Review

Environmental implications of gene flow from sugar beet to wild beet – current status and future research needs

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Gene flow via seed or pollen is a basic biological process in plant evolution. The ecological and genetic consequences of gene flow depend on the amount and direction of gene flow as well as on the fitness of hybrids. The assessment of potential risks of transgenic plants should take into account the fact that conventional crops can often cross with wild plants. The precautionary approach in risk management of genetically modified plants (GMPs) may make it necessary to monitor significant wild and weed populations that might be affected by transgene escape. Gene flow is hard to control in wind-pollinated plants like beet (*Beta vulgaris*). In addition, wild beet populations potentially can undergo evolutionary changes which might expand their geographical distribution. Unintended products of cultivated beets pollinated by wild beets are weed beets that bolt and flower during their first year of planting. Weed beets cause yield losses and can delay harvest. Wild beets are important plant genetic resources and the preservation of wild beet diversity in Europe has been considered in biosafety research. We present here the methodology and research approaches that can be used for monitoring the geographical distribution and diversity of *Beta* populations. It has recently been shown that a century of gene flow from *Beta vulgaris* ssp. *vulgaris* has not altered the genetic diversity of wild *Beta vulgaris* L. ssp. *maritima* (L.) Arcang. in the Italian sugar beet seed production area. Future research should focus on the potential evolution of transgenic wild beet populations in comparison to these baseline data. Two monitoring models are presented describing how endpoints can be measured: (1) "Pre-post" crop commercialization against today's baseline and (2) "Parallel" to crop commercialization against GMP free reference areas/populations. Model 2 has the advantage of taking ongoing changes in genetic diversity and population dynamics into account. Model 1 is more applicable if gene flow is so strong that most areas/populations contain GMPs. Important traits that may change the ecology of populations are genes that confer tolerance to biotic and abiotic stress. An assessment of environmental effects can realistically only be based on endpoints and consequences of gene introgression, which may include economic values of biodiversity in littoral and other ecosystems containing wild beet. In general, there is still a great need to harmonize worldwide monitoring systems by the development of appropriate methods to evaluate the environmental impact of introgressed transgenes.

Keywords: plant biosafety / gene flow / gene introgression / sugar beet / wild beet / biodiversity / monitoring

Abbreviations: **GMPs**: Genetically Modified Plants

INTRODUCTION

Beets of the species *Beta vulgaris* are comprised of an extraordinarily variable group in which cultivated, wild and weed forms are often difficult to distinguish (Tab. 1). This is mainly due to the extensive use of sea beet (*Beta vulgaris* L. ssp. *maritima* (L.) Arcang.) gene resources in conventional breeding programs

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Table 1. The *Beta vulgaris* cultivar-wild plant complex from the view of naturalization status and taxonomic classification. *Beta macrocarpa* is included as inter-specific hybridization partner.

Habitat	Naturalization status	Taxonomic classification
Outside agricultural area		
Europe and Mediterranean area	Wild	*B. vulgaris* ssp. *maritima* *B. macrocarpa* Other *Beta* species
N. America, Australia	Weed	*B. vulgaris* ssp. *vulgaris* *B. vulgaris* ssp. *maritima* *B. macrocarpa*
Inside agricultural area		
Crop plantation	Cultivated	*B. vulgaris* ssp. *vulgaris*
Sugar beet field	Weed	*B. vulgaris* hybrids between ssp. *maritima x* ssp. *vulgaris*, *B. macrocarpa*
Outside sugar beet field	Ruderal	*B. vulgaris* ssp. *maritima* *B. macrocarpa*

(Ford-Lloyd and Williams, 1975; Ford-Lloyd, 1986; Lange et al., 1999). Sea beet 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 (Letschert, 1993). It also 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. Sea beet varies from self-compatible annuals to self-incompatible iteroparous perennials with a life span between one and more than eleven years (Hautekeete et al., 2002). Cultivated *B. vulgaris*, including Swiss chard, red garden beet and sugar beet, are biennial. The latter is partially self-incompatible due to the extensive use of male sterility genes in sugar beet breeding. The oldest cultivation form – Swiss chard and red garden beet – have been grown for more than 2000 years, and the youngest form – sugar beet – has now been cultivated for 200 years (Lange et al., 1999). The cultivars have not shown unwanted ecological effects despite the introduction and spread of this European species to the New World (Bartsch and Ellstrand, 1999).

