Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations

Detlef Bartsch[1], Marc Lehnen[1], Janet Clegg[2], Matthias Pohl-Orf[1], Ingolf Schuphan[1], and Norman C. Ellstrand[2]

[1] Chair of Biology V, Ecology, Ecochemistry and Ecotoxicology, Aachen University of Technology – RWTH Aachen, D-52056 Aachen, Germany
[2] Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124, USA

Keywords for indexing purposes:
Beta vulgaris, plant conservation genetics, gene flow, intogression, genetic diversity, risks of genetically modified plants

Correspondence to:
Detlef Bartsch, Tel.: (241) 806676, Fax: (241) 8888-182,
e-mail: bartsch@rwth-aachen.de

Abstract:
Gene flow and introgression from cultivated plants may have important consequences for the conservation of wild plant populations. Cultivated beets (sugar beet, red beet, and Swiss chard: *Beta vulgaris* ssp *vulgaris*) are of particular concern because they are cross compatible with the wild taxon, sea beet (*B. v.* ssp *maritima*). Cultivated beet seed production areas are sometimes adjacent to sea beet populations; the numbers of flowering individuals in the former typically outnumber those in the populations of the latter. In such situations, gene flow from cultivated beets has the potential to alter the genetic composition of the nearby wild populations. In this study we measured isozyme allele frequencies of 11 polymorphic loci in 26 accessions of cultivated beet, in 20 sea beet accessions growing near a cultivated beet seed production region in north-eastern Italy, and 19 wild beet accessions growing far from seed production areas. We found one allele that is specific to sugar beet, relative to other cultivated types, and a second that is in much higher frequency in Swiss chard and red beet than in sugar beet. Both alleles are typically rare in sea beet populations that are distant from seed production areas, but both are common in those that are near the Italian cultivated beet seed production region – supporting the contention that gene flow from the crop to the wild species can be substantial when both grow in proximity. Interestingly, the introgressed populations have higher genetic diversity than those that are isolated from the crop. The crop-to-wild gene flow rates are unknown, as are the fitness consequences of such alleles in the wild. Thus, we are unable to assess the long term impact of such introgression. However, it is clear that gene

flow from a crop to a wild taxon does not necessarily result in a decrease in the genetic diversity of the native plant.

Introduction

Domesticated plants often have the potential to spontaneously hybridize with those wild relatives that are growing in close proximity (Ellstrand et al., 1999). Such hybridization may lead to gene flow -- "the incorporation of genes into the gene pool of one population from one or more populations" (Futuyma 1998). If new or locally rare alleles from the domesticate persist in wild populations, gene flow may lead to significant evolutionary change in the recipient populations (e.g. Anderson 1949). Crop-to-weed gene flow will have important practical and economic consequences if it promotes the evolution of more aggressive weeds (e.g. Anderson 1949, Barrett 1983). Hybridization with domesticated species has also been implicated in the extinction of certain wild crop relatives (e.g. Ellstrand & Elam 1993, Small 1984). Also, because gene flow tends to genetically homogenize populations (reviewed by Slatkin 1987) and because crops are typically genetically depauperate compared to their wild relatives (Ladizinsky 1985), overwhelming gene flow from crops is expected to deplete genetic diversity in wild populations (Ellstrand et al. 1999).

Over the last decade, much attention has been focused on crop-to-weed hybridization as a potential avenue for the escape of crop transgenes into natural populations (e.g. Colwell et al. 1985, Dale 1994, Darmency 1994). Although that narrow issue has been the topic of numerous theoretical, empirical, and synthetic publications, the general issue of the population genetic consequences of the flow of alleles from domesticated plants (whether genetically engineered or not) to their wild relatives has received scant attention.

Empirical work has been largely focused in two areas: experiments addressing whether a crop and a wild relative are able to hybridize under field conditions (e.g., Arias & Rieseberg 1994, Arriola & Ellstrand 1996, Darmency et al. 1998, Langevin et al. 1990) and descriptive studies addressing whether introgression from crops has occured in populations of adjacent wild relatives (e.g., Oka & Chang 1961, Wendel & Percy 1990, Whitton et al. 1997, Bartsch & Ellstrand, in press). However, we are not aware of any study that addresses how population genetic diversity of natural populations is altered under gene flow from a crop relative.

