as temperature, photoperiod and precipitations might have a small effect on individual plants, but a significant effect on the ability of individual plants to build viable populations and colonise new sites.

Since climate change is a relatively recent phenomenon it could not trigger the distribution shift of *L. serriola* towards Northern Europe before the second part of the 20th century, when warming effects first became noticeable. By modelling the distribution of the species' climatic niche, we indeed showed that the climate warming increased the number of suitable sites for the species in Northern Europe and western part of Great Britain. We have shown in this paper that *L. serriola* responded to climatic changes by migration towards northern latitudes zones to maintain its distribution range in equilibrium with its climatic niche. The same phenomenon was demonstrated for other species at their Northern latitudinal limit (e.g. Walther et al. 2005 for Holly) and toward higher altitudes (Dobbertin et al. 2005; Senften et al. in press). Thus, *L. serriola* northward shift in distribution during the 20th century matches the associated climate change during the same period. Sufficiently mobile species can effectively be expected to track the geographical position of their suitable climatic requirements through dispersal (Pearson et al. 2003).

The climatic models predict a decrease in the habitat suitability over the last century at the southern limit of the distribution (Southern France) and Western Europe. This decrease is not confirmed by the current knowledge about the distribution of the species in these regions. This decrease in habitat suitability is obviously a modelling artefact due to the fact that we did not account for the entire distribution of the species to calibrate the model. More precisely, we did not encompass Spanish populations. If such south-western populations would have been taken into account, these region would have probably been situated in the middle of the response curve and would have been less affected by the climate shift.

Still, our model did not predict the presence of *L. serriola* in northern Scandinavia. Possible explanations are either that our resolution (50 km) did not permit to detect all the favourable sites for the species, or that factors other than climate accounted for the rapid spread of the species observed in our data. A third explanation is that most of the herbarium sheets in Scandinavia were collected around urbanised centres, where temperatures are always higher than the natural environment. Thus, such sites only suitable at a local scale, may not have been highlighted with an inappropriate resolution for the recent period.

Distribution limit can also extend without relation with climate and/or the biology. For instance, in epidemic models, the increase of susceptible sites near to, but beyond a plant's distribution limit, could displace its climatic equilibrium distribution limit without change in the climate or in the biology (Carter et al. 1981). Thus, the availability of colonisable habitats and their accessibility are also predominant factors to understand the spread of a species. It is therefore of high importance to consider the interaction between landscape structure and climate change, to understand in time and space a plant distribution limit.

Shift of ecological amplitude

L. serriola might have changed ecologically along the time span it has been conquering Europe. Hooftman et al. (2006, in press) have shown that *L. serriola* is found in a wider range of plant communities in the Netherlands. Its habitat type has on average become less continental, cooler and more humid. Thus, in addition to the ruderal and thermophilous vegetation types, *L. serriola* also occurs in more closed structure. Each year a new generation is present and changes in ecological position may be among the reasons for the recent spread.

Disturbance, habitats availability and dispersal

Non climatic influences of global change, such as habitats modification, may dominate locally and are of great importance to understand the spread of species (Parmesan et al. 2003). Yet, no data is available to quantify the impact of disturbance on the population dynamics of *L. serriola*, but it has been noticed that a population generally establishes within one year. Its persistence depends then on the continued availability of open areas uncolonised by plants of later successional stages (Carter et al. 1985). The influence of disturbance on population expansion as well as on seed germination and establishment has already been shown for other ruderal plants (Steinlein et al. 1996; Bossard 1991). The response of *L. serriola* (expansion) to anthropogenic urbanised ecosystems is discernable from occurrence data (Bowra 1992; Senften et al. in press; Prince et al. 1985; personal observation) and historical records.

The opportunity to be transported from one available habitat to another is a key factor that enables colonization. In many areas of the world, *L. serriola* as well as other alien and invasive plant species thrive close to roads and railways. The modern expansion of international traffic is therefore likely to be accompanied by an expansion in the range of aggressive road-side weeds (Clifford 1959). In consequence, they should be the earliest species to shift their ranges (Dukes et al. 1999; Parendes et al. 2000; Landolt 2001). Central and Northern Europe witnessed a tremendous development of human- made habitats and transport network during the past 250 years (construction of motorways, rails, airports, canals, builded up areas, agriculture). Roads and railways interconnect anthropogenic urbanised ecosystems and facilitate expansion of plant species, both by providing corridors along which the species can migrate in a landscape and by supporting the anthropogenic disturbance (Parendes et al. 2000). They allow “r” strategist, ruderal (R strategy; Grime 1979) and urbanophile plant species like *L. serriola* to build viable populations (Lebeda et al. 1999; Dolezalovà et al. 2001; Landolt 2001; Lebeda et al. 2004; Hill et al. 2002; Dukes et al. 1999; Parendes et al. 2000; Wittig 2002; Kowarik 2003; Forman et al. 1998; Trombulak et al. 2000) and to quickly disperse their seeds over long distances. The seeds can be easily transported from one site to another by motor vehicles in the mud attached to most cars and trucks and/or the by transport of material over long distance (Pitelka et al. 1997).

Occupation of new regions occurs through passive seed dispersal and establishment of seedlings in sites where conditions are suitable (Davis et al. 2001). However, the ability of

species to migrate rapidly across large distances could be driven primarily by infrequent long-distance dispersal events that are difficult to quantify (Higgins 1999; Malcolm et al. 2002). Prince (1985) already stressed the importance of long-distance seed-dispersal events for building new *L. serriola* colonies. From our results, it is impossible to prove long distance dispersal events. However, several first records of the species in short time interval, but at long distances from each others, could indicate that these kinds of events might have happened.
Such long dispersal events could lead to the formation of outlier populations exerting a continual outward pull. This could results in a more rapid migration than that along a single population front (rapid in-filling of the intervening space) and could increase the colonization rate. However, outlier populations may fail to build viable populations making the frequency of introduction and the amount of seeds introduced beyond the distribution limit of great importance. In this context, the growth of the volume of trade along commercial routes greatly increased the frequency to which introductions were repeated (Perrings et al. 2005). Moreover, the seed bank formed one year can substitute a lack of immigrant seeds the next year; one single plant of *L. serriola* can indeed produce a huge amount of wind-dispersed seeds and potentially form a short term seed bank (1 to 3 yr) (Weaver et al. 2003).

Gene flow and hybridization

Hybridization and introgression of crop trait to wild relative could promote colonization and dispersal (Stewart et al. 2003) as well as invasiveness (Paterson et al. 1995; Ellstrand et al. 1999; Ellstrand et al. 2000). Frietema (1994) questioned whether the recent massive spread of *L. serriola* in Europe can be linked to the cultivation of crop lettuce. The two species are extremely closely related and differ only in characters connected to domestication (Frietema de Vries et al. 1994). Our field experiments indicated that crop-to-wild hybridisation does occur at a significant rate although floral mechanisms of *Lactuca* ensure a high degree of self-fertilization (unpublished data). The hybrids fertility is good and often better than in the parents line (Lindqvist 1960 a; Hooftman et al. 2005). They perform well in ruderal habitats (personal observation) and could potentially become invasive because of substantial differences in vital rates and seeds returned per seed sown (Hooftman et al. 2005). Moreover, the plants of second-generation hybrids are as fit as the wild species (Hooftman et al. 2005).

Few ancient reports showing that spontaneous hybridization took place occasionally between *L. sativa* and *L. serriola* are available (Pammel 1918; Ownbey et al. 1949) Mc Collum, 1953 *in* (Lindqvist 1960 c). Hybrids can be phenotypically distinguished from wild parents only at the rosette stage and would remain unnoticed thereafter. Thus, hybrids could be much more frequent than observed. Our field experiments on hybridization between the two species indicate that the two species hybridize at a non negligible rate in every location where they occur in sympatry. Furthermore, the incidence of hybridization (i.e. the percentage of mother plants that underwent at least one hybridization event) was very high (Chapter 3). Recently, a hybrid has been observed in Val d’Aosta (personal observation). This indicates that hybridization is still an ongoing process.

The basis for introgression is present, even though no or few evidences of introgression of cultivated markers in the wild species could be highlighted (C. van de Wiel, personal communication). Such events remain difficult to assess because *L. serriola* is probably the direct or one of the direct ancestors of *L. sativa* (Lindquist 1960, Körber-Grohne 1995, de Vries 1990, de Vries 1997) and the two species are genetically very closely related.

Crop traits that could potentially increase prickly lettuce colonization ability if transferred to wild populations can be pathogen resistance, cold resistance or other unexpected crop traits as for instance rapid growth with low root allocation or shallow root system that may be of significant importance in northern countries where growing season is reduced and water availability is increased. As far as Downy Mildew (*Bremia lactucae*) concern, one of the most important pathogen affecting the two species, it seems that its presence had no substantial influence on *L. serriola* expansion (Lebeda, personal communication). Furthermore, the virulence structure of *B. lactucae* populations, originating from crop and wild pathosystems are completely different from each other and there is clear genetic affinity to the original host species (Petrzelova et al. 2004; Petrzelova et al. 2004). However, there is no detailed information about the resistance structure of natural populations of prickly lettuce to *B. lactucae* (Petrzelova et al. 2004) and on geographical distribution and virulence variation of the pathogen in wild populations of *L. serriola* (Lebeda 2002). Further investigation could tell us to which extent crop traits could be implicated in recent prickly lettuce spread.

Conclusion

Our results highlight the possible impact of elements of global change as climate warming or habitat disturbance on the expansion of Mediterranean plant species like *L. serriola* towards Northern regions of the European continent. Our work shows the importance of historical, floristic and herbarium data for a global understanding of the spread of a species. Data witnessing the past events are the basis for detailed studies and for the management of modern environmental problematics as the influence of changing environmental factors on species reaction, invasion or biodiversity changes. Finally, the study presented here, using *L. serriola* as a model species to study the colonization routes in Europe, will help to better foresee and manage future expansion of species in the case of dramatic changes in our region. These data could also serve as a basis for further molecular studies on migration routes and genetic diversity of this species in Europe.

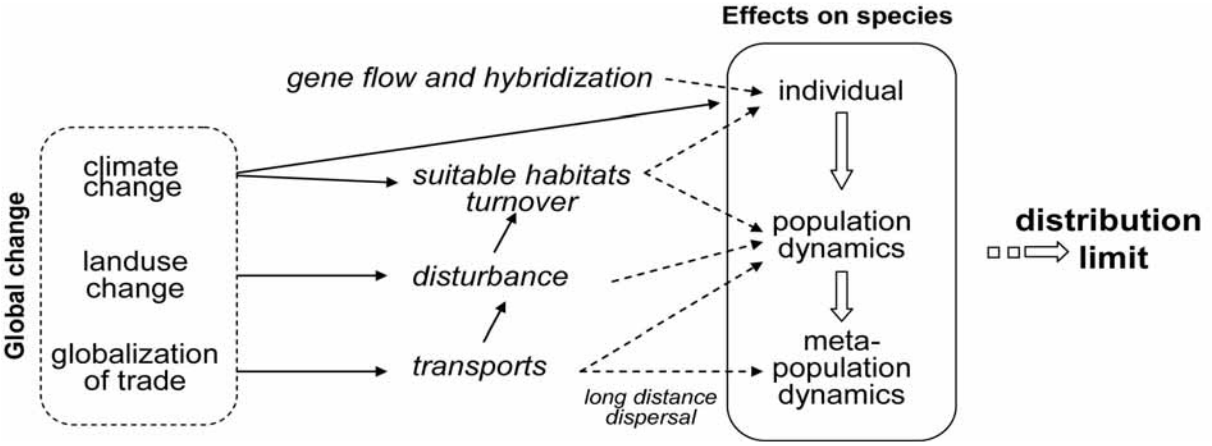

Figure 7. Factors influencing *L. serriola* distribution limit

Acknowledgments

The authors would like to thank Ales Lebeda for its helpful remarks and its cooperation and all ANGEL members for fruitful discussions. This study is part of the EC-funded project "ANGEL" (EU-QLK-CT-2001-01657).

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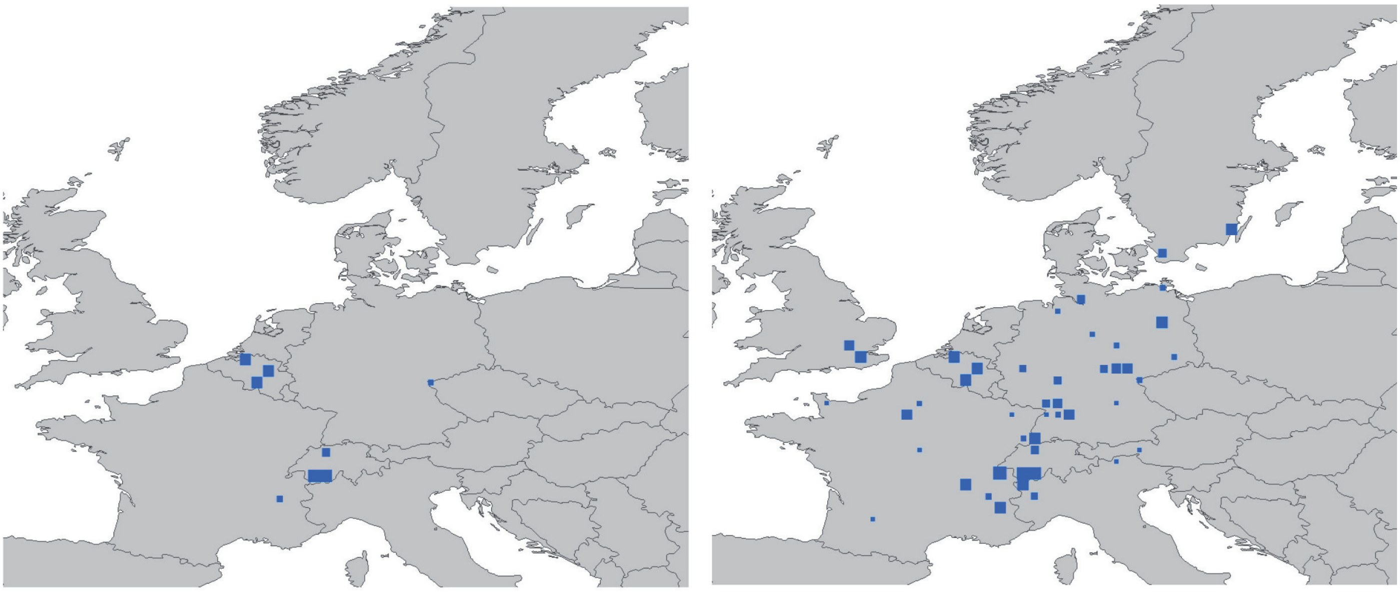

Fig 1a : 1765-1820

Fig 1b : 1821-1850

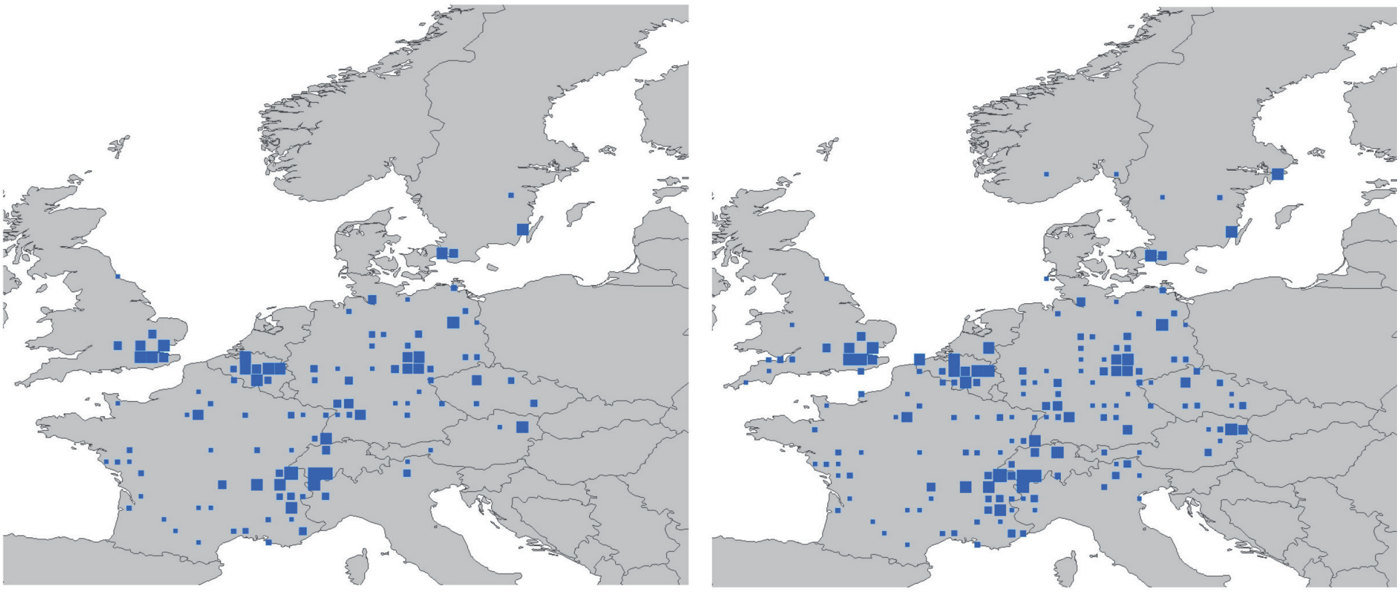

Fig 1c : 1851-1880

Fig 1d : 1881-1910

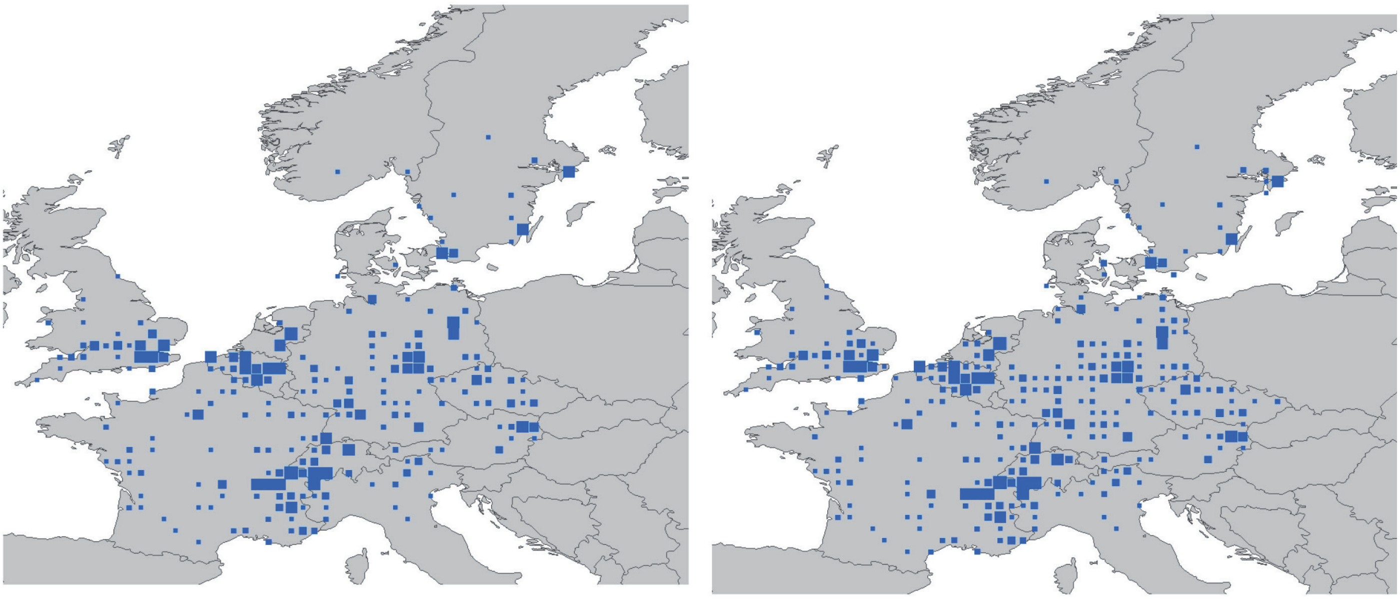

Fig 1e : 1911-1940

Fig 1f : 1941-1970

Figure 1 a to f. Distribution pattern of detailed from herbarium data from 1765 to 1970. The square size increase with the number of the occurences (for the legend, see next figure)

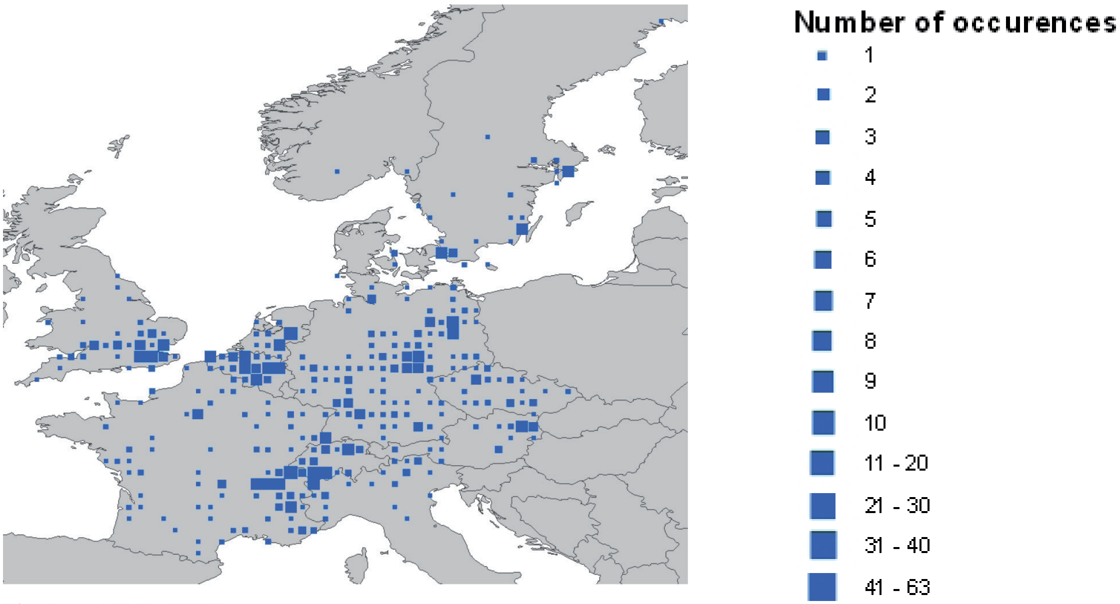

Fig 1g : 1971-2000

Figure 1g. Distribution pattern of *L. serriola* detailed from herbarium data from 1971 to 2000

Figure 2 a to f. Distribution pattern of *L. serriola* in Austria detailed from literature data from 1632 to 2000.

● Absent

☐ No data

░ Present whithout indication of abundance

▒ Very rare to scarce

▓ Scatterd

█ Common to frequent

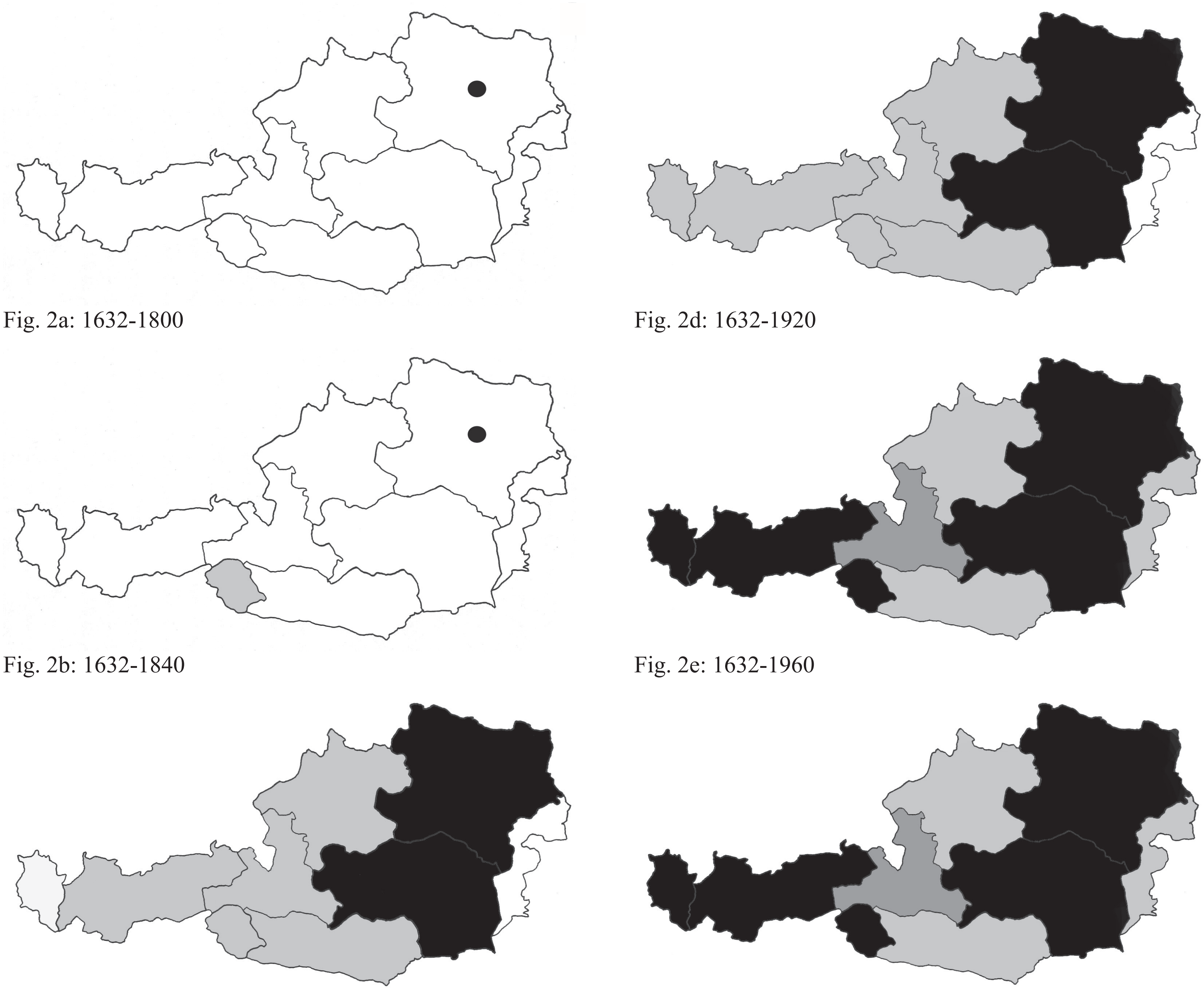

Fig. 2a: 1632-1800

Fig. 2b: 1632-1840

Fig. 2c: 1632-1880

Fig. 2d: 1632-1920

Fig. 2e: 1632-1960

Fig. 2f: 1632-2000

Figure 3 a to f. Distribution pattern of *L. serriola* in Germany detailed from literature data from 1632 to 2000.

