Beta section *Beta*:
Biogeographical patterns of variation, and taxonomy.

15

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Beta section *Beta*: biogeographical patterns of variation, and taxonomy.

proefschrift
ter verkrijging van de graad van doctor in de landbouw- en milieuwetenschappen, op gezag van de rector magnificus dr. H.C. van der Plas, in het openbaar te verdedigen op vrijdag 26 februari 1993 des namiddags te vier uur in de aula van de Landbouwuniversiteit te Wageningen.

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STELLINGEN

1. Integratie van biosystematisch onderzoek met onderzoek naar oecologische kenmerken geeft een beter begrip van soortvormingsprocessen.

2. *Beta patula* is in fylogenetisch opzicht geen intermediair tussen de 'primitieve' sectie *Procumbentes* en de 'afgeleide' sectie *Beta* maar een soort die vrij recent is afgescheiden en geisoleerd van continentale populaties van *Beta vulgaris* subsp. *maritima*.

   V.I. BURENIN & I.P. GAVRILYNK, 1982. Trudy po Prikladnoi Botanike, Genetike i Selektsii 72(3): 3-12 (German Translation).

3. De geïntensiveerde exploitatie van de locaties in Portugal waar zoutwinning uit zeewater plaats vindt, de zogenaamde salinas, vormt een bedreiging voor vrijwel alle daar voorkomende populaties van *Beta macrocarpa*.

   L. FRESE, E. DE MEYER & J. LETSCHERT, 1990. Zuckerind. 115 (11): 950-955.

4. Voor veel belangrijke cultuurplanten – waaronder *Beta vulgaris* L. – behoort niet de reconstructie en strikte interpretatie van het Linneaans concept uitgangspunt te zijn bij typificatie van de naam, maar moet afgewogen worden in hoeverre consolidatie van de hedendaagse namen mogelijk is.

   J. HELM, 1957. Kulturpflanze 5: 55-74. D.O. WIJNANDS, 1986. Acta Horticulturae 182: 67-78.

5. *Beta vulgaris* subsp. *provulgaris* Ford-Lloyd & Williams is een hypothetisch taxon geconstrueerd ter illustratie van een evolutionair scenario en heeft als zodanig geen empirische basis.

   B.V. FORD-LLOYD & J.T. WILLIAMS, 1975. Bot. J. Linn. Soc. 71: 89-102.

6. Het oprichten van nieuwe, flexibele categorien binnen het raamwerk voor infraspecifieke classificatie van de ICBN voor de formele beschrijving van de resultaten van biosystematisch onderzoek is niet aanbevelenswaardig, vooral omdat de gebruikswaarde in de praktijk zeer laag zal zijn.

   H. DEN NIJS, 1983. Biosystematic studies of the *Rumex acetosella* complex. stelling 6 bij de dissertatie.

7. Het voorstel van Santoni & Bervillé *Beta* sectie *Corollinae* op te delen in twee subsecties op basis van slechts één extra restrictie site *Bam*HI B_4 is geen bijdrage tot het oplossen van de taxonomische problemen in deze groep.

   S. SANTONI & A. BERVILLÉ, 1992. Theor. Appl. Genet. 83: 533-542.

8. De bewering van Santoni & Bervillé dat met de nucleair ribosomaal DNA unit type V-11-2.9 voor het eerst een moleculaire marker is vastgesteld waarmee de belangrijkste cultuurvormen van de biet kunnen worden onderscheiden van de wilde taxa in *Beta* sectie *Beta* is onjuist gelet op de resultaten die vermeld staan in tabel 2 van hun artikel.

S. SANTONI & A. BERVILLÉ, 1992. Theor. Appl. Genet. 83: 533-542.

9. Behalve zich te vergewissen van de exacte herkomst en correcte identificatie van hun plantenmateriaal zouden moleculair systematici en moleculair genetici er goed aan doen voucher specimens vast te leggen die controle achteraf mogelijk maken. De zeer onvolledige documentatie van het onderzoeksmateriaal in het artikel van G. Mita et al. maken de aan RFLP onderzoek ontleende conclusies aangaande de systematiek van het genus *Beta* van betrekkelijke betekenis.

P. GOLDBLATT, P.C. HOCH & L.M. MCCOOK, 1992. Ann. Missouri Bot. Gard. 79: 969-970; G. MITA, M. DANI, P. CASCIARI, A. PASQUALI & E. SELVA, 1991. Euphytica 55: 1-6.

10. In plaats van boos naar de milieubeweging te wijzen na het door de Raad van State binnen twee maanden vernietigen van zeven vergunningen voor het doen van veldproeven met transgene planten, zouden de gedupeerde instituten en bedrijven er beter aan doen hun lichtvaardige inschatting van de publieke wens tot controle van nieuwe technologieën bij te stellen, en er toe over moeten gaan een werkelijk serieuze analyse te maken van de risico's van verspreiding van transgenen in het milieu.

Bionieuws 15, 16, 17 & 18; De Volkskrant 30-12-92; Landbouwkundig Tijdschrift, December 1992; F.T. de Vries, R. van der Meijden en W.A. Brandenburg, 1992. Gorteria Supplement 1, Botanical files.

11. De opleiding van de AIO in de taxonomie en systematiek zou moeten zijn opgebouwd volgens een vakgerichte scholing in de eerste twee jaar van aanstelling, en in de laatste twee jaar, wegens het ontbreken van enig perspectief op een baan, mogelijkheden moeten bieden voor omscholing tot een ander beroep.

12. Realisatie van de plannen voor 'natuurontwikkeling' vormt een serieuze bedreiging voor de nederlandse weidevogels.

13. 75 jaar Landbouwuniversiteit heeft het bijna failliet van de nederlandse akkerbouw niet kunnen afwenden.

Stellingen behorend bij het proefschrift van J.P.W. Letschert; *Beta* section *Beta*: biogeographical patterns of variation, and taxonomy.

Wageningen, 26 februari 1993.

In herinnering aan mijn vader
voor Josė

Curriculum vitae

Josephus Petrus Wenceslaus Letschert werd op 10 Januari 1960 geboren te Amsterdam. Van 1978 tot 1986 studeerde hij biologie met hoofdvak oecofysiologie en bijvakken milieukunde en zoögeografie aan de Vrije Universiteit te Amsterdam. Van 1988 tot 1992 was hij als assistent in opleiding (A.I.O) werkzaam bij de vakgroep Plantentaxonomie van de Landbouwuniversiteit te Wageningen.

Contents

Biogeografische variatiepatronen en taxonomie van *Beta* sectie *Beta*

Achtergrond en doel van de studie

De zes hoofdstukken van dit proefschrift vormen het verslag van een kwantitatief biosystematisch onderzoek van soorten direct verwant aan de geteelde biet *Beta vulgaris* subsp. *vulgaris*.

Het onderzoek had tot doel de taxonomische situatie rond de wilde taxa van sectie *Beta* op te helderen, – de resultaten van dit onderzoek zijn dan ook verwerkt in een taxonomische revisie van sectie *Beta* – en tevens een analyse te maken van morfologische, genetische en ecologische variatiepatronen op infraspecifiek niveau.

Het project had de vorm van een samenwerkingsverband tussen de vakgroep Plantentaxonomie LUW enerzijds en de genenbank CGN van het CPRO-DLO anderzijds. De methode van biosystematisch onderzoek en de toepasbaarheid van biosystematisch onderzoek voor het werkterrein van de genenbank worden hier kort toegelicht.

Effectieve representatie van wild Beta materiaal in genenbankcollecties

De wilde soorten die verwant zijn aan de cultuurbiet bezitten allerlei eigenschappen die interessant zijn voor de plantenveredeling. De teeltkundig gunstige eigenschappen lopen uiteen van resistentie tegen bacteriën, virussen, schimmels, aaltjes en andere ziekten en plagen, tot resistentie tegen doorschieten en tolerantie voor koude. Verder worden bij de wilde soorten natuurlijke polyploiden aangetroffen. In de veredeling maakt men graag gebruik van mannelijk steriele lijnen. Cytoplasmatische mannelijke steriliteit (CMS) komt van nature voor in wilde populaties. Men is reeds lang op zoek naar cytoplasma's die een alternatief kunnen vormen voor het in de veredeling gangbare Owen-cytoplasma.

Alvorens de gewenste eigenschappen in het gewas kunnen worden gebracht is het van belang deze eigenschappen te localiseren en vervolgens genetisch te karakteriseren. De specifiek nuttige genetische informatie waarnaar men op zoek is, is soms aanwezig in meerdere verwante soorten, soms specifiek terug te vinden in één wilde soort, soms ook slechts terug te vinden in een klein aantal populaties van een wilde soort. De genetische overerving van de gewenste eigenschap kan sterk varieren: voor een aantal kenmerken zoals ziekteresistenties is slechts een klein aantal, betrekkelijk simpel overervende genen en allelen verantwoordelijk. De meeste eigenschappen zijn echter polygeen gecontroleerd, en de expressie is kwantitatief van aard. Verschillende eigenschappen zijn bovendien niet alleen afhankelijk van het samenspel van meerdere loci en allelen, maar staan ook sterk onder invloed van de milieucondities.

Teeltkundig gunstige genencombinaties worden daar aangetroffen waar specifiek geadapteerde genotypen onder speciale klimaats-, bodem-, of ziekte-omstandigheden voorkomen. Vanuit de optiek van de genenbank is het gewenst dergelijke genotypen op te sporen en in collecties onder te brengen, waarbij het de bedoeling is een optimale representatie van de totale 'gene pool' te creëren. Het is duidelijk dat het in kaart brengen van morfologische groepen en het aangeven van clinale variatie in relatie tot klimaatsovergangen bijdraagt aan de samenstelling van een zo heterogeen mogelijke verzameling genotypen.

Ook de informatie uit allozymanalyses kan gebruikt worden voor het verzamelen van zoveel mogelijk verschillende genotypen. De verspreiding van bepaalde allozymvarianten geeft een idee over de mate van recent contact tussen regionale populaties. Neemt men infraspecifieke verschillen in de populatiedifferentiatie en populatiestructuur van de soorten waar, dan kan dit aanleiding zijn de verzamelstrategie aan te passen. Zo kan men op basis van de kennis uit allozymdata kiezen voor het bemonsteren van veel of weinig individuen per populatie of het bemonsteren van meer of minder populaties per regio.

Taxonomische problematiek: het afgrenzen van soorten

Het beschrijven van taxonomische groepen in sectie *Beta* en zo mogelijk onderscheiden van soorten vormde een andere doelstelling van dit onderzoek. Sinds Linnaeus in 1753 *Beta vulgaris* vaststelde, zijn een groot aantal soorten gepubliceerd en in sectie *Beta* geplaatst. In Tabel 1.1 zijn voor sectie *Beta* 15 soorten vermeld. Naar de mening van sommige auteurs diende dit aantal drastisch gereduceerd te worden. De meest recente revisies (Ford-Lloyd & Williams 1975, Ford-Lloyd 1986) gaven aan dat sectie *Beta* in feite slechts één soort omvat, *B. vulgaris*, waarin zowel wilde vertegenwoordigers als de cultuurbiet thuis hoorden. De analyse van materiaal uit een groot deel van het verspreidingsgebied had het beeld opgeleverd van de soort als *een morfologisch continuum van biotypen*. Ford-Lloyd & Williams (1975) en Ford-Lloyd (1986) stelden vast dat de taxa beschouwd moesten worden als deel uitmakend van een gemeenschappelijke 'gene pool'. De enorme vormenvariatie was naar hun mening het gevolg van fenotypische plasticiteit in relatie tot groei-omstandigheden. Halofytische groei-omstandigheden zijn aanleiding voor graduele verschillen in succulentie. Klimaatsfactoren veroorzaken een fysiologisch effect op de bloei, als gevolg waarvan eveneens een grote variatie aan groeivormen optreedt. Tevens veronderstelden zij dat er sprake was van een verminderde genenuitwisseling tussen populaties, en dat er sprake was van 'micro-evolutie'.

Ford-Lloyd (1986) was van mening dat het sterk variabele zuidelijk 'ecotype' afgegrensd konden worden van de uniforme noordelijke strandbiet. De noordelijke vorm noemde hij *B. vulgaris* subsp. *maritima*, het veelvoud aan zuidelijke vormen werd gereduceerd tot *B. vulgaris* subsp. *macrocarpa*.

Ford-Lloyd gaf niet expliciet aan in hoeverre beide geografische groepen als subspecies ook taxonomisch onderscheiden en geidentificeerd konden worden aan de hand van morfologische kenmerken. Ook de status van sommige in het verleden beschreven taxa werd niet opgehelderd. Was het 'lumpen' van deze

taxonomische groepen gerechtvaardigd? Voorts bleef er veel verwarring bestaan met betrekking tot het gebruik van infraspecifieke categorieën. In taxonomisch opzicht bestond op dit vlak een additioneel probleem aangezien binnen *B. vulgaris* cultuurvormen van de bieten en wilde vormen zijn vertegenwoordigd. De vraag hoe deze naast elkaar staande groepen adekwaat in één systeem te classificeren diende opgelost te worden.

Het geheel van de taxonomische literatuur samenvattend kan men stellen dat in sectie *Beta* aanvankelijk te veel soorten geclassificeerd zijn waarbij de omgrenzing van deze soorten problematisch is gebleken, en dat later een meer realistische behandeling van de variatie heeft plaats gevonden, maar dat vervolgens door verregaande simplificatie niet altijd even effectief de complexe biosystematische relaties helder tot uiting zijn gekomen.

Taxonomische groepen – of deze nu aangeduid worden als soorten, ondersoorten, variëteiten of formae, zijn gekarakteriseerd door overeenkomstige uiterlijke kenmerken, en kunnen op basis daarvan worden geidentificeerd. Voor het identificeren van zulke groepen zijn enerzijds de relevante publicaties met de beschrijvingen en het oorspronkelijk herbariummateriaal bestudeerd, anderzijds werd hiertoe wild plantenmateriaal van verschillende herkomst opgekweekt en in veldproeven geëvalueerd. Morfometrische waarnemingen werden statistisch geanalyseerd, en op basis hiervan werden conclusies getrokken m.b.t. de onderscheidbaarheid van zulke groepen. Sommige taxa vertoonden ten opzichte van elkaar genoeg verschillen om ze als aparte soort te erkennen.

Factoren belangrijk voor soortsvorming

Er is niet alleen een beschrijving van soorten gegeven, maar tevens is een poging gedaan de soortenvariatie nader toe te lichten in termen van soortsvormingsprocessen en evolutionaire aanpassing aan de standplaats.

Aspecten van de reproductiestrategie zijn van direct belang voor het verkrijgen van inzicht in het soortvormingsproces. Evolutionaire divergentie kan bevorderd worden doordat soorten reproductief van elkaar geisoleerd raken. Teneinde dit te onderzoeken zijn in de kas in beperkte mate soortskruisingen uitgevoerd. Het bleek dat bij de soorten van sectie *Beta* vrijwel geen kruisingsbarrieres aanwezig zijn; in het algemeen werden fertiele F_1 en F_2 nakomelingen verkregen in kruisingen tussen *B. vulgaris* subsp. *vulgaris* en *B. macrocarpa* en tussen *B. patula* en *B. vulgaris* subsp. *maritima*.

Met behulp van enzymelectroforese zijn kenmerken van de reproductiestrategie van de afzonderlijke taxa nader aan het licht gekomen. Met de allozymtechniek werd informatie over de genotypische constitutie van de individuen in een populatie verkregen. Aldus kon de populatieheterogeniteit worden bepaald, ondermeer naar voren komend in de balans tussen homozygote en heterozygote genotypen. Het bleek mogelijk de soorten te karakteriseren aan de hand van de populatiestructuur. De heterozygotiefractie in de populaties verschafte aanwijzingen omtrent reproductiestrategieën van de soorten. Bij taxa met sympatrische populaties konden, ondermeer aan de hand van zeldzame en soortspecifieke

allozymen introgressieverschijnselen bestudeerd worden. Tenslotte was het mogelijk ook binnen één soort genetische verschillen te kwantificeren. Populaties van verschillende regio's konden worden vergeleken aan de hand van similariteitscoëfficienten.

Geografische standplaats en demografische variatiepatronen

Het areaal van de strandbiet strekt zich uit van noord Europa, het gehele mediterrane gebied, tot en met het Indisch schiereiland. De soort overschrijdt aldus verschillende klimatologische zones. In noord en west Europa is ze vrijwel beperkt tot de kustgebieden. Het klimaat is daar koel, regenrijk, met soms lage temperaturen in de winter. In het Middellandse Zee gebied wordt de strandbiet niet alleen langs de kust aangetroffen, maar ook op ruderale plaatsen in het binnenland. Het mediterrane klimaat wordt vooral gekenmerkt door zomerdroogte. Het aride klimaat van het Nabije en verre Oosten kent soms extreme hitte.

Dat de strandbiet in staat is geweest zich uit te breiden over dit enorme areaal komt waarschijnlijk doordat de natuurlijke standplaats langs de kust (dijken, zand- en kiezelstranden, rotskusten, estuariën, kwelders) een effectieve lineaire dispersie toelaat van de zaden via land en water, vrijwel niet onderbroken door grote barrières. Van belang is ook dat de strandbiet een 'koloniserende soort' is, samengesteld uit veel locale populaties die slechts korte tijd op één plek gedijen. Het onkruidachtige karakter van de strandbiet maakt de soort voortdurend afhankelijk van het vinden van nieuwe geschikte locaties. Deze demografische instabiliteit kan overigens aanleiding zijn voor een differentiatie van strandbietpopulaties op regionale schaal, maar het is vaak moeilijk hierin enige structuur te herkennen, zie bijvoorbeeld Hoofdstuk 6: tussen aangrenzende populaties afkomstig van de regio Sicilie bleek vaak een hoge mate van variatie in kwantitatief morfologische kenmerken aanwezig te zijn. Echter, wanneer populaties van verschillende subregio's werden vergeleken, dan was voor de gemeten variabelen geen duidelijk geografisch patroon aanwijsbaar.

Voortdurende kolonisatie en het stichten van steeds nieuwe populaties zorgt voor veel 'genetic drift', die een (tijdelijke) genetische differentiatie mogelijk maakt, maar het lijkt dat deze evolutionaire krachten juist weer worden tegengewerkt door krachten die hernieuwde genenuitwisseling stimuleren: het binnendringen van immigranten van andere populaties en eigenschappen van de strandbiet als relatief sterke zelfincompatabiliteit. Op een wat grotere schaal heeft natuurlijke selectie wel geresulteerd in geografische verschillen tussen populaties. De differentiatie in de populaties verloopt dan meer volgens een clinaal patroon, waarbij geleidelijke overgangen van kenmerken optreden. Dit is met name gebleken uit de analyse van een aantal demografische kenmerken van strandbiet populaties. In dit onderzoek werd een analyse gemaakt van de geografische variatie binnen *B. vulgaris* subsp. *maritima* voor bloei-aanvang, en de verschillen tussen populaties wat betreft de behoefte aan vernalisatie voor bloei-inductie werden nader onderzocht. Tevens werd het effect van vernalisatie op bloei-aanvang vast-

gesteld. Bij de meeste van deze parameters bleken clinale (noord-zuid dan wel west-oost) overgangen aanwezig te zijn. Zeer opvallend was de divergentie van de overige soorten van sectie *Beta* m.b.t. deze parameters.

Samenvatting van de resultaten

In hoofdstuk 1 wordt aan de hand van een literatuuroverzicht ontwikkelingen in de taxonomische classificatie van het genus *Beta*, en in het bijzonder de classificatie van *Beta* sectie *Beta* besproken.

Het genus *Beta* is onderverdeeld in 4 secties. Behalve de type sectie zijn dit sectie *Corollinae*, met 3 tot 5 soorten, de monotypische sectie *Nanae* en sectie *Procumbentes*, waarin 3 soorten zijn opgenomen. De secties vormen systematisch en ecologisch aparte eenheden en tonen onderling beperkte verwantschap. Op basis van experimenteel onderzoek is vastgesteld dat de sectie *Corollinae* meer verwant is aan sectie *Beta* dan de overige secties.

Bij de behandeling van sectie *Beta* wordt nadrukkelijk de classificatiegeschiedenis van de wilde taxa besproken. Een belangrijke stap om tot een meer stabiele classificatie te komen werd gedaan door de Linneaanse namen *B. vulgaris* L. en *B. maritima* L. te typificeren. Tot op heden was het onduidelijk gebleven naar juist welke specimen deze voor zowel cultuur- als wilde planten veel gebruikte namen verwijzen. Onderzoek in de Linneaanse herbaria bracht aan het licht dat er geen origineel door Linnaeus geannoteerd materiaal beschikbaar is. Er werd besloten tot lectotypificatie van de naam *B. vulgaris* met een exemplaar aanwezig in het door Linnaeus geraadpleegde herbarium van Adriaan van Royen in Leiden. Een lectotype werd vastgesteld voor de Linneaanse variëteit *cicla* met een exemplaar uit het Clifford herbarium. Voor *B. maritima*, *B. vulgaris* var. *perennis* en *B. vulgaris* var. *rubra* werden neotypes aangewezen. De consequentie van de gevolgde handelswijze was dat de namen *vulgaris* en *maritima* heterotypisch werden vastgesteld, en dat aldus aansluiting werd gevonden bij het gangbare gebruik van de namen waarbij de cultuurbiet is opgenomen in *B. vulgaris* subsp. *vulgaris* en de wilde vorm van de soort vertegenwoordigd wordt door ondermeer *B. vulgaris* subsp. *maritima* (L.) Arcang.

Aansluitend werd in hoofdstuk 1 een voorstel gedaan van een nieuwe taxonomische indeling van sectie *Beta*. Dit gebeurde op basis van de experimentele gegevens voornamelijk gepresenteerd in hoofdstuk 2, 3 en 4, en op basis van een evaluatie van oorspronkelijk herbarium materiaal.

Er werden drie soorten vastgesteld in sectie *Beta*. Behalve *B. vulgaris* zijn dit *B. patula* Ait. en *B. macrocarpa* Guss.

B. patula is een soort die geisoleerd voorkomt in de nabijheid van het eiland Madeira. De soort onderscheidt zich door de veelbloemige vruchtkluwen en de smal langwerpige bracteeën.

B. macrocarpa is een soort die een mediterrane verspreiding heeft en veelal samen voorkomt met *B. vulgaris* subsp. *maritima* in matig tot sterk saliene kust habitats. Karakteristiek voor de soort zijn de opstaande randen van het operculum (dit is het dekseltje waarmee de vrucht openspringt) en de stijve afstaande bloemblaadjes.

Het aantal infraspecifieke taxa in *B. vulgaris* is sterk gereduceerd. Op grond van literatuuronderzoek en herbariumstudie werd geconcludeerd dat veel van de tot dan toe erkende botanische variëteiten in *B. vulgaris* niet gehandhaafd konden worden, omdat zij zijn gebaseerd op slechts een klein aantal exemplaren, of omdat de diagnostische kenmerken te zwak bleken, of omdat geen rekening werd gehouden met het variatiepatroon van de soort als geheel. In de revisie van *Beta* sectie *Beta* is afgezien van classificatie op het niveau van de botanische variëteit.

Populaties afkomstig van de Egeische eilanden en de Turkse kust werden aangemerkt als een karakteristieke groep, morfologisch homogeen en afwijkend van *B. vulgaris* subsp. *maritima*. Zij zijn geclassificeerd als *B. vulgaris* subsp. *adanensis*. Deze ondersoort onderscheidt zich van *B. vulgaris* subsp. *maritima* op meerdere kenmerken van de bloem en de vlezige bracteeën. Deze zijn onderaan de stengel opvallend groot, de apicale bracteeën daarentegen zijn sterk gereduceerd.

In een aantal uitgebreide veldproeven werden 79 accessies (monsters van wild plantmateriaal van verschillende geografische herkomst) geëvalueerd waarbij kwantitatieve morfometrische waarnemingen werden verricht. De resultaten zijn vermeld in Hoofdstuk 2. Een analyse werd gedaan waarbij de accessies werden beschouwd als Operational Taxonomic Units (OTU's). Een objectieve groepering van de accessies op basis van de 19 bepaalde variabelen werd verkregen met Clusteranalyse. Met behulp van Principale Componenten analyse werd een ruimtelijk beeld verkregen van de afstanden tussen OTU's, en de mate waarin taxonomische groepen segregeerden. Met dezelfde methode werd onderzocht in hoeverre *Beta vulgaris* subsp. *maritima* accessies volgens geografische patronen konden worden gerangschikt. Accessies van orientaalse herkomst (India, Pakistan, Iran) onderscheidden zich van accessies uit het mediterrane gebied.

In hoofdstuk 3 wordt verslag gedaan van de allozymvariatie van sectie *Beta*. 11 enzymsystemen waarvan tenminste één locus polymorf was, werden bestudeerd, en 76 accessies werden in het onderzoek betrokken. Nei's genetic distance, mean genetic diversity H_e en mean observed heterozygosity H_o werden berekend op basis van 9 loci en 59 accessies. Een groot aantal accessies behorend tot *B. vulgaris* subsp. *maritima* werd bestudeerd en de allozymsamenstelling van de overige taxa kon hieraan worden gerelateerd. Bij *B. vulgaris* subsp. *vulgaris*, *B. vulgaris* subsp. *adanensis* en *B. patula* werden geen allozymen tot expressie gebracht die afweken van de bij populaties van *B. vulgaris* subsp. *maritima* gevonden allozymen. Wel bleken de frequenties van de verschillende allozymen in de populaties van *B. patula* en *B. vulgaris* subsp. *adanensis* sterk af te wijken van die van *B. vulgaris* subsp. *maritima*. *B. macrocarpa* bleek unieke allozymen te hebben voor de loci *Lap1*, *Acp1*, *Pgm2* en *Px2*.

De genetische diversiteit en de waargenomen heterozygotie bleek sterk te variëren van soort tot soort. De genetische diversiteit berekend over een aantal accessies van *B. patula* en *B. macrocarpa* was 0,01 respectievelijk 0,07. Bij *B. vulgaris* subsp. *maritima*, *B. vulgaris* susbp. *vulgaris* en *B. vulgaris* subsp. *adanensis* was

de genetische diversiteit beduidend hoger namelijk tussen 0,28 en 0,32.

De fractie heterozygote genotypen was duidelijk hoger in de populaties van *B. vulgaris* subsp. *maritima* dan in die van *B. macrocarpa*, *B. patula* en *B. vulgaris* subsp. *adanensis*. Deze verschillen in heterozygotieniveau zijn gecorreleerd aan een verschil in reproductiestrategie tussen de taxa. In het geval van *B. macrocarpa* werd een sterke neiging tot autogamie vastgesteld. Bij *B. patula* en *B. vulgaris* subsp. *adanensis* is waargenomen dat ze zelf-compatibel zijn, maar ook dat ze gemakkelijk kruisen. Op basis van de allozymgegevens kan worden aangenomen dat *B. vulgaris* subsp. *maritima* een hoger niveau van kruisbestuiving vertoont dan *B. vulgaris* subsp. *adanensis* en de soorten *B. macrocarpa* en *B. patula*.

Het voorkomen en de verspreiding van algemene en minder algemene allozymen werd onderzocht. Allozymen met een regionale verdeling waren *Acp1*-7 (hoofdzakelijk mediterraanse *maritima*), *Mdh1*-1 (hoofdzakelijk Griekse en Siciliaanse *maritima* en *B. macrocarpa*), *Lap1*-5 (hoofdzakelijk Griekse en Siciliaanse *maritima*) en *Acp1*-2 (Atlantische *maritima* en *B. patula*). *Mdh1*-3 was een allozym met lage frequentie in een beperkt aantal populaties maar het werd aangetroffen verspreid over het gehele gebied.

Hoofdstuk 4 beschrijft de levenscyclusvariatie en de variatie in bloeitijd van de soorten van sectie *Beta*. De levenscyclusvariatie uit zich ondermeer in het voorkomen van éénjarige planten (annuals) en overblijvende planten (perennials). De categorie van overblijvende planten kon nog verder worden onderverdeeld in een groep die reeds in het eerste jaar tot bloei kwam (early perennials), en een groep die in het eerste jaar *niet* tot bloei komt (late perennials).

Om tot een goede omschrijving te komen van de levenscyclusvariatie is getracht een antwoord te verkrijgen op de volgende vragen. In hoeverre is vernalisatie noodzakelijk voor bloei-inductie? In hoeverre is er tussen en binnen accessies genetische variatie voor vernalisatiebehoefte? In hoeverre is lange dag noodzakelijk voor bloei-inductie? In hoeverre treedt regeneratie op na bloei? In veldexperimenten en kasproeven werd de generatieve ontwikkeling van een groot aantal accessies vastgelegd, en werd het effect van een vernalisatiebehandeling en lange/korte dag condities op de generatieve ontwikkeling vastgesteld.

Kwantitatief bleken er aanzienlijke geografische verschillen te zijn m.b.t. het in bloei komen van planten in het eerste jaar na kieming. Zo werden in een veldproef accessies afkomstig van Ierland en de Atlantische kust van Frankrijk (Bretagne) en Portugal getest. De Ierse planten bleven in rozet, van de Franse planten kwam uiteindelijk slechts 40% in bloei, terwijl de Portugese planten allemaal bloeiden in de veldproef. Ook accessies met een mediterrane oorsprong werden geëvalueerd in een veldproef. Alle planten met mediterrane oorsprong in de veldproef bloeiden in het eerste jaar na kieming. Een klein deel van de mediterrane planten die in de kas werden opgekweekt kwam echter niet in bloei. De waarneming van niet bloeiende planten in de kas gaf de mogelijkheid aan van een vernalisatiebehoefte bij een deel van de mediterrane planten. Vernalisatiebehoeftige planten schenen afwezig te zijn bij accessies afkomstig van Pakistan, en accessies van Griekenland. De aanwezigheid van vernalisatiebehoeftige

planten en niet vernalisatiebehoeftige planten in de mediterrane accessies duiden mogelijk op een genetische factor die de vernalisatiebehoefte uitschakelt.

Voor de noord Atlantische accessies werd onderzocht in hoeverre een vernalisatiebehandeling de generatieve ontwikkeling beinvloedde. Planten van Ierse, Nederlandse en Franse (Bretagne) herkomst werden gezaaid in het najaar en overwinterden in een vernalisatiekas. In het volgende voorjaar werden de planten in een veldproef opgesteld. De vernalisatiebehandeling bleek zowel het doorschieten als het uiteindelijk tot bloei komen te beinvloeden. Bij de Ierse planten bleek dat in vergelijking tot de niet gevernaliseerde planten meer planten doorschoten en meer planten bloeiden. 26% van de planten kwam uiteindelijk tot bloei. De accessies met de meeste bloeiende planten waren van zuid-Ierse oorsprong. In een aantal van de meest noordelijke accessies bleef, opmerkelijk genoeg, ook na vernalisatie zowel doorschieten als bloei achterwege. Van de Nederlandse en Franse accessies kwam na vernalisatie uiteindelijk 91% respectievelijk 99% in bloei.

Van de planten die tot bloei kwamen werd het tijdstip van bloei-aanvang gekwantificeerd. Het bleek dat Ierse en Nederlandse planten gemiddeld 18 dagen later startten met bloeien dan planten uit Bretagne. Met betrekking tot mediterrane en orientaalse accessies van *B. vulgaris* subsp. *maritima* werd vastgesteld dat de orientaalse accessies gemiddeld twee weken eerder doorschoten en bloeiden.

Planten van *B. vulgaris* subsp. *maritima* behielden vitale delen na de bloei, slechts een enkele accessie kon gekwalificeerd worden als een annuel. Het betrof planten in de Peloponnesische accessie 3300 en 6% van de orientaalse planten.

Alle gegevens m.b.t. *B. vulgaris* subsp. *maritima* in aanmerking nemend werden twee geografische tendensen vastgesteld: (i) vernalisatiebehoefte lijkt afwezig te zijn in de accessies van orientaalse oorsprong en althans een deel van de oost mediterrane accessies. Vernalisatiebehoefte is aanwezig in westelijk mediterrane en Atlantische accessies. (ii) Betreffende de planten met een vernalisatiebehoefte bestaat een zuid-noord clinaal verloop in de gevoeligheid voor vernalisatie.

B. macrocarpa en *B. vulgaris* subsp. *adanensis* onderscheidden zich van *B. vulgaris* subsp. *maritima* door de vroege bloei en de eenjarigheid. *B. macrocarpa* was strict eenjarig, had geen vernalisatie nodig, en bloeide ook onder korte dag omstandigheden. *B. vulgaris* subsp. *adanensis* was eenjarig, maar onder bepaalde condities overblijvend. *B. vulgaris* subsp. *adanensis* had geen vernalisatiebehoefte, maar bleef onder korte dag omstandigheden in de rozetfase zonder door te schieten.

De bloeiaanvang van *B. patula* is in het algemeen later en vergelijkbaar met de bloei-aanvang van mediterrane *B. vulgaris* subsp. *maritima*. *B. patula* is in de kas een overblijvende soort. *B. patula* onderscheidde zich van *B. vulgaris* subsp. *maritima* doordat een rozetfase vrijwel ontbreekt: de meeste planten schieten snel door. De soort bloeide in principe zonder vernalisatie, echter, een gedeelte van de planten kwam na door schieten *niet* in bloei en bleef gedurende het eerste jaar vegetatief.

In hoofdstuk 5 wordt de zaadkieming van de verschillende soorten besproken. Bij *Beta* zijn de zaden gevat in zogenaamde zaadkluwens (glomerulae). Een zaadkluwen kan één of meer bloemen bevatten. Het vlezige bloemdek is vergroeid met het vruchtbeginsel. In de vruchttijd raken zowel vruchtbeginsel als bloemdek verhard, het omhulde zaad springt open met een dekseltje (het operculum).

Het kiemingspatroon van *B. vulgaris* subsp. *maritima* werd bestudeerd aan de hand van zaadaccessies afkomstig van het noordelijk gedeelte van het verspreidingsgebied, en zaadaccessies afkomstig van het mediterrane gebied. De hypothese werd gesteld dat er (verschillende) seizoensoptima voor kieming in het gematigde respectievelijk mediterrane habitat zijn, en dat kieming in ongunstige seizoenen wordt voorkomen. Het mechanisme ter beperking van kieming in ongunstige perioden wordt samengevat in de term kiemrust. Met betrekking tot de noordelijke populaties werd kiemrust verwacht, omdat na vruchtzetting de zaden een ongunstige wintertijd wacht met grote kans op inundatie en lage temperaturen. Voor mediterrane accessies kan een kiemruststrategie voordelig zijn gedurende zomerdroogte, wanneer voorkomen moet worden dat kieming optreedt ten gevolge van een incidentele regenbui. Behalve *B. vulgaris* subsp. *maritima* werden ook zaadaccessies van *B. macrocarpa* en *B. vulgaris* subsp. *adanensis* betrokken in het experiment. Omdat beide taxa nauw verwant zijn aan *B. vulgaris* subsp. *maritima*, en ze in hetzelfde mediterrane habitat voorkomen, werd verwacht dat het patroon van kieming grotendeels overeen zou komen met dat van de mediterrane *B. vulgaris* subsp. *maritima* accessies.

In de experimenten werd de aanwezigheid van kiemrust in de zaadaccessies getest door zaadaccessies gedurende korte of lange tijd te stratificeren bij 4 °C en de kiemingsrespons te vergelijken met niet gestratificeerde zaden, en door het kiemingsgedrag te bestuderen van zaden die werden geëxposeerd aan verschillende temperaturen: respectievelijk een constante temperatuur 5°, 10°, 15°, 20°, 25° en 30 °C en wisseltemperaturen 5/15 °C en 5/25 °C.

De noord Atlantische accessies gaven optimale kieming te zien bij de hogere temperaturen 20-30 °C, bij lagere temperaturen (5° en 10 °C) was vrijwel geen kieming. Bij deze temperaturen trad wel enige kieming op wanneer zaden enige weken werden gestratificeerd. Deze waarnemingen duiden op de aanwezigheid van kiemrust in de zaden. Adaptatie van de zaden bij wisseltemperatuur 5/25 °C gaf een geleidelijke opheffing van de kiemrust te zien.

De mediterrane *B. vulgaris* subsp. *maritima* accessie van Cyprus vertoonde relatief veel kieming bij alle temperatuurregimes, met name ook bij de lage temperaturen. Het stratificeren van de zaden bleek in het algemeen geen duidelijke verhoging van de kieming te geven. De brede temperatuurreeks waarover kieming kon plaatsvinden bij deze accessie gaf aan dat kiemrust in de zaden afwezig was.

Lage kiemingspercentages bij vrijwel alle temperatuurregimes werden vastgesteld voor *B. vulgaris* subsp. *adanensis*. In het algemeen trad geen verbetering op van de kieming onder invloed van stratificatie. Temperatuur bleek alleen van invloed te zijn op de snelheid waarmee de zaden kiemden. Voorzover er sprake was van kiemrust in de niet kiemende zaden, kon deze niet door de temperatuursbehandelingen worden opgeheven.

Voor *B. macrocarpa* was de optimale kiemingstemperatuur 20 °C, maar ook bij lagere temperaturen vond nog kieming plaats. Stratificatie leidde niet tot een verhoging van de totale kieming. Opvallend bij deze soort was dat de zaden traag kiemden, dat wil zeggen dat de inductietijd voor kieming relatief lang was, ongeacht de temperatuur. Niet zozeer de temperatuurscondities als wel andere factoren lijken de kieming bij *B. macrocarpa* te vertragen. Het operculum is bij *B. macrocarpa* steviger verbonden met de pericarp en springt niet gemakkelijk open. Bovendien waren er aanwijzingen dat de absorptie van vocht minder snel verloopt, en dat bovendien substanties op de kluwens aanwezig zijn die mogelijk remmend werken op de kieming.

In hoofdstuk 6 werd een analyse gemaakt van het patroon van variatie in een Siciliaanse genenbankcollectie. De classificatie van de collectie in een groep van 'kustpopulaties' en een groep van 'binnenlandpopulaties' werd aan de hand van morfometrische waarnemingen opnieuw beoordeeld. Aan de hand van discriminantanalyse werd aangetoond dat een dergelijke onderverdeling in principe mogelijk is, maar dat deze slechts een zwakke basis heeft. Grote verschillen in kwantitatief morfologische kenmerken tussen aangrenzende kustpopulaties waren aanwezig, een discrete geografische structuur met betrekking tot zulke variabelen ontbrak echter.

De benadering van het variatieprobleem vanuit de hierboven besproken invalshoeken resulteert uiteindelijk in een concrete strategie voor het effectief verzamelen van de genetische variatie in sectie *Beta*.

Het ruimtelijk patroon van variatie in *B. vulgaris* subsp. *maritima* kan als volgt worden samengevat: op macrogeografische schaal is er differentiatie in morfologische kenmerken en demografische kenmerken, maar het is moeilijk hierin scherpe overgangen te ontwaren. Een genetische basis voor vernalisatiebehoefte en een rol voor zg. 'bolting genes' is waarschijnlijk, maar het ontbreekt aan (moleculaire) markers voor dergelijke genen om de geografische variatie nauwkeurig in beeld te brengen.

De macrogeografische verspreiding van bepaalde allozymen laat zien dat gesproken kan worden van een 'Atlantische gene pool' en een 'mediterrane gene pool'.

Het voorkomen van het allozym *Acp1*-2 in de meeste Atlantische populaties en ontbreken ervan in alle mediterrane accessies markeert vrij scherp de twee groepen. De hogere genetische diversiteit van zuid-oost en middenmediterrane accessies en de hogere frequentie waarmee bepaalde zeldzamere allozymen (*Lap1*-5, *Mdh1*-1, *Acp1*-7) worden aangetroffen in zuid-oost en middenmediterrane accessies gaf aan dat een dergelijke indeling niet misplaatst is.

Binnen bepaalde geografische subregio's is de allozymdifferentiatie beperkt. De genetische diversiteit in *B. vulgaris* subsp. *maritima* is min of meer gelijkelijk verdeeld over de individuele planten in een populatie en over aangrenzende populaties. Het verzamelen van planten uit één grote populatie binnen zo'n subregio zou daarom bijna even effectief zijn als het verzamelen van planten afkomstig van verscheidene populaties.

Kenmerkend voor *B. macrocarpa* is de genetische homogeniteit binnen accessies. Enerzijds werd fixatie van karakteristieke allozymen over het gehele verspreidingsgebied gevonden, anderzijds werd vastgesteld dat tussen buurpopulaties soms grote verschillen aanwezig zijn (bijvoorbeeld de fixatie van twee verschillende allozymen *Acp1*-1 en *Acp1*-3 bij zuidspaanse populaties). De allozymcompositie van *B. vulgaris* subsp. *adanensis* is meer variabel. Er is meer heterogeniteit, niet alleen *tussen* populaties, maar ook *binnen* populaties. Deze heterogeniteit kwam tot uiting in een relatief lage genetische identiteit tussen de populaties (I = 0,76 tussen acht accessies, vergelijk met *B. vulgaris* subsp. *maritima* I = 0,91 tussen 34 accessies, en *B. macrocarpa* I = 0,91 tussen tien accessies).

B. macrocarpa en *B. vulgaris* subsp. *adanensis* vertoonden ten opzichte van *B. vulgaris* subsp. *maritima* een sterk verhoogde homozygotie, en deze waarneming werd gerelateerd aan een reproductiesysteem van zelfbevruchting. Zelfbevruchting reduceert genenuitwisseling tussen populaties zodat eerder adaptatie aan locale ecologische en klimaatsomstandigheden kan optreden.

Het is opvallend dat populaties van beide taxa aangetroffen kunnen worden in verschillende habitats; *B. vulgaris* subsp. *adanensis* is als overblijvende plant te vinden op kiezelstranden, maar vestigt zich ook als éénjarig onkruid in akkers. *B. macrocarpa* is een soort van extreem zoute gronden (zuid Portugal) of ruderale binnenlandhabitats (zuid Spanje). Op grond van deze informatie mag geconcludeerd worden dat het verzamelen van genotypische variatie in *B. vulgaris* subsp. *adanensis* en *B. macrocarpa* pas doeltreffend is wanneer zoveel mogelijk populaties van zo divers mogelijke habitats worden bezocht. Hierbij kan volstaan worden met het bemonsteren van een kleiner aantal individuen per populatie.

1 Classification and taxonomy

1.1 Introduction

The species of *Beta* section *Beta* have been variously treated and, to date, no entirely satisfactory classification has been proposed for the group. There are several reasons for the difficulties in the classification of the section:

(i) Confusion was created because both wild and cultivated plant groups are present in the section. Wild and cultivated taxa were treated as different species or at infraspecific levels. Several separate systems for wild and cultivated material evolved, with different levels of infraspecific categories, or with different interpretations of infraspecific categories by both plant breeders and taxonomists.

(ii) As far as the wild taxa were concerned, classification of minor variants took place in the early taxonomic treatments, based on incomplete knowledge of the continuous variation pattern over a large distribution area (Ulbrich 1934, Aellen 1938).

(iii) Recent taxonomic treatments present classifications that attempt to recognise most entities at the rank of subspecies or variety. These consider wild *Beta vulgaris* L. to be a very variable taxon, in which 'clinal variation' and the formation of 'biotypes' (Ford-Lloyd 1986) in parts of the distribution area is prevalent. In the past considerable doubt about the distinction of several of the taxa has been expressed (Ford-Lloyd 1986), while others, e.g. Buttler (1977[b]), have argued that relationships between the numerous taxa described are still far from clear, that there is a lack of information from parts of the distribution area, and that the naming of wild material is often impossible.

In order to acquire more insight in the taxonomic structure of this complex group, and the structure of genetic diversity in the widespread *B. vulgaris* subsp. *maritima* (L.) Thell., it seems best to use an approach in which plants from wide geographical origin are grown under uniform conditions and evaluated simultaneously (Chapter 2). This approach should also clarify the relations between the so called 'annuals' and 'perennials' (Buttler 1977[b], cf. Chapter 4).

The results obtained in Chapter 2, 3 and 4 are used in the present Chapter to delimit taxonomic groups. Before taxonomic groups in section *Beta* are described, an account is given of the historical subdivision of the genus and its sections, and the relations of the sections are discussed. An overview is given of classification in section *Beta* from Linnaeus onwards. The taxonomic treatment of the wild material by various authors is critically analysed. Emphasis is given to classification of wild forms, and the relation to cultivated *Beta* is also discussed.

Typification of the Linnaean names *B. vulgaris* L. and *B. maritima* L. is attempted for the benefit of a stable nomenclature in the section. Conclusions

are drawn with respect to the taxonomy of section *Beta*. The treatment of the taxa recognised further comprises the synonymy, literature reference, descriptions, illustrations and reference to herbarium specimens examined.

1.2 Classification in the genus *Beta*

1.2.1 Taxonomic history and subdivision of the genus Beta

In 1927 Transhel divided the genus in three 'gruppa'. Ulbrich (1934) elaborated on the work of Transhel and transformed these 'gruppa' to sections concordant with the rules of botanical nomenclature. Transhel had considered *B. nana* Boiss. & Heldr. to belong to his gruppa *Vulgares*, but Ulbrich decided to propose a fourth section *Nanae*, because he considered it to be an aberrant species. *B. nana* is known from a few localities in the Parnassos mountains in Greece, and it is both taxonomically and ecologically isolated.

For unexplained reasons Ulbrich amended Transhel's gruppa *Patellares* and named it section *Procumbentes*. Historically the species concerned have always been referred to as the '*Patellares*' species – particularly in plant breeding literature – but the correct name is *Procumbentes*, as Ulbrich typified the section on *B. procumbens* Smith. Zosimovic (1940) transformed Transhel 's gruppa *Patellares* to section *Patellares*, but this was superfluous, since Ulbrich had already proposed a legitimate name for the section. Williams, Scott & Ford-Lloyd (1977) raised the section to generic level, naming it *Patellifolia*, but up till now their view has not been widely accepted.

As was noted by Buttler (1977[b]), the correct name for the type section of the genus, which includes the type species *B. vulgaris*, is section *Beta*. Ulbrich's section *Vulgares* should therefore be referred to as section *Beta*.

1.2.2 Short description of the sections Corollinae, Nanae and Procumbentes

Species relationship within *Beta* is expressed through subdivision of the genus into four sections (Table 1.1). Morphological coherent groups in the genus are fairly easy to recognise. Moreover, these groups of species are distributed in separate geographical regions, and show specific habitat preferences.

Section *Corollinae* Ulbrich

Species of section *Corollinae* are all perennial and develop a robust tap root. The inflorescence is a large, lax, long-branched terminal panicle of up to 1.5 m tall. The bracts are linear-lanceolate. The perianth is corolla-like, green or whitish in colour. Species are either monogerm or polygerm, with trigerm glomerules occurring most frequently. Within section *Corollinae* from three to six species have been classified in the past. Initially, the species *B. lomatogona* Fisch. & Meyer, *B. trigyna* Wald. & Kit. en *B. macrorhiza* Steven were recognised on morphological grounds. Russian taxonomists, in particular Transhel (1927) and

Table 1.1 Species published in the genus *Beta*.

1.	section *Beta*	
	gruppa *Vulgares* Transel	Bull. Appl. Bot. Pl. Breed. (Leningrad) 17-2:208 (1927)
	sectio *Vulgares* Ulbrich	in: Engler & Prantl Natürl. Pflanzenfam. ed. 2 16c: 459 (1934)
	species	
	Beta vulgaris L. (typus)	Species plantarum, ed 1 p 222 (1753)
	Beta maritima L.	Species plantarum ed 2 p 322 (1762)
	Beta cicla L.	Syst. Nat. ed 12 p 195 (1767)
	Beta hortensis Miller	Gard. dict. ed 8 (1768)
	Beta patula Aiton	Hort. Kew. p 315 (1789)
	Beta sativa Bernh.	Syst. Verz. Erfurt p 162 (1800)
	Beta orientalis Roth	Nov. pl. Ind. Or. p 181 (1821)
	Beta macrocarpa Gussone	Fl. Sicul. Prod. I p 302 (1827)
	Beta bengalensis Roxburgh	Fl. Ind. 2: p 59 (1832)
	Beta stricta Koch	Linneae Bd. p 18 (1849)
	Beta bourgaei Cosson	Not. Pl. Crit. p 44-45 (1849)
	Beta atriplicifolia Rouy	Rev.Sci. Nat. Ser. 3 p 246 (1883)
	Beta adanensis Pamukçuoglu apud Aellen	Notes R.B.G. Edinb. 28: p 29 (1967)
	Beta trojana Pamukçuoglu apud Aellen	Notes R.B.G. Edinb. 28: p 29 (1967)
	Beta palonga Basu & Mukkerjee	Can. J. Bot. 53: p 1166 (1977)
2.	section *Corollinae* Ulbrich	in: Engler & Prantl Natürl. Pflanzenfam. ed.2 16c: 462 (1934)
	gruppa *Corollinae* Transel	Bull. Appl. Bot. Pl. Breed. (Leningrad) 17-2:215 (1927)
	species	
	Beta macrorhiza Steven (lectotype)	Mem. Soc. Nat. Moscou 3: 257 (1812)
	Beta trigyna Wald. et Kit.	Desc. et Icones Pl. rar. Hung. I p 34 (1812)
	Beta lomatogona Fischer & Meyer	in: Hohenacker, Bull.Soc. Nat. Moscou 3, Enum. Pl. Talysch., p 360 (1838)
	Beta foliosa ex siccati Haussknecht	Sched. P. It. Orient. 27 (1890)
	Beta corolliflora Zosimovic ex Buttler	Mitt. Bot. Staatssamml. München 12: 289 (1975)
3.	section *Nanae* Ulbrich	in: Engler & Prantl Natürl. Pflanzenfam. ed. 2 16c: 463 (1934)
	species	
	Beta nana Boissier & Heldreich (typus)	Diag. Pl. Orient, Ser. I p 82 (1846)
4.	section *Procumbentes* Ulbrich	in: Engler & Prantl Natürl. Pflanzenfam. ed.2 16c: 463 (1934)
	gruppa *Patellares* Transel	Bull. Appl. Bot. Pl. Breed. (Leningrad) 17-2: 215 (1927)
	section *Patellares* Zosimovic	Sveklovodstvo 1: 18 (1940)
	genus *Patellifolia* Williams, Scott & Ford-Lloyd	Taxon 26: 284 (1977)
	species	
	Beta procumbens Smith (typus)	in: Horn. Hort. Hafn. Suppl., p 57 (1849)
	Beta webbiana Moquin	Chen. Enum. p 16 (1840)
	Beta patellaris Moquin	in: DC Prod. XIII, p 57 (1849)

Zosimovic (1939) have dealt with section *Corollinae* extensively. When more plant material from the Sovjet Union became available, and cytological research demonstrated the existence of di-, tetra-, and hexaploid cytotypes, the section was extended with *B. foliosa* Haussknecht and *B. corolliflora* Zosimovic. In the most recent treatment of the group, Buttler (1977[a]) accepted three species as the core for a revision of the section. *B. lomatogona*, *B. macrorhiza* and *B. corolliflora* were taken as the 'basic species' (basis-Arten) since they were morphologically and ecologically well defined and sexually reproducing. The taxonomic position of the others, apomicts and 'hybrid species', is still unsolved.