Therefore, the aim of this study is to examine how gene flow and introgression from cultivated beet (*Beta vulgaris* ssp. *vulgaris* (DC.) HELM) to sea beet (*B. v.* ssp. *maritima* (L.) ARVANG) has impacted the genetic diversity of effected wild populations. So far no population genetic study has been conducted in this gene flow direction in *Beta*, but there are several reports of gene flow from wild to

cultivar populations. Weed beets have appeared in sugar beet fields in France (Boudry et al. 1993, Desplanque et al. 1999) and United Kingdom (Hornsey & Arnold 1979, Ford-Lloyd & Hawkes 1986) as a result of natural crosses between sugar beet and sea beet in sugar beet seed production areas, with sea beet as the pollen parent.

The genus *Beta* is endemic to the Old World. Cultivated beets have been known for more than 2000 years in the eastern Mediterranean region (Ford-Lloyd and Williams 1975). In Europe, wild *Beta vulgaris* ssp. *maritima* is largely a coastal taxon, with a wide distribution from the Cape Verde and Canary Islands in the west, northward along Europe's Atlantic coast to the North and Baltic Seas. It extends eastward through the Mediterranean region into Asia where it occurs in Asia Minor, in the central and outer Asiatic steppes and desert areas as far as western India (Letschert 1993). There is no crossing barrier between wild and cultivated forms of *B. vulgaris* (Bartsch & Pohl-Orf 1996.

This study focuses on one of Europe's most important sugar beet seed production district in north-eastern Italy (Fig.1). Domesticated beet seed production has been going on here for more than 100 years in this region, with an intensification since the 1950's. All subspecies of *B. vulgaris* are usually wind-pollinated, but also insect pollination is possible. In north-eastern Italy, commercial sugar beet seeds are produced on 4.500 ha, each ha containing approximately 50.000 flowering plants. Furthermore, small farmers in the region grow red beet and Swiss chard for private seed production, which may be an additional source of gene flow. Wild sea beet populations occur on the nearby coastal plain, sometimes within 1000 m of the cultivated fields. All red beet and Swiss chard cultivars are diploid like the wild sea beet. The most common varieties of sugar beet are diploid. However, some tetraploid sugar beet cultivar lines are used to breed triploid varieties by crosses with diploids (Barocka 1985). Previous studies demonstrated that genetically based morphological markers that are crop-specific occasionally occur in the wild populations, revealing some gene flow occurs in this region from 2.25×10^8 flowering sugar beets into populations of approximately 4×10^4 flowering wild beets over an area of 4000 km² (Bartsch & Schmidt 1997, Bartsch & Brand 1998).

We used allozymes to characterize the genetic variation within accessions of cultivated beet (sugar beet, Swiss chard, and red beet), accessions of wild sea beet adjacent to the cultivated beet seed production region of north-eastern Italy, and accessions of wild sea beet from elsewhere in its distribution (but far from cultivated beet production areas). We first assessed the genetic relationship of these accessions and groups. We then identified alleles that are typically common in cultivated beet but typically rare in sea beet accessions that are isolated from cultivated beet to permit us to identify which types of cultivated beet contributed to the introgression into the Italian sea beet populations. Finally, we

compared the genetic diversity of the sea beet populations with a history of introgression from cultivated beet with the genetic diversity of sea beet populations with no history of recent contact with cultivated beet.

Material and Methods

I: Plant materials

We assayed allozyme diversity in 69 wild and cultivated *Beta* accessions. We obtained samples from seed companies (mostly diploid, but also one triploid, sugar beet material), from international plant genetic resource collections or from collecting directly from wild populations (Table 1a,b). Accessions were selected to represent a wide geographical range of wild beets (Fig. 1). Precise locations (in latitude and longitude) are available on request from the senior author. We concentrated on selecting cultivated accessions of sugar beet, Swiss chard, and red beet, because these were the types most likely to hybridize with sea beet in north-eastern Italy.