● Absent

□ No data

░ Present whithout indication of abundance

▒ Very rare to scarce

▓ Scatterd

■ Common to frequent

Fig. 3a: 1632-1800

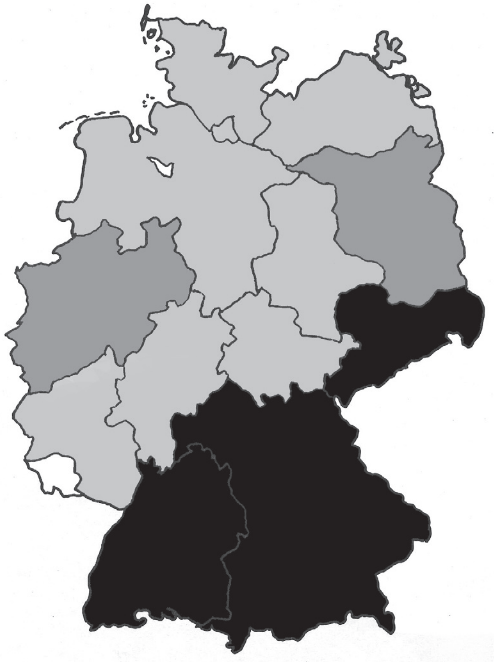

Fig. 3b: 1632-1840

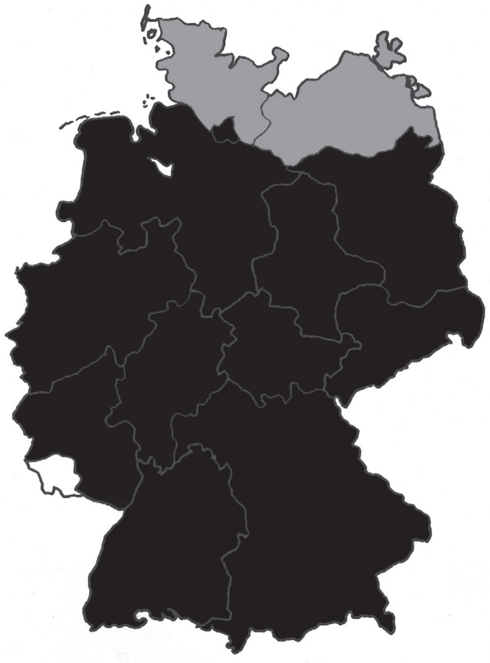

Fig. 3c: 1632-1880

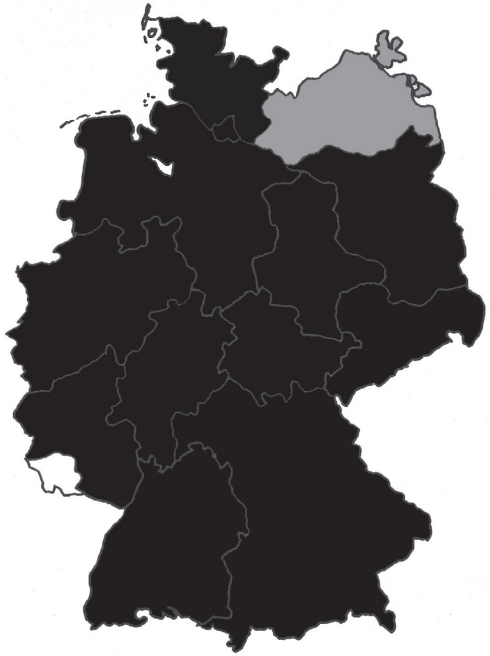

Fig. 3d: 1632-1920

Fig. 3e: 1632-1960

Fig. 3f: 1632-2000

Figure 4 a to f. Distribution pattern of *L. serriola* in Great Britain detailed from literature data from 1632 to 2000.

- ● Absent
- □ No data
- ▨ Present whithout indication of abundance
- ▨ Very rare to scarce
- ▨ Scatterd
- ■ Common to frequent

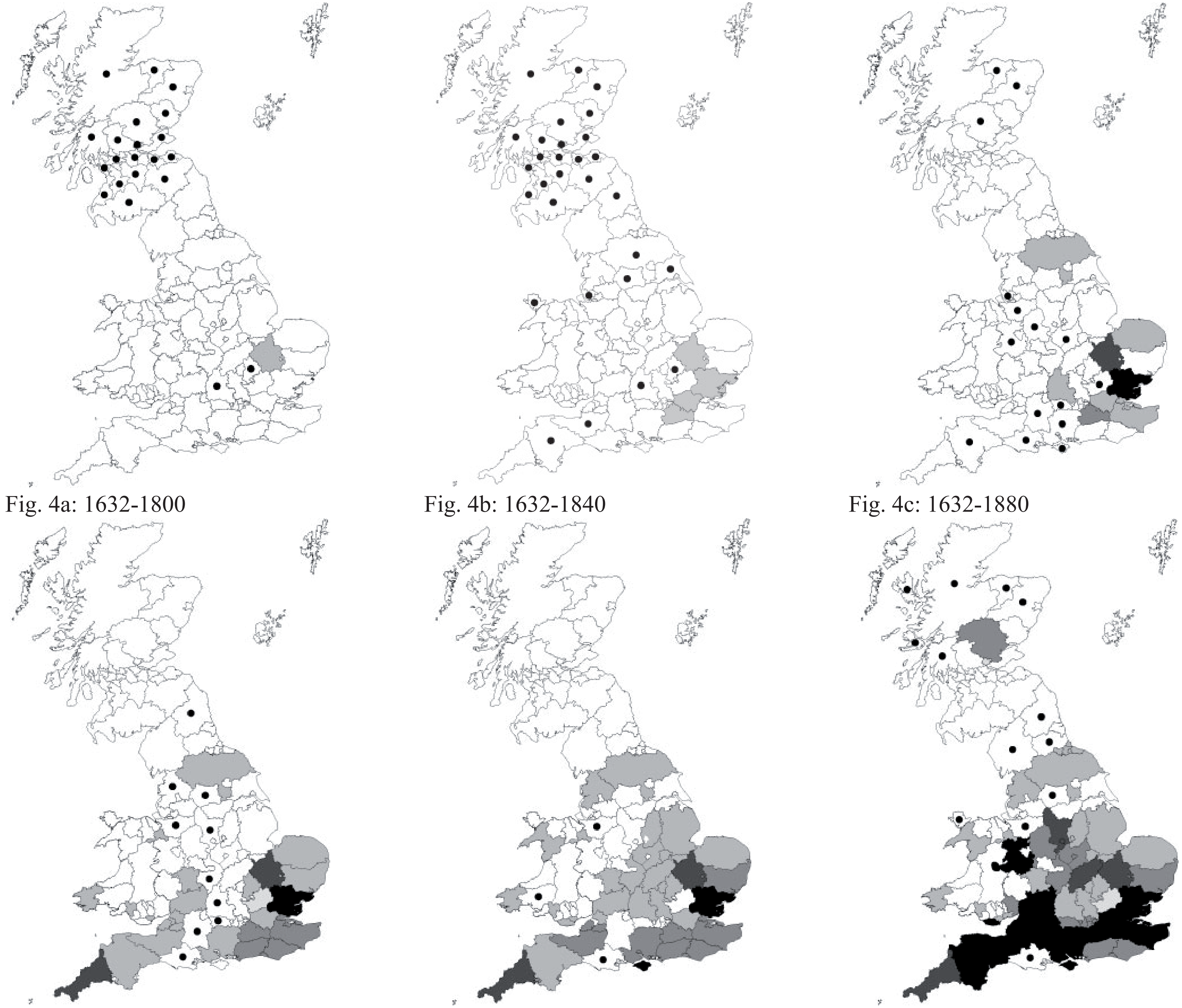

Fig. 4a: 1632-1800

Fig. 4b: 1632-1840

Fig. 4c: 1632-1880

Fig. 4d: 1632-1920

Fig. 4e: 1632-1960

Fig. 4f: 1632-2000

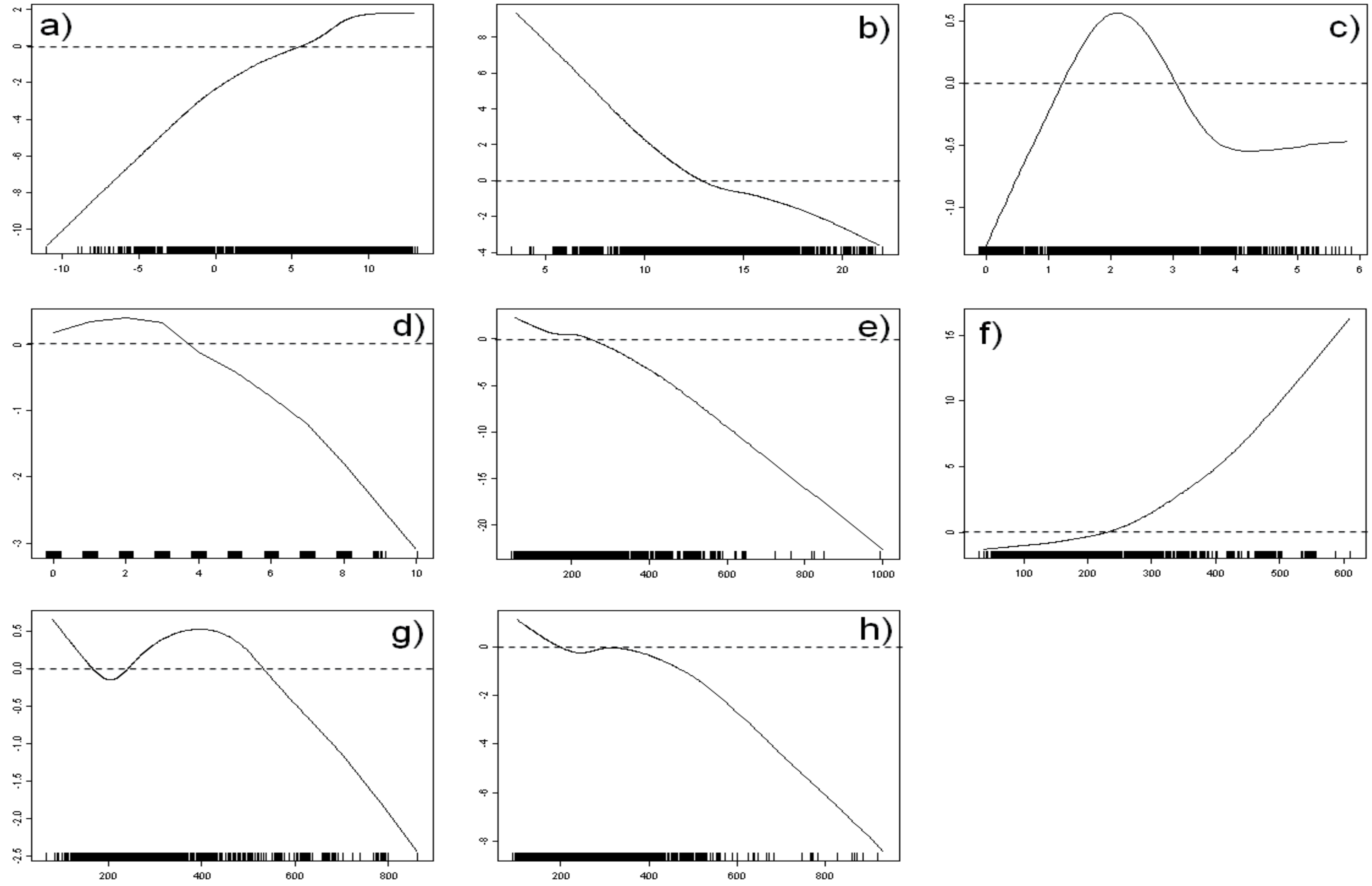

Figure 5. The curves represent the response curve of the species to the a) mean temperature in spring and, b) summer, c) number of month with temperature > 10°C, d) number of month with frost, e) sum of precipitation during winter, f) spring, g) summer and, h) autumn. Parts of the curves above the dashed lines indicate a positive influence of the predictor on the presence of the species, whereas parts of the curves under the dashed lines indicate a negative influence.

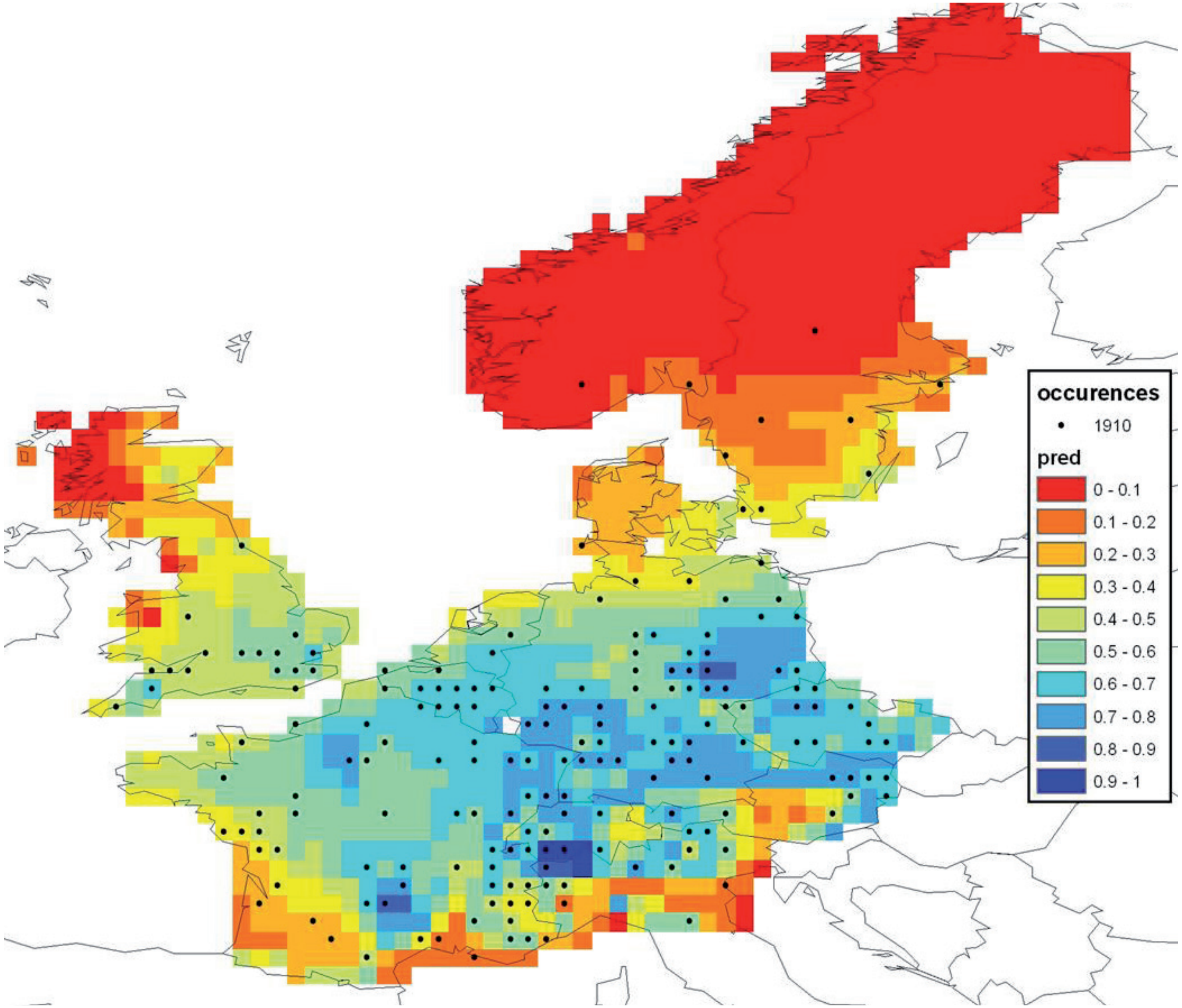

Figure 6a . Potential distribution maps. Prediction for 1910. The dots correspond to herbarium records before 1920.

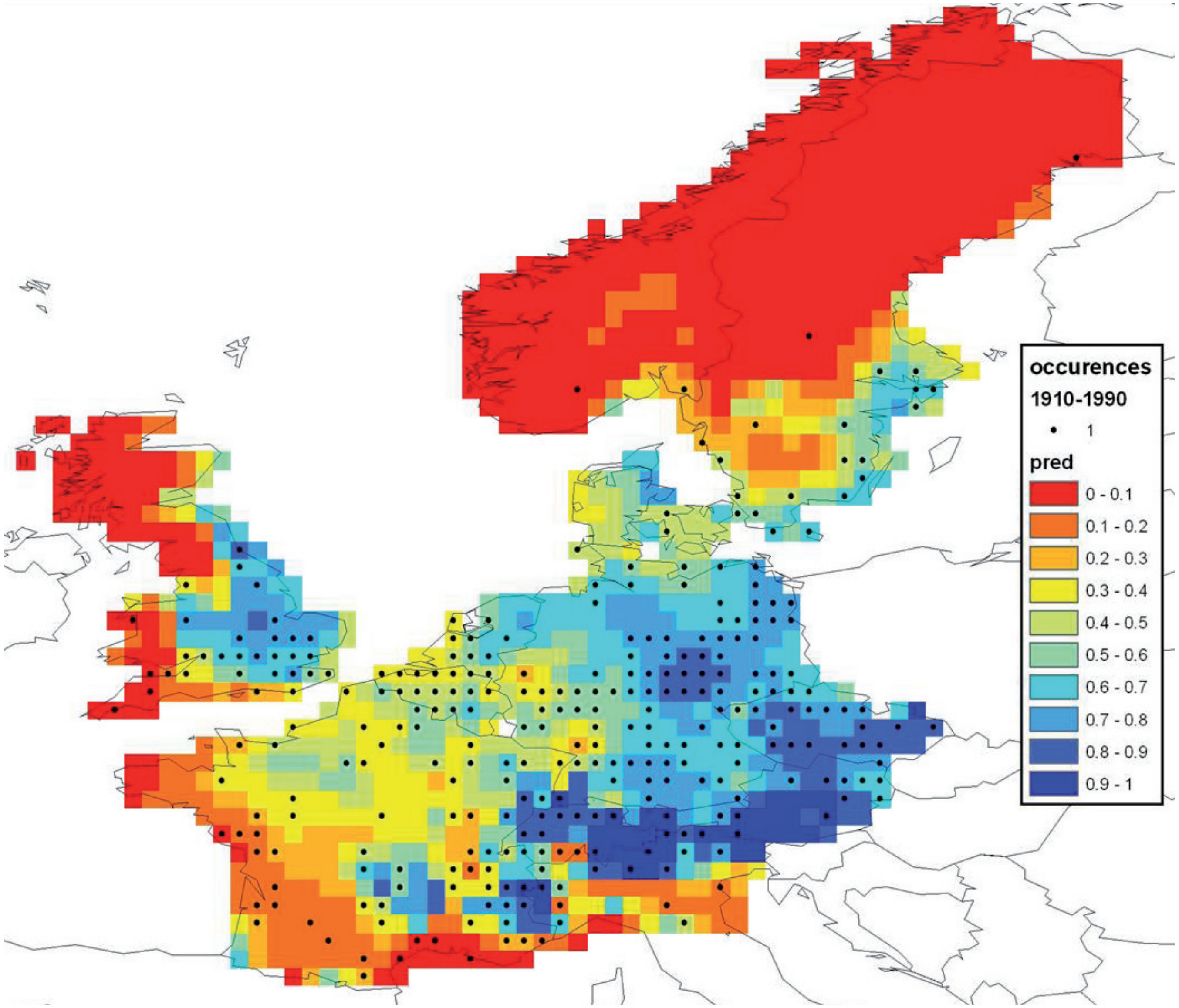

Figure 6b. Potential distribution maps. Projection for 1981-2000. The dots correspond to herbarium records until 2000.

CHAPTER 2

MOLECULAR BIOGEOGRAPHY OF PRICKLY LETTUCE (*L. SERRIOLA* L.) IN EUROPE BASED ON NUCLEAR MICROSATELLITES AND CHLOROPLAST DNA POLYMORPHISM

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Abstract

Lactuca serriola, the wild relative of crop lettuce, is a large spring or winter annual herb pioneer of open habitats. This Mediterranean species has its original distribution in southern Europe, northern Africa and western Asia. The species expanded throughout northern Europe during the last two centuries. Nowadays, it is frequent in disturbed areas over all Europe.

We performed a population genetic analysis on 101 wild populations from western and central Europe representing 2622 individuals. We used chloroplast (PCR-RFLP) and nuclear (Microsatellites, SSR) markers, which are respectively uniparentally and biparentally inherited. SSR results revealed strong genetic differentiation between populations. Low genetic variation and high inbreeding coefficient was found within populations. A clear geographical pattern of isolation by distance was found. Despite this, only a weak pattern of correlation between genetic diversity and geographical distance was highlighted at the continental scale.

Genetic structure was in accordance with the autogamy of the species. Moreover, it supported on one hand a historical diffusion of the species, and on the other hand, several founder events coupled with short and long distance migrations, possibly at different periods.

This information could also be useful to identify potential zones of high genetic diversity in the perspective of conservation of wild germplasm for future programmes of crop genetic improvement.

Keywords: *L. serriola*, biogeography, microsatellites, chloroplast PCR-RFLP, population genetic, spatial autocorrelation, global change, kriging.

Introduction

Patterns of population genetic structure within a plant species over a wide distribution range are shaped by the interaction of many biological, ecological and historical factors. Some of those factors are intrinsic to the species' life history (breeding system, modes of seed dispersal, pollination, and ecology) that often defines the strategy used by a species to colonise a new available habitat. Other factors are extrinsic like external perturbations induced by natural processes (environmental stochasticity) or by human activity (global change).

The genus *Lactuca* (Asteraceae) is distributed in temperate and warm regions of the northern hemisphere as well as northern Africa, North and South-America (Zohary 1991; Frietema de Vries 1992). Central Asia and the Mediterranean basin are considered to be the main centres of diversification of the whole genus (Stebbins 1953; Vuilleum 1973; Feràkovà 1977; Mejias 1993). *L. serriola* is the most variable and widely distributed species of the genus (Lebeda et al. 2004). It is native from Mediterranean climate (Gallardo et al. 1996) and has its original distribution in southern Europe, northern Africa and western Asia, but it has been widely introduced elsewhere and has now a synanthropic worldwide distribution (Carter et al. 1985; Zohary 1991). It is considered to be a drought tolerant species (Werk et al. 1986) and grows mainly in anthropogenic habitats such as roadsides, railways, dumps and urban areas. It occurs also as a weed in a variety of crops where no-till or conservation tillage system are used, orchards, vineyards, pastures and turfgrass (Weaver et al. 2003; Lebeda et al. 2004).

Lactuca serriola is a large spring or winter annual herb growing mostly on sunny exposure. It is considered to be a “r” strategist (R strategy; Grime 1979) as its evolution has tended towards a short life cycle, strong self-fertilization ability, good adaptation for wind dispersal, quick germination and yellow flowers (Lebeda et al. 2001; Frietema de Vries 1992; Mejias 1993; Mejias 1994). The species spread is closely related to human activities, mainly to transports (Lebeda et al. 2001). From herbarium and floristic data, we showed that the species has expanded throughout Northern Europe during the past two centuries (Chapter 1) like many other ruderal plants from the Mediterranean area (Carter et al. 1985; Zohary 1991; Landolt 2001).