The distribution of the section is continental, from central Europe and the Balkan to Anatolia, the Caucasus, southern Russia, and Iran. The species occur mainly as weeds in arable land, *B. lomatogona* growing particularly along the edges of wheat and barley fields. *B. trigyna* and *B. corolliflora* are found more often within arable fields rather than along their margins. *B. macrorhiza* is more typical a species of roadsides and ruderal habitats (Buttler 1977[a]).

Section Nanae Ulbrich

B. nana Boiss.& Heldr., constituting a monotypic section (Table 1.1), is a perennial species occurring on mountain slopes in Greece. The plants form a small rosette, not more than 10 cm in diameter. The inflorescence is prostrate, and comparatively small, up to 15 cm. Flowers are solitary (monogerm), the perianth is green and short. Recently allozyme patterns of *B. nana* have been analysed (Nagamine & Ford-Lloyd 1989). The presence of many unique allozymes confirms the isolated position of this species.

Section Procumbentes Ulbrich

Three species belong to section *Procumbentes*. The plants are perennial. They are distinct from section *Beta* by the monogerm nature, the fact that perianth segments are very short and the globular fruit shape. *B. patellaris* Moq. is a tetraploid cytotype and it is distinct in its leaf shape. *B. procumbens* Smith and *B. webbiana* Moq. are very similar, and Curtis (1968) has cast doubt on their status as separate species. Their complete interfertility and intergradation of morphological characters suggests them to be extreme variants within a single species. Wagner et al. (1989) have given support to this hypothesis with evidence from isozyme analysis. The distribution of the species is limited to the Canary Islands. In addition, *B. patellaris* is found in southern Spain and Morocco. The species occur over a wide range of inland and coastal habitats. In southern Spain *B. patellaris* is found in the frontline of the vegetation at the seashore (Frese et al. 1990) and the globular fruits can be found in vast amounts on the beaches blown by the wind. On the Canary Islands the species is also found at low altitudes of 200 m in inland mountain ranges, as well as in the coastal spray zones (Ford-Lloyd & Williams 1975).

1.2.3 Relations of section Beta with the other sections

Analysis of the relationships of the *Beta* sections has been approached by means of comparative morphology, artificial hybridization, isozyme analysis, cytogenetics and molecular DNA genome analysis. On morphological grounds section *Procumbentes* and section *Nanae* were thought to be more diverged from section *Beta* than is section *Corollinae*. Krassochkin (1959) considered *B. maritima*, *B. procumbens* and *B. macrorhiza* as species connecting the sections of *Beta*. However, no arguments are presented for this opinion.

A theory on the phylogenetic history of the genus *Beta* has been put forward by Burenin & Gavrilynk (1982). These authors postulated a hypothetical ancestral beet *Protobeta* and emphasised the peculiarities of *B. patula* as those of a species bridging section *Beta* and section *Procumbentes*. *B. patula* is assumed to be a relict of the ancestral *Protobeta*. Species of the section *Procumbentes* still possess many of the 'primitive traits' of *Protobeta*, while *B. patula* is seen as the most primitive representative of section *Beta* with similar Macaronesian distribution, and thus connecting the two sections. The status and distribution of the apomorphic and plesiomorphic characters in the genus *Beta* is not made explicit in the paper of Burenin and Gavrilynk (1982), so it is difficult to comment on their theory. The results of the present investigations (Chapter 2 and 3) point to close affinities of *Beta patula* with other members of section *Beta*. *Beta patula* is not believed to be a primitive taxon intermediate between section *Beta* and section *Procumbentes*.

There is a long history of interspecific hybridizations of wild species of *Beta* carried out by plant breeders (Coons 1975, De Bock 1986, review in Van Geyt et al. 1990[a]). Hybridisation of cultivated beets with species of section *Beta* do not run up against genetical incongruency, and hybrids are generally fertile (Coons 1975, McFarlane 1975, Dale & Ford-Lloyd 1983). Abe et al. (1987[a]) reported hybrid pollen sterility and seed abortion in crosses between *B. macrocarpa* and *B. vulgaris*, *B. maritima* or *B. atriplicifolia*. Hybridisations between *B. vulgaris* and species of section *Corollinae* are very difficult, mainly due to the disparity of chromosome numbers between the parents. Chromosome numbers in *Corollinae* species are $2n = 18$, $2n = 36$ and $2n = 54$. Successful crosses were reported, but the hybrids were generally sterile or showed apomictic reproduction. Jassem & Jassem (1971) reported absence of pairing of chromosomes between *B. vulgaris* and *B. lomatogona*, but Cleij et al. (1976) reported bivalent formation. Van Geyt et al. (1990[a]) concluded that there were no indications for introgression through natural recombination of *B. vulgaris* with *Corollinae* species.

It is virtually impossible to hybridise the three species of section *Procumbentes* with *B. vulgaris*. Lethality, high hybrid sterility, irregular meiosis and inadequacy of chromosome pairing were reported by several authors (reviewed by Van Geyt et al. 1990[a]). Crosses of *B. vulgaris* with *B. nana* have not been reported so far.

Comparison of allozyme expression in species of section *Nanae* (Nagamine & Ford-Lloyd 1989) and section *Procumbentes* (Oleo et al. 1986, Van Geyt et al. 1988, Wagner et al. 1989) with allozyme expression in section *Beta* revealed a high degree of differentiation (viz. unique allozymes) at several loci.

Fritzsche et al. (1987) performed restriction endonuclease analysis and molecular cloning of plastid DNAs involving species of three sections. Based on polymorphism in two endonucleases a genetic tree was constructed which revealed a higher degree of homology of *Corollinae* species to section *Beta* species than of *Procumbentes* species to section *Beta*. Within section *Beta* restriction patterns were identical for *B. vulgaris*, *B. macrocarpa* and *B. orientalis*. Interrelationships of the sections have also been investigated from the point of chloroplast DNA variation. Kishima et al. (1987) detected seven distinct ctDNA types. *Procumbentes* species differed in 8 out of the 11 distinct SmaI bands. Jung & Pillen (1991) investigated RFLP patterns in members of all four sections. Once more it was concluded that *Procumbentes* species and *Beta nana* are more distantly related to *B. vulgaris* than are species of section *Corollinae*.

1.2.4 Taxonomic history of section Beta wild forms

An account of the variation of *B. vulgaris* affiliated wild forms and their distribution was not available until the beginning of the 20th century. Classification of wild taxa in section *Beta* was attempted by Transhel (1927) and Ulbrich (1934). The separation of wild and cultivated beet in two or more species was abandoned. Ulbrich (1934) described a wild subspecies *perennis* with six varieties in *B. vulgaris* and recognised one more wild species *B. macrocarpa* Guss. (Table 1.2). The two species *B. patula* Ait. and *B. atriplicifolia* Rouy recognised by Ulbrich were originally put by him in section *Corollinae*, but this was later corrected by Coons (1954). In 'Die Orientalische *Beta*-Arten' Aellen (1938) added more infraspecific taxa to the section. Special emphasis was put on the taxa from the eastern Mediterranean region, the Middle East and the Indian subcontinent. Aellen (1938) considered *B. vulgaris* subsp. *perennis* var. *glabra* (Del.) Aellen as synonymous with *B. vulgaris* var. *perennis* L., the wild beet of Linnaeus. Helm (1957[a]) renamed the taxon as *B. vulgaris* subsp. *maritima* var. *maritima*.

Aellen suggested *B. vulgaris* subsp. *macrocarpa* (Guss.) Thell. as a possible ancestor of cultivated beet forms, more particularly the primitive leaf beets, referring to the similarity in perianth structure between the two, and the differences in perianth structure between subsp. *maritima* and subsp. *vulgaris*. Aellen's comment on *B. vulgaris* subsp. *macrocarpa* is disputable. The results shown in Chapter 2 and 3 show that this taxon should be considered as a separate species *Beta macrocarpa* and that it is very unlikely that it has had anything to do with the domestication of *Beta vulgaris*.

In his treatment of *Beta* in the Flora of Turkey (Aellen 1967), Aellen added two more species (*B. adanensis* Pamuk. and *B. trojana* Pamuk. respectively) and one more subspecies, *B. vulgaris* subsp. *grisea* Aellen, to the section (Table 1.2).

Table 1.2 Treatment of wild taxa in section Beta.

authority	*date*	number of wild taxa	species	subspecies	varieties	formae
Linnaeus	1753	1	vulgaris		perennis	
Linnaeus	1762	1	maritima			
Linnaeus	1767	1	maritima			
Ulbrich	1934	7	vulgaris	perennis	maritima	
					erecta	
					debauxii	
					pilosa	
					brevibracteata	
					trigynoides	
			macrocarpa*			
Aellen	1938	8	vulgaris	perennis	perennis	stricta
						euperennis
						annua
					foliosa	
					pilosa	
				orientalis		
				lomatogonoides		
				macrocarpa		
Helm (= Aellen in Hegi	1957 1960)	8	vulgaris	maritima	maritima	stricta
						euperennis
						annua
					foliosa	
					pilosa	
				orientalis		
				lomatogonoides		
				macrocarpa		
Aellen in Davis	1967	5	maritima	maritima		
				pilosa		
				grisea		
			adanensis			
			trojana			
Coons	1954	4	maritima			
			macrocarpa			
			patula			
			atriplicifolia			
Krassochkin	1959	7	maritima	mediterraneum	prostrata	
					erecta	
					macrocarpa	
					atriplicifolia	
				danica		
			patula			
			orientalis			

Table 1.2 continued.

authority	*date*	number of wild taxa	species	subspecies	varieties	formae
Ford-Lloyd & Williams	1975	11	vulgaris	maritima	maritima erecta prostrata atriplicifolia macrocarpa trojana	
				orientalis adanensis provulgaris lomatogonoides patula		
Ford-Lloyd	1986	2	vulgaris	maritima macrocarpa		

* Note: Ulbrich placed Beta patula and Beta atriplicifolia in section Corollinae.

While discussing the wild taxa of genus *Beta*, Coons (1954) classified members of section *Beta* as separate species (Table 1.2). Coons collected plants in Europe and cultivated this material in a greenhouse in the US. He noted that all species would hybridise readily with *B. maritima*. Nevertheless he opposed the suggestion that the wild beet was simply an infraspecific form of *B. vulgaris*. Pointing to the distinct growth habit, the thick leaves and sprangled roots, Coons concluded that 'no useful purpose seems to be served by classifying *B. maritima* as variety or subspecies of *B. vulgaris* unless all other members of this section are similarly classed as subspecies'. Apparently, for Coons criteria of species delimitation were based on minor distinctions. However, in a later publication Coons (1975) considered *B. adanensis* and *B. trojana* as variants of *B. maritima*.

Based on the works of Transhel (1927) and Zosimovic (1939), Krassochkin (1959) revised the genus and he established new taxa. The author acknowledged the divergence between the domesticated and wild beets, and treated them as separate species. Evaluation of *Beta* accessions collected in the time of Vavilov made the researchers aware of the geographical variation present. Geographical patterns were seen both in the wild material as well as in the cultivated plants of various origins. Krassochkin identified a European and an Asiatic beetroot gene pool and consequently proposed *B. vulgaris* subsp. *europea* Krassochk. and *B. vulgaris* subsp. *asiatica* Krassochk. Similarly, taxa were established in the leaf beet species *B. cicla* L. for Cretan, Tunisian, west European, and Anatolian-Caucasian origins. Geographical distinction in *B. maritima* resulted in the proposal of subspecies *mediterraneum* Krassochk. and subspecies *danica* Krassochk. for southern and northern origins of the species respectively (Table 1.2).

For their revision of section *Beta*, Ford-Lloyd & Williams (1975) included numerical techniques to investigate taxonomical meaningful groups. Grown under uniform (greenhouse) conditions, both wild, primitive cultivated and modern cultivated representatives of *B. vulgaris* sensu lato were analysed morphometrically. The authors concluded that the variation among the groups was continuous and high variation within accessions was present. The largest range of variation was noticed in the wild and foliage beets. It was assumed that a large amount of gene exchange had occurred in the plants raised under more primitive farming conditions leaving the genetic relationships of different types of beet like beetroot, primitive leaf beets and wild beet plants quite obscure. Field observations on the heterogeneous complex of cultivated beet found in Turkey, yielded a form believed by the authors to be the remains of an ancestral subspecies. Regarded as somewhat intermediate between truly wild *B. vulgaris* subsp. *maritima*, and the non-swollen-root types of cultivated beet *B. vulgaris* subsp. *cicla* (L.) Koch, it was established as a new subspecies *B. vulgaris* subsp. *provulgaris* Ford-Lloyd & Williams. It is a weed (or sometimes primitively cultivated) in certain parts of Turkey. It was described on rather poorly discriminating characters such as: bracts 15-25 mm long not showing much variation, and roots always swollen and fleshy, fibrous but never woody. The newly proposed subspecies fits a theory regarding the evolution of *B. vulgaris* sensu lato. According to the authors domestication started with simple selection from maritime beet or truly wild inland beet by man. A partly cultivated, partly weedy beet resulted, represented by *B. vulgaris* subsp. *provulgaris*.

With respect to other taxa that have been described in section *Beta* Ford-Lloyd & Williams (1975) reached no definite conclusions, but they inferred from (i) the lack of agreement in the classification in the past of *B. macrocarpa*, *B. patula* and *B. atriplicifolia*, (ii) the apparent similarities between herbarium specimens of these taxa, as well as (iii) the reported crossability with other members of section *Beta*, that these taxa are the result of adaptive micro-evolution. A process of ecotypic differentiation and a certain degree of inbreeding within populations had caused morphological diversification. The authors pointed specifically to *B. adanensis*, and to a lesser extent to *B. trojana* as the products of such an evolutionary trend.

These 'micro-evolutionary' trends neccessitated relegation of *B. maritima*, *B. adanensis* and *B. patula* to the rank of subspecies within *B. vulgaris*, and the classification of a number of other subspecies: *B. vulgaris* subsp. *orientalis* (Roth) Aellen, *B. vulgaris* subsp. *lomatogonoides* Aellen (Table 1.2). Within *B. vulgaris* subsp. *maritima* the existence of a number of varieties was acknowledged: var. *maritima*, var. *trojana* (Pamuk.) Ford-Lloyd & Williams, var. *macrocarpa* (Guss.)Moq., var. *prostrata* Krassochk., var. *erecta* Krassochk., and var. *atriplicifolia* (Rouy) Krassochk. (Table 1.2). Many of these taxa were commented upon without detailed reference to the original plant material, but exclusively based on interpretation of work of earlier authors.

Classification of a multitude of separate subspecies and varieties in *B. vulgaris*

was abandoned by Ford-Lloyd in 1986. The establishment of a semi-wild, semi-cultivated *B. vulgaris* subsp. *provulgaris* as a morphological unity was no longer tenable and consequently it was withdrawn. It was defended that any formal classification below the subspecies level should be avoided. According to Ford-Lloyd section *Beta* is better characterised as a morphological continuum of biotypes, than as a collection of distinct morphological and geographical entities. The absence of any genetical barrier to crossing between the wild taxa was believed to give further evidence of close evolutionary affinities.

Provenances from the northern distribution area appeared homogeneous morphologically, and were regarded as a distinct infraspecific taxon *B. vulgaris* subsp. *maritima*. The more southern Mediterranean 'ecotypes' of beet were more variable, and they included all the Mediterranean taxa earlier described. According to Ford-Lloyd (1986) the southern taxa should be seen as one common gene pool, and an attempt to classify them below the species level or subspecific level was considered useless. The author therefore chose to unite them in one subspecies *B. vulgaris* subsp. *macrocarpa*, geographically separated from the northern subspecies.

From the taxonomic point of view the relegation of all earlier described taxa of southern origin into one single subspecies may be questioned. Using the name *macrocarpa* to classify all Mediterranean forms seems rather arbitrary. How does this *B. vulgaris* subsp. *macrocarpa* in Ford-Lloyd 1986 relate to *B. vulgaris* subsp. *maritima* var. *macrocarpa* in the classification of Ford-Lloyd & Williams 1975? The author pointed specifically at *B. vulgaris* subsp. *orientalis*, *B. macrocarpa*, *B. patula*, *B. adanensis*, *B. trojana*, and *B. atriplicifolia* to be included in *B. vulgaris* subsp. *macrocarpa*. What about the taxa the author does not comment on, viz.: *B. vulgaris* subsp. *lomatogonoides*, or *B. vulgaris* subsp. *maritima* var. *erecta* (Table 1.2)? Are they joined in *B. vulgaris* subsp. *macrocarpa* as well, and on what grounds?

Summing up, in the early taxonomic treatments overclassification of minor variants took place, and recognition at the subspecies or variety level (or even at the species level) has been primarily a matter of personal opinion. Phenotypic variation and the uncomprehended relation between annual and perennial taxa caused confusion. Lumping of some of the taxa seems necessary, but it should be done with great care. It is neccessary to study the original type material, and to sample more plant material for a better understanding of geographical variation patterns (Chapter 2). Buttler (1977[b]) drew attention to conspicuous variation in shape of perianth segments and discovered a tetraploid cytotype originating from the Canary Islands. This finding demonstrated that systematic knowledge of the section was still incomplete and that there was a need for more biosystematic work regarding wild forms in section *Beta*.

1.3 Typification of the Linnaean names *Beta vulgaris and Beta maritima*

1.3.1 Linnaeus' concept of Beta vulgaris

Linnaeus changed his concept of the species a few times during the course of his life, as judged from several of his publications. The first valid publication of the binomial *B. vulgaris* is in *Species plantarum* ed. 1, 1753, p 222 (Fig. 1.1a). In the first edition of *Species plantarum* (1753) eight varieties are listed under *B. vulgaris* with the usual Greek alphabet characters. Linnaeus specified three of the eight varieties giving them a name: var. *perennis*, var. [β] *rubra* and var. [η] *cicla*.

In the protologue of *Species plantarum* (1753) Linnaeus combined cultivated and wild plants in *Beta vulgaris*. The treatment of the species is started with var. *perennis*. Initially Linnaeus regarded cultivated beets to have been developed from wild plants (cf. Wijnands 1986), and that var. *perennis*, *'Habitat in* Angliae & Belgii *litoribus maris'*, is the core of the material. The variety *perennis* is synonymous with *Beta sylvestris maritima* as recognised by the pre-Linnaean botanist Caspar Bauhin (Bauhin 1623).

In the second edition of *Species plantarum* in 1762, Linnaeus takes out the var. *perennis* to render it the status of species under the new name *B. maritima* L. By doing so Linnaeus put the wild beets, *Beta caulibus decumbentibus* (beets with decumbent branches), apart from the domesticated varieties, *Beta caule erecto* (beets with ascending branches) (see Fig. 1.1b). Separation of the wild beets caused substitution of the Greek alphabetical designation of the varieties remaining in *B. vulgaris* L. It meant that all varieties in *Species plantarum* ed. II, 1762 had shifted one position: so var. [β] *rubra* (L. 1753) = var. [α] *rubra* (L. 1762).

In *Systema naturae* (ed. XII, 1767, p 195) Linnaeus again separated a variety to give it the status of species. The var. [η] *cicla* was established as *B. cicla* L. Some of the unnamed varieties (like *B. vulgaris* var. [ϑ]) may have been included in *B. cicla*. Since in this publication varieties are not mentioned, neither for *B. vulgaris* nor for *B. cicla*, Linnaeus final concept of *B. vulgaris* is somewhat difficult to grasp.

It is important to keep in mind that most of the varieties Linnaeus originally mentioned were known to him solely through the literature of Bauhin. According to Helm (1957[a]) the varieties *perennis*, [η] *cicla* and [β] *rubra* must have been (more) familiar to Linnaeus because they were specifically provided with a phrase. Since both var. *perennis* and [η] *cicla* were separated from *B. vulgaris*, the identity of var. [β] *rubra* (L. 1753) is of interest, as this is the 'typical' element in *Beta vulgaris* (1762) according to Helm (1957[b]). In a review of pre-Linnaean names in *Beta* Helm (1957[a]) suggested that the identity of [β] *rubra* L. could be traced back through comparison and interpretation of the publications of Bauhin and some of the old herbalists (Chapter 1.3.2).

The aim of Helm was to do historical justice to Linnaeus' concept of *Beta*

maritima. 1. BETA caulibus decumbentibus.
Beta caulibus decumbentibus, foliis triangularibus petiolatis. *Mill. dict.*
Beta ſylveſtris maritima. *Bauh. pin.* 118. *Raj. angl.* 4. *p.* 127.
Habitat in Angliæ, Belgii *littoribus maris.*

vulgaris. 2. BETA caule erecto.
Beta. *Hort. cliff.* 83. *Hort. upſ.* 56. *Mat. med.* 113. *Roy. lugdb.* 220.
rubra. α. Beta rubra vulgaris. *Bauh. pin.* 118
β. Beta rubra major. *Bauh. pin.* 118.
γ. Beta rubra, radice rapæ. *Bauh. pin.* 118.
δ. Beta lutea major. *Bauh. pin.* 118.
ε. Beta pallide virens major. *Bauh. pin.* 118.
Cicla. ζ. Beta alba vel palleſcens, quæ Cicla officinarum. *Bauh. pin.* 118.
η. Beta communis viridis. *Bauh. pin.* 118.
Habitat - - - - - , ♂, *forte a maritima, in exoticis, prognata.*

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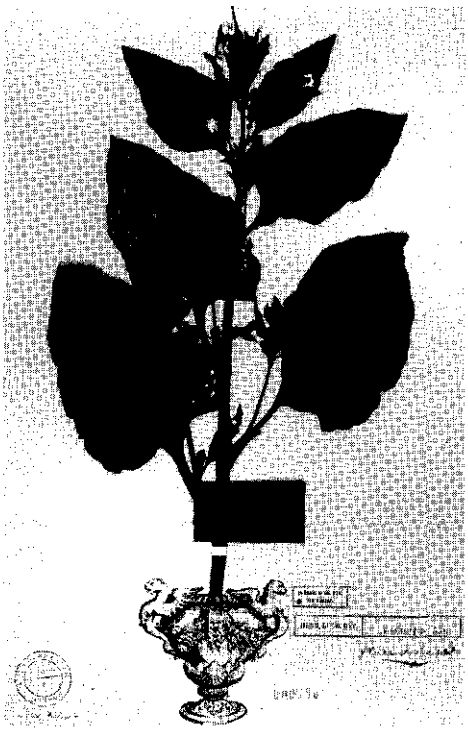
vulgaris. 1 BETA. *Hort. cliff.* 83. *Hort. upſ.* 56. *Mat. med.* 113. *Roy. lug lb.* 220.
perennis. Beta ſylveſtris maritima. *Bauh. pin.* 118.
rubra. β. Beta rubra vulgaris. *Bauh. pin.* 118.
γ. Beta rubra major. *Bauh. pin.* 118.
δ. Beta rubra, radice rapæ. *Bauh. pin.* 118.
ε. Beta lutea major. *Bauh. pin.* 118.
ζ. Beta pallide virens major. *Bauh. pin.* 118.
Cicla. η. Beta alba vel palleſcens, quæ Cicla officinarum. *Bauh. pin.* 118.
ϑ. Beta communis viridis. *Bauh. pin.* 118.
Habitat in Angliæ & Belgii *littoribus maris.* ♂

Figure 1.1. Protologue Species plantarum ed 1 (1753) p 222 (a) and Species plantarum ed 2 (1762) p 322 (b).

vulgaris. Although the extensive works of Helm are very valuable for understanding which type of crop is indicated by the Linnaean variety names, the intentions of Helm were not to actually designate a type specimen as the nomenclatural type of the species *Beta vulgaris*, (or to designate type specimen to its varieties) according to the rules of the International Code of Botanical Nomenclature (Greuter 1988).

Following the ICBN rules the name should be typified by a specimen seen by Linnaeus and still present in one of the Linnaean herbaria, or the species should be lectotypified with a specimen which may have served as a representative *Beta vulgaris* plant in the time of Linnaeus. At the same time, such a choice should preferably not be in contradiction with the present interpretations of these names.



Photograph 1.1 *Beta vulgaris* L. Lectotype: specimen no. 899213-556 herb. van Royen (L)

1.3.2 Typification of Beta vulgaris

Unfortunately there is no original material of *B. vulgaris* annotated by Linnaeus. In *Species plantarum* (1753) Linnaeus cites *Hortus Cliffortianus* (1738), *Hortus Upsaliensis* (1748), *Materia Medica* (1749) and van Royen's *Flora Leydensis prodromus* (1740). The reference to *Hortus Cliffortianus* is vouchered by a specimen which apparently relates to var. *cicla* (Photograph 1.1). The sheet includes the Bauhinian polynomial '*Beta alba vel pallescens, qua Cicla officinarum*'. This specimen seems to be useful because its association with the Bauhinian polynomial makes it a syntype.

No relevant specimen to be used for typification could be traced from any of the general Linnaean herbaria LINN, UPS, S, MW, and H. LINN contains three *Beta* sheets: LINN 314.1, is collected in Spain, and LINN 314.2, with the name '*Beta maritima*', is from the Middle East. LINN 314-3, from 'Aegypto' appears to be cultivated material. Neither of these sheets contain a *Species plantarum* account number, and so appear to be post 1753. The sheets therefore are not original for the name *Beta vulgaris*, and hence unsuitable for selection as a lectotype. The herbarium in Uppsala, arranged by Burser according to the system of Bauhin, was consulted frequently by Linnaeus. There are no specimens in the Burser herbarium, for the *Beta* sheets would have been in the lost volume V, destroyed by fire. The Stockholm herbarium holds plants cultivated in the Uppsala garden. Two sheets (Fiches S109.9: *Beta vulgaris* and S109.13: *Beta*

Photograph 1.2 *Beta vulgaris* var. *cicla* L. Lectotype: specimen no. p 83.1.ß(2) Clifford herb. (BM).

maritima) are present, but the annotation is not by Linnaeus.

The reference to van Royen is vouchered by specimen no. 899213-556 van Royen *herb. Lugd. Bat.* (L) (Photograph 1.2). The plant is mounted in a van Royen 'pot' (see Wijnands & Heniger 1991). The plant is erect, not decumbent, and since the label does not contain any reference to a place of collection, it is probably a cultivated plant. The label mentions *1. Beta vulgaris*, in two different hand writings: *Beta* is written by Adriaan van Royen, and *vulgaris* was added later, probably by David van Royen (pers. comm. Mr. M. Sosef and dr. J.F. Veldkamp). A full citation of the prodromus is lacking , but this is not unusual on a van Royen sheet. The sheet can be considered a syntype.

The Clifford sheet and the Van Royen sheet are both acceptable as a choice of lectotype for *Beta vulgaris*. The specimens of both sheets provide good diagnostic characters. The Clifford sheet has the explicit reference to var. [η] *cicla* and the Bauhinian polynomal *Beta alba vel pallescens, qua Cicla officinarum.*

The name *cicla* is still used in the recent taxonomic literature on *Beta* (cf. Helm 1957[a], Ford-Lloyd & Williams 1975, Ford-Lloyd 1986). Ford-Lloyd (1986) discriminated between subsp. *cicla* (leaf beets, spinach beets and Swiss chards) and subsp. *vulgaris* (sugar beet, fodder beet, garden beet) as two distinct morphological entities. It will be noted in Chapter 1.5.2 that it is rather superfluous to maintain the two morphological groups as separate subspecies, and that cultivar nomenclature would be more appropriate to classify cultivated beets.

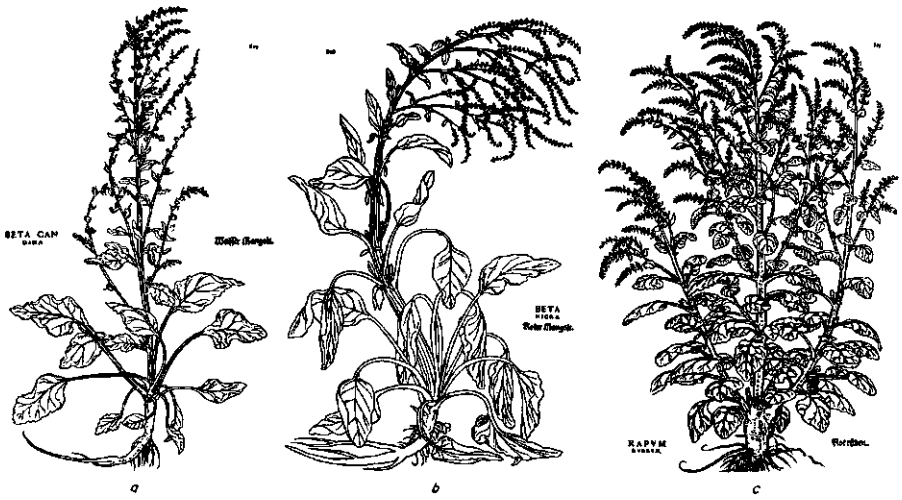


Figure 1.2. Representation of *Beta* forms in L. Fuchs, De historia stirpium. Basel, 1542. *Beta candida*, (Weisser Mangold, p 805) *Beta nigra* (Roter Mangold, p 806) *Rapum rubrum* (Rotrübe, p 213). Reprinted from Helm (1957[b]) p 66.

Anyhow, it would be advantageous to maintain the names *Beta vulgaris* var. *vulgaris* and *Beta vulgaris* var. *cicla* heterotypic and this can be achieved if *Beta vulgaris* is lectotypified with the Van Royen sheet, and *B. vulgaris* var. *cicla* is lectotypified with the Clifford sheet.

For the remaining varieties published in 1753 by Linnaeus no syntype material is available, and consequently these need to be neotypified. Helm (1957[b]) investigated the identity of the varieties, and his suggestions are followed here to neotypify the variety specifically phrased as var. [β] *rubra*.

The variety [β] *rubra* L., extracted from Bauhin's *Beta rubra vulgaris*, is, according to Helm, a leafy beet with red pigmentation in the roots and with red, sometimes dark (dark red/ dark green) leaves. Referring to Morison (1699) and Lonicerus (1679) Helm concludes that it is only a colour variant in the group of white mangolds (var. [η] *cicla*), and that it is synonymous to *Beta rubra* mentioned in Dodonaeus (1583) and Lonicerus (1679) or *Beta nigra* in the works of Matthiolus (1571) and Fuchs (1542), and *Beta rubra vulgatior* in Lobelius (1570). *Beta rubra* and *Beta nigra* are red mangolds in the descriptions of the herbalists Lobelius and Dodonaeus but there are also descriptions of *Beta rubra* or *Beta nigra* characterising it as a primitive garden beet with swollen roots. E.g. in the herbalist books of Matthiolus (1571) and Tabernaemontanus (1664) plates of *Beta rubra* are shown with thickened roots. A plant with a thick swollen root is shown in the work of Matthiolus (p 230), and in the work of Tabernaemontanus (p 814) a plant with a slightly swollen root is shown. In Fuchs (1542) a representation of *Beta nigra* is given (Fig. 1.2b). Similar to *Beta candida*, (Fig. 1.2a), it has ordinary roots. Another plate (Fig. 1.2c) shows *Rapum rubrum* with swollen roots.

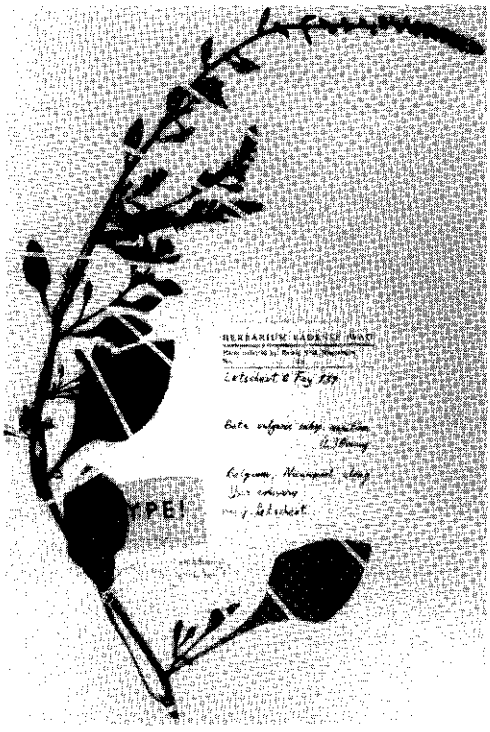
The representation of *Beta nigra* in Fuchs (1542) agrees with the description given above. Therefore the plate of *Beta nigra* in Fuchs (Fig. 1.2b) is designated here to be the neotype of var. [β] *rubra*.

Conclusions
Beta vulgaris L. Lectotype: specimen no. 899213-556 *herb. van Royen* (L) lectotype, designated here.
Beta vulgaris var. *cicla* L. Lectotype: specimen p 83.1.ß(2) Clifford *Clifford herb. BM*. lectotype, designated here.
Beta vulgaris var. *rubra* L. Neotype: Plate *Beta nigra* (Fuchs, 1542, p 806). Neotype, designated here.

1.3.3 Typification of Beta maritima

The first valid publication of *B. maritima* is by Linnaeus in *Species plantarum* ed II, p 322 (Fig 1.1b). Through the synonymy with the Bauhinian polynomial *Beta sylvestris maritima* (Bauhin 1623) it is clear that the species is derived from *B. vulgaris* var. *perennis* L., 1753. Any original material relating to *Beta maritima* or to *B. vulgaris* var. *perennis* is absent and neotypification of this element is necessary.

The diagnosis states that the species has decumbent stalks, (contrary to *B. vulgaris* which has erect stalks) and triangular petiolate leaves. Linnaeus cites



Photograph 1.3 *Beta maritima* L. Neotype: Nieuwpoort, Belgium, Letschert & Fey 137 (WAG).

Miller and Ray in the protologue. The account of Miller (1741) mentions that the species grows naturally on the banks of the sea, and in salt marshes in diverse parts of England. There is no illustration in Millers dictionary of the wild beet. The account of Ray (1686 page 157, not 127) is very short, but he says; 'Plentifully around Nottingham; Mr J. Sherard'. The Sherardian herbarium in Oxford has three sheets with *B. maritima* specimens, but there is no evidence that these were collected by Sherard 'around Nottingham'.

The LINN sheets 314.1, a beet originating from Spain, and 314.2, from the Middle East, are not suitable as a choice of neotype, because they conflict with '*Hab. in Angliae, Belgii*', in Linnaeus' protologue.

It is proposed to neotypify the name *B. maritima* and the name *B. vulgaris* var. *perennis* with recently collected material from the population in Nieuwpoort along the Yser estuary on the Belgian coast. The specimen is collected under number Letschert & Fey 137 and is available from WAG (Photograph 1.3).

Conclusions
B. maritima L. neotype: specimen Letschert & Fey 137 (WAG)
neotype designated here.
B. vulgaris var. *perennis* neotype: specimen Letschert & Fey 137 (WAG)
neotype designated here.

1.4 Taxonomic criteria and taxonomic conclusions

Species definition

In this revision a species is defined as a group of individuals that share a set of morphological features different from those of other such groups (Grant 1981, Stuessy 1990). Generally, morphological similarity points to common ancestry and cohesion of the species due to sexual reproduction. Thus, the species is defined in terms of comparative morphology, and discontinuities in morphological features are used to distinguish species (Chapter 2). Furthermore, in the context of population structure and patterns of reproductive behaviour information is provided (Chapter 3) which allows a comprehension of genetic isolation of the species and the potential for gene flow between species.

Infraspecific categories

In this study populations of a species are considered over a wide geographic range. Not unexpectedly, the observations presented in Chapter 2, 3 and 4 show different stages of gradual diverging of populations in different geographical regions. Morphological differences between populations, and between geographical groups of populations, are noticed (Chapter 2), but many should not be emphasised taxonomically, since they merely express the potential for ecotypic differentiation of the plant group. Taxonomic classification of clinal variation or the classification of minor variants is considered useless. However, some of the morphological parameters illustrate local discontinuities between infra-

specific groups worthwile of taxonomic recognition. The infraspecific category *subspecies* (subsp.) is used in this taxonomic treatment to designate a geographically coherent group of populations, which is morphologically clearly distinguishable.

In addition the subspecies category is used to combine cultivated plants and wild relatives in a biological species (Chapter 1.5). It must be understood that in this context the subspecies level does not indicate geographical entities in the biosystematic sense.

Taxonomic conclusions

Through a combination of morphological characters and additional experimental evidence three species in section *Beta* are distinguished: *Beta vulgaris*, *Beta macrocarpa* and *Beta patula*. *B. patula* and *B. macrocarpa* are readily separated from *B. vulgaris*, using both vegetative and generative morphological characters. By morphological criteria they could be recognised at the species level next to *B. vulgaris*. In the context of reproductive behaviour, the Linnaean species in section *Beta* do not equate with the biological species, defined by Mayr (1957) as groups of reproductively isolated entities, as the species hybridise readily in the greenhouse (Chapter 3). However, in zones of contact in the field sympatric populations of *B. macrocarpa* and *B. vulgaris* maintain their identity. Evidence from allozyme analysis (Chapter 3) revealed that *B. macrocarpa* has diverged at a number of loci from *B. vulgaris*.

B. patula is a geographical isolate. Substantial morphological differentiation is not associated with allozymic divergence from *B. vulgaris*. *B. patula* is characterised by specific allozymes at high frequencies and low allozyme variation compared to populations of *B. vulgaris* subsp. *maritima*.

In the Aegean distribution area groups of populations are morphologically diverging, but distinction was insufficient to consider them to be separate species. A group of early flowering, semi-annual plants, with large glomerules, succulent bracts and a reduced number of flowers per glomerule was recognised, which should be considered as infraspecific in *B. vulgaris*. *B. vulgaris* subsp. *adanensis* is distributed on the Peloponnesus, the Aegean islands and the south and west coast of Turkey. Populations of *B. vulgaris* subsp. *maritima* and *B. vulgaris* subsp. *adanensis* are frequently found close together. Generally the plants retain their identity in such places of overlap between the members of both groups. The information given in Chapter 3 points to divergence in allozyme frequencies between *B. vulgaris* subsp. *adanensis* and *B. vulgaris* subsp. *maritima*, suggesting autogamy for the former. Taking into account all the relevant information, it seems appropriate to identify this group of plants as a distinct subspecies in *B. vulgaris*.

1.5 Classification of cultivated beet

It is common practise to link cultivated plants with their wild relatives using the level of subspecies (Harlan & de Wet 1971, de Wet 1981, Pickersgill 1986).

In this context the subspecies level does not indicate geographical entities in the biosystematic sense. Present views on the classification of cultivated plants propagate the use of the cultivar and cultivar group category, thereby discarding the subspecies level for classification of cultivated plants (cf. Brandenburg & Schneider 1988). As a consequence the species name applies to the wild plant, and the species should preferably be typified with a wild plant.

If such a line would be followed for *B. vulgaris*, the precise nomenclatural consequences would be a disruption to the current usage of the names.

The wild plants, currently well known as *B. vulgaris* subsp. *maritima*, would then have taxonomic status as *B. vulgaris* subsp. *vulgaris*. Abolition of the almost universally used name *B. vulgaris* subsp. *maritima* is not very attractive. Therefore, maintaining the distinction of cultivated plants in *Beta* at the subspecies level seems preferable, leaving *Beta vulgaris* subsp. *vulgaris* for the cultivated plants, and *Beta vulgaris* subsp. *maritima* for the wild plants.

In the past classification systems of cultigens in *Beta* were characterised by vast amounts of taxa of many hierarchical ranks. Even though wild and cultivated taxa were treated at infraspecific levels from the beginning, separate systems for wild and cultivated plants evolved, with different infraspecific categories. Examples can be found in Ulbrich (1934), who recognised two subspecies and 10 varieties, Zosimovic (1939) (2 subspecies, 6 proles, 2 subproles, 24 varieties), Krassochkin (1959) (2 species, 6 subspecies, 11 varieties), Helm (1957[a]) (one subspecies, two convarieties, six provarieties, 17 formae), and Ford-Lloyd & Williams (1975) (two subspecies, 2 varieties). Mansfeld (1986) partly adopted the classification of Helm (1957[a]). Helm produced a classification based on morphological characters only. The ranks in his hierarchical system have but little biological significance since genetic relationships among the crop beets was not considered. At present it is assumed that the evolutionary affinities among the cultivar groups are very close and that there is no need for extensive use of formal categories. An alternative to the hierarchical way of classifying was proposed by Ford-Lloyd & Williams (1975) and Ford-Lloyd (1986), and discussed at an IBPGR crop meeting on *Beta* (IBPGR 1987). It was suggested to refer cultigens directly to categories such as the cultivar group – traditional commodity groups like Garden beet, Mangel, Fodder beet, Sugar beet, Chards, Spinach beet etc. – and the cultivar. Conservation of two subspecies was suggested by Ford-Lloyd (1986) to emphasise separate geographical origin of domestication of leaf beets in the Mediterranean and beetroot in northern Europe respectively. A relatively older and evolutionary more primitive gene pool, the leaf beets, is placed by Ford-Lloyd (1986) in *B. vulgaris* subsp. *cicla* (L.) Koch, and the youngest domesticated crops, beet roots, are placed in *B. vulgaris* subsp. *vulgaris*.

The distinction of more than one subspecies by Ford-Lloyd (1986) for classification of cultivated *Beta* can be challenged: if it is agreed that any formal taxonomic level should preferably be discarded in classification of cultivated plants, and that the cultivar group and cultivar are better categories, it seems rather superfluous to maintain the groups of leaf beets and beet roots as separate subs-

pecies. As indicated earlier, these subspecies do not represent geographical entities in the biosystematic sense, although Ford-Lloyd (1986) stated that some of the crops have local significance.

Occurrence and classification of weed beets

The close affinities between wild and cultivated beets have lead to the occurrence of weed beets. Weed beets[1] are the products of recent hybridisation between wild and cultivated plants (Ford-Lloyd & Hawkes 1986, Hornsey & Arnold 1979, Ford-Lloyd 1986). Hybridisation may be followed by introgression into either the beet crop plant or the wild plant (Evans & Weir 1981).

Classification of these plants should not lead to the creation of a separate subspecies for weedy plants. More or less permanent populations with introgression genes from cultivated plants should be taxonomically treated as *Beta vulgaris* subsp. *maritima*.

1.6 Taxonomy of section Beta

1.6.1 Genus and section

Genus *Beta* L., Species plantarum ed 1 p 222 (1753) Genera plantarum ed 5 (1754) (*Patellifolia* Williams, Scott et Ford-Lloyd, Taxon 26 (1977) 284)

type of the genus: *Beta vulgaris* L.

Annual or perennial, monoecious herbs or shrubs. Leaves alternate, entire, the basal leaves long petiolate. Flowers hermaphrodite, single or fused with few other flowers in axillary clusters (glomerulae). Perianth segments five, green or whitish, keeled. Stamens five, stigmas two or three. Ovary semi-inferior. Fruit opening by a lid (operculum).

Beta section *Beta*

(section *Vulgares* Ulbrich in Engler & Prantl Natürl. Pflanzenfam. ed. 2 p 462 (1934))

Annual or perennial herbs, usually in coastal habitats. Perianth always green. Perianth segments narrowly keeled, sometimes broadened, or with distinct appendages at the base.

1.6.2 Key to the wild taxa of Beta section Beta

1. Operculum convex, sometimes thickened. Perianth segments thin, sometimes spongy. At maturity perianth segments bent, sometimes covering the opercu-

[1] wild *Beta vulgaris* behaving as weeds in arable fields are sometimes referred to as weed beets too, cf. Ford-Lloyd & Hawkes 1986.

lum. Perennial, sometimes annual. 2
Operculum depressed, margins elevated. Perianth segments erect, spongy, at maturity perianth segments patent or contiguous to the operculum. Glomerules (2–) 3 (–7) flowered. Glomerules spaced on the inflorescence. Bracts large, upper bracts sometimes 3X-5X diameter of glomerule. Annual species. . . . . **Beta macrocarpa**

2. Perianth segments narrow, usually 1,5 X longer than broad. After fructification tips of segments bent, covering the operculum. Glomerules (1–) 3 (–7) flowered. Upper bracts smal, 1.5-2X length of glomerule. 3
Perianth segments short, between 2.2 and 3.2 mm, and relatively broad, between 2.0 and 2.8 mm. Segments generally not longer than broad (mean ratio tepal length/tepal width = 1.12). After fructification operculum rises up above the short perianth segments. Tips of perianth segments not contiguous in fruit. Glomerules (1–) 2 (–4) flowered. Glomerules spaced on inflorescence, proximal bracts large and succulent, distal bracts very small, hardly exceeding length of glomerule. . . . . **Beta vulgaris** subsp. **adanensis**
3. Glomerules with (1 -) 3 (- 7) flowers. 4
Glomerules many flowered (2–) 7 (–12). Proximal glomerules usually with 8 or more flowers. Glomerules spaced on inflorescence. Leaves and bracts linear or lanceolate, glabrous. . . . . **Beta patula**
4. Glomerules usually crowded apically and the inflorescence compressed. Perianth segments bent in fruit, contiguous to the operculum, less than 7 mm long. Upper bracts small linear or rhombic.
Leaves ovate or deltoid, sometimes lanceolate or rhombic, glabrous or moderately pubescent, or occassionaly densely covered with hairs. Leaves sometimes waxy. Glomerules crowded apically. Plants erect or decumbent. . . . . .**Beta vulgaris** subsp. **maritima**
Glomerules spaced on the inflorescence. Flowers large, perianth segments of proximal flowers erect, spongy, longer than 7 mm. At maturity perianth segments patent or contiguous to the operculum. Upper bracts large, rhombic. Plants from the Canary Islands . . . . . **Beta macrocarpa**

1.6.3 Species descriptions

Beta vulgaris L., Species plantarum ed. 1 p 222 (1753).

B. vulgaris subsp. **vulgaris**
Sugar beets, garden beets and fodder beets. Leaf beets for consumption of leaves: foliage beet with abundant leaf material, leaf beets with swollen midribs.

B. vulgaris subsp. **maritima** (L.) Arcangeli, Comp. Fl. Ital. 593 (1882).
basionym: *B. maritima* L. Species plantarum ed. 2 p 322 (1762)

Selected synonymy of *B. vulgaris* L. subsp. *maritima* (L.) Arcang.

Beta vulgaris subsp. *perennis* (L.) Species plantarum ed. 1 p 222 (1753)
B. orientalis Roth, Novae Plantarum Species praesertim Indiae Orientalis. p 181 (1821)

B. bengalensis Roxburgh Fl. Ind. 2: p 59 (1832)
B. vulgaris var. *orientalis* (Roth) Moq. in A. Decandolle., Prodr. 13: (2) p 56 (1849)
Beta vulgaris subsp. *cicla* var. *maritima* (L.) Alef. Ldw. Fl.: p 278 (1866).
B. atriplicifolia Rouy Rev. Sci. Nat. Ser. 3: p 246 (1883)
B. vulgaris subsp. *perennis* var. *maritima* Ulbrich in Engl. u. Prantl, Nat. Pfl.-Fam.., ed.2, 16c p 460 (1934)
B. vulgaris subsp. *lomatogonoides* Aellen, Ber. Schweiz. Bot. Ges. 48: p 478 (1938)
B. vulgaris subsp. *maritima* var. *glabra* (Del.) Aellen, Ber. Schweiz. Bot. Ges. 48: p 479 (1938)
B. vulgaris subsp. *maritima* var. *foliosa* (Ehrenb.) Aellen, Ber. Schweiz. Bot. Ges. 48: p 478 (1938)
B. vulgaris subsp. *maritima* var. *pilosa* (Del.) Aellen, Ber. Schweiz. Bot. Ges. 48: p 479 (1938)
B. vulgaris subsp. *orientalis* (Roth) Aellen, Ber. Schweiz. Bot. Ges. 48: p 479 (1938)
B. maritima subsp. *danica* Krassochkin, Trudy prikl. Bot. Genet. Selec. 32 (3): p 34 (1959)
B. maritima subsp. *mediterraneum* var. *prostrata* Krassochkin, Trudy prikl. Bot. Genet. Selec. 32 (3): p 27 (1959)
B. maritima subsp. *mediterraneum* var. *erecta* Krassochkin, Trudy prikl. Bot. Genet. Selec. 32 (3): p 28 (1959)
B. maritima subsp. *mediterraneum* var. *atriplicifolia* Krassochkin Trudy prikl. Bot. Genet. Selec. 32 (3): p 27 (1959)
B. trojana Pamukçuoglu in Aellen, Notes R.B.G. Edinb. 28: 29 (1967)
B. vulgaris subsp. *maritima* var. *grisea* Aellen, Notes R.B.G. Edinb. 28: p 30 (1967)
B. vulgaris subsp. *maritima* var. *atriplicifolia* (Rouy) Krassochkin, Fl. Cult. Pl. 19: p 30 (1971)
Beta vulgaris subsp. *provulgaris* Ford- Lloyd & Williams, Bot. J. Linn. Soc. 71:p 99 (1975)
B. palonga Basu and Mukherjee, Can. J. Bot. 53: p 1166-1175 (1975)

Description
Habit perennial, sometimes annual, erect or prostrate. Branches subopposite from the base, ascending or decumbent, green or red striate, occasionally orange.
Leaves narrowly ovate or deltoid, glabrous or slightly hairy, lower leaves long petiolate, upper leaves sessile.
Bracts lower bracts elliptic – ovate, apical bracts linear.
Glomerules flowers occasionally solitary, usually united in groups of 2-8 in glomerules in paniculate inflorescences. Glomerules crowded in the upper part of the inflorescence branches, these sometimes long, up to 50 cm.
Perianth segments five, green, spathulate or triangular, thickened, more or less flat or sometimes thin, with hyaline margins on the back, to a variable degree hooded. Perianth segments sometimes strongly carinate on the back, with large spongy swellings on the bases of the flowers.
Operculum usually flat or slightly convex.

Chromosome number
Beta vulgaris subsp. *maritima* is diploid with 2n = 18. (Coons 1954, De Bock 1986).

Distribution
B. vulgaris subsp. *maritima* occurs along the Atlantic coasts of western Europe including the British Isles, and on the Azores, and it is common and widespread along the coasts of nearly all Mediterranean countries. It occurs in the countries of the Middle East and extends to the Indian subcontinent.

In the north the species is known from several localities in Sweden, each ac-

commodating only few individuals. A number of fairly large populations are found on the west coast of Sjaelland, Denmark (Rasmussen 1932, Tjebbes 1933, Simmons 1930). The species is rare on the coasts of Germany, The Netherlands and Belgium, but fairly common on the coasts of the British Isles (Doney et al. 1990) and western France (Frese 1991).

In the northern part of the distribution area the sea beet is found in a narrow band along the coast usually within 10 to 20 m of the high water mark. Favorable habitats are rocky cliffs and gravel beaches. Less favorable habitats are dense grass lands, sandy beaches, salt marshes or disturbed sites.

In the Mediterranean and Middle East region populations are primarily coastal but habitats are more diverse and inland populations are frequently found. Inland populations are also reported from Sicily (Toll & Hendriksen 1982) and from south-eastern Spain (Frese et al. 1990), where populations occur either at ruderal sites or in closed vegetation on mountain slopes up to 800 m. Niches of occurrence are sheltered sites on disturbed grounds, shingles, sandy or loamy beaches, as well as salt marshes where salinity can be extremely high.

