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O. H. Bark · M. J. Havey

Similarities and relationships among populations of the bulb onion as estimated by nuclear RFLPs

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Abstract Random nuclear restriction fragment length polymorphisms (RFLPs) were used to assess similarities and relationships among open-pollinated (OP) populations of the cultivated bulb onion (*Allium cepa*). Seventeen OP populations and 2 inbreds of contrasting day-length response [termed by convention as long (LD) and short (SD) day], 1 shallot (*A. cepa* var. *ascalonicum*), and one cultivar of bunching onion (*Allium fistulosum*) were examined with 104 cDNA clones and two to four restriction enzymes. Sixty (58%) clones detected at least 1 polymorphic fragment scorable among the OP populations and were used for analyses. The average number of polymorphic fragments per polymorphic probe-enzyme combination was 1.9, reflecting that numerous monomorphic fragments were usually present. Similarities were estimated as the proportion of polymorphic fragments shared by 2 populations. Average similarity values among LD, among SD, and between LD and SD OP populations were 0.79, 0.67, and 0.68, respectively. Relationships among the OP populations were estimated by parsimony, cluster analysis of similarities using the unweighted-pair-group method (UPGMA), and multivariate analysis using principle components. Parsimony analysis generated a strict consensus tree that grouped all but 1 LD onion with unresolved relationships to the SD OP populations. The UPGMA analysis placed together the LD storage OP populations. Principal component analysis grouped all but 2 LD onions; the other OP populations were dispersed. The results suggest that LD and SD onions do not represent distinct germ plasm, but that LD storage onions represent a derived group selected for production at higher latitudes. If it is assumed that the sampled populations are representative of all onion OP populations, the lower similarities among SD OP populations indicate that their collection and maintenance in germ plasm collections is important for the preservation of genetic diversity.

Key words *Allium cepa* · Day-length response Parsimony · Phylogenies · Restriction fragment length polymorphisms

Introduction

Allium cepa L. is an outcrossing diploid (2n = 2x = 16) biennial that has been classified into the 'common-onion' (blub onion) and 'aggregatum' (potato onion and shallot) groups (Hanelt 1990). Both the bulb onion and shallot are economically important world-wide. For the bulb onion, there is considerable phenotypic variation among open-pollinated (OP) populations for size, shape, and pungency of bulbs; skin and flesh color; maturity; solidity; storage quality; and day-length response (Magruder et al. 1941). Many of these characteristics have significant genetic components, e.g., bulb color is controlled by five major genes (El-Shafie and Davis 1967) or bulb shape and solidity are quantitatively inherited (McCollum 1966, 1968). Other traits, e.g., bulb size, are largely environmentally determined (McCollum 1968, 1971). Bulb formation is affected by temperature and length of night (Magruder and Allard 1937). By convention, onion populations have been classified according to the length of day, as opposed to length of night, at which normal bulbing occurs. Although onion populations require daylengths of or greater than 10 h to induce bulbing (Magruder and Allard 1937), long-day (LD) populations form bulbs under longer days (14–16 h) than short-day (SD) populations (12–14 h). This distinction is relative and ignores the important role of temperature in bulbing. Nevertheless, onion breeders routinely use LD versus SD to

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O. H. Bark
Department of Horticulture, University of Wisconsin, Madison WI 53706, USA

M. J. Havey (✉)
Department of Horticulture and USDA-ARS Vegetable Crops Unit, University of Wisconsin, Madison, WI 53706, USA

classify bulbing response. LD populations grown at the tropical and subtropical low latitudes may never bulb; SD populations grown at the temperate high latitudes quickly form bulbs that are too small to successfully flower after vernalization.

In some crops, contrasting phenotypes reflect divergent genetic backgrounds. The northern-flint and southern-dent phenotypes of maize are distinguished by divergent backgrounds as evidenced by isozymes, restriction fragment length polymorphisms (RFLPs), and significantly greater heterosis in crosses among inbreds (Melchinger et al. 1991; Smith et al. 1990). The concept of heterotic groups in maize was established by relating combining abilities among inbreds of known genetic backgrounds (Hallauer and Miranda 1988). Greater heterotic combinations have also been observed by crossing unrelated lines of rapeseed (Lefort-Buson et al. 1987) and *Drosophila* (Ehiobu and Goddard 1990) from different geographic regions. However, in onion, no significant heterosis was observed for yield per unit area, bulb weight, size, and quality in a wide cross between Japanese autumn-sown and European spring-sown cultivars (Dowker 1990).

Although significant heterosis has been observed among onion inbreds (Jones and Davis 1994; Joshi and Tandon 1976; Hosfield et al. 1977), bulb-onion germ plasm has not been systematically evaluated for the relationship between heterotic response and genetic distance. Kalkman (1984) examined C-karyotypes of plants from three populations ('Downing Yellow Globe', 'Lemi', 'Italian Red Torpedo') and reported infraspecific variations in telomeric bands, amount of heterochromatin, relative chromosome arm lengths, and centromere indices. He suggested the potential use of the C-banding for analysis of spontaneous and artificial hybrids and relationships among cultivated populations. Maggini et al. (1978) and Sato (1981) investigated the number of nucleolus organizing regions (NOR) and observed an extra one on chromosome arm 8CS in addition to that normally on 6CS. None of these cytological markers have been used to systematically characterize the relationships among bulb-onion populations. Only one isozyme marker (Peffley and Castillo 1987) and no RFLPs in chloroplast and nuclear 45s ribosomal DNA (Havey 1992) have been observed among bulb-onion OP populations.