Gene flow is by definition the active or passive dispersal of genes via seed, pollen or clonal parts of a plant into the environment (Fig. 1). Since risk is a product of both exposure and hazard, it is clear that biosafety research on environmental effects of genetically modified plants (GMP) should not only target the probability of gene flow, but must also focus on the

consequences (and potential hazards) of transgene flow to relatives of transgenic crops (Bartsch and Schuphan, 2002). Today, transgenic sugar beet with recombinant
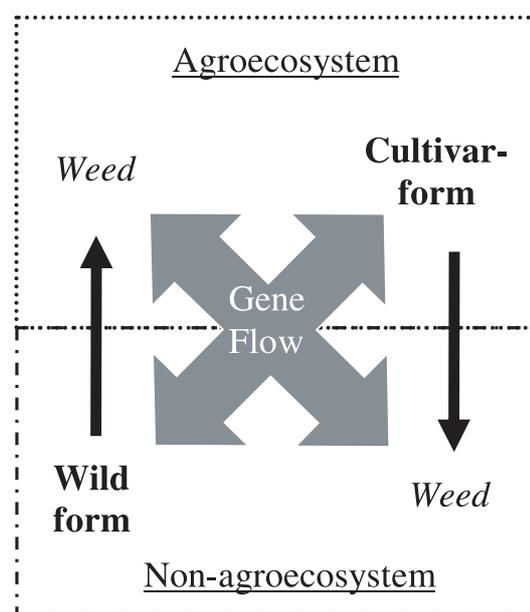
Figure 1. Intra-specific gene flow in a wild-weed-cultivar system: thin black arrows represent the spread of beet plants into unwanted habitats, where they are regarded as weeds. Weeds may also evolve due to gene flow. Wild and cultivar forms of a single species can be protected as genetic resources in one place and eradicated as weed in another.

herbicide and virus resistance genes is the focus of biosafety research, since, worldwide, regulations ask for risk assessment of this new technology (Bartsch et al., 2001; Crawley et al., 2001; Desplanque et al., 2002). The only realistic way of assessing the environmental effect of transgenic beets is a comparison with the performance and evolutionary history of classically bred cultivars (Bartsch et al., 1996). The aim of this paper is first to summarize our knowledge of past environmental effects of conventional *Beta vulgaris* gene flow and what additional impact transgenes might have. We start with some general aspects of sugar beet, since this variety is most important in the *Beta* cultivar-wild plant complex. Second, we highlight geographic hot spots of gene flow in Europe and America, since GMPs will be grown in these areas in the future. Third, we summarize the results of past biosafety research based on an ecologically relevant trait. Since biosafety research cannot be expected to address every possible anticipated and unanticipated risk, we discuss the role of monitoring in risk assessment of GMPs. Finally, we give an outlook on future research needs.

GENERAL ASPECTS OF SUGAR BEET GENE FLOW

For the wind pollinated sugar beet (*Beta vulgaris* L. ssp. *vulgaris* Sugar beet group) gene escape via hybridization is most likely in "off-type" plants that flower during the first year of growth when sugar beet is grown vegetatively or, during the second year of growth after mild winter cold vernalization for commercial seed production. All cultivated forms of beet are more or less biennials. They are sown after spring frosts and their roots and leaves are harvested at the end of their first vegetative season. If young beet plants are grown at low temperatures (0 °C to +10 °C) for several days, this results in vernalization of the biennial cultivars (Van Dijk et al., 1997). This physiological response has been widely used for commercial seed production. Beets for seed production are sown at the end of the summer, germinate in autumn, become vernalized during winter and set seeds in temperate climate regions of southwestern France and northeastern Italy at the end of spring (Desplanque et al., 2002). The vernalization requirements for wild and cultivated forms differ among populations. Boudry et al. (2002) found evidence for an adaptive significance of this trait according to geographical climate conditions. In contrast to cultivated forms, the life span in wild *Beta vulgaris* is genetically fixed according to their demographic distribution range, with

up to 11 years maximum in north Brittany (Hautekeete et al., 2002). Even the shortest period observed comprises a one year 2-season span, as found in southwestern France.