II: Allozyme electrophoresis

Fresh leaf material of wild and cultivated beet was extracted from greenhouse grown plants. Starch gel electrophoresis was performed on crude protein extracts of young leaf tissue. Approximately 100 mg of tissue from each individual was ground in 0.5 ml extraction buffer [0.1 M Tris-HCl pH 7, 4% polyvinylpyrrolidone (PVP), 0.1% dithiothreitol (DTT), and 0.1% ascorbic acid]. Our nine enzyme systems revealed 12 loci: aspartate amino transferase (*Aat*; E.C. 2.6.1.1), aconitase (*Aco*; E.C. 4.2.1.3), glutamate dehydrogenase (*Gdh*; E.C. 1.4.1.2), leucine aminopeptidase (*Lap*; E.C. 3.4.11.1), NAD^+ malate dehydrogenase (*Mdh1*, *Mdh2*; E.C. 1.1.1.37), phosphoglucomutase (*Pgm1*, *Pgm2*; E.C. 5.4.2.2), shikimate dehydrogenase (*Skd*; E.C. 1.1.1.25), triose phosphate isomerase (*Tpi1*, *Tpi2*; E.C. 5.3.1.1), and uridine diphosphoglucose pyrophosphorylase (*Udp*; E.C. 2.4.1.1). To resolve these isozymes, we used three different electrophoretic buffer systems: tris-EDTA-borate pH 8.8 (Heywood 1980) for *Gdh, Lap,* and *Udp*, lithium-borate pH 8.0 (Rieseberg & Soltis 1989) for *Aat, Pgm,* and *Tpi*, and morpholine-citrate pH 7.0 (O'Malley et al. 1980) for *Aco, Mdh,* and *Skd*. Staining techniques are as described by Devlin and Ellstrand (1989) and Wendel & Weeden (1989).

Genetic interpretations of allozyme variation patterns were based on previously published reports for *Beta* (Abe & Tsuda, 1987, Nagamine et al. 1989, Letschert 1993, Raybould et al. 1996). Loci encoding the less anodally migrating allozyme for each enzyme system were designated ”1”, with additional loci numbered sequentially in order of increasing mobility. This nomenclature is identical to Nagamine et al. (1989), but inverted relative to locus/allele designations for allozymes of Letschert (1993).

III: Data analysis

Standard population genetic parameters were used to estimate genetic polymorphism and population genetic structure for individual accessions and groups of accessions, including the proportion of polymorphic loci (*P*), the mean number of alleles among all loci (*A*) and among polymorphic loci (A_P), the mean number of alleles per accession U_m and the absolute number of alleles per accession group *U*, and Nei's (1978) genetic diversity (*H*). Genetic distances were computed according to Nei (1978). Computations were facilitated by the PC-based program POPGENE[1].

Results

For the 9 enzyme systems, we resolved 12 loci and 36 alleles (ca. 3.0 alleles per locus). Allele frequencies for individual accessions are available on request. A summary of the loci and alleles resolved in major accession groups (taxonomically and geographically sorted) is provided in Table 3. One locus (*Tpi1*) was monomorphic, 3 loci *(Aco1, Lap1* and *Udp*) had only 2 alleles per locus. Three loci (*Gdh1*, *Mdh1, Pgm1*) were tri-allelic, and the remaining loci were multi-allelic, displaying up to 5 alleles per locus.

As a species, our sample of *B. vulgaris* (wild + domesticated) has a moderately high value for *H* (0.21). Averaged across loci, the mean estimated heterozygosity for individual groups of *B. vulgaris* accessions ranged from a high of approximately 0.25 for sugar beet to a low of 0.16 of red beet (Table 4). Of the cultivated forms, the sugar beet is the most polymorphic. This trend was evident in the summary statistics (Table 4) and allelic frequencies for groups of *B. vulgaris* (Table 3). The cultivated forms posses fewer of the total 36 alleles found in *B. vulgaris* (sugar beet 29, Swiss chard 23, red beet 26) than both sea beet groups (33 each).