Illustrations, see Ulbrich 1934, Hegi 1960, Aellen in Davis 1967, Valdes et al. 1987.

B. vulgaris subsp. **adanensis** (Pamukçuoglu) Ford-Lloyd & Williams, *Bot. J. Linn. Soc. 71:* p 100 (1975)

type: Karatas to Adana 29.05.1963 Pamukçuoglu (holo:IZ, iso:G-PAE 7422-1!)
basionym: *Beta adanensis* Pamukçuoglu in Aellen, Notes R.B.G. Edinb. 28: 29 (1967)

Description:
Habit annual or perennial herb, prostrate or erect.
Leaves basal leaves long wedge-shaped. Stem leaves fleshy, the lower broadly obovate and petiolate, the median and upper sessile, obovate, lanceolate or rhombic in outline. Sometimes leaves curled.
Bracts lower bracts large, up to eight times the glomerule diameter, fleshy, broadly rhombic-spathulate, the upper narrowly obovate or glomerules ebracteate.
Glomerules composed of groups of two, three or more flowers. Glomerules spaced, not crowded on the inflorescence as in *Beta vulgaris* subsp. *maritima*. Glomerules on the main stem with more flowers than glomerules on the lateral stems.
Perianth segments short from a broad base, the apex flat or strongly keeled, spongy. Towards maturity of the fruit the segments more or less curved and contiguous to the operculum.
Operculum in large fruits the operculum thickened and strongly convex.

Chromosome number
A diploid subspecies with 2n = 18 (Buttler 1977[b]).

Distribution Greece: Peloponnesus, Aegean Islands Kos, Leros, Samos, Chios, Crete, Rhodos. Cyprus, south and west coast of Turkey, Syria. On Cyprus *B. vulgaris* subsp. *adanensis* is dispersed diffusely and continuously on gravelled beaches or at roadsides quite near the sea. Large populations were seen near irrigated crop fields within one km from the sea. *B. vulgaris* subsp. *adanensis* was an important weed here.

Illustrations Fig. 1.3 and in Buttler 1977[b], p 129, Fig 4e

B. vulgaris subsp. *adanensis* is distinguished from *B. vulgaris* subsp. *maritima* by its broader, shorter perianth segments. The glomerules are generally bigerm, and the flowers are more flat than in *B. vulgaris* subsp. *maritima*. Bracts, especially in the proximal part, are large and fleshy. Only a small rosette is formed, flowering already starts from the seventh node. The glomerules are spaced on the inflorescence stem, not closely packed as in *B. vulgaris* subsp. *maritima*. Difficulty in identifying herbarium plants is due to the fact that specimen are sometimes collected too early, that is before diagnostic characters of the perianth are developed. Perianth structure is not fully differentiated before the time of fruit ripening.

Synonymous names in wild B. vulgaris

The variation of *B. vulgaris* in its wide geographical range has provoked a number of authors to further subdivide the taxon in a large number of infraspecific taxa (Chapter 1.2.4). Many names have been published based on few herbarium specimens, weak taxonomic characters and without sufficient consideration of phenotypic variability. Geographical continuity was found absent for many of the variants described (Chapter 2). After examining the original materials in several herbaria and the published descriptions it was concluded that the use of many of these names should be discontinued and put into synonymy with *B. vulgaris* subsp. *maritima*. Some of the variants are evaluated here.

In its most northernly distribution area *B. vulgaris* subsp. *maritima* is characterised by perennial plants with decumbent flowering stems and thick glaucous dark green leaves with long petioles. The plants are large and fairly homogenous compared to Mediterranean subsp. *maritima*. This northern form (or ecotype, a qualification by Tjebbes (1933)) has been given subspecific names such as *B. vulgaris* subsp. *perennis* Ulbrich or *B. vulgaris* subsp. *danica* Krassochkin. Krassochkin (1959) considered annual and perennial Mediterranean plants with ascending stems to be more heterogeneous than subsp. *danica*, especially with regard to time of flowering, growth habit and leaf morphology. Consequently he described a number of varieties in his subspecies *mediterraneum*. Plants from Spain, Italy and Dalmatia representing var. *erecta* had thin, light green leaves and ascending stems. Algerian plants with rather small glomerules and glaucous leaves were typical for his var. *prostrata*. Krassochkin went on to classify some weedy plants or what may be primitive leaf beets from 4 geographical regions in subspecies of *B. cicla*. It is not clear whether these subspecies only refer to

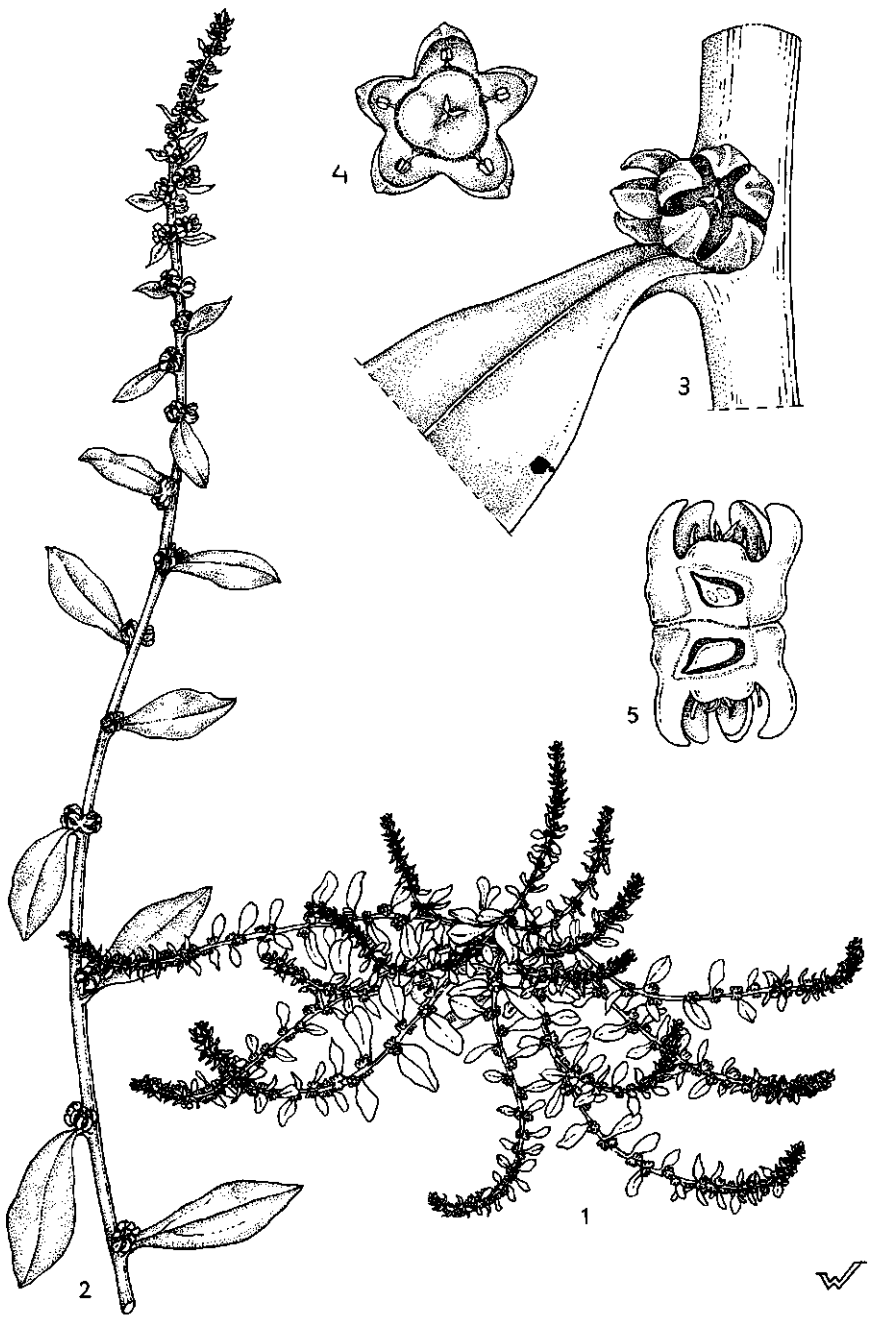


Figure 1.3. *B. vulgaris* subsp. *adanensis*. 1. plant habitus, 2. fruiting stem, 3. glomerule in axil of bract, 4. flower, overall view, 5. glomerule, longitudinally cut. culta from seed (gene bank passport number 1502), Greece; Leros, Plefouti. Letschert 114 (WAG).

cultivated plants or also to wild plants which could be weeds or escapes from cultivation. There is no reference to herbarium material or type specimens in the publication by Krassochkin (1959), and consequently the names are not validly published.

Aellen (1938, 1967) revised the oriental taxa of section *Beta* (Table 1.2). He studied material from Turkey, Cyprus, the Near East and inland origins from Syria, Iraq, Iran, Pakistan and India. His species and subspecies are based on differences in glomerule size, glomerule morphology, bract size and leaf pubescence. The type specimen of the various taxa recognised by Aellen were examined from the herbarium Aellen in Geneva (HPAE).

Beta trojana Pamuk. in Aellen (1967) is described having 'perianth segments strongly carinate on the back' and with 'large spongy swellings on the bases of the flowers'. In the Flora of Turkey *B. trojana* is keyed out by having perianth segments 'broadly triangular, strongly keeled, the keel almost winged, with distinct appendages at the base' (illustration in the Flora of Turkey p 317). It was noted that among the herbarium material only the type specimen exhibited the characteristic larger glomerules. All other specimens had only small monogerm glomerules. The large root stocks and long petioled rosette leaves indicated that the plants were presumably perennials.

Plants with strongly keeled, spongy perianth segments with conspicious outgrows at their base are common in *B. macrocarpa* and *B. vulgaris* subsp. *maritima* throughout the whole distribution area. The traits are expressed in plants which develop comparatively large fruits. It is seen more often in plants from populations from the Mediterranean than in plants from Atlantic populations. *B. trojana* should be put in synonymy of *B. vulgaris* subsp. *maritima* because it lacks distinctive characteristics.

Aellen described *B. vulgaris* subsp. *lomatogonoides* from Irak and Iran on the basis of spathulate perianth segments typically being as long as the the diameter of the receptacle (illustration in Aellen 1938). Glomerules are usually biflowered and the perianth is described as 'elegant'. The habitus of the plants is like *Beta lomatogona*. The type specimen in the collection of Aellen shows only part of an inflorescence. The inflorescence has biflowered glomerules, the perianth segments are connivent and rather short (tepal length ca. 3 mm, tepal width ca. 2 mm). The inflorescence of Aellen 7422/17 was very *maritima*-like. The plants show no special leaf characteristics. It could not be determined whether the plant was perennial or annual. There is one specimen (originating from France (?)) annotated by Aellen as subsp. *lomatogonoides* which is undoubtedly perennial. It had remarkable small monogerm glomerules, but distinctive characters could not be found. It was concluded that subsp. *lomatogonoides* should not be recognised.

Plants with variable degrees of pubescence have been classified in var. *glabra* (no hairs), var. *pilosa* (described already by Ulbrich (1934): plants from Egypt) and subsp. *grisea*. Herbarium plants are classified as subsp. *grisea* but sometimes

as var. *grisea*. Aellen comments that subsp. *grisea* is characterised by leaves with scattered long hairs and a felt of smaller crisped hairs. In contrast, var. *pilosa* plants have scattered hairs concentrated on the back side of the rosette leaves.

There is no reason to maintain all varieties mentioned above, since leaf pubescence is very variable, sometimes even within populations, and it is not a very reliable taxonomic trait.

B. atriplicifolia was described by Rouy in 1883 as an endemic species collected near Murcia, Spain. It could be found at calcareous soils in the coastal hills at altitudes over 700 m. Descriptions of the species are few and limited, but the herbarium material shows that the plants are rather fluffy, caused by the fact that the leaves are covered by a thick layer of hairs. The leaf shape is rhombic, more or less *Atriplex*-like, and the stems are tri-angular. The glomerules show no special characteristics.

Ulbrich (1934) and Coons (1954) considered *Beta atriplicifolia* to be a species distinct from *Beta vulgaris*. Krassochkin (1959, 1971) classified it as a variety. Results of this study (Chapter 2 and 3) indicate that the taxon should be considered nothing more than a peculiar form in *Beta vulgaris* subsp. *maritima*. Ecotypic development is apparent from the taxon's non-coastal distribution and restriction to calcareous soils.

The type specimen of *Beta orientalis* Roth was examined in the herbarium of Aellen. It contains few seeds. An illustration of the glomerules is given in Aellen (1938). *Beta orientalis* is keyed out by its tiny, monogerm and bigerm glomerules. Perianth segments are spathulate/oblong and thin, to a variable degree hooded and sometimes narrowly membranous margined on the back. In 1821 Roth described *Beta orientalis* as an annual weedy herb which is growing wild in India. Moquin, in his revision of the *Salsolaceae* in 1849 reduced *B. orientalis* to a variety of *B. vulgaris*, and this was accepted by Ulbrich in 1934. In 1832 Roxburgh had described the same type of plant as *B. benghalensis* and commented that he was not certain whether it was sufficiently distinct from *B. vulgaris* to describe it as a separate species. Hooker (1886) considered Roxburgh's *B. bengalensis* as a synonym of *B. vulgaris* and reports that it is only a form with more erect branches, different from *B. maritima* which has decumbent branches. Nayar and Ramamurthy (1977) reported on *B. vulgaris* var. *orientalis* that it is cultivated in the north of India and that it is known as Indian spinach or Palong (Palang, Palak etc.). The leaves are eaten as a vegetable in salads or in stews. Basu & Mukherjee (1975) reported on collection sites of Indian *Beta* at 11 localities in and around Calcutta, near Bombay, and from Allahabad, Chandigarh, Cuttack, Delhi, Lucknow, Nainital, Patna, and Tamil Nadu. Basu & Mukherjee (1975) attempted to investigate the taxonomic status of the Indian spinach beet through the application of cytological methods and data on phenolic substances in addition to morphology. Annual growth habit, differences in chromosome morphology and variation in phenolic components in the leaves were presented as evidence that *B. vulgaris* and Indian leaf beet were actu-

ally two distinct species. However, it seems that no structural differences were found and consequently the proposed species *B. palonga* Basu & Mukherjee is rejected here.

Accessions originating from India, Pakistan and Iran evaluated in the field trial (Chapter 2) answered to the descriptions of *B. orientalis* Roth. Primarily based on quantitative differences in number of flowers per glomerule and in perianth morphology accessions originating from India, Pakistan and Iran could be grouped together. It is considered useless to give a formal taxonomic rank to this group, but they will be referred to here and in the following chapters as 'the oriental accessions'.

Specimens examined:

Beta vulgaris subsp. *maritima*

IRELAND: Dublin, Howth, 19-07-1958, de Wilde & de Kock (WAG).

THE NETHERLANDS: Schiermonnikoog, paal Q, Westhoff (WAG).

GERMANY: Sleeswijk Holstein, 1838, Hansen 821 (WAG).

FRANCE: Avignon, 23-07-1961, Aellen 7422/50, 7422/51 (G, herb. Aellen). Bretagne, le Faon, 26-07-1970, Marquardt, herb. Aellen 7422/18 (G).

Charente inferieure, Marennes, 27-06-1960, Personnat 3191 (WAG). Somme St. Valery, 09-07-1959, Boerboom (WAG). Cote Sauvage, Quiberon (WAG). Corsica, St Florent, W beach 18-07-1956, Segal 416. Corsica, Ajaccio (WAG).

ITALY: Venice, Auf dem Lido bei Venedig, Kellner von Koellenstein 2452 (WAG). Liguria, 15-08-1970, strandboschung, Baumgartner 3524 herb. Aellen 7422/49, (G).

YUGOSLAVIA: Dalmatia, Trau, 1843, Prior (K). Dalmatia, Lesina, 04-1903, Prior (K). Dalmatia, Split, am Meere Spalato, 04-1870 (K).

SPAIN: Mallorca, Sa. Parossa 13-04-1966, Duvigneaud 66E242 herb. Aellen 7422/39, 7422/40 (G). San Lucar de Barrameda, 1849, Bourgeau 426 (K, WAG). Cartagena, Veler rubio, 05-1989, Letschert & de Meyer 23 (WAG). Cartagena, Champs au bords de la mer, 20-04-1850, Bourgeau 859 (K, WAG) (var. *atriplicifolia*). Cartagena, 24-04-1926, Sandwith 333 (K) (var. *atriplicifolia*). Route de Puerto de Lombreras a Velez Rubio, Almeria, 08-06-1882, herb. A. Guillon, (P) (var. *atriplicifolia*). Murcie, Sierra Tercia, vers la Hoya, V et VI, Jeronimo 7372, (P) (var. *atriplicifolia*).

PORTUGAL: Azores, Terceira, S. Pedro Areias Brancas, between Silveira and Fanal, 22-07-1986, E. Dias 579, (herbario da universidade dos Açores). Azores, Terceira, Angra, Negrito, 13-04-89, B. Gorreseres, (herbario da universidade dos Açores).

MOROCCO: Greater Atlas, Siksaua, 05-1871, Hooker (K). Fedhala, 03-1933, Treltewy (K). Essaouira, 26-03-1972, Branwell & Murray 198 (K). between Rabat and Temara, 14-03-1970, Andreas 5975 (WAG).

EGYPT: Alexandria, between El Meks and lake Mariut, 08-04-1908, Bornmuller 10964 (B). Mariut, 5 km. E Abu Sit on the lake bed, Davis 64746, herb. Aellen 7422/43 (var. *pilosa*) (G). Alexandria, between El Meks and Lake Mariut, 08-04-1908, Bornmuller 10964 (B)

ISRAEL: Jaffa, 18-03-1873, Dringler (B). Jericho, Dead Sea, 10-03-1913, Meyers & Dinsmore (K). Between Jaffa and Larona, 22-05-1904, Kneucker 332, herb. Bornmuller (B). Wadi Malkhah, Jordan Valley, 30-03-1928, Gabrielith (K).

LEBANON: 11 km a Beirut orientem versus supra Yamhour, 09-05-1922, herb. Aellen 7422/24 (G).

SYRIA: Beinit, 07-05-1877, apud Colleg. Syriens. Protest., Postian (K). Damascus, Ruderalflache Südlich Kisoue, 20-04-1965 (M). Berges de la riviere Afrin, 16-05-1911, Haradjian (K). Environments de Homs, 05-1910, Haradjian 3242 (K).

TURKEY: Izmir, from Bergama to Ali Aga, 1 km before Ali Aga, 16-06-1956, Demirez 3077, herb. Aellen 7422/42 (var. *glabra*), (G). Izmir, Aliaga plaji, 03-06-1962, Pamukçuoglu (G). Anatolia, 1867, Wiedemann, herb. Hookerianum (var. *orientalis*) (K). Cannakkale, Çiplak köyu 04-07-1962,

Pamukçuoglu, herb. Aellen 7422/44, 7422/45, 7422/46, 7422/47 (G). Cannakale, Çiplak köyu 11-06-1961, Pamukçuoglu, 7422/32 to 7422/34 (typus subsp. *grisea*) (G). Eski kumkale, 05-07-1962, herb. Aellen 7422/37, /38. Izmir, Aliaga Plaji, 03-06-1962, herb. Aellen 7422/35. Izmir, C. kale, herb. Aellen 7422/4 to 7422/15 (typus *B. trojana*) (G).

GREECE: in halipedo Phaleri, 19-05-1891, De Heldreich herbarium Graecum Norm. 1184, herb. Aellen 7422/41.

IRAN: Musjid-i-Sulimani, 2000 feet, 1927, Macmillan 255, Macmillan 256, herb. Aellen 7422/16 (typus subsp. *lomatogonoides*) (K,G). Culta Wageningen, 22-08-1989, IDBB 5399, Letschert 126 (WAG).

IRAQ: Gatt al Dwat, near Amara, 22-04-1934, herb. Aellen 7422/17 (var. *orientalis*) (G).

PAKISTAN: Culta Wageningen, 22-08-1989, IDBB 5383, Letschert 111, 118 (WAG).

INDIA: Bengalen (culta Leatherhead), 1945, Burkill (K) (*B. bengalensis*). Ex horto propio et ex India orientali a Clario, 1816, D. Beny Heyne, herb. Aellen 7422/48, (typus *B. orientalis*) (G).

B. vulgaris subsp. *adanensis*

GREECE: Rhodos, Kamiros, 26-04-1972, Fagersten (herb. Greuter). Thrakia, Nea Chili, 28-05-1967, Bauer & Spitzenberger 1542 (M, herb. Buttler, herb. Greuter). Aegaea, insula Hios, ad sinum Karfas, 30 m, 15-04-1973.

CYPRUS: Paphos camping site near beach, 07-06-1989, Letschert 54 (WAG). Kissonerga, pebble beach, 07-06-1989, Letschert 59,62 (WAG). Polis, Pomos, end of village, farm field near the sea, 13-06-89, Letschert 70 (WAG).

TURKEY: Adana, Karatas, 29-05-1963, leg. A. Pamukçuoglu, (G, Herb. Aellen 7422/1, *isotype*). Culta Geneve, seeds from Pamukçuoglu, 31-07-1964, (G, herb. Aellen). Adana, NW of Karatas, 1.5 km W of Golkaya köyu, 09-05-1969, Buttler 12914 & Uzunoglu 0403 (M, herb. Buttler). Adana, 9 km SW of Karatas, 11-07-1972 Uzunoglu 0101 (M). Hatay, 1.3 km W of Sarkkonak köyu near Diba plajiari, 13-05-1969, Buttler 12990 & Uzunoglu 0403 (M, herb. Buttler).

Beta macrocarpa Gussone, *Fl. Sic. Prodr. 1: 302* (1827)

type locality Agrigento, Sicily, Italy. Type specimen not available.
B. vulgaris var. *macrocarpa* Moquin, *Chenop. Monogr. Enum.* p 14 (1840)
B. vulgaris subsp. *macrocarpa* Thellung, *Mem. Soc. Nat. Sci. Cherbourg 38:* p 190 (1912)
B. vulgaris subsp. *mediterraneum* var. *macrocarpa* Krassochkin, *Trudy prikl. Bot. Genet. Selec. 32(3):* p 26 (1959).
Heterotypic synonym:
B. bourgeaei Cosson, Not. Pl. Crit. 44-45 (1849) *Type:* Spain, Cadiz, Sa. Maria 05-1849 Bourgeau 858 (P)

Description:

Habit annual, 15 to 60 cm high, prostrate or erect. Branches subopposite from the base, semi-erect or decumbent. Stems green, usually veins red striated.

Leaves glabrous and entire, green, sometimes with little pigmentation, lower leaves 6 to 35 cm long, obovate, occasionnally fleshy.

Bracts the lower bracts up to 15 cm long, petiolate, ovate or obovate. Bracts decreasing in size towards the apex, but often 3 or 5 times longer than the glomerule diameter. Upper bracts narrowly obovate or lanceolate.

Glomerules containing (2)-3-(7) flowers, spaced on the inflorescence stem, not apically packed.

Perianth segments spongy, usually triangular from a broad base, sometimes narrow oblong and strongly keeled. Towards maturity of the fruit the tepals either patent and not contiguous to the operculum, or tepals connivent and appressed to the operculum (Fig. 1.4).

Figure 1.4 *B. macrocarpa.* plant habitus, glomerule in axil of bract, (top) (bottom right) flower, overall view, ovary and operculum (bottom left). (Valdes et al. 1987, p 182).

Operculum the center of the operculum depressed, as the margins of the operculum rise up at maturity of the fruit. Contrasting with the elevated operculum margins is the deep groove around the operculum.

Chromosome numbers

Beta macrocarpa is diploid with 2n = 18. Natural polyploidy in *Beta macrocarpa* was reported by Buttler (1977[b]) in origins from the Canary Islands. All collections made from three different islands had the tetraploid number 2n = 36.

Distribution

Portugal, south of Lisbon, Algarve. South and south-east part of Spain, Baleares, Canary Islands (tetraploid cytotype), Morocco, Algeria, south of France, Sicily, Greece, Cyprus, Israel, Turkey.

Beta macrocarpa is a fairly common species of ruderal habitats such as field margins, garbage places, slopes of terraces, orchards and roadsites between Almeria and Murcia in southern Spain. It is usually found on chalky or stony

clay soils with varying levels of salinity. In south and west Portugal *Beta macrocarpa* is a less common species almost completely restricted to the dry parts of salt marshes. In particular those sites are inhabited where dams have been constructed for salt exploitation (salinas). Only once a population was found outside this characteristic habitat (Frese et al. 1990).

Illustrations Fig. 1.4 and Coste (1906) *Flore de la France* p 180. Buttler (1977[b]) p. 129, fig. 4 a, b and c.

Specimens examined:

GERMANY: Düsseldorf, (adv), 23-09-1928, (G, Herb. Aellen 7422/22).

ITALY: Sicily, Agrigento, Lido Rosello near Realmonte, 16-04-1976, Buttler 20556 & Zielonkowski (M, herb. Buttler).

SPAIN: Sables maritimes á Cartagena, 23-04-1852, Bourgeau (WAG) Murcia, Cartagena, 17-04-1850, Bourgeau (K). Almeria, Sierra Alhamilla, 25-04-1928, Ellman & Sandwith (K). Cadiz, Coto marshes, 05-1957, Huxley (K). Cadiz, Sa. Maria, 05-1899 Bourgeau 858 (K). Formentera, San Fernando, 05-06-1972, Kuhbier & Finschow 1726, herb. Aellen 7422/23 (G). Murcia, La Hoya, near railway station, saline clay and calcerous field edge, Letschert & de Meyer 131 (WAG).

CANARY ISLANDS: Gomera, NE of San Sebastian de la Gomera, la Gallarda, 02-05-1968, van der Maesen 297 (WAG). Lanzarote, montana de Famara, 10-04-1964, F. Markgraf (G-herb. Aellen 7422/20). Gran Canaria, Las Palmas, San Christobal, 90 m., 16-01-1967, Kunkel 9772 (M). Near Agaete, 120 m, 28-02-1967 Kunkel 10421 (M). Tenerife, Punta Hidalgo, au NW de Bajamar, 04-04-1972, Duvigneaud 72E553 (G).

ALGERIA: Biskra, -05-1853, Balansa 1042 (K). Oran, Muley-abd-el-Kader, pres de Sidi bel-Abbes, ravins argilo-sales, 20-05-1877, Warrion (K). Biskra Mou, 05-1853, Balansa 1042, (K, WAG). Pied des falaises de la batterie Espagnole, 09-05-1852, Balansa 422, (WAG).

MOROCCO: 35 km E of Taourirt road to Oudja-Taza, 400 m, 08-04-1967, Merxmuller 22226 & Oberwinkler (M). 15 Miles up road to Sebu 20-06-87 (K).

GREECE: Crete, Irakleion, L. Hersonisou, 16-05-1983, Larsen 38124 & Larsen (B). Aegaea or., insula Psara, in Planitie Ahladhokambos, 20m, 19/25-04-1973, Greuter 10867 (herb. Greuter). Attica, 5 km. SE Vugliameni on road Piraeus-Kap Sunion, 15-05-1967, Podlech 13922 (M, herb. Podlech, herb. Buttler). Athens, prope Phalerum, 19-04-1891, de Heldreich, herb. Graecum Norm. 1185 (G,M).

TURKEY: in insula Kibihu, (Sinus Pers. Aust.) in palmetis, 22-05-1893, Bornmuller, (B). NW Anatolien, Bolu, Akcakoca am Schwarzem Meer, Steilufer, Wagenitz & Beug 207 (B).

CYPRUS: Limassol, Ziyyi, end of village, eroding lime cliff, 09-06-89, Letschert 67 (WAG).

UNIDENTIFIED: (*Beta vulgaris* s.l. or *Beta macrocarpa*)

EGYPT: oasis Aicur-Musa 17-05-1903 Kneucker (B).

UK: Leathorpe limes, British Isles, 08-08-1958 (K).

SWEDEN: Goteborg, Ringon, in ruderatis 22-08-1953, Carl Blom (G-herb. Aellen 7422/19) (adv. annual).

SPAIN: Lluch Mallorca 19-08-1971, Duvigneaud 71E603, (G).

TURKEY: Umgebung von Trapezunt, 1843, K. Koch (B)

Beta patula Aiton, Hort. Kew. p 315 (1789)

Type specimen not available

B. vulgaris subsp. *patula* Ford-Lloyd & Williams Bot. J. Linn. Soc. p 100 (1975)

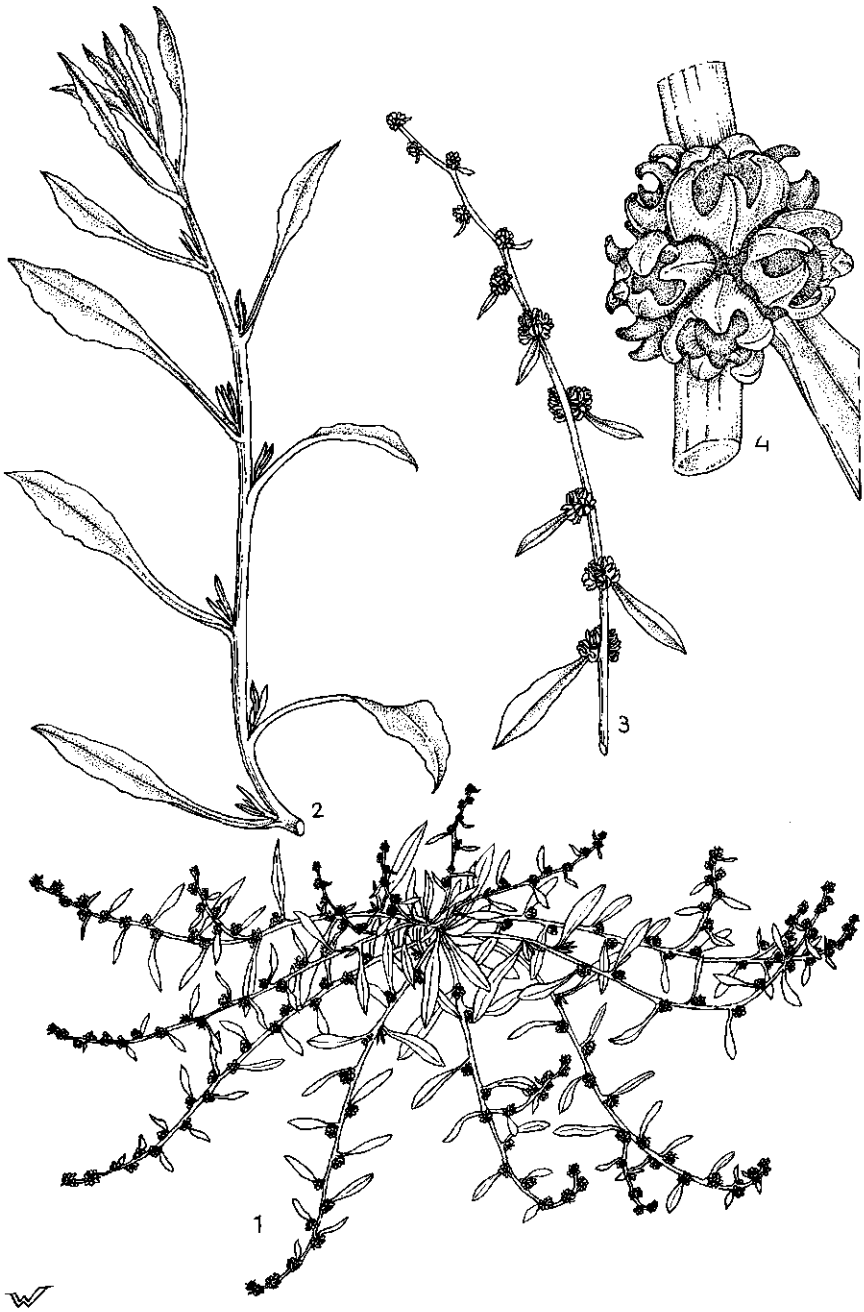


Figure 1.5 *B. patula*. 1. plant habitus, 2. fruiting stem, 3. glomerule in axil of bract, 4. flower, overall view, 5. glomerule, longitudinally cut. culta from seed (gene bank passport number 6963), Portugal; Madeira 01643W- 03236O. Letschert 136 (WAG).

Description:
Habit perennial, 15-60 cm high, prostrate or semi-erect.
Branches decumbent. Stems green, occassionally striate-red.
Leaves alternate, glabrous and entire, lower leaves 3 – 8 cm long, rhombic, lanceolate or oblong, non succulent, bright green usually without red except for occassional slight traces at the veins or margins. Upper leaves narrow linear to oblong.
Bracts linear, as upper leaves. Lower bracts large (up to 10 times the diameter of glomerule), gradually decreasing in size to the apex of the inflorescence.
Glomerules more or less loosely packed on the inflorescence. Glomerules with on average 7 flowers, the proximal glomerules large, with up to 12 flowers.
Flowers in the cluster variable in size, green.
Perianth segments thin or slightly thickened with hyaline margins, strongly hooded. Toward maturity the segments bent over to cover the operculum to a variable degree.
The *operculum* thin, flat or convex.

Chromosome number 2n = 18 (Coons 1954, De Bock 1986).

Distribution
Very limited distribution: The species is found at Ilheu dos Embarcaderos, a small Island near Madeira. According to Frese (pers. comm.) not present anymore on the main island of Madeira.

Illustration Fig. 1.5.

Specimens examined:
PORTUGAL: Madeira, St. Laurenti, 03-1832, Lowe 698 (K). Madeira, 1865, 1866 G. Mandon 211 (K). Cult hort. Par. Ex herb. A. Jamain (P). Cult. in h. dorpot, 1823, ex herb. A. de Bunge. Madeira,?, Itb. Pers. no. 899.213-537 (L).

2 Morphometric analysis of variation in *Beta* section *Beta*

2.1 Introduction

In the present chapter morphological similarities and differences between the taxa of *Beta* section *Beta* are discussed and the geographical variation of *Beta vulgaris* is analysed. Morphological variation in *Beta* is highly influenced by environmental conditions, therefore the simultaneous evaluation of living plants is required. Environmental conditions not only influence developmental parameters like earliness of flowering and annuality, but they may also affect morphology and size of leaves and flowers. Therefore, quantitative aspects of the variation were studied using plants grown under equal conditions.

2.2 Materials and methods

2.2.1 Materials and methods of plant cultivation

The plant accessions used in this study were obtained from gene banks (accessions originating from Turkey, Greece, Iran, Pakistan, India, Tunisia, Algeria, Spain, Portugal, France, Italy, Yugoslavia) and from collections made by the author in Portugal, southern Spain and Cyprus (Table 2.1). All accessions were analysed in the same field trial in 1989, except for accessions 7036, 7045, 7052, 7086, 7091, and 6963 which were analysed in a field trial one year later.

Some of the accessions received as 'wild' *B. vulgaris* proved to contain plants with characteristics of cultivated beets. This could be deduced from a number of parameters e.g. intense pigmentation of the leaves (accessions 5236, 5250, 5398, 3412) and sometimes thickened hypocotyl. Large leaved, erect plants, sometimes with broad petioles were found mixed with wild plants in accessions 3359, 3372 and 3378. Accessions containing cultivated beets were excluded from multivariate analysis. Morphological heterogeneous accessions were encountered in the analysis. Subsets of the heterogeneous accessions 1502 (Leros, Greece), 1180 (Izmir, Turkey), and accessions 463 and 473 from Cyprus were treated as separate units in the analysis.

After sowing, plants were temporarily grown in the greenhouse where temperature was controlled between 15 and 20 °C. The accessions were sown in the first week of April, and stayed approximately one month in the greenhouse. In the first week of May the plants were transplanted to an experimental field in Wageningen. Plants were arranged in three fully randomised blocks with 8 – 10 plants per accession per block. A randomised block design was used to minimise the effect of environmental gradients within the experimental field.

Table 2.1. Characters and character states used in the study.

1. Growth habit and branching: plant erect, main stem prominent, branching below and above, lower branches long, erect or procumbent (1) plant erect, main stem not prominent, branches long exceeding central stem (2) plant spreading and prostrate, main stem and branches prostrate (3) plant prostrate, main stem short and erect, branches prostrate (4).
2. Leaf pubescence: absent (1) moderate (2) strong (3).
3. Pigmentation: present in the petioles, leaves and glomerules (1) present as perpendicular stripes on the leaves (2) absent (3).
4. Leafiness of inflorescence: leaves few and little reduced (1) leaves many and much reduced (2) leaves few and much reduced (3).
5. Glomerule spacing: widely spaced (1) crowded together (2).
6. Degree of tepal keeling: little (1) moderate (2) strong (3).
7. Tepals: with appendages (1) smooth (2).
8. Tepal position in fruit: connivent, contiguous (1) connivent, not contiguous (2) connivent and patent (3).
9. Operculum: smooth, flat (1) smooth, convex, thickened (2) center depressed, margins raised (3).
10. Number of nodes: measured from the first leaf pair to first glomerulus on main axis.
11. Number of flowers per glomerule: measured as the number of flowers in the first 20 proximal glomerules of the first branche.
12. Tepal length (0.1 mm).
13. Tepal width (0.1 mm)
14. Ratio tepal length / tepal width.
15. Flower diameter.
16. Fresh weight: above ground fresh weight.
17. Time of bolting: measured as the length of the bolting stem 36 days after germination.
18. Flowering start: number of days from germination to first flowering.
19. Flowering end: number of days from germination to last flowering

2.2.2 Methods of numerical analysis

Variation within accessions was considered by univariate statistics (mean, variance, standard deviation, T test). Multivariate numerical analysis was performed using Cluster Analysis and Principal Component Analysis (PCA). SPSS/PC 4.0 statistical package including subroutines Cluster Analysis and Factor Analysis was used for all computations. The OTU (operational taxonomic unit) was an accession that consisted of 8 to 24 plants from which the average value of 19 characters (Table 2.1) was determined. Passport data of the accessions used for numerical analysis are given in Table 2.2.

Character standardisation

The 19 by 79 datamatrix was standardised for a number of quantitative characters, viz. characters 10 to 19 (Table 2.1). Characters were standardised using the method of Blackburn (1978). The data were ranked and divided into seven equal sized states. The state containing the lowest value was ranked 1, the state containing the highest value was ranked 7. This method of standardization is not greatly affected by outlying values, which is the problem with the usual method of standardization to zero mean and unit variance (Hill 1980).

Data matrices

Two data matrices were used in this study: (1) A matrix consisting of 79 OTUs

representing accessions of *B. vulgaris* s.l., *B. macrocarpa* and *B. patula*. (2) A matrix from which the accessions of *B. macrocarpa* and *B. patula* were omitted.

Cluster analysis

Squared Euclidian Distance was computed as the measure of similarity between OTUs. The resulting distance matrix was subjected to average linkage (UPGMA) agglomerative clustering (Sneath & Sokal 1973).

Principal Component Analysis (PCA)

PCA was performed on the character x character data matrix. The initial factor matrix was rotated (varimax rotation) to achieve easier interpretation of factors and variables. The first three eigenvectors extracted from this matrix were used to obtain projections of the OTUs onto the principal axes. The results of this analysis are presented in the form of two-dimensional graphs showing the relative positions of the OTUs in component space.

2.3 Results

2.3.1 Variation of morphological parameters

Growth habit

Growth habit in section *Beta* is determined by a number of factors: presence or absence of a main shoot, mode of branching and earliness of bolting. Plants from the north Atlantic coasts produce a large flat rosette with thick glaucous, long petiolate leaves. At the time of bolting lateral inflorescence stems, prostrate or slightly ascending, grow out. Secundary rosettes may develop on these stems. In populations from southern latitudes erect plants, in which bolting and flowering is initiated from a central main stem, are more frequent. Early bolting plants develop a small rosette which soon withers. Inflorescence stems may be developed as several equivalent shoots with an erect, decumbent or prostrate growth habit, or as an erect main stem with several decumbent off shoots.

Leaf morphology

It was noted that there was considerable inter- and intra-population variation in parameters of leaf size, (lamina length and width), leaf thickness, petiole length and petiole width. For the purpose of describing species or infraspecific groups quantification of leaf and bract characters is not very useful. Leaf morphology and other vegetative characters are strongly determined by the life cycle stage at the time of measurement. Because of the wide variation in time needed for generative development, both within and between populations as well as between different taxa, measurements of mature leaves are difficult to standardise. The position of a particular leaf is highly variable between plants. Thus, a standard leaf, for instance a leaf of the sixth leaf pair, can be a rosette leaf when the plant is still in the vegetative stage at the time of measurement. It can be a leaf on the inflorescence stem when the plant is an early bolter. It is also possible that the plant has started to flower before the sixth leaf pair has

Table 2.2. Geographic origin of the accessions included in the study and taxonomic identification.

IDBB	country	district	location	longi-	lati-	altitude	taxonomic label
463	Cyprus	Larnaka	Ayos Theodoros	03325E	03445N	0	*B. vulgaris*
470	Cyprus	Famagusta	Paralimni	03403E	03501N	6	*B. vulgaris* subsp. *maritima*
473	Cyprus	Famagusta	Ayia Thekla	03356E	03458N	2	*B. vulgaris*
497	Cyprus	Limassol	Evdhimou bay	03247E	03439N	2	*B. vulgaris* subsp. *adanensis*
1180	Turkey	Izmir	Altinova, 50 km N of Bergama	02647E	03912N	1	*B. vulgaris* subsp. *maritima*
1185	Turkey	Izmit	Karamursel, 2km E of	02937E	04042N	5	*B. vulgaris* subsp. *maritima*
1328	(India?)		(received as: *B. orientalis*)				*B. vulgaris* subsp. *maritima*
1483	Greece	Rhodos	Kalavarda, 5km s of	02815E	03621N		*B. vulgaris* subsp. *adanensis*
1488	Greece	Rhodos	Kritinia			6	*B. vulgaris* subsp. *adanensis*
1494	Greece	Kos	Kos, 2km sw of	02718E	03652N	2	*B. vulgaris* subsp. *adanensis*
1497	Greece	Kos	Kos, 5km e of	02720E	03653N	2	*B. vulgaris* subsp. *adanensis*
1502	Greece	Leros	Plefouti, outskirts			2	*B. vulgaris* subsp. *adanensis*
1513	Greece	Samos	Potami, outskirts			1	*B. vulgaris* subsp. *adanensis*
1520	Greece	Samos	Iraon village, 2km e of			1	*B. vulgaris* subsp. *adanensis*
1536	Greece	Chios	Giossonas village			1	*B. vulgaris* subsp. *adanensis*
1555	Greece	Lemnos	Agios Karalamlis			20	*B. vulgaris* subsp. *maritima*
1567	Spain	Murcia	Lorca, outskirts	00141W	03740N	0	
			(received as *B. atriplicifolia*)				*B. vulgaris* subsp. *maritima*
1570	Usa	California	Imperial Valley			0	*B. macrocarpa*
1571	Spain	Tenerife	Buena Vista	01654W	02819N	0	*B. macrocarpa*
1662	Spain	Tenerife	Los Silos	01644W	02819N	10	*B. macrocarpa*
1668	Spain	Tenerife	San Andres	01613W	02832N	25	*B. macrocarpa*
2193	Greece	Peloponnese	Scarpeti	02100E	03732N	0	*B. vulgaris* subsp. *maritima*
2199	Greece	Rhodos	Kalavarda	02816E	03621N	2	*B. vulgaris* subsp. *adanensis*
2673	Greece	Peloponnese	Kaminia	01913E	03808N	0	*B. vulgaris* subsp. *maritima*
3101	Greece	Rhodos	Fanes, Delphini restaurant	02815E	03621N	1	*B. vulgaris* subsp. *adanensis*
3183	Spain	Tenerife		01611W	02830N		*B. macrocarpa*
3193	Tunisia			01041E	03654N		*B. macrocarpa*
3194	Tunisia						*B. macrocarpa*
3195	Algeria						*B. macrocarpa*
3196	Turkey						unidentified
3283	Greece	Peloponnese	Paralia Kallonis	02057E	03732N	0	*B. vulgaris* subsp. *maritima*
3284	Greece	Peloponnese	Ermioni,3km E of	02056E	03724N	0	*B. vulgaris* subsp. *maritima*
3290	Greece	Peloponnese	Karavostasi	02253E	03642N	0	*B. vulgaris*
3294	Greece	Peloponnese	Skala,6km SW of	02241E	03648N	2	*B. vulgaris* subsp. *maritima*
3296	Greece	Peloponnese	Agios Nikolaos	02218E	03659N	2	*B. vulgaris* subsp. *maritima*
3300	Greece	Peloponnese	Pilos, SW of	02021E	03655N	10	*B. vulgaris* subsp. *maritima*
3304	Greece	Peloponnese	Tsoukaleika	02140E	03808N	0	*B. vulgaris* subsp. *maritima*
3314	Greece	Peloponnese	Agios Vasilios, E of	01929E	03819N	0	*B. vulgaris* subsp. *maritima*
3331	Greece	Leros	Plefouti	02649E	03711N	2	*B. vulgaris* subsp. *maritima*
3353	Greece	Lesbos	Mitilini, Agios Georgios	02347E	03911N	6	*B. vulgaris* subsp. *maritima*

IDBB	country	district	location	longi-	lati-	altitude	taxonomic label
3370	Greece	Zakinthos	Kipi Zakinthos	02054E	03747N		*B. vulgaris* subsp. *maritima*
3371	Greece	Cephalonia	Katelios beach	02039E	03815N	3	*B. vulgaris* subsp. *maritima*
3373	Greece	Levkas	Nikitas beach	02042E	03850N	2	*B. vulgaris* subsp. *maritima*
3374	Greece	Aitoloakarn	Astakos	02104E	03832N	4	*B. vulgaris* subsp. *maritima*
4665	Spain	Gran Canaria	Aracas	01532W	02808N		*B. macrocarpa*
5165	India						*B. vulgaris* subsp. *maritima*
5236	India						*B. vulgaris* subsp. *maritima*
5353	India						*B. vulgaris* subsp. *maritima*
5383	Pakistan						*B. vulgaris* subsp. *maritima*
5399	Iran						*B. vulgaris* subsp. *maritima*
5429	Pakistan						*B. vulgaris* subsp. *maritima*
5432	Pakistan						*B. vulgaris* subsp. *maritima*
5442	India						*B. vulgaris* subsp. *maritima*
5641	Italy	Po delta		01200E	04500N		*B. vulgaris* subsp. *maritima*
5642	Italy	Po delta		01200E	04500N		*B. vulgaris* subsp. *maritima*
5701	Italy	Sardinia	Alghero, 1km S of Sassari	00819E	04035N	20	*B. vulgaris* subsp. *maritima*
5760	France	Corsica	Ajaccio, on beach	00838E	04153N	1	*B. vulgaris* subsp. *maritima*
5951	Turkey	Adana	9km SW of Karatas, to Fevziye	03510E	03700N	20	*B. vulgaris* subsp. *adanensis*
5952	Turkey	Hayay	Iskenderun, 7km west of	03600E	03637N	0	*B. vulgaris* subsp. *adanensis*
6203	Turkey	Izmir	Aliaga	02659E	03849N		*B. vulgaris* subsp. *maritima*
6206	France	Marseille		00505E	04332N		*B. vulgaris* subsp. *maritima*
6300	France	Bouche du rhone	Logis-Neuf. d'Allauch	00050E	04320N		*B. vulgaris* subsp. *maritima*
6315	Italy		Lago di Varano, southern shore				*B. vulgaris* subsp. *maritima*
6370	Yugoslavia	Istria	Secovce	01352E	04452N		*B. vulgaris* subsp. *maritima*
6371	Greece	Aegean isls	Karpathos	02711E	03531N		*B. macrocarpa*
6954	Italy	Calabria	Punto Alice, 1km N of	01328E	03934N	3	*B. vulgaris* subsp. *maritima*
6956	Spain	Murcia	Santa Pola (received as *B. atriplicifolia*)	00032W	03812N		*B. vulgaris* subsp. *maritima*
6957	Spain	Murcia	Santa Pola, Alhama near Murcia (received as *B. atriplicifolia*)				*B. vulgaris* subsp. *maritima*
6963	Portugal	Madeira	(received as *B. patula*)				*B. patula*
7036	Spain	Murcia	La Hoya	00136W	03743N	270	*B. vulgaris* subsp. *maritima*
7045	Spain	Almeria	Agua Amarga, N332 to village	00159W	03657N	150	*B. macrocarpa*
7052	Spain	Almeria	Albox, east of Huercal Overa	00208W	03723N	300	*B. macrocarpa*
7067	Portugal	Mafra	Ericeira bay	00925W	03957N	10	*B. vulgaris* subsp. *maritima*
7078	Portugal	Aljezur	Carrapateira beach	00854W	03709N	5	*B. vulgaris* subsp. *maritima*
7085	Portugal	Olhao	Fuseta, salt winning area	00746W	03704N	3	*B. macrocarpa*
7086	Portugal	Tavira	Tavira, saline	00737W	03707N	5	*B. macrocarpa*
7089	Spain	Puerto Real	Puerto Real to San Fernando	00610W	03630N	1	*B. vulgaris* subsp. *maritima*
7091	Spain	San Fernando	Chiclana, saline	00609W	03627N	2	*B. macrocarpa*
7119	Cyprus	Paphos	Paphos camping, crop field	03223E	03445N	10	*B. vulgaris* subsp. *adanensis*
7127	Cyprus	Limassol	Ziyyi	03321E	03443N	10	*B. macrocarpa*

developed, as is the case in early flowering taxa. Leaf morphology is extremely variable from the base to the apex of the plants. Rosette leaves are morphologically different (viz. cordate with long petioles) from the leaves on the bolting stem (rhombic or obovate and with shorter petioles). Leaves from the rosette are frequently shed when bolting starts.

Bracts show extreme variation with respect to size and shape. In early flowering Mediterranean taxa bracts are large. In *B. vulgaris* subsp. *maritima* both shape and size are variable. Variation ranges from plants which are almost ebracteate to plants with relatively large bracts in the distal part of the inflorescence (the latter sometimes taxonomically assigned to *B. vulgaris* subsp. *maritima* var. *foliosa* (Ehrenb.) Aellen.

Pigmentation

With respect to pigmentation great variability is seen. Individual plants range from purely green to moderately red, sometimes pigmentation is orange. The shoots of most plants have red vertical stripes. The red may extend to the petioles and the laminas. Characteristically the glomerules are red tinged at their base in some accessions of *B. macrocarpa* and *B. vulgaris* subsp. *adanensis*.

Leaf pubescence

The presence of leaf hairs is a phenomenon with diffuse occurrence in section *Beta*. In some Greek accessions (notably the accessions 2193, 3283, 3284, 3300 (Peloponnesus), 1555 (Lemnos) and accession 470 (Cyprus)) the phenomenon of leaf pubescence was particularly remarkable. The hairs are present on both sides of the leaf as well as on the petioles. Often leaf pubescence was accompanied by a special pattern of pigmentation on the leaf (red stripes perpendicular to the veins). It is particulary present on the lower leaves before bolting. Leaves on the inflorescence have less pubescence, or they are glabrous.