This spread is thought to be caused by elements of global change such as climate change, globalization of trade and travel and by the increase of disturbed habitat through the development of trade roads and urban areas. These factors had direct consequences on ecosystems and therefore acted directly on population and metapopulation dynamics of *L. serriola.*

L. sativa and *L. serriola* are closely related and are often considered as conspecific (Lindqvist 1960 a; De Vries 1990; Whitaker 1939), or ecospecies (De Vries et al. 1994; Lindqvist 1960 a) differing only for characters connected to domestication.

Conservation of biodiversity of plant genetic resources for food and agriculture, including wild relatives of crop species, is considered to be one of the most important milestones for the new millennium (Lebeda et al. 2004). Wild relatives, such as *L. serriola*, and progenitors of cultivated plants play an important role in plant breeding; they often represent a broad genetic base and a reservoir of characters that may be important for genetic improvement of the crop, such as resistance to pathogens or other physiological traits (Lebeda et al. 2001).

Recently, several studies have demonstrated the close relatedness of all *Lactuca* species using morphological characteristics (de Vries & van Raamsdonk 1994), chromosome banding patterns (Koopman et al. 1993), RFLPs (Kesseli et al. 1991), RAPDs (Wycott and Fort 1994), and microsatellites (van de Wiel et al. 1998).

Microsatellites, also called simple sequence repeats (SSRs), have been shown to be excellent molecular markers for the study of genetic diversity (Karp et al. 1997) and phylogenetic relationships at intra- and inter-specific levels (Markert et al. 1999; Heuertz et al. 2004; Ge et al. 2005). Nuclear microsatellites have been isolated from crop lettuce, *Lactuca sativa* (van de Wiel et al. 1999), and provide a powerful tool to establish relationships between accessions/cultivars, to identify lettuce species of the subsection *Lactuca* and to assess polymorphism in genebank accessions of *L. serriola* (van de Wiel et al. 1998).
Chloroplast DNA has a slower rate of sequence and structural evolutionary change than SSRs (Palmer et al. 1988) and is maternally inherited in most plant species (Birky 1995). Specific amplification of cpDNA fragment followed by digestion with restriction enzymes (restriction length polymorphism, RFLP) has been widely applied to examine cpDNA variation (Besnard et al. 2002; Panda et al. 2003; Pharmawati et al. 2004) and represents an excellent complement to the analysis of more polymorphic SSR markers.

In the present paper, we study historical processes and population structure of *L. serriola.* Our main objective is to perform a population genetic analysis of spontaneous wild population samples from western and central Europe with uniparentally (cpDNA) and biparentally (nDNA) inherited markers. More specifically, we aim to:

1) Characterize the genetic diversity and the genetic structure of *L. serriola* in Europe. This is the first report of SSR and PCR-RFLP analyses for that species.

2) Document the migration of *L. serriola* into north-western Europe from the geographical distribution of genetic variation. In particular, we want to determine whether colonisation happened through several foundation events or whether it occurred through diffusion from the east and/or from the Mediterranean region.

Material and Method

Plant material

Seeds were sampled in 101 populations from seventeen countries along a North-South and an East-West transects in Europe in 2001, 2002 and 2003. Nine populations from the UK, sampled for another project (GENE-MINE,QLK5-2000-00722) were added to this dataset (Table 1, Fig.1). Plants were grown from seeds and total DNA was extracted from fresh young leaves following the QUIAGEN® extraction kit protocol.

Microsatellite analysis

A subset of 101 populations totalizing 2622 individuals that represented whole Europe was selected for simple sequence repeats (SSRs) analysis. Ten polymorphic SSR primers (LsD106, LsE003, LsB101, LsB104, LsA001, LsA004, LsD109, LsD108 LsE011, LsE018) described in (van de Wiel et al. 1999) were performed on each individual. SSR analysis for loci LsD106, LsE003, LsB101, LsB104, LsA001, LsA004 were performed by Plant Research International in Wageningen (the Netherlands). PCR were performed in two multiplex reactions, both using 12 µl of PCR mix and 8 µl of DNA template (to a final concentration of about 2 ng/reaction) in a total volume of 20 µl; end concentrations of primer pairs were optimized to 1.6 pMol/reaction of LsA001 (NED label), 1.2 pMol/reaction of LsA004 (FAM label) and 0.6 pMol/reaction of LsD106 (HEX label) for multiplex "A", and 1.6 pMol/reaction of LsB101 (NED label), 1.6 pMol/reaction of LsB104 (FAM label), 2 pMol/reaction of LsD103 (FAM label) and 4 pMol/reaction of LsE003 (HEX label) for multiplex "B", respectively, from stock solutions of 10 pMol/µl. The PCR reaction was performed on an MJ PTC200 thermocycler, using a heated lid, as follows: 1 cycle 94^0C for 3 min, followed by 30 cycles 94° C for 30 sec, 50° C for 30 sec (ramp 1^0C/sec to Ta), and 72° C for 45 sec (ramp 1^0C/sec to Tp), ending with one cycle of 72^0C for 10 min. After separation of PCR products on Sephadex 50, samples were prepared for loading on an ABI 3700 Sequencer by mixing 1.5 µl of a 1:1 mixture of both PCR reactions with 2.5 µl ultrapure formamide, 0.5 µl Loading buffer (PE Biosystems) and 0.5 µl Size Standard with Rox label. The samples were denatured for 2 minutes at 90° C and then immediately put on ice; 1.25 µl sample was loaded in the sequencer. For detection, the filterset D was used on the ABI Sequencer 3700, which was calibrated for the colour labels Ned (yellow), Fam (blue), Hex (green) and Rox (red, size standard).

SSR analysis on loci LsD109, LsD108, LsE011, LsE018 were performed by EBOLAB, University of Neuchâtel in Switzerland on an LI-COR DNA analayser IR^2. PCR reactions were performed using 5 µl of PCR mix and 1.5 µl of DNA template (to a final concentration of about 5 ng/reaction) in a total volume of 5.5 µl; end concentrations of primer pairs were optimized to 10 pMol/µl. PCR amplification were performed on a Whatman Biometra T gradient, using a heated lid, as follows : 1 cycle of 15 min at 95°C to activate the Hotstar Taq polymerase linked to 12 cycle with a touch down of 0.5°C per cycle of 30s at 94°C, 1 min 30 at 65°C (LsE 018, LsE011) 60°C (LsD109) 67 °C (LsD 108) , 1 min at 72°C, followed by 17 cycles of 30s at 94°C, 1 min 30 at 59°C (LsE 018, LsE011) 54°C (LsD109) 61 °C (LsD 108), 1 min at 72°C, followed by 3 min at 72°C to complete primer extension. Samples were prepared for loading in a acrylamide gel in the LI-COR DNA analyser IR^2 by mixing 1 µl of PCR reactions with 5 µl desionised, 5 µl blue dye containing formamide. The samples were denatured by 2 minutes at 90° C and then directly put on ice; 1.5 µl sample was loaded in an acrylamide gel in the sequencer. For detection, LI-COR DNA analayser IR^2 was calibrated for the wavelength 700 nm (LsD109, LsD108) and 800nm (LsE011, LsE018).

Sizing was performed with the software LICOR Saga Microsatellite analysis.

Chloroplastic PCR-RFLPs

In order to investigate intraspecific variation of the chloroplast DNA, thirty-two combinations of primers pairs and restriction enzymes were tested on a subset of about 30-34 individuals from 17 different populations representing the whole European range of distribution. Amplification of cpDNA was carried out using 10 universal primer pairs: VL, TrnVS, TrnST, TrnSM, TrnQB, TrnTL, Hk, psbCS, FV, atpb; all defined in (Dumolin-Lapegue et al. 1997). Chloroplastic PCR-RFLP analyses were performed using 23 µl of PCR mix and 1.5 µl of DNA template containing 50ng of DNA template. PCR amplification of chloroplast DNA fragments were performed on a Whatman Biometra T gradient, with the following protocol: 1 cycle of 15 min at 95°C to activate the Hotstar Taq polymerase linked to 35 cycle of 30s at 94°C, 45s at 50°C, 1 min 30 at 72°C, followed by 5 min at 72°C to complete primer extension. Restriction was performed, in a separate reaction for each enzyme, using the enzymes *Taq*I, *Alu*I, *Rsa*I, *Hae*III, *Pst*I, *Bam*H, according to the provider's recommendation, using 5 µl of amplified DNA and incubating for 4 hours at 37° C. Samples were prepared for loading by mixing the reaction product and a formamide-based loading dye in a 1:1 ratio. After denaturation (2 minutes at 95° C), sample were loaded and separated in a 5% acrylamide gel. The variation was visualized using Ethidium Bromide.

Data analysis

For every microsatellites locus, we recorded the total number of alleles K, the range of allele sizes and the total gene diversity Ht to assess overall polymorphism. Additionally, we computed Wright's inbreeding coefficient F_{IS}, which provides information on inbreeding. Global statistics based on allele identity (*F*-statistics) and allele size (*R*-statistics) were used to analyse the differentiation between populations, for every locus and over all loci. The contribution of stepwise-like mutations to the population differentiation was investigated by testing whether the observed R_{ST}-value was significantly larger than the observed F_{st}-value. R_{st} and F_{st} have the same expected value when differentiation is caused solely by drift, whereas R_{ST} is expected to be larger than F_{st} when stepwise mutations contribute to the differentiation (Hardy et al. 2003). The contribution of stepwise mutations can be tested by permuting allele sizes over alleles within populations; for these computations, we used the software SPAGEDI (Hardy et al. 2002). The number of permutations used for this test, and for all other permutation tests, was 9999.

We used FSTAT version 2.9.3 (Goudet 2001) to compute an estimation of the gene diversity H_E, the total genetic diversity Ht, the observed proportion of heterozygotes Ho as well as Nei’s (1987) G-statistics (G_{IS}, G_{ST}).

AMOVA was computed with the program GENALEX version 6 (Peakall et al. 2006) http://www.anu.edu.au/BoZo/GenAlEx.

Isolation by distance

One of the main objectives of this study was to investigate whether there was a relationship between the genetic composition and the geographic distribution of the individuals. To test for such isolation by distance, we used simple Mantel tests (Mantel 1967) and Mantel correlograms (Sokal 1986). To this end, we calculated a matrix of pairwise Fst values between all populations as well as a matrix of geographic distances between all populations, taking into account the curvature of the earth. Additionally, we looked for trends in the genetic variability of the populations, by calculating the gene diversity for every population and testing in a Mantel test for an association between the difference in gene diversity between pairs of populations and geographic distance. Mantel tests and Mantel correlograms were performed using GenoDive version 2.0a (Meirmans et al. 2004).

Spatial organisation of Genetic Diversity

In order to get insights into the spatial organisation and distribution of populations genetic diversity we analysed the dataset using the program "PCAgen" version 1.2.1 by J. Goudet (available at http://www2.unil.ch/popgen/). This program performs a Principal Components Analysis, using populations as observations and the allele frequencies of the populations as variables; instead of the percentage of explained variance, an Fst value is given for each axis. The significance of each axis can be tested through permutations by randomizing the individuals over the populations and then comparing the Fst value for the randomised dataset with the original.

Kriging

To get a better overview of the distribution of the Gene Diversity a map was produced using the technique of kriging. The stochastic approach, based on kriging leads to a quantification of uncertainty for *L. serriola* genetic diversity spatial distribution. Kriging was interpolated for the Gene Diversity with a cell size of 0.5 x0.5 cm, on Arc Map 9.0 (ESRI®). Using the Arc Map grid-analyst, the maps were extracted from the grid into new maps corresponding to the study area. Having fitted a model to the experimental variogram, an estimation of our variable can be made at all points of a regular grid by the method of standard kriging. Simulations represent a method of estimation which reconstruct the real variability of the variable and allow calculation of probability maps (Chilès et al. 1999).

Table 1. Origin of plant material. Coordinates are given in decimal degrees.

Country	Code	Location	Site description	Analysis	Longitude (dd)	Latitude (dd)
Austria	A03	St. Valentin	road side	SSR/RFLP	14.517	48.150
Austria	A05	Salzburg (n. Eugendorf)	along pavement	SSR/RFLP	13.083	47.850
Austria	A07	Wörgl	ruderal site (old industry area, parking)	SSR	12.250	47.417
Austria	A08	Insbruck	fallow ground	SSR/RFLP	11.500	47.333
Austria	A09	Landeck	road side near industrial area	SSR	10.500	47.100
Austria	A10	Bludenz	industrial area	SSR	9.900	47.150
Austria	A11	Hohenems	industrial area, old parking	SSR	9.767	47.367
Bulgaria	PK1	Road to Samokov		SSR/RFLP	23.744	42.490
Bulgaria	PK2	South of Varna.	fallow	SSR/RFLP	27.950	43.200
Bulgaria	PK3	West of Samokov	fallow	SSR/RFLP	23.744	42.490
Czech Republic	LSC 2	Pribram (4), 6 kms SE of P, close to Milin	edges of Brassica field	SSR	13.896	49.634
Czech Republic	LSC 4	Suchdol, between "Eurosport" and road crossing	verge between road and grassland	SSR/RFLP	14.232	50.143
Czech Republic	LSC 6	Ceske Budejovice-Pisek (20), (second) junction to Pistin	edges moist grassland, extensive management	SSR/RFLP	14.325	49.048
Denmark	DEN_O	Fyn, Odense. At the road margin on the South side of E20, departure Odense West	ruderal site.	SSR/RFLP SSR	10.550 11.000	55.483 55.700
Denmark	DEN_R	Zealand, Kalundborg. At the sandy/stony beach facing Kalundborg Fjord; 1 km from Gisseløre	sandy/stony beach			
Denmark	DEN_S	Zealand, Slagelse. At the junction between E20 and road 22; at the road margin on the North side of E20	ruderal site	SSR/RFLP	11.383	55.400
France	F10	Rochefort - Montagne, N 89	verge of road	SSR/RFLP	2.806	45.693
France	F12	N 117 direction Orthez	ruderal site	SSR/RFLP	0.846	43.511
France	F6	Les Hauts de Céret going down to Le Céret	orchard	SSR	2.749	42.480

Table 1 (*continued*)

Country	**Code**	**Location**	**Site description**	**Analysis**	**Longitude (dd)**	**Latitude (dd)**
France	F8	N89 (E70) 1 km after exit 26 of motorway going around Bordeauy direction Périgueux	ruderal site	SSR	0.477	44.866
France	FF1	St-Gilles-Croix-de-Vie	parking les Cyprès	SSR/RFLP	-1.083	46.683
France	GM1	Nîmes area, road D	road side	SSR/RFLP	4.083	43.917
France	LS-11	Contrexéville - Neufchateau (54, Fr): D164, hameau Villars, 3 km before Neufchateau	former railway emplacement and embankment; rubble heaps	SSR	5.704	48.324
France	LS-13	Briey - Longyon (54, Fr): RN43 junction Bertrameix	road verges to Triticum field and grassland	SSR/RFLP	5.784	49.326
France	LS-02	Mirabel-et-Blacon/Crest (26, Fr), 1.5 km E Mirabel	verge between road and arable field	SSR	5.116	44.704
France	LS-03	Chavanay-Pélussin (69, Fr): D7 road bar 3.8	clearing of Quercus *pubescens / petraea* mixed forest	SSR	4.713	45.415
France	LS-07	Buxy - Givry (71, Fr): D981 at Rosey	verge between road and arable field	SSR	4.716	46.739
France	LS-09	Is-sur-Tille - Selongey (21, Fr): D903 just before round about	grassland, former arable field	SSR/RFLP	5.129	47.536
Germany	D10	Wolfenbüttel (Exit 7 from A 395, Northbound), in left aside terrain right	very dense vegetation	SSR	10.494	52.140
Germany	D12	Brodenbach, c. 10 kms W of Koblenz	Mosel river side	SSR/RFLP	7.452	50.226
Germany	D2	Mosbach - Buchen (B27); 2.0 km before Heidersbach	edges shrubby plantations around road side restaurant	SSR	9.250	49.446
Germany	D5	Karlstadt - Hammelburg (B27); 0.3 km before Obereschenach	ruderal site	SSR/RFLP	9.831	50.094
Germany	D7	Bebra - Sontra, c. 0.7 km from exit Bebra-Nord (B27)	dense grass vegetation	SSR	9.816	50.994
Germany	LBM-1	Herrenberg (D)	building area	SSR/RFLP	8.883	48.600

Table 1 (*continued*)

Country	**Code**	**Location**	**Site description**	**Analysis**	**Longitude (dd)**	**Latitude (dd)**
Germany	LBM-2	Biberach (D)	ruderal site around of an abandoned hotel	SSR	9.767	48.083
Germany	LS 17	Schwerin-Wismar (B106), in village Medewege, 3 kms N of Schw.	fallow field vegetable garden close	SSR/RFLP	11.406	53.661
Germany	LS 21	Ahrensbök, c. 20 kms NW of Lübeck (B432), Gewerbegebiet Barghorst	building grounds	SSR	10.577	54.022
Germany	LS 22	Altmühlmünster	verges between road	SSR/RFLP	11.700	48.950
Greece	PK4		Edge of road	SSR/RFLP	21.000	40.050
Holland	Hol01	Gendt	Between bricks of river Dike	SSR	5.955	51.868
Holland	Hol12	Valburg	Between bricks of river dike	SSR	5.754	51.886
Holland	Hol16	Westerlee	verge between “hollow” road and arable field	SSR/RFLP	4.219	51.975
Holland	Hol17	Beesd	Verge of road and arable field	SSR	5.188	51.903
Holland	Hol20	Braamt	Verge of road and arable field	SSR/RFLP	6.269	51.923
Holland	Hol21	Alkmaar	Construction site	SSR	4.773	52.653
Hungary	H01	Kosd (Vac) (H)	road verge	SSR/RFLP	19.167	47.750
Hungary	H03	Emöd (Miskolc) (H)	road verge	SSR/RFLP	20.800	47.967
Hungary	H04	Budapest (H)	industry area with railway	SSR	19.067	47.383
Hungary	H06	Kisbér (H)	fallow ground	SSR	18.033	47.500
Hungary	H08	Mosouszolouk (H)	fallow ground along road	SSR/RFLP	17.167	47.867
Italy	Ao-2	Val D’Aosta , direction Nus next to a bus stop	ruderal site. LD *L serriola* var *integrifolia* + *serriola*	SSR	7.614	45.863
Italy	Ao-3	Val D’Aosta, road direction Châtillon	ruderal site over vine	SSR/RFLP	7.604	45.942

Table 1 (*continued*)

Country	**Code**	**Location**	**Site description**	**Analysis**	**Longitude (dd)**	**Latitude (dd)**
Italy	Ao-4	Val D'Aosta, road direction Emarese from St-Vincent	edge of road; mixed with *L. augustana*	SSR	7.773	45.913
Italy	Ao-6	Val D'Aosta, Châtillon direction Ussel before an underway which cross the motorway	edge of road / only *L. augustana*	SSR/RFLP	7.865	45.965
Italy	Ao-9	Val D'Aosta, road direction Arvier	Edge of road close to an orchard	SSR/RFLP	7.380	45.789
Italy	I-10-02	Altamara (I)	industrial area	SSR	16.550	40.833
Italy	I-11	Fidenza (I)	ruderal site between garage and grassland, rubble heaps	SSR/RFLP	10.100	44.900
Italy	I-13	S. Giuliano (I)	embankment between parking and water stream	SSR	9.250	45.383
Italy	I-02	Roseto degli Abruzzi (I)	edge of corn field	SSR	14.017	42.650
Italy	I-03	Ancona (I)	road verge to fallow ground	SSR/RFLP	13.367	43.567
Italy	I-3-02	Aquasanta (I)	ruderal site	SSR	13.417	42.783
Italy	I-04-02	Avezzano (I)	parking area	SSR/RFLP	13.467	42.067
Italy	I-05	Riccione (I)	fallow ground with newly turned soil for construction	SSR/RFLP	12.617	43.983
Italy	I-06-02	Vairano Scalo (I)	ruderal site	SSR	14.100	41.317
Italy	I-07	Faenza (I)	fallow ground	SSR/RFLP	11.850	44.317
Italy	I-08-02	Picerno (I)	road side	SSR/RFLP	15.633	40.633
Italy	I-09	Modena (I)	ruderal site around old house	SSR/RFLP	10.983	44.567
Italy	LBS4	Casarano (I)	road verge to vineyard	SSR/RFLP	18.150	40.033

Table 1 (*continued*)

Country	**Code**	**Location**	**Site description**	**Analysis**	**Longitude (dd)**	**Latitude (dd)**
Luxembourg	LS-14	Arlon - Bastogne (Luxembourg, Be): A26, parking area after junction Vaux-sur-Sure	verges and embankments parking lot	SSR	5.644	49.940
Poland	PL01	Rzepin (PL)	road verge	SSR/RFLP	14.817	52.333
Poland	PL10	Brzeziny (PL)	road verge	SSR	19.000	50.317
Poland	PL03	Pniewy (PL)	parking in industrial area	SSR	16.217	52.517
Poland	PL06	Jarocin (PL)	road side	SSR	17.517	51.983
Poland	PL08	Bakow (n. Kobylagor	along railway	SSR/RFLP	18.183	50.967
Portugal	P02	After Santiago de Cadem direction Grandola	verge of road	SSR	-8.674	38.022
Portugal	P03	N 118 After Samora Correia	verge of road	SSR/RFLP	-8.859	38.937
Portugal	P04	IC 2 direction Porto behind the restaurant " O cruzamento"	kitchen garden / with *L.sativa*	SSR	-8.446	40.288
Portugal	P05	idem than P4	construction site /	SSR/RFLP	-8.446	40.288
			with *L.virosa*	SSR	-8.616	40.892
Portugal	P07	N 109 direction Porto	verge of road / 2 stems of *L.virosa*			
Slovakia	SVK-1	near Chyzne (SVH)	road verge	SSR/RFLP	19.700	49.383
Slovakia	SVK-2	Dolny Kubin (SVK)	parking in industrial area	SSR	19.300	49.233
Slovakia	SVK-3	Banska Bystryca (SV	arable field road	SSR	19.150	48.750
Slovakia	SVK-4	Krupina (SVK)	field road	SSR/RFLP	19.117	48.400
Spain	E10	N 324 just before A 92 direction Granada	verge of road	SSR/RFLP	-2.898	37.168
Spain	E13	N 340 direction Cadiz after Tarifa	verge of road	SSR	-5.764	36.191
Spain	E14	E1 direction Portugal after Hueva	verge of road	SSR	-7.056	37.325
Spain	E15	N 550 direction Santiago de Compostela after Padròn	verge of road and crane depot close	SSR	-8.651	42.777
Spain	E16	C 642 after Ortigueira	verge of road and kitchen garden	SSR/RFLP	-7.788	43.714
Spain	E17	N 634 (E 70) direction Aviles	verge of road /	SSR	-6.674	43.544

with *L.virosa*

Table 1 (*continued*)

Country	**Code**	**Location**	**Site description**	**Analysis**	**Longitude (dd)**	**Latitude (dd)**
Spain	E18	N 634 (E 70) 20 km before Aviles	verge of road / with *L.virosa*	SSR	-6.045	43.533
Spain	E21	N 240 direction Vitoria Gasteiz	surroundinggs of a peat industry	SSR/RFLP	-2.675	42.991
Spain	E3	C25 direction Lleida	verge of road	SSR/RFLP	1.913	41.789
Spain	E6	Cabanes (Cv 10 direction Castello)	orchard	SSR	0.039	40.161
Spain	E8	Xabià direction Gata/Benitatxell	ruderal site on the verge of road	SSR/RFLP	0.153	38.781
Sweden	SW-10	Karlskrona, Verkö	industrial area	SSR	15.059	56.164
Sweden	SW-12	Ystad	railroad/ industrial area.	SSR	13.087	55.436
Sweden	SW-14	Gävle	industrial area outside of factory.	SSR/RFLP	17.025	60.659
Sweden	SW-2	Åkersberga	construction area.	SSR	18.029	59.473
Sweden	SW-5	Norrköping	industrial area.	SSR	16.008	58.621
Sweden	SW-7	Vimmerby	parking-lot, in central of city.	SSR/RFLP	15.087	57.664
Switzerland	CH1	Bevaix	ruderal site	SSR	6.803	46.931
Switzerland	CH-12	St Gallen (CH)	road side	SSR/RFLP	9.333	47.333
Switzerland	CH-15	Bellinzona (CH)	verges and embankment of newly constructed parking	SSR	9.033	46.200
Switzerland	CH-16	Quinto (CH)	road verge and ruderal site	SSR/RFLP	8.733	46.500
Switzerland	CH-17	Rapperswil (CH)	edge around gravel-pit	SSR/RFLP	8.800	47.250
United Kingdom	UK03	Chapel Farm, Cardington 27(Bedfordshire)	field margin	SSR	-0.4028	52.1214
United Kingdom	UK04	Blackford Mill Farm, Henley in Arden (Warwickshire)	area around farm buildings and field margins	SSR	-1.7761	52.2833
United Kingdom	UK05	A605 near turnings to Elton Hall and Wigsthorpe (Northamptonshire)	bank of ditch on roadside	SSR	-0.4281	52.5056

Table 1 (*continued*)

Country	**Code**	**Location**	**Site description**	**Analysis**	**Longitude (dd)**	**Latitude (dd)**
United Kingdom	UK06	Panks Farm (Mr Cundell), Bedford Level; first on RHS of B1443 from A1073 to Newborough (Cambridgeshire)	dyke embankment 5m deep SSR	SSR	-0.2017	52.6436
United Kingdom	UK07	Fincham's Farm (Bill Natrass), Sixteen Foot Bank B1098, Stonea, March (Cambridgeshire)	field margin	SSR	0.1478	52.5153
United Kingdom	UK08	Chesterton; 200m from village towards motorway bridge (Warwickshire)	set aside field	SSR	-1.4933	52.2203
United Kingdom	UK09	Southam, east on the road to Priors Marston (Warwickshire)	derelict building	SSR	-1.3797	52.2492
United Kingdom	UK11	Newton Park, Bath BA2 9BN, grounds of Bath Spa University College (North Somerset)	disused walled garden	SSR	-2.4386	51.3700
United Kingdom	UK12	Bristol	ruderal edge of tarmac path near dock wall of floating harbour	SSR	-1.0889	52.8703

Results

Overall diversity

The total number of allele observed per locus ranged from 9 to 38, with an overall total of 232 alleles scored over the 10 loci (Table 2). The size range of PCR products corresponding to these alleles were comprised between 61-327 nucleotides. Some populations mainly consisted of a single genotype so the average gene diversity within population (Hs), which equals to the expected heterozygosity, is relatively low (0.35). Observed heterozygosity (Ho) differed between loci, but was always lower than Hs as reflected by the overall Ho (0.014). Populations that presented heterozygotes individuals were distributed overall Europe (Fig. 2). Overall inbreeding coefficient (Gis) value that is close to 1 (0.96), indicating deficiencies of heterozygotes within populations, in accordance with the predominantly self-pollinated mating system of *L. serriola*. However, four populations presented a lower Gis indicating outcrossing (Fig. 3). These populations were respectively from Holland (Hol 12, Gis = 0.51), Denmark (DEN O, Gis = 0.38), Sweden (SW 02, Gis = 0.29) and Bulgaria (PK 2, Gis = 0.53). The same pattern, but less contrasted, emerged for population allele richness (Fig. 4). Non frequent alleles (alleles present in 25 % or less of the populations) were very rare in northern and western populations (Fig. 5). Only few populations presented private alleles (Fig. 6). Loci were in linkage disequilibrium; as a result of the high selfing rate of the species, leading to a deficit of heterozygotes.

