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# Genetic evidence for the origin of Californian wild beets (genus *Beta*)

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**Abstract** The genus *Beta* L. is a morphologically and genetically variable group composed of wild, weedy, and domesticated forms that are used for sugar production or as vegetables. In this study, we have evaluated genetic variation in 64 germplasm accessions of wild and domesticated beets and examined the origin of wild beet accessions in California using allozyme analysis. UPGMA analysis showed overall that domesticated and wild beets form genetically coherent groups. Wild beets in California have two different origins, from European *Beta vulgaris* or from *Beta macrocarpa*. Population-level patterns of allozyme variation for wild California beets related to *B. vulgaris* suggest that those populations evolved from naturalized populations of the cultivated *B. vulgaris* ssp. *vulgaris* which had hybridized to varying degrees with the sea beets *B. vulgaris* ssp. *maritima*. Wild California beets related to *B. macrocarpa* are essentially genetically identical to European accessions. In addition, we found substantial evidence for hybridization and introgression of *B. vulgaris* alleles in one *B. macrocarpa* accession in California. The obligate outcrosser *B. vulgaris* exhibits more allelic diversity than the self-compatible *B. macrocarpa*. *Beta vulgaris* ssp. *maritima* exhibits more genetic diversity than domesticated *B. vulgaris* ssp. *vulgaris*.

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## Introduction

Wild beets are presently found in California from the San Francisco Bay area to the Mexican border (Fig. 1). It is unclear when wild *Beta* became part of the California flora. The first herbarium specimen of wild beet was reported as early as 1893 in Southern California (Rancho Santa Ana Botanical Garden). Carsner (1928) was the first to report that wild beet had become a weed of sugar beet in the Imperial Valley of California's Imperial County. He reported other occurrences in Santa Clara, Ventura, San Bernardino, Los Angeles, and Orange Counties. Carsner identified these beets as probable descendents of crosses between sugar beet and sea beets (*Beta vulgaris* ssp. *maritima* Arcang.), which may have arrived from Europe as a contaminant of imported seed. However, Dahlberg and Brewbaker (1948) suggested that California's wild beets could be descendents of sugar beets or vegetable beets which had escaped from cultivation. In field experiments Johnson and Burtch (1959) observed the evolution of wild beet from cultivated sugar beet in a few generations. Later, McFarlane (1975) used morphology to assign the wild beets of California's Imperial Valley both to *B. vulgaris* ssp. *maritima* and to *Beta macrocarpa* Gussone. He also found plants that were morphologically intermediate between sugar beet and *B. macrocarpa*, suggesting hybridization had occurred between the crop and the wild species. It is known from experimental crosses that certain wild beet species, including *B. macrocarpa*, can hybridize with cultivated *B. vulgaris* (Abe et al. 1986, 1987). Thus, there are three hypothesis for the origin of wild beet in California: (1) direct introduction of wild taxa, (2) naturalization of escaped cultivated sugar beet or vegetable beet, and (3) hybridization and subsequent introgression of cultivated beet with *B. vulgaris* ssp. *maritima* or *B. macrocarpa*.

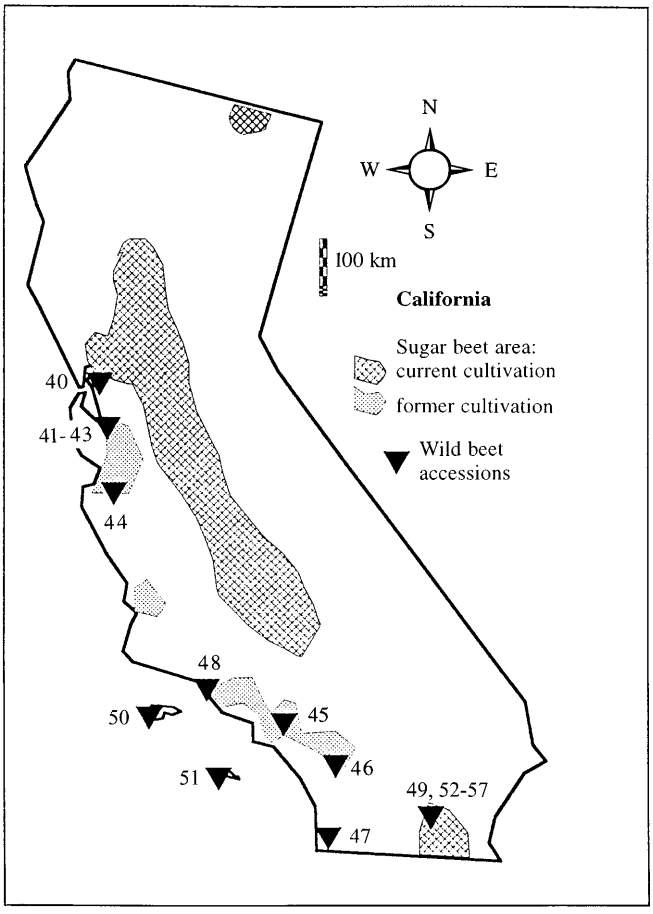


**Fig. 1** Sugar beet cultivation areas and wild beet accessions in California

So far no genetic study has been conducted to evaluate the different hypotheses on the origin of wild beets in California. Genetic analysis with molecular markers has proven useful for identifying the origins of weedy crop relatives, including the possibility of introgression of crop alleles into wild populations (Doebley 1989; Smartt and Simmonds 1995). Therefore, we have used allozymes to characterize the genetic diversity of wild beet populations in California and to compare it with the diversity of the cultivated *Beta* and putative wild progenitors. This comparison provides evidence for the evolutionary origin of Californian wild beets.

## Materials and methods

### Plant materials

For this study, allozyme diversity was assayed from 64 wild and cultivated accessions of the genus *Beta*. Samples were obtained from seed companies, from international plant genetic resource collections or from collecting directly from wild and cultivated California populations (Table 1). Accessions were selected so that most of the geographical range of wild and cultivated beets in Europe and California was represented. We concentrated on selecting *Beta* accessions of *B. vulgaris* and *B. macrocarpa*, as these were the species to most likely be naturalized in California. We chose one accession each of *Beta webbiana* Moquin, *Beta patellaris* Moquin, and *Beta procumbens* Smith accessions for outgroup comparison. Because these species hybridize with each other, but are cross-incompatible with *B. vulgaris*, they provide an independent gene pool (Coons 1975; Van Geyt et al. 1990).

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 *Beta vulgaris* ssp. *maritima* is largely a coastal species, 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). *B. macrocarpa* also occurs in the coastal Mediterranean region and west to the Atlantic coast of Portugal and the Canary Islands (Coons 1954; Letschert 1993). *B. webbiana*, *B. patellaris*, and *B. procumbens* are centered in the western Mediterranean region, including south-eastern Spain and Morocco, and also occur in the Cape Verde, Canary, Salvage and Madeira Islands (Ford-Lloyd and Williams 1975). The geographical range of the above mentioned wild species overlaps between the Canary Islands and south-eastern Spain.