Wild *B. vulgaris* ssp. *maritima* beets often show an annual habit, since they start flowering in their first year of growth, and usually die after seed set due to unfavourable environmental conditions during hot and dry summers in southern Europe. Flowering in the first season without vernalization is mediated by genetic adaptation to different environmental factors. First year flowering is genetically related to a single locus called B (B = bolting), and the inheritance of this trait is only partly Mendelian (Boudry et al., 2002). In sugar beet, genes for cold sensitivity have been selected against so that the plants exhibit low levels of bolting. This is based mainly – but not exclusively – on homozygosity of "bb" in diploid or "bbb" in triploid sugar beet varieties. In summary, the B allele removes vernalization requirement, but it is not unambiguously dominant.

Concerning biosafety related issues of flowering and gene flow, there are three main cropping systems that can enhance gene escape from GM sugar beet:
1. Hybridization of bolting sugar beet with flowering forms of sea beet in seed production areas (Boudry et al., 1993; Mücher et al., 2000).
2. Hibernation and vernalization of old vegetative beet parts remaining on the field after harvest (Pohl-Orf et al., 2000).
3. Vernalization of young seedlings due to unexpected low temperatures below 10 °C during the early spring growing season (Van Dijk et al., 1997; Pohl-Orf et al., 2000).

HOT SPOTS OF SUGAR BEET GENE FLOW

Gene flow and introgression from cultivated to wild plants may have important consequences for the conservation of wild plant populations (Ellstrand et al., 1999). Cultivated beets (*B. vulgaris* ssp. *vulgaris*) are of particular concern because they are cross-compatible with wild taxa like *B. vulgaris* ssp. *maritima* (Darmency, 1994) and *B. macrocarpa* (Bartsch and Ellstrand, 1999). Geographical "hot spots" for hybridization are located in the seed production areas of eastern England, southwestern France and northeastern Italy (Ford-Lloyd and Hawkes, 1986; Boudry et al., 1993; Bartsch et al., 1999; Stevanato, 1999). For this reason, biodiversity surveys of local wild beet populations and their habitat characteristics are necessary for the assessment of any effect related to the establishment of transgenic beets

and their offspring (Bartsch and Schuphan, 2002). In addition, the unintended products of hybridization between wild and cultivated beets are weed beets that also bolt and flower during their first year of planting. Weed beets are found in sugar beet fields all over Europe and California, often causing serious yield losses (Ford-Lloyd and Hawkes, 1986; Mücher et al., 2000; Soukup et al., 2002).