l. Alleles that characterize major groups of *Beta vulgaris* ssp. *vulgaris*

We found no allele unique to cultivated beets. However, we found one allele (*Mdh*2-1) that occurs in all sugar beet cultivars (at frequencies of 0.07 to 1.00, with a mean of 0.52), that was absent in our Swiss chard accessions, and that was extremely rare in our red beet accessions (in only one of 7 accessions at a frequency of 0.07 with a mean of 0.01 over all accessions). This allele is quite rare in sea beet accessions from areas isolated from sugar beet seed production; it is present in only 4 of the 19 accessions examined with a mean frequency of 0.02 over all accessions (Table 5). Only one population had the allele in high frequency (0.28); not surprisingly, this accession (#35) is from the English Channel region, the region in which sea beet populations were originally sampled in the 18th century for the ancestor of sugar beet (Fischer 1989). In contrast, *Mdh*2-1 is much more common in our sea beet accessions from north-eastern Italy growing close to sugar beet seed production; it is present in 12 of the 20 accessions exampled with a mean frequency of 0.10 over all accessions. These

[1] http://www.ualberta.ca/~fyeh/index.htm

data support the contention that introgression has occured from flowering sugar beet into at least some of the nearby natural populations of sea beet.

We also found one allele (*Aco*1-2) that occurs in substantial frequencies in all examined accessions of Swiss chard and red beet (at a frequency of 0.34 - 1.00 with means of 0.72 in Swiss chard and 0.73 in red beet). This allele is typically absent or rare in most sugar beet cultivars (in only 8 of the 14 accessions, with a mean of 0.07 over all accessions). This allele is quite rare in sea beet accessions from areas isolated from sugar beet seed production; it is present in only 11 of the 19 accessions examined with a mean frequency of 0.08 over all accessions (Table 5). In contrast, *Aco*1-2 is much more common in our sea beet accessions from north-eastern Italy growing close to the beet seed production region; it is present in all of the accessions exampled with a mean frequency of 0.60 over all accessions. These data support the contention that introgression has occured from flowering Swiss chard and/or red beet into the nearby natural populations of sea beet.

II. Genetic diversity of sea beet: Comparison of 'seed production' accessions with 'control' accessions

In terms of Nei's estimated diversity (*H*) and the fraction of polymorphic loci, sea beet accessions from the beet seed production area had a higher diversity than the control sea beet accession (Table 4). The number of alleles per accessions of the seed production wild populations was also slightly higher with 20.6 alleles compared to 20.0 alleles in the control accessions, leading to an average of 1.58 alleles per locus in the sea beets affected by gene flow and 1.54 in the control group, respectively. Only the average number of polymorphic alleles in both groups was in the same range with approximately 2.2 alleles.

As a group, *B. vulgaris* included 36 alleles at 12 loci, with an average of 1.6 alleles per locus (2.2 alleles per polymorphic locus). Seven of these alleles (*Aat*1-1, *Aat*1-2, *Gdh*-3, *Pgm*1-1, *Pgm*2-1, *Tpi*2-1, *Tpi*2-4) were unique to sea beet. The diversity of *B. vulgaris* alleles depends overall on the wild sea beet subspecies *B. vulgaris* ssp *maritima*, in which all of the 36 alleles could be found. The accessions that comprise the seed production area populations showed the highest overall diversity within the *B. vulgaris* group with 33 total alleles / 12 loci and a per accession avarage A = 1.6, A_P = 2.2 and H = 0.21. The wild control accession group had slightly less polymorphism with 33 alleles/12 loci (A = 1.5, A_P = 2.2, H = 0.17). The mean number of alleles per accession was higher for the seed production area (20.6 alleles) than the number in the control sea beet accessions (20.0 alleles).

Discussion

We found substantial genetic evidence for gene flow from domesticated beet seed production fields into nearby wild sea beet populations in north-eastern Italy. Two

alleles that are common in cultivars -- but otherwise typically quite rare in wild beets -- were found in unusually high frequencies in the natural populations. Our data are supported by a previous study (Bartsch & Schmidt 1997) which reported that these wild populations had a substantial number of individuals displaying morphological traits that are common in cultivars but typically rare in the wild subspecies.