Fresh leaf material of wild and cultivated beet was sampled at several locations in California (UCR 1998 collections, Fig. 1) and transported at 4°C for immediate extraction in the laboratory. We also extracted greenhouse-cultivated plant material grown from seed donated by USDA-ARS (WRPIS Pullman, Washington, USA, and NPA, Fort Collins, Colorado, USA), the Beet Genetic Resources Collection (BGRC, FAL Braunschweig, Germany), Spreckels Sugar (Mendota, California, USA), Betaseed (Tangent, Oregon, USA), KWS (Einbeck, Germany), the RWTH seed collection (University of Technology, Aachen, Germany), the HHU Botanical Garden (University Oldenburg, Germany), and Dr. M. Zayed (El-Menoufia University, Egypt). We bought commercial seed of the Swiss chard and red beet varieties from W. Atlee Burpee and Co. (Warminster, Pennsylvania, USA), Los Angeles Seed

**Table 1** Species and accessions of beet surveyed in this study: $N_i$=number of individuals examined, Underlined accessions are recommended by Dr. Lee Panella for standard use (Fort Collins)

| No. | Species | Subspecies | Variety/type | Accession | Origin | Location | $N_i$ |
|---|---|---|---|---|---|---|---|
| 1 | *B. vulgaris* | *vulgaris* | sugar beet | FC172 | USA | (Reg. by Hecker and Ruppel 1986) | 15 |
| 2 | | | | KWS-2N1009 | Germany | | 30 |
| 3 | | | | KWS-Kavetina | Germany | | 49 |
| 4 | | | | KWS-Rizor | Germany | | 48 |
| 5 | | | | KWS-246 | Italy | | 39 |
| 6 | | | | KWS-247 | Italy | | 41 |
| 7 | | | | Betaseed-4035 | California | | 25 |
| 8 | | | | Betaseed-4581 | California | | 24 |
| 9 | | | | Betaseed-4776 | California | | 31 |

**Table 1** (Continued)

| No. | Species | Subspecies | Variety/type | Accession | Origin | Location | $N_i$ |
|---|---|---|---|---|---|---|---|
| 10 | | | | Spreckels-HH103 | California | | 29 |
| 11 | | | | Spreckels-IV2R | California | | 30 |
| 12 | | | | Spreckels-NB2 | California | | 34 |
| 13 | | | | Spreckels-SS781 | California | | 85 |
| 14 | | | | Spreckels-NB7R | California | | 26 |
| 15 | | | | UCR-NB | California | Imperial County, Brawley | 70 |
| 16 | | | | UCR-BB | California | Imperial County, Brawley | 61 |
| 17 | *B. vulgaris* | *vulgaris* | Swiss chard | Dark Green | USA | | 70 |
| 18 | | | | Chard Fordhook | USA | | 21 |
| 19 | | | | Chard Lucullus | USA | | 21 |
| 20 | | | | Chard Rhubarb | USA | | 39 |
| 21 | *B. vulgaris* | *vulgaris* | Red beet | GB W300 C | USA | (USDA Ft Collins Standard) | 32 |
| 22 | | | | Burpee | USA | | 78 |
| 23 | | | | Detroit Dark Red | USA | | 16 |
| 24 | | | | Red Ball | USA | | 25 |
| 25 | | | | Tall Top | USA | | 29 |
| 26 | *B. vulgaris* | *maritima* | Sea beet | Zayed collection | Egypt | Alexandria | 21 |
| 27 | | | | PI 504266 | France | Corsica, Ajaccio | 26 |
| 28 | | | | PI 540575 | France | Gironde County, Andernons Bains | 21 |
| 29 | | | | PI 540588 | France | Charante Marit. County, Brouage | 30 |
| 30 | | | | HHU | Germany | Oldenburg, Botanical Garden | 85 |
| 31 | | | | RWTH – 1 | Greece | Chalkidiki | 7 |
| 32 | | | | RWTH – 2 | Greece | Crete | 21 |
| 33 | | | | RWTH – 3 | Greece | Peleponnes | 20 |
| 34 | | | | PI518398 | Ireland | Kerry County, Dingle | 37 |
| 35 | | | | BGRC 54228 | Ireland | (Standard) | 26 |
| 36 | | | | RWTH collection | Italy | Ravenna County, Cervia | 57 |
| 37 | | | | RWTH collection | Italy | Goriza County, Grado | 10 |
| 38 | | | | RWTH collection | Italy | Venice County, Venice | 60 |
| 39 | | | | PI518310 | UK | East Sussex County | 48 |
| 40 | *B. vulgaris* | | Wild | UCR – 01 | California | Contra Costa County, Martinez | 15 |
| 41 | | | (*v.*-type) beet | UCR – 02 | California | Alameda County, Fremont | 20 |
| 42 | | | California | UCR – 03 | California | S. Clara County 1, Mountain High | 20 |
| 43 | | | | UCR – 04 | California | Santa Clara County 2, San Jose | 17 |
| 44 | | | | UCR – 05 | California | San Benito County, Hollister | 17 |
| 45 | | | | UCR – 06 | California | Los Angeles County, Pomona | 25 |
| 46 | | | | UCR – 07 | California | Riverside County, Wildomar | 40 |
| 47 | | | | UCR – 08 | California | San Diego County, Chula Vista | 25 |
| 48 | | | | UCR – 09 | California | Santa Barbara County, S. Barbara | 40 |
| 49 | *B. macrocarpa* | | Wild | UCR – 10 | California | Imperial County (Introgressed pop.) | 158 |
| 50 | | | (*m.*-type) beet | UCR – 11 | California | Ventura County, Santa Cruz Island | 30 |
| 51 | | | California | UCR – 12 | California | L. Angeles County, Catalina Island | 40 |
| 52 | | | | UCR – 13 | California | Imperial County | 348 |
| 53 | | | | PI 546448 | California | Imperial County | 38 |
| 54 | | | | PI 546449 | California | Imperial County | 35 |
| 55 | | | | PI 546450 | California | Imperial County, Imperial | 7 |
| 56 | | | | PI 546454 | California | Imperial County, Imperial | 13 |
| 57 | | | | PI 546455 | California | Imperial County | 35 |
| 58 | *B. macrocarpa* | | Wild | BGRC 53034 | Israel | Athlistean Plain | 26 |
| 59 | | | Mediterranean | BGRC 57644 | Cyprus | Larnaca | 13 |
| 60 | | | | BGRC 57664 | Spain | Cartagena | 70 |
| 61 | | | | BGRC 57676 | Spain | Granada | 15 |
| 62 | *B. webbiana* | | Wild | PI 564064 | unknown | | 11 |
| 63 | *B. patellaris* | | Wild | PI 566900 | unknown | | 10 |
| 64 | *B. procumbens* | | Wild | PI 564059 | unknown | | 2 |

Co. (California, USA), and Advance Seed Co. (Fulton, Kentucky, USA), for analyses and use as standards.