Plants from the west Mediterranean are usually glabrous. Accessions from France (e.g. accession 6300) were slightly hairy and accessions from south east Spain (accessions 1567, 6956 and 6957 labeled as *B. atriplicifolia*) exhibited leaves with dense pubescence.

Number and size of flowers

Taxonomically useful characters are found in number, size and shape of the flowers. Hermaphrodite flowers of all species of section *Beta* are united in clusters (glomerules) in the axils of leafy bracts. These glomerules contain a variable number of flowers. The number of flowers per glomerule is largest in the proximal part of the inflorescence stem of the plant and decreases to three, two or single flowers in the distal part of the inflorescence stem. When more flowers are united in a glomerule, variation in flower size is seen. In a cluster of three or more flowers one of them is usually better developed and can be twice as big as the other flowers. In all cases the largest flower was taken for measurements.

Morphology of flower and fruit

During flowering the 5-parted perianth is green and patent. The tepals are shaped triangular, spathulate or thin lanceolate. The tepals can either be thin and flexible with hyaline margins or succulent and rigid, more or less hooded. A narrow keel is formed by the main nerve on the back of the tepals. When the fruit is maturing, the tepals expand and swell, increasing in size and, to a varying degree, bend to cover the stigmatic lobes. The base of the perianth becomes wider, sometimes with spongy appendages. After seed maturing the vestiges of the perianth form a corky pericarp firmly attached to the seed. The seed is covered by a lid (operculum) which may be lifted off at the time of germination. The shape of the lid is of taxonomic importance. Generally it is more or less convex with three radial ridges corresponding to the stigmatic lobes. In large flowers it is seen that the lid is surrounded by a marginal groove.

2.3.2 Distinction of B. vulgaris, B. macrocarpa and B. patula

Cluster analysis revealed a total of four gross morphological groups, the phenogram is shown in Fig. 2.1. Two separate clusters (cluster A and Cluster D, cf. Fig. 2.1) were formed by OTUs a priori indicated to belong to *B. vulgaris*. OTUs a priori considered to belong to *B. macrocarpa* clustered together in cluster C. *B. patula* clustered separately from *B. vulgaris* and *B. macrocarpa* (cluster B). OTUs of *B. vulgaris* cluster D were in fact more similar to OTUs in cluster C, with OTUs a priori designated as *B. macrocarpa*, than to OTUs in cluster A. One OTU in cluster D was distinct from all others: it was recognised as accession 5952 from the region of Iskenderun in Turkey.

In cluster C 16 OTUs were combined. Four of these clustered separately, and they could be identified as the tetraploid cytotype of this taxon. In cluster C one OTU was placed which could not be identified beforehand (accession 3196 from Turkey). Accession 473 from Cyprus was a priori identified as *B. vulgaris*, but it was placed in cluster C. Three accessions labeled beforehand as *B. macrocarpa* were not placed in cluster C, but they were clustered in one of the two *B. vulgaris* clusters. They represented accession 7085 from Portugal, which clustered in cluster A, and, clustering in cluster D, accession 7119 from Cyprus and accession 3183 from Tenerife.

Principal component analysis was carried out to compare *B. vulgaris* with *B. patula* and *B. macrocarpa* (PCA 1). The first five factors have individual eigenvalues greater than 1.00, indicating that the variables are not too highly correlated. The first three factors account for 59.2% of the variance. High factor loadings (f) for factor 1 (33.6% of variation) are primarily rate of generative development flowering start ($f=0.71$) and flowering end ($f=0.87$), glomerule spacing ($f=0.84$) and glomerule position on the inflorescence ($f=0.78$), fresh weight ($f=0.73$) and shape of the operculum ($f=-0.70$). Time of bolting ($f=0.83$) contributed to factor 2. High loadings for factor 3 were from tepal length ($f=0.88$), ratio tepal width/tepal length ($f=0.86$), and degree of tepal keeling ($f=0.63$).

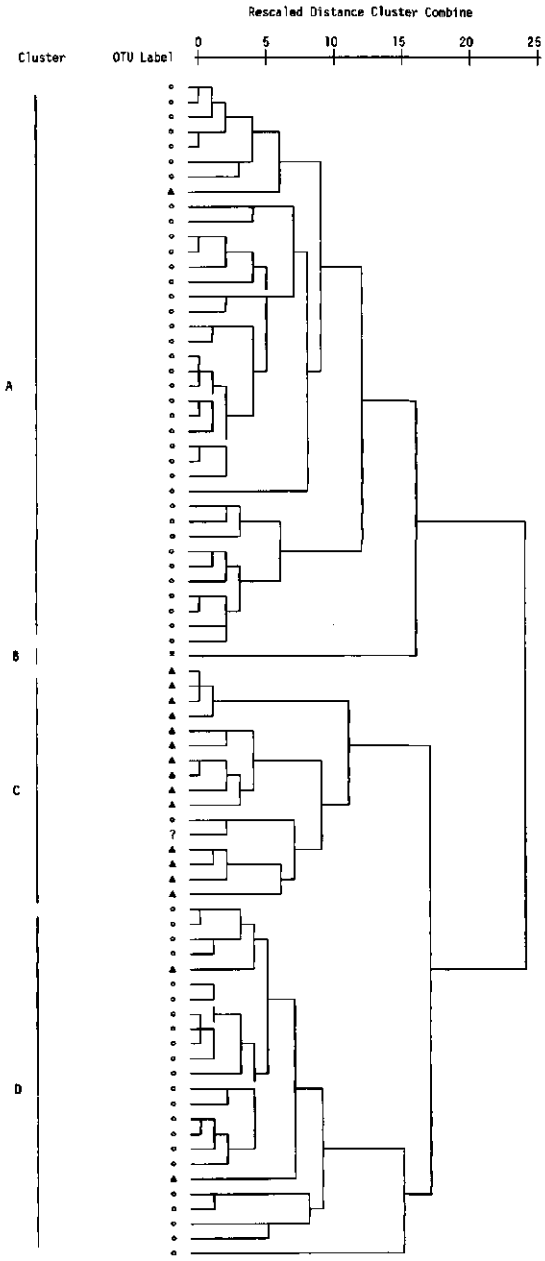


Figure 2.1 Correlation phenogram (UPGMA) composed of 79 OTUs belonging to *B. vulgaris* s.l., *B. macrocarpa* and *B. patula*.
Labels indicate a priori identification.
OTUs were given labels as follows:

- o *B. vulgaris* s.l.
- * *B. patula*
- ▲ *B. macrocarpa*
- ? OTU not identified beforehand

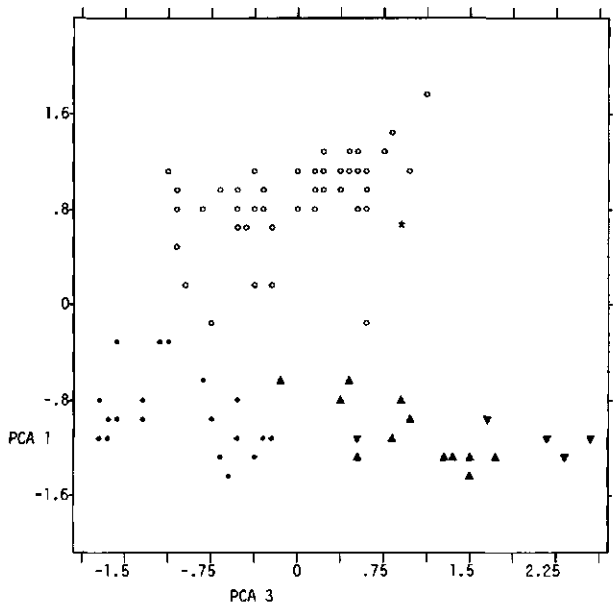


Figure 2.2 PCA I. Projections of OTUs onto the first principal component (ordinate) and the third principal component (absciss).
Groups are coded as follows:
○ *B. vulgaris* (OTUs in Cluster A)
● *B. vulgaris* (OTUs in Cluster D)
* *B. patula*
▲ *B. macrocarpa* diploid cytotype
▼ *B. macrocarpa* tetraploid cytotype

The projections of OTUs onto the principal axes reveal trends in the data that are complementary to the results of the cluster analysis. Because the third component gave a better separation of OTUs than the second component, in Fig. 2.2 the first and third principal component are plotted with projections of OTU scores. In principal, the positioning of OTUs in component space corresponded with the clusters defined from the cluster analysis. The distinctiveness of *B. macrocarpa* in component space is evident. *B. patula* however is not very well separated from *B. vulgaris* cluster A OTUs. The results further show the distinctiveness of *B. vulgaris* cluster A OTUs from *B. vulgaris* cluster D OTUs, the groups are separated primarily on the first component axis. At the same time, the relative closeness of *B. vulgaris* cluster D OTUs with *B. macrocarpa* OTUs is apparent. Distinction between the latter groups is realised only at the third component axis.

Diagnostic features for *B. macrocarpa* can be found in the morphology of the operculum. In all diploid *B. macrocarpa* the operculum is characteristic on account of the fact that the margins of the operculum are exserted towards maturity of the fruit, causing a depression of the centre of the operculum. In contrast

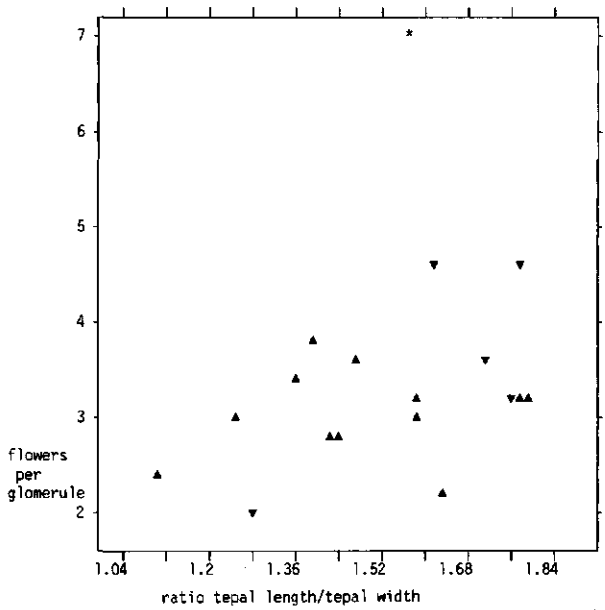


Figure 2.3 Plot of number of flowers per glomerule with tepal length/width ratio.
accessions are labeled as follows:

* *B. patula*

▲ *B. macrocarpa* diploid cytotype

▼ *B. macrocarpa* tetraploid cytotype

Symbols represent accession means. Standard deviations around each mean are omitted for reasons of clarity.

to the raised operculum margins is the deep groove around it. This contrast between lid margins and groove, which is best visible in the larger fruits, has diagnostic value for this species. Additionally, in some plants the operculum becomes remarkably red at the time of fructification. In mature seeds the operculum remains visible as a corky ring covering the seed. This feature is less pronounced in the tetraploid accessions. In tetraploid accessions from the Canary Islands the lid is flat or just a little convex, no marginal exsertion of the lid is developed.

Flowers of *B. macrocarpa* are sometimes larger than those of *B. vulgaris*, but differences are not significant at the 0.05 level. There is variation in length / width ratio of tepals and number of flowers per glomerule in *B. macrocarpa* (Fig. 2.3). Maximum tepal length is found in tetraploid *B. macrocarpa* accessions from the Canary Islands. In the diploid accessions however, tepals are not significantly longer than in *B. vulgaris*. The tepals are spongy, usually triangular from a broad base, sometimes narrowly oblong and strongly keeled. Towards maturity of the fruit the tepals generally stay free and patent, contrasted to *B. vulgaris* subsp. *maritima* in which the tepals become contiguous after fructification. However, in some accessions of *B. macrocarpa* the perianth is connivent and the tepals are contiguous above the operculum. This is seen in all the tetraploid accessions of *B. macrocarpa* from the Canary Islands and occasionally in diploid

accessions. *B. macrocarpa* produces a small rosette, and flowering starts early. Glomerules are present on the lower parts of the stem, starting from the fourth node. In *B. macrocarpa* bracts are usually glabrous and ovate. The bracts are large in the proximal part, gradually diminishing in size in the distal part of the inflorescence.

B. patula is a rather small, elegant plant, 15 – 60 cm in length. The stems and branches are decumbent, green, occasionally striate-red. *B. patula* is morphological distinct by its lanceolate shaped leaves and linear, somewhat curly bracts. The lower bracts are large, up to 10 times the diameter of the glomerule. The glomerules are widely spaced on the inflorescence. Glomerules contain on average seven flowers, with a maximum of twelve flowers. The number of flowers per glomerule distinguishes *B. patula* from all other taxa (Fig. 2.3). The flowers are very variable in respect to size. The tepals are green, thin and strongly hooded. At maturity the tepals cover the operculum. The operculum is thin and concave.

2.3.3 Infraspecific groups in B. vulgaris

Both PCA 1 and cluster analysis 1 suggested additional grouping of OTUs which were a priori designated as accessions belonging to *B. vulgaris*. OTUs identified as *B. macrocarpa* and *B. patula* were omitted from the matrix, to allow a better scattering of the remaining OTUs. The cluster procedure and PCA procedure was repeated. The results of the cluster analysis are shown in Fig. 2.4. Two large clusters 1 and 2 are distinguished. These two groups already emerged in the first cluster analysis with the initial OTU matrix as cluster A and D, respectively.

Cluster 1 is subdivided in a cluster 1a with OTUs identified as *B. vulgaris* subsp. *maritima*. The accessions originate from middle and east Mediterranean countries: Spain, France, Italy, Yugoslavia, Greece and Turkey. The three accessions representing *B. atriplicifolia* are included in this cluster. Cluster 1b contains OTUs which originate primarily from oriental countries such as India, Pakistan and Iran. However, accessions from Turkey, Cyprus and France are included in this cluster as well. Cluster 2 has accessions from Cyprus, Greece, the Aegean Islands Kos, Rhodos, Samos, Chios, Leros, and from the Turkish west and south coast. In cluster 2 the subspecies *adanensis* is identified. As was the case in cluster analysis I the accession 5952 (Iskenderun, Turkey) is somewhat deviant from the rest and makes a separate cluster 2b. Subvariants of the heterogeneous accessions 1180 from Izmir, Turkey and accessions 473 from Cyprus are divided over the clusters 1 and 2. Subvariants of the heterogeneous accessions 1502 from Leros, Greece and 473 from Cyprus all clustered in cluster 2.

A principal component analysis on the reduced datamatrix was performed. In PCA 2 the first five factors have individual eigenvalues greater than 1.00. The first three factors account for 69.8% of the variance. High loadings for factor 1 (40.9 % of the variance) are primarily ratio tepal length / tepal width (f = 0.88), fresh weight (f = 0.82), rate of generative development (f = 0.80, f = 0.72), tepal

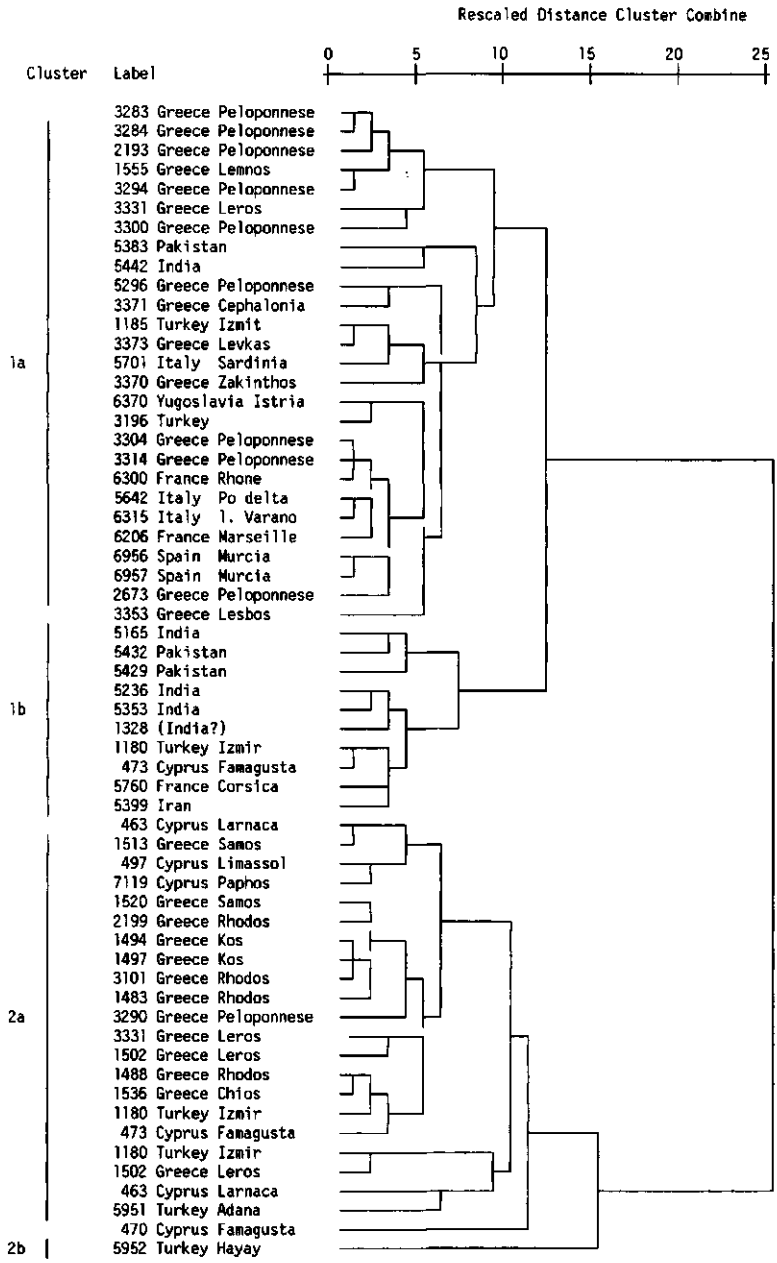


Figure 2.4 Correlation phenogram (UPGMA) composed of 60 OTUs belonging to *B. vulgaris* s.l. The labels indicate IDBB number, origin country, and district.

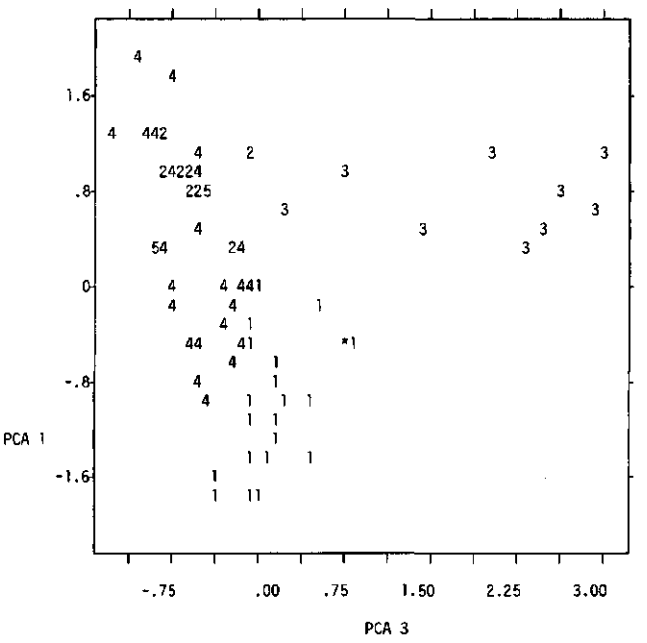


Figure 2.5 PCA 2. Projections of OTUs onto the first principal component (ordinate) and the third principal component (absciss).

Groups are coded as follows:
1 *B. vulgaris* subsp. *adanensis*
2 *B. vulgaris* subsp. *maritima* middle Mediterranean accessions
3 *B. vulgaris* subsp. *maritima* oriental accessions
4 *B. vulgaris* subsp. *maritima* east Mediterranean accessions
5 OTUs received as *B. atriplicifolia*
* type locality accession of *B. vulgaris* subsp. *adanensis*.

length (f=0.73), and leafiness of the inflorescence (f=0.77). Flower diameter (f=-0.85) and leaf pubescence (f=0.56) contributed much to factor 2. High loadings for factor 3 were from tepal shape (f=0.79), bolting rate (f=0.74), number of flowers per glomerule (f=-0.50) and tepal position in fruit (f=0.79).

In Fig. 2.5 the first and third principal components of PCA 2 are plotted with projections of OTU scores on the component axes. The first principal component separates the two subspecies *B. vulgaris* subsp. *maritima* and *B. vulgaris* subsp. *adanensis* (cf. Fig. 2.2; the first component of PCA I separates *B. vulgaris* cluster A representing largely *B. vulgaris* subsp. *maritima* from *B. macrocarpa* and *B. vulgaris* cluster D, representing *B. vulgaris* subsp. *adanensis*). In Fig. 2.5 the accession which represents the type locality of this taxon (accession 5951 Adana, Turkey) is indicated.

B. vulgaris subsp. *adanensis* is diverged from *B. vulgaris* subsp. *maritima* in

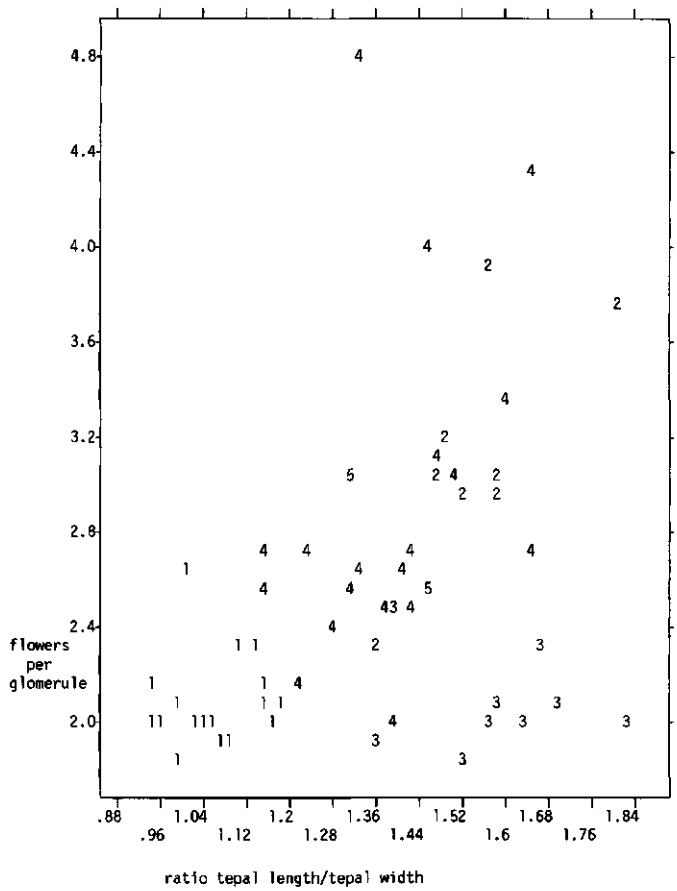


Figure 2.6 Plot of number of flowers per glomerule with tepal length/width ratio.

Groups are coded as follows:
1 *B. vulgaris* subsp. *adanensis*
2 *B. vulgaris* subsp. *maritima* middle Mediterranean accessions
3 *B. vulgaris* subsp. *maritima* oriental accessions
4 *B. vulgaris* subsp. *maritima* east Mediterranean accessions
5 OTUs received as *B. atriplicifolia*
Numbers represent population means. Standard deviations about each mean are omitted for reasons of clarity.

a number of traits. The subspecies is characterised by a very short vegetative period. The glomerules and bracts are more spaced on the inflorescence than in *B. vulgaris* subsp. *maritima*. In *B. vulgaris* subsp. *adanensis* bracts are wedge-shaped, ovate or obovate and characteristically succulent and fleshy. Bracts are relatively large (seven to eight times the size of the glomerule) in the proximal part of the inflorescence. In the distal part the bracts gradually diminish in size,

almost indistinguishable from the tepals. Tepals of *B. vulgaris* subsp. *adanensis* are spongy and short, generally as broad as they are long. Most typical for *Beta vulgaris* subsp. *adanensis* is the shape of the perianth after fructification. In fruit the tips of the short tepals slightly bend, but remain free, they do not touch as in *Beta vulgaris* subsp. *maritima*.

B. vulgaris subsp. *adanensis* lacks the depressed operculum characteristic for *B. macrocarpa*. Instead the operculum is smooth, usually green, and convex. In large fruits the tissue of the operculum is thickened, sometimes exserted above the tepals, and it is surrounded by a deep groove. In *B. vulgaris* subsp. *adanensis* the number of flowers per glomerule is highly constant – the mean number is two – and significantly different from the number of flowers per glomerule in *B. macrocarpa* and Mediterranean *B. vulgaris* subsp. *maritima* (Fig. 2.3 and Fig. 2.6). Plant habit is characteristic because the main inflorescence stem is erect, and the lateral stems are prostrate.

Both cluster analysis and PCA separate a group of OTUs referable to as *B. vulgaris* subsp. *maritima* oriental accessions – accessions from India, Pakistan and Iran. In Fig. 2.5 this geographical group is distinguished on the third principal component axis. Within the Mediterranean gene pool no geographical variation could be demonstrated. OTUs originating from the west and middle Mediterranean area (Spain, France, Italy, Yugoslavia) overlap with OTUs originating from the east Mediterranean countries Greece, Turkey and Cyprus. Oriental *B. vulgaris* subsp. *maritima* is differentiated from Mediterranean *B. vulgaris* subsp. *maritima* with regard to growth habit (plants are more erect), and with regard to rate of bolting and flowering. Quantitative differences in number of flowers per glomerule and characteristics of the tepals further demonstrate the geographic variation (Fig. 2.6). On average plants have three flowers per glomerule. Accessions from India, Pakistan and Iran (and *B. vulgaris* subsp. *adanensis*) have significantly less flowers per glomerule. In addition, flower tepals of oriental plants are more narrow compared to Mediterranean plants.

2.4 Discussion

For a description of morphological variation individual accessions were used as OTUs. Initially, some (qualitative) characters were found useful for taxonomic description of taxa. Thus, a tentative identification of each accession was performed during the course of the experiment, which could later be tested through multivariate analysis. With the help of cluster analysis an objective grouping of accessions was made drawing attention to relatively stable units which exhibited a combination of similar morphological characteristics. With the help of principal component analysis a better picture was achieved of the true distances between OTUs and between clusters of OTUs than a cluster phenogram gives.

B. macrocarpa is a geographically variable taxon and the characters considered most diagnostic are difficult to see in some of the plants. Transitions between the extreme types of the 'operculum' character are frequent. Additional characters like tepal shape, the spacing of the glomerules and annuality (cf. Chapter 4) are needed to recognise east Mediterranean and the Canary Islands material. Buttler (1977[b]) identified two morphological types but he was not sure whether they represented taxonomic different groups. He classified material from the Canary Islands, Spain, Sicily, northern Africa, Greece and Jordan in a type 1 group with thin tepals. A form with spongy tepals from Attica and Karpathos (Greece) was believed to be different and was included in a type 2 group. Examining the herbarium material referred to by Buttler and additional material from the east Mediterranean region, it seems that this material has to be included in *B. macrocarpa*. The confusion partly arises by polymorphism of the taxon and possible intergradation with *B. vulgaris* s.l. in the east Mediterranean distribution of this species. Accession 7127 from Cyprus is polymorphic and consists of plants with patent, thin and long tepals as well as plants with connivent, short and fleshy tepals. Plants with characteristics intermediate between *B. macrocarpa* and *B. vulgaris* subsp. *adanensis* (viz. fewer flowers per glomerule, shorter and darker coloured tepals, operculum flat) were encountered in the field. However, when seeds of these intermediate plants were analysed both in the field trial and by allozyme analysis (Chapter 3) they could be attributed to either of the two species. Thus, it could not be demonstrated that hybridization or introgression were responsible for the morphological polymorphism of the accession from Cyprus. However, plants with hybrid and introgression characteristics (*B. macrocarpa* x *B. vulgaris*) were frequent in accession 3196, an accession from Turkey (cf. Chapter 3).

B. vulgaris subsp. *adanensis* has centers on the Aegean islands and the west and south coast of Turkey. It could be concluded from the data that populations of *B. vulgaris* subsp. *adanensis* and *B. vulgaris* subsp. *maritima* are frequently found close together, and that heterogeneous accessions from Cyprus, Leros and Izmir, Turkey, contained plants of either group. Plants in one accession (accession 3290 from the Peloponnesus) were transitional between *B. vulgaris* subsp. *adanensis* and *B. vulgaris* subsp. *maritima*. Primarily based on quantitative differences in number of flowers per glomerule and in perianth morphology accessions originating from India, Pakistan and Iran could be grouped together. These oriental accessions had fewer flowers per glomerule and more narrow tepals, but otherwise no discrete differences exist if compared with Mediterranean accessions. In the past *Beta* accessions from these regions have been treated taxonomically as *B. orientalis*, *B. bengalensis*, or *B. palonga* (Chapter 1). It is doubted whether it is useful to give taxonomic ranking to this group. It is very difficult to apply a reliable key character for identification of these plants. In the cluster analysis some Mediterranean accessions clustered into the group of oriental accessions. PCA showed the proximity of a few oriental accessions to *B. vulgaris* subsp. *maritima*.

In the literature *B. atriplicifolia* is described as an endemic species from the

area near Murcia, in south eastern Spain (Rouy 1883). The accessions analysed in the field trial that should represent this taxon had a patulous growth habit, a peculiar leaf shape (small lanceolate or rhombic) and moderate leaf pubescence. It is questioned whether these morphological traits justify taxonomic separation. They certainly do not qualify for recognition as a distinct species. Parameters of vegetative and generative development, glomerule size and morphology of flowers revealed no special characteristics separating this taxon from *B. vulgaris* subsp. *maritima*.

3 Patterns of allozyme differentiation in *Beta* section *Beta*

3.1 Introduction

Allozyme analysis using gel electrophoresis is an often applied tool in the evaluation of genetic relations between plants. Allozyme analysis has proved to be an accurate quantitative method for measuring the genetic variation in plant populations and for estimating the apportion of variation between populations (Brown 1978, Brown & Weir 1983, Nevo et al. 1984, Brown 1990). Additionally it has been applied to solve problems regarding the systematic relationships of plant species (Crawford 1983, Gottlieb 1977, 1981).

With the establishment of large collections of accessions in gene banks it has become more urgent to meet with criteria of efficient sampling and preserving. Brown (1989) and Marshall & Brown (1983) formulated that a collection should have a maximum amount of useful genetic variation within a strictly limited number of samples. To achieve this, it is important to analyse taxonomic relationships, to study the morphological characteristics and the environmental adaptibility of single species, to compare accessions from different collection sites for specific properties and to estimate distribution of genetic variation within and gene flow among populations.

In this chapter the result of an electrophoretic survey of taxa of *Beta* section *Beta* is reported. The objective was, firstly, to learn more about the distribution of allozymes in these taxa, secondly, to analyse the within population variation and, thirdly, to look for geographical patterns in the distribution of the variation.

The analysis of allozyme variation in the cultivated beet was started by Van Geyt & Smed (1984). Abe & Tsuda (1987) performed genetical analysis of five enzyme systems; Oleo et al. (1986) applied eight more systems to investigate inter-species hybrids. Abe & Shimamota (1989) reported on species relationships in the genus *Beta* on the basis of allozyme variation. Jung et al. (1986), Smed et al. (1989), Van Geyt et al. (1988) and Van Geyt et al. (1990[b]) extended the number of enzyme systems in their work on nematode resistance and alien additions involving wild *Beta* species. Nagamine et al. (1989) studied phenotypic variation of 13 enzyme systems in fodder beet, sugar beet and wild species. Wagner (1990) described linkage relationships of allozymes in the sugar beet. The enzyme systems used in present work conform with the systems of the previous authors.

3.2 Materials and methods

3.2.1 Plant material

The majority of the seed samples was received from various gene bank collections. Material from Portugal, Spain, Cyprus and The Netherlands was collected in the field during collection missions in cooperation with the Dutch gene bank CGN (Frese et al. 1990). In total 76 accessions have been screened for allozyme diversity; 59 accessions out of these have been used for calculating genetic variation measurements. In Appendix I the populations used in this study are presented along with locality data and IDBB collection numbers.

Morphological identification of the material preceded biochemical characterisation. Taxa in section *Beta* are morphologically separated from each other (Chapter 1 & 2). They are *B. vulgaris* L. subsp. *maritima* Arcang., *B. patula* Ait., *B. macrocarpa* Guss. (including both diploid and tetraploid representatives) and *B. vulgaris* subsp. *adanensis* (Pamuk.) Ford-Lloyd & Williams. The only available accession that should represent *B. atriplicifolia* Rouy was compared electrophoretically to the other taxa. The taxonomic status of this species is highly uncertain. For the purpose of allozyme evaluation this accession is referred to as *B. vulgaris* accession 6956. Cultivated material (*B. vulgaris* subsp. *vulgaris*) was represented by primitive Greek landraces of leaf beet (accession 3359 and 3372) and Italian leaf beet 'Costa Argentata' (no accession number).

3.2.2 Electrophoresis

From bulked seed 25 to 50 plants per population were grown in the greenhouse. The exact number of plants used per accession is mentioned in Appendix II. Fresh leaves of 6 weeks old plants were used for extraction of enzymes.

NAD Dependent Malate Dehydrogenase (MDH; E.C.1.1.1.37), Phosphoglucomutase (PGM; E.C.5.4.2.2), Shikimic Acid Dehydrogenase (SKDH; E.C. 1.1.1.25), Phosphoglucoisomerase (PGI; E.C.5.3.1.9), Isocitrate Dehydrogenase (ICD; E.C.1.1.1.42), cathodal Peroxidase (PX; E.C.1.11.1.7) and 6-Phosphogluconate Dehydrogenase (6-PGDH; E.C.1.1.1.44) were studied on horizontal starch gel electrophoresis following methods of Hendriksen & Jelnes (1980) and Van Geyt & Smed (1984) with minor alterations. Leaf samples were homogenized in 0.05 M Tris-HCl extraction buffer containing 0.14 M NaCl 0.02 M $NaNO_3$, 1% (w/v) DOWEX Cl^-, 0.10% (w/v) dithioerythrol and 0.1% (v/v) 2-mercapthoethanol. Extracts were centrifuged for 5 minutes at 10,000 rpm. Filter paper wicks were saturated with supernatant and loaded into the cut gel. The 12% starch gel was prepared with a 5 mM histidine monohydrochloride gel buffer (pH = 7) and sucrose added 1:1 with the starch. The electrode buffer was 0.2 M trinatrium-citrate.$2H_2O$ (pH = 7). Electrophoresis was carried out at constant 55 mA for 4 hours at 4 °C.

Leucine Aminopeptidase (LAP; E.C.3.4.11.1), Acid Phosphatase (ACP; E.C.3.1.3.2.) and Glutamate Oxaloacetate Transaminase (GOT; E.C.2.6.1.1.)

were resolved on a contineous vertical acrylamide system. The 6.25% acrylamide gels were made with a 0.06 M Tris-Boric acid gel buffer (pH = 7.8). The electrode buffer was identical to the gel buffer. The gel was run for 2 hours at a maximum of 800 volts (ca. 110 mA) at a constant temperature of 16 °C.

Procedures used for the visualization of all enzymes followed Vallejos (1983) with minor modifications. Gene and locus symbols are written in small letters in italics, sometimes followed by allozyme number (e.g. *Acp1*-1). Enzymatic activity which is not yet genetically identified is written in capitals (e.g. PGI etc.). All allozymes migrated anodally, except for PX which migrated to the cathode. The locus specifying the fastest migrating allozyme was designated as 1, the next 2, etc. At each locus allozymes were numbered 1,2,3, etc. with increasing migration from the origin. The migration distance was measured as the distance in millimeters from a reference band expressed by the monomorphic control accession 1570. Estimation of genetic diversity and genetic distances according to the formulae of Nei (1978) were performed based on 59 accessions for which the loci *Acp1*, *Lap1*, *Mdh1*, *Pgm1*, *Pgm2*, *Icd1*, *Icd2*, *Pgi2* and *Got3* were scored.

Genetic diversity is here defined as one minus the sum of squared allelic frequencies[2]:

$$h_e = 1 - \Sigma (p_i)^2$$

where p_i is the frequency of the i^{th} allele.

The mean genetic diversity per locus H_e is the sum of h_e over all loci divided by the total number of loci. the monomorphic locus *Icd2* was included in H_e. The observed heterozygosity (h_o) is the fraction of heterozygous individuals per locus. The mean observed heterozygosity H_o is the sum of h_o over all loci divided by the total number of loci. Nei's coefficient of genetic identity between two taxa (or populations) was given by:

$$I = \frac{\Sigma x_i y_i}{\sqrt{(\Sigma (x_i)^2 \, \Sigma (y_i)^2)}}$$

where x_i and y_i are the frequencies of the i^{th} allele in taxa x and y respectively. Genetic distance was calculated as $D = -\ln I$. The hypothesis of each population to be in Hardy-Weinberg equilibrium was tested. The Chi^2 goodness of fit test was performed on the variables H_o and H_e using every variable locus. The Fixation Index was calculated estimating the deficiency or the excess of observed heterozygotes. Biosys-1 computer programme package (Swofford & Selander 1989) and SPSS (Norusis 1990) were used for calculations.

[2] The quantity was introduced by Marshall & Allard (1970) as 'the polymorphic index', and is known also as the index of 'Gene diversity' (Nei 1973). It is the calculated heterozygosity based on allele frequencies in (Hardy-Weinberg) populations (Weir 1990).

3.3 Results

3.3.1 Analysis of gene loci and allozymes

Allozyme phenotypes are described, and the variation patterns are discussed in terms of allelic variation of loci. Allelic variation is discussed with reference to allozyme segregation data in the literature. In addition crosses of different species aided in genetic interpretation of zymogrammes. The allozyme genotypes of the parents and the F_1 are shown in Table 3.1. The cross of *B. macrocarpa* with *B. vulgaris* gave 17% species hybrids. *B. macrocarpa* expressed a strong tendancy for autogamy. The hybrids showed vigorous growth and normal seed fertility. A segregating F_2 population was variable with respect to growth habit and morphology of the leaves and seed balls. A small amount (4%) of chlorotic plants segregated which died in the cotyledon stage. The amount of pollen produced by the F_2 plants was comparable to the amount produced by *B. macrocarpa*.

Table 3.1 Survey of crossing experiments.

parents (receptor x pollinator) species, IDBB accession number, origin

B. macrocarpa 3193 Tunisia x *B. vulgaris* subsp. *vulgaris* (breeding strain CPRO-DLO)
B. patula 6963 Portugal x *B. vulgaris* subsp. *maritima* 6942 Greece

parents genotypes	F_1 genotypes (number of plants)		percentage of species hybrids
B. macrocarpa x *B. vulgaris* subsp. *vulgaris*			17%
Lap1 –11 × *Lap1* –44	*Lap1* –11 (109)	*Lap1* –14 (22)	
Acp1 –33 × *Acp1* –55	*Acp1* –33 (109)	*Acp1* –35 (22)	
Mdh1–11 × *Mdh1*–22	*Mdh1*–11 (89)	*Mdh1*–12 (20)	
Got3 –11 × *Got3* –22	*Got3* –11 (88)	*Got3* –12 (13)	
Icd1 –11 × *Icd1* –11	*Icd1* –11 (111)		
Pgi2 –11 × *Pg12* –11	*Pgi2* –11 (108)		
Pgm2–22 × *Pgm2*–22	*Pgm2*–22 (95)		
Px1 –11 × *Px1* –33	*Px1* –11 (15)	*Px1* –13 (3)	
B. patula x *B. vulgaris* subsp. maritima			84%
Lap1 –33 × *Lap1* –44	*Lap1* –44 (8)	*Lap1* 34 (41)	
Mdh1–22 × *Mdh1*–22	*Mdh1*–22 (49)		
Acp1 –22 × *Acp1* –66	*Acp1* –22 (5)	*Acp1* 26 (38)	
Acp1 –22 × *Acp1* –65	*Acp1* –22 (8)	*Acp1* 26 (15)	
		Acp1 –25 (15)	
Got3 –22 × *Got3* –22	*Got3* –22 (49)		
Icd1 –11 × *Icd1* –11	*Icd1* –11 (49)		
Pgi2 –11 × *Pgi2* –22	*Pgi2* –11 (5)	*Pgi2* –12 (44)	
Pgm2–22 × *Pgm2*–22	*Pgm2*–22 (45)		

The cross of *B. patula* with *B. vulgaris* subsp. *maritima* resulted in 84% hybrid plants. The hybrids were intermediate between the parents and perennated. A F_2 generation showed no segregation of chlorotic plants and normal pollen fertilities.

Acid Phosphatase (ACP)

ACP was expressed on a polyacrylamide gel system. In the upper zone of the anodal gel 8 to 12 bands are located at close distances from each other. The variation pattern of these bands is such that as a group little differences in migration rate were noted among genotypes. Interpretation of this row of bands in terms of enzyme coding loci was not possible. Abe & Tsuda (1987) referring to Endo (1981) have suggested that modification of the gene products takes place and that epigenetic events are responsible for the unconventional variation pattern.

In the lower part of the gel a polymorphic locus *Acp1* was studied. In crosses the locus showed monomeric segregation (Table 3.2a). *B. macrocarpa* (*Acp1*-33) was crossed with *B. vulgaris* subsp. *vulgaris* (*Acp1*-55). The genotype of the hybrids was *Acp1*-35. *B. patula* (*Acp1*-22) was crossed with *B. vulgaris* subsp. *maritima* (*Acp1*-55 and *Acp1*-66). The F_1 hybrids had *Acp1*-25 and *Acp1*-26 respectively.

Up to seven allelic forms were identified for representatives of section *Beta*. Electrophoretic determinations were made by comparing the distance of all bands relative to the reference band 3 (Table 3.2a). On few occasions (and only after long staining) a very weak additional band was seen a little below the allozymes of *Acp1*. As it did not interfere with *Acp1* allozyme expression it was assumed that this band belonged to another gene.

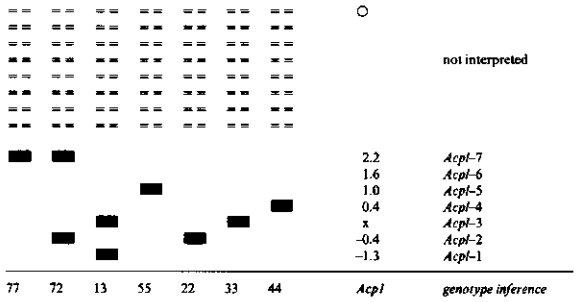
Leucine aminopeptidase (LAP)

Resolution of LAP allozymes was superior on acrylamide gels compared to starch gels. After resolution on polyacrylamide gels two LAP activity zones were encountered. The slower bands in the upper gel part gave weak expression. When expression was clear two bands were seen not showing any variation in migration rate. These bands were not analysed genetically. In the lower part of the gel a clear banding zone could be visualized. The reference band for LAP given by the control accession 1570 was the fastest migrating band. A total of five allozymes were noted for representatives of *Beta* section *Beta* (Table 3.2b), allozyme bands *Lap1*-3 and *Lap1*-5 being extremely rare. The different allozymes of this *Lap1* locus were expressed as monomers (Table 3.2b). Genotypes with one or with two bands were observed. *B. macrocarpa* (*Lap1*-11) crossed with *B. vulgaris* subsp. *vulgaris* (*Lap1*-44) gave F_1 hybrids *Lap1*-14. *B. patula* x *B. vulgaris* subsp. *maritima* hybrids showed *Lap1*-34.

NAD depending malate dehydrogenase (MDH)

Starch gels stained for MDH displayed three zones of enzyme activity. Very close to the origin bands were visible, sometimes very vague. The reference acces-

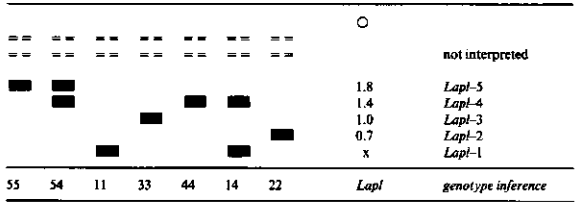
Table 3.2a Schematic representation of ACP isozyme patterns.



phenotype	distance	locus and allozyme
	○	
		not interpreted
	2.2	*Acpl–7*
	1.6	*Acpl–6*
	1.0	*Acpl–5*
	0.4	*Acpl–4*
	x	*Acpl–3*
	–0.4	*Acpl–2*
	–1.3	*Acpl–1*
77 72 13 55 22 33 44	*Acp1*	*genotype inference*

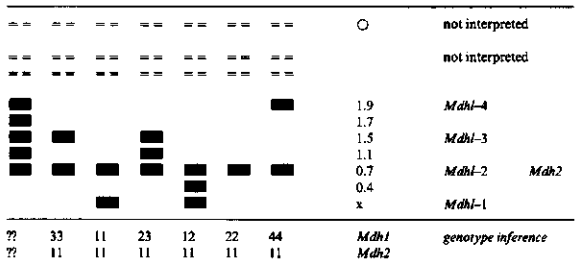
Note: reference band is indicated as x. Migration distance from x is given in cm. Gel origin indicated as ○.

Table 3.2b Schematic representation of LAP isozyme patterns.



phenotype	distance	locus and allozyme
	○	
		not interpreted
	1.8	*Lap1–5*
	1.4	*Lap1–4*
	1.0	*Lap1–3*
	0.7	*Lap1–2*
	x	*Lap1–1*
55 54 11 33 44 14 22	*Lap1*	*genotype inference*

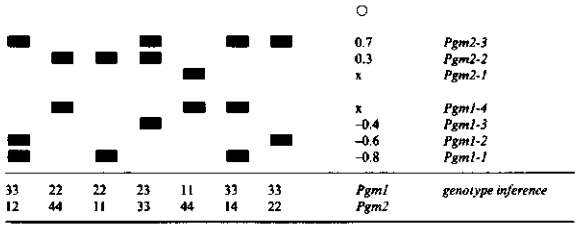
Table 3.2c Schematic representation of MDH allozyme patterns.



phenotype	distance	locus and allozyme
	○	not interpreted
		not interpreted
	1.9	*Mdh1–4*
	1.7	
	1.5	*Mdh1–3*
	1.1	
	0.7	*Mdh1–2* *Mdh2*
	0.4	
	x	*Mdh1–1*
?? 33 11 23 12 22 44	*Mdh1*	*genotype inference*
?? 11 11 11 11 11 11	*Mdh2*	

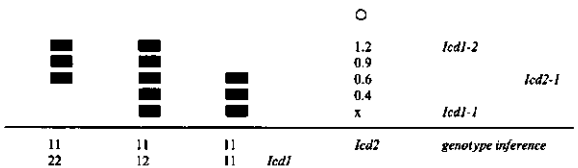
The *Mdh2* band overlaps wit the *Mdh1-2* electromorph. Activity of locus *Mdh2* is variable. *Mdh2* is usually invariant but Phenotype 1 expressing 5 bands bay be explained assuming polymorphism of both loci.

Table 3.2d Schematic representation of PGM isozyme patterns.



phenotype	distance	locus and allozyme
	○	
	0.7	*Pgm2-3*
	0.3	*Pgm2-2*
	x	*Pgm2-1*
	x	*Pgm1-4*
	–0.4	*Pgm1-3*
	–0.6	*Pgm1-2*
	–0.8	*Pgm1-1*
33 22 22 23 11 33 33	*Pgm1*	*genotype inference*
12 44 11 33 44 14 22	*Pgm2*	

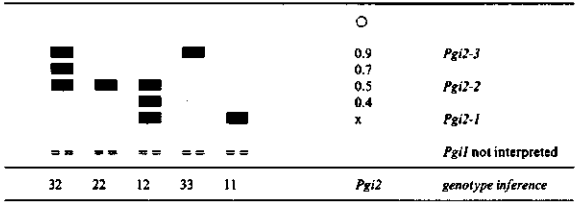
Table 3.2e Schematic representation of ICD isozyme patterns.



phenotype	distance	locus and allozyme
	○	
	1.2	*Icd1-2*
	0.9	
	0.6	*Icd2-1*
	0.4	
	x	*Icd1-1*
11 11 11	*Icd2*	*genotype inference*
22 12 11 *Icd1*		

Icd2 is invariant and migrates to a position between the *Icd1* allozymes.

Table 3.2f Schematic representation of PGI isozyme patterns.



phenotype	distance	locus and allozyme
	○	
	0.9	*Pgi2-3*
	0.7	
	0.5	*Pgi2-2*
	0.4	
	x	*Pgi2-1*
		Pgi1 not interpreted
32 22 12 33 11	*Pgi2*	*genotype inference*

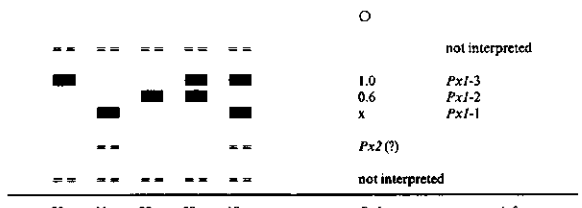
Table 3.2g Schematic representation of SKDH isozyme patterns.

phenotype	distance	locus and allozyme
	○	
		not interpreted
	0.9	*Skdh1-3*
	1.6	*Skdh1-3*
	x	*Skdh1-3*
	0.4	*Acpl–4*
33 22 32 31 11	*Skdh1*	*genotype inference*

Table 3.2h Schematic representation of GOT isozyme patterns.

phenotype	distance	locus and allozyme
	○	
	1.3	*Got3-3*
	1.0	
	0.7	*Got3-2*
	0.4	
	x	*Got3-1*
		Got2 not interpreted
		Got2 not interpreted
33 22 12 23 11	*Got1*	*genotype inference*

Table 3.2i Schematic representation of PX isozyme patterns.



phenotype	distance	locus and allozyme
	○	
		not interpreted
	1.0	*Px1-3*
	0.6	*Px1-2*
	x	*Px1-1*
		Px2 (?)
		not interpreted
33 11 22 23 13	*Px1*	*genotype inference*

sion expressed two clear bands. The expression of the tested accessions was generally weak and the variation pattern of the slow migrating bands could not be interpreted genetically. Clear enzyme activity was visible in the middle of the gel. The individual phenotypes either produced one intensely stained band, two bands or three bands. Very rarely phenotypes produced five bands (Table 3.2c). A cross between *B. macrocarpa* and *B. vulgaris* subsp. *vulgaris* confirmed that the one-banded and three-banded phenotypes can be explained genetically assuming a dimeric structure of the *Mdh1* locus. *B. macrocarpa* expressed the *Mdh1*-11 band, and *B. vulgaris* expressed *Mdh1*-22. Hybrids expressed *Mdh1*-12, and an additional heterodimeric band in between (Table 3.2c).