Another interesting example of crop-to-wild gene flow occurs in California, where local wild beets belong to two different taxa and have at least three different origins. Bartsch and Ellstrand (1999) found wild beet evolved from escaped Swiss chard or red beet cultivar varieties, and *B. macrocarpa*, presumably introduced from Spain. In addition, both *B. vulgaris* ssp. *vulgaris* and *B. macrocarpa* hybridized locally. Although wild sea beet (*B. vulgaris* ssp. *maritima)* probably played some role in the origin of California wild beets, genetic information is insufficient to determine the extent to which hybridization of cultivated beet with sea beet and/or direct introduction of sea beet from Europe contributed to contemporary *B. vulgaris*-type wild beets in California. A special hot spot for gene flow is the Imperial Valley in Southern California. Here, *B. macrocarpa* plants grow as weeds in sugar beet fields. Sugar beet is grown in winter culture, and vernalization of the biennial plants is a common phenomenon ("bolting") due to moderate cold winter temperature. A 1998 examination based on 15 sugar beet fields (representing an area of approximately 200 ha) showed a sugar beet bolting rate of 0.6 plants.m^{-2}. An extraordinary cool winter 1997/98 contributed to this phenomenon with periods below freezing (0 °C) in some parts of the area. The density of the annual weed *B. macrocarpa* was in the range of 2.7 plants.m^{-2} (representing an area of approximately 100 ha of sugar beet plantation examined). Although the annual *B. macrocarpa* usually flowers earlier than sugar beet bolters, a flowering time overlap was detected in May 1998. Based on 9 species-specific isozymes, introgression of crop alleles in this area was detected at a rate of 2% in wild beet populations (13 of 594 examined plants), which were morphologically similar to *B. macrocarpa*, but had isozyme alleles specific to *B. vulgaris* (Fig. 2). This past gene flow has led to a *de facto* increase of genetic diversity in the *B. macrocarpa* weed (Tab. 2). A question remains: can Californian wild beet be regarded as a plant genetic resource? If wild beet in this area is only regarded as a common weed (according to Fig. 1), genetic diversity monitoring endpoints are not relevant. However, in Europe genetic
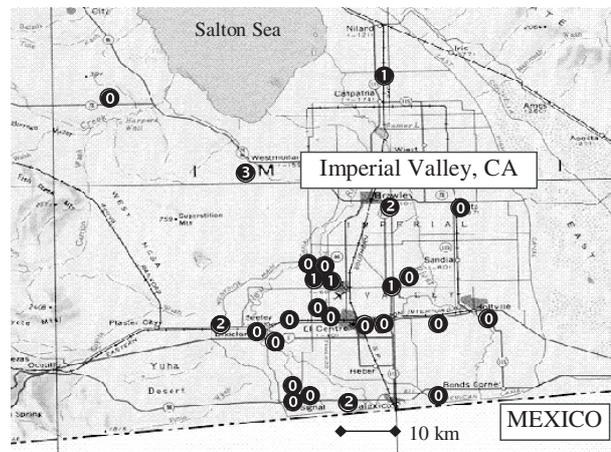
Figure 2. Gene flow from cultivar to weed in California's genus *Beta*: circles indicate number of individuals of a *B. macrocarpa* population with gene introgression from *B. vulgaris*. Total number of populations: 20; sample size was 20 individuals per population (data re-calculated from Bartsch and Ellstrand, 1999).

monitoring in agricultural areas will also have an important role, since the populations of wild beet occur in both agricultural and uncultivated environments.

BIOSAFETY RESEARCH ON TRANSGENIC VIRUS-RESISTANT BEET

Transgenic sugar beets have been the target of intensive biosafety studies. Beside the insertion of various herbicide resistance genes into the beet genome, the transformation of beet to give resistance to the soil-born virus, *beet necrotic yellow vein virus*, which causes a serious disease called rhizomania, has been targeted extensively (Bartsch and Schuphan, 2002). In particular, rhizomania-resistant genotypes were examined for sugar beet as well as for sugar beet – Swiss chard hybrids. The beet's ecological performance was compared under various environmental conditions with regard to parameters such as competitiveness, winter hardiness and seed production. No difference was found in seedling performance even under virus infestation (Bartsch et al., 1996). The competitive performance of beet was tested against *Chenopodium album*, a common weed in sugar beet fields and young fallow. Field experiments carried out between 1993–2001 demonstrated that transgenic sugar beets often grew better than virus-susceptible beets, but only when the virus was present. The difference between susceptible and resistant beets declined as more competing weeds were placed nearby. No differences

Table 2. Genetic diversity data of Californian wild beet in comparison to other *Beta* groups.

	A	AP^2	P^3	H^4	U^5
B. vulgaris USA/Europe ($n_{all}^1 = 47$)	2.92	3.08	0.923	0.330	36
Sugar beet USA/Europe (n = 16)	2.23	2.42	<u>0.923</u>	<u>0.343</u>	29
Swiss chard USA (n = 4)	1.85	2.30	0.769	0.248	24
Red beet USA (n = 5)	2.15	2.60	0.769	0.250	27
Sea beet Europe (n = 13)	<u>2.69</u>	<u>2.83</u>	<u>0.923</u>	0.304	<u>35</u>
Sea beet California (n = 9)	**2.38**	**2.64**	**0.846**	**0.284**	**30**
B. macrocarpa **California (n = 9)**	**2.31**	**2.89**	**0.682**	**0.125**	**28**
B. macrocarpa Europe (n = 4)	1.62	2.13	0.615	0.145	13