High values of Gst were found (overall Gst = 0.572), indicating that a large percentage of the total genetic variation was distributed among populations. However, the value of Gst is dependent on the level of within-population variation, making comparison between studies difficult when markers with different mutation rates are used (Hedrick 2005). Thus, a standardized G'st, independent of the level of within subpopulation genetic variation, as described in Hedrick (2005), was calculated by dividing the usual Gst value by the maximum level possible given the observed amount of genetic variation (labeled Gst(max), which is approximated by 1-Hs). G'st is therefore equal to the proportion of Gst(max) that the observed Gst represents. G'st yielded even higher values than Gst with an overall value of 0.880.

Gene diversities were very similar for each locus with the exception of LsE018 that showed less than half the polymorphism of other loci. Differentiation among populations taking into account the allele size, Rst = 0.629, was significantly larger than differentiation based on allele identity, Gst = 0.558 ($p< 0.001$), after a progressive Bonferroni correction. This indicated that stepwise mutations contributed to overall among-population differentiation.

Table 2. Genetic variation within and among prickly lettuce wild populations analysed with nuclear microsatellites and chloroplastic PCR-RFLP. K, total number of alleles; Ho, observed heterozygosity; HS, within population gene diversity; HT, total gene diversity; GIS, inbreeding coefficient; GST estimation of fixation index FST; GST (max) maximum possible GST given the current within population variation, GST (max) = 1-Hs; G'ST, Standardized GST corrected by the variation within population; RST, relative among population differentiation based on allele size; P(H1: RST > RST perm.), one sided test of information content of allele size from (Hardy et al. 2003): ns, non significant; ** p ≤ 0.01 ; p***p<0.001.

Locus	K	Allele size	HO	HS	HT	GIS	GST	GST(max)	G'ST	RST	P(H1: RST > RST perm.)
LsA001	34	133-209	0.019	0.406	0.926	0.954	0.564	0.592	0.949	0.575	ns
LsA004	25	168-226	0.013	0.375	0.925	0.965	0.597	0.623	0.955	0.661	ns
LsD106	12	140-188	0.008	0.3	0.803	0.972	0.628	0.698	0.897	0.625	ns
LsB101	27	187-239	0.015	0.398	0.874	0.963	0.547	0.6	0.909	0.598	ns
LsB104	33	155-235	0.015	0.401	0.917	0.963	0.565	0.597	0.943	0.736	***
LsD109	23	252-327	0.02	0.365	0.822	0.946	0.558	0.633	0.879	0.582	ns
LsD108	38	61-211	0.013	0.428	0.905	0.97	0.518	0.56	0.906	0.649	**
LsE018	9	154-205	0.005	0.132	0.319	0.958	0.587	0.867	0.676	0.565	ns
LsE011	16	251-302	0.014	0.391	0.87	0.964	0.553	0.607	0.908	0.633	**
LsE003	15	134-196	0.02	0.306	0.806	0.934	0.622	0.692	0.896	0.551	ns
Overall	22.6	232	0.014	0.35	0.817	0.96	0.572	0.65	0.880	0.629	***
Chloroplast				0.127	0.315		0.598	0.873	0.685		

Patterns of isolation by distance

The Mantel test between the pairwise populations Fst matrix and the matrix of geographic distances between all populations was highly significant (p = 0.001) and gave a relatively high coefficient of correlation (Mantel's r) of 0.266 (Table 3). The corresponding Mantel correlogram is shown in Figure 7. The average Fst, instead of the usual Mantel's r, was used as an autocorrelation statistic as it is more informative in a population genetic context. The horizontal line is the overall mean pairwise Fst. The relationship between Fst and distance is approximately linear. The first four classes (up to 777 km) and the three last one (since 1368 km) were significant (Table 4). The correlogram show a clear geographic pattern in the distribution of the genetic variation: the positive autocorrelation in the smaller distance classes indicates that populations in close spatial proximity are genetically more similar than on average. Reversely, the negative correlations of more distant classes indicate that distant populations are more dissimilar than on average. This suggests that isolation by distance might be a factor of population differentiation.

The used Mantel test assumed a linear relationship between Fst and distance. However, according to population genetic theory, in a two-dimensional system of populations, such a linear relationship is not expected, but rather a linear relationship between Fst/(1-Fst) and the logarithm of the geographic distance (Rousset, 1997). Transforming the matrices accordingly and testing this relationship yielded a Mantel's r of 0.063, which was only marginally significant at p = 0.0118. Clearly, the Mantel test performed on untransformed matrices explained much more of the total variance, indicating that the stepping-stone model, on which the theoretical expectations were based, may not be applicable to *L. serriola*.

Table 3. r-statistics calculated from the Mantel permutation test (9999 permutations); ***p<0.001.

Comparison	Correlation
Pairwise Fst values between all populations *Vs* Geographic distance between all populations	0.26^{***}

Table 4. Classes and mean Fst per class used for Mantel correlogram; ns, non significant; ** $p \leq 0.01$; ***$p<0.001$.

Class	Upper limit (km)	mean Fst	Mantel's r	
1	327.9	0.479	-0.129	***
2	506.2	0.485	-0.119	***
3	655.1	0.513	-0.075	***
4	776.8	0.520	-0.065	***
5	897.0	0.536	-0.039	ns
6	1016.4	0.576	0.023	ns
7	1172.3	0.573	0.018	ns
8	1367.6	0.585	0.038	ns
9	1588.1	0.616	0.086	***
10	1889.3	0.637	0.119	***
11	3164.8	0.650	0.140	**
	overall	0.561		

Despite the high overall Gst and the significant pattern of isolation by distance, only a very weak pattern was displayed by the PCAgen analysis (Fig. 8). The first axis had an associated Fst of only 0.0716 meaning that it explained only about 7.16 % of the total genetic variation, or 13 % of the total between populations variation. The first axis separated mainly the UK-populations plus one from Denmark from the rest. These were mainly dominated by a single genotype with some mutational variants, even though they were separated by up to 200 kilometres within the UK and almost 1000 kilometres between the UK and the Danish population. Several populations from Holland (Hol 16, 17, 20), Luxembourg (LS 21) were dominated by the same genotype.

Geographic structure of genetic diversity

The AMOVA performed on groups based on bioclimatic zones in Europe explained only 5% of the total amount of genetic variance between populations (results not shown).

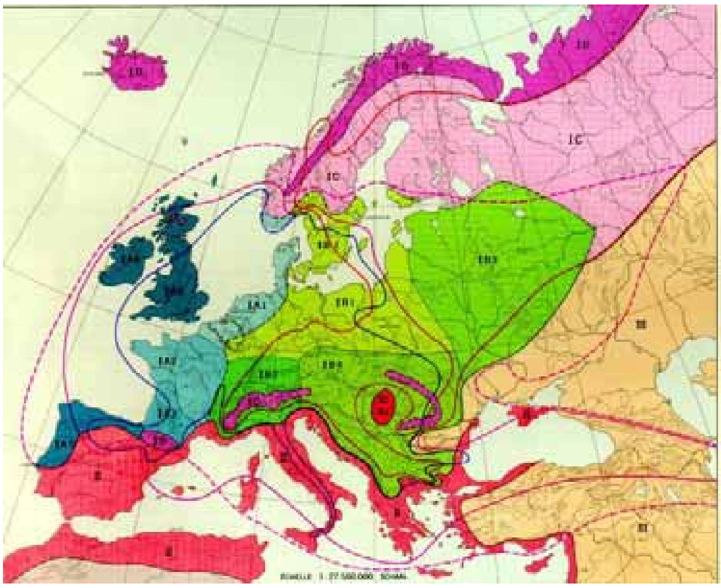

Figure 9. Bioclimatic zones used for AMOVA: IA1 (boreo-atlantic zone), IA6 (Great-Britain), IB1 (baltico-rhénan zone), IB2 (alpine zone), IB4 (west-pontique zone) , II (Mediterranean zone)

The highest amount of genetic diversity was found in central Europe: Hungary, Slovakia, Czech Republic and Poland, while populations from west Mediterranean (Portugal and Spain), South-Italy, Great-Britain, the Alps and Southern-Scandinavia possessed generally lower gene diversities. Discrepancies were present in Scandinavia with some polymorphic populations and a monomorphic one (Fig. 10).

Mantel test between differences in gene diversity between pairs of populations and geographic distance was not significant (r = 0.036, p = 0.152). However, a correlogram indicated that there was indeed some spatial pattern in the variability, with classes 1, 2 (up to 506 km) and , 7 and 8 (between 1016 km and 1368 km) being significant after a progressive Bonferroni correction (Table 5, Fig. 11). Thus, for low distances, differences of genetic diversity were significantly negatively correlated. On the contrary, they were significantly positively correlated for distance of classes 7 and 8.

A standard correlation test reveals a significant correlation between the gene diversity and longitude (r = 0.27), but not with latitude (r = -0.13). However, excluding UK populations, the correlation with longitude was no longer significant.

Kriging of gene diversity values showed that Eastern Europe and South of France and North of Italy represented areas of high genetic diversity (Fig. 12a and b). A region of lower value is found around the Alps. The gene diversity decreased westwards and northwards from these areas and was the lowest in Great Britain and Portugal.

Table 5. Classes and Mantel's r per class used for Mantel correlogram; ns, non significant; ** $p \leq 0.01$; ***$p<0.001$.

Class	Upper limit (km)	Mantel's r
1	327.9	-0.097 ***
2	506.2	-0.046 ***
3	655.1	-0.028 ^{ns}
4	776.8	-0.011 ^{ns}
5	897.0	0.008 ^{ns}
6	1016.4	0.006 ^{ns}
7	1172.3	0.051 ***
8	1367.6	0.060 ***
9	1588.1	0.015 ^{ns}
10	1889.3	0.001 ^{ns}
11	3164.8	0.040 ^{ns}

Chloroplastic PCR-RFLPs

Only one combination enzyme-primer was polymorphic (psbCS-Alu1) with 2 haplotypes (Table 6). It was used to analyze 771 samples from 58 different populations. The pattern of distribution of the haplotypes is shown in Figure 13.

Thirty seven populations from Europe and Egypt were monomorphic for haplotype 1 and two populations from Germany were monomorphic for haplotype 2. A total of nineteen populations, from all countries studied except Sweden, Slovakia, Spain and Greece, were polymorphic for this maternally inherited trait. Haplotype 2 was absent from the Mediterranean basin and from the Iberian Peninsula, while it was present in Eastern Europe, close to the Black Sea. Italy possessed both haplotypes in its northern part. Most polymorphic populations were located in central Europe.

Gst was quite similar to that for the SSRs (Table 2). Because of the low variability, it was not possible to compute a matrix of pairwise Fst-values, as Fst is undefined when all populations are fixed for the same allele. Instead, we calculate a distance matrix of the difference in the frequency of haplotype 1, and tested whether there was any spatial structure. This was not the case in a simple Mantel test (Mantel's r: -0.052162, $p = 0.2781$).

Table 6. Cytoplasmic DNA fragments – restriction enzymes combinations investigated for PCR-RFLP inter-populations markers

Primers	**Enzymes**	**Primers**	**Enzymes**
atpb	Pst1	**TrnHA**	EcoR1
	BamH		Msp1
TrnFT	EcoR1		RSAI
	Alu1		HaeIII
psbCS	EcoR1		Taq1
	Taq1		BamH
	Alu1		Pst1
	Msp1		HindIII
TrnHK	EcoR1	**Nad1**	EcoR1
	Msp1		RSAI
	Alu1		HaeIII
TrnST	EcoR1		Taq1
	Alu1		BamH
	Msp1		Pst1
	Taq1		HindIII
TrnTL	EcoR1		
	Msp1		

Table 7. Haplotype frequencies per populations

Population	Haploltype 1 (%)	Haplotype 2 (%)	Population	Haploltype 1 (%)	Haplotype 2 (%)
A3	58	42	P2	100	0
A5	83	17	P5	100	0
A9	87	13	PK1	67	33
A03	100	0	PK2	7	93
AO6	100	0	PK3	80	20
AO9	100	0	PK4	100	0
CH1	100	0	PL1	100	0
CH16	100	0	PL8	67	33
CH17	93	7	SVK1	100	0
D12	67	33	SVK4	100	0
D5	100	0	SW14	100	0
DENO	100	0	SW7	100	0
DENS	87	13	US1	100	0
E10	100	0	US2	100	0
E16	100	0			
E21	100	0			
E3	100	0			
E8	100	0			
EGY1	100	0			
EGY2	100	0			
F10	23	77			
F12	100	0			
FF	100	0			
GM	100	0			
H1	100	0			
H3	60	40			
H8	80	20			
HO16	100	0			
HO20	0	100			
I11	40	60			
I3	100	0			
I4	100	0			
I5	100	0			
I7	100	0			
I8	100	0			
I9	100	0			
LBM1	71	29			
LBS4	100	0			
LS13	9	91			
ls17	0	100			
LS9	25	75			
LSA	7	93			
LSC4	53	47			
LSC6	100	0			

Discussion

Overall diversity

The genetic differentiation between populations was very high (G'st =0.880). Most of the genetic variation was present between populations, and only little within (Hs = 0.35). This is in accordance with the life-history of *L. serriola* which is an autogamous annual species that lives in temporary habitats. Populations undergo generally a rapid turnover. Empty patches are generally colonised by few seeds giving rise to few founders. Each of them can produce from 10'000 to 100'000 wind-dispersed seeds that further fill the habitats.

Mutational events probably played a significant role in producing individual variations since individuals could have genotypes that only differ at a single or few loci. Moreover, some variant of widespread multilocus genotypes actually only differed by one or two mutation steps, which would indicate a stepwise-like mutation (SMM) behaviour. We found that differentiation among populations taking into account the allele size was significantly larger than differentiation based on allele identity. Thus even if SMM is probably not accurate to explain the mutational process in *L. serriola*, it gives nevertheless a closer approximation to what really happens than the infinite allele model (IAM).

Patterns of isolation by distance

Isolation by distance was demonstrated. Thus, close populations tended to be more genetically similar than distant ones. It is in accordance with the hypothesis of progressive spread of the species from one suitable site to the other.

Local genetic correlation may result from diffusion from a source population to nearby habitats. Moreover, isolation by distance probably result from migrants characterized by different genotypes.

The low amount of heterozygote (Ho = 0.014) and the very high overall inbreeding coefficient (Gis = 0.96) indicate indeed a high selfing rate and an extremely low level of gene flow between populations. Moreover, the similarity between Gst obtained from both nuclear and chloroplastic markers indicated that the nuclear genes were practically maternally inherited. This is not naturally the case except for strict selfers. Thus the high degree of self-fertilization ensured by the floral mechanisms of *Lactuca serriola* (Jones 1927; Lindqvist 1960 a; De Vries 1990) is confirmed and contributed probably highly to population isolation and structuring.

Geographic structure of genetic diversity

Despite the high overall Gst and the significant pattern of isolation by distance, only a very weak pattern was displayed by the PCAgen analysis. Similarly, simple Mantel test revealed that there was no structure in the distribution of the frequency of cp haplotype 1. The lack of any structure at the continental scale probably reflects the high mobility of the species and its recent rapid spread over Europe. *L. serriola* is a ruderal plant species that thrives close to roads and that grows mainly in synanthropic disturbed habitats. Its dispersal is therefore very closely related to human activities and transports, which strongly increased during the past few centuries. Nowadays, anthropogenic urbanised ecosystems represent patches interconnected by a very dense transport network. They allow "r" strategist, ruderal plant species like *L. serriola* to build viable populations (Lebeda et al. 1999; Dolezalovà et al. 2001; Landolt 2001; Lebeda et al. 2004; Dukes et al. 1999; Parendes et al. 2000; Hill et al. 2002; Wittig 2002; Kowarik 2003; Forman et al. 1998; Trombulak et al. 2000) and to quickly disperse their seeds over long distances. Polymorphic populations for a maternally inherited trait as shown in figure 13 suggest that some areas are colonized through repeated introductions. The presence of two haplotypes in a single population indicates indeed the presence of at least two founder events.

A remarkable observation is that British populations were mainly dominated by a single genotype even though they were separated by more than 200 km (Fig.8). This genotype was also found in one population from Denmark, Luxembourg and several Dutch populations. The situation in Great Britain could be explained by a recent colonisation through a founder event. The fact that *L. serriola* f. *integrifolia* is the dominant form in the British Isles (Carter et al. 1985; Lebeda et al. 2001) probably shows that few events of introduction are at the origin of the colonisation of Great Britain. In the same way, the discrepancies found in Scandinavia with southern polymorphic populations and northern monomorphic ones are possibly issued from a single founder event (Fig.14).

At the continental scale, no correlation between gene diversity and longitude or latitude could be shown. However, the positive correlation between geographical distance and difference in gene diversity for populations distant between 1016 km and 1368 km showed that gene diversity is not equally distributed over Europe (Fig.11). Indeed, as figure 9 shows, most of the diversity is found in south-eastern populations and in central Europe. Allelic richness is lower in northern population (Fig.5) and less common alleles (Fig.4) were almost absent in these populations.

Kriging of gene diversity suggests a diffusion of the Gene Diversity northwards and westwards from Eastern Europe (Fig.13) that would indicate a colonisation of the continent from the South-East. This is not surprising as Eastern Europe is part of its original distribution. It is still not clear whether Southern France was colonised by the East along the Alps or from Northern Africa, which is a possible way of migration. The Alps probably stopped the northern progression of Italian populations. Interesting enough is that the Iberian Peninsula had a low amount of gene diversity; even though it was considered a potential area of origin and/or diversification of the species. Moreover, the allelic compositions of its populations were rather different than the ones from South-France (Fig.14).

Conclusion

Our study demonstrate that nuclear microsatellite and chloroplastic PCR RFLPs markers are adequate tools for the analysis of population genetic structure and the inference of population history at broad geographical scales. Our molecular results corroborate the recent and rapid colonisation of Europe by the species already shown in our historical biogeography (Chapter 1). They support on one hand a historical diffusion of the species, and on the other hand, several founder events coupled with short and probably long distance migrations possibly at different periods. The molecular analysis of wild populations and a better understanding of the geographical repartition of the genetic diversity will also serve in the perspective of a more efficient wild germplasm sampling for gene bank collection.

Acknowledgments

We wish to thank Danny A.P. Hooftman, Hans C.M. den Nijs, Gerard B. Oostermeijer, Rikke Bagger Jørgensen, Nicola Schoenenberger, Simon Riat, Marc Hulmann for the sampling. We also like to thank all other ANGEL members for fruitful discussions. We are very grateful to Jean-Marie Ndoumvé, Anouk Béguin and Sarah Mamie for their precious help in the lab and to Dr. Mahmoud Bouzelboudjen for its help with kriging. We thank also the botanical garden of Neuchâtel for the cultivation of plants. This study is part of the EC-funded project "ANGEL" (EU-QLK-CT-2001-01657).