Up to four different allozymes could be assigned to a locus *Mdh1*. The phenotypes with two or five bands are harder to account for. Abe & Shimamoto (1989) and Van Geyt et al. (1988) suggested an additional non polymorphic locus *Mdh2* migrating to the same position as the most common *Mdh1* allele (*Mdh1*-2 in Table 3.2c). This could explain the two-banded phenotypes to be a homozygous genotypes for *Mdh1* and additional expression of an independent locus *Mdh2* respectively. However, expression of a comigrating band of the additional locus *Mdh2* is variable. Homozygous *Mdh1*- 1 plants (nearly all *B. macrocarpa* accessions and some plants in accessions belonging to *B. vulgaris* subsp. *maritima*) were frequently deficient of the *Mdh2* band. Whereas one would expect to see two bands (one for each locus), these phenotypes only expressed one. Apparently expression of *Mdh2* is not constant, and this observation is confirmed by Van Geyt et al. (1988) who reported that only seedlings and young plants showed activity at this locus and that individual variation occurred frequently. On the other hand, the five-banded phenotypes which occurred in accession 3304, make it likely that this was caused by *Mdh2* activity and that this locus is infrequently polymorphic. The five-banded phenotypes can only be explained assuming polymorphism for both genes *Mdh1* and *Mdh2*. Due to uncertain expression of *Mdh2*, genetic interpretations were performed concerning locus *Mdh1* only. As with all other enzymes the position of the different bands was compared to that of the reference accession 1570.

Phosphoglucomutase (PGM)

This enzyme could only be visualized on starch gels. Some of the PGM phenotypes for section *Beta* representatives are given in Table 3.2d. The maximum number of bands in one individual is four (not shown in Table 3.2d), the minimum number of bands is two. PGM is controlled by two loci with monomeric segregation (Smed et al. 1989). Both loci were found to be polymorphic. The fast migrating bands were designated to locus *Pgm1*, the slower migrating bands were designated to locus *Pgm2* . Variation for *Pgm1* is expressed by four different allozymes. In all three different allozymes could be appointed to the second locus. The reference accession 1570 displayed the slow allozyme *Pgm1*-4 and allozyme *Pgm2*-2. Activity of *Pgm2* bands was found to decrease in older plants.

Isocitrate dehydrogenase (ICD)

One activity zone was present in the gel and not more than three phenotypes were observed. Two three banded phenotypes with different gel positions and a five banded phenotype were noticed. Bands of the latter phenotype were identical with the bands of the three banded phenotypes (Table 3.2e). Smed et al. (1989) studied segregation of different ICD genotypes, and concluded that two loci control expression. Only one locus is responsible for the polymorphism. *Icd1* has different allozymes at two different positions in the gel, while *Icd2*, being not polymorphic, migrates to a position intermediate of the *Icd1* allozymes. Heteromeric bands between the two loci allozymes are formed. The two homozygotic genotypes expressed three bands, and the heterozygotic genotypes expressed five bands.

These three phenotypes were also described by Van Geyt & Smed (1984), Van Geyt (1986) and Wagner (1990). Nagamine et al. (1989) reported additional phenotypes, one phenotype with three bands migrating faster, and another phenotype with four bands.

Glucose phosphate isomerase (PGI)

In most plant species PGI is dimeric (Tanksley & Orton 1983). The species from section *Beta* make no exception (see Smed et al. 1989, analysing segregation of PGI in crosses and Nagamine et al. 1989). All plants expressed two activity fields on the starch gel. The gene coding for the faster allozyme was designated *Pgi1*. The expression of this gene was invariant. In the field nearer to the origin polymorphism was present. Hybrids in the cross between *B. patula* and *B. vulgaris* subsp. *maritima* were heterozygous and expressed three bands. The homozygous parent plants expressed one band (Table 3.1). Three allozymes were detected for locus *Pgi2* (Table 3.2f).

Shikimate dehydrogenase (SKDH)

SKDH activity on the gel is distributed over two zones. The upper zone had very low activity and no variation was found. The lower zone was polymorphic with monomeric gene expression (Table 3.2g). Though only a limited number of accessions were screened (Appendix II) three allozymes were noted. The reference accession 1570 was fixed for the *Skdh1*-2 allozyme. *Skdh1* polymorphism in *Beta* is also reported by Nagamine et al. (1989), Van Geyt (1986) and Wagner (1990).

Glutamate oxaloacetate transaminase (GOT)

In the literature different opinions exist as to the number of GOT loci present in *Beta*. Abe & Tsuda (1987) and Wagner (1990) recognize two loci, a fast monomorphic and a slow polymorphic locus. According to Oleo (pers. comm.) three loci are involved. The dispersion of GOT bands sometimes leads to overlapping bands for certain combinations of allozymes from different loci. Three allozymes could be identified at the locus *Got3* (Table 3.2h). Polymorphism at locus *Got2*, which is active close to *Got3*-1, could not be determined. The activity of locus

Got1 is clearly separated from the other loci, but it is usually monomorphic. Variation in this band is very rare, and heterodimeric band expression was not observed. The reference accession 1570 displayed a typical fast allozyme at *Got3*. This allozyme was denoted *Got3*-1 (Table 3.2h). The most frequent allozyme for *Got3* was found to be the intermediate *Got3*-2.

The cross of *B. macrocarpa* (*Got3*-11) with *B. vulgaris* subsp. *vulgaris* (*Got3*-22) gave hybrid plants with a blurred activity band. Due to overlap with *Got2* bands and inadequate resolution it was sometimes impossible to distinguish the heterodimeric *Got3* genotypes. Under good gel conditions *Got3* and *Got2* dispersed well enough to make it possible to identify the heterodimeric bands.

Peroxidase (PX)

PX expression was observed in the cathodal starch gel (Table 3.2i). A fast migrating band expressed very clear activity, but it did not show any polymorphism. There was also PX activity close to the origin, but due to poor resolution no discrete banding could be determined. In addition, only plants older than two months expressed enzyme activity at this part of the gel. Variation was observed in the middle part of the gel. Phenotypes with one or with two bands occurred. Smed et al. (1989) have described a polymorphic locus with two allelic forms for PX in sugar beet. Abe & Tsuda (1987) have found resolution of more PX bands (a total of 11 bands) in representatives of both wild and cultivated beet. The band of the *Beta macrocarpa* reference plants of accession 1570 was migrating faster than the two bands that were commonly seen (Table 3.2i, *Px1*-1). According to Abe & Tsuda (1987) *B. macrocarpa* invariably shows two migrating bands whereas in the present studies only one fast migrating band could be determined. The test strain 1570 was used by Abe & Tsuda (1987) as a reference accession as well, so the incongruent results could only be caused by differences in gel resolution. F_2 segregations of the PX isozymes in crosses between *B. vulgaris* and *B. macrocarpa* made Abe & Tsuda (1987) conclude that the fast PX bands represents a monomorphic locus *Px2* (see Table 3.2i) only present in *B. macrocarpa* which is linked tightly to the polymorphic locus *Px1*.

The present results suggest that the fast migrating band is not just present in *B. macrocarpa* but also could be seen in accessions of *B. vulgaris* subsp. *adanensis* and *B. vulgaris* subsp. *maritima*, and represents an allelic variant in *Px1* (Table 3.2i, Appendix II). The cross of *B. macrocarpa* (*Px1*-11) with *B. vulgaris* subsp. *vulgaris* (*Px1*-33) gave hybrid genotypes *Px1*-13. A verification with respect to number of loci involved is needed for this isozyme.

6-phosphogluconate dehydrogenase (6-PGDH)

6-PGDH isozymes were resolved on a starch gel medium as well as on polyacrylamide. Starch gel zymograms expressed an intensily stained band and two or three thin bands. The thick band was found invariable and could represent a non polymorphic locus. Polymorphism in the zone with weak band expression was found rarely. In one instance segregation was observed in a population when a test was performed on a polyacrylamide gel with tris-glycine gelbuffer. Too

few individuals were tested with this system to be sure of the nature of the segregation. Resolution on starch may be inadequate. Only a few accessions were screened for this isozyme.

Nadh dehydrogenase (NADHDH)

The activity of this isozyme was constant on a starch gel, but it expressed only limited variation. The most frequently observed phenotypes expressed one band or two close bands with equal migration distance. Very rarely phenotypes with four bands were seen.

3.3.2 Allozyme distribution in taxa of section Beta

Table 3.3 gives, in terms of presence/absence, the distribution of allozymes in the different taxa. Generally, the so called 'common' allozymes (allozymes on line 1 in Table 3.3) with relatively high frequency in many of the accessions belonging to *B. vulgaris* subsp. *maritima* were the common allozymes in the leaf beets, in *B. vulgaris* acc. 6956, in *B. vulgaris* subsp. *adanensis*, and in *B. patula*

Table 3.3. Allozymes observed in species of section *Beta*.

locus	*B.vulgaris* subsp. *maritima*	*B. vulgaris* subsp. *vulgaris*	*B. vulgaris* acc. 6956	*B.vulgaris* subsp. *adanensis*	*B. patula*	*B. macrocarpa*
Acp1	4	4	4	6	2	3
	2,3,5,6,7	6,7	6,7	4,5		1
Lap1	4	4	4	4	4	1
	2,5	2	2	2,3,5		4
Mdh1	2	2	2	2	2	1
	1,3,4	1,3	3	1,3		2
Pgm1	2	2	2	2	2	4
	1,3	3	3	3		2
Pgm2	2	2	1	2	2	2
	1,3		2			
Icd1	1	1	1	1	1	1
	2	2	2	2		
Pgi2	1	1	1	1	1	1
	2	2	2	2,3		2
Skdh1	2	–	–	2	2	2
	1,3					
Got3	2	2	2	1	2	1
	3	3		2		2
Px1	2	–	3	1	–	1
	1,3			2,3		
number of accessions	56	3	1	8	1	11

Note: the most common allozyme is on line 1. Allozymes which occur in lower frequencies are on line 2. The number of accessions evaluated for each taxon is given below.

as well. *B. macrocarpa* had a number of unique allozymes at several loci and, in addition, exhibited shifts in allozyme frequencies at other loci.

In populations of *B. vulgaris* subsp. *adanensis* some rare allozymes occurred not present in any of the subsp. *maritima* populations. In the population 2199 from Rhodos a LAP variant was found (referred to as *Lap1*-3). Accession 3105 from Crete was characteristic for the variant *Pgi2*-3. In addition, shifts in allozyme frequencies were observed for GOT. *Got3*-1 was observed in six of the eight *B. adanensis* populations screened. This allozyme is similar to the *B. macrocarpa* allozyme.

The allozyme composition of *B. patula* was highly monomorphic. Only the 'common' allozymes were present in this species, with one exception: *B. patula* deviated at locus *Acp1* where it expressed the fast migrating allozyme *Acp1*-2. This allozyme is restricted to some of the populations of *B. vulgaris* subsp. *maritima* originating from the Atlantic coast (Chapter 3.3.8).

Diploid *B. macrocarpa* had the most divergent allozyme composition. It is differentiated from the other species by a number of allozymes. *Lap1*-1 and *Pgm2*-4 separate this species from all other taxa and they may be considered as diagnostic. In addition fixation for characteristic allozymes *Acp1*-1 and *Acp1*-3, *Mdh1*-1, *Px1*-1 and *Got3* emphasize the distinct nature of this taxon.

The tetraploid cytotype of *B. macrocarpa* partly expressed the allozymes characteristic for diploid *B. macrocarpa* and partly expressed allozymes resembling common *maritima* allozymes. In tetraploid plants codominant expression of both '*macrocarpa*' and '*maritima*' allozymes was observed for some of the loci. The tetraploid accessions were uniform in *Lap1* and *Mdh1*. For these loci a codominant expression of '*macrocarpa*' allozymes *Lap1*-1 and *Mdh1*-1 and '*maritima*' allozymes *Lap1*-4 and *Mdh1*-2 was observed in all individuals tested. Formation of additional heterodimeric bands at *Mdh1* resulted in three banded genotypes. There was some variation between the two tetraploid accessions tested. Accession 3183 was fixed for the *macrocarpa Acp1*-3 allozyme, while accession 1668 expressed both allozymes *Acp1*-3 and *Acp1*-5. Heterozygosity for *Acp1* was complete in accession 1668 (but only four plants were tested). In addition GOT variation between accessions was observed, accession 1668 only expressed the *Got3*-2 allozyme band, while accession 3183 expressed both allozymes *Got3*-1 and *Got3*-2.

3.3.3 Genetic diversity in the populations

As can be deduced from Table 3.4, *Beta vulgaris* sensu lato (including *B. vulgaris* subsp. *maritima*, the leaf beets, *B. vulgaris* subsp. *adanensis* and *B. vulgaris* acc 6956) was more heterogeneous than *Beta patula* and *Beta macrocarpa*. Mean genetic diversity ranged from 0.28 to 0.32 in *Beta vulgaris* s.l., and was 0.01 and 0.07 in *Beta patula* and *Beta macrocarpa* respectively.

Genetic diversity in most of the *B. macrocarpa* populations was low (Appendix III). This monomorphism of *B. macrocarpa* populations does not neccessarily mean that as a whole it is a uniform species. The variation between populations

Table 3.4. Analysis of genetic variability in taxa of section *Beta*.

Taxon	Number of accessions	Mean sample size per locus	Percentage of loci polymorphic*	Heterozygosity H_o	Genetic diversity H_e
B. vulgaris subsp. *maritima*	34	741 ± 27.4	89%	.14 ± .037	.28 ± .072
B. vulgaris subsp. *vulgaris*	3	61 ± 4.5	78%	.14 ± .049	.32 ± .086
B. vulgaris accession 6956	1	26 ± 2.6	77%	.19 ± .068	.28 ± .070
B. vulgaris subsp. *adanensis*	8	159 ± 4.9	89%	.05 ± .017	.32 ± .078
Beta patula	1	32 ± 2.6	22%	.01 ± .007	.01 ± .007
Beta macrocarpa	10	154 ± 6.9	44%	.00 ± .002	.07 ± .040

Note:
(*) Genetic variability at loci *Acp1*, *Lap1*, *Mdh1*, *Pgm1*, *Pgm2*, *Icd1*, *Icd2*, *Pgi2* and *Got3*. A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

can be considerable, even for neighbouring populations. E.g. accessions 7085 and 7036 from Spain were fixed for *Acp1*-1 while all other diploid *B. macrocarpa* populations were fixed for *Acp1*-3 (Appendix II).

Within population polymorphism in *B. macrocarpa* was seen only in accession 3193 from Tunisia ($H_e = 0.058$) accession 3792 from Israel ($H_e = 0.233$) and accession 3196 from Turkey. The Israel population was remarkable for its high level of genetic diversity. Macromorphologically this population was very uniform and it was not different from any other *macrocarpa* population. The allozyme composition of the population was composed of plants either expressing the typical *macrocarpa* allozymes *Got3*-1, *Acp1*-3, *Lap1*-1 and *Pgm2*-4, or expressing the common *maritima* allozymes *Got3*-2, *Acp1*-4, *Lap1*-4 and *Pgm2*-2. Although genetic diversity is high, $H_e = 0.233$, the number of actual observed heterozygotic genotypes in this accession was very small, $H_o = 0.007$ (Appendix III). In accession 3196 originating from Turkey the individual protein phenotypes revealed, in contrast to accession 3792, a high level of observed heterozygosity (58% of the genotypes was heterozygotic). A combination of '*macrocarpa*' alleles and common '*maritima*' alleles was found. Macro-morphologically, the plants were difficult to classify, or showed hybrid characteristics. The high variation could have been caused by contamination of the seed sample during rejuvenation. In consequence, the accession was not included in the calculation of genetic variation coefficients. Nine diploid *B. macrocarpa* accessions had low genetic diversity. Contrary, genetic diversity in the tetraploid Tenerife accessions of *B. macrocarpa* was remarkably higher: H_e was 0.180 and 0.282 for the two accessions tested.

3.3.4 Deficiency of heterozygotes

In most taxa and accessions mean observed heterozygosity H_o is lower than mean genetic diversity H_e (Table 3.4, Appendix III).

Table 3.5. Coefficient F for heterozygote deficiency or excess in taxa of section *Beta*.

Taxon	Locus								all
	Acp1	*Lap1*	*Mdh1*	*Pgm1*	*Pgm2*	*Icd1*	*Pgi2*	*Got3*	loci
B. vulgaris subsp. *maritima*	0.58	0.24	0.33	0.61	0.65	0.53	0.48	0.77	0.52
B. vulgaris subsp. *vulgaris*	0.77	–0.08	0.45	0.27	–	0.90	0.35	–	0.47
Beta vulgaris acc. 6956	0.05	–	1.00	–0.11	0.08	0.43	0.48	–	0.41
B. vulgaris subsp. *adanensis*	0.99	0.87	0.85	0.71	–	–	0.77	1.00	0.87
B. patula	–	–	–	–	–	–	–	–	–
Beta macrocarpa	0.97	1.00	–	–	–	–	0.94	–	0.97

In *Beta vulgaris* subsp. *maritima* and the leaf beets, the frequency of observed heterozygotes H_o is about half the frequency of the number expected H_e. Values for H_o in *B. vulgaris* subsp. *maritima* are relatively high compared to *B. vulgaris* subsp. *adanensis* and species *B. patula* and *B. macrocarpa*. The lack of heterozygotes is most apparent in accessions of *B. vulgaris* subsp. *adanensis*.

A coefficient F, the fixation index, was calculated estimating the deficiency (positive values for F) or the excess (negative values for F) of observed heterozygotes. F values are reliable only when the number of expected heterozygotes is not close to zero. As a criterium F values were calculated only if the number of expected heterozygothic genotypes was five or more. Table 3.5 shows that deviations from Hardy-Weinberg genotype frequencies were numerous. High values of F (close to 1.0) were calculated for *B. macrocarpa* and *B. vulgaris* subsp. *adanensis* indicating fixation of homozygotes. Despite the fact that many heterozygote genotypes were present in populations of *B. vulgaris* s.l., there still remains a deficit when compared with Hardy-Weinberg distributions.

3.3.5 Genetic similarities

In Table 3.6 and 3.7 genetic similarity between and within the taxa is expressed as Nei's genetic identity coefficient. Averaged values were based on a population to population comparison.

Genetic similarities between species

Comparison of I between species revealed low similarity of diploid *B. macrocarpa* with *B. vulgaris* subsp. *vulgaris*, (I = 0.45), with *B. vulgaris* subsp. *maritima* (I = 0.46), with *B. vulgaris* subsp. *adanensis* (I = 0.52) and with *Beta patula* (I = 0.44). *B. patula* and *B. vulgaris* subsp. *maritima* were highly similar I = 0.87.

Genetic similarity between conspecific populations

I between *B. vulgaris* subsp. *adanensis* and *B. vulgaris* subsp. *maritima* was 0.78. The population range was large 0.54 – 0.99. Genetic identity of *B. vulgaris* acc 6956 with *B. vulgaris* subsp. *maritima* was high, I = 0.93. The similarity of the leaf beet accessions with *B. vulgaris* subsp. *maritima* ranged from 0.72 to 0.99.

Table 3.6. Genetic Identity between taxa of section *Beta*.

taxonomic group	number of pops.	1 maritima	2 adanensis	3 patula	4 macrocarpa 2x	5 macrocarpa 4x	6 vulgaris	7 acc. 6956
1 B. vulgaris subsp. maritima	34	.91 (.75-1.00)						
2 B. vulgaris subsp. adanensis	8	.78 (.54-.99)	.76 (.61-.94)					
3 B. patula	1	.87 (.75-.98)	.72 (.54-.88)	** (**-**)				
4 B. macrocarpa 2x	10	.46 (.32-.69)	.52 (.34-.70)	.44 (.34-.57)	.91 (.78-1.00)			
5 B. macrocarpa 4x	2	.80 (.68-.92)	.72 (.54-.85)	.80 (.72-.86)	.74 (.55-.89)	.87 (.87-.87)		
6 B. vulgaris subsp.vulgaris	3	.88 (.72-.99)	.74 (.51-.98)	.80 (.72-.85)	.45 (.33-.65)	.76 (.64-.87)	.84 (.76-.91)	
7 B. vulgaris acc. 6956	1	.93 (.84-.98)	.79 (.62-.97)	.89 (.89-.89)	.44 (.35-.60)	.80 (.75-.86)	.93 (.90-.97)	** (**-**)

coefficient Nei (1978) unbiased genetic identity

Table 3.7. Genetic identity between accessions of *B. vulgaris* subsp. *maritima* from different geographical regions.

REGION	number of pops.	1 Ireland	2 Netherlands	3 France	4 Portugal	5 Sicily	6 Greece
1 Ireland	5	.89 (.83-.95)					
2 Netherlands	3	.88 (.79-.99)	.89 (.84-.95)				
3 France	4	.91 (.81-.99)	.93 (.81-.98)	.96 (.94-.97)			
4 Portugal	5	.90 (.78-.99)	.90 (.83-.96)	.94 (.85-.99)	.91 (.82-.97)		
5 Sicily	13	.90 (.80-.98)	.91 (.77-.98)	.92 (.83-.99)	.91 (.82-.97)	.94 (.85-1.00)	
6 Greece	4	.86 (.75-.94)	.89 (.77-.97)	.90 (.85-.96)	.88 (.80-.94)	.91 (.80-.96)	.89 (.80-.95)

Average Genetic identity among the 34 populations belonging to *Beta vulgaris* subsp. *maritima* was high: $I_{within} = 0.91$. Table 3.7 summarizes genetic similarities within and between geographical groups of accessions in *B. vulgaris* subsp. *maritima*.

Similarity within geographical clusters is high ($I_{within} > 0.88$), but average similarity between these geographical clusters was high also: $I_{between} > 0.86$. The lowest I value was calculated for populations from Ireland and from Greece.

The populations of diploid *B. macrocarpa* were highly similar. Average similarity of diploid *B. macrocarpa* with the tetraploid accessions was 0.74 (range 0.55-0.89). Note that average similarity of *B. vulgaris* subsp. *maritima* with tetraploid *B. macrocarpa* was 0.79 (ranges 0.68-0.92), values are almost equal.

In *B. vulgaris* subsp. *adanensis* a broad range in genetic identity values between populations is seen. I ranged from 0.61 to 0.94, indicating allozyme divergence over ample distance.

Population estimates of genetic identity within the species *B. patula* were absent since only one accession of the species was analysed.

Population clustering

From all pairwise combinations of 59 accessions a matrix of I values was computed. An UPGMA cluster analysis effectively reduced the amount of information and a phenogram of similarity relations of all populations was extracted. Inspection of the phenogram revealed strong clustering of all diploid *B. macrocarpa* populations (Appendix IV). The overall similarity with all other populations was only 0.48. The tetraploid accessions associated in a larger subsp. *maritima* group. Five populations belonging to *B. vulgaris* subsp. *adanensis* aggregated to form another cluster at an overall similarity of 0.70 with the rest of the populations. The *B. vulgaris* subsp. *adanensis* populations from Kos, Adana and Leros (IDBB 1497, 5951 and 3331 resp.) were not included in this cluster but aggregated separately. Weak clustering patterns were seen in the geographic groups of subsp. *maritima* origins: accessions from Sicily aggregated as did the Greek *maritima* accessions. No cluster of a group of Atlantic origins could be extracted.

3.3.6 Genetic distance between the taxa of section Beta

Genetic distances between the taxa of section *Beta* are presented in Fig. 3.1. Genetic distance between diploid *B. macrocarpa* and the cluster which includes *Beta vulgaris* sensu lato and *Beta patula* is 0.68. Mean genetic distance between populations of *B. vulgaris* subsp. *adanensis* and *B. vulgaris* subsp. *maritima* is small, $D = 0.11$.

3.3.7 Macrogeographic allozyme distribution patterns

As opposed to the 'normal' or 'common' allozymes that are found widespread and usually with high population frequency, other categories of allozymes may

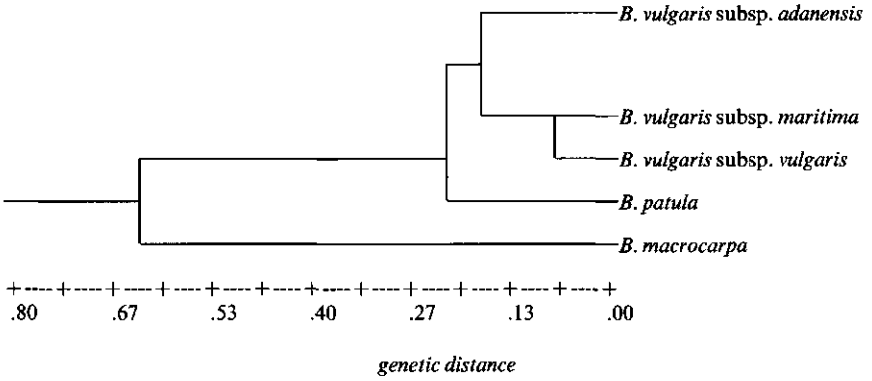


Figure 3.1. Cluster analysis using unweighted pair group method of five taxa in section *Beta*. The dendrogram is based on Nei's unbiased genetic distance coefficient (*D*). The cophenetic correlation is 0,969.

be thought of as 'rare and localized', or 'widespread but with low frequency' allozymes. The first category has been partly discussed earlier. As was demonstrated allozymes with restricted occurrence could be found in the taxa *B. macrocarpa* and *B. vulgaris* subsp. *adanensis* (notably *Acp1*-1, *Pgi2*-3, *Lap1*-3). A rare and relatively low frequent allozyme *Got3*-3 was expressed in accession 7078 from Portugal. This allozyme was found in few other accessions from Sicily and one from Ireland, but always at a very low frequency (Appendix II).

A second category of allozymes was found to be those that were relatively common in the Mediterranean Basin, but absent or only of low frequency in Atlantic populations. An example is *Acp1*-7 which is found with fairly high frequency in Mediterranean *maritima* accessions (mainly Sicily, Calabria, Yugoslavia and the Peloponnesus) as well as in the leaf beet accessions and *B. vulgaris* accession 6956. This allozyme is encountered with very low frequency in various Atlantic populations – mainly Portugal, a few from France (Fig. 3.2b).

If the distribution of the fast migrating *Mdh1*-1 allozyme is considered, it can be seen from Fig. 3.2c that its occurrence is more or less restricted to the southern accessions. This allozyme is fixed in the majority of the *B. macrocarpa* populations and occurs with low or medium high frequency in Mediterranean subsp. *maritima*. Two Atlantic accessions carried *Mdh1*-1, but with very low frequency. A similar distribution pattern as for *Mdh1*-1 was observed for the rare *Lap1*-5 allozyme (Fig. 3.2d) which was most frequent in Sicilian, Greek and Turkish subsp. *maritima* accessions. The distribution of the *Mdh1*-3 allozyme showed that, though it is a low frequency allozyme, it is fairly widespread (Fig. 3.2e).

A characteristic of Atlantic *maritima* populations is found in a fast migrating allozyme named *Acp1*-2. *Acp1*-2 is geographically restricted to the Atlantic: none of the Mediterranean beet populations examined displayed this specific band. It is clear from Fig. 3.2f that *Acp1*-2 reaches its highest frequency in the Irish

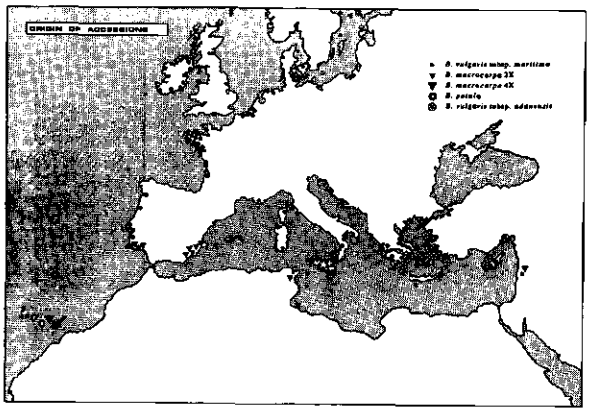


Figure 3.2a. Map with accessions origin.

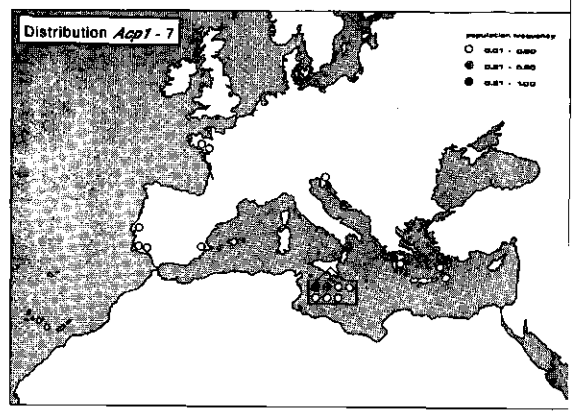


Figure 3.2b. Geographical distribution of *Acp1*-7.

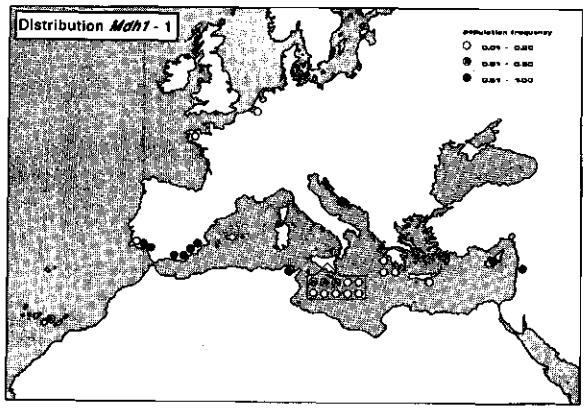


Figure 3.2c. Geographical distribution of *Mdh1*-1.

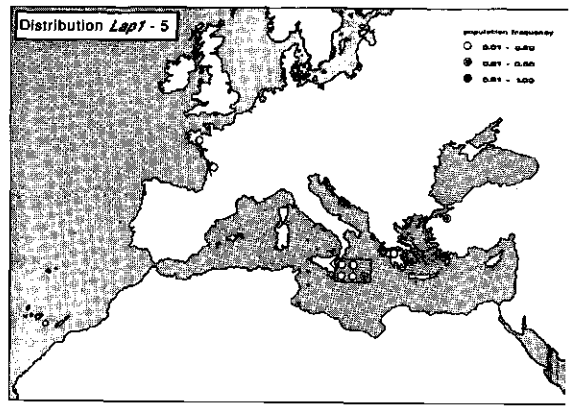


Figure 3.2d. Geographical distribution of *Lap1*-5.

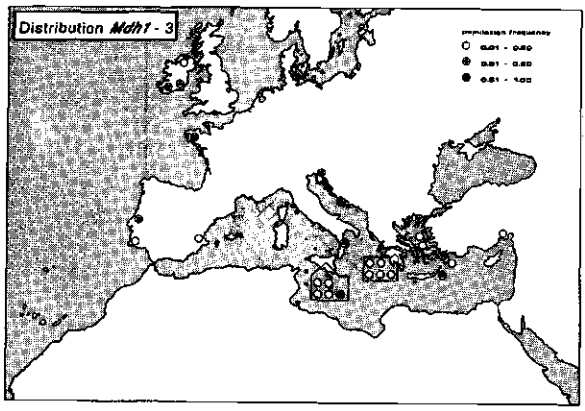


Figure 3.2e. Geographical distribution of *Mdh1*-3.

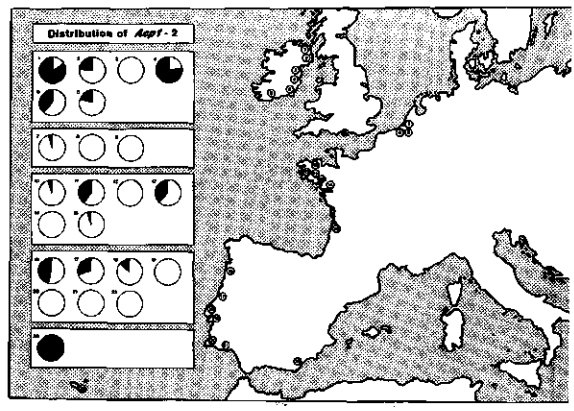


Figure 3.2f. Geographical distribution of *Acp1*-2.

Figure 3.2. The macrogeographic distribution pattern of a number of allozymes.

Table 3.8. Genetic diversity in *Beta vulgaris* subsp. *maritima* averaged over populations from different localities.

Geographical region	Genetic diversity H_e	Number of accessions
Ireland	0.17	5
The Netherlands	0.13	3
France	0.14	4
Portugal	0.18	5
Italy	0.25	13
Greece	0.28	4

populations. It may also reach a fairly high frequency in some French and Portuguese populations. *Acp1*-2 is not found any more in subsp. *maritima* populations south of Lisbon. However, it is completely fixed in *B. patula* from the Madeira region.

3.3.8 Regional differences in genetic diversity

Sofar most of the allozyme variation in *B. vulgaris* subsp. *maritima* was shown to be concentrated in the mediterranean accessions. The Atlantic accessions seemed to be less polymorphic. In Table 3.8 accessions are evaluated for genetic diversity in relation to geographical origin. Higher levels of diversity were expressed in the south eastern and middle mediterranean accessions than in the Atlantic accessions (T test: $t = 4.90$, significant at 0.01 level).

Subgeographical differences for H_e were analysed using Scheffé's multiple comparison test. The variation in the samples was tested region by region. Comparison of H_e means from different regions revealed that no two regions were significanty different at the 0.05 level. Genetic diversity in a few Atlantic accessions (cf. accession 7100 and 7078 from Portugal, Appendix II) was comparable to the high level of genetic diversity in separate mediterranean accessions. A subdivision of *B. vulgaris* subsp. *maritima* in six geographical clusters cannot be justified with the present data.

3.4 Discussion

3.4.1 Taxonomic differentiation

Interspecific population divergences have occurred primarily at *Lap1*, *Acp1*, *Pgm2* and *Px1*. At these loci unique allozymes were found in *B. macrocarpa*. In addition divergence in allelic frequencies were found at loci *Mdh1*, *Got3*, and *Icd1*. Genetical divergence of *B. macrocarpa* from the other taxa of *Beta* section *Beta* was further illustrated by the low values for I and high values for D.

B. macrocarpa had I values 0.52 or less which indicates genetical separation from the other taxa. Among congeneric species I is on average 0.67 (Gottlieb 1977, 1981, Crawford 1983, Brown 1990; Some caution should be taken comparing I levels of different species, since I is biased by sample size, number of analysed loci, life history characters and mode of reproduction, see below).

Ranges characteristic for conspecific taxa are reported between 0.80 and 1.00 (Crawford 1983). The I values between conspecific populations of *Beta macrocarpa* and *Beta vulgaris* s.l. were for the largest part more than 0.80.

Indicated by a mean $I_{between}$ of 0.78, it is justified to consider subsp. *adanensis* conspecific with subsp. *maritima*. The more so, since accessions of *Beta vulgaris* subsp. *adanensis* expressed a rather variable allozyme composition when compared to each other ($I_{within} = 0.76$).

The allozyme data on *Beta patula* indicated that it has not diverged very much from *B. vulgaris* subsp. *maritima*. $I_{between} = 0.87$, which falls in the class of conspecific populations.

The allozymes observed in tetraploid *B. macrocarpa* resembled the common allozymes found in diploid *B. macrocarpa* and the common allozymes found in subsp. *maritima*. Fixed heterozygosity was observed for loci *Mdh1* and *Lap1* in both accessions tested. Lange & De Bock (1989) concluded from crossing experiments involving tetraploid *B. macrocarpa* and diploid and tetraploid *B. vulgaris* that the genomes of both species are highly homologous. According to the authors an alloploid origin of the tetraploid *B. macrocarpa* cytotype is likely in view of the diploidised meiosis. Thus the tetraploid might have originated from hybridisation between *B. vulgaris* subsp. *maritima* and diploid *B. macrocarpa*. The conservation of allozyme alleles from both genome donors also suggests an alloploid origin and very little genetical divergence from the progenitors. The fixed heterozygosity for *Lap1* and *Mdh1* gives further evidence for the polyploid to be an allopolyploid (Crawford 1985).

Remarkable little allozymic differentiation existed within accessions of *B. macrocarpa*, considering that accessions have been used from a large geographical distribution range. This intrapopulational uniformity is concordant with evidence found for nearly complete autogamy in this species. Under greenhouse conditions *B. macrocarpa* is a self compatible species. The same holds for *B. vulgaris* subsp. *adanensis* and *B. patula* (see also Dale & Ford-Lloyd 1983, Abe et al. 1987[a], Lange & De Bock 1989). The calculated fixation indices (Table 3.5) indicate that selfing is a predominant reproduction mode in *B. macrocarpa* and *B. vulgaris* subsp. *adanensis*. Low levels of observed heterozygosity, even in accessions with intermediate allozyme frequencies (accession 3290 and 3331 *B. vulgaris* subsp. *adanensis*; accession 3792 *B. macrocarpa*) give evidence that outbreeding under natural conditions is limited in these taxa. *B. macrocarpa* in Portugal was usually found mixed in *B. vulgaris* subsp. *maritima* populations (Frese et al. 1990). Judged both from observations on site and after allozyme analysis no hybridisation could be demonstrated in these associated stands. It should be noted however that other mechanisms may obstruct gene flow between

the two species, e.g. differences in flowering time (Chapter 4) and sterility in hybrid plants (Abe et al. 1987[a]). Genetic diversity was demonstrated in the case of *B. macrocarpa* accession 3792 from Israel and accession 3196 from Turkey. In both accessions a mixture of *B. macrocarpa* alleles and *B. vulgaris* subsp. *maritima* alleles was present. However, it is not certain whether the observed variation is natural in *B. macrocarpa*, and the east Mediterranean makes up a centre of diversity for this species, or, as an alternative, there is a higher chance of hybridisation with other species in this part of the distribution area, possibly by breakdown of introgression barrieres and simultaneous flowering time between the local species.

Allozyme variation in *B. patula* is extremely low. However, it can not be assumed that in fact it is a monomorphic species until more provenances are sampled. The allozyme composition demonstrates the proximity of *B. patula* to Atlantic *B. vulgaris* subsp. *maritima*, specifically to those accessions expressing the *Acp1*-2 allozyme. It appears that in *B. patula* substantial morphological differentiation is not associated with allozymic divergence. The negligible isozymic variation may evidence the rapid morphological change of a dispersed *maritima* population that lost contact with continental populations. It can be hypothesized that subsequent to morphological speciation there has not been enough time for allozyme divergence.

As allozyme polymorphism in the accession is almost nil, nothing can be said about the natural reproduction mode in *B. patula*. Under greenhouse conditions however, the species is fully self compatible.

B. vulgaris accession 6956 is morphologically distinct from *B. vulgaris* subsp. *maritima* in such morphological features as leaf morphology and patulous growth habit. However, from the perspective of allozyme composition and genetic diversity, this accession is not different from any other Mediterranean accession belonging to *B. vulgaris* subsp. *maritima*.

The allozyme composition of the three leaf beet accessions was very similar to the patterns observed in *B. vulgaris* subsp. *maritima*. Allozyme frequency deviations were seen occasionally for some loci, but much of the variation was shared with the wild subspecies. In a study by Nagamine et al. (1989) the conclusion was reached that the cultivated *B. vulgaris* still holds a great deal of allozyme variation, even in the more modern crop gene pools of sugar beet and fodder beet.

3.4.2 Geographical patterns and beet sampling strategies

Estimation of allelic frequencies on a population to population base is biased, because the accessions have been collected as bulk by many different people, and sometimes information about the size of the population and the number of plants that have been harvested in the population is lacking. Interpretation

of allozyme distribution should therefore be based primarily on criteria of presence/absence of allozymes, rather than on interpretation of the observed allelic frequencies.

The present data provide a general impression of macrogeographical distribution of allozymes and an estimate of variation in geographical subareas. The information can be used for formulating a strategy for the collection of wild *Beta* populations.

In the Mediterranean species *B. macrocarpa* and *B. vulgaris* subsp. *adanensis* increased homozygosity compared with subsp. *maritima* populations was measured and this observation was related to a predominantly selfing breeding system. Inbreeding may have caused the fixation of many of the allozyme alleles by which the populations of both species differ. Inbreeding reduces gene flow between populations so that localised adaptations can occur in response to ecological and climatic adaptations. Dispersion of *B. macrocarpa* in southern Portugal is more or less restricted to an extreme type of habitat in the salt marshes which are exploited for sea salt. In other parts of the distribution area *B. macrocarpa* inhabits less salty environments, and the species can even be found inland (Frese et al. 1990). Similarly *B. vulgaris* subsp. *adanensis* inhabits diverse environments like pebble beaches and it is found also as a weed on arable land.

Considering the homozygosity of many of the populations, the fixation of unique allozymes in adjacent populations and the adaptation to different habitat conditions, it may be concluded from the point of collecting variation in *B. macrocarpa* and *B. vulgaris* subsp. *adanensis* that it is essential to sample as many populations as possible from as many different environments as possible.

Within *B. vulgaris* subsp. *maritima* a contrast in the amount of allozymic heterogeneity between Atlantic and Mediterranean accessions was indicated. Higher levels of allozymatic variation were demonstrated in south eastern and middle Mediterranean populations, grading to lower values in the south Atlantic and north Atlantic populations (Table 3.8). However, a further subdivision of *B. vulgaris* subsp. *maritima* in six geographical clusters could not be justified with the present data. Localised allozymes were basically absent, and low frequency allozymes were noticed for Atlantic accessions (*Acp1*-2) as well as for Mediterranean accessions (*Lap1*-5, *Mdh1*-3). Heterozygosity levels agreed with an outbreeding reproduction mode in *B. vulgaris* subsp. *maritima*.

In *Beta vulgaris* subsp. *maritima* genetic diversity is more or less equally divided between individual plants in a population and between neighbouring populations. Therefore, extensive sampling of one large population within a geographical region can be as adequate as sampling many populations.

From the perspective of sampling allozyme variation in *B. vulgaris* subsp. *maritima*, it would be more efficient to screen Mediterranean populations, although some Atlantic accessions express high variation as well.

4 Generative development and life cycles in *Beta* section *Beta*

4.1 Introduction

4.1.1 Defining life cycles

Differences between taxa and differences between 'ecotypes' may be expressed through differences in their inherited growth rhythms. Species of section *Beta* display remarkable variation in life cycles. An inspection of the literature regarding *Beta* species (Chapter 1) reveals that the genus is highly polymorphic for this trait, and the different life cycles of the species are summarised as annual, biennial or perennial strategies. However, the use of these terms is often confusing and inconsequent. For example, in the breeding literature cultivated *B. vulgaris* is a biennial species, apparently to indicate that no generative development takes place in the first growth season. However, breeders are acquainted with recalcitrant bolters in the crop, which shows that first year flowering is common in the cultivated beet.

In the Flora Europaea (Tutin et al. 1964) the wild beet *B. vulgaris* subsp. *maritima* is a perennial or an annual species. In some of the taxonomic literature the northern wild beet *B. vulgaris* subsp. *maritima* is classified as perennial, and sometimes the name *perennis* is used. In contrast, the Mediterranean wild beet is considered annual. Annual wild beet has been described as a distinct taxon *B. vulgaris* subsp. *maritima* var. *maritima* forma *annua* (Asch. and Gr.) Aellen (Aellen 1938). Others consider the Mediterranean wild beet annual as well as perennial, depending upon environmental conditions. Beets from inland sites in the Near East and from India and Pakistan, are reported to be annuals (Basu & Mukherjee 1975). Buttler (1977[b]) considers some Mediterranean *Beta* species to be annuals which however have closely related perennial counterparts.

The confusion in the literature points to the need for clarity in the terminology to be used. The main problem is the fact that the terms annual, biennial and perennial are poorly defined and that in fact two different demographic parameters are involved. These parameters refer to a) *longevity of the plant* and b) *earliness of flowering*.

Assuming that germination takes place in spring, an *annual* beet can be defined as a plant which flowers and sets seed in the year of germination and does not survive until the next year. *Biennial* beets are vegetative in their first year, flower in the second year and die after flowering once. *Perennial* beets are able to flower repeatedly during several years. Van Dijk & Boudry (1991) state that for *Beta* it is desirable to distinguish between *early flowering perennials* and *late flowering perennials*. Early flowering perennials start flowering for the first time in the

year of germination. In late flowering perennials flowering is delayed until the second or the third year.

Using these definitions it is not always possible to classify a plant in the field as either annual or an early or late perennial because information on time of germination is not available. Size of the plants and robustness of the root system is often an indication of age, but in fact these criteria are inadequate. Moreover, environmental conditions e.g. water availability, soil fertility and competition from other species may cause a plant to wither prematurely.

4.1.2 Factors important for flowering in B. vulgaris

It is a well known fact that, to induce flowering, the cultivated beet must be vernalized. Vernalization means the exposure of the plant to low temperatures for some time. The optimal vernalization temperature for the cultivated beet is between 5° and 9°C, and the time needed for vernalization is between 3 and 5 weeks (Wiebe 1989). In addition to vernalization a long daylength is required for flower induction. Vernalization and daylength are both quantitative parameters which can intensify each others effects in particular conditions. To induce flowering in non-vernalized beets daylength has to be at least 14 hours (Van Dijk & Boudry 1991).

Not all *B. vulgaris* plants need to be vernalized to be able to flower. Munerati (1931) reported on such genotypes which he had discovered in a commercial cultivar. It appeared from crossing experiments that a major gene, now known as the *bolting gene B*, was involved (Abegg 1936). This gene is located on the YRB linkage group (Abegg 1936) on chromosome II (Butterfass 1964), to which isozyme markers GOT and ICD are linked also (Abe et al. 1987[b], Wagner 1990). The mechanism of the gene is such that *BB* or *Bb* genotypes can flower without vernalization, while *bb* genotypes require vernalization. Whether the gene *B* is responsible for synthesis of flowering stimulating substances such as giberellines is not known. Giberellines cannot replace the effect of the gene *B* (pers. comm. H. van Dijk).

4.1.3 Induction of flowering in wild beets

Similar to cultivated *B. vulgaris*, wild beets may require vernalization for flowering (cf. Van Dijk & Boudry 1991). However, little is known about the conditions required for vernalization of wild plants and the influence of day length on flowering. Field observations suggest that there are huge differences in the time needed for attaining the flowering stage between populations of geographically distinct regions. Populations of *B. vulgaris* subsp. *maritima* in the northern part of the distribution area are known to be late flowering. Plants from southern latitudes (e.g. Mediterranean plants) seem to flower earlier, but it is not known whether these plants have any requirement for vernalization.

In this chapter it is investigated to what extent accessions from different geographical regions yield bolting plants and flowering plants in the first growth

season. Regarding the plants which flower in the first growth season, it is investigated whether they could be classified as annuals or as perennials. Furthermore, it is a point of interest to learn about the plants that have attained the bolting stage, whether they will also start flowering in the same year. Consequently, in addition to time of bolting start of flowering is quantified. Regarding the plants which did not flower in the first growth season, it is investigated to what extent controlled vernalization during the winter months yields flowering plants in the subsequent growth season.

4.2 Materials and methods

Flowering and life cycles in *Beta* were studied in several field trials at the CPRO/DLO experimental station in Wageningen. Accessions taxonomically grouped in *B. vulgaris* subsp. *maritima* were sorted according to geographical origin.

4.2.1 Atlantic accessions

Accessions with Atlantic distribution are subdivided in accessions from Ireland, the Netherlands, the west coast of France and the west coast of Portugal. Generative development in Atlantic accessions was evaluated in two ways:

(A) Generative development of one calendar year plants.

Atlantic accessions from Ireland and Brittany (France) were evaluated for first year flowering in the field trial of 1988. In this field trial accessions from Sicily were taken as Mediterranean reference plants. Atlantic accessions from Portugal were grown simultaneously with a Mediterranean accession from Spain (accession 7089 Puerto Real) in the field trial of 1990.

Seeds were sown in the first week of April. Plants were temporarily grown in the greenhouse where temperature was controlled between 15 and 20 °C. The plants were taken to the field in the first week of May.

(B) Flowering after a vernalization treatment: Generative development of two calendar year plants.

Atlantic accessions from Ireland, the Netherlands and the west coast of France were evaluated for flowering after vernalization in the field trial of 1990. Seeds had been germinated in the preceding year in the first week of October. Young rosette plants were taken to a vernalization room in the first week of December. The vernalization temperature in this room fluctuated between 5° and 10 °C and the plants received 14 hours light during the winter period. In the second week of February plants were put outside and received additional vernalization. The accessions were planted in the field in three fully randomised blocks (with 8 plants per block) in the first week of May.

4.2.2 Mediterranean and oriental accessions

Observations referring to accessions of *B. vulgaris* subsp. *maritima*, *B. vulgaris*

subsp. *adanensis*, *B. macrocarpa* and *B. patula* were carried out in the 1989 field trial with a few additional observations in 1990. Accessions of *B. vulgaris* subsp. *maritima* were subdivided in geographical groups. Accessions from Pakistan, Iran and India were brought together in the group of oriental accessions. Accessions from Turkey and Greece represented the east Mediterranean group. A group of accessions from southern France, Corsica and Italy were grouped in the middle Mediterranean group. The passport data of the accessions are given in Table 2.2 (Chapter 2).

The accessions analysed in the field trial were sown in the first week of April, and stayed approximately one month in the greenhouse. In the first week of May the plants were transplanted to an experimental field in Wageningen. Plants were arranged in three fully randomised blocks with 8–10 plants per accession per block.

A number of parameters were measured characterizing generative development and life cycles. *Bolting* was quantified as the number of plants having bolted the day the plants were taken out of the greenhouse, 36 days after germination. Additionally the length of the generative stem was measured at this stage. *Flowering start* was quantified as the number of days between germination and opening of the first flowers. Measuring the lowest node with a glomerule on the main stem, counting from the first pair of leaves, was used as an estimate of earliness of flowering (parameter *glomerule position*). *Flowering end* was recorded when the flowers had stopped producing pollen. Observations on *withering* and *survival after flowering* were recorded until 169 days after germination of seeds.

Seven accessions representing different taxa or geographical groups were evaluated for comparison of generative development in the greenhouse. The plants were evaluated in long day and in short day conditions. These accessions were germinated in the greenhouse in the first week of April and in the third week of August, respectively. Observations continued until 19 weeks after germination.

4.3 Results

4.3.1 Flowering and life cycle of B. vulgaris subsp. maritima

4.3.1.1 First year flowering of Atlantic accessions

Comparative observations on the three life cycle stages (rosette stage, bolting and flowering) were carried out for Atlantic accessions (Table 4.1). Plants from Irish accessions did not bolt and flower during the whole observation period. Most of the plants only expressed vegetative growth and produced huge rosettes. From the rosettes of some of the plants lateral stems would grow out towards the end of the growth season in late August. However, no flowers were formed on these creeping stems. Instead, new secondary rosettes developed on the lateral stems.