[1] Number of populations examined (n); [2] proportion of polymorphic loci (*AP*); [3] the mean number of alleles among all loci (*A*) and among polymorphic loci (*AP*); [4] estimated heterozygosity (*H*); and [5] total number of alleles found within a population group (*U*). Highest values within *B. vulgaris* are <u>underlined</u>.

were observed in most cases if the virus was absent, but occasionally potential costs of resistance were reported for some transgenic events in sugar beet (Bartsch et al., 2001). Some of the experiments focused on over-wintering of transgenic and non-transgenic sugar beet at different locations in Europe representing mild to cold winters in the years 1994–1999. No survival differences were found even under virus infestation conditions. Also, in one experiment, the potential for weed beet production was addressed. It was found that the transgenic genotype had a much "safer" performance because it was less likely to bolt early than the isogenic control (Bartsch et al., 2001). This phenomenon was either caused by somaclonal variation or by a pleiotropic effect of the transformation event. In conclusion, this experiments addressed primarily the ecological consequences of gene flow in a hybrid environment, since crop-variety hybrids were used as a model for crop-wild crosses in the experiments. By complementary use or transgenic and near-isogenic genotypes, direct comparisons were made in experiments, so that any difference measured was caused by the transgenic event. For all cases examined, increased fitness effects were not found based on transgenic rhizomania-resistance genes.

Transgenic attributes can be transferred via gene flow to sexually compatible relatives. One pre-requisite condition is sympatric occurrence of cultivars and their hybridization partners. Transgenic attributes are genetically dominant in heterozygotes, and inherited like conventional genes in wild beet populations (Saeglitz et al., 2000). In the GM rhizomania-resistant beet, no difference was found in the hybridization ability of transgenic plants in comparison to non-transgenic

controls. The resulting hybrids between sugar beet and wild or weedy relatives were also the subject of studies of germination, competitiveness, winter hardiness and seed production. No special transgenic effect was observed except a potential cost of resistance that was associated with some transformation events (Pohl-Orf et al., 2000). Generally, transgenic sugar beet plants behaved in an ecologically similar manner to non-GMP, if the modified trait confers no advantage under particular environmental or experimental conditions. However, the GMP may perform better than non-GMP when challenged by ecological conditions to which the modified trait confers tolerance. In order to address this issue, Bartsch and Brand (1998) demonstrated that rhizomania is absent in saline wild beet habitats, and concluded that this virus disease cannot play an ecological role there. Only GM weed beet growing in virus infested land will benefit from resistance genes, whether they are of transgenic or of classical breeding origin. This will also apply to herbicide-resistant weed beets in crop rotations using the complementary herbicide.

BEYOND BIOSAFETY: THE ROLE OF MONITORING

Environmental monitoring is used for post-commercialization assessment of the fate or effects of transgenes in the environment (Suter, 1993). Monitoring requires baseline data of the flux and evolution of a given (eco-) system structure, systems and processes (Fig. 3). Both indirect and direct methods of measurement can be used to detect possible impacts of transgenes or their products. Environmental monitoring of GM agricultural crops and
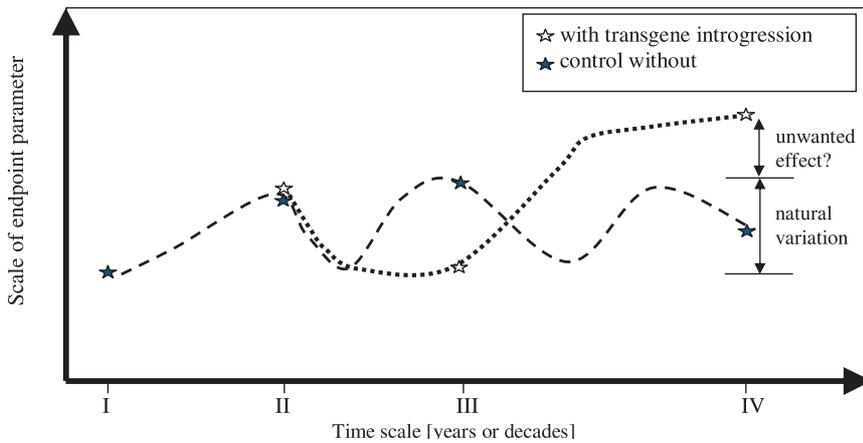
Figure 3. Hypothetical monitoring model: critical points for measuring the potential impact of transgene escape into a wild plant population: I. before transgene release; II. at first commercial transgene release; III. and IV. representative time after transgene release. Suggestions for endpoint parameters are listed in Table 3.

their production practices is generally needed, not because of any specific, identified risk, but to understand the potential contribution or impact of GM crops on sustainable crop production practices.