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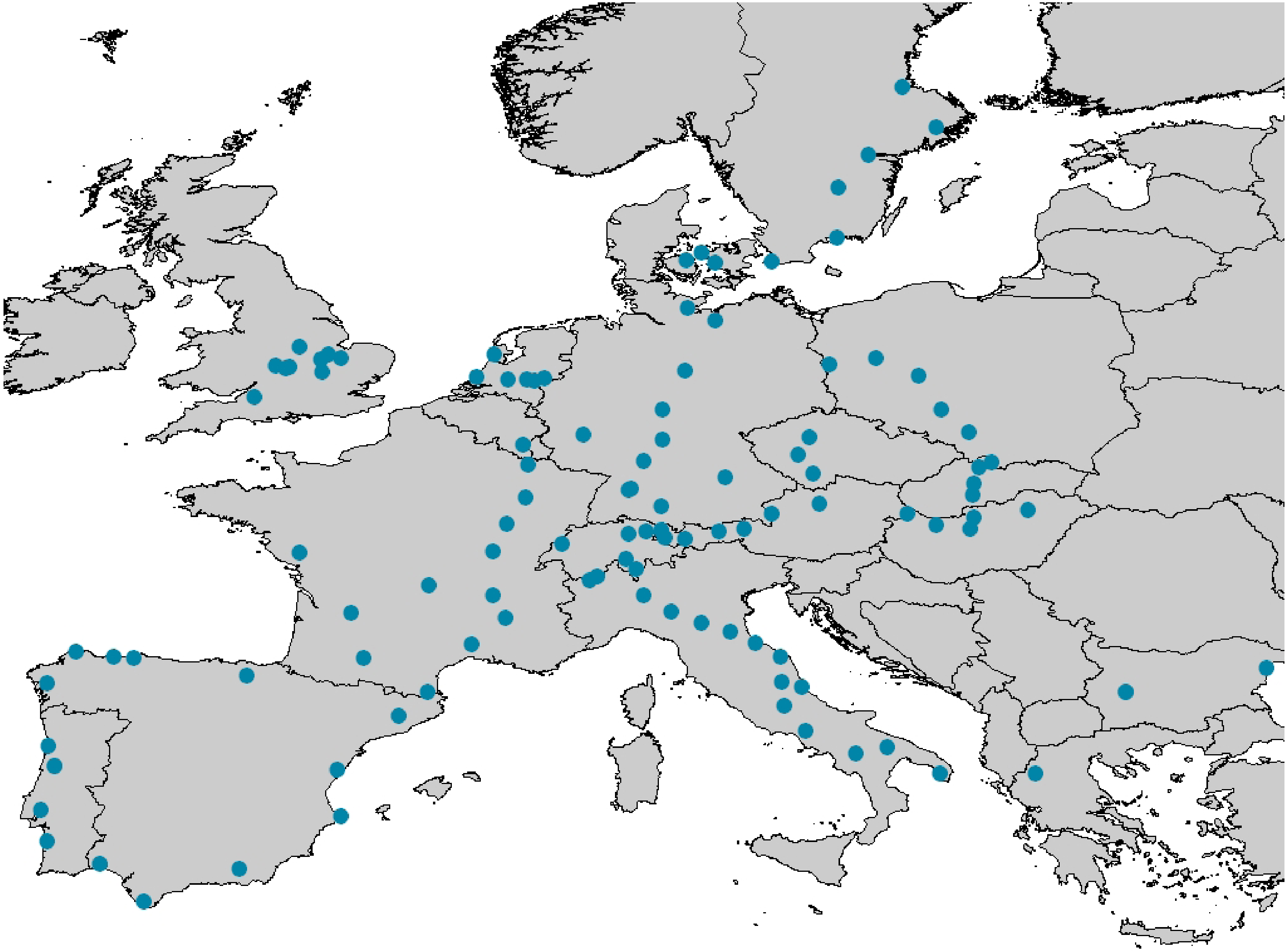

Figure 1. Wild populations of *L. serriola* analysed

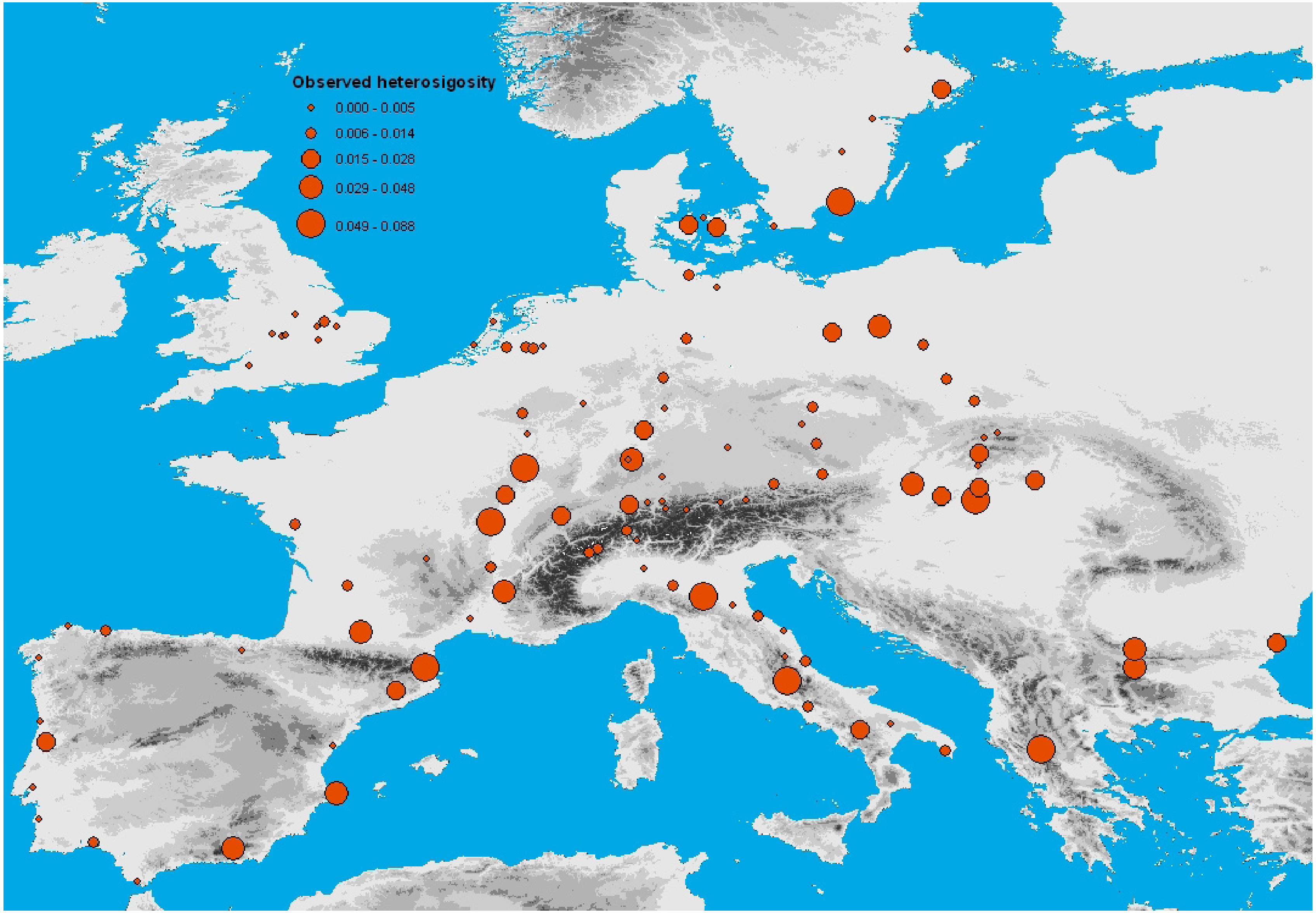

Figure 2. Observed heterozygosity values

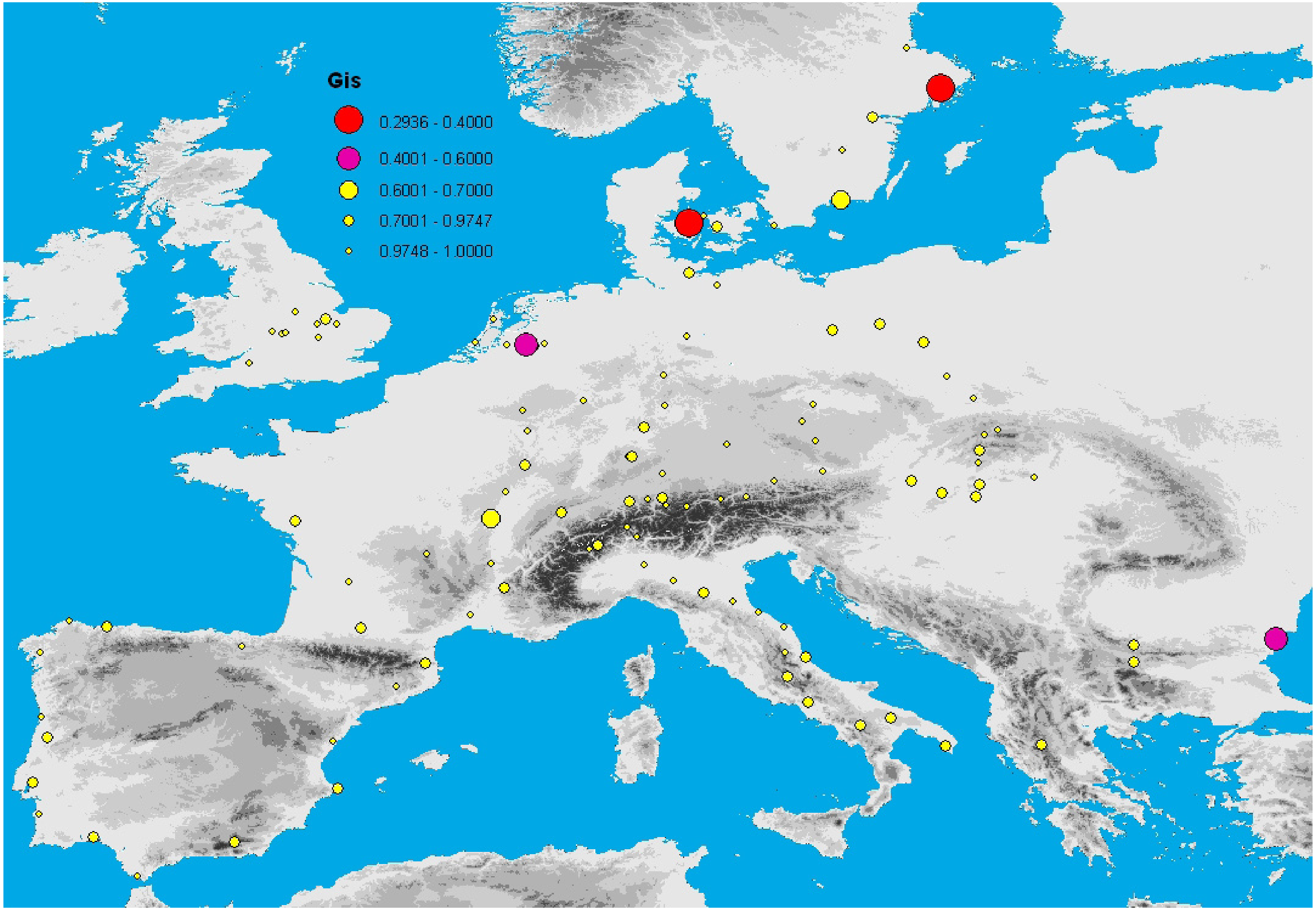

Figure 3. Inbreeding coefficient values

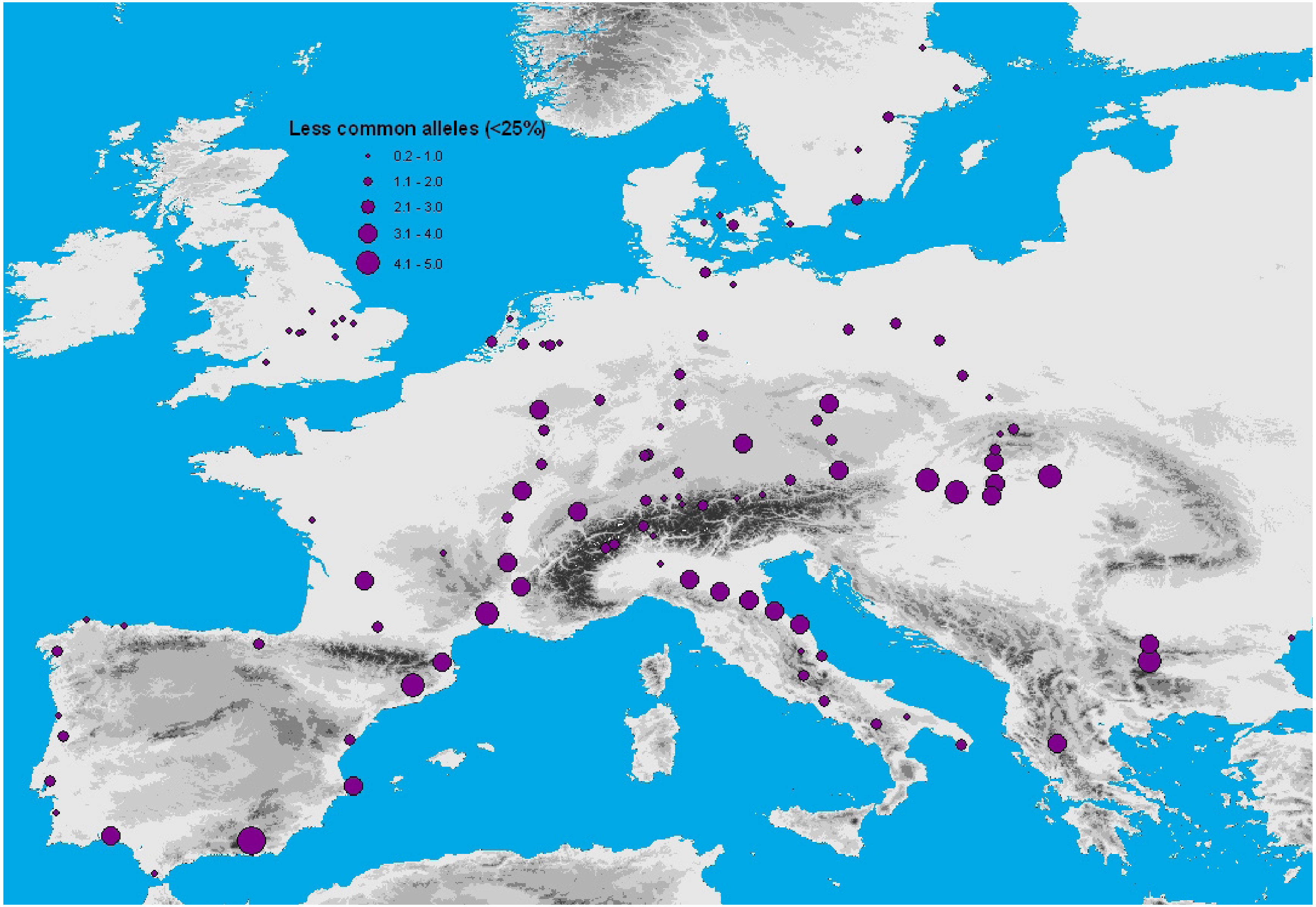

Figure 4. Distribution of the less common alleles (present in less than 25 % of the populations).

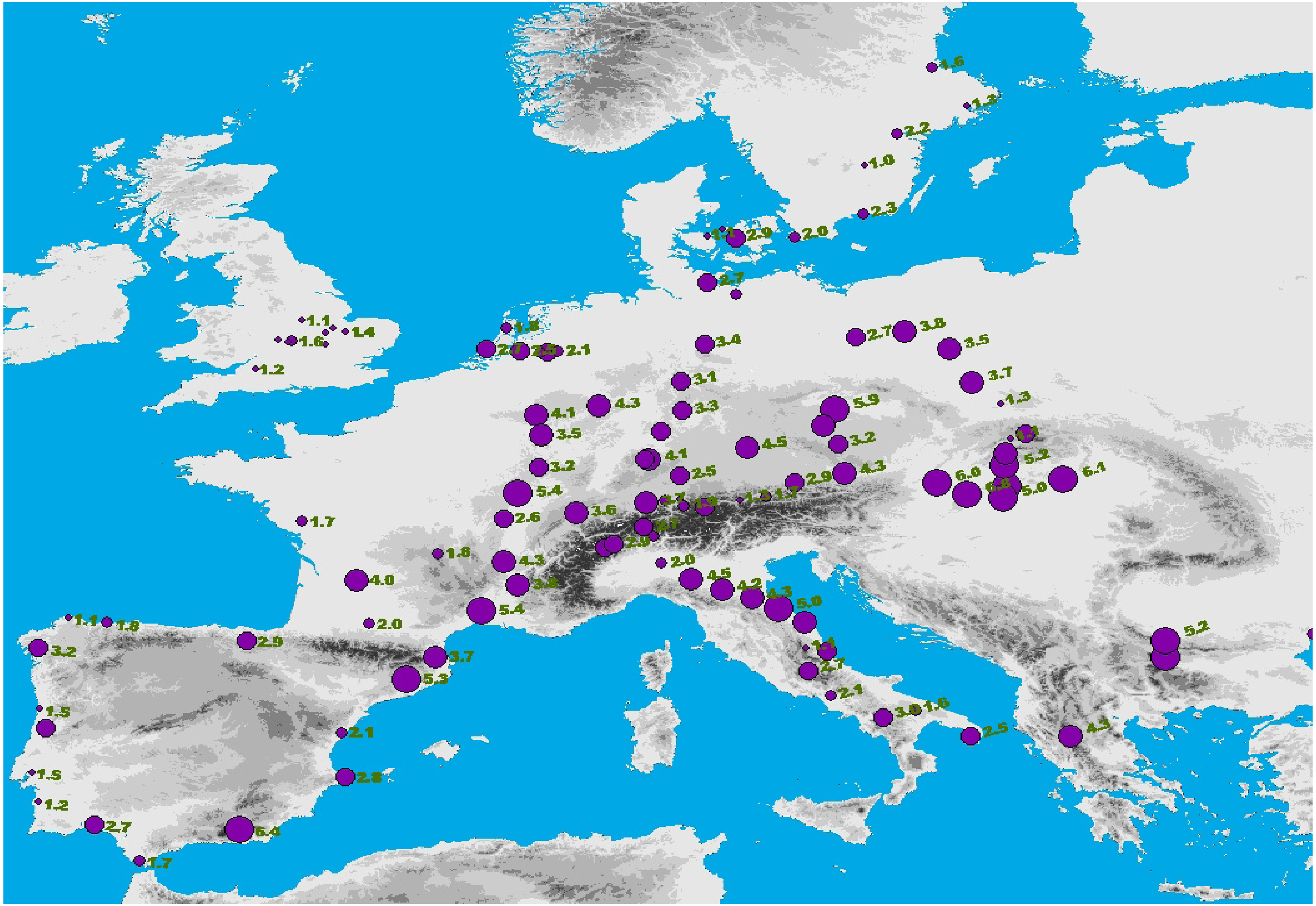

Figure 5. Mean number of alleles per population.

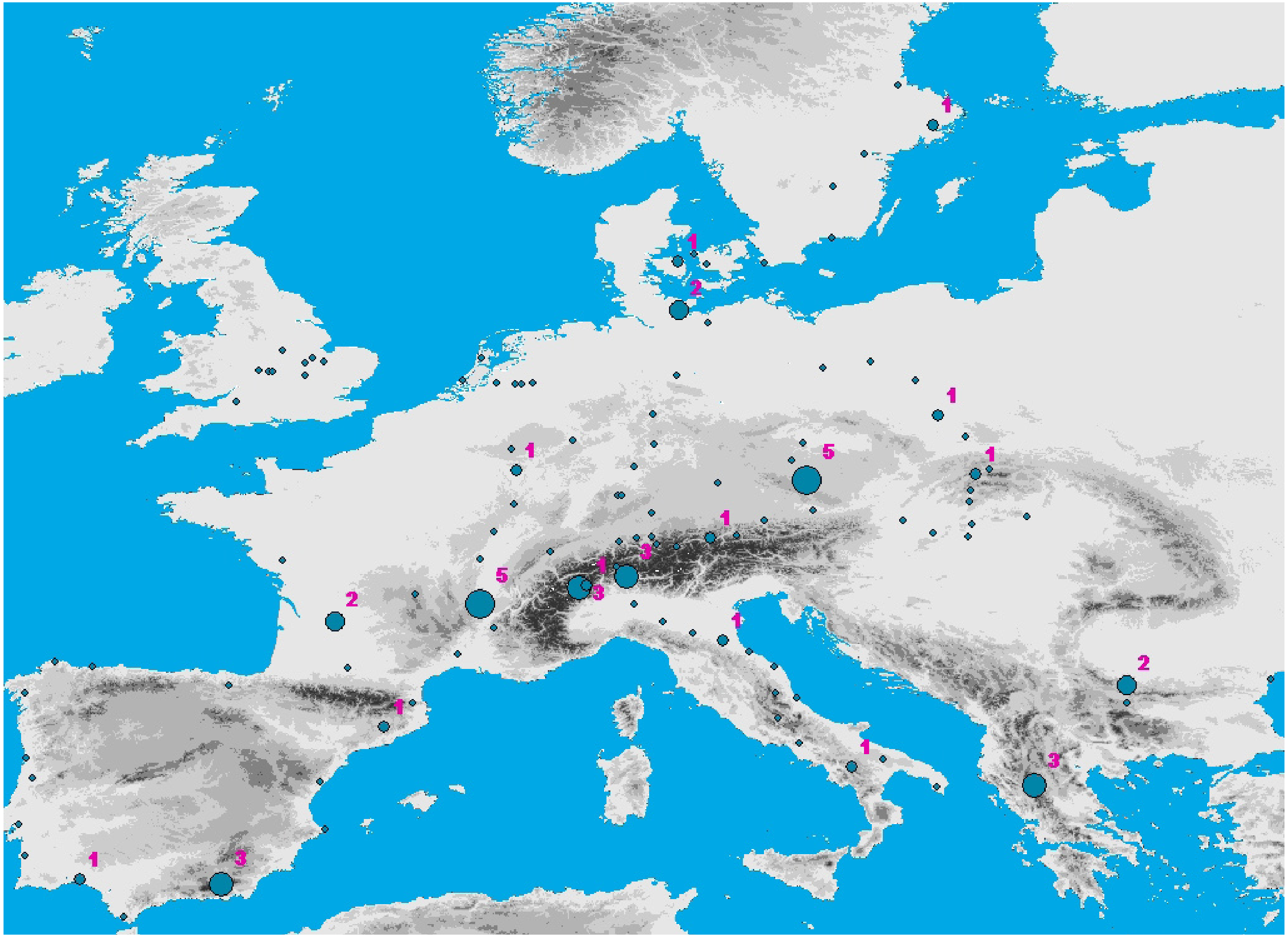

Figure 6. Distribution of the private alleles.

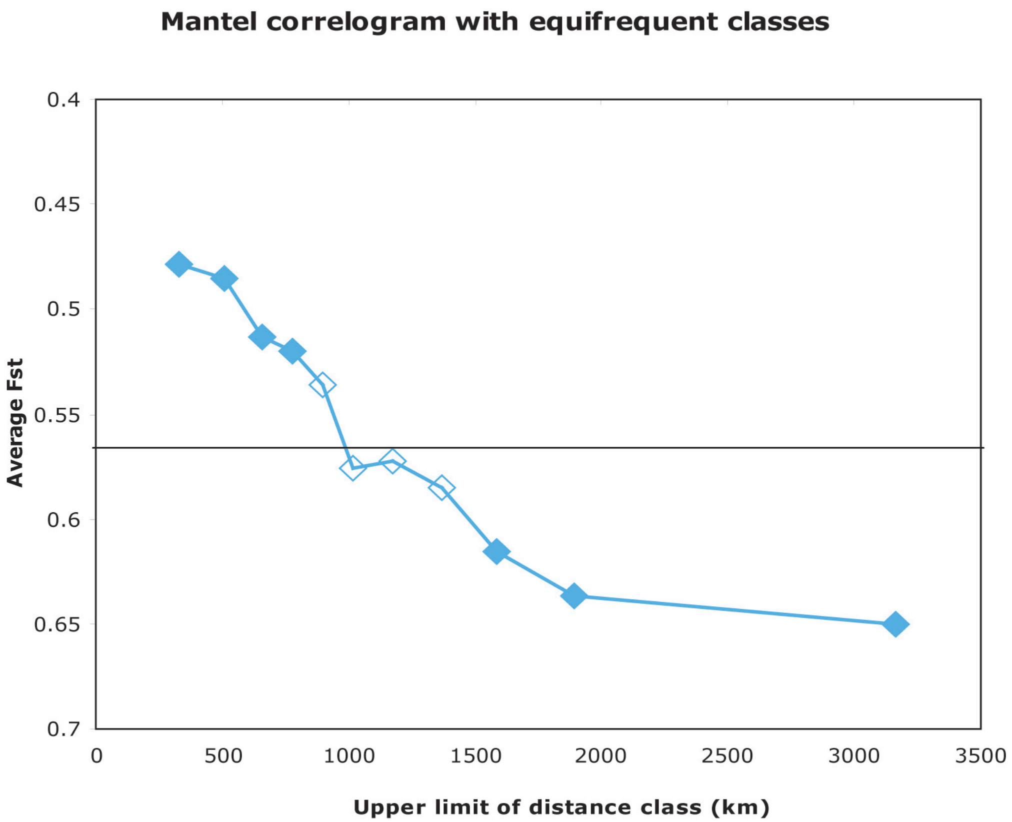

Figure 7. Mantel correlograms with equifrequent classes relating the pairwise Fst value between pop to the distance between populations. Statistically significant auto-correlations (at a Bonferroni corrected 5 % level) are represented with filled symbol.

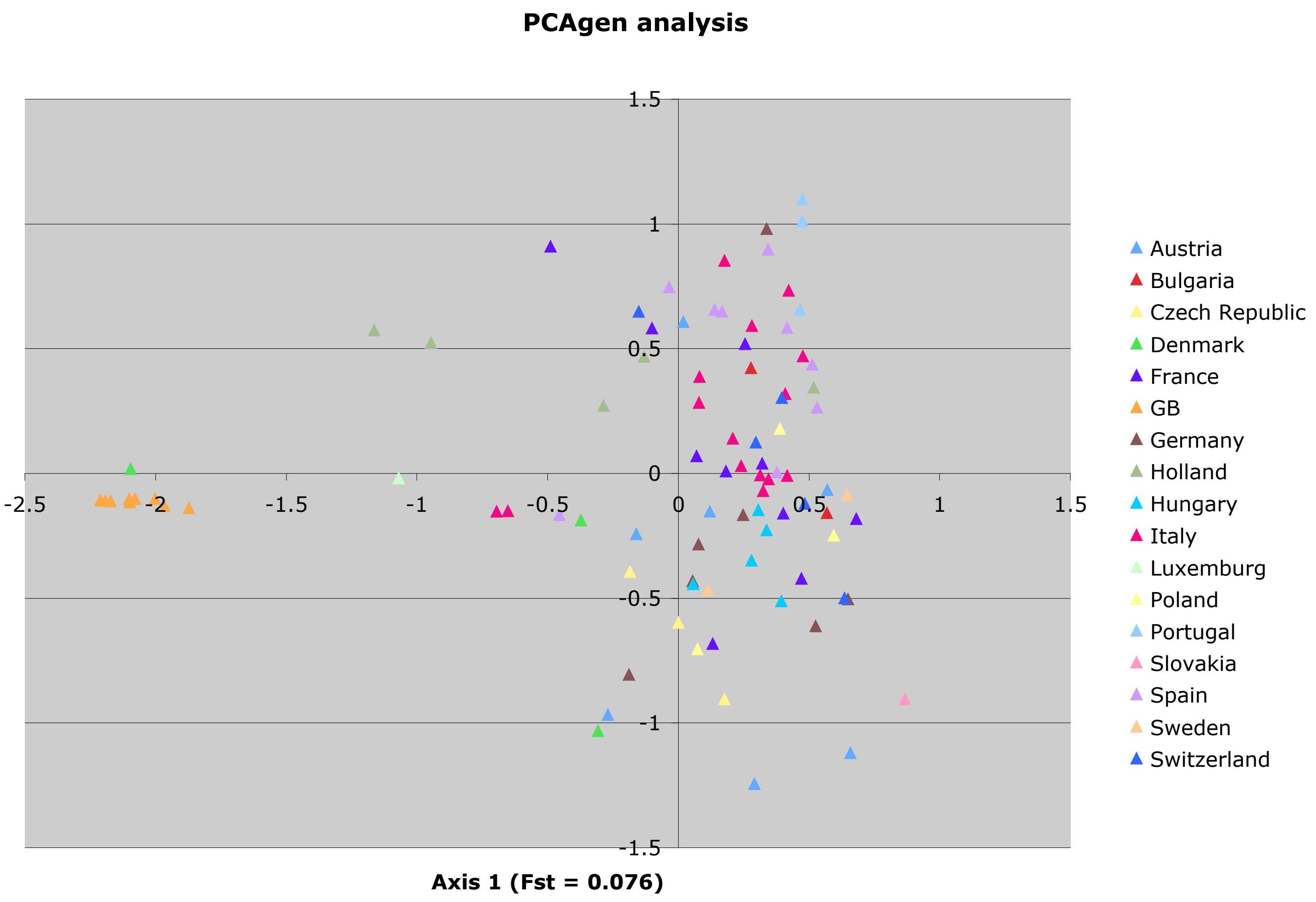

Figure 8. PCAgen analysis showing the distribution of genetic variation. Only the first axis was significant with an associated Fst of 0.0716 (p = 0.04). The second axis was not significant.