Table 4.1. First year generative development in populations of *B. vulgaris* subsp. *maritima* with Atlantic origin.

accession origin (1)		number of plants	generative stage (2)						days to flowering (4)	
			rosette		bolting		flowering			
			I	II	I	II	I	II	mean	st.d.
Ireland	1988									
3852 Wexford	Bannow Island	10	100	100	0	0	0	0	–	–
Tramore	Tramore	5	100	100	0	0	0	0	–	–
3888 Ardmore	Ardmore	10	100	100	0	0	0	0	–	–
3889 Kerry	Tralee, Ballyhaigue	10	100	100	0	0	0	0	–	–
3884 Down	Blackrock	10	100	100	0	0	0	0	–	–
	total/mean (3)	45	100	100	0	0	0	0	–	–
France, Brittany	1988									
6111 Finistere	Pointe de Penvins	10	60	10	40	30	0	60	x	x
6110 Morbihan	Kermagen-pleubian	10	90	20	10	70	0	10	x	x
6118 Finistere	Saint Colombier	10	100	10	0	80	0	10	x	x
6117 Finistere	Pors-Meurs Plouescat	10	50	0	50	40	0	60	x	x
6113 Finistere	Lepouldu-Santec	10	10	10	90	20	0	70	x	x
	total/mean	50	62	10	38	50	0	40	x	x
Portugal	1990									
7067 Mafra	Ericeira bay	22	0	0	100	0	0	100	105	14.9
7078 Aljezur	Carrapateira beach	22	0	0	100	0	0	100	93	11.6
7072 Lissabon	Lissabon	20	0	0	100	0	0	100	95	11.2
7084 Olhao	Fuseta salt marshes	16	0	0	100	0	0	100	91	7.9
7096 Alcochete	Alcochete	23	4	0	96	0	0	100	89	7.5
7100 Figueira dF	saline	24	8	0	92	0	0	100	97	11.1
7102 Viana C	Areosa beach	23	0	0	100	0	0	100	103	7.1
	total/mean	150	3	0	97	0	0	100	96	10.7

Notes: (1) accession origin: geographical subregion, year of evaluation, IDBB number, district, location.
(2) percentage of plants in rosette stage, bolting, or flowering in two different periods of evaluation of generative stage:
Period I: Generative stage 55 – 62 days after germination (30 May).
Period II: Generative stage 113 – 120 days after germination (30 July).
(3) means per region.
(4) mean number of days from germination to flowering.
Mean number of days based on flowering plants only.
(x) indicates that no quantitative information was recorded.

Description of first year generative development in plants from Brittany is based on five accessions (Table 4.1). In the end of May 38% of the plants had bolted and 62% was still in rosette stage. There was variation for earliness of

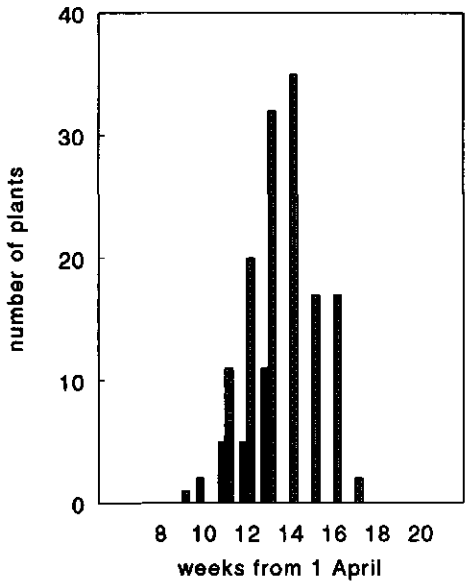


Figure 4.1. Variation for start of flowering in *B. vulgaris* subsp. *maritima*.
First year flowering. Plants originate from Portugal (hatched) and south west Spain (black).

bolting between accessions. All plants of accession 6118 had remained in rosette stage during period I, whereas 90% of the plants of accession 6113 had bolted in this period. The other accessions had both rosette plants and bolting plants (Table 4.1). At the end of July/ first week of August 10% of the Brittany plants were still in rosette stage. At the same time 40% of the plants were flowering. Flowering had started in June for early plants. Late plants started flowering in August. Some plants flowered as late as September, but unfortunately these events were not recorded quantitatively.

Populations from Portugal were analysed in the 1990 field trial. Nearly all plants (97%) had bolted 8 weeks after germination (Table 4.1). Flowering started between 9 weeks and 17 weeks after germination (Fig. 4.1). All plants flowered. The accessions 7067 and 7102 were the latest flowering Portugese populations (approximately two weeks later: see Table 4.1).

4.3.1.2 Atlantic accessions; flowering after a vernalization treatment

Accessions from Ireland, The Netherlands and France were given a vernalization treatment. Generative development is described in Table 4.2.

Most Irish accessions bolted, but bolting was relatively late. The plants that bolted, developed long, prostrate lateral stems, occasionally an erect central stem (accession 3863). Bolting before May 10th was absent in four out of the seven Irish accessions. Plants from accession 5871 did not bolt at all, and only two plants from accession 3882 bolted.

Table 4.2 also presents the amount of plants that reached the flowering stage

Table 4.2. Generative development of *B. vulgaris* subsp. *maritima* (Atlantic populations) after a vernalization treatment.

accession origin (1)				percentage of plants bolting 10 May	percentage of plants flowering 20 August	days to flowering (2)		
						mean	st.d.	n
Ireland 1991								
3880	Down, Killard Point	05419 N	24	16	16	101	22.7	4
3882	Down, Rossglass	05413 N	24	0	0	–	–	–
5871	Meath, Laytown	05370 N	24	0	0	–	–	–
5870	Dublin, Malahide	05327 N	24	0	16	126	0.0	4
3889	Kerry, Tralee	05240 N	23	0	17	107	10.5	4
3852	Wexford, Bannow Island	05212 N	24	42	58	99	19.1	14
3863	Ardmore, Ardmore Bay	05157 N	24	4	75	109	13.9	18
	total/mean		167	9	26	106	17.0	44
The Netherlands 1991								
6523	Schouwen	05140 N	23	17	100	97	17.7	23
6522	Noord Beveland	05135 N	23	17	100	108	9.8	23
6519	Zeeuws Vlaanderen	05121 N	22	19	100	102	12.3	22
6520	Zeeuws Vlaanderen	05122 N	24	8	83	119	6.8	20
6521	Walcheren	05126 N	24	25	67	103	16.8	16
	total/mean		116	17	91	106	15.0	104
France 1991								
6116	Finistere, Kerdeniel	04840 N	20	19	95	88	15.3	19
6117	Finistere, Pors-meurs	04840 N	23	6	100	85	13.5	23
6119	Finistere, St. Mathieu	04821 N	22	46	100	93	21.0	22
7104	Morbihan, Saille	04718 N	22	58	100	88	12.7	22
6109	Morbihan, Kergroix-	04731 N	23	63	100	81	9.6	23
6112	Finistere, Perros-	04731 N	22	54	100	81	10.9	22
6115	Morbihan, P. de Penlan	04731 N	22	46	100	95	12.1	22
7105	Morbihan, Manemeur	04729 N	24	38	100	88	14.3	24
6106	Charente maritime	04600 N	22	50	100	91	12.0	22
	total/mean		200	44	99	88	14.4	199

Notes: (1) accession origin: geographical district, year of evaluation, IDBB number, District (Location), northern latitude of accession origin, number of plants evaluated.
(2) start of flowering: number of days from 1 April. Mean based on flowering plants only. st.d. = standard deviation, n = number of flowering plants.

subsequent to vernalization. In total 26% of all Irish plants flowered subsequent to vernalization. There was variation between accessions with respect to the amount of flowering. The frequency of flowering plants was highest in the south-

ern Irish accessions from Wexford and Ardmore (Table 4.2). Five accessions of the south-west part of The Netherlands were analysed. All plants bolted, most plants after May 10th. 91% of all plants started to flower. Mean start of flowering was July 16th, on average the same as the Irish accessions.

Nine accessions from the French west coast were analysed. Eight accessions were from Brittany, one accession originated from the district of Charente maritime, which is more to the south. Vernalization induced bolting and flowering in nearly all plants of the accessions from the French west coast (Table 4.2).

A relation between flowering development and geographical origin of the plants can be deduced from Table 4.2 where accession latitude is given.

In two of the most northern populations (Meath: 53°70'N, and Down: 54°13'N) flowering plants were absent. Incomplete flowering occurred in populations sampled between 54°19' and 51°22' northern latitude. Less than 20% flowering was found in populations sampled between 54°19'N and 52°40'N. More than 50% flowering frequency was observed in accession samples starting from the south coast of Ireland (Wexford: 52°12'N, Ardmore: 51°57'N) and two of the Dutch accessions more to the south. Complete flowering was found in three Dutch accessions and in all accessions which were sampled south of 48°40'N on the west coast of France.

The parameter *start of flowering* (Table 4.2) reveals another geographical trend. *Start of flowering* was estimated for the plants in the accessions that actually started flowering within the period of observation. Table 4.2 shows that the average flowering start in the French accessions was 18 days earlier than

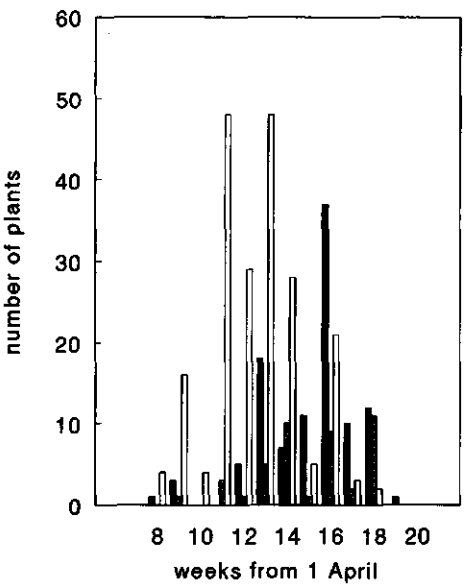


Figure 4.2. Variation for start of flowering in *B. vulgaris* subsp. *maritima* after a vernalization treatment (second year flowering). Plants originate from the Netherlands (black), Ireland (hatched), and the Atlantic coast of France (white).

Table 4.3. First year generative development in populations of *B. vulgaris* subsp. *maritima* with Mediterranean origin.

Accession origin (1)		number of plants	rosette (2)		bolting (2)		flowering (2)		days to flowering (3)	
			I	II	I	II	I	II	mean	st.d.
Spain	1990									
7089	Puerto Real	23	0	0	100	0	0	100	85	7.3
Italy, Sicily	1988									
2207	Trabia beach	30	14	0	73	7	3	93	88	6.9
2208	Caccamato to Roccapalumba	27	0	0	100	0	0	100	94	12.4
2212	Aquedelei E of St Stefano	25	0	0	92	0	8	100	95	14.7
	Mazara 10 km to Salemi	25	20	0	48	0	32	100	70	17.5
2691	Pergusa to piazza Amerina	21	5	5	81	10	14	86	102	22.7
	total/mean	137	9	1	80	3	10	96	90	15.4

Notes: (1) accession origin: geographical subregion, year of evaluation, IDBB number, district, location.
(2) percentage of plants in rosette stage, bolting, or flowering in two different periods of evaluation of generative stage:
period I: Generative stage 55 – 62 days after germination (30 May).
period II: Generative stage 113 – 120 days after germination (30 July).
(3) mean number of days from germination to flowering (based on flowering plants only).

in the accessions from Ireland and The Netherlands. However, flowering start of individual plants is highly variable (Fig. 4.2). Occasionally the difference between early and late plants in a single accession is 8 weeks (the difference between the latest and the earliest plant is 11 weeks, cf. Fig. 4.2). Moreover, the earliest flowering plants are from Dutch accessions, and among the latest flowering plants are plants from Brittany.

A direct comparison of the parameter *flowering start* for vernalized and non-vernalized plants is difficult to make. Unfortunately, exact quantitative data on *start of flowering* in non vernalized plants from Brittany are not available.

4.3.1.3 First year flowering of Mediterranean and oriental accessions

Because a number of accessions was evaluated simultaneously, flowering in Mediterranean accessions can be compared here to first year flowering in Atlantic accessions. The data show that the rate of generative development in plants from the Mediterranean is most similar to the rate of development in the Atlantic accessions from Portugal (compare Table 4.3 with Table 4.1).

Of the Sicilian plants 80% had bolted by the end of May and 10% had started flowering in period I. The remaining rosette plants (9%) bolted in the second period except for a few plants (5%) from accession 2691; 96% of the plants was flowering in the second half of July.

All plants of the Mediterranean accession 7089 (Puerto Real, south west

Table 4.4. Early bolting in *Beta* section *Beta*. Number and percentage of plants bolting in the greenhouse, 36 days after germination.

Taxon/ geographical group	plants bolting	plants in rosette	bolting percentage	length bolting-stem (cm)	
				mean	st.d.
B. vulgaris subsp. *maritima*					
oriental accessions	145	23	86	7.3	3.3
east Mediterranean accessions	100	270	27	1.6	2.1
middle Mediterranean accessions	43	115	27	1.5	2.1
B. vulgaris subsp. *adanensis*	305	23	93	3.1	2.7
B. macrocarpa 2x	113	3	97	4.7	2.5
B. macrocarpa 4x	88	0	100	2.4	1.3
B. patula	24	0	100	3.0	0.3

Table 4.5. Start of flowering in *Beta* section *Beta*.

taxon	number of plants	flowering start (1)		glomerule position (2)	
		mean	st.d.	mean	st.d.
B. vulgaris subsp. *maritima*					
oriental accessions	158	66.8	7.0	8.5	1.5
east Mediterranean accessions	375	77.7	12.4	9.2	2.2
middle Mediterranean accessions	143	80.3	7.2	9.7	2.3
B. vulgaris subsp. *adanensis*	320	52.4	5.0	7.2	1.4
B. macrocarpa 2x	114	50.8	3.8	4.4	1.1
B. macrocarpa 4x	88	54.1	3.8	4.1	0.9
B. patula	24	84.1	0.3	7.5	1.0

Notes: (1) flowering start: number of days from germination.
(2) glomerule position = number of nodes to first glomerule

Spain) flowered. Mean time of flowering start was on average 11 weeks after germination, which was about one to two weeks earlier than Portugese plants (Table 4.3, Fig. 4.1).

In Table 4.4 and Table 4.5 the earliness of bolting and flowering in the other geographical groups of *B. vulgaris* subsp. *maritima* accessions is given. Table 4.4 shows the percentage of plants that started to bolt already in the greenhouse, before transference of the plants to the field. 86% of the oriental origins of *B. vulgaris* subsp. *maritima* had started to bolt already in the greenhouse. Among the groups of accessions from east and middle Mediterranean origin 73% were still in rosette after 36 days. All plants bolted after transfer of the plants to the field. Individual accessions were distinct with respect to bolting development. Some of the accessions were relatively late bolting, notably accessions 1555

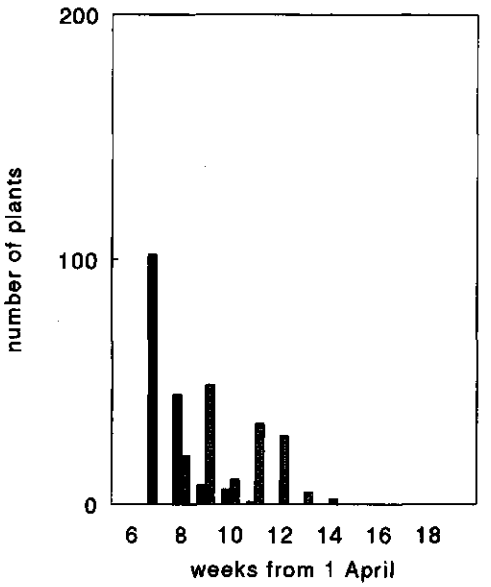


Figure 4.3. Variation for start of flowering in oriental accessions of *B. vulgaris* subsp. *maritima* (hatched), and accessions of *B. macrocarpa*. First year flowering.

(Lemnos, Greece) and 2193 (Peloponnesus, Greece), while accession 470 (Famagusta, Cyprus) and 2673 (Peloponnesus, Greece) were very early.

The group of oriental accessions started flowering between ca. eight and twelve weeks after germination (Fig. 4.3). Flowering in Mediterranean plants was significantly later between 10 and 13 weeks after germination (Fig. 4.4, Table 4.5). The earliest flowering populations in this group were from Cyprus (acces-

Table 4.6. Termination of flowering.

taxon	number of plants N	terminated flowering (1) %	duration of flowering (2) days	st.d.	withering (3) %	not surviving (4) %
B. vulgaris subsp. *maritima*						
oriental accessions	151	52	–	–	43	6
east Mediterranean accessions	370	46	–	–	11	3
middle Mediterranean accessions	158	47	–	–	16	0
B. vulgaris subsp. *adanensis*	313	97	55	13.2	89	42
B. macrocarpa 2x	109	100	57	10.1	95	64
B. macrocarpa 4x	87	100	68	8.8	98	57
B. patula	24	40	–	–	0	0

Notes (1) percentage of plants having terminated flowering at day 150.
(2) duration of flowering: mean number of days.
(3) percentage of plants withering after flowering.
(4) percentage of plants not surviving after flowering.

Table 4.7. Generative development of accessions in a greenhouse trial. Plants not vernalized. Plants grown in long day (l) and short day (s) conditions.

time from germination (1) (weeks)	hours daylight		flowering stage (2)	*B. vulgaris* subsp. *maritima* 3331 Greece		5383 Pakistan		504199 Italy	
	l	s		l	s	l	s	l	s
5.5	15.3	12.5	R	100	100		45	72	89
			B			100	55	28	11
			F						
7	16.0	12.0	R	100	100		45	72	78
			B			100	55	28	22
			F						
8	16.2	11.4	R	11	100		45	44	78
			B	89		100	55	56	22
			F						
10	16.4	10.3	R		100		45	17	78
			B	89			55	78	22
			F	11		100		6	
12	16.4	9.4	R		100		45	17	78
			B	11			45	44	22
			F	89		100	10	39	
19	14.5	7.5	R		88		10	11	78
			B		12		80	6	22
			F	100		100	10	83	
number of plants				18	14	18	13	18	18

Notes: (1) seeds were sown 1 April (long day plants) and 15 August (short day plants)
(2) Percentage of plants in each of the generative stages: R = rosette, B = bolting stage, F = plants flowering.

sion 470), Turkey (6203) and the Peloponnesus (3300). These accessions flowered on average 10-13 days earlier than the others.

Towards the end of the observation period about half of the plants had completed flowering (Table 4.6). Plants from accession 6206 (Marseille, France) restarted vegetative growth after flowering, and secondary rosettes were produced on the generative branches. In all the populations which completed flowering relatively early, vegetative growth proceeded very slowly and sometimes withering of the plants was noted. Plant withering was observed especially in the oriental accessions of *B. vulgaris* subsp. *maritima*. Some of the plants had died (Table 4.6), but the root system of the majority of the plants that were checked were robust enough to suppose perenniality. The early flowering accession 3300 (Peloponnesus) showed an annual habit: many plants withered and died after seed ripening.

Three accessions were grown in the greenhouse (Table 4.7) in long day and in short day conditions. In long day conditions plants of accession 5383 (oriental

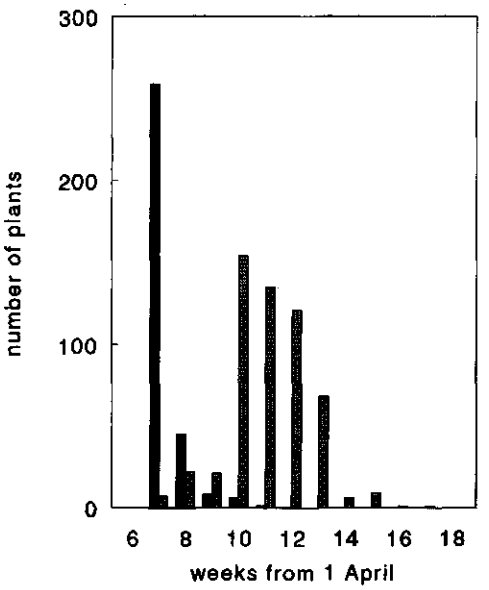


Figure 4.4. Variation for start of flowering in Mediterranean accessions of *B. vulgaris* subsp. *maritima* (hatched), and accessions of *B. vulgaris* subsp. *adanensis*. First year flowering.

accession) flowered within 10 weeks. Complete flowering was reached in accession 3331 (Greece) as well. Only 39% of the accession from Italy started to flower after 12 weeks. After 19 weeks 83% flowered, but 11% of the plants did not bolt.

In short day conditions half of the plants of accession 5383 (oriental accession from Pakistan) stayed in rosette, about half of the plants bolted after five weeks (Table 4.7). However, after bolting generative development in these plants stagnated. Only one plant started to flower in the 12th week of observation. Most of the plants of accession 504199 (Italy) stayed in rosette phase during the whole period of observation. Only 27% of the plants bolted after five to seven weeks. Generative development in accession 3331 (Greece) followed the same pattern as accession 504199 (Italy); none of the plants reached the flowering stage.

4.3.2 Flowering and life cycle of affiliated taxa

4.3.2.1. B. vulgaris subsp. adanensis

B. vulgaris subsp. *adanensis* started bolting within 36 days after germination. Only 7% of the plants did not bolt already in the greenhouse (Table 4.4). Flowering start is concentrated in the seventh and eighth week (Table 4.5, Fig. 4.4). On average flowering continued for 55 days (Table 4.6). Many of the plants withered after flowering, and 42% of the plants were not alive anymore at the end of the observation period. However, in a number of populations (accessions 1180, 1502, 2199, 3331 and 3290) withered plants produced new branches from

the beginning of September onwards. These plants flowered for the second time in late September. Apparently, the root parts of some of the plants survived and new branches were produced.

Accession 5951 was grown in the greenhouse. Under long day conditions, all plants reached the flowering stage in 8 to 10 weeks, but under short day conditions, nearly all plants stayed in rosette (Table 4.8). Only one plant bolted and flowered. Bolting of this plant took place in the beginning of the experiment, when daylength was relatively long. During the 19 weeks period of observation all other plants showed only vegetative growth. This was strongest in the first weeks, afterwards it gradually slowed down.

Field observations gave indications both for annuality as for perenniality in *B. vulgaris* subsp. *adanensis*. On Cyprus small populations of *B. vulgaris* subsp. *adanensis* were encountered on pebble beaches and ruderal sandy places near the sea. Pebble beach plants had long roots penetrating through the layer of pebbles. These plants were suspected to be perennials. Both withered plants and green flowering plants were present in the first week of June. Repeated flowering within one year seemed to be occurring also. A few plants had branches with withered leaves and ripened seed as well as fresh branches on which flowers were developing. At ruderal sites near roads and fields small annual plants dominated. Most of the plants were dead and seeds had been dropped.

4.3.2.2 B. macrocarpa

In early development *B. macrocarpa* produced a small rosette consisting of four or five leaf pairs. Bolting started soon. Nearly all plants bolted within 36 days after germination. Late bolting plants were rare (Table 4.4). Between *B. vulgaris* subsp. *maritima* and *B. macrocarpa* variation in flowering start was clear (Fig. 4.3). East Mediterranean plants (Cyprus, Greece) and plants from Tunisia started flowering ca. seven weeks after germination. This was on average four weeks earlier than Mediterranean *B. vulgaris* subsp. *maritima* (Table 4.5, Fig. 4.3). Variation for early flowering among individual plants was limited. In 1989 more than 90% of the plants flowered in the seventh week, the latest plants started flowering in the tenth or eleventh week (Fig. 4.3).

Earliness of flowering was estimated by counting to the lowest node with a glomerule. In *Beta macrocarpa* the first glomerule is already formed at the fourth or the fifth node. The position of the glomerule on the main stem illustrates the habit of early flowering in *B. macrocarpa*.

In tetraploid *B. macrocarpa* accessions bolting and flowering start was similar to diploid *B. macrocarpa* accessions, but duration of flowering was longer. The tetraploid cytotype flowered on average 11 days longer (Table 4.6). Plants from both cytotypes withered immediately after flowering. At the end of the field trial less than 50% of the plants were still alive.

Two diploid accessions were grown in the greenhouse for evaluation of generative development in long day and in short day conditions. Table 4.8 shows that bolting and flowering takes place in *B. macrocarpa* both in long day as in short day conditions. In long day conditions all plants from both accessions

Table 4.8. Generative development of accessions in a greenhouse trial. Plants not vernalized.
Plants grown in long day (l) and short day (s) conditions.

time from germination (weeks)	hours daylight l	s	flowering stage (1)	*B. patula* 6963 Madeira l	s	*B. vulgaris* subsp. *adanensis* 5951 Turkey l	s	*Beta macrocarpa* 7127 Cyprus l	s	1570 USA l	s
5.5	15.3	12.5	R		6	100	94				22
			B	100	94		6	100	100	78	67
			F							22	12
7	16.0	12.0	R				94				
			B	100	100	83	6	50	95	78	88
			F			17		50	5	22	12
8	16.2	11.4	R				94				22*
			B	100	100	11	6		50		39
			F			89		100	50	100	39
10	16.4	10.3	R				94				22
			B	89	100		6		50		22
			F	11		100		100	50	100	39
			W								17
12	16.4	9.4	R				94				22
			B	17	100		6		36		22
			F	83		100		100	61	100	39
			W						3		17
19	14.5	7.5	R				94				22
			B	17	94		6				33
			F	83	6	89			11	56	22
			W			11		100	89	44	22
number of plants				18	16	14	18	18	19	18	18

Notes: (1) percentage of plants in each of the generative stages: R = rosette, B = bolting stage, F = plants flowering, W = plants withering.
(*) bolting stagnating, new rosette leaves are formed

flowered within eight weeks. In short day conditions considerable variation in the flowering response of individual plants is seen. All plants of accession 7127 flowered. For some of the plants of accession 7127 it took more than 12 weeks to reach the flowering stage, but after eight weeks ca 50% of the plants flowered. Size of the flowers and number of flowers produced were small and the plants withered readily. 63% of the plants of accession 1570 did not reach the flowering stage. In some of the plants bolting stagnated and vegetative growth was reinforced after some time.

In Algeria *B. macrocarpa* flowers in March and April (Lechevalier, 1968). In southern Spain and Portugal *B. macrocarpa* flowers from March until May (Valdes et al., 1987). Populations in the province of Almeria completed seed ripening in the first half of May and in the second half of May all plants had withered (Frese et al. 1990). No living plants were seen in the populations of Portugal and south west Spain in August. On Cyprus the populations of *B. macrocarpa* had completed their life cycle before the first week of June.

4.3.2.3 B. patula

B. patula is a very early bolting species. The seedling plants form no rosette and will grow out directly to form an inflorescence stem. However, plants analysed in the field trial flowered not before day 84 (Table 4.5), and therefore *B. patula* started flowering approximately at the same time as the accessions belonging to *B. vulgaris* subsp. *maritima*.

When plants were grown in the greenhouse flowering start was equal to flowering start in the field. Of the plants germinated in April in long day conditions flowering started in the twelfth week for most of the plants, but 17% did not show any signs of flowering. In short day conditions plants bolted, but flowers had not developed in the majority of the plants. After 19 weeks only one plant flowered (Table 4.8).

In the field trial *B. patula* flowered until the end of the observation period and the plants showed no signs of withering. Five greenhouse plants survived beyond two years of cultivation. These observations suggest a perennial strategy for *Beta patula*.

4.4 Discussion

4.4.1 Need for vernalization in B. vulgaris subsp. maritima

Table 4.9 summarises the results of the field trials with respect to flowering parameters in *B. vulgaris* subsp. *maritima* and *B. vulgaris* subsp. *adanensis*. In Fig. 4.5 the results of Table 4.9 are integrated with data from other sources (see below) to show trends in vernalization need for different geographical groups of accessions.

It was shown in the field trials that accessions from Ireland, the Netherlands and the Atlantic coast of France contained plants that do not flower in the first

Table 4.9. Summary of flowering parameters in *B. vulgaris* subsp. *maritima* and *B. vulgaris* subsp. *adanensis*.

Geographical group	first year flowering (percentage)	second year flowering (*) (percentage)	days to flowering (from 1 April)
B. vulgaris subsp. *maritima*			
northern Ireland	0	0	–
Ireland	0	26	106 (*)
the Netherlands	0	91	106 (*)
Brittany, France	40	99	88 (*)
Portugal	100	×	96
Spain, France, Italy (middle Mediterranean)	100	×	82
Greece, Turkey (east Mediterranean)	100	×	78
Iran, Pakistan, India (oriental)	100	×	67
B. vulgaris subsp. *adanensis*			
Greece, Turkey	100	×	52

(*) Note: flowering after vernalization treatment

year after germination. Irish accessions showed a total absence of bolting and flowering plants in the first year. In the field trial 40% of the plants from Brittany, France, flowered. All plants from Mediterranean origin and from Atlantic Portugal flowered in the first year, suggesting no vernalization requirement. Table 4.7 gives an indication of flowering in three accessions grown in the greenhouse. Under greenhouse conditions non-flowering plants were recorded in a single Mediterranean accession (504199 from Italy). However, all plants of 3331 (Greece) and 5383 (Pakistan) were flowering.

Concluding from these observations, a vernalization requirement is present in the north Atlantic plants and part of the plants from Brittany. A few Mediterranean plants show a need for vernalization in the greenhouse, but not in the field trial.

These results are compared to observations on flowering by Van Dijk & Boudry (1991). It is noted that Van Dijk & Boudry (1991) grew their plants in a greenhouse where temperature was controlled above the critical vernalization range. The authors observed that a considerable amount (25%) of plants from the French Mediterranean coast did not flower in the first year. In addition, Van Dijk & Boudry (1991) recorded only 0% to 6 % first year flowering in accessions from Brittany, and accessions from the French Atlantic coast south of Brittany. The authors concluded that the majority of the Atlantic plants and part of the Mediterranean plants have a vernalization requirement.

The discrepancy between the observations by Van Dijk & Boudry (1991) and observations reported in the present paper can be explained when it is assumed that a considerable number of plants from Mediterranean latitudes and nearly all plants from Brittany require vernalization to flower, but the vernalization need is very moderate and can be satisfied even in spring. (The temperate climate

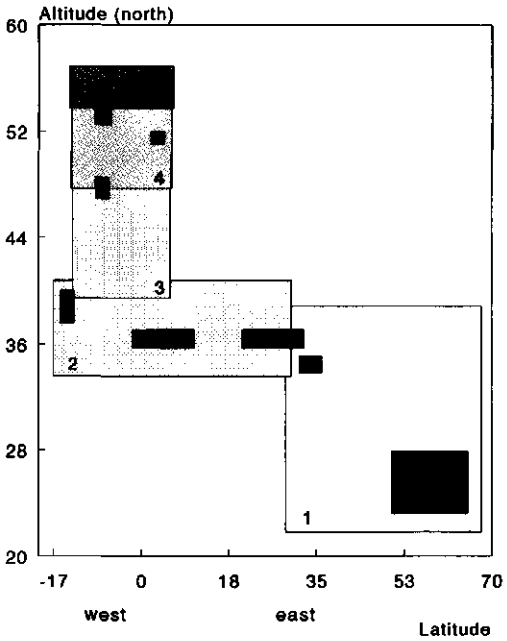


Figure 4.5. Schematic presentation of transitions in vernalization need among groups of accessions. Geographical groups are shown as black rectangles indicating the altitudinal and latitudinal distribution of the accessions. The represented geographical regions are from north to south and from west to east:
Ireland, the Netherlands, Brittany (France), Portugal, west and middle Mediterranean, east Mediterranean (*B. vulgaris* subsp. *maritima*), east Mediterranean (*B. vulgaris* subsp. *adanensis*), oriental accessions (*B. vulgaris* subsp. *maritima*).
Pattern boxes indicate geographical transitions in vernalization need. Area boxes are numbered by levels of increasing vernalization need:
Area 1. area with plants capable of bolting without vernalization.
Area 2. area with plants having unknown (no or moderate) vernalization need.
Area 3. area with plants having moderate vernalization need.
Area 4. area with plants having strong vernalization need.
Area 5. area with plants having very strong vernalization need.

in the Netherlands has cold nights even into the second half of May). Apparently, during the first weeks in the field the rosettes of the plants were big enough to receive adequate vernalization for bolting.

At this point two remarks should be made. Firstly, in order to estimate the precise vernalization requirement of a particular plant it is neccessary to grow the plant in a greenhouse where temperature is controlled. Secondly, to respond to the question whether plants will flower in the first year it is necessary to observe them in their original habitat. Potentially, in their indigenous environment Mediterranean plants could be unable to bolt in spring, but at the same time they could be all bolting during spring in the Netherlands.

All plants used in the present study were grown in the greenhouse (at 18-20 °C)

for 36 days before they were transferred to the field. As far as the plants from southern latitudes are concerned, reinspection of Table 4.4 can give a minimum estimate of plants without a vernalization requirement. *B. patula*, *B. macrocarpa* and *B. vulgaris* subsp. *adanensis* had bolted completely in the first 36 days. Regarding *B. vulgaris* subsp. *maritima* 27% of the Mediterranean plants showed signs of unvernalized bolting in the greenhouse. At least 86% of the oriental plants had no vernalization requirement (Fig. 4.5).

4.4.2 Presence of gene B

The presence of a genetic factor for bolting in natural populations was analysed by Van Dijk & Boudry (1991). In crosses of first year flowering plants, segregation for flowering and non-flowering plants suggested that a gene *B* for bolting was present, the same as or similar to the gene *B* in cultivated *Beta vulgaris*.

Some general conclusions can be drawn with regard to interpretation of the described variation in terms of genotypic polymorphism for gene *B*. In the north Atlantic region (i.e. accessions from Ireland and the Netherlands) all plants require vernalization and consequently polymorphism for gene *B* is not likely. With regard to the accessions from Brittany the presence of *bb* or *Bb* genotypes in this region cannot be excluded given the results of the field trials, but is highly unlikely in view of the results of Van Dijk & Boudry (1991).

As far as accessions of the Atlantic coast of Portugal and the west and middle Mediterranean are concerned no definite conclusions for the presence of gene *B* can be drawn from the results presented here. The fact that there are bolting and non-bolting plants under greenhouse conditions could indicate the presence of gene *B*. With regard to the accessions of the oriental region and some east Mediterranean accessions a vernalization requirement is almost certainly absent in *B. vulgaris* subsp. *maritima*. *B. vulgaris* subsp. *adanensis* has no vernalization requirement either. It is believed that *bb* genotypes do not occur in the plants from the oriental and east Mediterranean distribution areas because the climate of the east Mediterranean and the climate of Pakistan and India may be too hot for *bb* genotypes to bolt. There could be other reasons for strong selection against *bb* genotypes. Van Dijk & Boudry (1991) report that an inland wild beet population in the south of France was almost fixed for *B*. In inland habitats the plants behave as weeds in or next to arable fields with a high risk of being weeded and removed. The authors assume that delaying flowering until the next year will be highly selected against, because winter survival is very low.

4.4.3 North-south clinal variation in vernalization need

Accessions from Ireland, the Netherlands and Brittany were given a vernalization treatment. A vernalization treatment affected bolting in most accessions (Fig 4.5). It was curious however, that part of the plants of northern accessions did not react at all: e.g. no bolting occurred in accession Meath 5871 (northern

Ireland), and only two plants of accession Down 3882 (northern Ireland) bolted. Some of the Irish plants developed lateral prostrate stems very late in the season. But this type of bolting is not followed by flowering. Many plants, instead of producing flowers, developed secondary rosettes on bolting stems. Perhaps under the influence of shortening daylength, further generative development is blocked, and inefficient or 'too late' flowering is prevented. Accessions from southern Ireland bolted but some showed an incomplete flowering response. In contrast, nearly all plants from Brittany and the Netherlands flowered due to the vernalization treatment.

The time and intensity of vernalization was equal for all accessions, yet it was too short or otherwise inadequate for the non-bolting and non-flowering plants from Ireland. Apparently, the northern accessions have an increased need for vernalization. (As an alternative, *devernalization*: too rapid elevation of temperature after vernalization could have occurred in the northern accessions). Insensitivity to vernalization (or relative sensitivity to devernalization) may act as a threshold to premature flowering and consequently could be interpreted as an adaptation to the relatively harsher climate from the north. Prolonged vegetative growth gives rise to more vigorous plants which are more competitive in the temperate, maritime Atlantic vegetation and more stable during gales and floods.

4.4.4. Variation in time of flowering

A north-south gradient for the parameter *start of flowering* is evident, mainly due to the differences in vernalization need between the accessions. The accessions from the Atlantic coast which actually started flowering after vernalization differed considerably in flowering start (Fig. 4.5). The plants from Brittany flowered on average 18 days earlier than plants from the Netherlands and Ireland, but there was large variation for individual plants within these geographical regions regarding the start of flowering. The synchronising effect of a vernalization treatment seems to be limited.

At southern latitudes an east-west gradient is apparent regarding the parameter *start of flowering* (Fig. 4.5).

4.4.5 Evolutionary significance of life cycle divergence in the affiliated taxa

With respect to flowering and life cycles, the affiliated taxa *Beta macrocarpa, Beta patula* and *B. vulgaris* subsp. *adanensis* have diverged from *B. vulgaris* subsp. *maritima* in two ways. Firstly, these taxa lack a period of vegetative growth before bolting or this period is strongly reduced. Secondly, these taxa tend to behave more like annuals than do accessions of *B. vulgaris* subsp. *maritima.*

Beta patula is peculiar for the fact that it bolts without producing a rosette, independent of vernalization or daylength. The species needs long days to flower.

Beta macrocarpa is the most readily flowering taxon. The species has no vernalization need and flowers even under short day conditions. *B. macrocarpa* is an obligatory annual species, whereas *B. vulgaris* subsp. *maritima* and *B. vulgaris* subsp. *adanensis* seem to be perennial or facultative annual. *B. patula* is more or less perennial.

Concluding from the observations presented here it seems that life-cycle divergence of the taxa from *B. vulgaris* subsp. *maritima* involves not only fixation of '*B* genes' cancelling vernalization need for bolting, but also fixation of genes cancelling sensitivity for daylength, genes responsible for time of flowering, and genes controlling annuality. Abe et al. (1987[b]) suggested that the annual habit in *Beta macrocarpa* is controlled by at least two or three pairs of genes homologous to the gene *B* in *Beta vulgaris*. In addition, the presence of a major gene controlling early flowering in this species was reported.

5 Germination ecology of some *Beta* species

5.1 Introduction

The type of germination behaviour which is exhibited by a given species is expected to have evolved in response to the characteristics of the habitat of that species (Angevine & Chabot 1979, Grime 1979). Furthermore, it may be expected that within a species, seed accessions of different geographical origin can have different germination strategies, determined by the different conditions found in the germination habitat of the accessions.

Taxa of section *Beta* are widely distributed occupying the littoral zone of Europe, the Middle East and the Indian subcontinent (Chapter 1). *B. vulgaris* subsp. *maritima* is distributed along the Atlantic coasts of western Europe, and it is common along Mediterranean beaches. Considering populations which are geographically separated, it may be hypothesised that the germination characteristics of such populations have diverged. *B. vulgaris* subsp. *adanensis* and *B. macrocarpa* are species from Mediterranean coastal habitats. On Cyprus populations of these taxa are found sympatric with *B. vulgaris* subsp. *maritima*. Phylogenetically, the species are closely related. Since these species also share the same habitat, it is expected that their germination requirements are quite similar.

In the Mediterranean environment taxa of section *Beta* face a severe stress period: summer drought, high temperatures and hypersalinity. Summer rainfall varies considerably between years. Showers are unpredictable and may be followed by long dry spells. The conditions for germination in this season seem unfavourable. Rainfall is more abundant in autumn and winter when temperature is moderate.

In northern regions the climate is temperate and the environment may be more suited for germination in spring and summer. Drought and hypersalinity occur only rarely in the summer period. However, on Atlantic coasts the environment will be more unpredictable due to floods occurring throughout the year (but more frequent in the autumn and winter). A very important limiting factor for germination in autumn or winter is the risk of seedling mortality when temperature drops below zero. Seedlings at this stage are also sensitive to other stress factors typical for the winter season such as inundation and salinity.

Prevention of germination in unfavourable seasons can be realised through seed dormancy. Innate or primary dormancy is the inability of the seeds to germinate at certain environmental conditions. When dormancy is induced, no germination is possible, or germination is restricted to a fixed temperature regime (Karssen 1982, Bouwmeester 1990). The relief of dormancy is accompanied by a broader range of temperatures at which germination is possible (Karssen 1982,

Bouwmeester 1990). Induction and relief of dormancy can be influenced by temperature fluctuations in the environment. Cold stratification of the seeds may stimulate the release of dormancy. Thus, both the expression and the control of dormancy are regulated by temperature (Bouwmeester 1990).

Studies on germination in weed species and littoral species of temperate regions including *Chenopodium album, Cakile maritima, Crambe maritima, Atriplex littoralis* and *Atriplex hastata* have shown that mechanical factors and chemical factors may be related to dormancy. In the littoral species *Cakile maritima* and *Crambe maritima* a tight pericarp prevents the radicle to protrude (Binet 1960, 1968, Baron 1961).

As was demonstrated by Heydecker et al. (1971) germination of fruit balls of *B. vulgaris* may be obstructed both by chemical factors as well as by mechanical factors. The fruit balls of *B. vulgaris* contain an inhibitor complex involving several phenolic acids. Additionally the fruit coat contains high concentrations of inorganic salts which account for most of the inhibitory effects (Junttila 1976). This inhibitor complex may interact with the fruit ball microflora. The respiration of the embryo is possibly affected by one of the factors or by the interaction of both factors. Germination may be depressed mechanically by the operculum which is tightly appressed to the pericarp.

In the present study the germination patterns of geographically distinct accessions of *B. vulgaris* subsp. *maritima* are described. Seed accessions originating from the Netherlands were selected to represent the north Atlantic gene pool. Populations in the Netherlands are dispersed at the northern limit of distribution of the species. A dormancy pattern is expected as these accessions must overcome an unfavorable winter season. Mediterranean seed accessions originated from southern France and Cyprus. A comparison is made of the germination ecology of the three taxa *B. vulgaris* subsp. *maritima*, *B. macrocarpa* and *B. vulgaris* subsp. *adanensis*. For these accessions seed dormancy can be a meaningful characteristic during summer drought. Seeds may sometimes react to sparse rainfall and germinate. However, species from semi-arid habitats are usually adapted to prevent germination after small rainfall events or to avoid germination before the next wet season (Angevine & Chabot 1979).

Experiments were designed in order to investigate the germination condition of the fruit balls and to see whether any form of innate or primary dormancy is present in the seeds. The expression of dormancy is tested by exposing variously pretreated fruit balls to different temperature regimes. The experimentally established germination response of the accessions is discussed in relation to field observations.

5.2 Materials and methods

5.2.1 Seed collection and storage

Table 5.1 lists the origins of the fruit balls used in the experiments. After collection the fruit balls were dried and divided in two batches. One batch was stored at 4 °C. The other batch was used for rejuvenation and for obtaining fresh fruit balls. Plants were raised in the greenhouse and fruit balls were harvested in August 1991. The accessions from het Zwin and Burghsluis were sampled in two years. Freshly harvested fruit balls from these accessions were dried and used instantly in the germination experiments.

5.2.2 Pretreatment of fruit balls

Inhibiting effects of phenolic substances and mechanical factors could (partly) be overcome by taking some precautions before and during the experiment. To remove external phenolic substances and to loosen the operculum the fruit balls were soaked in tap water (for a period of three hours) and dried overnight. Fruit balls were washed after harvesting, the treatment was repeated one day before the experiment started.

5.2.3 Experiments

For each treatment a hundred fruit balls per population were germinated. Two petri-dishes per population, each of fifty fruit balls, were used in the experiments. Fruit balls were germinated on 9 cm petri-dishes with a layer of filter paper, to which 3.5 ml aquadest was added. Excess water (free water not absorbed by the filter paper) was removed. Moisture loss by evaporation was checked during the experiment. Germination was determined every two days until 32 days from imbibition.

Table 5.1. Date and place of collection of the fruit balls used in the study.

species / accession origin	IDBB number	date of collection
B. vulgaris subsp. *maritima*		
The Netherlands, Burghsluis	6523	September 1990 / October 1991
The Netherlands, het Zwin	6519	September 1990 / October 1991
France, Perpignan	–	August 1990
Cyprus, Famagusta	470	June 1986
B. vulgaris subsp. *adanensis*		
Cyprus, Limassol	7128	June 1989
B. macrocarpa		
Cyprus, Limassol	7127	June 1989

For reference of germination capacity a hundred fruit balls per population were germinated in the green house in soil (a mixture of sand and compost). The effect of light and dark on germination was tested using two different light regimes. In the dark, and in 12 hours dark, 12 hours light per day, germination was tested at a constant temperature of 20 °C.

Temperature experiments were performed in dark temperature boxes, recordings were taken under green light. The temperature response of the six accessions under study was tested under two fluctuating temperature regimes and five regimes with constant temperature. Germination was assayed at 10 °C, 15 °C, 20 °C, 25 °C, and 30 °C. Additionally, alternating temperatures 5 °/15 °C and 5 °/25 °C (16 h/8 h) were applied.

Germination capacity was tested using the original seed sample, using fruit balls that were stratified for eight weeks at 4 °C, and using fruit balls that had been dry stored at 4 °C for more than one year.

Mean number of days to germination day was calculated as:

$$\frac{\Sigma \text{ (day of germination) (number of germinating seeds)}}{\text{(total number of germinating seeds)}}.$$

The number of seeds per fruit ball is variable between accessions (Chapter 2), the number of viable seeds varies between one, and, less frequent, two or three. Emergence of one radicle was considered as germination of the fruit ball.

For the purpose of statistical analysis each fruit ball is considered as a single replicate, and thus each treatment has 100 replicates. Statistics for this situation are derived from the binomial distribution. The standard deviation of the proportion of fruit balls germinating (i.e. percentage germination) is calculated as $\sqrt{pqn}$ where p is the proportion of fruit balls germinating, q = 1-p, and n = 100 (the number of replicates). Significance intervals for the different percentages were calculated. For the purpose of comparing the data a difference between two germination percentages of 10% or more is in any case significant at the 0.05 level.

5.3 Results

5.3.1 The effect of light on germination

A possible effect of light on germination was investigated in an experiment in which fruit balls were germinated in diurnal darkness and in a regime of 12 hours light, 12 hours darkness. The results of the experiment are presented in Table 5.2.

Germination occurred in complete darkness as well as in light, for all accessions. A general quantitative effect of light could not be determined in this experiment. More germination was observed in the Perpignan population and in the population from het Zwin in light. Less germination was observed in *B. vulgaris* subsp. *maritima* from Cyprus. Light did not seem to influence germination of

Table 5.2. *Beta* species at two light regimes, two different substrates, and at a constant temperature of 20°C. Germination percentage after 32 days.

species / population	diurnal dark period (hours) / germination substrate			
	12 / soil	12 / petri-dish	24 /soil	24 / petri-dish
B. vulgaris subsp. *maritima*				
The Netherlands, Burghsluis	72	59	67 (3)*	65 (0)
The Netherlands, het Zwin	73	66	55 (2)	42 (1)
France, Perpignan	35	39	22 (8)	27 (1)
Cyprus, Famagusta	47	48	69 (5)	56 (0)
B. vulgaris subsp. *adanensis*				
Cyprus, Limassol	41	48	44 (0)	34 (2)
B. macrocarpa				
Cyprus, Limassol	77	40	67 (0)	40 (1)

* Seeds which did not germinate within 32 days in diurnal darkness were taken to 12 hours light conditions conditions. After 7 days additional germination was counted (results in parentheses).

B. macrocarpa, B. vulgaris subsp. *adanensis* and some of the *B. vulgaris* subsp. *maritima* accessions. Upon transferring non germinated fruit balls from dark conditions to light, a small number of fruit balls germinated (Table 5.2), but the reaction was not significant.

5.3.2 The effect of substrate on germination

Germination in soil was compared to germination on petri-dishes under two light conditions (Table 5.2). Soil was a better substrate for the accessions from het Zwin and Burghsluis. On average 10% more germination was observed. More fruit balls of *B. macrocarpa* germinated using soil as a substrate, with an average difference of 32%, when compared with petri-dish germination. The fruit balls of *B. macrocarpa* are tougher and more spongy than the fruit balls of *B. vulgaris* subsp. *maritima* and those of *B. vulgaris* subsp. *adanensis*. *B. macrocarpa* may be less capable of absorbing water from the filter paper than the other species, because the stiff erect tepals prevent optimal contact with the moist filter paper. Furthermore, the composition of the fruit ball microflora may be different, or the amount of germination inhibiting substances on the fruit balls is higher: even after repeated washing a brown extract leaked out readily from the fruit balls on the filter papers. Furthermore, at high germination temperatures (see below) infection with fungi occurred sometimes in petri-dishes with *B. macrocarpa* fruit balls, a phenomenon that was observed less frequently in fruit balls of the other species.

Table 5.3. Mean number of days to germination of fruit balls at various temperature regimes.

species / population	Temperature (°C)						
	10	15	20	25	30	5/15	5/25
Beta vulgaris subsp. *maritima*							
The Netherlands, Burghsluis	23.0	12.3	8.5	8.9	7.6	25.5	24.0
The Netherlands, het Zwin	20.9	14.9	11.4	9.4	9.2	23.8	18.9
France, Perpignan	17.0	10.2	6.9	4.6	7.3	16.4	12.5
Cyprus, Famagusta	12.2	6.2	9.3	7.8	3.0	16.8	13.9
B. vulgaris subsp. *adanensis*							
Cyprus, Limassol	19.6	10.7	6.6	6.9	6.3	19.5	16.0
B. macrocarpa							
Cyprus, Limassol	15.5	12.6	15.2	10.5	–	19.9	13.6

5.3.3 The effect of temperature on germination

Results of the temperature response of the six accessions under study are presented in Table 5.3, Fig. 5.1 and 5.2.

Fruit balls of *B. vulgaris* subsp. *maritima*, accessions Burghsluis and het Zwin, showed optimum germination in the range of higher temperatures 20 °C to 30 °C. Remarkably germination at 30 °C was rather high (Fig. 5.1). Freshly harvested fruit balls of both accessions showed almost no germination at 10 °C. At 15 °C germination was low.

When fruit balls were stratified for eight weeks germination improved especially at the lower temperature ranges 10 °C and 15 °C (Fig. 5.1). Similarly, the long stored fruit balls germinated better at low temperature ranges, the response being most pronounced at 10 °C.

Fig. 5.2 shows the induction time for germination at the various regimes. At 10 °C induction of seed germination is seen only after 10 to 12 days of imbibition (Fig. 5.2a and 5.2c). Afterwards germination proceeded gradually and still continued at the end of the observation period, when no more germination was observed at the other temperature regimes. A slow rate of low temperature germination was characteristic of the northern accessions: mean number of days to germination at 10 °C was between 20.9 and 23.0 days for accession Burghsluis and het Zwin (Table 5.3).