Monitoring of transgenes is conducted to achieve four specific objectives: (1) confirm compliance with regulatory requirements; (2) collect information necessary for controlling and stewardship of GMPs so as to limit environmental impact; (3) detect and assess parameter changes identified ("known" to be important) by prior environmental impact assessment and (4) detect "unexpected" and potentially damaging effects (Suter, 1993). Monitoring may be recommended to reduce uncertainty remaining from risk assessments, confirm conclusions with additional data, or provide informational feedback on system status or condition (Nickson and Head, 1999). Monitoring is not a substitute for biosafety research or environmental risk assessments, but can be used to validate or verify risk assessments and environmental risk assessments. Monitoring is integrated with research and risk assessment to ensure that ecological systems and processes of value are being protected. A decision to require monitoring is ideally based on the scientific information provided in the risk assessment or some other scientific rationale that a risk is possible. Monitoring resources should be concentrated to areas identified as having a risk potential.

Nickson and Head (1999) have divided monitoring of GMPs into two basic approaches: general or specific. General monitoring, which is also referred to as surveillance in the new EU directive 18-2001, is not necessarily based on any specific hypothesis of risk. It could be accomplished using expertise and infrastructure already present in agricultural systems and within conservation efforts. By gaining familiarity and experience with GMPs through general monitoring, one can conduct "range-finding" and possibly better define the nature of a perceived risk and benefit. Specific monitoring must be based on a scientific hypothesis. It is science-based monitoring where a protocol with specific interpretable endpoints is used. Eventually, information from general monitoring could be refined through the development of specific monitoring protocols designed to determine what, if any, correlations exist between practices, technologies, activities, etc. used in agriculture and the overall condition of the system.

In the case of beet, any monitoring should focus on the bio-geography and genetic diversity of transgenic wild and weed beet populations in comparison to baseline data, and it will depend on the phenotype of the new traits. Several studies have already delivered useful baseline data at a local scale (Laporte et al., 2001; Raybould and Mogg, 1996; Raybould et al., 1997; Raybould et al., 1998; Tufto et al., 1998; Viard et al., 2002). Two monitoring models can be discussed on how endpoints could be measured: (1) "Pre-post" crop commercialization against today's baseline and (2) "Parallel" to crop commercialization against transgene free reference areas/populations. Model 2 has the advantage of taking ongoing changes in genetic diversity and population dynamics into account (Fig. 3). Model 1 is more applicable if gene flow is so strong that most areas/populations contain genetically modified plants, and natural variation has been measured as baseline beforehand (Fig. 3). In every case we need a baseline in order to detect deviations from natural variation of certain endpoint parameters (Tab. 3).

Causal analytic approaches need to compare transgenic gene flow with conventional gene flow effects (Bartsch and Schuphan, 2002; Reboud et al., 1999).

Table 3. Suggestions for endpoint parameters on a time scale according to Figure 3.

Time/stage	I	II	III	IV	
Endpoint Parameter	Pre commercialization		Post commercialization		Remarks and special tasks
a. Population size	C	C	C[2] T[1]	C T	Population monitoring
b. Geographic range	C	C	C T	C T	Estimation of spread
c. Genetic diversity	C	C	C T	C T	Population monitoring
d. Level of gene flow	C	C T	C T	C T	Cause–effect analyses
e. Fitness		C T		C T	Mostly experimental

Shaded cells represent areas where data are available for the genus *Beta* (see Sect. Biosafety research ... of this paper). Abbreviations: [1] T = population(s) with transgene introgression; and [2] C = control population(s) with no transgene introgression.

In addition, the measurable effect of any transgene needs a broader assessment as to whether the effect is unwanted or acceptable both ecologically (Darmency, 1997; Raybould and Gray, 1993) or legally.