### Allozyme electrophoresis

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 of extraction buffer [0.1 M Tris-HCl pH 7.4% polyvinylpyrrolidone (PVP), 0.1% dithiothreitol (DTT), and 0.1% ascorbic acid]. Gel buffers are cited below, and stain techniques are as described by Devlin and Ellstrand (1989) and Wendel and Weeden (1989). Our nine enzyme systems revealed a minimum of 13 isozymes: aspartate amino transferase (*Aat1, Aat2*; 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 and Soltis 1989) for *Aat, Pgm* and *Tpi*, and morpholine-citrate pH 7.0 (O'Malley et al. 1980) for *Aco, Mdh* and *Skd*.

Genetic interpretations of allozyme variation patterns were based on previously published reports for *Beta* (Abe and Tsuda 1987; Nagamine et al. 1989; Letschert 1993; Raybould et al. 1996). Banding patterns of polymorphic loci were congruent with typical angiosperm zymograms, and were interpreted according to Weeden and Wendel (1989). Loci encoding the less anodally migrating allozyme for each enzyme system was designated "1", with additional loci numbered sequentially in order of increasing mobility. This nomenclature is inverted relative to the locus/allele designations for allozymes of Letschert (1993).

### 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 ($A_P$), and estimated heterozygosity (H). Genetic distances were computed according to Nei (1978). Computations were facilitated by the PC-based program POPGENE[1].

## Results

For the nine enzyme systems, we resolved 13 loci (approximately 1.4 loci per enzyme system) and 53 alleles (approximately 4.1 alleles per locus). Allele frequencies for individual populations and individual accessions are available on request from the senior author. A summary of the loci and alleles resolved in ten major accession groups (taxonomically and geographically sorted) is provided in Table 2. Three loci (*Aat2, Tpil*, and *Udp*) had only two alleles per locus. Three loci (*Aco, Gdh, Tpi2*) were tri-allelic, and the remaining seven (*Aat1, Lap, Mdh1, Mdh2, Pgm1, Pgm2*, and *Skd*) were multi-allelic, displacing up to six alleles per locus.

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

### Origin of Californian wild beets

To evaluate the origin of Californian wild beets, we first constructed a UPGMA dendrogram to elucidate the genetic relationships among 64 wild and cultivated beet populations based on Nei's (1978) genetic distances (Fig. 2). The dendrogram generally separates the accessions according to their taxonomic designations. All sugar beet accessions are separated as one group from other *B. vulgaris* subspecies. Swiss chard and red beets are clustered mostly within their own groups, with the exception of the red beet 'GBW 300C', 'Burpee', and the Swiss chard 'Rhubarb' accession. Sea beet is at the base of the *B. vulgaris* branch of the dendrogram, which fits well with its role as putative ancestor of the cultivated beets. The other *Beta* species are quite distant and distinct from *B. vulgaris*. *B. macrocarpa* is widely separated from the *B. vulgaris* group. *B. webbiana, B. patellaris*, and *B. procumbens* are clustered together in a common subgroup branch.

In the tree, Californian wild accessions either clustered within the *B. vulgaris* group (which we designate '*v.*-type') or within the *B. macrocarpa* group (which we designate '*m.*-type'). The wild *v.*-type is divided into three subgroups. We tested whether the three California *B. vulgaris* wild groups exhibit significant differentiation using the GENEPOP program (Raymond and Rousset 1995). We combined all the populations of a group into a single population and recalculated the allele frequencies. Then, we tested the hypothesis that the allelic distribution is identical across populations. For each of eight loci (*Mdh*1, *Aco, Lap, Aat*1, *Skd, Udp, Pgm*2, *Gdh*), the unbiased *p*-value was lower than 0.05, indicating significant genetic differentiation. Two loci distributions (*Pgm*1, *Mdh*2) were not significantly different; the remaining three loci (*Aat*2, *Tpi*1, *Tpi*2) could not be tested due to null alleles or a lack of allelic variation.

One group representing collections from the southwestern part of California (accessions 45–47) is closely related to red beet and Swiss chard, suggesting naturalization directly from vegetable beet ancestors. The second *v.*-type subgroup (representing the San Francisco Bay area and adjacent southern areas, accessions 40–44) appears in between the vegetable red beet/Swiss chard group and the sea beet accessions. This subgroup may have emerged from hybridization of cultivated beet with sea beets introduced from the Old World. The third *v.*-type subgroup consisted of the Santa Barabara coastal population (accession 48), which was related to the sea beet accessions and the one aberrant Swiss chard accession. Because the third California wild beet subgroup was most closely related to the Swiss chard accession, its origin remains unclear. If we compile the different accessions in a single *v.*-type group, then that group clusters closely with the Old World sea beet (Fig. 3), strongly supporting the hypothesis that at least some of California's wild beets originated directly from sea beet. We cannot exclude the possibility that feral populations may have evolved the allele frequencies of Old World sea beets. However, the morphologies of the *v.*-

**Table 2** Mean allele frequencies for ten major groups of the genus *Beta* (with accession number) examined in this study. Loci showing evidence for gene introgression in Californian wild beets of *B. macrocarpa* are underlined. Locus/allele nomenclature is described in Materials and methods. * See text for explanations of these designations