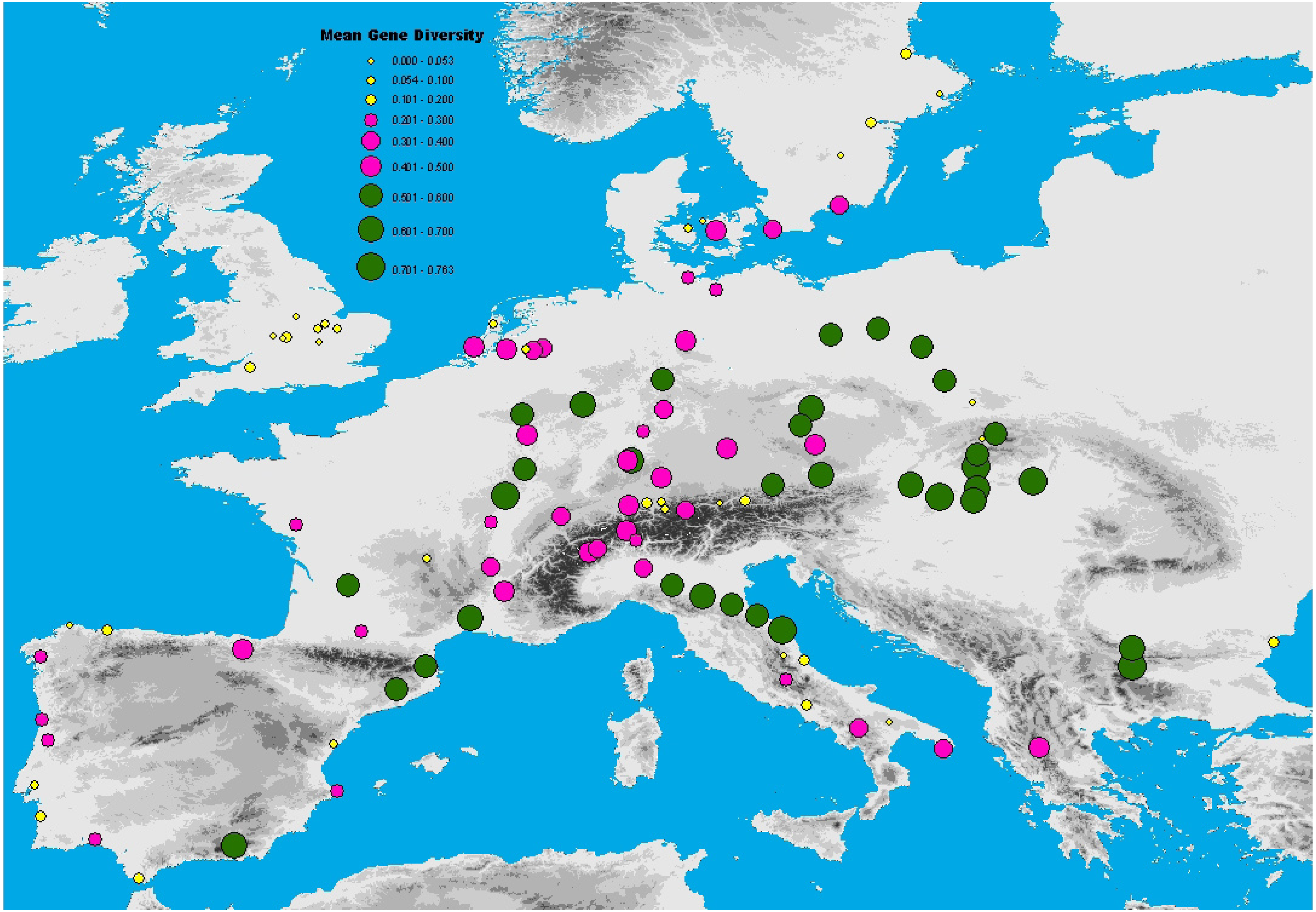

Figure 10. Overall Gene Diversity for all the populations analysed

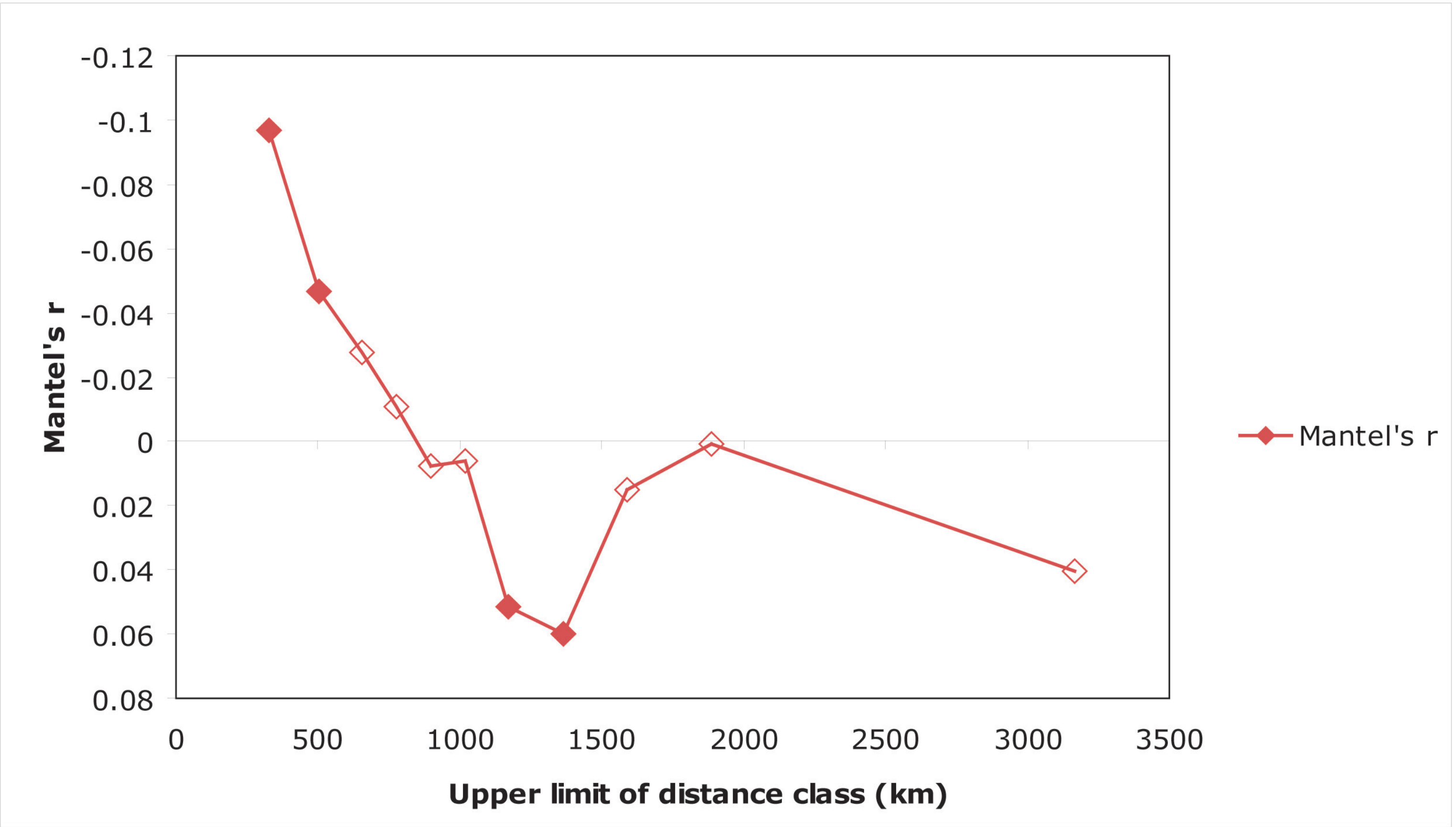

Figure 11 . Mantel correlograms relating the genetic diversity of the populations to (a) the distance between populations. Statistically significant auto-correlations (at a Bonferroni corrected 5 % level) are represented with filled symbol.

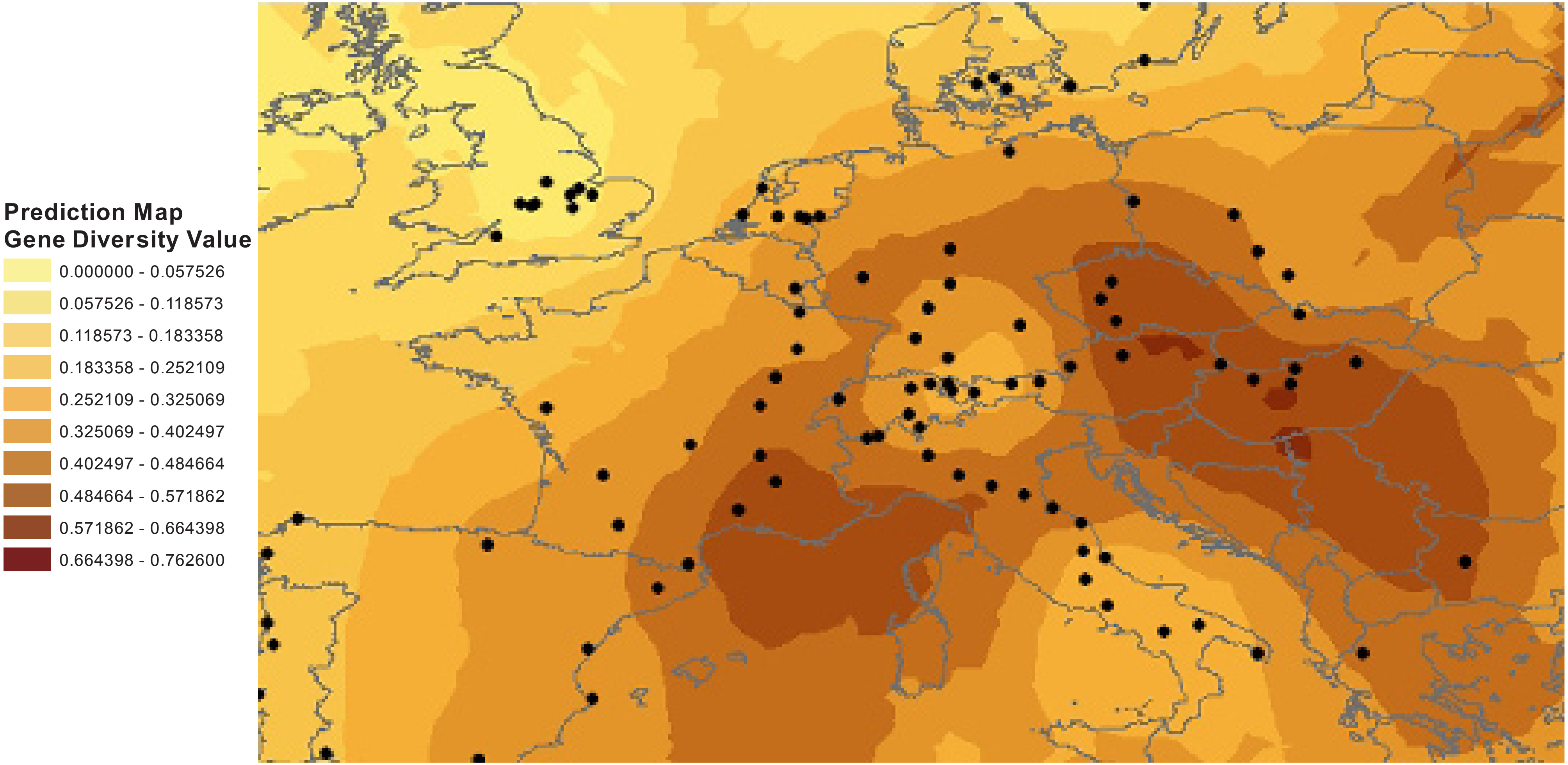

Figure 12 a. Kriging of Gene Diversity (prediction map)

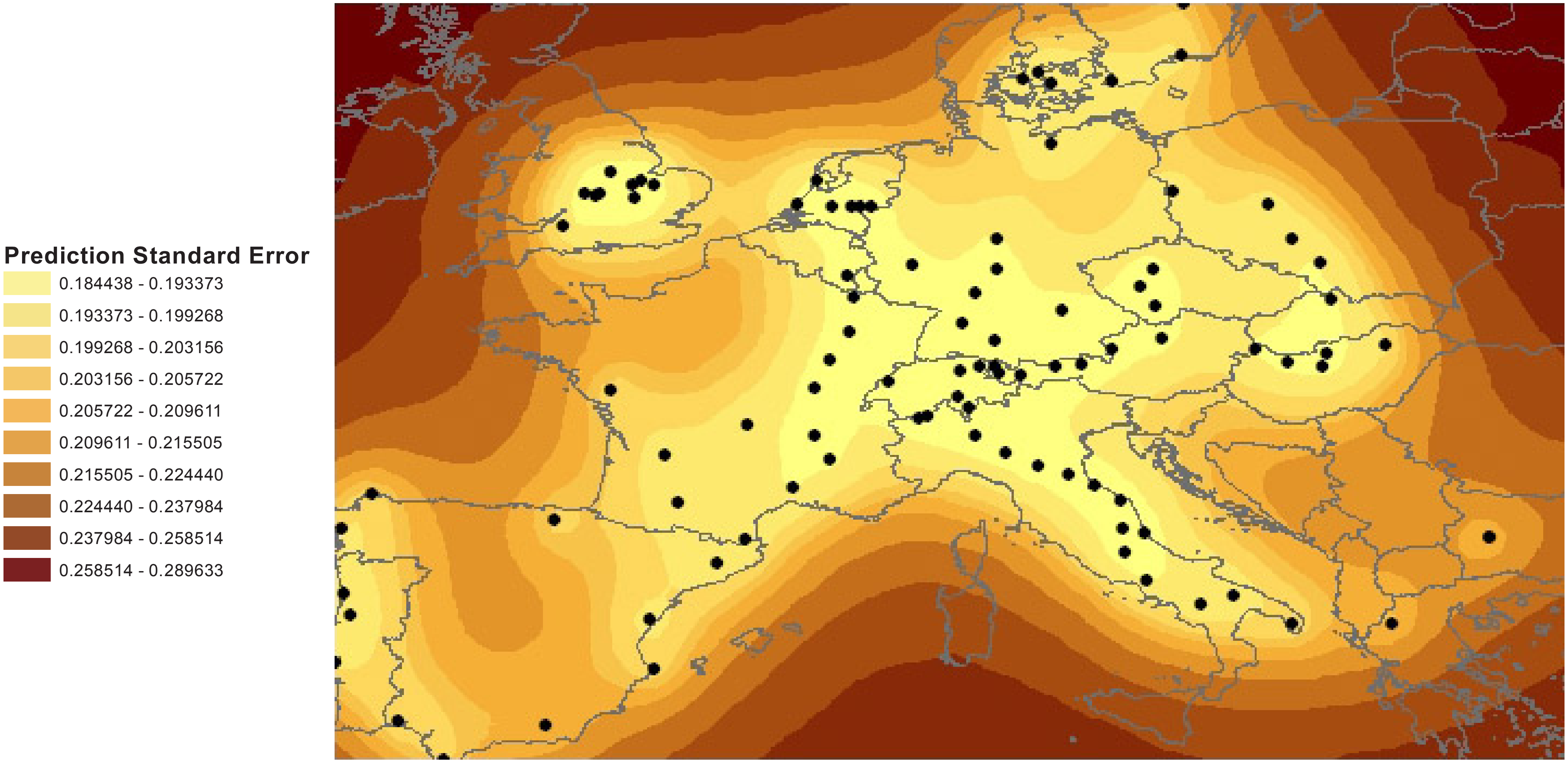

Figure 12 b. Standard error of Gene Diversity kriging

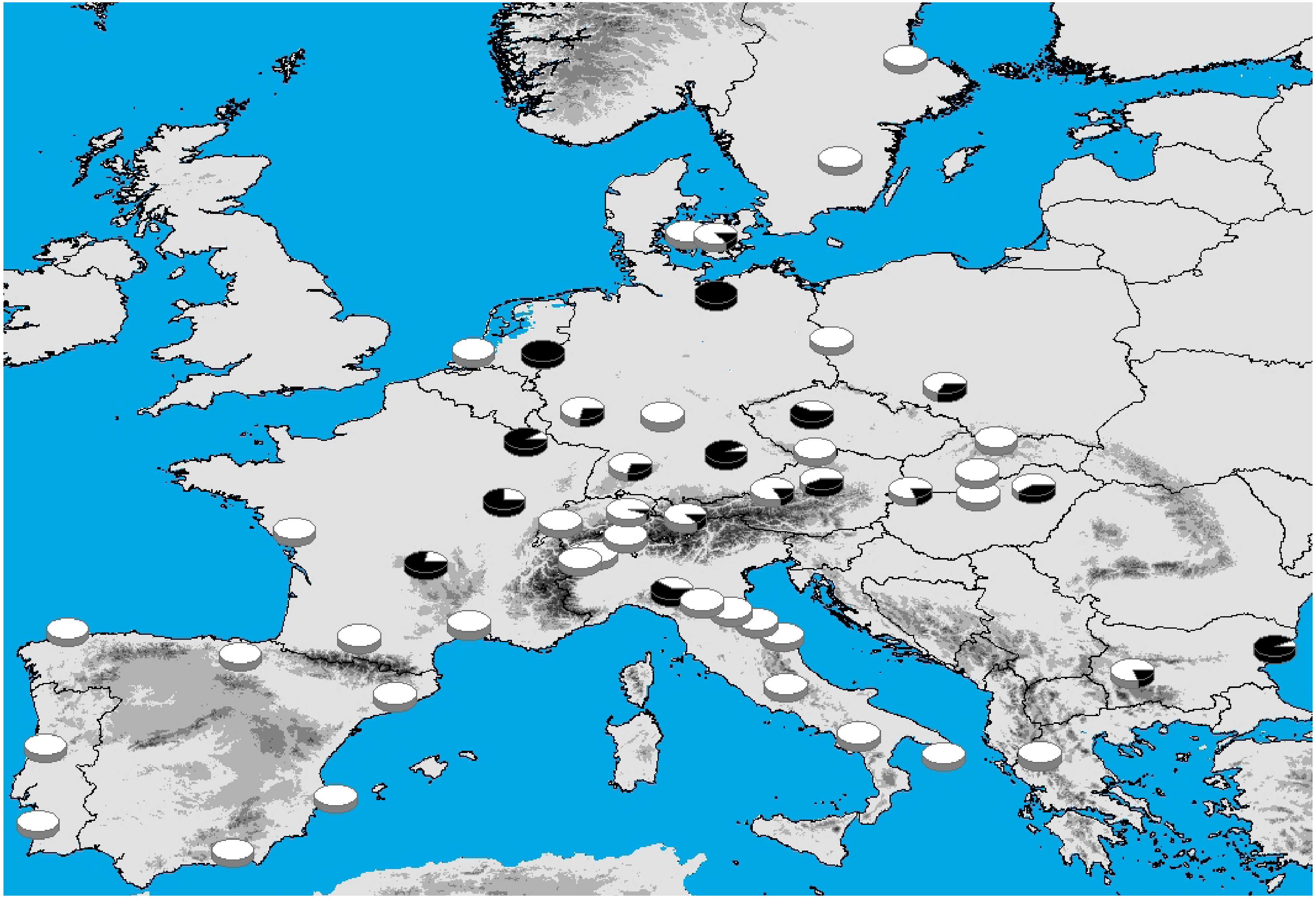

Figure 13. Distribution of the two cp haplotypes. Haplotype 1 = white and haplotype 2 = black.

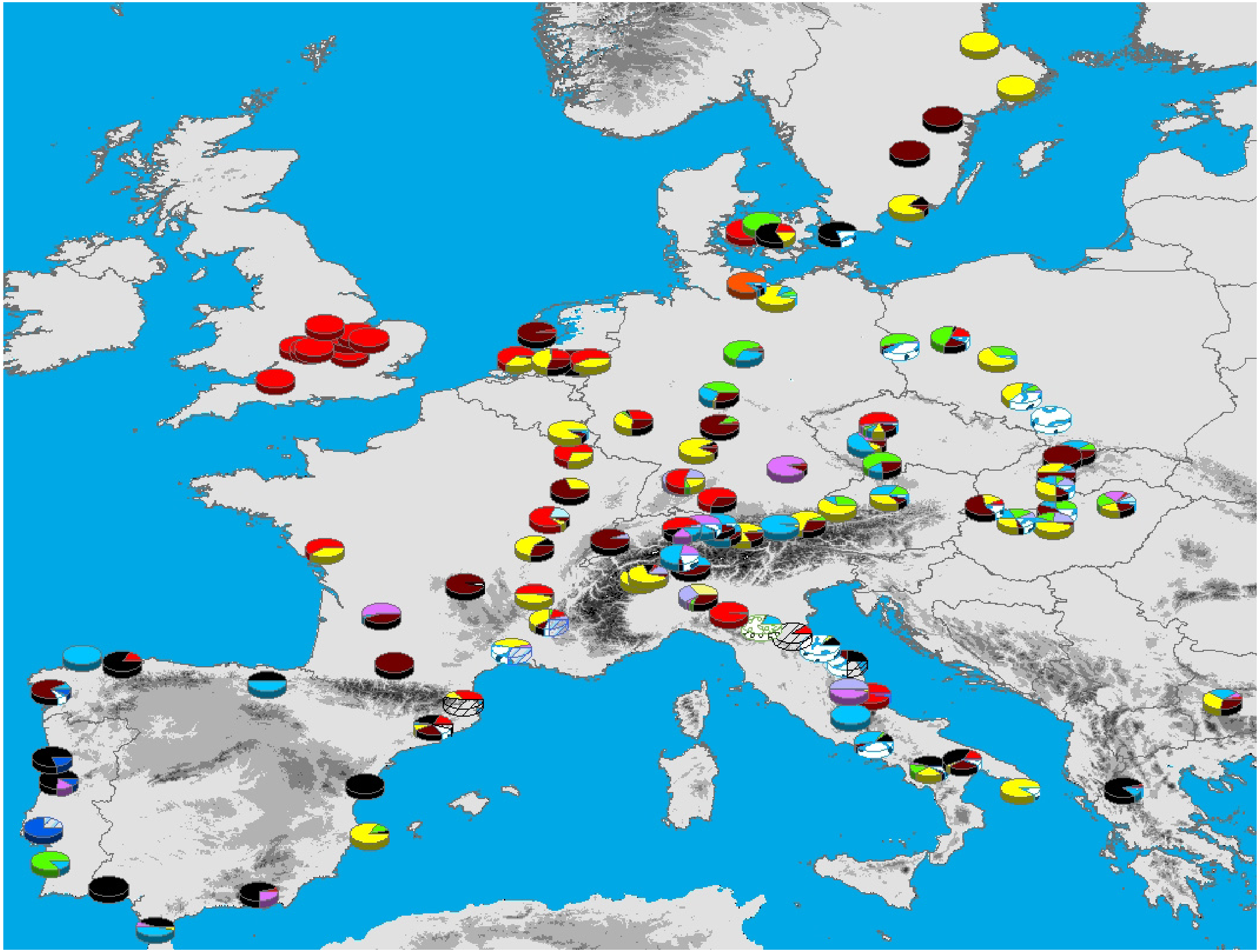

Figure 14. Distribution of nuclear SSR alleles for locus LsE 011

CHAPTER 3

DIRECT EVIDENCE OF GENE FLOW BY POLLEN FROM CULTIVATED LETTUCE (*LACTUCA SATIVA* L.) TO ITS WILD RELATIVE PRICKLY LETTUCE (*L. SERRIOLA* L.).