Contrary to predictions that crop-to-wild gene flow should result in decreased genetic variation (Ellstrand et al. 1999), we found, for most parameters, a slight increase in per-accession genetic variation in our Italian wild beet populations compared to their counterparts from elsewhere in the range of the wild subspecies. The difference is most profound in Nei's H, which averaged about 30% higher than that of the accessions growing far from the seed production region.

It is possible that north-eastern Italy represents a "center of diversity" *for Beta vulgaris* ssp *maritima* and that the diversity we observed has nothing to do with gene flow from the crop. However, it is unusual for a small portion of a wild taxon's range (on the order of a few hundred square kilometers, Table 1) to hold as much or more diversity as the vast majority of its range (encompassing tens of thousands of square kilometers). Given that sea beets in north-eastern Italy grow so close to overwhelming numbers of cross-compatible, wind-pollinated crop plants, gene flow seems likely the most parsimonious explanation.

Indeed, we can suggest several reasons why gene flow from the crop has not lead to the erosion of genetic diversity in this particular system. First, although most crops examined have low genetic diversity compared to their wild relatives (Ladizinsky 1985, Doebley 1989), beet cultivars typically hold a level of genetic diversity roughly equivalent to that of their wild progenitor. In fact, allozyme diversity of sugar beet cultivars is substantially higher than that of the wild beet accessions (Table 4). Thus, if evolution from sugar beet gene flow continued to equilibrium, we would expect diversity to increase to the level of the crop.

Second, sea beets in Italy have received gene flow from many cultivars over the last century as new varieties have emerged (van Geyt et al. 1990). If each new cultivar contained less variation than the previous one, we might expect an erosion in diversity in the wild populations receiving gene flow. But if the new cultivars were well-differentiated from the prior ones, we might see at least a temporary accumulation of different alleles in the wild populations.

Third, in this particular system, sea beets have received gene flow from both sugarbeet and red beet/swiss chard, as our data have demonstrated. These different groups of cultivars are genetically distinct (Table 2). A population

receiving gene flow from two well-differentiated sources would be expected to evolve more diversity than one receiving gene flow from a single source.

Finally, the system might not be in equilibrium. Under high levels of unidirectional gene flow from a source fixed for a novel allele, recipient populations would experience a transient increase in variation as that allele accumulated over generations. Fixation of that allele would eventually occur if not opposed by natural selection, mutation, or gene flow from an alternate source.

Whether these populations are in equilibrium or not, we do note that a century of crop-to-wild gene flow has had a limited evolutionary effect on the wild populations. They have not been so overwhelmed by gene flow as to evolve into cultivated beets. Although natural selection undoubtedly plays a role in limiting the establishment of certain domesticated alleles, we acknowledge that gene flow may be somewhat limited as well. A previous study has shown that prevailing winds would more frequently carry pollen from the wild plants to the crop than the other way around (Bartsch & Schmidt 1997).

Many descriptive genetic studies on other crop species have provided evidence for crop-to-wild introgression (reviewed by Ellstrand et al. 1999; Doebley 1989). However, we are not aware of any previous studies that are comparable to this study, that is, any that directly compare levels of genetic diversity in natural populations with a history of hybridization with a crop versus those that have no such history of hybridization. Such studies on other crops would be valuable to determine the generality of our findings.

We have evidence that a few of our ”control” accessions of sea beet might also be contaminated with gene flow events from cultivated beet. Especially Accession #29 has the highest frequency of the Aco1-2 alleles (0.7) in the control group and was originally identified as a landrace of leaf beet[2], but our morphological examination showed a stronger affinity to sea beet. Accession #44 has a weed beet origin and is therefore known to have a hybridization history with sugar beet (Bartsch, unpublished data). A history of gene flow might account for the occurence of our cultivar ”markers” (*Mdh*2-1 and *Aco*1-2) in a few of our other control accessions. However, the rarity of those common cultivar alleles in the control accessions clearly demonstrate the extraordinary level of gene flow in the north-eastern Italy accessions.