| Allele | | Sugar beet (1–16) | Swiss chard (17–20) | Red beet (21–25) | Sea beet (26–39) | Wild (*v.*) beet Ca.* (40–48) | Wild (*m.*) beet Ca.* (49–56) | *B. macrocarpa* (57–61) | *B. webbiana* (62) | *B. patelaris* (63) | *B. procumbens* (64) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Aat1* | –1 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.409 | 0.000 | 0.000 |
| | –3 | 0.533 | 0.500 | 0.496 | 0.541 | 0.515 | 0.010 | 0.027 | 0.000 | 0.500 | 0.500 |
| | –4 | 0.000 | 0.000 | 0.004 | 0.225 | 0.067 | 0.986 | 0.973 | 0.000 | 0.000 | 0.000 |
| | –5 | 0.466 | 0.500 | 0.500 | 0.234 | 0.415 | 0.004 | 0.000 | 0.409 | 0.000 | 0.000 |
| | –6 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.182 | 0.500 | 0.500 |
| *Aat2* | –1 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 | 1.000 | 0.989 | 1.000 | 1.000 | 0.000 |
| | –2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.011 | 0.000 | 0.000 | 0.000 |
| *Aco* | –1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 | 1.000 |
| | –2 | 0.914 | 0.176 | 0.254 | 0.742 | 0.612 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 |
| | –3 | 0.086 | 0.824 | 0.746 | 0.258 | 0.388 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *Gdh* | –1 | 0.824 | 0.933 | 1.000 | 0.690 | 0.802 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –2 | 0.176 | 0.067 | 0.000 | 0.217 | 0.198 | 0.008 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –3 | 0.000 | 0.000 | 0.000 | 0.093 | 0.000 | 0.990 | 1.000 | 1.000 | 1.000 | 1.000 |
| *Lap* | –1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.500 | 1.000 |
| | –2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.500 | 0.000 |
| | –3 | 0.390 | 0.725 | 0.551 | 0.867 | 0.791 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –4 | 0.610 | 0.275 | 0.449 | 0.173 | 0.201 | 0.007 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.992 | 1.000 | 0.000 | 0.000 | 0.000 |
| *Mdh1* | –1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.864 | 1.000 | 0.500 |
| | –2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.136 | 0.000 | 0.500 |
| | –3 | 0.765 | 0.525 | 0.827 | 0.881 | 0.760 | 0.691 | 0.782 | 0.000 | 0.000 | 0.000 |
| | –4 | 0.157 | 0.443 | 0.005 | 0.022 | 0.152 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –5 | 0.078 | 0.032 | 0.168 | 0.097 | 0.088 | 0.308 | 0.218 | 0.000 | 0.000 | 0.000 |
| *Mdh2* | –1 | 0.536 | 0.000 | 0.004 | 0.018 | 0.080 | 0.005 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –2 | 0.464 | 0.993 | 0.965 | 0.928 | 0.912 | 0.496 | 0.500 | 0.000 | 0.000 | 0.000 |
| | –3 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.499 | 0.500 | 0.500 | 0.000 | 0.000 |
| | –4 | 0.000 | 0.007 | 0.031 | 0.054 | 0.000 | 0.000 | 0.000 | 0.500 | 0.500 | 0.000 |
| | –5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.500 |
| | –6 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.500 | 0.500 |
| *Pgm1* | –1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.409 | 0.000 | 0.000 |
| | –2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.500 | 0.000 |
| | –3 | 0.000 | 0.000 | 0.000 | 0.049 | 0.000 | 0.986 | 0.991 | 0.591 | 0.000 | 0.000 |
| | –4 | 0.998 | 1.000 | 1.000 | 0.933 | 0.995 | 0.014 | 0.009 | 0.000 | 0.000 | 1.000 |
| | –5 | 0.002 | 0.000 | 0.000 | 0.018 | 0.005 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –6 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.500 | 0.000 |
| *Pgm2* | –1 | 0.000 | 0.000 | 0.000 | 0.012 | 0.000 | 0.993 | 0.833 | 0.000 | 0.000 | 0.000 |
| | –2 | 0.266 | 0.028 | 0.009 | 0.103 | 0.069 | 0.002 | 0.051 | 0.000 | 0.000 | 0.000 |
| | –3 | 0.686 | 0.964 | 0.991 | 0.821 | 0.902 | 0.004 | 0.116 | 0.000 | 0.000 | 1.000 |
| | –4 | 0.048 | 0.008 | 0.000 | 0.064 | 0.029 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 |
| *Skd* | –1 | 0.036 | 0.000 | 0.061 | 0.052 | 0.024 | 0.041 | 0.000 | 1.000 | 0.000 | 0.000 |
| | –2 | 0.005 | 0.000 | 0.010 | 0.078 | 0.024 | 0.952 | 0.991 | 0.000 | 0.500 | 0.500 |
| | –3 | 0.826 | 0.973 | 0.822 | 0.766 | 0.841 | 0.008 | 0.009 | 0.000 | 0.000 | 0.000 |
| | –4 | 0.133 | 0.027 | 0.107 | 0.104 | 0.111 | 0.000 | 0.000 | 0.000 | 0.500 | 0.500 |
| *Tpi1* | –1 | 0.499 | 0.500 | 0.500 | 0.475 | 0.500 | 0.500 | 0.535 | 0.500 | 0.000 | 0.500 |
| | –2 | 0.501 | 0.500 | 0.500 | 0.525 | 0.500 | 0.500 | 0.465 | 0.500 | 1.000 | 0.500 |
| *Tpi2* | –1 | 0.000 | 0.000 | 0.000 | 0.062 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –2 | 0.971 | 1.000 | 0.911 | 0.882 | 1.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 |
| | –3 | 0.029 | 0.000 | 0.089 | 0.056 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *Udp* | –1 | 0.556 | 0.482 | 0.831 | 0.197 | 0.520 | 0.000 | 0.000 | 0.046 | 0.500 | 0.500 |
| | –2 | 0.444 | 0.518 | 0.169 | 0.803 | 0.480 | 1.000 | 1.000 | 0.954 | 0.500 | 0.500 |