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Abstract

Hybridisation and subsequent introgression from cultivated crop into wild relatives could have important evolutionary and ecological consequences like respectively, erosion of natural gene pools or increased invasiveness. In the present study, we investigated several aspects of natural hybridisation between *L. sativa* and its wild relative *L. serriola*, two cross-compatible, predominantly autogamous and insect pollinated species.

During summer 2003 and 2004, an experiment was carried out in two locations in North-Switzerland in order to test if crop to wild gene flow occurs in natural conditions. In each field trial, we planted 216 *L. serriola* individuals in 12 rows of 18 plants at 0, 1, 5, 15, 25, 40 m North and South of a 25 x 1 m plot composed of 400 plants of crop lettuce (*L. sativa*). All the seeds of the wild species were collected and sown. This progeny was screened both, morphologically, and with RAPD markers, in order to detect natural hybridisation events.

Hybridisation occurred up to the maximal distance tested (40 m) and individual hybridisation rates varied between 0 to 26%. It decreased as a function of the distance. More than 80 % of the wild plants underwent a cross fertilisation (incidence of hybridization, IH) event at 0 m and 1 m. IH ranged from 32 % to 62 % at 5 m and from 5 to 21 % at 15 m. The incidence of hybridization was higher at 25 meters than at 15 m with ranges from 12 to 22 %. Even at 40 m, 4 to 5 % of the wild plants produced hybrids.

In sympatric crop-wild populations, pollen flow from cultivated lettuce to its wild relative has to be seen as the rule rather than the exception, at least at close distances. Moreover, despite the floral mechanisms ensuring self-fertilisation in both species, incidence of hybridization can be relatively high even at long distances. Common assumption that autogamy, at least in lettuce, will hamper crop (trans-)gene transfer to wild species should probably be revised.

Keywords: gene flow, hybridization, hybrids, autogamy, risk assessment, wild relatives, RAPD.

Introduction

In the last decade, the extensive cultivation and commercialization of Genetically Modified (GM) crops has exacerbated the arguments about crop-to-wild gene flow and its subsequent putative negative environmental effects. A major concern is the potential changes in the ecology of crop related wild species, due to the introgression of transgenes into wild population living in agricultural areas (Ellstrand et al. 1999; Wolfenbarger et al. 2000). This could lead to an increased invasiveness and/or weediness of introgressants (Snow et al. 1997), both ending in economic threat for the cropping system if super-weeds are created and/or in environmental threat if competitors are dispersed in natural assemblage (Klinger et al. 1991). Other consequences of gene flow as genetic erosion or pollution of natural gene pools leading to the extinction of wild taxa through out-breeding or swamping have also been stressed out (Ellstrand et al. 1999). Alien species or indigenous weeds invading agricultural systems and/or colonizing natural areas are now a subject of concern for both the scientific community and the international organizations dealing with biodiversity maintenance and weed control. Furthermore, these risks are likely to be higher where wild plant show already weedy tendencies (Snow et al. 1997). In this context, *L. serriola* is already described as an invasive weed in Canada and in Argentina, where it occurs in a variety of crops where no-till or conservation tillage systems are used (Weaver et al. 2003) and in many European countries, where it occupies ruderal places (Lebeda et al. 2004). Moreover, in the United States as well as in Australia herbicide-resistant weedy populations of prickle lettuce have already been found (http://www.weedscience.org/in.asp).

Clearly, the risk of (trans)gene escape increases when the flowering periods of a sexually reproducing crop and of its wild relatives overlap and when no pre or postzygotic barriers to hybridization exist. Both conditions are met for *L. sativa* and *L. serriola*, since the two species are extremely closely related and are often considered as conspecific (Lindqvist 1960 a; De Vries 1990), (Whitaker 1939), or ecospecies (De Vries et al. 1994) (Lindqvist 1960 a). Crop lettuce differs from prickly lettuce only for characters connected to domestication, like rapid growth, short life-cycle, absence of dormancy, and no or few prickles on the leaves and on the stem (Frietema de Vries et al. 1994). The absence or disabling of dispersal mechanisms have been found to provide a very reliable mean to distinguish between *L. sativa* (erect involucre) and *L. serriola* (reflexed involucre) (Frietema de Vries et al. 1994). The two species are diploid, and their chromosome length and genetic background is equivalent (Lindqvist 1960 a; Koopman et al. 2001).

The quantification of crop-by-crop or crop-by-wild hybridization is not new to plant breeders and seed producers, who have been concerned with it for a long time. In particular, studies on seed purity maintenance produced considerable results on gene flow, on pollen dispersal and on required isolation distances to minimise it (Bateman 1947). Durst (1930) found that cross-fertilization in *L. sativa* is a rare event. Thompson (1948) observed about 1% cross pollination between different lettuce varieties grown in adjacent rows (spaced of 1.2 m) and an average amount of cross-pollination of 2.9 % for one variety surrounded by plants of another one. Watts (1958) found that cross pollination was higher late in the season, and explained this observation by a seasonal increase in the hover-fly population. It is true indeed, that since the pollen is not windblown, pollinators contribute substantially to the effective transfer of pollen to the stigma, within, and likely between, flower heads. Not surprisingly, distance and pollinators availability have been found to be the most relevant factors for pollen migration between different *L. sativa* varieties (Thompson et al. 1958; Goubara et al. 2003; Goubara et al. 2004). While the case of wildXcrop hybridization cannot be inferred directly from these results, we can assume that the factors influencing the most the pollination of prickly lettuce by the crop are probably the same.

Indeed, F1 hybrids are obtained easily, independently on the species acting as the female parent (Lindqvist 1960 a; De Vries 1990) and are completely fertile. Moreover, although only few studies have been carried out to understand the role of pollinators on gene flow in lettuce (Goubara et al. 2003; Goubara et al. 2004), hybrids are usually the result of insect pollination.

The risks of (trans-)genes escape through pollen flow are not expected to be different for biotech crop than for similar conventional crops (http://www.bio.org/foodag/background/ geneflow.asp). In order to assess the risk of gene transfer from GM or conventional crop to wild relative through gene flow, quantifying potential hybridization is a key issue. These risks are often minimized in predominantly autogamous crop-wild complexes like that of lettuce, because of their supposed tendency to avoid cross pollination. However, recent studies have shown that even low out-crossing rates could not prevent cultivated genes to move in sympatric wild populations, neither at very short nor at longer distance from the crop. Interestingly, these studies include autogamous and predominantly insect pollinated species like soybean or cotton (IbarraPerez et al. 1997; Nakamaya et al. 2002; Van Deynze et al. 2005), as well as wind pollinated autogamous species as rice or foxtail millet (Wang et al. 1997; Song et al. 2003; Zhang et al. 2003).

Although the cross compatibility of crop and prickly lettuce is known (Frietema de Vries et al. 1994), the chances for cross pollination have often been considered as low, because *L. serriola* has always been considered as highly autogamous. To our knowledge, the quantification of hybridization between *L. sativa* as the male parent and its wild relative *L. serriola* as the female is addressed here for the first time.

The objectives of this study were 1) to estimate natural rates of hybridization between *L. sativa* and *L. serriola* and 2) to evaluate the influence of distance on these hybridization rates. An additional goal was to produce basic data on isolation distances, useful for decision making in the context of cultivation of genetically engineered lettuce.

Materials and methods

Field trials

Our cross pollination experiments were performed in "natural" conditions using the same experimental design, in 2003 and 2004, in two locations of northern Switzerland. Field trial 1 was located in Bevaix (canton Neuchâtel) (46°56′ N, 6°49′ E) and field trial 2 in Séprais (canton Jura) (47°20′ N, 7°12′ E). Both experimental plots were oriented North-South but possessed a rather different topography and ecology. In trial 1, a steep slope separated the northern-upper part from the southern flat part; vegetation was typical grassland. Trial 2 was located on a homogenous slope in a typical meagre pasture. The floristic richness was greater in trial 2 than in trial 1.

Experimental design

In the middle of each field trial, a 25 m x 1 m plot was used as crop pollen source and was planted with 400 *L. sativa* plants, distributed in 4 rows (Fig.1). Hundred individuals of the 4 different varieties were planted alternatively and spacing between plants was 25 cm. *L. serriola* individuals were planted in 12 parallel rows, 6 North and 6 South of the pollen source at 0 m, 1 m, 5 m, 15 m, 25 m and 40 m distance. In each row, 6 plants of 3 different populations were planted at 1 m distance and randomly distributed.

Plant material

As local pollen source we planted 400 *L. sativa* plants of four different cultivars: Reine de mai (butterhead lettuce with green leaves), Laitue de Morges (romaine lettuce, with dark leaves), Laitue de St-Blaise (romaine lettuce, with green leaves), Feuille de Chêne rouge (leaf lettuce with red leaves). Three different wild populations of *L. serriola* collected in South Germany (LBM5: 9.0833 E, 47.8333 N; D20: 8.6167 E, 48.1833 N) and North Switzerland (CH18: 8.7667 E, 47.5000 N) have been used as local pollen sink. All the plants were sown and grown to a rosette stage in the botanical garden and then planted in the field.

In both trials, in 2003, *L. serriola* were field planted three weeks after *L. sativa* cultivars. In 2004, in order to optimize the overlapping of the flowering period, wild plants were planted three weeks sooner than the cultivated, except for those located at 0 m of trial 2, which were planted simultaneously.

Seeds of each individual of the wild species were collected separately every two days, when allowed by the meteorological conditions. Due to rather different climatic conditions, the harvesting period was the whole month of July in 2003, while in 2004 plants reached maturity in August.

Data scoring

Morphological screening

In 2003, only few plants survived in both field trials and all seeds were sown. In 2004, for each survived mother plant, we sowed 1050 seeds individually, which roughly represents the seed set by 70 capitula (15 florets/ capitula). All the seeds were sown for the plants that set less than 1050.

At the rosette stage (4 to 5 leaves), hybrids look like the paternal *L. sativa* variety and are easily recognisable. At the end of the rosette stage, they are more similar to *L. serriola*, with thicker leaves and a row of spine under the leaves. When hybrid plants start to set seeds, it becomes very difficult to distinguish them from pure wild plants. Their morphology resembles to *L. serriola*, with entire or lobed leaves possessing a row of spine on the midrib of the underside of the leaves and oriented vertically; the stem becomes also prickle. The inflorescence look like *L. serriola* and the involucral bracts reflex at maturity. For these reasons, we screened morphologically the progeny of the wild mother plants at the rosette stage. 89 mother plants in 2003 representing 44’321 seeds and 328 mother plants in 2004 representing 252’345 seeds.

Individual hybridization rates (HR) were calculated as the percentage of hybrids in the sowed progeny of each mother plant, while mean HR were calculated as percentage of hybrids of the pooled progeny of all the individuals of the same row (e.g. all the individuals at 5 m etc.). The incidence of hybridization (IH) was measured as the proportion of *L. serriola* mother plants producing at least one hybrid.

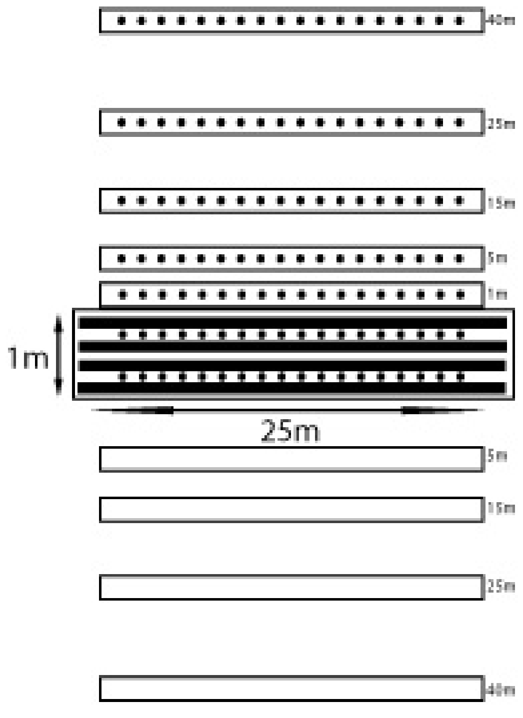

Figure 1. Experimental design

(●) represent an individual of *L. serriola*; 18 wild plants from 3 different populations randomly distributed at 1 m intervals in a row. Twelve rows were planted, 6 North from centre and 6 South (0m, 1m, 5m, 15m, 25m, 40m).

Fulled rectangles represent the rows of *L. sativa* (100 plants per row)

Molecular confirmation of morphological screening

In order to confirm our hybrid morphological screening, we tested 23 RAPD primers to find specific cultivar markers absent in wild populations of *L. serriola*. Eleven primers totalizing 17 reliable markers which could differentiate all the used cultivars from the wild populations (Fig. 2) were selected for further analysis. Total DNA was extracted from fresh young leaves following the QIAGEN® extraction kit protocol. RAPDs amplifications were performed in 25µl final volume, with the following final concentrations: 1X PCR buffer, 0.2 mM dNTP, 0.4 µM primer, 0.75 U Taq polymerase (Qiagen AG, Basel) and 20-40 ng template DNA. Amplifications were carried out in a Biometra T3 thermocycler as follow: 4 min initial denaturation at 94°C, then 37 cycles of 93°C: 60s; 41°C: 60s and 72°C: 60s. Final extension was 5 min at 72°C. PCR products were mixed with 1/3 volume loading buffer and loaded onto 1.5% (w/v) agarose gels stained with ethidium bromide. Electrophoreses were carried out at 100V.

Fifty plants scored morphologically as wild progenies of *L. serriola* and 50 plants scored as hybrids from 10 different mother plants were used to verify the morphological screening. All the 50 F1 hybrids scored morphologically amplified the specific *L. sativa* cultivars RAPD markers, while none of the crop markers were amplified in wild S1 or F1 *L. serriola* (Fig.3). Thus, we considered morphological screening as reliable and used it for hybrid detection in the rest of the progeny. In order to avoid the overestimation of the hybridization rate, all plants whose identification was too doubtful were scored as *L. serriola.*

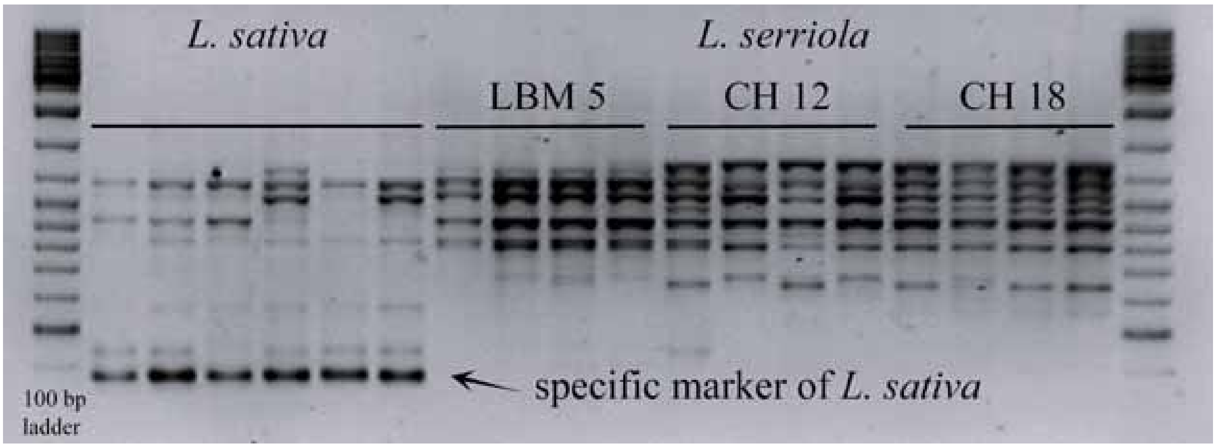

Figure 2. RAPD amplification with primer OPB-12.
The figure shows the DNA amplification of *L. sativa* cultivar and wild populations of *L.* ***serriola.*** *L. sativa*: 1 individual per cultivar. Arrow: specific marker of cultivated lettuce.
L. serriola: four individual for each of the 3 wild populations (LBM 5, CH 12, CH 18) used for the field experiment.

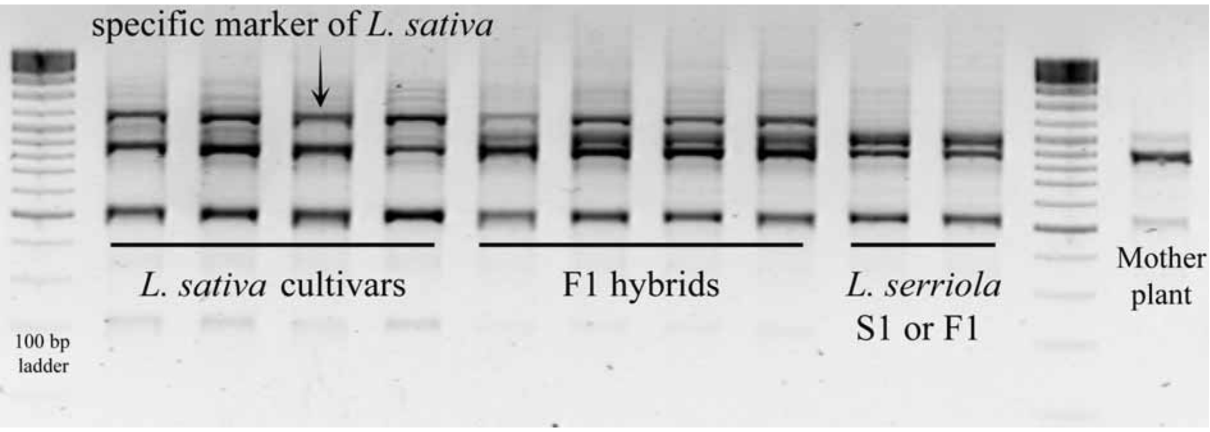

Figure 3. RAPD amplification with RAPD primer OPT-06
The figure shows the DNA amplification of the four *L. sativa* cultivars used in the experiment (pollen source), the F_1 hybrids and the S_1 or F_1 *L. serriola* and *L. serriola* mother plants (pollen sink). Note that amplification of F_1 hybrids DNA revealed the specific markers of both, *L. sativa* and *L. serriola*, while mother plants and S_1 *L. serriola* possess only the wild fragments.

Pollinators

While the survey of *Lactuca* pollinators was not the goal of the study, the most frequent insects that were active on the flowers have been captured in trial 2 and determined, in order to have at least some qualitative data on the factors influencing pollination.

Statistical analysis

As many plants died the first year, HR and IH were compared between 2003 and 2004 for both sites with Mann-Withney U tests with SPSS (SPSS Inc., Chicago, Illinois). Bonferoni's correction (Rice 1989) was applied in order to calculate significance levels adjusted for multiple comparisons performed on the same data set.

As the data did not fit a normal distribution and were not comparable between years, logistic regressions analyses between HR, IH and the logarithm of the distance from the pollen source, were carried out for 2004 only.

In order to test if the origin of the populations of *L. serriola* influenced the HR or IR, three logistic regressions were successively carried out removing each time one population. All the comparisons were not significant. Therefore, the origin of the populations of *L. serriola* was removed from the analyses. Logistic regression analyses were carried out with SAS software (SAS Institute Inc., Cary, NC, USA) and graphical representation with S-Plus® (Insightful Corp.) software.

Results

Plant survival, overlap in flowering time and seed collection

The average plant survival was much higher in 2003 than in 2004 (Table 1 and 2, respectively). In 2003, Switzerland and most of the European countries suffered exceptionally high temperatures and severe drought. A very intensive watering of the plants could only be done in field trial 1, but only 37 % of the plants reached maturity (80 plants). In field trial 2, where the conditions were even more severe and watering was impossible, only 7 % of the wild plants reached maturity (16 plants). Overall, flowering of the two species overlapped poorly. In 2004, conditions were optimal for plant growth. However, *L. serriola* plants suffered competitions of perennial grasses in trial 1; even tough grasses were cut in June around the plants. *L. serriola* plants were spindlier and hidden between the grasses. The plants located at 15 m North from the crop pollen source suffered from drought and almost all the plants died. In total, 85 % of the wild plants reached maturity in trial 2 (184 plants), and 65 % in trial 1 (142 plants). Flowering of crop and wild lettuce in both field trials overlapped completely.

Data scoring

For each site, HR and IH were compared between both years. For trial 1, IH was globally significantly higher in 2003 than in 2004. For trial 2, HR was significantly higher in 2003 than in 2004. Because of the lack of data in 2003 (Table 1) and the differences of results between years, only the data of 2004 were further analysed.

In 2004, the data from the two different trials did not differ significantly for the hybridization rates (Table 2), while significant differences were observed for the incidence of hybridization.

Hybridization rate (HR)

In 2003, maximum individual HR was 16 % at 0m and 26 % at 1m in trial 1, while maximum was between 12 % and 14 % at 1m in trial 2. Despite the limited sampling, due to the very low survival of wild plants, the HR clearly decreased as a function of the distance. The mean hybridization rate, mean HR, reached also high level as 7.37 % at 1 m or 3 % at 5 m.

In 2004, the values of HR were lower than those obtained in 2003. The HR decreased as a function of the distance but not in a constant manner (Fig. 4). Individual HR varied considerably between plants, ranging from 0 to 12 % at 0 m, from 0 to 3.15 % at 1 m, from 0 to 0.56 % at 5 m, from 0 to 0.29 % at 15 m, from 0 to 1.33% at 25 m and from 0 to 0.11 at 40 m. Overall, the mean HR varied between 1.9 % and 2.75 % inside the cultivated plot and between 0.69 to 0.74 % at 1 m. It was lower than 0.15 % at further distances. Finally mean HR was higher than zero at all distance.