Sugar beet is an important crop of Europe, North America, the Near East, Chile, and Japan[3]. Consequently, it has become an object of transformation by

[2] see http://ars-grin.gov/cgi-bin/npgs/html/acchtml.pl?1399108 (USDA-ARS GRIN database on PI504172)
[3] see http://apps.fao.org/ (FAOSTAT statistics database)

recombinant DNA technology. For example, two different sugar beet cultivars engineered for herbicide tolerance have been recently deregulated in the United States by USDA-APHIS[4]. There has been concern that transgenic traits may cause unwanted effects after they escape via hybridization into sea beet populations (Bartsch & Pohl 1996, Bartsch et al. 1996, Dietz-Pfeilstetter & Kirchner 1998). Transgenes may be more likely to alter the fitness of hybrid or introgressed individuals than supposedly neutral alleles like allozymes. Therefore, the introgression of transgenes into wild populations may change their niche relationships (Ellstrand 1992). Given that crop alleles apparently move with ease into populations of the sea beets of north-eastern Italy, we suggest that these populations should be monitored after seed production of transgenic beets starts in this region. Our study here provides baseline data on the current genetic diversity prior to the introductions of GMOs.

It is hard to judge the ecological genetic impact of a century of gene flow from traditionally bred cultivated beets into the sea beet populations of north-eastern Italy. We have not quantified the crop-to-wild gene flow rates in this system, nor have we quantified the fitness consequences of crop alleles in the wild. Therefore, we do not have the parameters to assess the long term evolutionary impact of unilateral crop gene flow into the wild beet popultions (Ellstrand et al. 1999). However, it is clear that gene flow from a crop to a wild relative does not necessarily result in a decrease in the genetic diversity of the wild plant.

References

Abe J, Tsuda CH (1987) Genetic analysis for allozyme variation in the section *Vulgares*, genus *Beta*. *Japanese Journal of Breeding* **37**, 253-261.

Anderson E (1949) *Introgressive Hybridization* New York: John Wiley & Sons.

Arias DM, Rieseberg LH (1994) Gene flow between cultivated and wild sunflowers. *Theoretical and Applied Genetics* **89**, 655-660.

Arriola PE, Ellstrand NC (1996) Crop-to-weed gene flow in the genus *Sorghum* (Poaceae): spontaneous interspecific hybridization between johnsongrass, *Sorghum halepense*, and crop sorghum, *S. bicolor*. *American Journal of Botany* **83**, 1153-1160.

Barocka, KH (1985) Zucker- und Futterrüben. in: Fischbeck G, Plarre W, Schuster W (eds): Lehrbuch der Pflanzenzüchtung landwirtschaftlicher Kulturformen, Bd, 2, Spezieller Teil. Paul Parey Berlin/Hamburg: 245-287.

Barrett SCH (1983) Crop mimicry in weeds. *Economic Botany* **37**, 255-82.

Bartsch D, Pohl-Orf M (1996) Ecological aspects of transgenic sugar beet: Transfer and expression of herbicide resistance in hybrids with wild beets. *Euphytica* **91**, 55-58.

[4] see http://www.aphis.usda.gov/bbep/bp/petday.html(USDA-APHIS Current Status of Petitions)

Bartsch D, Schmidt M (1997) Influence of sugar beet breeding on populations of *Beta vulgaris* ssp. *maritima* in Italy. *Journal of Vegetation Science* **8**, 81-84.

Bartsch D, Brand U (1998) Saline soil condition decreases rhizomania infection of *Beta vulgaris*. *Journal of Plant Pathology* **80**, 219-223.

Bartsch D, Ellstrand NC (in press) Genetic evidence for the origin of Californian wild beets (genus *Beta*). *Theoretical and Applied Genetics.*

Bartsch D, Schmidt M, Pohl-Orf M, Haag C, Schuphan I (1996) Competitiveness of transgenic sugar beet resistant to beet necrotic yellow vein virus and potential impact on wild beet populations. *Molecular Ecology* **5**, 199-205.