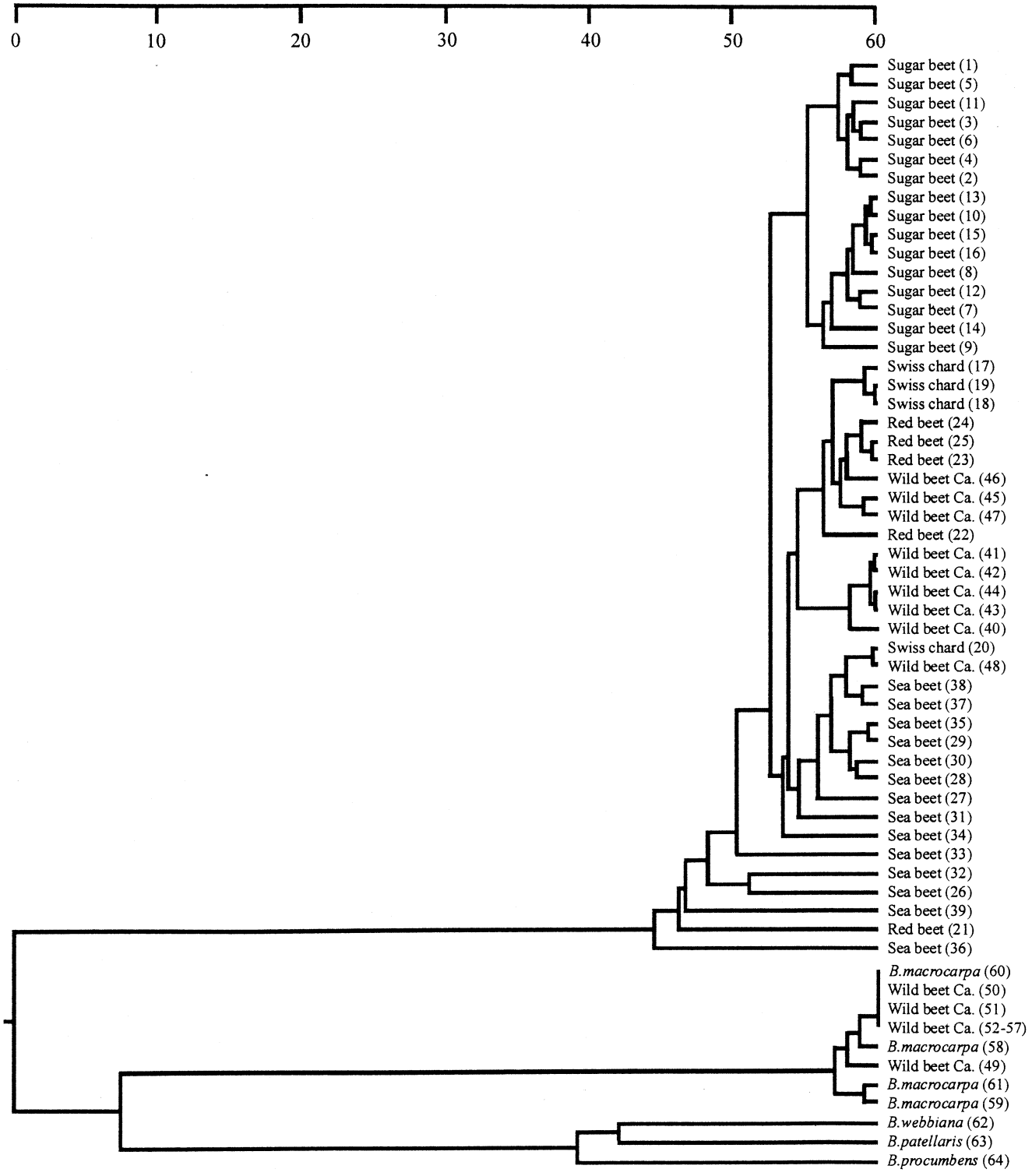


**Fig. 2** UPGMA dendrogram of systematic relationships among 64 wild and cultivated beet populations based on Nei's (1978) genetic distances derived from allele frequencies at 13 polymorphic allozyme loci. Population numbers are described in Table 1

type plants also suggest a wild-cultivated hybrid ancestry (D. Bartsch, unpublished data).

Non-bolting (accession 15) and bolting (accession 16) California sugar beets had nearly identical allele frequencies, suggesting a climatic induction of early flowering of sugar beet. There was no evidence for hybridization or introgression of annual wild beet germplasm in these genotypes.

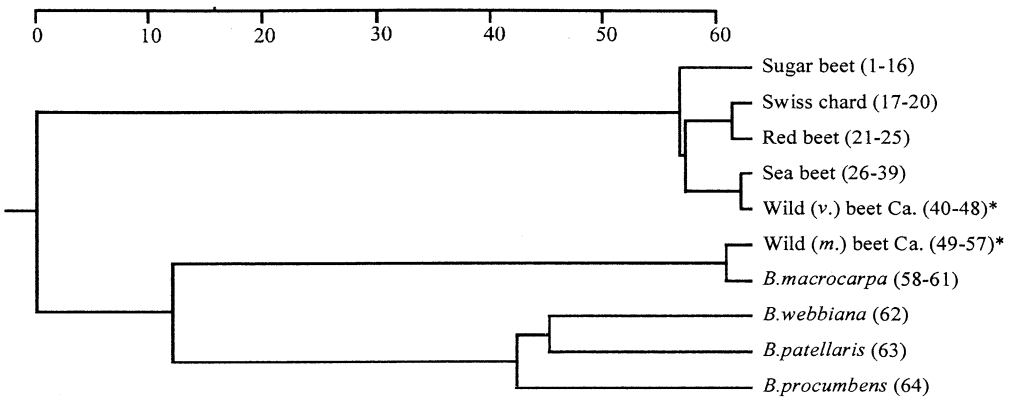


**Fig. 3** UPGMA dendrogram of systematic relationships among ten major groups (with accession number) of wild and cultivated beet based on Nei's (1978) genetic distances derived from allele frequencies at 13 polymorphic allozyme loci. * See text for explanations of these designations

With one exception, the Californian wild beet accessions (50–56) are genetically identical with a Spanish *B. macrocarpa* from the Mediterranean area of Cartagena. One wild *m.*-type beet accession (accession 49 – Imperial Valley) had some plants with one or more of ten alleles that are otherwise specific to *B. vulgaris* (*Aat*1–6, *Gdh*1–1, *Gdh*1–2, *Lap*–3, *Lap*–4, *Mdh*1–4, *Mdh*2–1, *Pgm*2–4, *Skd*–1, *Tpi*2–2, Table 2) although all plants were morphologically similar to *B. macrocarpa* and had genomes that were otherwise quite similar to *B. macrocarpa*. This is substantial evidence of introgression from the crop in the area (a major cultivated beet-growing region of California). About 2% of the wild *m.*-type beets in the Imperial Valley have introgressed genomes (13 of all 594 examined plants, accessions 49–56 in Table 2). If we exclude those apparently introgressed individuals of accession #49 showing alleles otherwise found only in *B. vulgaris*, the accession is essentially the same as the other *m*-type accessions.

In summary, our genetic evidence suggests that Californian wild beets belong to two different taxa, and have at least three different origins. We found wild beet evolved from: (1) escaped Swiss chard or red beet, (2) *B. macrocarpa*, presumably introduced from Spain, and (3) hybridization of *B. vulgaris* with introduced *B. macrocarpa*. Although wild sea beet probably played some role in the origin of California wild beets, our genetic information is insufficient to determine the extent to which hybridization of cultivated betwith sea beet and/or direct introduction of sea beet from Europe contributed to contemporary *v.*-type wild beets in California.

## Genetic relationships among different groups in the genus *Beta*

The genetic relationships among *B. vulgaris* and other species of the genus *Beta* (including *B. macrocarpa, B. patellaris, B. webbiana, B. procumbens*) were evaluated for ten major groups (Table 2). The Californian wild beet accessions were lumped into *v.*-type and *m.*-type according to their evolutionary origin. We recognize this lumping merges the disparate histories of single accessions and therefore note that any interpretations must be made with caution.

Genetic relationships among the major groups of the *Beta* accessions examined were evaluated by calculating the genetic identity (*I*) coefficient of Nei (1978, Table 4). Within *B. vulgaris*, the highest genetic identities were observed in pairwise comparisons between sea beet and wild *v.*-type beet (*I*=0.98), and the lowest were between sea beet and red beet (*I*=0.85). Swiss chard and red beet also had a high identity (*I*=0.96). As expected, the wild *m.*-type beet and the *B. macrocarpa* group had a high identity (*I*=0.95). In the various *B. vulgaris* groups, the two wild beet groups are closest to the other *Beta* species, especially to the two *B. macrocarpa* groups (*I*=0.46 and 0.54). The lowest genetic identity was observed between red beet and *B. webbiana* (*I*=0.11). Among the other species, *B. procumbens* had some affinity with *B. webbiana* (*I*=0.69) and *B. patellaris* (*I*=0.68).