Before the post-commercialization monitoring can start, we need in the case of beet a combination of experimental biosafety research, ecological field observation, and population genetic analysis that will be continued at appropriate time intervals (Saeglitz and Bartsch, 2002). Wild sea beet habitats are found in naturally and man-made disturbed areas (Doney et al., 1990; Driessen et al., 2001; Letschert, 1993). Many populations have rarely more than 100 individuals, as well as very high dynamics in population size (Tab. 4). Year-to-year monitoring data demonstrate the difficulty on how to find significant effects within a great natural variability of population dynamics, but population genetic data can be used to study the evolutionary history of wild populations. As an example, Bartsch et al. (1999) studied population genetics of populations that were exposed to gene flow from traditionally bred cultivated beets into the wild sea beet populations of northeastern Italy. They demonstrated 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, despite the fact that cultivated beets are less diverse and outnumbered the wild relatives by the factor 10 000 to 1. These data support the view that gene flow alone should be not regarded as an adverse environmental effect of transgenic plants. In addition, no relationship between genetic diversity (Fig. 4, in terms of heterozygosity) and population size was found. An explanation for this phenomenon can be a high amount of gene flow, the great temporal variation in population size, and the existence of a seed bank. Founder effects or genetic bottlenecks seem to play a minor role in the nature of *Beta* populations in Italian coastal areas.

In Germany, coastal areas wild *B. vulgaris* ssp. *maritima* is actually expanding its geographical distribution. This phenomenon takes place without any influence of transgenic introgression, since transgenic cultivars are still not grown in this region even for small scale field test releases (Driessen et al., 2001). These examples make clear that any observation of GMP spread can only be assessed by help of baseline knowledge of natural population genetics and dynamics. Many annual and biannual species occur as metapopulations (Eriksson, 1996) – making rigorous monitoring difficult to carry out. Important factors influencing high variability of population size and diversity are crop rotation, seed bank dynamics and habitat fragmentation. Monitoring should therefore focus on genetic diversity parameters like isozyme allele frequency or other molecular techniques (Bartsch et al., 1999; Driessen et al., 2001).

OUTLOOK: RESEARCH PRIORITIES FOR TRANSGENIC SUGAR BEET

Gene flow is not a new phenomenon of crop-wild plant interaction in *Beta* spp., and research into the historical context of plant breeding implications is helpful to answer risk assessment questions. Future research may

Table 4. Population size of representative sea beet (*B. vulgaris* ssp. *maritima*) populations in northeastern Italy. This area is of special concern since gene flow from sugar beet seed production affects wild beet populations (Bartsch and Schmidt, 1997).

	Location	Year	1994	1995	1996	1997	1998	1999	2000	2001	Sum/years
1	Cervia Saline		0	?	?	200	400	30	8	6	644
2	Boccasette		70	87	51	20	21	5	14	100	368
3	Scanarello		5	4	0	6	3	4	6	25	53
4	Porto Levante (sea dike)		5	70	1	1	0	0	30	35	142
5	Porto Levante (harbor)		3	3	6	13	32	23	25	21	126
6	Albarella (harbor)		2	27	6	6	1	6	8	2	58
7	Albarella (near P. Levante)		18	?	2	2	12	?	4	12	50
8	Albarella (yachting club)		5	5	29	49	53	?	150	100	391
9	Albarella (inland dike)		100	100	28	61	83	5	55	107	541
10	Chioggia		100	30	100	17	200	?	?	46	495
11	Pellestrina		23	30	12	12	0	?	0	88	165
12	Porto di Malamocco		70	?	8	18	20	?	20	54	190
13	Fusina		60	89	10	124	203	?	?	123	609
14	Cimitero San Michele		5	?	601	46	200	?	90	170	1112
15	San Erasmo (sea dike)		?	?	20	38	12	?	?	200	270
16	San Erasmo (inland field)		?	?	?	3000	400	?	?	3000	6400
17	Torcello		?	?	71	600	1450	?	101	3000	5222
18	Punta Sabbioni		10	1	1	0	0	0	?	0	12
19	Bilione (Valpelina)		?	?	1200	500	60	?	?	27	1789
20	Ausa Corno		18	4	20	12	0	2	?	66	122
21	Grado (sea dike)		50	200	100	51	65	?	5	49	522
	Approx. sum over locations		546	652	2269	4778	3215	?	?	7232	