Boudry P, Mörchen M, Saumitou-Laprade P, Vernet P, Van Dijk H (1993) The origin and evolution of weed beets: consequences for the breeding and release of herbicide-resistant transgenic sugar-beets. *Theoretical and Applied Genetics* **87**, 471-478.

Colwell RK, Norse EA, Pimentel D, Sharples FE, Simberloff D (1985) Genetic engineering in agriculture. *Science* **229**, 111-12.

Dale PJ (1994) The impact of hybrids between genetically modified crop plants and their related species: general considerations. *Molecular Ecology* **3**, 31-36.

Darmency H (1994) The impact of hybrids between genetically modified crop plants and their related species: introgression and weediness. *Molecular Ecology* **3**, 37-40.

Darmency H, Lefol E, Fleury A (1998) Spontaneous hybridizations between oilseed rapes and wild radish. *Molecular Ecology* **7**, 1467-1473.

Desplanque B, Boudry P, Broomberg K, Saumitou-Laprade P, Cuguen J, Van Dijk H (1999) Genetic diversity and gene flow between wild, cultivated and weedy forms of Beta vulgaris L. (Chenopodiaceae), assessed by RFLP and microsatellite markers. *Theoretical and Applied Genetics*, in press

Devlin B, Ellstrand NC (1989) Transmission genetics of allozyme loci in *Raphanus sativus* (*Brassicaceae*): stress dependent non-Mendelian segregation. *American Journal of Botany* **76**, 40-46.

Dietz-Pfeilstetter A, Kirchner M (1998) Analysis of gene inheritance and expression in hybrids between transgenic sugar beet and wild beets. *Molecular Ecology* **7**, 1693-1700.

Doebley J (1989) Isozymic evidence and the evolution of crop plants. In: Soltis DE, Soltis PS (eds) *Isozymes in Plant Biology*. Dioscorides Press, Portland, Ore., 87-105.

Ellstrand NC (1992) Gene flow by pollen: implications for plant conservation genetics. *Oikos* **63**, 77-86.

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* **24**, 217-242.

Ellstrand NC, Prentice HC, Hancock JF (1999) Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics,* in press.

Fischer HE (1989) Origin of the 'Weisse Schlesische Rübe' (white Silesian beet) and resynthesis of sugar beet. *Euphytica* **41**, 75-80.

Ford-Lloyd BV, Hawkes JG (1986) Weed beets, their origin and classification. *Acta Horticultura* **82**, 399-401.

Ford-Lloyd BV, Williams JT (1975) A revision of *Beta* section *Vulgares* (*Chenopodiaceae*), with new light on the origin of cultivated beets. *Botanical Journal of the Linnean Society* **71**: 89-102

Futuyma DJ (1998) *Evolutionary Biology*. 3rd edn. Sinauer Press, Sunderland.

Heywood JS (1980*) Genetic correlates of edaphic differentiation and endemism in Gaillardia*. PhD thesis. University of Texas, Austin.

Hornsey KG, Arnold MH (1979) The origin of weed beet. *Annuals of Applied Biology* **92**, 279-285.

Ladizinsky G (1985) Founder effect in crop-plant evolution. *Economic Botany* **39**, 191-199.

Langevin S, Clay K, Grace JB (1990) The incidence and effects of hybridization between cultivated rice and its related weed red rice (*Oryza sativa* L.) *Evolution* **44**, 1000-1008.

Letschert JPW (1993) *Beta* section *Beta*: biogeographical patterns of variation and taxonomy. *Wageningen Agricultural University Papers* **93**, 153pp.

Nagamine T, Catty JP, Ford-Lloyd BV (1989) Phenotypic polymorphism and allele differentiation of allozymes in fodder beet, multigerm sugar beet and monogerm sugar beet. *Theoretical and Applied Genetics* **77**, 711-720.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583-590.

O'Malley DM, Wheeler NE, Guries RP (1980) *A manual for starch gel electrophoresis*. University of Wisconsin Madison – Department of Forest Staff Paper Series.