To evaluate relationships among groups of accessions, we constructed a UPGMA dendrogram based on Nei's (1978) genetic distances (Fig. 3). According to this pattern, the Californian wild beets have major affinities to both *B. vulgaris* and *B. macrocarpa*. In the tree, sea beet clustered with the wild *v.*-type beet, and the wild *m.*-type clustered with Old World accessions of *B. macrocarpa*. By incorporating the three different outgroup species, the unique nature of the two distinct wild *Beta* species in California is clearly apparent. The genetic distance obtained for *B. vulgaris* and *B. macrocarpa* is nearly as great as the distance of each of them to the outgroups of *B. procumbens, B. webbiana,* and *B. patellaris* (all of which are cross-incompatible with *B. macrocarpa* and *B. vulgaris*). It is remarkable that, despite their substantial distances, hybridization between *B. vulgaris* and *B. macrocarpa* is still possible, as both allozyme markers in this study demonstrated and prior literature (Coons 1975; Abe et al. 1986) has suggested.

In summary, we found strong evidence for the classification of *B. macrocarpa* as a separate species from *B. vulgaris.* Although the two species are cross-compatible, they are clearly differentiated at the molecular level.

**Table 3** Genetic diversity statistics for ten major groups of the genus *Beta*. * See text for explanations of these designations

| Group | $N^a$ | $A$ | $A_p$ | $P$ | $H$ | $U$ |
|---|---|---|---|---|---|---|
| *B. vulgaris* (all) | 34.5 | 2.92 | 3.08 | 0.923 | 0.330 | 36 |
| Sugar beet (1–16) | 39.8 | 2.23 | 2.42 | 0.923 | 0.343 | 29 |
| Swiss chard (17–20) | 37.8 | 1.85 | 2.30 | 0.769 | 0.248 | 24 |
| Red beet (21–25) | 36.0 | 2.15 | 2.60 | 0.769 | 0.250 | 27 |
| Sea beet (26–39) | 33.5 | 2.69 | 2.83 | 0.923 | 0.304 | 35 |
| Wild (*v.*) beet Ca. (40–48)* | 24.3 | 2.38 | 2.64 | 0.846 | 0.284 | 30 |
| Wild (*m.*) beet Ca. (49–56)* | 88.0 | 2.31 | 2.89 | 0.682 | 0.125 | 28 |
| *B. macrocarpa* (57–61) | 31.0 | 1.62 | 2.13 | 0.615 | 0.145 | 13 |
| *B. webbiana* (62) | 11.0 | 1.38 | 2.17 | 0.462 | 0.222 | 6 |
| *B. patellaris* (63) | 10.0 | 1.31 | 2.00 | 0.462 | 0.273 | 9 |
| *B. procumbens* (64) | 2.0 | 1.31 | 2.00 | 0.462 | 0.273 | 6 |

[a] Abbreviations for gene diversity statistics include $N$ (average number of plants sampled per accession), $A$ (average number of alleles per locus), $A_p$ (average number of alleles per polymorphic locus), $P$ (Proportion of loci polymorphic), $H$ (estimated heterozygosity), and $U$ (number of unique alleles per group with the *B. vulgaris* alleles)

**Table 4** Nei's genetic identity (*I*: above diagonal) and genetic distance (*D*: below diagonal) for major groups of cultivated beet (genus *Beta*) and related wild species. * See text for explanations of these designations

| Type | Sugar beet (1–16) | Swiss chard (17–20) | Red beet (21–25) | Sea beet (26–39) | Wild (*v.*) beet Ca.* (40–48) | Wild (*m.*) beet Ca.* (49–56) | *B. macrocarpa* (57–61) | *B. webbiana* (62) | *B. patellaris* (63) | *B. procumbens* (64) |
|---|---|---|---|---|---|---|---|---|---|---|
| Sugar beet (1–16) | **** | 0.8705 | 0.8913 | 0.8590 | 0.8861 | 0.3151 | 0.3865 | 0.1435 | 0.3908 | 0.1691 |
| Swiss chard (17–20) | 0.1387 | **** | 0.9630 | 0.8639 | 0.9120 | 0.2343 | 0.3059 | 0.1394 | 0.3906 | 0.1498 |
| Red beet(21–25) | 0.1151 | 0.0377 | **** | 0.8465 | 0.9065 | 0.2348 | 0.2963 | 0.1104 | 0.3998 | 0.1561 |
| Sea beet (26–39) | 0.1520 | 0.1463 | 0.1667 | **** | 0.9781 | 0.4967 | 0.5433 | 0.2902 | 0.3876 | 0.2960 |
| Wild beet Ca. (40–48) | 0.1209 | 0.0921 | 0.0982 | 0.0221 | **** | 0.3973 | 0.4596 | 0.2446 | 0.3841 | 0.2711 |
| *B.macrocarpa* (49–56) | 1.1549 | 1.4512 | 1.4492 | 0.6998 | 0.9231 | **** | 0.9523 | 0.4314 | 0.2989 | 0.3841 |
| *B.macrocarpa* (57–61) | 0.9506 | 1.1844 | 1.2164 | 0.6100 | 0.7775 | 0.0489 | **** | 0.4138 | 0.2683 | 0.3657 |
| *B.webbiana* (62) | 1.9416 | 1.9701 | 2.2037 | 1.2374 | 1.4081 | 0.8408 | 0.8823 | **** | 0.6344 | 0.6949 |
| *B.patellaris* (63) | 0.9395 | 0.9401 | 0.9168 | 0.947 | 0.9568 | 1.2078 | 1.3155 | 0.4551 | **** | 0.6778 |
| *B.procumbens* (64) | 1.7774 | 1.8982 | 1.8572 | 1.2176 | 1.3051 | 0.9569 | 1.0058 | 0.3640 | 0.3889 | **** |

## Genetic variability in *B. vulgaris*

As a species, our sample of *B. vulgaris* (wild+domesticated) has a moderately high value for *H* (0.33). Averaged across loci, the mean estimated heterozygosity for individual groups of *B. vulgaris* accessions ranged from a high of approximately 0.34 for sugar beet to a low of 0.25 for red beet (Table 2).