Under conditions of fluctuating temperatures population het Zwin gave low germination response. On the contrary, 5/25 °C was the optimum for the Burghsluis population with more than 80% germination. Lowering the night temperature resulted in more complete, but slower germination. A specific induction time of 12 to 16 days was noted at 5 °/15 °C. The induction time was approximately 10 days at 5 °/ 25 °C (Fig. 5.2b and 5.2d). Mean number of days to germination

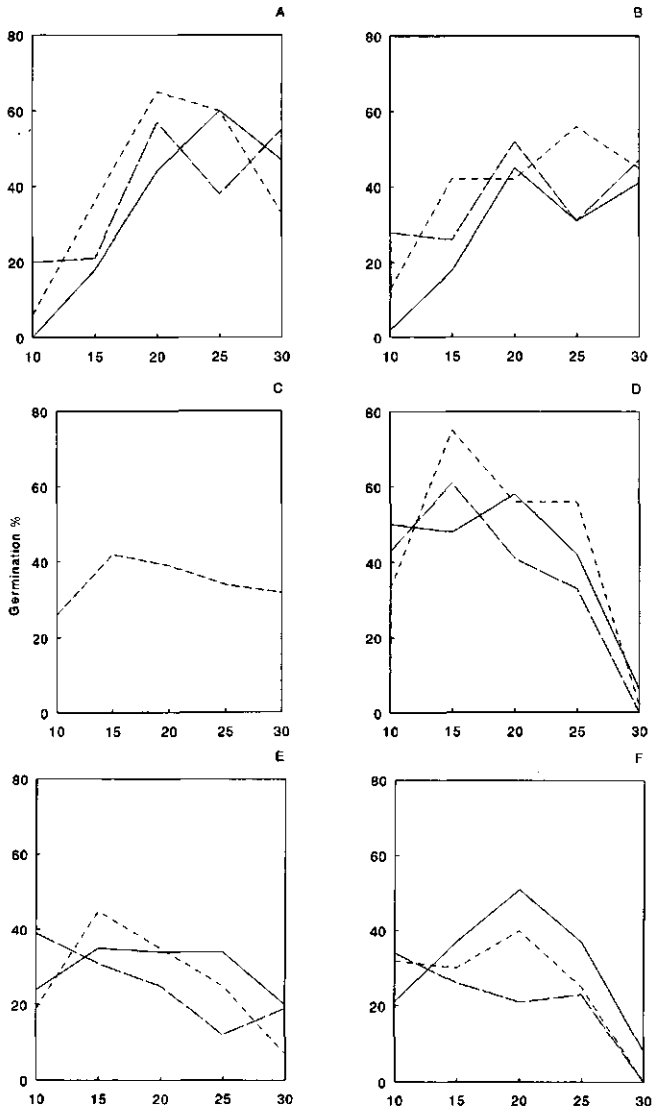


Fig. 5.1. Final germination of accessions at different temperatures

Germination response of fresh fruit balls (treatment A), fruit balls cold stratified for 8 weeks at 4°C (treatment B), and fruit balls stored for more than year at 4°C (treatment C).

Fig. 5.1.a *B. vulgaris* subsp. *maritima* accession Burghsluis
Fig. 5.1.b *B. vulgaris* subsp. *maritima* accession het Zwin
Fig. 5.1.c *B. vulgaris* subsp. *maritima* accession Perpignan
Fig. 5.1.d *B. vulgaris* subsp. *maritima* accession Cyprus
Fig. 5.1.e *B. vulgaris* subsp. *adanensis*
Fig. 5.1.f *B. macrocarpa*

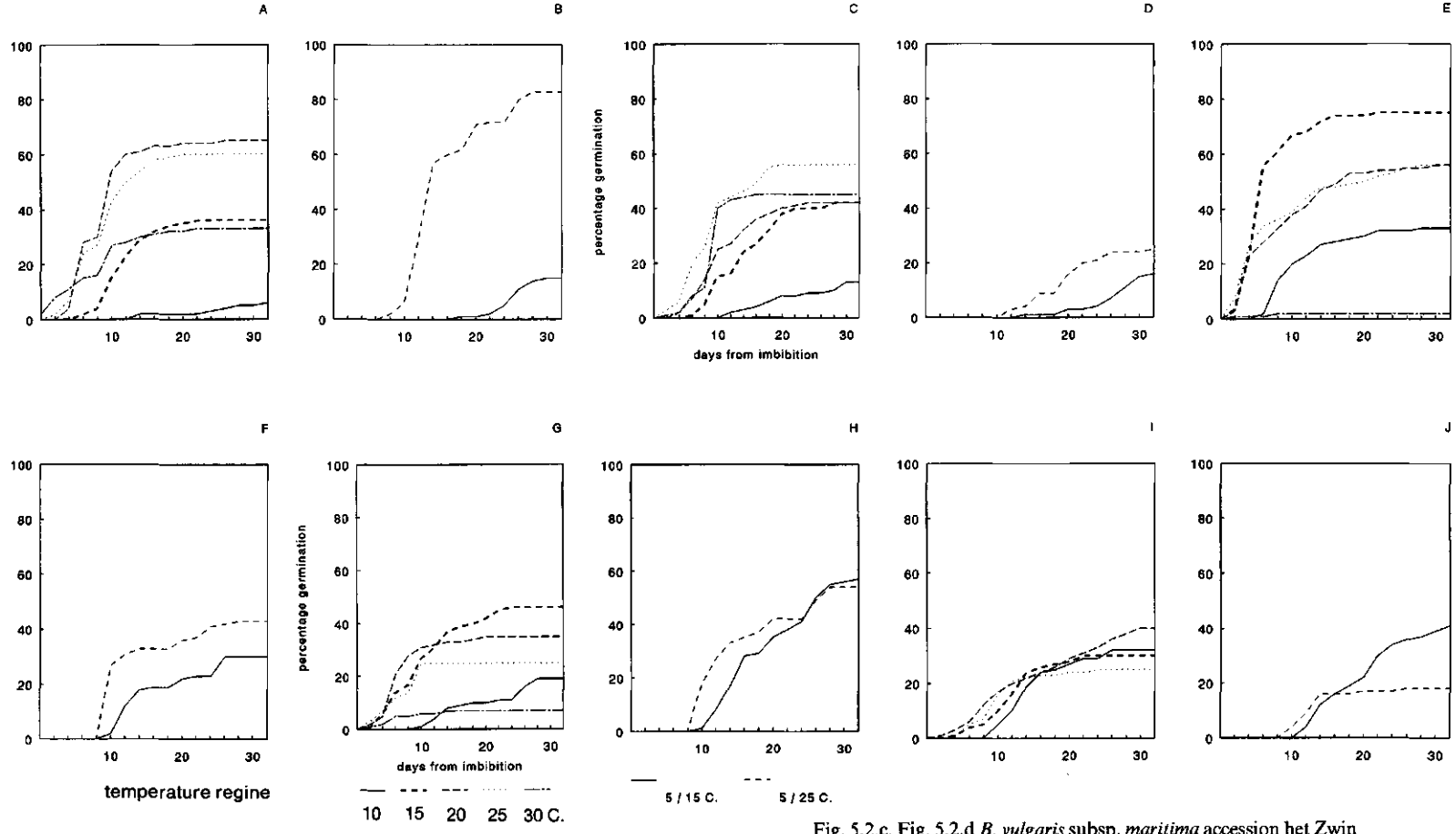


Fig. 5.2. Cumulative germination of accessions at various temperature regimes.
Fig. 5.2 a,c,e,g,i: temperature regime constant, fruit balls cold stratified.
Fig. 5.2 b,d,f,h,j: temperature regime fluctuating.
Fig. 5.2.a, Fig. 5.2.b *B. vulgaris* subsp. *maritima* accession Burghsluis
Fig. 5.2.c, Fig. 5.2.d *B. vulgaris* subsp. *maritima* accession het Zwin
Fig. 5.2.e, Fig. 5.2.f *B. vulgaris* subsp. *maritima* accession Cyprus
Fig. 5.2.g, Fig. 5.2.h *B. vulgaris* subsp. *adanensis*
Fig. 5.2.i, Fig. 5.2.j *B. macrocarpa*

was delayed by ca. 15 days (Burghsluis) respectively 9.5 days (het Zwin) as compared with constant 25 °C regime (Table 5.3).

The subsp. *maritima* population from Perpignan was tested only with long stored fruit balls. Any stratification effect could not be determined. The fruit balls tested responded with moderate germination at all temperature regimes without any clear optimum (Fig. 5.1). Highest germination, 42%, occurred at 15 °C. Low temperature (10 °C) and alternating temperatures retarded germination on an average of seven to ten days (Table 5.3).

B. vulgaris subsp. *maritima* from Cyprus showed no specific optimum germination temperature. At all temperatures germination was between 40% and 60% except at the 30 °C temperature regime when germination was almost absent (Fig. 5.1). The effect of stratification of the fruit balls was a small increase of germination at 15 °C, but a decrease at 10 °C. At the higher temperature regimes no clear effect was seen. Long-stored fruit balls gave an almost identical germination response as the non-stratified fruit balls: high germination occurred at 10 °C, 15 °C and 20 °C, and low germination at 30 °C. In addition, the population was responsive to fluctuating temperature regimes as ca. 40% germination occurred at 5/15 °C and 5/25 °C (Fig. 5.2).

The rate of germination was relatively high, both at the higher and the lower temperatures. After two days of imbibition germination is rather explosive, at 15 °C, 20 °C, 25 °C and 30 °C. Under fluctuating temperatures start of germination is 4 to 6 days later than at constant temperature regimes (Fig. 5.2). The Cyprus accession has the lowest Mean Germination Day (Table 5.3). At higher temperatures (20 °C and 25 °C) mean number of days to germination was 9.3 and 7.8 respectively, but at 15 °C mean number of days to germination was shorter, 6.2 days.

For *B. vulgaris* subsp. *adanensis* no specific temperature optimum was noted. Germination was low, not more than 35%, and almost equal at all constant temperature regimes. (Germination was only little reduced at 10 °C and 30 °C, Fig. 5.1). *B. vulgaris* subsp. *adanensis* gave higher responses at fluctuating temperatures (Fig. 5.2). Little stimulus for germination after seed stratification could be observed (Fig. 5.1). Germination was enhanced at 15 °C, but final germination was still below 50%. Long stored seeds gave 15% more germination at 10 °C, but at the other temperature regimes germination was less.

Optimum temperature for germination for *B. macrocarpa* is 20 °C (Fig. 5.1). Similar germination response was observed at 15 °C and 25 °C. At 10 °C still 21% germination occurred, but germination was less than 10% at 30 °C. Seed stratification did not lead to higher germination. 11% more germination was observed at 10 °C, but germination was less at other temperatures.

Long stored fruit balls gave a rather low germination response. At 20 °C germination was only half the amount of the treatment with fresh, non stratified

fruit balls. However, better germination was seen at the lower temperatures. In contrast to the *B. vulgaris* subsp. *maritima* accessions, more germination at 5/15°C (41%) than at 5/25°C (18%) was observed. At 5/15°C germination started after 10 days and continued gradually. At the end of the observation period fruit balls were still germinating (Fig. 5.2). At all other temperature regimes germination was relatively slow also. Induction time for germination is longer, and the seed population is more polymorphic for time of germination, irrespective of temperature conditions.

5.3.4 Germination in the field

Field observations on germination pattern in the north Atlantic populations of het Zwin and Burghsluis showed that germination of fruit balls may happen in autumn. On October 15th germination was observed in both populations. In population het Zwin it was observed that besides seedling plants, rosette plants in all sorts of size classes were present (Table 5.4). The presence of small rosettes indicated that germination had proceeded continuously, may be even from July onwards.

In the population of Burghsluis thousands of seedlings (all in cotyledon stage) were present at the time. It was clear that fruit balls germinated immediately after fructification in autumn. Germination from an older seed bank could be ruled out, since all seedlings were of the same age. Adult plants with seeds had been mown along with the adjacent dyke vegetation, and produced mass germination. Due to incomplete development of the seeds, induction of dormancy

Table 5.4. Field germination and age structure in *Beta vulgaris* subsp. *maritima* populations from the Netherlands.

		population			
stage	rosette size (diameter), number of leaf pairs	het Zwin*		Burghsluis**	
		15 Oct. 1991	6 March 1992	15 Oct. 1991	6 March 1992
seedlings		113	–	> 10,000	–
non-flowering plants					
	5 cm, 5	76	138	210	1051
	10 cm, 12-16	86	(77)***	18	(26)
	20 cm, 16-25	45	(32)	14	(14)
	35 cm, 25-35	23	(38)	3	(4)
	60 cm, > 25	4	(14)	1	(5)
flowering plants		95	–	5	0

* het Zwin: total population
** Burghsluis: 2 × 2 meter transect
*** ( ): roughly estimated from number and size of old leaves

in the fruit balls had probably not occurred. When both populations were visited again in March 1992, no seedlings were present. In Burghsluis the 5 cm rosette size class was predominant (Table 5.4). It is certain that these rosettes were the surviving seedlings from the previous year. After a relatively moderate winter, many rosettes had survived.

Observations in the field show that *B. vulgaris* subsp. *adanensis* is capable of germination immediately after seed ripening. Accessions which were evaluated in a field trial in Wageningen (Chapter 2) readily dehisced seeds after flowering and instantaneous germination (acc. 2199 and 1497 in particular) was observed in the beginning of September. (In contrast: *B. macrocarpa* accessions in the field trial showed less than 0.01% immediate germination after ripening). Additional observations from Cyprus confirmed instantaneous germination of *B. vulgaris* subsp. *adanensis* if moisture conditions were favourable. The species occurs on Cyprus on pebble beaches and in ruderal sandy places near the sea, but also in irrigated agricultural fields close to the sea. In June no seedlings were found on the beaches and ruderal sites where most plants had withered. At these sites a seed bank with high densities of fruit balls was present. In the irrigated fields many seedling plants, rosette plants, flowering as well as fruiting plants were seen, indicating continuous germination under adequate moisture conditions.

B. macrocarpa flowers in March and April. It was noted during seed collection in Portugal and south western Spain in the second half of May that *B. macrocarpa* had completely ripened fruit balls and that all plants had withered (Frese et al. 1990). No signs of young plants, seedlings or germinating fruit balls were encountered at the time of visit in May and during a later visit in August. This was also the case for the population visited on Cyprus in the first week of June. Apparently field germination of *B. macrocarpa* is restricted to late autumn, winter and early spring.

5.4 Discussion

The results show that proportions of fruit balls of all accessions tested are able to germinate shortly after flowering and seed set. Generally, the difference between the north Atlantic populations of *B. vulgaris* subsp. *maritima* on the one hand, and the Cyprus populations of *B. vulgaris* subsp. *maritima*, *B. vulgaris* subsp. *adanensis* and *B. macrocarpa* on the other hand seems to be the fact that the latter taxa are relatively less sensitive to temperature effects on germination.

5.4.1 Response of the north Atlantic accessions

The observation that the northern accessions het Zwin and Burghsluis were unable to germinate at low temperatures may indicate the presence of dormancy in the seeds. Further evidence for dormancy is given by the fact that stratification at 4°C generally enhanced germination, especially at the lower temperature

regimes, and most clearly in the fruit balls which were long stored. Apparently, dormancy was released in the non-stratified fruit ball batch which was adjusted to a 5°/25°C regime. Fig. 5.2 showed that it took 10 days of imbibition before any germination could be observed, but then the fruit balls rapidly germinated to a final 83%.

In the northern populations induction of flowering depends on vernalization (Chapter 4). All north Atlantic plants require intense vernalization for flowering induction. Whether or not a plant will flower in the next season, will be decided by the time of germination, and the state of development of the rosettes before the time of vernalization. Before the winter a rosette of adequate size can be developed in summer or autumn seedlings which receive enough vernalization to induce flowering in the next growth season. Spring seedlings will not render flowering plants in the same growth season.

5.4.2 Response of Mediterranean accessions

There are no clear indications for a temperature-controlled dormancy pattern in the fruit balls of the Mediterranean *B. vulgaris* subsp. *maritima* accessions. The Cyprus accession of *B. vulgaris* subsp. *maritima* showed relatively high germination at all temperatures regimes, specifically the low temperatures, and no clear response following seed stratification. Similarly, the Perpignan accession showed unreduced germination at the low temperature regimes. The response of the Mediterranean accessions to germination at low temperatures clearly deviated from the response of the temperate climate accessions het Zwin and Burghsluis in the experiments.

In *B. vulgaris* subsp. *adanensis* germination is low under any temperature regime. Stratification pretreatment or alternating temperature regimes did not clearly enhance germination. The influence of different temperature conditions on germination was apparent only in the *rate* of germination (induction time) of the seed samples. Compared with the other annual species *B. macrocarpa*, in *B. vulgaris* subsp. *adanensis* the rate of germination is higher at high temperature regimes (Fig. 5.2).

No indications were found that germination in the species *B. macrocarpa* was influenced by temperature pretreatment of the fruit balls or by alternating temperatures during the germination test. In *B. macrocarpa* germination is slow, irrespective of temperature conditions (Fig. 5.2).

If it is not dormancy that regulates germination in *B. macrocarpa*, there must be other mechanisms that prevent germination directly after seed set. Rather than temperature conditions mechanical factors may limit germination in *B. macrocarpa*. The operculum of this species is fixed more tightly to the pericarp, preventing easy protrusion of the radicle. The penetration of moisture to the seed through the tough pericarp is limited, and thus the species may avoid germination after incidental rainfall.

In *B. vulgaris* subsp. *adanensis* the operculum is thickened and more succulent,

enabling quicker absorption of moisture. The operculum is more readily released from the pericarp after absorption of moisture, admitting moisture to the seed. Despite this case, the species has a high remnant percentage of dormant fruit balls, which do not react to favourable moisture conditions. The dormancy in *B. vulgaris* subsp. *adanensis* is not quickly relieved by temperature as was shown, and it is not known which other factor is involved. Perhaps there is a high proportion of non viable seeds in this seed accession.

It was noted during collection visits that *B. macrocarpa* is occasionally a species of highly saline habitats. The distribution of the species in Portugal is almost restricted to the 'Salinas', salt marshes which are sometimes commercially exploited (Frese et al. 1990). The highly saline environment requires that fruit balls of *B. macrocarpa* must be able to germinate in relatively high concentrations of sea water, or they must be able to retain the capacity to germinate at the onset of lowered salinities in rainy periods which occur mainly in winter time. *B. macrocarpa* shows some adaptive features which could be related to postponement of seed germination and which make the species distinct from *B. vulgaris* s.l. Firstly, the fruit balls of *B. macrocarpa* stay attached to the maternal plant, even long after withering of the plants. In late August only limited fruit ball dehiscence was observed in *B. macrocarpa*. Secondly, it is believed that after dehiscence, most of the fruit balls remain at the site of dispersal. In the close vicinity of much larger maternal plants many tiny plants were found crowded together. The fruit balls of *B. macrocarpa* are heavier and less rounded than those of *B. vulgaris* s.l., due to the fact that tepals stay erect and patent. These features cause dispersal of fruit balls by wind or by (sea) water to be less efficient. With reduced dispersal a very localised seed bank of dormant fruit balls is built. For an annual species the presence of such a dormant seed bank can be of adaptive significance as it ensures local survival of very small populations over several years, even in years with high salinity and unfavourable rainfall. The other annual subspecies, *B. vulgaris* subsp. *adanensis*, seems to follow a more opportunistic strategy, as it is adapted to invest arable fields where, due to irrigation, germination conditions are not limited to a specific period of the year.

6 Analysis of morphological variation in wild beet (*Beta vulgaris* L.) from Sicily*

6.1 Introduction

Wild relatives of the sugar beet are increasingly used in breeding programmes to improve the pest and disease resistance of the crop (Van Geyt et al. 1990[a]). Samples of wild beet are collected and preserved by genebanks. Currently, more than 2300 accessions of wild *Beta* section *Beta* germplasm are stored world-wide in various *Beta* holdings (Frese 1992). To what extent this amount of germplasm provides a reasonable and economic representation of existing genetic diversity is not known. Today, the collecting strategy for wild beets mainly relies on taxonomical and geographical criteria. More detailed information on the structure of genetic variation at the population level would allow a more efficient preservation of the genetic resources. However, neither the extent of genetic differentiation nor the factors involved in this process are fully understood as yet.

The sea beet *Beta vulgaris* subsp. *maritima* (L.) Arcang. is distributed along the Atlantic and Mediterranean coasts. Macro-geographic variation patterns of morphological characters were described by Doney (1992) and Letschert (this thesis). The north Atlantic sea beet is later flowering, has more succulent leaves, fewer leaf hairs, shows less seed shattering, and a more procumbent to prostrate growth habit than the Mediterranean forms.

Morphological evidence (Doney 1992) and the distribution pattern of a specific allozyme *Acp1*-2 (Chapter 3) suggest the existance of a spatially isolated Atlantic and Mediterranean gene pool. Within a more limited geographic area variation patterns are less clear. Doney (1992) found significant morphological differences between neighbour populations distributed along the French coast line. However, mean values for morphological characters were usually not unique to specific collection sites, but could be found at one time in distant populations. Furthermore, considerable variation occurs in large populations at undisturbed sites (Doney 1992, Frese et al. 1990). Such populations may encompass a major part of the total genetic variation present within a geographic entity, i.e. an island or coast intercept.

The sea beet originating from Mediterranean localities, shows considerable morphological variation. The dynamic and variable habitat is probably one of the factors to cause this morphological polymorphism.

6.1.1 The habitat

Wild beets mainly occur in a restricted zone near the sea shore. They show a preference for heavy alluvial soils and clays at disturbed sites. They are found

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at the upper part of beaches where wastage is deposited by the high tide. Populations of beet may also be found at more sandy soils or at rocky coasts where plants are dispersed vertically as seeds and branches may be carried up by the water and deposited in crevices at considerable height. On pebble beaches, on moderate saline grounds or in salt marshes prostrate growing plants with small succulent leaves may be seen more frequently than elsewhere. (Doney et al. 1990, Frese et al. 1990). On Sicily in particular, wild populations of beet may be found inland, far away from the marine environment. They thrive as weeds on waste grounds, at roadsides, at field and plantation borders and in dry river beds (Toll & Hendriksen 1982). The environment may be open or densely vegetated. In closed vegetation beet plants tend to be more vigorous and erect, enabling them to compete with other species.

Differences in habitats may bring about different selective forces acting upon gene frequencies in populations. It may lead to shifts in morphological and developmental characters and to the establishment of discrete populations. Maintenance of differences between locally adapted populations will depend strongly on the relative importance of selection and on the potential for gene flow between adjacent populations.

6.1.2 The potential for gene flow

Both wild and cultivated representatives of *Beta vulgaris* are outbreeding, self-incompatible, wind-pollinated species (Larsen 1977, Dale & Ford-Lloyd 1983). However, through a system of pseudo-compatibility (Larsen 1983) isolated plants may temporarily turn to selfing, permitting survival of small populations. Plants produce huge amounts of seeds which are distributed by seawater. Thallasochorous dispersion of seeds is very effective in enabling a species to colonize new localities along the sea shore. The seeds may float for days in the sea before reaching land, without loosing germination capacity (Tjebbes 1933). Besides an effective dispersal of seeds, pollen may be dispersed over considerable distances (Tyldesley 1978). Thus it is likely that gene flow among sea shore populations is not obstructed. However, the actual extent of gene flow was found to be low in *B. vulgaris* subsp. *maritima* populations from the Atlantic coast of France (Cuguen et al. 1992). The dispersal of germplasm to inland areas may be even more difficult and it can be hypothesized that inland populations are isolated from coastal populations.

6.1.3 Ecogeographical diversity on Sicily

In 1981 Toll & Hendriksen (1982) collected 98 samples of wild beet populations and 8 samples of cultivated beet on Sicily. The island is described as ecogeographically highly diverse. The samples were gathered from coastal areas and from inland mountainous sites, up to 1000 meter above sea level. Collecting wild *Beta* germplasm, Toll & Hendriksen (1982) noted that 'variability in morphological characters was greater between populations of beet than within populations'. During the expedition they classified populations as 'maritime wild'

Table 6.1. Collection sites of 35 accessions of *Beta vulgaris* ssp. *maritima* from Sicily.

Enr.	Rec.nr.	BGRCnr.	Collec.nr.	Location	Long.	Lat.	Alt.
01	869202	028924	si/81 078	Palermo	01320E	03807N	50
02	869204	028926	si/81 088	Trabia beach	01339E	03800N	0
03	869205	028927	si/81 089	Caccamato to Roccapalumba	01339E	03755N	520
04	869209	028931	si/81 096	Aquedelei e of St. Stefano	01437E	03803N	0
05	869269	028909	si/81 055	Mazara, 10 km from, to Salemi	01240E	03744N	175
06	869191	028913	si/81 063	East of Favignana	01220E	03756N	0
07	869197	028919	si/81 070	Terrasini W of Palermo	01304E	03810N	0
08	869252	028892	si/81 030	Vittoria, strada buffa, casa mazza	01439E	03654N	25
09	869148	028951	si/81 120	Sperling west of Nicosia	01432E	03746N	650
10	869257	028897	si/81 036	Frigintini to Giarratana	01448E	03657N	520
11	869259	028899	si/81 039	Chiaramonte, nearby, to Comisa	01442E	03702N	450
12	869260	028900	si/81 041	Comunelli river, mouth, Gela to Licata	01408E	03706N	0
13	869261	028901	si/81 042	Marina di Palma	01344E	03710N	0
14	869239	028879	si/81 013	Fontane bianche south of Syracruse	01514E	03658N	50
15	869240	028880	si/81 016	Calabernado s of Avola	01508E	03658N	0
16	869243	028883	si/81 020	Syracuse, teatro greco	01516E	03706N	50
17	869244	028884	si/81 021	la Maddalena south of Syracuse	01518E	03702N	25
18	869245	028885	si/81 022	Syracuse, capo Murro di Porco	01520E	03700N	25
19	869246	028886	si/81 023	Fontane bianche to Avola	01511E	03658N	40
20	869249	028889	si/81 027	Marina di Ragusa to Donnalucata	01434E	03650N	0
21	869250	028890	si/81 028	Irminio river/rd. San Croco	01438E	03648N	75
22	869251	028891	si/81 029	Camarina, mouth of river Ippari	01426E	03652N	0
23	869215	028937	si/81 105	Milazzo, close to station	01515E	03813N	120
24	869216	028938	si/81 106	Tono (Milazzo)	01515E	03815N	0
25	869217	028939	si/81 107	Oliveri near Falcone	01504E	03808N	0
26	869230	028870	si/81 002	Borsalino near Belpasso	01500E	03728N	50
27	869234	028874	si/81 007	Palagonia, 5km before junct. Catan.-Gela	01452E	03723N	0
28	869238	028878	si/81 012	Riposto, 1km from, to Catania	01513E	03743N	0
29	869212	028934	si/81 101	Cesaro, 5km from, to Randazzo	01443E	03751N	1150
30	869213	028935	si/81 102	San Domenica Vittoria	01458E	03755N	1027
31	869265	028905	si/81 047	Serradifalco to San Cataldo	01355E	03702N	550
32	869223	028945	si/81 114	Geracello, Enna to Barrofranco	01414E	03727N	500
33	869226	028948	si/81 117	Alimena, 2km from, to Petralio	01408E	03743N	600
34	869227	028949	si/81 118	Fasano, Alimena to Petralio	01407E	03747N	830
35	869228	028950	si/81 123	Pergusa to Piazza Amerina	01420E	03731N	650

Enr. = number of the population in the analysis.
Rec. nr. = receipt number of the Centre for Genetic Resources (CGN), Wageningen, the Netherlands.
BGRC nr. = genebank accession number of the Institute for Crop Science (FAL), Braunschweig, Germany. Coll. nr. = Colllection number by Toll & Hendriksen (1982).
Long., Lat., = geographical longitude and latitude of the sample site.
Alt. = sample site altitude.

or as 'inland wild'on the basis of certain morphological characteristics. However, later they doubted the validity of doing so, because they were unable to estimate the influence of environmental conditions at the collection site on these characters, as for instance drought, salinity and soil type.

In this paper the variation pattern within a subset of the Sicilian *Beta* collection is analysed. Accessions from Sicily were grown under controlled conditions and were evaluated morphometrically. The objective was to identify geographical distribution patterns of morphological characters and to reassess the putative classification of the collection into 'maritime' and 'inland' populations.

6.2 Materials and methods

A selection of 35 accessions from the collection made by Toll & Hendriksen (1982) was evaluated. The accessions originated from coastal areas in the north, the south and the east, and from inland sites (Table 6.1 for relevant passport data). Seed was sown in the greenhouse in April and seedlings transplanted to the field in May. A block design with three replications and ten plants per replication was chosen. A total of 15 characters were studied, 10 of which were used for later numerical analysis. All characters and their mode of assessment are given in Table 6.2. Each character was assessed in all three replications, except for character Stem diameter, which was only assessed in two replications. An univariate analysis was conducted on ten characters. In addition to univariate analysis a canonical variate analysis was attempted to examine the extent to which hypothetical groups could be discerned. In a multiple discriminant analysis (SPSS/PC programme package) the probability of an accession to belong to either an 'inland' type of wild beet, or a 'maritime' type was assessed. Thus, groups were defined on the basis of population origin: Populations collected at inland sites a priori were designated to group 1 (14 populations). Populations from coastal areas were dessignated to group 2 (15 populations).

Six accessions were treated separately, because they contained plants that were collected in the vicinity of beet cultivation areas or in private gardens[4]. These accessions were not classified in group 1 or group 2, but they were named 'ungrouped' and are referred to as group 3. In later instance the variation pattern of these accessions was compared to that of the 'wild' accessions from group 1 or 2.

[4] In a few accessions plants with characteristics of a Swiss Chard cultivar were present: Plants with large darker green leaves and broad white petioles and plants with light green leaves and long petioles.

According to Toll & Hendriksen (1982) the cv. Costa Argentata is cultivated on Sicily on a very limited scale. These plants are left to set seed and can become escapes. Escapes from cultivation or spontaneous plants resulting from hybridization with wild plants are not rare phenomenona in beet (Ford-Lloyd & Hawkes 1986, Evans & Weir 1981, Hornsey & Arnold 1979). However, Toll & Hendriksen (1982) state that introgression from cultivated to wild plants is unlikely on Sicily, because large scale cultivation is very limited. However, they draw attention to the habit of local people to grow wild beet plants for consumption in their gardens.

Table 6.2. Characters and character states used in the study.

1.	Pigmentation	
	– slight	
	– intermediate	
	– strong	
2.	Leaf pubescence	
	– glabrous	
	– very sparse	
	– hairy	
	– very hairy	
3.	Bract shape	
	– elliptic/rhomboic	
	– obovate	
4.	Multigermicity	
	(number of flowers per glomerule)	
	– one or two flowers per glomerule	
	– up to 4 flowers per glomerule	
	– up to 7 flowers per glomerule	
5.	Growth habit	
	plant erect, main stem prominent, branches in the upper region short, lower branches long and erect.	1
	plant erect; main stem not prominent branches erect and long, exceeding central stem.	2
	plant spreading; main stem prominent, long and erect. branches long and decumbent.	3
	plant spreading and prostrate, main stem not prominent, branches decumbent.	4
	plant prostrate; branches long and prostrate.	5
6.	Flowering stage in period I (Flowering I)	
	period I: 55 – 62 days after germination of plants	
7.	Flowering stage in period II (Flowering II)	
	period II: 113 – 120 days after germination of plants	
	scores for 6 and 7:	
	plants vegetative (rosette stage)	1
	bolting stems developing first stage	2
	bolting stems developing second stage	3
	flowering less than 10%	4
	flowering 10 to 50%	5
	more than 50% or flowering completed	6
	seed set completed	7
8.	Lamina length (cm)	
9.	Lamina width (cm)	
10.	Lamina thickness (0.01mm)	
11.	Petiole length (cm)	
12.	Petiole width (cm)	
13.	Stem diameter (mm)	
14.	Biomass (kg)	
15.	Plant height (cm)	

6.3 Results

6.3.1 Variation of the characters

Variation was observed in a number of qualitative parameters like Pigmentation, Leaf pubescence, Bract shape and Multigermicity (number of flowers per glomerule). Plants of most accessions had slight pigmentation in the stems. A

few plants had intermediate pigmentation with the red extending into the leaves. No plants showed strong pigmentation. Moderately pubescent plants could be found in the majority of the accessions. Strongly pubescent plants were absent. Differences in bract shape were small between and among accessions. Multigermicity was not a variable character in accessions from Sicily. The most common class was represented with plants having up to four flowers per glomerule. Plants with more flowers were present in about 20% of the accessions. Five morphological types were noted with respect to character growth habit. Predominance of a main shoot and formation of first order and second order offshoots results in a erect type of plant, while predominance of equivalent first order offshoots may lead to a more patulous, bushy type of plant. Differences in branching pattern were also accentuated by the relative length of the offshoots and the way they vertically or horizontically spread from the main shoot. In many instances different types could be found in one accession. Type 2 and 3 were the most common in the accessions and type 4, which was very distinct was found at a low frequency in many type 2 accessions as well as in accessions which were predominantly type 5. The prostrate type 5 plants were characteristic for south east Sicilian coastal populations (accessions numbered 14, 15, 17, 19, 22; Table 6.1) and a number of inland populations (accessions numbered 5, 26, 27 and 35).

A variance analysis showed high variation for characters Lamina length, Lamina width, Lamina thickness, Petiole length, Petiole width, Stem diameter, Biomass (fresh weight), Plant height, Flowering I, and Flowering II (Table 6.3). A few of the Group 3 accessions accounted for considerable skewness in the frequency distribution of characters Petiole width, Biomass, Flowering stage and Stem diameter. These accessions were not included when calculating the discriminant function.

Mean values for morphological characters are reported in Table 6.3. In Fig. 6.1 values for some of the characters are presented graphically for neighbouring populations. Significant differences between adjacent populations can be found in many of the characters. Large fluctuations between adjacent coastal populations prevail for characters Petiole length, Leaf length, Leaf width and Biomass. Comparing coastal northern, eastern and southern populations a tendency towards decreasing Leaf length and decreasing Leaf width is seen. However, population 19 and 12 do not fit in the trend. For character Leaf thickness and character Flowering II fluctuations between the populations were less pronounced. No single variable unambiguously reflects a regional or geographical pattern.

6.3.2 Discriminant analysis

A few characters showed significant variation between group 1 (inland) and group 2 (maritime). Maritime populations bolted and flowered earlier than inland populations (univariate F ratios for Wilks' lambda were significant at the 0.05 level for character Flowering I and Flowering II. The pooled within groups correlation between the characters Flowering I and Flowering II was 0.48).

Table 6.3. Measurements and variability of the characters.

Mean values, population 1 through 35.
Variables are (from left to right): Flowering period I, Lamina length, Lamina width, Lamina thickness, Petiole length, Petiole width, Stem diameter, Biomass, Plant height, Flowering period II.

Entry	FlowI	LLen	LWid	LThi	PLen	PWid	Stem	Biom	Heig	FlowII
01	2.40	8.39	6.23	0.553	7.70	3.67	12.01	1.931	96.6	5.60
02	2.43	6.56	4.95	0.565	4.83	2.91	8.89	1.221	86.0	5.70
03	2.40	5.93	4.83	0.588	7.63	2.87	7.39	1.193	70.8	5.63
04	2.70	8.56	6.75	0.567	7.97	4.29	16.59	1.613	98.6	5.97
05	2.00	4.74	4.05	0.775	7.18	2.72	4.83	1.128	49.8	5.93
06	2.20	6.10	5.21	0.816	5.56	3.12	6.18	1.020	53.8	5.53
07	2.17	8.64	6.86	0.628	5.25	3.43	13.08	1.974	88.9	5.80
08	2.33	8.96	7.27	0.582	6.63	3.70	12.60	2.110	93.7	5.70
09	1.00	11.87	8.31	0.461	7.26	5.05	10.00	2.379	56.5	1.03
10	2.27	5.25	4.25	0.613	6.60	2.77	6.34	1.451	63.7	6.07
11	2.50	7.74	6.19	0.599	5.72	3.13	10.46	1.397	86.5	5.87
12	2.57	6.68	5.17	0.638	6.07	3.06	9.77	1.551	77.5	5.93
13	2.80	4.60	3.58	0.628	6.34	2.72	4.78	.624	39.5	5.90
14	2.63	5.32	4.10	0.628	5.14	2.40	7.57	1.087	66.7	5.80
15	2.93	4.62	4.06	0.659	4.45	2.47	4.88	.675	42.3	5.97
16	3.10	5.21	4.10	0.594	4.80	2.71	10.07	1.014	73.2	5.97
17	2.70	5.26	4.10	0.583	5.58	2.38	7.28	.859	60.9	5.77
18	2.67	5.52	4.31	0.820	6.37	2.75	7.96	1.191	70.8	6.00
19	2.23	8.30	6.00	0.652	7.52	3.16	8.65	1.518	89.0	5.50
20	2.07	10.30	7.49	0.681	7.42	4.77	21.66	2.184	97.0	4.97
21	2.17	7.43	5.66	0.537	6.33	3.23	10.73	1.417	94.6	5.87
22	3.07	5.81	4.64	0.691	5.56	2.80	7.28	.996	50.5	5.77
23	3.60	8.24	6.51	0.540	5.27	3.80	10.18	1.895	89.7	6.00
24	2.67	7.04	5.60	0.638	7.13	3.80	10.44	1.568	87.1	5.90
25	2.73	7.95	5.79	0.503	5.58	3.49	11.69	1.415	84.6	5.97
26	2.33	4.86	3.47	0.578	4.62	2.26	4.95	.719	55.6	5.67
27	2.23	5.03	4.21	0.585	7.36	2.73	5.51	.890	55.3	5.83
28	2.60	7.68	5.60	0.619	6.20	3.09	9.01	1.615	93.1	6.03
29	2.03	5.42	4.29	0.550	7.11	2.77	6.23	1.037	68.9	4.90
30	2.10	8.04	6.57	0.537	7.39	3.46	9.81	1.743	96.2	5.40
31	1.80	4.97	3.95	0.534	7.02	2.53	7.23	.990	69.8	5.10
32	1.63	8.65	6.52	0.587	7.64	6.10	19.22	1.730	89.7	4.83
33	1.27	9.97	7.78	0.566	6.24	6.10	28.36	2.038	89.3	2.93
34	1.70	6.20	5.08	0.592	6.57	3.76	17.26	1.352	84.3	4.63
35	2.03	5.32	4.31	0.570	7.18	2.74	5.28	.731	47.5	5.93
LSD(0.05)	0.37	0.88	0.74	0.056	0.99	0.58	3.79	.408	11.0	0.43

Variability of the characters

Character	minimum	mean	maximum	m.s.$_{(acc)}$	m.s.$_{(resid)}$	F
Flowering period I	1.00	2.35	3.60	7.78	0.54	14.34*
Lamina length (cm)	4.60	6.89	11.87	102.27	3.02	4.57*
Lamina width (cm)	3.58	5.37	7.78	50.47	2.15	23.43*
Lamina thickness (0.01 mm)	4.6	6.1	8.2	1.8	0.10	14.76*
Petiole length (cm)	4.45	6.38	7.97	30.43	3.83	7.95*
Petiole width (cm)	2.26	3.35	6.10	26.74	1.33	20.17*
Stem diameter (mm)	4.78	10.03	28.36	518.47	37.46	13.84*
Biomass (kg)	0.62	1.38	23.79	23.59	22.64	9.98*
Plant height (cm)	39.53	74.71	98.60	9651.30	476.0	20.28*
Flowering period II	1.03	5.47	6.07	28.42	0.72	39.68*

d.f. (acc) = 34, d.f. (resid) = 945, except for character Stem diameter where d.f. (resid) = 630.
* = significant at $P_{(0.001)}$.

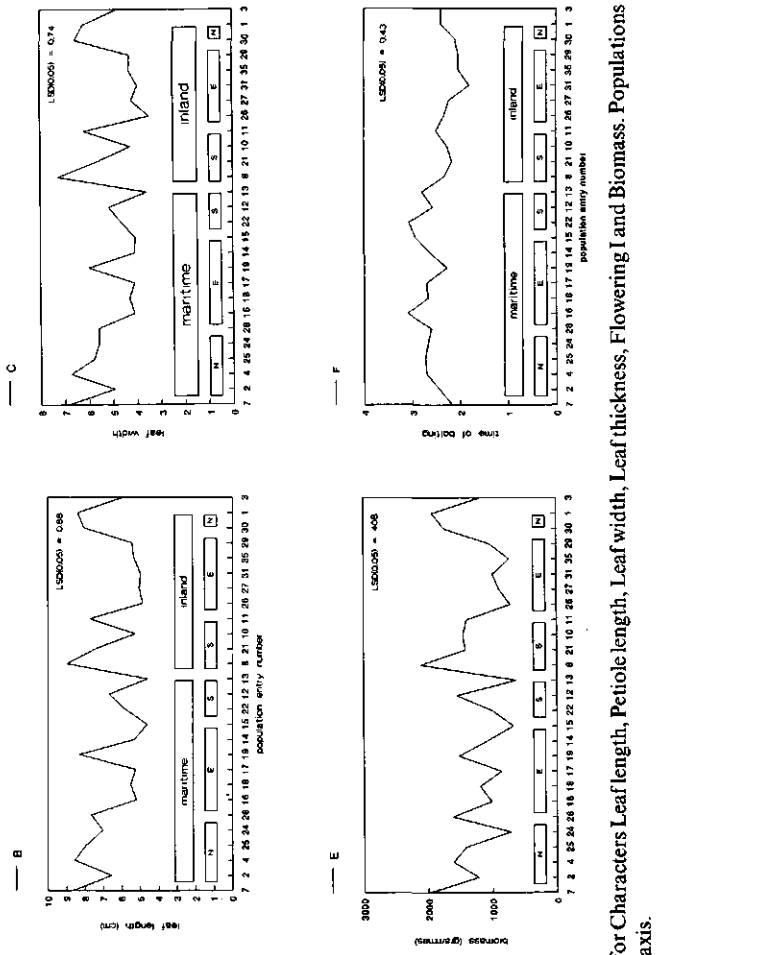

Fig. 6.1. Variability among adjacent populations for Characters Leaf length, Petiole length, Leaf width, Leaf thickness, Flowering I and Biomass. Populations are indicated by Entry number (Table 1) on the X-axis.

Table 6.4. The selected variables for discriminant analysis and the pooled within groups correlation between the variables and the canonical discriminant function.

character	correlation	character	correlation
Flowering I	0.40	Leaf length	0.05
Petiole length	–0.28	Biomass	–0.04
Flowering II	0.28	Petiole width	0.03
Leaf thickness	0.25	Plant height	0.02
Stem diameter	0.16	Leaf width	0.01

Group means for all other variables only slightly differed and variation between the two groups was non-significant (Wilks' lambda, univariate F ratio's).

On the basis of 10 variables a canonical discriminant function was calculated for the two putative groups of accessions. It appeared that characters Flowering I, Petiole length, Flowering II and Leaf thickness had the highest grouping value as could be deduced from their correlation with the discriminant function. The order of importance of the characters from the set chosen for use in the analysis can be read from Table 6.4.

As was indicated earlier, parameters of generative development were important for separation of the groups. In addition, Petiole length, Leaf thickness and Stem diameter contributed to the discriminant function. Inland populations showed a remarkable uniformity (population 26 excluded) for character Petiole length (cf. Fig. 6.1). Compared to coastal accessions the inland accessions had longer and more narrow petioles, and less succulent leaves.

The one canonical variate separating the two groups of wild populations is shown in Fig. 6.2. Complete separation was not obtained, and there is some overlap between groups. In fact, inland population 26 has a higher chance of belonging to the maritime group (cf. Table 6.4). No entries originally placed

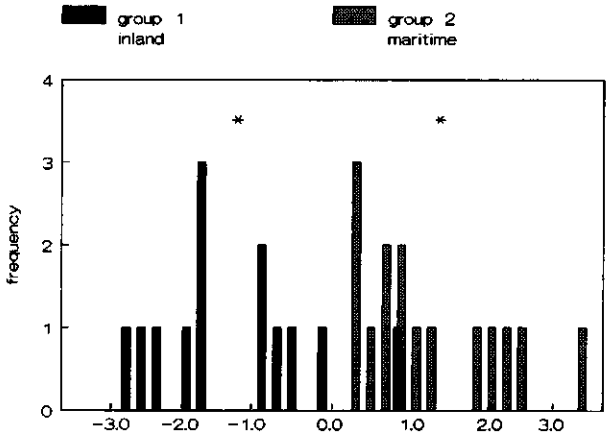

Fig. 6.2. Ordination of the groups on the discriminant axis.

in group 2 were found to be classified incorrectly in this group. A total level of correct classification of 96.43% of grouped entries was achieved.

6.3.3 Classification of the 'ungrouped populations'

The reference entry 33 was a Swiss Chard cultivar collected by Toll & Hendriksen (1982) in a vegetable garden. Classification along the discriminant axis resulted in a exceptionally high score, indicating that it did not belong to any of the two groups (Table 6.5). Entry 20 and 32 had moderately high scores falling outside the range of group 1 and 2. Both accessions carried escaped plants from Swiss Chards. Entry 20 was a highly variable population with large erect plants and prostrate plants with maritime characteristics. It can not be concluded with certainty whether this heterogenous accession had introgression genes from cultivated beet. Entry 6 was reported to be collected in a back garden and therefore not a priori classified in group 1 or 2. The apparent wild nature of this entry according to its discriminant score agreed with the assumption of Toll & Hendriksen (1982) that the plants originated from a wild population. Their comment on entry 9, collected in a mountaneous area at 650 m altitude, was that the plants looked 'maritime in habit' and they assumed that the rocks among which the plants grew were imported from a coastal area. Their impression that the plants might have a maritime origin is in agreement with the classification of entry 9 in the maritime group. A similar explanation may hold for the ungrouped accession 34, which was collected inland near a beet cultivation centre, but was classified as maritime. The ungrouped accession 30 was classified as belonging to the inland group.

6.4 Discussion

The analysis of morphological characters supports the idea that there is only limited genetic and spatial isolation between Sicilian wild beet accessions. Discriminant analysis partly succeeded in separating a group of inland beet populations from coastal populations but the overall morphological differences were small. In addition to the morphological character Petiole length, the rate of generative development was found to be important for distinguishing between the groups.

The slight difference between the maritime and inland group may be interpreted as the result of a shift in gene frequencies. Under natural conditions a sharper contrast between both groups may be observed, attributable to the considerable phenotypic plasticity of the species. Merely, the limited morphological differentiation noted under Wageningen standard conditions shows that inland populations are genetically not much different from coastal populations. The existence of inland populations on Sicily shows that beet populations quickly adjust to different ruderal habitats. Inland areas may be colonized step by step through a range of transitional habitats like river beds, road sites and irrigated fields. Moreover, seeds of wild beets transported by man as described by Toll

Table 6.5. Classification of inland (group 1), maritime (group 2) and ungrouped (ungrpd) population. Actual group membership is compared to that predicted by the discriminant score. The group to which a population actually belongs is listed in the column labeled "actual group". The most likely group for a population based on the discriminant score is indicated. The population that was misclassified using the discriminant score is flagged with asteriks.

Entry Number	Actual Group	Highest Probability Group	P(D/G)	P(G/D)	2nd Highest Group	P(G/D)	Discrim Scores
1	1	1	.40	.75	2	.25	–.5313
2	2	2	.45	.79	1	.21	.4350
3	1	1	.59	.99	2	.01	–1.8966
4	2	2	.43	.99	1	.01	1.9659
5	1	1	.67	.90	2	.10	–.9554
6	Ungrpd	2	.03	.99	1	.00	3.2505
7	2	2	.54	.84	1	.15	.5815
8	1	1	.68	.98	2	.01	–1.7719
9	Ungrpd	2	.98	.96	1	.04	1.3523
10	1	1	.17	.99	2	.00	–2.7385
11	1	1	.21	.51	2	.48	–.1197
12	2	2	.62	.88	1	.12	.6968
13	2	2	.82	.93	1	.06	.9666
14	2	2	.98	.96	1	.04	1.2051
15	2	2	.67	.89	1	.10	.7669
16	2	2	.27	.99	1	.00	2.2842
17	2	2	.36	.72	1	.28	.2785
18	2	2	.03	.99	1	.00	3.2947
19	2	2	.64	.88	1	.11	.7216
20	Ungrpd	2	.00	1.00	1	.00	8.7523
21	1	1	.74	.91	2	.08	–1.0456
22	2	2	.19	.99	1	.00	2.4795
23	1	1	.50	.82	2	.17	–.7053
24	2	2	.30	.65	1	.34	.1631
25	2	2	.56	.99	1	.01	1.7550
26	1**	2	.71	.91	1	.09	.8171
27	1	1	.21	.99	2	.00	–2.6147
28	2	2	.32	.67	1	.32	.2026
29	1	1	.32	.99	2	.00	–2.3520
30	Ungrpd	1	.00	1.00	2	.00	–4.0415
31	1	1	.46	.99	2	.00	–2.0978
32	Ungrpd	2	.00	1.00	1	.00	7.6304
33	Ungrpd	2	.00	1.00	1	.00	13.1974
34	Ungrpd	2	.00	1.00	1	.00	3.9967
35	1	1	.67	.98	2	.01	–1.7853

Entry Number: number of the accession (Table 6.1).
Actual Group: 1 = inland origin, 2 = maritime origin.
Highest Probability Group: highest probability group for the population based on the discriminant score.
P(D/G): probability of the discriminant score for a population, assuming the population to belong to the actual group.
P(G/D): probability of of the discriminant score of a population after designation of the population to the most likeley (or second most likely) group.
Discrim Scores: score of the population on the discriminant axis.

& Hendriksen (1982) will promote gene flow to inland sites.

Bogdyo et al. (1980) reported a discrete patterning of morphological characters for a Sicilian wheat collection, but apparently beet populations are less sharply differentiated ecogeographically. Although adjacent coastal populations exhibit significant variation for particular morphological characters, an evident discrete structure was not perceived. Beet populations distributed along an 1800 km island coast line probably form a polymorphic complex easily interchanging genetic material. The dynamics of the sea shore habitat will cause populations to diminish or go extinct but under favorable conditions adapted genotypes may quickly colonize new sites. In both situations populations will experience a loss of genetic variation. The often large differences between adjacent populations can perhaps be explained by genetic drift through extinction of a part of the population or through founder effects. It would be interesting to learn whether the accessions exhibiting little within population variation may have descended from a few well established polymorphic accessions. If so, a great deal of the Sicilian gene pool will be present in one or a few accessions and gene banks could limit the number of accessions needed to maintain the total genetic diversity to a few highly polymorphic populations. Rationalization would lower the costs of germplasm maintainance, particularly in the case of an outbreeding, wind-pollinated species. On the other side, distinct accessions with limited genetic variation could contain agronomically interesting characters at higher frequencies than polymorphic populations. Hence, it could be easier and less expensive to recover interesting traits from a collection of separate accessions than from bulked material. Bulking will destroy the differentiation in the samples brought about by natural selection.

Although the findings in this chapter point to a lack of a systematic variation pattern of morphological characters, no evidence is provided for a high genetic similarity of groups of populations, and more information on genetic relationships is needed. A rationalization of the Sicilian collection by bulking accessions would be premature, and it would be better to keep the accessions as they were collected.