Oka HI, Chang WT (1961) Hybrid swarms between wild and cultivated rice species, *Oryza perennis* and *O. sativa*. *Evolution* **15**, 418-430.

Raybould AF, Mogg RJ, Clarke RT (1996) The genetic structure of *Beta vulgaris* ssp. *maritima* (sea beet) populations: RFLPs and allozymes show different patterns of gene flow. *Heredity* **77**, 245-250.

Rieseberg LH, Soltis DE (1989) Assessing the utility of allozyme number for determining ploidal level: evidence from *Helianthus* and *Heliomeris* (Asteraceae). *Aliso* **12**, 277-286.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* **236**, 787-792.

Small E (1984) Hybridization in the domesticated-weed-wild complex. In: *Plant Biosystematics* (ed. WF Grant), pp. 195-210. Academic Press, Toronto.

Van Geyt JPC, Lange W, Oleo M, De Bock ThSM (1990) Natural variation within the genus *Beta* and its possible use for breeding sugar beet: A review. *Euphytica* **49**, 57-76.

Wendel JF, Percy RG (1990) Allozyme diversity and introgression in the Galapagos Islands endemic *Gossypium darwinii* and its relationship to continental *G*. *barbadense*. *Biochem. Ecol. Syst.* **18**, 517-528.

Wendel JF, Weeden NF (1989) Visualization and interpretation of plant allozymes. In: *Isozymes in Plant Biology* (eds Soltis DE, Soltis PS), pp 5-45, Dioscorides Press.

Whitton J, Wolf DE, Arias DM, Snow AA, Rieseberg LH (1997) The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization. *Theoretical and Applied Genetics* **95**, 33-40.

Acknowledgements

We thank the following for assistance during early phases of this project: K. Meyerholz (KWS Einbeck), A. Schröter (KWS Italia), J.R. Stander (Betaseed), L. Panella (Fort Collins), R. Whitkus (UC Riverside), L. Frese (FAL Braunschweig), E. Biancardi (ISC Rovigo), Cancelliere Rosario (Isola Albarella), B. Heinrich (Helgoland), C. Morak, H Gluth, B. Witte, S. Driessen, T. Mücher, P.R. Hesse, C. von Soosten (RWTH-Aachen), U. Lansing, M. Lansing (Dorsten), M. Zayed (El-Menoufia University, Egypt). This study was supported by The German Ministry of Science and Technology (Grant #0310532 and #0310785) and University of California DANR Competitive Grant (#1997-980069).

Author Information Box

D. Bartsch is a plant ecologist with general interest in plant invasiveness and the ecological behavior of genetically modified species. His working group for practical biosafety research on transgenic organisms includes a plant ecologist (M. Lehnen) and a plant physiologist (M. Pohl-Orf). I. Schuphan is an ecotoxicologist working on the environmental impact of chemicals and transgenic organisms. J. Clegg is an isozyme analytic specialist, N. C. Ellstrand is an applied population geneticist interested in gene flow between wild and cultivated species as well as the role of gene flow in plant population genetics.

Figures

Figure. 1: Geographical distribution of sea beet accessions surveyed in this study (see Table 1).

Tables

Table 1a: Cultivated *Beta vulgaris* accessions surveyed in this study: N = number of individuals examined

Table 1b: Wild *Beta* accessions surveyed in this study: N = number of individuals examined, WBN = World Beta Network, accessions are usually diploid

Table 2: Nei's genetic identity (*I*: above diagonal) and genetic distance (*D*: below diagonal) for major groups of cultivated beet (genus *Beta*) and related wild taxa

Table 3: Mean allele frequencies for major groups of *B. vulgaris* and *B. macrocarpa* (with accession numbers) examined in this study. Locus/allele designation is described in Materials and Methods.

Table 4: Genetic diversity statistics for major groups of genus *Beta*

Table 5: Gene flow from cultivated beet to wild beets: Frequency variance of *Mdh2-1* (common sugar beet allele) and *Aco 1-2* (common Swiss chard/red beet allele) and frequency (F) per group.