As expected, the wild beets of *B. vulgaris* (including both sea beet and *v.*-type beet) were more polymorphic than domesticated beet. Of the cultivated forms, the sugar beet is the most polymorphic. This trend was evident in the summary statistics (Table 3) and allelic frequencies for groups of *B. vulgaris* (Table 2). As a group, *B. vulgaris* included 36 alleles at 13 loci, with 2.9 alleles per locus (3.1 alleles per polymorphic locus). Five of these alleles (*Gdh*-3, *Pgm*1–3, *Pgm*1–5, *Pgm*2–1, *Tpi*2–1) were unique to wild beet (*v.*-type and sea beet, Table 3). The accessions that comprise the Old World sea beet showed the highest overall diversity within the *B. vulgaris* group with 35 total alleles/13 loci (A=2.7, $A_p$=2.8) and a *H* value of 0.31. The wild *v.*-type beet had less polymorphism with 30 alleles/13 loci (A=2.4, $A_p$=2.6, H=0.28). Among the groups of cultivated beet, sugar beet was most polymorphic with 29 alleles/12 loci (A=2.2, $A_p$=2.4, H=0.34). Red beet and Swiss chard had 27 alleles/12 loci (A=1.9, $A_p$=2.6, H=0.25) and 24 alleles/12 loci (A=2.2, $A_p$=2.3, H=0.26) respectively. Among all groups of *B. vulgaris*, sea beet had four unique alleles (*Gdh*-3, *Pgm*1–3, *Pgm*1–5, *Pgm*2–1, *Tpi*2–1), and wild *v.*-type beet had one (*Aat1*–6). One additional allele (*Aat*2–1) is unique to both wild beet groups. We found no allele unique to the cultivated beet group, although the sugar beet group showed a very high frequency of the *Mdh*2–1 allele. Both Swiss chard and red beet share a high frequency of the *Aco*–3 allele.

## Genetic variability in *B. macrocarpa*

The Old World *B. macrocarpa* accession group seems to be less genetically variable than any of the *B. vulgaris* groups. This result must be regarded carefully because we analysed only four accessions. Both the wild *m.*-type beet and *B. macrocarpa* (Table 3) are generally weakly polymorphic; seven of nine polymorphic loci of the wild *m.*-type beet and six of eight polymorphic loci of the Old World group had allele frequencies >0.9 for the most common allele. Consequently, as a species, both of our *B. macrocarpa* groups had a moderately low estimated heteorzygosity (*H*=0.13 and *H*=0.15 respectively, Table 2).

Wild *m*.-type beet is more polymorphic than the Old World group, as one might expect from our evidence of gene introgression from *B. vulgaris* (see section "Origin of Californian wild beets"). Wild *m*.-type beet has 30 alleles at 13 loci, an average of 2.3 alleles per locus (2.9 alleles per polymorphic locus). This group shared 23 of the 36 alleles found in *B. vulgaris* alleles. Ten of these 23 alleles (*Aat*1–5, *Gdh*1–1, *Gdh*1–2, *Lap*-3, *Lap*-4, *Mdh*1–4, *Mdh*2–1, *Pgm*2–4, *Skd*-1, *Tpi*2–2) were taxon specific for *B. vulgaris* relative to pure *B. macrocarpa* (Table 2) and occurred in an extremely low frequency in the California populations of *B. macrocarpa* (<0.042, average 0.008). The Old World *B. macrocarpa* group, lacking these ten *B. vulgaris*-specific alleles, had 21 alleles at 12 loci, an average of 1.6 alleles per locus (2.1 alleles per polymorphic locus). The two groups of *B. macrocarpa* shared one unique allele (*Lap*-5). We also found high frequency of two alleles which are rare or not present in other groups (*Aat*1–4, *Pgm*2–1), and one allele *Mdh*2–3, which *B. macrocarpa* shared only with *B. webbiana*. In summary, the *B. macrocarpa* species had a relatively low genetic variability, but was well-differentiated from the other *Beta* species by our allozyme markers.

### Genetic variability in the *B. procumbens*, *B. webbiana* and *B. patellaris* outgroup

The average number of alleles per locus for the *B. procumbens*, *B. webbiana*, and *B. patellaris* accessions (Table 3) are relatively low (A<1.4), but this is probably due to our relatively small number of individuals sampled. Less than half of the 13 loci examined are polymorphic ($p$=0.462). The estimated heterozygosity (H=0.22, H=0.27, and H=0.27 respectively, Table 2) is moderately high.

Members of this outgroup shared less than ten (6 or 9) of the total 36 alleles we found in *B. vulgaris*. A maximum of seven of the total 18 outgroup alleles are unique to this group compared with *B. vulgaris* and *B. macrocarpa*. All three species share three of these six unique alleles (*Aco*-1, *Lap*-1, *Mdh*1–1), the other 3–4 alleles were distributed differently (*Aat*1–2, *Mdh*1–2, *Pgm*1–1 for *B. webbiana*, *Lap*-2, *Mdh*2–6, *Pgm*1–2, *Pgm*1–6 for *B. patellaris*, and *Mdh*1–2, *Mdh*2–5, *Mdh*2–6 for *B. procumbens*, Table 2).

## Discussion

### Origin of wild beet in California

Our allozyme analysis gave substantial evidence for two independent genetic origins of wild beet in California. The evolutionary origin of the wild beet in the Imperial Valley and on the Channel Islands is *B. macrocarpa*; the allele frequencies of the Californian populations were almost identical to those of our Spanish accession. One potential introduction for *B. macrocarpa* was via contamination of cultivated beet seed. However, it is unlikely that the source was contaminated sugar beet seed. The first record of wild beet in the Imperial Valley (Carsner 1928) occurred before sugar beet came into this area in the 1930s, and sugar beet has never been grown on the Channel Islands (M. Hoefs and S. Chaney, personal communication). More likely is the contamination of vegetable cultivars of Swiss chard or red beet. A secondary introduction could have taken place from Mexico[2] via Spanish settlers as early as 1770 when Franciscan Fathers moved into California. This hypothesis is supported by McFarlane (1975), who suggested contaminated feed grain or agricultural seeds as the introduction source. Another source could have been contaminated ballast sand of ships. The historic link between California and the Spanish port city Cartagena – a departure point for ships with colonists bound for the New World[3] – may account for the close genetic identity of *B. macrocarpa* from this location and the accessions in California.

The other wild beets in California are the species *B. vulgaris*, as our allozyme data demonstrated. We found these to have evolved from as many as three different pathways: First, some populations may be directly descended from Swiss chard or red beet escapes. Escaped waifs are common for many crops (e.g. Hickman 1993) and have been reported for sugar beet in California (Johnson and Burtch 1959). Second, wild beets may be descendants of introduced sea beet. The source could have been the seed contamination of cultivar beets or the introduction in ballast sand of ships arriving from Europe. This latter probably accounts for the introduction of wild beets from the British Isles to the Baltic Sea coast in the 18th century (Christensen 1996). To support this hypothesis, the Californian accession most closely related to European sea beet was found directly on the Pacific coast of Santa Barbara. Third, hybridization of cultivated beet with sea beet may have occurred in California. This hybridization between wild and domesticated *B. vulgaris* is very common (Bartsch and Schmidt 1997) and has led to the evolution of weed beet populations in France (Boudry et al. 1993) and the United Kingdom (Hornsey and Arnold 1979; Ford-Lloyd and Hawkes 1986).