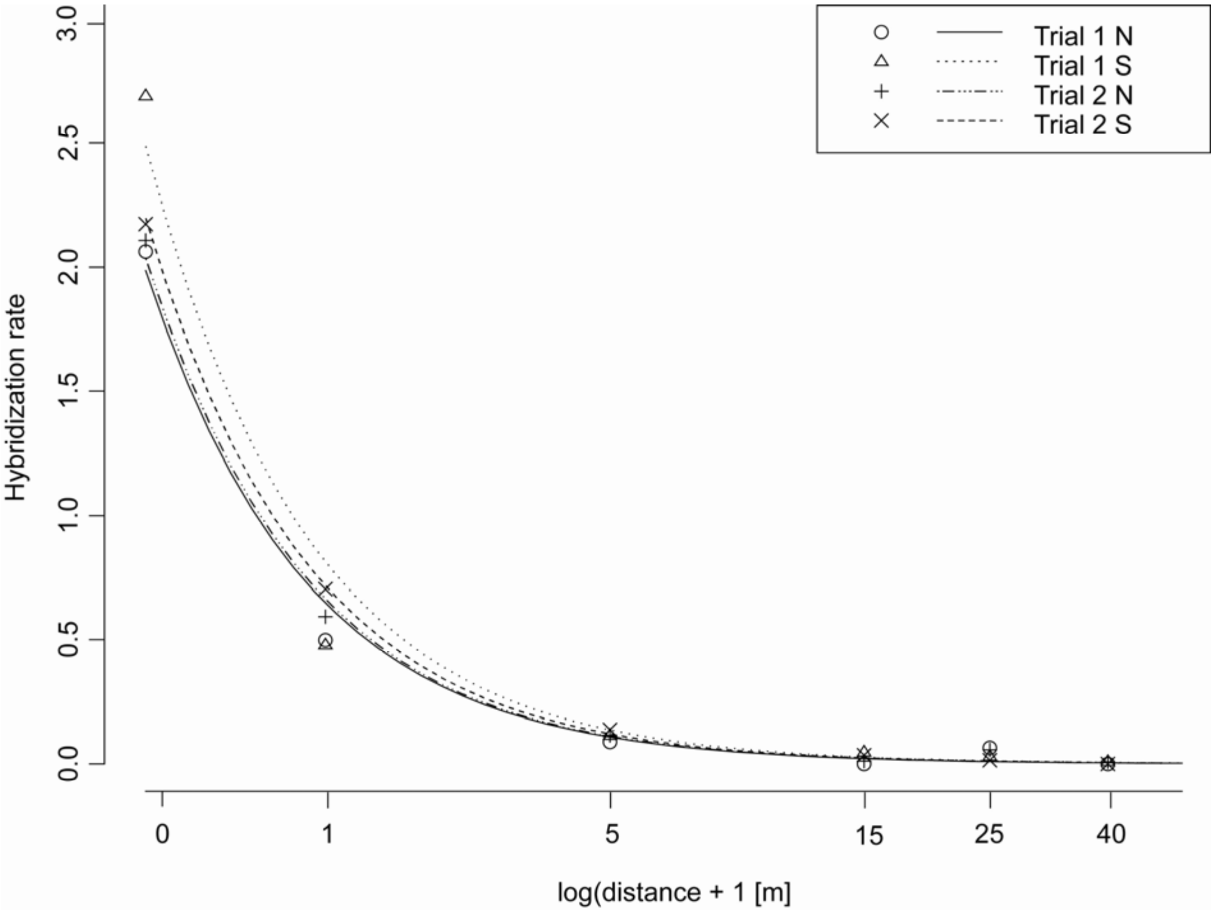

Figure 4. Representation of the hybridization rate as a function of the logarithm of the distance

Table 1. % survival, % average hybridization rates and % crossed mother plants at various distances for the two field locations for the year 2003

Field Site (orientation)	**distance from pollen source (m)**	**% survival (nb of surviving plants / 18)**	**average hybridization rate (%)**		**Incidence of Hybridization (%)**
Trial 1 North	0	28 (5)	0.48	(0-0.24)	40
(Bevaix)	1	39 (7)	0.62	(0-3.41)	29
(2003)	5	28 (5)	0		0
	15	39 (7)	0.11	(0-0.79)	14
	25	22 (4)	0		0
	40	39 (7)	0		0
Trial 1 South	0	44 (8)	4.5	(0-16.67)	50
(2003)	1	50 (9)	3.74	(0-26.24)	67
	5	39 (7)	1.02	(0-4.55)	43
	15	33 (6)	0		0
	25	33 (6)	0.02	(0-0.10)	17
	40	50 (9)	0.05	(0-0.42)	11
Trial 1 overall	0	36	2.49		94
(2003)	1	44	2.18		82
	5	33	0.51		32
	15	36	0.06		5
	25	28	0.01		12
	40	44	0.025		3
Overall survival		37			
Trial 2 North	0	0 (0)	ND		ND
(Séprais)	1	11(2)	6.07	(0-12.14)	50
(2003)	5	11 (2)	0.3	(0-0.6)	50
	15	0 (0)	ND		ND
	25	11 (2)	0		0
	40	11 (2)	0		0
Trial 2 South	0	0 (0)	ND		ND
(2003)	1	17 (3)	7.37	(0-14)	66
	5	22 (4)	3.04	(0-9.58)	75
	15	0 (0)	ND		ND
	25	5 (1)	0		0
	40	0 (0)	ND		ND
Trial 2 overall	0	0	ND		ND
(2003)	1	14	6.72		58
	5	17	1.67		62
	15	0	ND		ND
	25	8	0		0
	40	6	0		0
Overall survival		7			

Table 2. % survival, % average hybridization rates and % crossed mother plants at various distances for the two field locations for the year 2004

Field Site (orientation)	**distance from pollen source (m)**	**% survival (nb of surviving plants / 18)**	**average hybridization rate (%)**		**Incidence of Hybridization (%)**
Trial 1 North	0	89 (16)	2.21	(0.17-12.27)	100
(Bevaix)	1	61 (11)	0.7	(0-3.15)	82
(2004)	5	78 (14)	0.04	(0-0.22)	21
	15	5 (1)	0		0
	25	67 (12)	0.11	(0-1.33)	8
	40	67 (12)	0		0
Trial 1 South	0	100 (18)	2.56	(0-8.67)	89
(2004)	1	67 (12)	0.74	(0-2.67)	83
	5	50 (9)	0.11	(0-0.38)	44
	15	55 (10)	0.03	(0-0.29)	10
	25	67 (12)	0.03	(0-0.27)	16
	40	83 (15)	0.01	(0-0.11)	6
Trial 1 overall	0	94	2.39		94
(2004)	1	64	0.72		82
	5	64	0.08		32
	15	31	0		5
	25	67	0.07		12
	40	75	0.05		3
Overall survival		66			
Trial 2 North	0	67 (12)	2.75	(0-9.83)	92
(Séprais)	1	100 (18)	0.69	(0.1-3.11)	100
(2004)	5	89 (16)	0.12	(0-0.56)	44
	15	89 (16)	0.01	(0-0.1)	13
	25	67 (12)	0.06	(0-0.44)	33
	40	67 (12)	0.009	(0-0.1)	8
Trial 2 South	0	67 (12)	1.9	(0-5.11)	75
(2004)	1	94 (17)	0.72	(0-3)	94
	5	89 (16)	0.14	(0-0.36)	81
	15	94 (17)	0.03	(0-0.19)	29
	25	100 (18)	0.02	(0-0.25)	11
	40	100 (18)	0		0
Trial 2 overall	0	67	2.33		87
(2004)	1	97	0.71		97
	5	89	0.13		62
	15	92	0.02		21
	25	83	0.04		22
	40	83	0.0045		4
Overall survival		85			

Incidence of hybridization (IH)

In 2004, the IH decreased as a function of the distance (Fig. 5), but not as drastically as HR. In 2004, most of the wild plants underwent at least one cross fertilisation event at 0 m in trials 1 and 2 (87% and 94% respectively) and at 1 m (82% and 97%) (Table 2). At 5 m, IH was 32 % in trial 1 and 62% in trial 2, while it was 5 and 21 % respectively at 15 m. Surprisingly, IH was higher at 25 meters than at 15 m (except for trial 2 South); finally 4 and 5 % of the mother plants produced hybrids at 40 m.

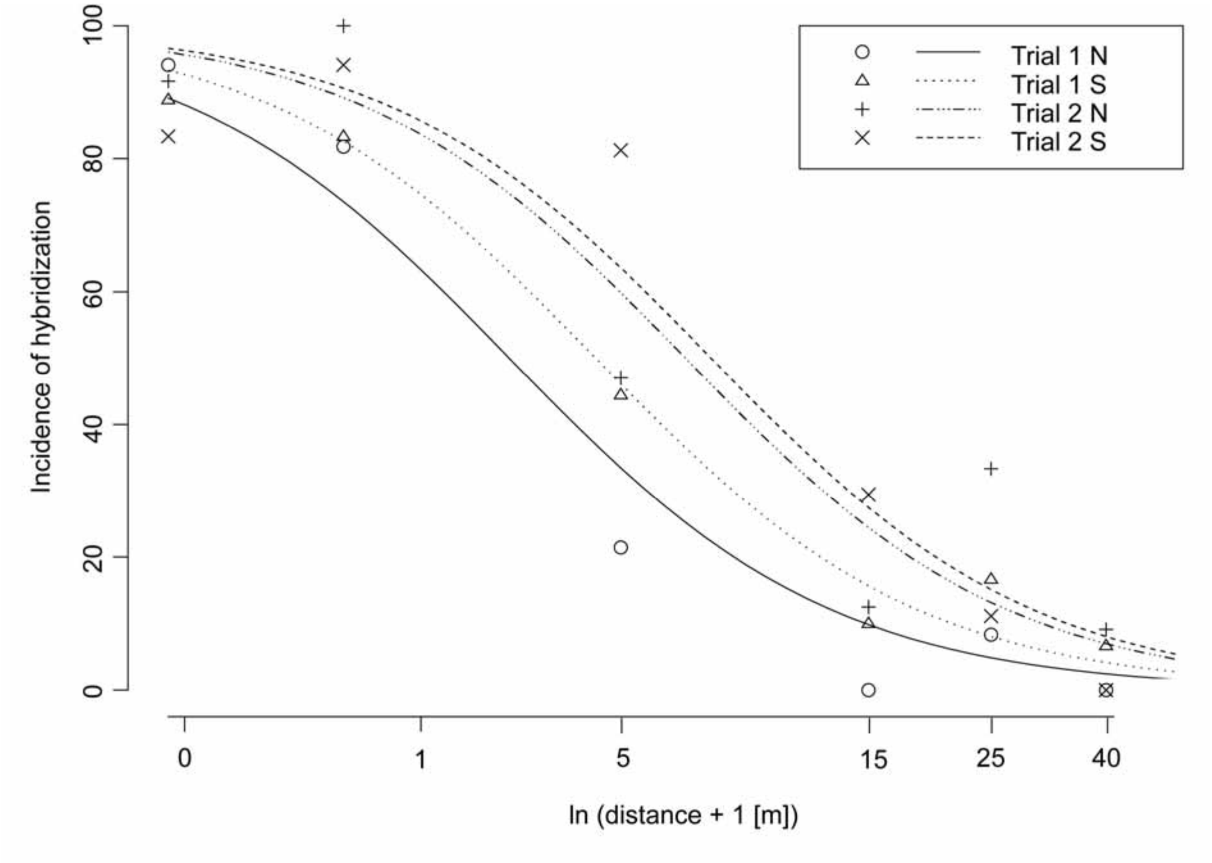

Figure 5. Representation of the incidence of hybridization as a function of the logarithm of the distance

Pollinators

A total of 12 insect species were captured on *Lactuca* flowers (Table 3). All of them are generalist pollinators and belong to Hymenopterans or Dipterans. Temporary foraging, as well as continual pollen gathering behaviours, were observed. Generally speaking, insects tended to move from one flower to another on a single plant, before moving to a different plant. In trial 2, where more flowering plants were present, more pollinators were observed, but in both trials, the highest density of pollinators was found in the crop plot.

Table 3: *Lactuca* pollinators captured in the field trials

Order	**Family**	**Genus**	**Species**
Hymenoptera	Apidae	*Apis*	*mellifera*
Hymenoptera	Apidae	*Bombus*	*lapidarius*
Hymenoptera	Andrenidae	*Andrena*	*sp.*
Hymenoptera	Halictidae	*Halictus*	*sp.*
Hymenoptera	Halictidae	*Lasioglossum*	*sp.*
Hymenoptera	Vespidae	*Vespula*	*vulgaris*
Hymenoptera	Sphecidae	*Ectemnius*	*continuus*
Diptera	Syrphidae	*Episyrphus*	*balteatus*
Diptera	Syrphidae	*Chrysotoxum*	*elegans*
Diptera	Syrphidae	*Sphaerophoria*	*scripta*
Diptera	Syrphidae	*Scaeva*	*pyrastri*
Diptera	Syrphidae	*Syrphus*	*vitripennis*

Logistical regression analysis

Results of 2003 could not be statistically analysed, due to the too high amount of missing data.

In our models, the residual sum of squares was always much lower when the log of the distance was used (0.202 for the hybridization rate and 1926 for the incidence of hybridization) instead of the distance (3.282 and 5535, respectively). Moreover, Chi-Square values were higher for the logarithm of the distance (952.12) than for the distance (395.16). Therefore, the model taking into account the log of the distance was retained. Among all covariables, the log distance had the greatest effect on HR and IH.

While no effect of the origin of wild lettuce population was observed, neither on HR, nor on IH (p>>0.05, results not shown), significant differences were observed for IH between field sites (p<0.0083). Overall, IH was indeed higher in trial 1. Finally, HR results from North *vs.* South lines in field trail 1 only were also significantly different (p=0.0019).

Table 4. Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Hybridization rate					
Intercept	1	-3.8352	0.0432	7867.7227	<.0001
site	1	0.0511	0.0545	0.8820	0.3477
logdist	1	-1.6350	0.0530	952.1215	<.0001
Bev_n_s	1	0.1145	0.0369	9.6119	0.0019
Sep_n_s	1	0.0394	0.0385	1.0470	0.3062
Incidence of hybridization					
Intercept	1	3.2691	0.3976	67.6142	<.0001
site	1	-0.8994	0.3409	6.9628	0.0083
logdist	1	-1.5580	0.1531	103.6179	<.0001
Bev_n_s	1	0.2662	0.2539	1.0996	0.2944
Sep_n_s	1	0.0792	0.2057	0.1483	0.7002

Discussion

The most relevant results of the present study are that 1) crop and prickly lettuce can hybridize at a rather high rate, especially for species that possess physical mechanisms favouring self-pollination and 2) that hybridization can reach substantial levels, even at a relatively long distance. These results have evident implications for both, the importance of crop-wild hybridization for the spread of prickly lettuce toward North of Europe and the ongoing controversy on the cultivation of genetically engineered crops. However, the results and the experimental design need to be discussed and compared, relatively to the context of current cultivation of crop lettuce.

In our experiment, a relatively large source of crop pollen (400 flowering plants) was available, which could intuitively be considered as clearly higher than what could be observed in fields cultivated with lettuce. It is true indeed that the crop is harvested well before it bolts. Yet, in most parts of Europe, crop fields and small-scale non-commercial vegetable gardens are often surrounded by roads, railways, waste places, embankments, ditches, fallows or ruderal habitats, where prickly lettuce commonly occurs. In 2005, 10 % of the Swiss production of the two most cultivated cultivars (mainly head type) was not harvested (information gathered at the Swiss central of gardening market), due to the low prices of lettuce on the market. In those cases, cultivated plants are left in the field, can bold and flower close to wild populations of *L. serriola*. This represents 140 ha that could potentially provide pollen available for cross pollination. It seems then realistic to consider our experimental conditions as intermediate between that of few cultivated lettuce bolting in private gardens and that of large scale lettuce cultivation, where the last plants are not harvested and left in the field. Moreover, both species have a very long blooming period, which naturally overlap for many weeks. Therefore, suitable conditions for cross pollination in agro-ecosystems seems to be frequently met in Switzerland, as well as in central and southern European countries.

Natural hybridization by pollen flow

The floral mechanism of reproduction of the two species is supposed to ensure a high degree of self-fertilization. The flowers show an incomplete protandry (Mejias 1994); the style emerges through the anther tube and branches when it is about 2 mm above the tube. These two branches curl back upon themselves, usually make contact with pollen grains on the sides of the style, which results in self-pollination. Despite this mechanism, we detected significant levels of gene flow from *L. sativa* to its wild relative *L. serriola.*

Individual hybridization rates reached high values, but the mean HR were comparable to out-crossing frequencies of lettuce varieties observed by Thompson (1958) and Watts (1958).
HR varied greatly between plants; however no evidence of population effect could be highlighted. The genetic background of the three different populations did not influence the hybrid production ($p>>0.05$, results not shown). This indicates that the difference in HR among plants depends more on environmental factors as climatic condition, pollinator

abundance or on genetic traits influenced by environmental conditions as induction of flowering (Prince et al. 1978).

This study revealed that the mean HR per row was negatively correlated with the distance. Higher mean HR were detected within the crop plot (0 m distance) or close to it. This represented also the most attractive region for pollinators because of the high density of flowers. Individual HR followed the same trend with values lower than 0.6 % at distances farther than 5 m, but hybridization was still detected at 40 m. However, the design was such that the furthest catch rows were separated from the pollen sources by several rows of catch plants at shorter distances. Thus, there will likely be an underestimation of the pollen transfer at long distances.

Results of both field locations were consistent; no influence of the location has been found on the HR values, which could indicate that hybridization between the two species is possible in every site where the two species occur in sympatry. The fact that generalist insect species, present all over the Swiss Plateau and Jura mountains have been observed foraging on the crop and its wild relative, support this view.

This study indicated that pollen flow was randomly distributed in trial 2, while orientation had an effect in trial 1. This is probably due to the greater heterogeneity of the field in trial 1. Plants beyond 15 m north were not visible from the cultivated plot. This possibly made the flowers in the southern part of the trial more attractive for pollinators, highlighting the influence of micro-topography on hybridization.

While data collected in 2003 do not allow reliable comparisons between years, some of the observed differences allow us to formulate several hypotheses and to make some remarks on spontaneous hybridization in exceptional climatic conditions. In 2003, extremely dry and warm climate in central Europe caused indeed a large mortality within our experimental wild plants. The main differences observed between 2003 and 2004 were higher HR in trial 2 and higher IH in trial 1. Such differences can be explained by both the patterns of activity of the pollinators and the floral mechanisms of crop and wild lettuce.

Although no evidence in the literature exists, showing that lettuce secretes, nectar, the importance of pollinators for the effective pollination of *Lactuca* flowers has been demonstrated (Fujita et al. 1997; Goubara et al. 2003; Goubara et al. 2004; Van Deynze et al. 2005). Jones (1927), showed that bagged flowers of *L. sativa* had significantly less or no pollen grains on their inner stigmatic surfaces, compared to open pollinated flowers. He concluded that cross-pollination between plants may be much more frequent than was formerly supposed. In another study, freely pollinated flower heads had always a higher seed set than flower head bagged before anthesis (Mejias 1994). However, it has also been shown that seed set of a capitula visited by a single pollinator is as high as that of selfed-pollinated flower head on a fine day (Goubara et al. 2003; Goubara et al. 2004; Van Deynze et al. 2005).

Since pollinators ensure both self- and allo-pollination, especially if crop and wild plants are visited alternately, their pollen gathering behaviour can largely affect gene flow in lettuce
The exceptionally high individual HR as well as the higher HR in trial 2 and higher IH in trial 1 observed in 2003 could then be explained by an increased activity of the pollinators, due to particularly warm and dry conditions. Indeed, in 2003 rain felt almost exclusively in late afternoon that is well after anthesis, while in 2004 rainy days were frequent. The opening of *Lactuca* flower heads occurs during the morning and is dependent on the temperature and rain. On warm bright days the flower heads open early in the mornings and remain open few hours. On cold days, flowers remain open longer, while on rainy day flowers they do not open at all. Since in rainy days flowers do not open, or open shortly, and insects do not fly, chances for cross pollination were lower in 2004.

Seasonal pattern of flowering can also affect the distance at which pollen can move. As the density of flowers declines within a population through a season, pollen can be transferred between plants at farther distance (Schmitt 1983). In 2003, only few crop lettuces were still flowering when *L. serriola* flowered. This could have made the central plot less attractive for pollinators, subsequently increasing their movement towards isolated but flowering wild plants.

Finally, most of the plants that had extremely high HR values in 2003 and 2004 suffered from drought or from competition. They presented scrawny phenotype, produced few flowers and consequently few seeds; it is reasonable to think that these plants also produced a lower amount of pollen. It is known that environmental stresses can influence pollen performance (Snow et al. 1996) and that temperature significantly influences positively in vivo pollen germination in cultivated lettuce (Eenink 1983). Therefore, the lower amount of "self" produced pollen of some wild plants, combined with the availability of crop pollen could have facilitated the formation of hybrids. Further investigations could tell us if the reduced fitness of the wild plants, due to environmental stresses, could lead to a reduced production of the pollen.

Incidence of hybridization

Our results indicated that the IH decreased with the distance, but less drastically than observed for the HR values. The IH values were significantly higher in trial 2, compared to trial 1; while the HR values were similar. The HR is probably influenced by the amount of crop pollen carried by insects, while the IH is more influenced by their abundance. Our observations on the abundance of pollinators and their activity are purely qualitative and do not allow to statistically explain the site differences observed for the IH values. However, the two sites presented a rather different vegetative composition, which could indirectly explain those differences, by its influence on the pollinators. Since the pollinator species and the number of plants was the same in the two trials, the amount of crop pollen carried by pollinators was probably the same, while the abundance of pollinators was greater in trial 2, due to a richer floristic diversity (data not shown).

For both field trials, IH ranged between 80 and 100 % for plants growing at distance up to 1 m. These results are much higher than those found in the self-fertilising and insect pollinated crop wild complex soybean (Nakamaya et al. 2002). Interestingly, our results are only slightly lower than those found in the out-crossing and insect pollinated crop-weed radish complex (Klinger et al. 1992), and higher than those found between mixed mating and insect-pollinated tobacco cultivars. At farther distances, the IH varied greatly, but frequencies as high as 81 % at 5 m, 29 % at 15 m or 33 % at 25 m were found. These very high values indicate that a single wild plant growing in proximity of blooming cultivated lettuce has high chances to produce hybrids. Furthermore, these results indicate that the autogamous reproductive system does not hinder a very high incidence of hybridization. Thus, pollen flow and cross fertilisation from cultivated lettuce to its wild relative has to be seen as the rule rather than the exception at least at close distances.

The distance to which pollen can be transferred between plants, and thus HR and IH, are highly dependent on all the factors that can affect pollinator guilds and their foraging strategies. These factors include the number of flowers produced per plants, the seasonal or annual changes in meteorological conditions, the spatial patterns of distribution of the sink population (the wild plants in our experiment) and their position relative to the pollen source (the crop species here) (Schmitt 1983; Van Deynze et al. 2005). In our study, the pollen could move up to the maximal distance (40 m) tested indicating that crop pollen can potentially be transported over long distances by insects. Not surprisingly, this indicates that even a single insect visiting a single plant relatively distant from the pollen source can produce a substantial number of hybrids.

Our data, like all data from empirical studies, are clearly related to specific experimental conditions. However, we performed these experiments in two different locations and in two different years. Although the comparison is made difficult by the lack of data in 2003, the general picture arising from the two sites and the two years is rather similar. Moreover, we did not influence the abundance of pollinators in the field trials, neither by growing attractive flowers, nor by placing beehives in close proximity. Furthermore, the closest beehives were located several kilometres distant from both field trials, and the pollinators we captured were generalist and most of them gathered pollen.

Hybrid characteristics and relevance for GE lettuce cultivation

Crop-to-wild gene flow has been proposed as a hypothesis to explain the recent massive spread of *L. serriola* in Europe (Frietema de Vries et al. 1994) but no study has been previously performed, in order to determine how easily the two species can hybridize. Yet, this is the first step toward the understanding of the mechanisms of introgression. In addition, *L. sativa* has already been engineered for many traits as herbicide resistance (Nagata et al. 2000; Lebeda 2002; McCabe et al. 1999), pathogen resistance (Okubara et al. 1997) and other agronomic traits (Curtis et al. 1999; Pileggi et al. 2001; Goto et al. 2000). Although this study does not address the question of crop-wild lettuce hybridization using transgenic varieties, our results have clear implications for the cultivation of these latter.

Morphologically, hybrids can be easily distinguished from wild parents only at the rosette stage, while at the adult stage the differences are less evident. Natural hybrids could then be more frequent than previously thought, based on superficial observations. Only, few ancient reports exist on the spontaneous hybridization between *L. sativa* and *L. serriola* (Pammel 1918; Ownbey et al. 1949) Mc Collum 1953 *in* (Lindqvist 1960 c). More recently, we observed an adult plant, recognized as a morphological hybrid growing in a wall next to a wild population of *L. serriola* and close to a private garden (where *L. sativa* was grown) in Val d'Aosta (Italy), and collected its seeds. We sowed and grew this progeny to maturity, and analyzed it using the same molecular markers of the present study. The presence of several crop lettuce specific RAPD markers confirmed its hybrid origin (unpublished data). While this observation does not tell anything about the frequency of hybridization between the two species, it clearly indicates that spontaneous hybridization occurs in natural conditions.

Even rare cross pollination events could have important consequences on the introgression of of conventional and/or genetically engineered traits. *L. serriola* can indeed easily produce from 10'000 to 100'000 seeds. Consequently, even a low individual hybridization rate of 0.1%, that is the HR observed in our experiment for the wild plants located at 40 m distance from the crop, would correspond to 10 to 100 viable hybrid seeds produced.
The F1 hybrids would likely combine the wild habitat adaptations of the wild parent and those to the agro-ecosystems of the cultivated parent. In further generations, natural selection would then favour the wild parent's characteristics but also traits of the cultivated parent that confer adaptability to man made habitats (Ladizinsky 1984). Compared to several other crop-wild systems, the transfer of crop (trans-) genes into the wild species could be potentially easier. The fertility of the hybrids is indeed at least as good, and often better, than that of the parental species (Lindqvist 1960 a; Hooftman et al. 2005). As observed in the maize-teosinte complex, since the hybrid pollen is as fit as that of the parents, hybrids could easily pollinate the wild parent (Guadagnuolo et al. in press).

Moreover, hybrids perform well in ruderal habitats and could potentially become invasive because of substantial increase in vital rates and seeds returned per seed sown, compared to the wild parent. The plants of second-generation hybrids are as fit as the wild species (Hooftman et al. 2005). In addition, hybrids self-pollinate and can produce numerous viable seeds, continual hybridization between the two species is thus not necessary for the persistence of crop genes in the wild.
Since the hybrid involucral bracts reflex at maturity and seeds scatter as in the wild parent, the seed dispersal is not expected to differ from that of *L. serriola*. The close relation of seed dispersal with human activities, mainly with vehicles (Lebeda et al. 2001), should not differ either. Not surprisingly, it has already been shown that crop genes can spread, via seed dispersal, over wide geographic areas (Snow et al. 1997). Finally, if seeds of lettuce

transgenic varieties will be commercialised and grown over large areas and/or available to the general public, the containment of transgenes will be very difficult if not impossible.

Conclusion

These findings clearly indicate a non negligible potential for crop-wild lettuce hybridization. Common assumption that autogamy, at least in lettuce, will hamper crop (trans-)gene transfer into wild species should probably be revised for numerous reasons. First, the two species live in sympatry on a wide area and both can be pollinated by generalist insects. Second, hybridization rates and incidence of hybridization remains considerable even at relatively long distances. Moreover, the huge amount of seeds produced by single plants and the effective seed dispersal, allow even low hybridization rates to produce significant numbers of hybrids. Finally, the fertility and the fitness of the hybrids and their progenies indicate that their persistence would only be lowered by the introgression of deleterious traits.

Acknowledgements

The author is grateful to Selina and Milo Stoecklin, Angelo, Lidia and Nadia D’Andrea, Christian Ethique, Christophe Mouhay, Isabelle Arpel, Lorraine Marligniez, Alexa Oppliger, Thomas Ferrantin for their precious help in the field, Anouk Béguin for her help in the lab and Jacqueline Moret for the statistical analyses. We wish to thank all the employees of the botanical garden of Neuchâtel, who made this study possible. This study is part of the EC-funded project “ANGEL” (EU-QLK-CT-2001-01657).

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