7 References

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8 Summary

In Chapter 1 an account is given of the historical subdivision of the genus *Beta* and its sections, and the relations of the sections are discussed. Emphasis is given to the taxonomic treatment of wild section *Beta* by various authors. The Linnaean names *B. vulgaris* L. and *B. maritima* L. are lectotypified, resp. neotypified as a basis for a new classification of the section. Based on the experimental evidence described in Chapters 2 and 3 conclusions are drawn with respect to the classification of section *Beta*. The revision of section *Beta* in Chapter 1 acknowledges three species, including *B. vulgaris*, *B. macrocarpa* and *B. patula*. *B. vulgaris* is subdivided in three subspecies. *B. vulgaris* subsp. *vulgaris* is preserved for classification of cultivated beets. Wild forms are classified as *B. vulgaris* subsp. *maritima* and as *B. vulgaris* subsp. *adanensis*. The number of infraspecific taxa in *B. vulgaris* is extensively reduced. The classification of minor variants in botanical variaties is abandoned.

In Chapter 2 morphological similarities and differences between the taxa of *Beta* section *Beta* are discussed and the geographical variation of *B. vulgaris* is analysed. Plants from wide geographical origin are grown under uniform conditions and evaluated simultaneously. Species relationships and geographical relationships are shown through multivariate cluster analysis and principal component analysis of 79 OTUs and 19 morphological variables.

In Chapter 3 allelic variation of isozymes in species of section *Beta* is evaluated. A selection of 76 accessions was surveyed for allozyme variability. A total of 11 isozymes, all polymorphic for at least one locus, were studied. Genetic variability coefficients were calculated based on 59 accessions and 9 loci.

In *B. vulgaris* subsp. *maritima* the greatest allozyme diversity is met in accessions originating from the Mediterranean Basin. Generally, *B. patula*, *B. vulgaris* subsp. *vulgaris*, and *B. vulgaris* subsp. *adanensis* express the same allozymes as *B. vulgaris* subsp. *maritima*. *B. patula* and *B. vulgaris* subsp. *adanensis* could be characterised by the expression of specific allozymes at high frequencies. *B. macrocarpa* has diverged at a number of loci notably *Lap1*, *Acp1*, *Pgm2* and *Px2*. Genetic diversity was low in *B. macrocarpa* ($H_e = 0.01$) and in *B. patula* ($H_e = 0.07$). In *B. vulgaris* sensu lato $H_e = 0.28$ or more. Interspecific differences in levels of observed heterozygosity could be related to the breeding system. The taxa *B. macrocarpa*, *B. patula* and *B. vulgaris* subsp. *adanensis* expressed low levels of observed heterozygosity confirming self-compatibility. A higher level of heterozygotic genotypes pointed to allogamous reproduction in *B. vulgaris* subsp. *maritima*.

The presence of common and rare allozymes was investigated. Allozymes

more or less restricted to geographical regions were *Acp1*-7 (primarily Mediterranean subsp. *maritima*), *Mdh1*-1 (primarily Greek and Sicilian subsp. *maritima* en *B. macrocarpa*), *Lap1*-5 (primarily Greek and Sicilian subsp. *maritima*) en *Acp1*-2 (subsp. *maritima*, Atlantic accessions only, and *B. patula*). *Mdh1*-3 was an allozyme with low frequency, but dispersed over the entire distribution area. The distribution of the particular allozyme *Acp1*-2 suggested a close relationship of *B. patula* with Atlantic *B. vulgaris* subsp. *maritima*.

It was concluded that the quantification of intrapopulational variability and the description of geographical variation patterns has offered useful information for sampling wild beet populations. Regarding *B. macrocarpa* and *B. vulgaris* subsp. *adanensis* it is essential to sample as many populations as possible from as many different environments as possible. In *B. vulgaris* subsp. *maritima* genetic diversity is more or less equally divided in individual plants in a population and between neighbouring populations. Differences in genetic diversity between geographical regions are weak.

In Chapter 4 the variation in life cycle and the variation in flowering time between accessions of *B. vulgaris* and affiliated taxa is described. The effect of external parameters on flowering initiation such as vernalization and daylength conditions was evaluated for a number of representative origins. A vernalization requirement was demonstrated for plants from north Atlantic regions. Although vernalization affected bolting in nearly all accessions, it was noted that the flowering response of the north Atlantic accessions upon vernalization was variable: after vernalization flowering was complete only in plants originating from Brittany, France. Plants from the Netherlands and from the Irish south coast responded by incomplete flowering in the season subsequent to vernalization. Irish north coast accessions seemed to be highly insensitive to vernalization, since even bolting plants were absent after vernalization. Thus, vernalization need and sensitivity to vernalization seem to follow a north – south cline.

B. macrocarpa, *B. vulgaris* subsp. *maritima*, and *B. patula* bolt readily without vernalization, and show a strongly reduced period of vegetative growth. *B. vulgaris* subsp. *maritima* needs long days both for bolting and flowering, *B. patula* bolts even in short day conditions, but cannot flower in such conditions. *B. macrocarpa* flowers readily even in short day conditions. *B. macrocarpa* seems to be the only obligatory annual taxon of section *Beta*.

In Chapter 5 the germination behaviour of *Beta* species is described in relation to seed dormancy. The expression of dormancy is tested by exposing variously pretreated fruit balls (glomerulae) to different temperature regimes. The germination pattern of *Beta vulgaris* ssp. *maritima* is analysed with accessions originating from the north part of the species distribution, and with accessions belonging to the Mediterranean gene pool. Fruit balls of northern accessions, showed optimum germination in the range of higher temperatures 20° to 30 °C. Freshly harvested fruit balls of both accessions showed almost no germination at 10° and

15 °C. Stratification of fruit balls enhanced germination at the lower temperatures. Mediterranean accessions showed no specific optimal germination temperature, but germination was reduced at 30 °C in most of the accessions tested. Generally, the Mediterranean accessions were less sensitive to temperature effects on germination.

Chapter 5 also describes the germination ecology of *B. macrocarpa*. Seed dormancy does not seem to be temperature controlled, but instead mechanical factors may limit germination in *B. macrocarpa*. Compared to *B. vulgaris* s.l. the penetration of moisture in the fruit balls of this species is more difficult, and the operculum is fixed more tightly to the pericarp, preventing easy protrusion of the radicle.

In Chapter 6 the variation pattern of a Sicilian *Beta* germplasm collection is analysed. Accessions from Sicily were grown under controlled conditions and were evaluated morphometrically. Geographical distribution patterns of morphological characters are identified and the putative classification of the collection into coastal and inland populations is reassessed. Between adjacent populations significant variation was found for Petiole length, Leaf length, Leaf width and Biomass, however not one variable unambiguously reflected a geographical pattern. Overall differences between coastal and inland populations were small. Petiole length and rate of generative development were found to be important for distinguishing between these groups. Interferences are drawn on how the collection from Sicily could be rationalized to avoid excessive duplication.

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Appendix I. List of accessions screened for allozyme diversity.

IDBB	origin country	region	taxonomic group
1106	France	Charente maritime	B. vulgaris subsp. maritima
1185	Turkey	Izmit	B. vulgaris subsp. maritima
1497	Greece	Kos	B. vulgaris subsp. adanensis
1570	USA	Imperial Valley	B. macrocarpa
1668	Spain	Tenerife	B. macrocarpa 4x
2199	Greece	Rhodos	B. vulgaris subsp. adanensis
2208	Italy	Sicily	B. vulgaris subsp. maritima
2212	Italy	Sicily	B. vulgaris subsp. maritima
2215	Italy	Sicily	B. vulgaris subsp. maritima
2220	Italy	Sicily	B. vulgaris subsp. maritima
2229	Italy	Sicily	B. vulgaris subsp. maritima
2234	Italy	Sicily	B. vulgaris subsp. maritima
2249	Italy	Sicily	B. vulgaris subsp. maritima
2691	Italy	Sicily	B. vulgaris subsp. maritima
2716	Italy	Sicily	B. vulgaris subsp. maritima
2717	Italy	Sicily	B. vulgaris subsp. maritima
2726	Italy	Sicily	B. vulgaris subsp. maritima
2728	Italy	Sicily	B. vulgaris subsp. maritima
3105	Greece	Crete	B. vulgaris subsp. adanensis
3183	Spain	Tenerife	B. macrocarpa 4x
3193	Tunisia	unknown	B. macrocarpa
3196	Turkey	unknown	unidentified
3290	Greece	Peloponnese	B. vulgaris subsp. adanensis
3294	Greece	Peloponnese	B. vulgaris subsp. maritima
3296	Greece	Agios Nikolaos	B. vulgaris subsp. maritima
3304	Greece	Peloponnese	B. vulgaris subsp. maritima
3331	Greece	Leros	B. vulgaris subsp. adanensis
3359	Greece	Thassos	B. vulgaris subsp. vulgaris leaf beet
3372	Greece	Levkas	B. vulgaris subsp. vulgaris leaf beet
3792	Israel	Lower Jordan Valley	B. macrocarpa
3853	Ireland	Wexford	B. vulgaris subsp. maritima
3863	Ireland	Ardmore	B. vulgaris subsp. maritima
3880	Ireland	Down	B. vulgaris subsp. maritima
3882	Ireland	Down	B. vulgaris subsp. maritima
5383	Pakistan	unknown	B. vulgaris subsp. maritima
5870	Ireland	Dublin	B. vulgaris subsp. maritima
5871	Ireland	Meath	B. vulgaris subsp. maritima
5951	Turkey	Adana	B. vulgaris subsp. adanensis
6081	Spain	Mallorca	B. vulgaris subsp. maritima
6106	France	Morbihan	B. vulgaris subsp. maritima
6109	France	Morbihan	B. vulgaris subsp. maritima
6115	France	Morbihan	B. vulgaris subsp. maritima
6116	France	Finistere	B. vulgaris subsp. maritima
6117	France	Finistere	B. vulgaris subsp. maritima
6119	France	Finistere	B. vulgaris subsp. maritima
6206	France	Marseille	B. vulgaris subsp. maritima
6519	Netherlands	Zeeuws Vlaanderen	B. vulgaris subsp. maritima
6520	Netherlands	Zeeuws Vlaanderen	B. vulgaris subsp. maritima
6521	Netherlands	Walcheren	B. vulgaris subsp. maritima
6522	Netherlands	Noord Beveland	B. vulgaris subsp. maritima

Appendix I. continued.

IDBB	origin country	region	taxonomic group
6942	Greece	Leros	B. vulgaris subsp. maritima
6951	Greece	Karpathos	B. vulgaris subsp. maritima
6952	Yugoslavia	Istria	B. vulgaris subsp. maritima
6954	Italy	Calabria	B. vulgaris subsp. maritima
6956	Spain	Murcia	B. vulgaris 'atriplicifolia'
6963	Portugal	Madeira	B. patula
7036	Spain	Murcia	B. macrocarpa
7045	Spain	Almeria	B. macrocarpa
7052	Spain	Albox	B. macrocarpa
7067	Portugal	Mafra	B. vulgaris subsp. maritima
7078	Portugal	Aljezur	B. vulgaris subsp. maritima
7084	Portugal	Olhao	B. vulgaris subsp. maritima
7085	Portugal	Olhao	B. macrocarpa
7086	Portugal	Tavira	B. macrocarpa
7089	Spain	Puerto Real	B. vulgaris subsp. maritima
7091	Spain	San Fernando	B. macrocarpa
7096	Portugal	Alcochete	B. vulgaris subsp. maritima
7100	Portugal	Figueira da Foz	B. vulgaris subsp. maritima
7102	Portugal	Viana Castello	B. vulgaris subsp. maritima
7103	France	Morbihan	B. vulgaris subsp. maritima
7104	France	Morbihan	B. vulgaris subsp. maritima
7105	France	Morbihan	B. vulgaris subsp. maritima
7119	Cyprus	Paphos	B. vulgaris subsp. adanensis
7127	Cyprus	Limassol	B. macrocarpa
7128	Cyprus	Limassol	B. vulgaris subsp. adanensis
–	Italy	Sicily	B. vulgaris subsp. vulgaris leaf beet

Accession numbers are taken from the International Data Base for Beta (IDBB). Accessions for which all 9 loci were interpreted, and for which genetic variability statistics were calculated, are reported in Appendix III.

Appendix II. Allele frequencies at 10 loci.

Allozyme frequencies by locus. Ht: frequency of observed heterozygotes for each locus. N: number of plants screened for each population.

1. Allozyme frequencies at locus Acp1

IDBB	*Acp1*-1	*Acp1*-2	*Acp1*-3	*Acp1*-4	*Acp1*-5	*Acp1*-6	*Acp1*-7	HTAcp	N
–	.00	.00	.00	.91	.00	.06	.03	.19	16
1185	.00	.00	.00	.13	.52	.25	.00	.21	24
1497	.00	.00	.00	.00	.00	1.00	.00	.00	24
1570	.00	.00	1.00	.00	.00	.00	.00	.00	32
1668	.00	.00	.50	.00	.50	.00	.00	1.00	4
2199	.00	.00	.00	.00	.00	1.00	.00	.00	22
2208	.00	.00	.00	.50	.00	.45	.00	.64	22
2212	.00	.00	.00	.53	.00	.47	.00	.19	16
2215	.00	.00	.00	.90	.00	.07	.03	.20	15
2220	.00	.00	.00	.50	.00	.50	.00	.00	20
2229	.00	.00	.00	.24	.09	.52	.15	.30	23
2234	.00	.00	.00	.38	.40	.00	.23	.55	20
2249	.00	.00	.00	.32	.14	.14	.41	.23	22
2691	.00	.00	.00	.46	.24	.11	.15	.65	23
2716	.00	.00	.00	.54	.08	.23	.15	.62	13
2717	.00	.00	.00	.69	.00	.24	.07	.34	29
2726	.00	.00	.02	.73	.00	.17	.08	.38	24
2728	.00	.00	.05	.64	.00	.20	.11	.45	22
3105	.00	.00	.00	.00	.00	1.00	.00	.00	14
3183	.00	.00	1.00	.00	.00	.00	.00	.00	31
3193	.00	.00	1.00	.00	.00	.00	.00	.00	34
3196	.00	.10	.12	.50	.00	.26	.02	.41	29
3290	.00	.00	.00	.52	.00	.48	.00	.00	29
3294	.00	.00	.00	.44	.00	.00	.56	.33	18
3296	.00	.00	.00	.75	.00	.25	.00	.33	12
3304	.00	.00	.18	.30	.00	.43	.09	.68	22
3331	.00	.00	.00	.66	.00	.34	.00	.00	29
3359	.00	.00	.00	.68	.00	.30	.02	.05	22
3372	.00	.00	.00	.26	.00	.50	.24	.26	27
3792	.00	.00	.77	.00	.02	.19	.00	.03	31
3853	.00	.21	.00	.00	.64	.14	.00	.19	21
3863	.00	.61	.00	.00	.00	.39	.00	.00	18
3880	.00	.86	.00	.14	.00	.00	.00	.00	21
3882	.00	.24	.00	.74	.01	.00	.00	.21	42
5870	.00	.00	.00	1.00	.00	.00	.00	.00	20
5871	.00	.78	.00	.22	.00	.00	.00	.22	18
5951	.00	.00	.00	.00	.00	1.00	.00	.00	23
6106	.00	.05	.00	.95	.00	.00	.00	.03	32
6109	.00	.00	.00	.47	.00	.53	.00	.27	15
6115	.00	.00	.00	.50	.00	.50	.00	.40	20
6116	.00	.33	.00	.20	.00	.20	.08	.15	20
6117	.00	.00	.00	.55	.00	.30	.15	.10	20
6119	.00	.05	.03	.89	.03	.00	.00	.16	37
6206	.00	.00	.00	.46	.54	.00	.00	.17	12
6519	.00	.00	.00	.86	.14	.00	.00	.11	46

1. continued

IDBB	*Acp1*-1	*Acp1*-2	*Acp1*-3	*Acp1*-4	*Acp1*-5	*Acp1*-6	*Acp1*-7	HTAcp	N
6520	.00	.00	.00	1.00	.00	.00	.00	.00	19
6521	.00	.05	.00	.00	.00	.95	.00	.10	21
6942	.00	.00	.00	.74	.00	.22	.04	.09	23
6951	.00	.00	.00	.40	.00	.54	.06	.12	26
6952	.00	.00	.00	.29	.00	.52	.19	.38	24
6954	.00	.00	.09	.13	.00	.35	.43	.74	23
6956	.00	.00	.00	.47	.00	.42	.11	.56	32
6963	.00	1.00	.00	.00	.00	.00	.00	.00	43
7036	1.00	.00	.00	.00	.00	.00	.00	.00	12
7045	.00	.00	1.00	.00	.00	.00	.00	.00	15
7052	.00	.00	1.00	.00	.00	.00	.00	.00	9
7067	.00	.09	.00	.36	.00	.50	.05	.36	11
7078	.00	.00	.03	.61	.35	.00	.00	.26	31
7084	.05	.00	.00	.23	.00	.59	.05	.18	11
7085	1.00	.00	.00	.00	.00	.00	.00	.00	9
7086	.00	.00	1.00	.00	.00	.00	.00	.00	10
7089	.00	.00	.00	.54	.00	.46	.00	.64	14
7091	.00	.00	1.00	.00	.00	.00	.00	.00	8
7096	.00	.00	.15	.11	.00	.43	.09	.11	27
7100	.00	.31	.00	.52	.00	.17	.00	.38	32
7102	.00	.47	.00	.53	.00	.00	.00	.31	32
7104	.00	.39	.00	.61	.00	.00	.00	.42	33
7105	.00	.00	.00	.90	.00	.10	.00	.15	41
7119	.00	.00	.12	.79	.00	.09	.00	.06	17
7127	.00	.00	1.00	.00	.00	.00	.00	.00	7
7128	.00	.00	.00	.00	1.00	.00	.00	.00	21

2. Allozyme frequencies at locus Lap1

IDBB	*Lap1*-1	*Lap1*-2	*Lap1*-3	*Lap1*-4	*Lap1*-5	HTLap	N
–	.00	.00	.00	1.00	.00	.00	31
1185	.00	.00	.00	.67	.33	.67	24
1497	.00	.00	.00	1.00	.00	.00	14
1570	1.00	.00	.00	.00	.00	.00	35
1668	.50	.00	.00	.50	.00	1.00	10
2199	.00	.00	1.00	.00	.00	.00	23
2208	.00	.19	.00	.76	.06	.33	27
2212	.00	.15	.00	.74	.11	.52	27
2215	.00	.03	.00	.97	.00	.06	16
2220	.00	.00	.00	.93	.07	.13	23
2229	.00	.00	.00	1.00	.00	.00	22
2234	.00	.05	.00	.95	.00	.00	20
2249	.00	.00	.00	1.00	.00	.00	22
2691	.00	.03	.00	.97	.00	.00	21
2716	.00	.00	.00	.63	.37	.47	15
2717	.00	.39	.00	.57	.04	.41	27
2726	.00	.09	.00	.87	.00	.09	23
2728	.00	.31	.00	.66	.00	.16	31
3105	.00	.00	.00	1.00	.00	.00	30

2. continued

IDBB	*Lap1*-1	*Lap1*-2	*Lap1*-3	*Lap1*-4	*Lap1*-5	HTLap	N
3183	.50	.00	.00	.50	.00	1.00	29
3193	1.00	.00	.00	.00	.00	.00	34
3196	.21	.12	.00	.67	.00	.38	29
3290	.00	.64	.00	.36	.00	.24	29
3294	.00	.00	.00	.63	.38	.25	20
3296	.00	.71	.00	.29	.00	.42	12
3304	.00	.02	.00	.80	.18	.41	22
3331	.00	.02	.00	.98	.00	.03	29
3359	.00	.16	.00	.84	.00	.32	22
3372	.00	.08	.00	.92	.00	.16	25
3792	.79	.00	.00	.21	.00	.03	31
3853	.00	.14	.00	.86	.00	.12	25
3863	.00	.03	.00	.98	.00	.05	20
3880	.00	.20	.00	.80	.00	.39	23
3882	.00	.07	.00	.93	.00	.05	43
5383	.00	.08	.00	.92	.00	.16	19
5398	.00	.10	.00	.90	.00	.20	10
5870	.00	.00	.00	1.00	.00	.00	20
5871	.00	.45	.00	.55	.00	.36	22
5951	.00	.00	.00	1.00	.00	.00	23
6081	.00	.92	.00	.08	.00	.17	12
6106	.00	.07	.00	.85	.08	.26	43
6109	.00	.03	.00	.97	.00	.07	15
6115	.00	.13	.00	.88	.00	.15	20
6116	.00	.11	.00	.86	.02	.27	22
6117	.00	.13	.00	.88	.00	.25	20
6119	.00	.07	.00	.93	.00	.14	36
6206	.00	.08	.00	.92	.00	.17	12
6519	.00	.50	.00	.50	.00	.51	37
6520	.00	.00	.00	1.00	.00	.00	23
6521	.00	.14	.00	.86	.00	.29	14
6522	.00	.02	.00	.98	.00	.04	23
6942	.00	.03	.00	.66	.38	.69	16
6951	.00	.30	.00	.70	.00	.44	25
6952	.00	.40	.00	.60	.00	.54	24
6954	.00	.20	.00	.80	.00	.39	23
6956	.00	.03	.00	.97	.00	.07	44
6963	.00	.01	.00	.99	.00	.02	46
7036	1.00	.00	.00	.00	.00	.00	10
7045	1.00	.00	.00	.00	.00	.00	10
7052	1.00	.00	.00	.00	.00	.00	9
7067	.00	.00	.00	1.00	.00	.00	11
7078	.00	.13	.00	.88	.00	.25	40
7084	.00	.12	.00	.88	.00	.23	13
7085	1.00	.00	.00	.00	.00	.00	15
7086	1.00	.00	.00	.00	.00	.00	20
7089	.00	.18	.00	.82	.00	.21	14
7091	1.00	.00	.00	.00	.00	.00	13
7096	.00	.00	.00	.91	.09	.10	29
7100	.00	.05	.00	.95	.00	.10	42
7102	.00	.00	.00	1.00	.00	.00	32

2. continued

IDBB	*Lap1*-1	*Lap1*-2	*Lap1*-3	*Lap1*-4	*Lap1*-5	HTLap	N
7103	.00	.22	.00	.72	.00	.11	18
7104	.00	.02	.00	.98	.00	.04	24
7105	.00	.42	.00	.58	.00	.59	39
7119	.00	.00	.00	.90	.10	.04	24
7127	.00	.00	.00	1.00	.00	.00	2
7127	1.00	.00	.00	.00	.00	.00	20
7128	.00	.00	.00	1.00	.00	.00	21

3. Allozyme frequencies at locus Mdh1.

IDBB	*Mdh1*-1	*Mdh1*-2	*Mdh1*-3	*Mdh1*-4	HTMdh	N
–	.48	.13	.39	.00	.00	23
1497	.00	.97	.03	.00	.06	16
1570	1.00	.00	.00	.00	.00	21
1668	.50	.50	.00	.00	1.00	10
2199	.00	.94	.06	.00	.11	18
2208	.18	.82	.00	.00	.07	14
2212	.11	.89	.00	.00	.21	19
2215	.34	.66	.00	.00	.14	22
2220	.08	.88	.05	.00	.15	20
2229	.12	.83	.05	.00	.24	21
2234	.37	.63	.00	.00	.42	19
2249	.00	1.00	.00	.00	.00	19
2691	.00	.36	.64	.00	.43	21
2716	.22	.78	.00	.00	.44	9
2717	.31	.69	.00	.00	.50	18
2726	.06	.76	.12	.06	.47	17
2728	.16	.79	.06	.00	.24	17
3105	.19	.81	.00	.00	.13	24
3183	.50	.50	.00	.00	1.00	24
3193	1.00	.00	.00	.00	.00	20
3196	.00	.54	.44	.02	.38	24
3290	.00	.88	.12	.00	.00	24
3294	.00	.88	.12	.00	.12	17
3296	.00	.96	.04	.00	.08	12
3304	.08	.72	.20	.00	.43	23
3331	.00	.80	.20	.00	.10	20
3359	.03	.77	.16	.03	.45	31
3372	.06	.60	.28	.06	.64	25
3792	.67	.33	.00	.00	.00	24
3853	.00	.67	.33	.00	.33	24
3863	.00	.67	.33	.00	.27	15
3880	.00	1.00	.00	.00	.00	9
3882	.00	.93	.07	.00	.14	28
5871	.00	1.00	.00	.00	.00	20
5951	.00	.97	.03	.00	.05	19
6116	.00	1.00	.00	.00	.00	20
6117	.00	1.00	.00	.00	.00	17
6119	.02	.74	.24	.00	.52	29

3. continued

IDBB	*Mdh1*-1	*Mdh1*-2	*Mdh1*-3	*Mdh1*-4	HTMdh	N
6519	.04	.96	.00	.00	.03	35
6520	.00	1.00	.00	.00	.00	20
6521	.00	1.00	.00	.00	.00	12
6951	.00	.73	.25	.02	.33	24
6952	.00	.63	.35	.02	.74	31
6954	.00	.60	.40	.00	.55	29
6956	.00	.89	.11	.00	.00	27
6963	.00	1.00	.00	.00	.00	31
7036	1.00	.00	.00	.00	.00	12
7045	1.00	.00	.00	.00	.00	11
7052	1.00	.00	.00	.00	.00	8
7078	.03	.96	.01	.00	.08	36
7084	.00	1.00	.00	.00	.00	14
7085	1.00	.00	.00	.00	.00	10
7086	1.00	.00	.00	.00	.00	11
7091	1.00	.00	.00	.00	.00	19
7096	.00	1.00	.00	.00	.00	15
7100	.00	.75	.25	.00	.36	44
7102	.00	1.00	.00	.00	.00	24
7105	.00	1.00	.00	.00	.00	36
7119	1.00	.00	.00	.00	.00	17
7127	1.00	.00	.00	.00	.00	6
7128	.00	1.00	.00	.00	.00	20

4. Allozymes frequencies at locus Pgm1.

IDBB	*Pgm1*-1	*Pgm1*-2	*Pgm1*-3	*Pgm1*-4	HTPgm1	N
–	.00	.93	.70	.00	.13	23
1497	.03	.97	.00	.00	.07	15
1570	.00	.00	.00	1.00	.00	25
1668	.00	1.00	.00	.00	.00	10
2199	.00	.33	.67	.00	.11	18
2208	.00	.43	.57	.00	.20	15
2212	.00	.45	.55	.00	.05	19
2215	.00	.52	.48	.00	.14	22
2220	.00	.65	.35	.00	.20	20
2229	.00	.19	.75	.06	.17	18
2234	.00	.89	.11	.00	.11	19
2249	.00	.83	.17	.00	.00	6
2691	.00	.32	.68	.00	.09	22
2716	.00	.44	.56	.00	.67	9
2717	.00	.33	.67	.00	.27	15
2726	.00	.50	.50	.00	.18	17
2728	.00	.69	.31	.00	.31	13
3105	.00	.98	.02	.00	.05	22
3183	.00	.23	.54	.23	.46	24
3193	.00	.00	.03	.97	.05	19
3196	.11	.36	.53	.00	.72	18
3290	.00	.14	.68	.18	.28	25

4. continued

IDBB	*Pgm1*-1	*Pgm1*-2	*Pgm1*-3	*Pgm1*-4	HTPgm1	N
3294	.00	.69	.31	.00	.38	16
3296	.00	.92	.08	.00	.00	12
3304	.00	.48	.52	.00	.56	25
3331	.00	.78	.23	.00	.35	20
3359	.00	.45	.55	.00	.37	19
3372	.00	.50	.50	.00	.36	25
3792	.00	.00	.00	1.00	.00	13
3853	.04	.90	.02	.00	.04	23
3863	.00	.25	.75	.00	.17	6
3880	.00	1.00	.00	.00	.00	19
3882	.00	1.00	.00	.00	.00	19
5871	.00	.43	.57	.00	.31	22
5951	.03	.97	.00	.00	.05	19
6116	.00	.93	.08	.00	.05	20
6117	.00	1.00	.00	.00	.00	17
6119	.00	1.00	.00	.00	.00	29
6519	.00	.63	.37	.00	.56	34
6520	.00	.90	.10	.00	.00	20
6521	.00	1.00	.00	.00	.00	20
6951	.00	.67	.33	.00	.65	23
6952	.00	.46	.54	.00	.17	24
6954	.00	.88	.12	.00	.00	17
6956	.00	.74	.26	.00	.43	21
6963	.00	.97	.03	.00	.06	31
7036	.00	.00	.00	1.00	.00	12
7045	.00	.00	.00	1.00	.00	15
7052	.00	.00	.00	1.00	.00	9
7078	.00	1.00	.00	.00	.00	40
7084	.00	1.00	.00	.00	.00	14
7085	.00	.00	.00	1.00	.00	10
7086	.00	.00	.00	1.00	.00	20
7091	.00	.00	.00	1.00	.00	8
7096	.00	.25	.75	.00	.07	14
7100	.00	1.00	.00	.00	.00	22
7102	.00	.93	.07	.00	.13	23
7105	.00	1.00	.00	.00	.00	36
7119	.00	.42	.58	.00	.08	13
7127	.00	.00	.00	1.00	.00	14
7128	.00	1.00	.00	.00	.00	21

5. Allozyme frequencies at locus Pgm2.

IDBB	*Pgm2*-1	*Pgm2*-2	*Pgm2*-3	HT*Pgm2*	N
–	1.00	.00	.00	.00	23
1497	1.00	.00	.00	.00	15
1570	1.00	.00	.00	.00	25
1668	1.00	.00	.00	.00	10
2199	.97	.03	.00	.06	18
2208	1.00	.00	.00	.00	15

5. continued

IDBB	*Pgm2*-1	*Pgm2*-2	*Pgm2*-3	HT*Pgm2*	N
2212	.87	.13	.00	.05	19
2215	1.00	.00	.00	.00	22
2220	1.00	.00	.00	.00	20
2229	1.00	.00	.00	.00	18
2234	1.00	.00	.00	.00	19
2249	1.00	.00	.00	.00	6
2691	1.00	.00	.00	.00	22
2716	1.00	.00	.00	.00	9
2717	1.00	.00	.00	.00	15
2726	1.00	.00	.00	.00	17
2728	1.00	.00	.00	.00	13
3105	1.00	.00	.00	.00	22
3183	1.00	.00	.00	.00	24
3193	1.00	.00	.00	.00	19
3196	.72	.28	.00	.33	18
3290	1.00	.00	.00	.00	25
3294	1.00	.00	.00	.00	17
3296	1.00	.00	.00	.00	12
3304	1.00	.00	.00	.00	25
3331	1.00	.00	.00	.00	20
3359	1.00	.00	.00	.00	22
3372	1.00	.00	.00	.00	25
3792	1.00	.00	.00	.00	13
3853	1.00	.00	.00	.00	23
3863	1.00	.00	.00	.00	6
3880	.95	.05	.00	.00	19
3882	.87	.13	.00	.26	19
5871	1.00	.00	.00	.00	22
5951	1.00	.00	.00	.00	19
6116	1.00	.00	.00	.00	20
6117	1.00	.00	.00	.00	17
6119	.86	.14	.00	.00	29
6519	.99	.01	.00	.03	34
6520	1.00	.00	.00	.00	20
6521	1.00	.00	.00	.00	20
6951	1.00	.00	.00	.00	23
6952	1.00	.00	.00	.00	24
6954	.88	.12	.00	.24	17
6956	.82	.18	.00	.27	22
6963	1.00	.00	.00	.00	31
7036	1.00	.00	.00	.00	12
7045	1.00	.00	.00	.00	15
7052	1.00	.00	.00	.00	9
7078	.45	.18	.21	.06	33
7084	1.00	.00	.00	.00	14
7085	1.00	.00	.00	.00	10
7086	1.00	.00	.00	.00	20
7091	1.00	.00	.00	.00	8
7096	1.00	.00	.00	.00	14
7100	.61	.23	.11	.42	31
7102	1.00	.00	.00	.00	23

5. continued

IDBB	*Pgm2*-1	*Pgm2*-2	*Pgm2*-3	HT*Pgm2*	N
7105	.93	.07	.00	.03	36
7119	1.00	.00	.00	.00	21
7127	1.00	.00	.00	.00	14
7128	1.00	.00	.00	.00	21

6. Allozymes at locus Icd_1.

IDBB	*Icd1*-1	*Icd1*-2	HT*Icd1*	N
–	1.00	.00	.00	19
1497	.59	.41	.09	11
1570	1.00	.00	.00	25
1668	1.00	.00	.00	10
2199	1.00	.00	.00	18
2208	1.00	.00	.00	15
2212	1.00	.00	.00	19
2215	1.00	.00	.00	21
2220	.95	.05	.00	20
2229	.84	.16	.21	19
2234	1.00	.00	.00	19
2249	1.00	.00	.00	23
2691	1.00	.00	.00	21
2716	.75	.25	.50	8
2717	.44	.56	.29	17
2726	1.00	.00	.00	17
2728	.85	.15	.29	17
3105	.98	.02	.04	24
3183	1.00	.00	.00	24
3193	1.00	.00	.00	19
3196	1.00	.00	.00	23
3290	1.00	.00	.00	25
3294	.42	.58	.11	19
3296	1.00	.00	.00	12
3304	.40	.60	.60	20
3331	1.00	.00	.00	19
3359	.90	.10	.20	15
3372	.00	1.00	.00	25
3792	1.00	.00	.00	24
3853	1.00	.00	.00	18
3863	1.00	.00	.00	15
3880	1.00	.00	.00	9
3882	1.00	.00	.00	27
5871	1.00	.00	.00	20
5951	1.00	.00	.00	19
6116	1.00	.00	.00	20
6117	1.00	.00	.00	17
6119	1.00	.00	.00	29
6519	.79	.21	.18	34
6520	1.00	.00	.00	20
6521	1.00	.00	.00	12

6. continued

IDBB	*Icd1*-1	*Icd1*-2	HT*Icd1*	N
6951	.48	.52	.59	22
6952	.47	.53	.31	16
6954	.92	.08	.16	32
6956	.68	.33	.25	20
6963	1.00	.00	.00	22
7036	1.00	.00	.00	12
7045	1.00	.00	.00	13
7052	1.00	.00	.00	7
7078	1.00	.00	.00	37
7084	.88	.12	.23	13
7085	1.00	.00	.00	10
7086	1.00	.00	.00	11
7091	1.00	.00	.00	8
7096	1.00	.00	.00	6
7100	.83	.18	.05	40
7102	1.00	.00	.00	24
7105	1.00	.00	.00	22
7119	1.00	.00	.00	20
7127	1.00	.00	.00	6
7128	1.00	.00	.00	20

7. Allozyme frequencies at locus Pgi2.

IDBB	*Pgi2*-1	*Pgi2*-2	*Pgi2*-3	HTPgi	N
–	.80	.20	.00	.22	23
1497	1.00	.00	.00	.00	16
1570	1.00	.00	.00	.00	21
1668	1.00	.00	.00	.00	10
2199	.39	.61	.00	.22	18
2208	.43	.57	.00	.29	14
2212	.50	.50	.00	.47	19
2215	.45	.55	.00	.18	22
2220	.75	.25	.00	.20	20
2229	.85	.15	.00	.18	17
2234	.55	.45	.00	.47	19
2249	.55	.45	.00	.37	19
2691	.79	.21	.00	.24	21
2716	.44	.56	.00	.00	9
2717	.46	.54	.00	.15	13
2726	.34	.66	.00	.47	19
2728	.71	.29	.00	.29	7
3105	.04	.25	.65	.13	24
3183	.75	.25	.00	.25	24
3193	.35	.65	.00	.23	13
3196	.65	.35	.00	.38	13
3290	.59	.41	.00	.06	16
3294	.92	.08	.00	.17	18
3296	.29	.71	.00	.08	12
3304	.81	.19	.00	.28	18

7. continued

IDBB	*Pgi2*-1	*Pgi2*-2	*Pgi2*-3	HTPgi	N
3331	.56	.44	.00	.25	16
3359	.66	.34	.00	.21	29
3372	.58	.42	.00	.54	13
3792	.33	.67	.00	.00	24
3853	.44	.56	.00	.29	24
3863	1.00	.00	.00	.00	15
3880	.63	.38	.00	.08	12
3882	.48	.48	.04	.59	27
5871	.31	.69	.00	.38	8
5951	.13	.87	.00	.16	19
6116	.73	.28	.00	.05	20
6117	.37	.63	.00	.07	15
6119	.93	.07	.00	.00	15
6519	.53	.35	.06	.35	17
6520	.38	.63	.00	.35	20
6521	.14	.86	.00	.09	11
6951	.36	.64	.00	.71	7
6952	.74	.26	.00	.35	23
6954	.53	.47	.00	.24	17
6956	.82	.18	.00	.16	19
6963	1.00	.00	.00	.00	24
7036	1.00	.00	.00	.00	12
7045	.00	1.00	.00	.00	4
7052	1.00	.00	.00	.00	18
7078	.50	.50	.00	.47	15
7084	1.00	.00	.00	.00	13
7085	1.00	.00	.00	.00	10
7086	1.00	.00	.00	.00	11
7091	1.00	.00	.00	.00	19
7096	.97	.03	.00	.07	15
7100	.61	.39	.00	.33	27
7102	.74	.26	.00	.14	21
7105	.74	.26	.00	.06	34
7119	.62	.38	.00	.31	13
7127	1.00	.00	.00	.00	14
7128	.60	.40	.00	.00	20

8. Allozyme frequencies at locus Skdh1

IDBB	*Skdh1*-1	*Skdh1*-2	*Skdh1*-3	HT*Skdh1*	N
1668	.00	.00	.75	.50	10
3882	.44	.31	.13	.63	8
6119	.00	.21	.52	.55	29
6519	.00	.68	.18	.26	19
6963	.00	.42	.46	.25	12
7078	.00	.25	.61	.28	32
7100	.00	.59	.32	.18	17
7102	.00	.96	.02	.04	23
7105	.00	1.00	.00	.00	19
7128	.00	1.00	.00	.00	20

9. Allozyme frequencies at locus Got3.

IDBB	*Got3*-1	*Got3*-2	*Got3*-3	N OO	
–	.00	1.00	.00	22	0
1497	.28	.72	.00	18	6
1570	1.00	.00	.00	25	0
1668	.00	1.00	.00	10	0
2199	1.00	.00	.00	16	4
2208	.00	1.00	.00	35	0
2212	.00	1.00	.00	29	0
2215	.00	1.00	.00	15	2
2220	.00	1.00	.00	22	1
2229	.00	.93	.07	42	2
2234	.00	.94	.06	16	6
2249	.00	.95	.05	21	2
2691	.00	.94	.06	16	7
2716	.00	1.00	.00	15	0
2717	.00	.93	.07	27	0
2726	.00	1.00	.00	19	4
2728	.00	1.00	.00	20	9
3105	1.00	.00	.00	19	5
3183	.50	.50	.00	14	0
3193	1.00	.00	.00	34	0
3196	.61	.39	.00	18	0
3290	1.00	.00	.00	16	4
3294	.00	1.00	.00	19	0
3296	.00	1.00	.00	17	0
3304	.18	.82	.00	17	0
3331	.00	1.00	.00	22	6
3359	.05	.95	.00	22	4
3372	.00	.96	.04	25	0
3792	1.00	.00	.00	20	0
3853	.00	1.00	.00	17	4
3863	.00	.95	.05	22	0
3880	.00	1.00	.00	20	1
3882	.00	1.00	.00	43	0
5871	.00	1.00	.00	18	0
5951	.00	1.00	.00	22	1
6116	.00	1.00	.00	32	0
6117	.00	1.00	.00	20	0
6119	.00	1.00	.00	40	0
6519	.00	1.00	.00	22	11
6520	.00	1.00	.00	19	0
6521	.00	1.00	.00	20	1
6951	.00	1.00	.00	11	0
6952	.00	1.00	.00	24	0
6954	.00	1.00	.00	21	2
6956	.00	1.00	.00	26	6
6963	.00	1.00	.00	31	0
7036	1.00	.00	.00	12	0
7045	1.00	.00	.00	16	0
7052	1.00	.00	.00	15	0
7078	.00	.73	.27	22	0
7084	.00	1.00	.00	15	3

9. continued

IDBB	*Got3*-1	*Got3*-2	*Got3*-3	N	OO
7085	1.00	.00	.00	15	0
7086	1.00	.00	.00	20	0
7091	1.00	.00	.00	10	0
7096	.00	1.00	.00	22	5
7100	.00	1.00	.00	28	7
7102	.00	1.00	.00	25	0
7105	.00	1.00	.00	25	7
7119	.95	.05	.00	22	0
7127	1.00	.00	.00	15	0
7128	1.00	.00	.00	12	15

In column OO the number of plants which could not be interpreted are given.

10. Allozyme frequencies at locus Px1

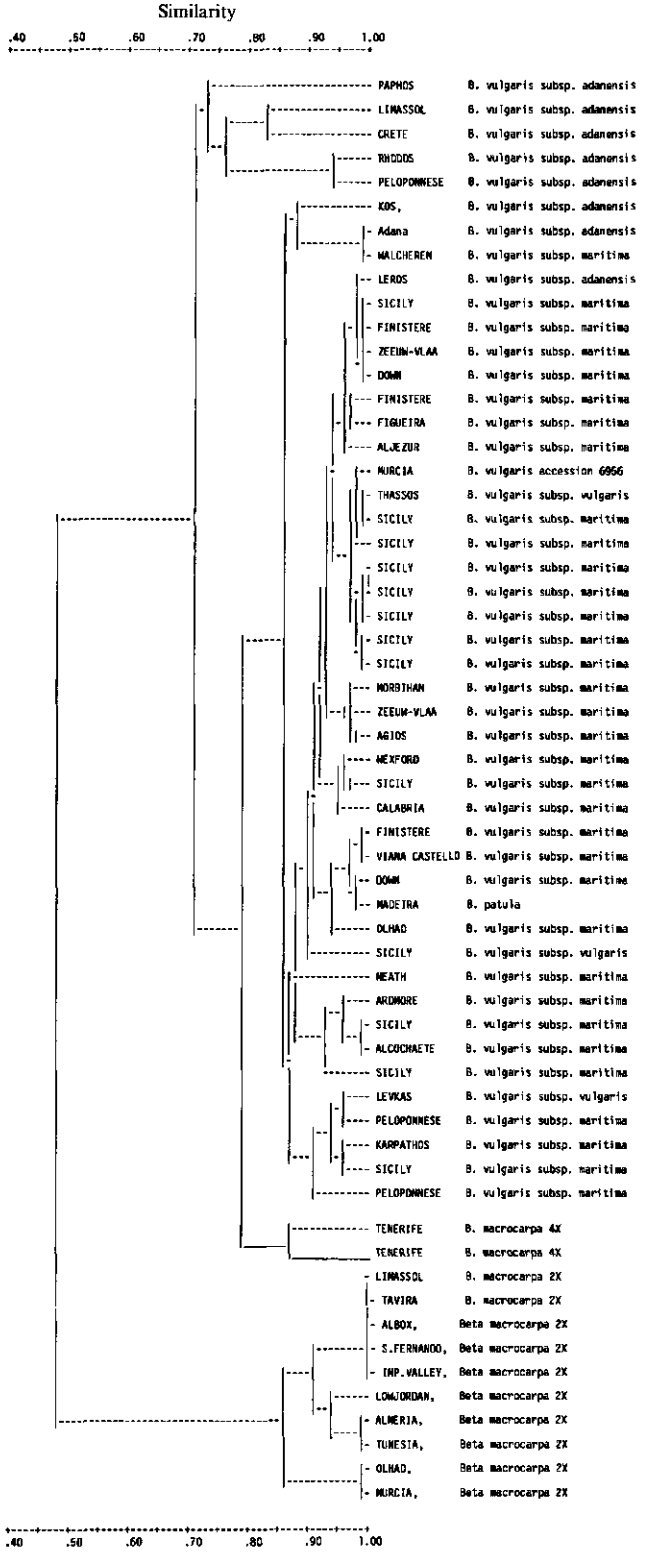
IDBB	*Px1*-3	*Px1*-2	*Px1*-3	HT*Px1*	N
2199	.06	.00	.94	.00	18
2249	1.00	.00	.00	.00	6
2726	.92	.08	.00	.00	12
3193	.00	.00	1.00	.00	11
3196	.42	.58	.00	.17	6
3290	.00	.00	1.00	.00	5
3304	.10	.90	.00	.00	10
3331	.20	.00	.80	.00	20
3359	.44	.56	.00	.00	9
3372	.28	.72	.00	.11	9
3863	.32	.68	.00	.36	14
5870	.92	.08	.00	.17	6
6116	.00	1.00	.00	.00	14
6951	.00	1.00	.00	.00	5
6952	.61	.33	.00	.00	18
6954	.16	.84	.00	.00	19
6956	1.00	.00	.00	.00	8
7036	.00	.00	1.00	.00	13
7052	.00	.00	1.00	.00	7
7084	.00	1.00	.00	.00	8
7096	.56	.44	.00	.38	8
7127	.00	.00	1.00	.00	16

Appendix III. Genetic variability at 9 loci in 59 populations

IDBB	Taxon	Sample size per locus		Alleles per locus		Loci polymorphic*	Genetic diversity**			
		value	s.d.	value	s.d	percentage	H_o	s.d.	H_e	s.d.
1497	*adanensis*	16.1	1.2	1.4	.2	22.2	.024	.012	.116	.066
1570	*macrocarpa* 2x	25.9	1.8	1.0	.0	.0	.000	.000	.000	.000
1668	*macrocarpa* 4x	9.3	.7	1.3	.2	33.3	.333	.167	.180	.090
2199	*adanensis*	18.8	.7	1.6	.2	33.3	.056	.026	.160	.076
2208	*maritima*	19.0	2.5	1.7	.2	55.6	.172	.076	.245	.081
2212	*maritima*	20.7	1.4	1.8	.2	66.7	.166	.068	.265	.077
2215	*maritima*	19.7	1.1	1.7	.2	44.4	.080	.028	.192	.078
2220	*maritima*	20.6	.4	1.8	.2	66.7	.076	.031	.235	.078
2229	*maritima*	21.6	2.6	2.0	.3	66.7	.126	.043	.212	.067
2234	*maritima*	18.9	.4	1.8	.2	66.7	.183	.077	.229	.085
2249	*maritima*	16.6	2.2	1.9	.4	44.4	.081	.053	.198	.084
2691	*maritima*	20.8	.6	1.9	.3	55.6	.160	.082	.240	.085
2716	*maritima*	10.7	.9	1.9	.3	66.7	.299	.097	.327	.086
2717	*maritima*	19.3	2.2	2.0	.2	77.8	.219	.063	.340	.075
2726	*maritima*	18.8	.9	1.9	.4	55.6	.168	.066	.215	.075
2728	*maritima*	16.2	2.4	2.1	.4	66.7	.194	.055	.290	.077
3105	*adanensis*	21.9	1.1	1.7	.3	22.2	.037	.018	.101	.060
3183	*macrocarpa* 4x	24.2	1.6	1.7	.2	55.6	.301	.142	.282	.091
3193	*macrocarpa* 2x	22.8	2.9	1.2	.1	11.1	.031	.026	.058	.052
3290	*adanensis*	22.8	1.8	1.8	.3	55.6	.065	.038	.245	.082
3294	*maritima*	18.0	.4	1.7	.2	66.7	.150	.048	.256	.076
3296	*maritima*	12.6	.6	1.6	.2	44.4	.102	.053	.166	.065
3304	*maritima*	21.1	1.0	2.3	.4	77.8	.329	.091	.356	.079
3331	*adanensis*	21.2	1.6	1.7	.2	44.4	.082	.044	.204	.079
3359	leaf beet	23.2	1.6	2.0	.2	66.7	.173	.056	.258	.066
3372	leaf beet	22.2	1.8	2.0	.3	55.6	.212	.080	.274	.092
3792	*macrocarpa* 2x	22.2	2.3	1.7	.2	55.6	.007	.005	.233	.076
3853	*maritima*	22.2	1.0	1.9	.4	55.6	.135	.057	.208	.078

3863	*maritima*	14.7	1.8	1.7	.2	33.3	.064	.032	.172	.076
3880	*maritima*	16.1	1.9	1.4	.2	44.4	.053	.043	.129	.061
3882	*maritima*	30.4	3.2	1.8	.3	55.6	.142	.068	.157	.064
5871	*maritima*	17.6	1.9	1.4	.2	44.4	.142	.058	.203	.081
5951	*adanensis*	20.2	.6	1.3	.2	11.1	.029	.018	.038	.026
6116	*maritima*	21.1	1.5	1.8	.4	44.4	.062	.033	.169	.085
6117	*maritima*	17.6	.7	1.4	.2	33.3	.046	.028	.145	.079
6119	*maritima*	28.8	2.9	1.9	.4	55.6	.091	.058	.123	.047
6519	*maritima*	30.7	3.3	2.0	.2	55.6	.196	.074	.248	.076
6520	*maritima*	20.1	.4	1.2	.1	22.2	.039	.039	.074	.055
6521	*maritima*	15.7	1.5	1.3	.2	22.2	.052	.032	.066	.036
6951	*maritima*	18.6	2.6	1.9	.3	66.7	.313	.099	.325	.082
6954	*maritima*	21.8	1.9	2.1	.3	77.8	.257	.087	.304	.086
6956	*atriplicifolia*	5.6	2.7	2.0	.2	66.7	.193	.068	.278	.070
6963	*patula*	31.4	2.8	1.2	.1	.0	.010	.007	.009	.007
7036	*macrocarpa* 2x	11.8	.2	1.0	.0	.0	.000	.000	.000	.000
7045	*macrocarpa* 2x	11.4	1.6	1.0	.0	.0	.000	.000	.000	.000
7052	*macrocarpa* 2x	11.3	1.5	1.0	.0	.0	.000	.000	.000	.000
7078	*maritima*	29.3	3.3	2.0	.3	55.6	.156	.055	.261	.084
7084	*maritima*	14.7	1.1	1.6	.3	33.3	.129	.078	.122	.076
7085	*macrocarpa* 2x	11.0	.8	1.0	.0	.0	.000	.000	.000	.000
7086	*macrocarpa* 2x	14.9	1.6	1.0	.0	.0	.000	.000	.000	.000
7091	*macrocarpa* 2x	12.4	1.7	1.0	.0	.0	.000	.000	.000	.000
7096	*maritima*	16.8	2.2	1.7	.3	33.3	.043	.018	.140	.076
7100	*maritima*	32.6	2.6	1.9	.3	55.6	.182	.062	.268	.084
7102	*maritima*	25.0	1.4	1.3	.2	33.3	.065	.073	.114	.066
7105	*maritima*	33.7	2.1	1.4	.2	44.4	.091	.064	.133	.063
7119	*adanensis*	16.9	1.4	1.7	.2	44.4	.054	.033	.182	.072
7127	*macrocarpa* 2x	12.2	1.6	1.0	.0	.0	.000	.000	.000	.000
7128	*adanensis*	19.6	1.0	1.1	.1	11.1	.000	.000	.055	.055
–	leaf beet	22.6	1.3	1.7	.3	44.4	.059	.031	.138	.071

* A locus is considered polymorphic if the frequency of the most common allele does not exceed .95.
** Unbiased estimate (see Nei 1978)



Appendix IV. Dendrogram of UPGMA cluster analysis of 59 populations using Nei's Genetic Identity Coefficient. The cophenetic correlation = 0.94. Populations are indicated by geographical origin and taxonomic group.