Individual numbers > 90 are in most cases estimations, missing data are marked as "?".
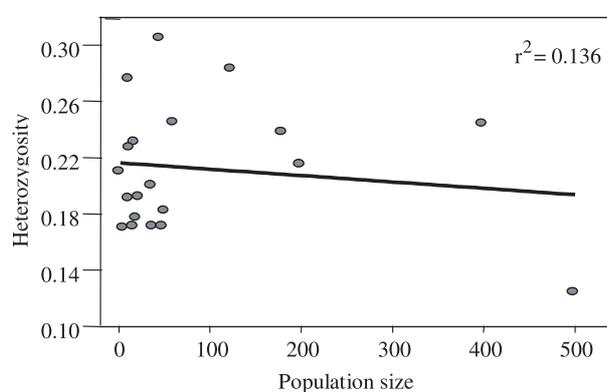
Figure 4. Relationship between heterozygosity calculated from isozyme data and population size of sea beet in NE Italy (data re-calculated from Bartsch et al., 1999).

include (a) identification of molecular markers for geographically distinct populations of wild and cultivated beet to identify distinct metapopulations and monitor the impact of gene flow on genetic diversity (Desplanque et al., 1999; Desplanque et al., 2000); (b) gene flow between weed and wild beet in coastal and agricultural areas (Bartsch et al., 1999; Darmency, 1994); (c) experimental measurement of ecological effects of different pests and pathogens on weed and wild beet (Bartsch and Brand, 1998; Ober and Luterbacher, 2002); (d) evaluation of weed beet control methods (Champion, 2000; Desplanque et al., 2002); and (e) more thorough identification and assessment of historical implications of beet breeding on unwanted ecological effects like loss of genetic diversity (Bartsch et al., 1999). However, in order to detect any effect, the establishment and spread of

specific transformation events in *Beta* has to be monitored.

There is a need to define what impacts of gene flow are unacceptable in terms of (a) nature conservation efforts; (b) protection of economic value; and (c) responsibility of stakeholders in cases where environmental or economic harm has occurred (Braun, 2002). In relation to (a), it is important that *Beta* plant genetic resources (gene pool) are protected from loss of diversity (Frese et al., 2001). In order to protect beet crops with relation to (b), the development and recombination of certain transgenic traits that may enhance fitness in natural wild beet habitats or develop aggressive weed beet genotypes by enhancing invasiveness should be avoided. Herbicide- and rhizomania-resistance genes seem to play a minor ecological role. So far weed beet is not a problem in crops other than sugar beet, but it might theoretically become more competitive if substantial fitness enhancing traits like stress tolerance are inserted. For (c), the outcome of EU legislation and discussion of threshold levels for transgene frequencies in seed and food products will affect the future biosafety research and monitoring tasks. At this moment there are severe constraints on field scale biosafety studies with novel constructs, due to concerns about gene flow to non-GM crops and the demands from some quarters for zero tolerance of unapproved GM events.

We need to study impacts on genetic diversity and genetic resources in wild *Beta* and impacts on niches currently occupied by *Beta* spp. There is also a need for harmonizing methods of utilizing molecular ecology at the different levels of biological organisation. The future task is causal analytic monitoring approach – a combination of experimental biosafety research, ecological field observation and population genetic analysis. Where monitoring confirms the possibility of an unanticipated adverse effect, further investigation is needed to establish a correlation, if present, between the use of the GM crop and the observed effect. "Gene flow" alone is from a scientific point of view definitely not a "damage". Instead, it is a significant risk component that has to be monitored but with a causal link to further environmental effects.

An assessment of environmental effects can realistically only be based on endpoints and consequences of gene introgression, which include socio-economic values of biodiversity in littoral and other ecosystems containing wild beet. In general, there is still a great need to harmonise worldwide monitoring systems by the development of appropriate methods to evaluate the environmental impact of introgressed transgenes.

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