### Genetic diversity and gene flow in wild beet

Most genetic comparisons of crops to their putative progenitors have shown that only a fraction of the total genetic variation present in an ancestral taxon is incorporated into the cultivated taxon (Doebley 1989). As new cultivars are propagated, additional genetic diversity may be lost through selection and genetic drift. Unless this process is ameliorated by gene flow, perhaps from wild or weed progenitors, loss of genetic variation is expected to continue during the domestication process. The degree and severity of genetic erosion ultimately depend on factors such as the intensity of selection, the preva-

[2] *B. macrocarpa* is also distributed in the Mexican Province of Baja California (Bartsch, personal observation)

[3] See Microsoft's Encarta Encyclopedia, Reference Suite '99

lence of drift, and the frequency with which new variation is introduced.

The historical development of beet cultivars has been documented by Ford-Lloyd and Williams (1975) and Mansfeld (1986). It is known that: (1) *Beta* was originally domesticated in the East Mediterranean area more than 2000 years ago, and Swiss chard and red beet are the oldest cultivated forms, (2) Asian cultivars were introduced to Western and Central Europe as early as the Roman Age, (3) Sugar beet is the youngest domesticated form originating from wild beet ancestors of the North European Channel Coast at the end of the 18th century (Fischer 1989). Each event may have imposed a genetic "bottleneck" on domesticated beet, so that the amount of genetic diversity in domesticates might be expected to be strongly associated with the degree of agronomic selection.

Estimates of the genetic variability from the present study indicate that wild *B. vulgaris* is more diverse genetically than domesticated *B. vulgaris*, the latter showing the predicted reductions in genetic diversity with increasing agronomic history (Table 3). When sea beets are compared with domesticated beets, the reduction in genetic diversity is manifested in three ways: as a reduction in allelic diversity ($A$ and $A_P$), as a reduction in the proportion of polymorphic loci ($P$), and the number of unique alleles per group with the *B. vulgaris* alleles ($U$). The reduction in estimated heterozygosity ($H$) is only manifested in comparisons of the sea beet with the Swiss chard and the red beet group.

Sugar beet is an exception to this pattern with more polymorphism than vegetable beets despite its recent origin. We can explain this paradox in part by the extensive breeding process used to create hybrid seed. Highly inbred self-incompatible (cms – 'cytoplasmatic male sterile') maternal lines are pollinated with an alternate highly homozygous inbred line in order to produce heterotic effects. Also, the higher diversity of sugar beet compared to the vegetable beets could also result from the regular use of sea beet germplasm for breeding improvement. This practice has "contaminated" the sugar beet gene pool with wild beet genes, which cannot completely be eliminated by backcrossing (Van Geyt et al. 1990).

The lower genetic diversity of the *B. macrocarpa* group compared with the *B. vulgaris* group can be explained by their difference in breeding system. *B. vulgaris* is highly outcrossed as it is usually self-incompatible and wind-pollinated (Barocka 1985). *B. macrocarpa* is self-compatible (Oldemeyer 1957). Selfing species typically have reduced genetic diversity relative to their outcrossed congeners. The relatively low degree of variability in the *B. macrocarpa* accessions could be the result of generations of self-pollination, bottleneck-effects, and genetic drift. Because of the weedy nature of *B. macrocarpa* (including the *m.*-type wild beet) and its ability to colonize disturbed soils, the genetic homogeneity in wild populations may reflect founder effects in recent or ecological or geographic expansion.

## Taxonomy of wild beets, *B. macrocarpa*, and other *Beta* species

Based on our allozyme data, the taxonomic placement of Californian wild beets was straightforward. We came to the conclusion that, as a group, Californian *v.*-type wild beets should be classified as *B. vulgaris* ssp. *maritima*; these are the accessions that, as a group, are nearly morphologically and genetically identical to the sea beet. Although our detailed genetic distance dendrogram suggested an ancestral role of domesticated beet for some of the *v.*-type wild beet accessions (Fig. 2), morphologically the *v.*-type beet is a genetic throwback to the wild sea beet, the primary ancestor of all domesticated beets. This interpretation is consistent with Letschert's (1993) that more or less permanent populations of wild beet with introgression of alleles from cultivated plants should be taxonomically treated as *B. vulgaris* ssp. *maritima*. But his suggestion is unworkable for our *m.*-type wild beets, which are essentially genetically identical with *B. macrocarpa*. Our study therefore has revealed that California wild beet comprises two separate species.

Because of the cross compatibility and morphological similarities of *B. macrocarpa* and *B. vulgaris*, some authors have classified the former as *B. vulgaris* ssp. *macrocarpa* (Helm 1957; Ford-Lloyd 1986) or as *B. vulgaris* ssp. *maritima* variety *macrocarpa* (Krassochkin 1959; Ford-Lloyd and Williams 1975). We found a strong genetic differentiation between *B. vulgaris* and *B. macrocarpa*, which supports the notion that the latter is a separate species. Other studies using DNA fingerprinting or sequencing techniques are concordant with these results (Jung et al. 1993; Shen et al. 1998a). Morphologically, these two species are safely distinguishable only by the convex operculum of *B. vulgaris* and the depressed operculum with elevated margins of *B. macrocarpa* (Letschert 1993).

We did not use the term 'weed beet' for any classification. The ecological behavior of a plant as a 'weed' is a functional and not a taxonomic term. We found *B. macrocarpa* as a widespread weed in a sugar beet field of the Imperial Valley in California. Hybrids between sugar beet and sea beet (both *B. vulgaris*) are known to be weeds in European sugar beet fields (Hornsey and Arnold 1979; Ford-Lloyd and Hawkes 1986; Boudry et al. 1993). A functional classification of these plants should not lead to the creation of a separate subspecies for weedy plants.

Based on our allozyme data, *B. webbiana, B. patellaris*, and *B. procumbens* all appear to be closely related. Perhaps they may even be subspecies of a single species. Our sample of accessions is too small to provide definitive information. All three species are perennial. They are distinct from *B. vulgaris* and *B. macrocarpa* by the monogerm seed nature, the very short perianth segments and the globular fruit shape (Letschert 1993). *B. patellaris* is a tetraploid cytotype, and it is distinct in its leaf shape. *B. procumbens* and *B. webbiana* are very similar (probably the two diploid parents of *B. patellaris*), and several authors (Curtis 1968; Letschert 1993; Shen et al. 1998b) have also cast doubt on their status as separate species.

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