RFLPs have been used to estimate genetic diversity and relationships among various plant populations (Song et al. 1988; Havey and Muehlbauer 1989b; Debener et al. 1990; Smith et al. 1990; Melchinger et al. 1991). Distance estimates based on RFLPs agreed with pedigrees in sweet (Gerdes and Tracy 1994) and field (Melchinger et al. 1991) maizes and offered a better resolution of relatedness among inbred lines than estimates based on morphological and isozyme markers (Smith and Smith 1989). In the study described here, we identified nuclear RFLPs and calculated similarities based on shared polymorphic fragments to determine if day-length response reflected divergent genetic backgrounds, and estimated relationships among OP populations of onion.

Materials and methods

Seventeen OP populations and 2 inbreds of onion, 1 shallot (*A. cepa* var. *ascalonicum*), and one cultivar of the Japanese bunching onion (*A. fistulosum* L.) were examined (Table 1). Eleven and 8 onions were selected as representatives of LD and SD germ plasm, respectively. A majority of the onions have been grown in the USA (13 of 19), and the remainder in Egypt, England, Holland, Italy, Japan, or Poland. We defined LD and SD as representing primary production areas in the northern hemisphere north and south of the 40th parallel, respectively.

A cDNA library of onion was constructed as a source of probes for nuclear RFLP evaluations. Isolation of polyadenylated mRNA from whole seedlings, synthesis of cDNAs, and cloning were as previously

Table 1 Sources and day-length requirement for bulbing of open-pollinated populations and inbreds of onion

Population	DL[a]	Origin	Source[b]	Lot
Alisa Craig 43 (AC)[c]	LD	England	Sunseeds	B32602
Brigham Yellow Globe 15-23 (BYG)[c]	LD	USA	Sunseeds	1750/2
Dorata di Parma (DdP)	LD	Italy	Asgrow	RNN385
Early Yellow Globe (EYG)	LD	USA	Sunseeds	
Mountain Danvers (MD)	LD	USA	Waldow	
Oregon Danvers (OD)	LD	USA	Crookham	
Rijnsburger (RIJ)	LD	Holland	Sunseeds	
Sapporo-ki (SKI)	LD	Japan	Shippo	
Southport Yellow Globe (SYG)	LD	USA	NSSL	
Wolska (WOL)	LD	Poland	Asgrow	RNC233
Yellow Globe Danvers (YGD)	LD	USA	Ohio Seed	
Crystal Wax (CW)	SD	USA	Sunseeds	
Giza #6 (G6)	SD	Egypt	Asgrow	S89A563
NuMex Sunlite (NMS)	SD	USA	NMSU	Unknown
Red Creole (RC)	SD	USA	PETO	
Sweet Spanish Colorado #6 (SSC)	SD	USA	Crookham	87H185
Texas Early Grano 502 (TEG)	SD	USA	Asgrow	
White Creole (WC)	SD	USA	Sunseeds	
Yellow Bermuda (YB)	SD	USA	NSSL	
Shallot (SLT)	—	France	Unknown	
Allium fistulosum (Ishikura)	—	Japan	NMSU	Unknown

[a] Day-length (DL) response assigned based on primary production area in the northern hemisphere located north (LD, long day) or south (SD, short day) of 40th parallel

[b] NSSL, USDA National Seed Storage Laboratory, Fort Collins, Colo., USA; NMSU, New Mexico State University, Las Cruces, N.M., USA. Unless indicated, seed lot same as reported by Havey (1993)

[c] Lines developed from inbreeding a single plant from the open-pollinated population for one to two generations.

described (Havey and Muehlbauer 1989a) except that the vector λgt10 (Promega, Madison, Wis., USA) was used. The restrictive host was infected with recombinant phage, and individual plaques were harvested into SM buffer (Sambrook et al. 1989). The cDNA inserts were amplified by the polymerase chain reaction (PCR) (Dorfman et al. 1989) using oligonucleotides that flank the cloning site and 30 cycles at 94 °C for 1.5 min, 37 °C for 1.5 min, 72 °C for 3 min. The amplified cDNA inserts were loaded onto 0.7% low-melting-point agarose gels in 1 × TAE (Sambrook et al. 1989). The DNA fragments were visualized with ethidium bromide, those between 0.5 and 2.0 kilobases (kb) cut out, and the agarose plug diluted at 3 ml of distilled water per gram of agarose. Tubes were boiled for 8 min to melt the agarose, shaken, and frozen at −20 °C.

Genomic DNA was extracted from lyophilized leaf tissue of approximately 100 seedlings and purified through cesium-chloride gradients (Murray and Thompson 1980). Concentrations were spectrophotometrically determined. Fourteen micrograms of DNA was digested with *Dra*I, *Eco*RI, *Eco*RV, or *Hin*dIII as recommended by the manufacturer (BRL, Gaithersburg, Md., USA) and electrophorized through 0.5% agarose gels in 1 × TBE (Sambrook et al. 1989) at 11 mA for 19 h. The DNA was denatured by soaking the gel in 0.4 *N* NaOH with 0.6 *M* NaCl for 15 min and transferred (Southern 1975) overnight to Zetaprobe filters (Biorad, Richmond, Calif., USA). Filters were neutralized in 1 × SSC (Sambrook et al. 1989) and dried by baking in a vacuum oven at 80 °C for 1 h. Probes were labelled with [P^{32}] by random priming (Feinberg and Vogelstein 1983) using a decamer kit (Ambion, Austin, Tex., USA). Filters were prehybridized in an excessive volume of 40% deionized formamide, 1 m*M* EDTA, 0.25 *M* Na_2HPO_4, and 7% SDS at 42 °C for 30 min in plastic containers. The labelled probes and unincorporated radionucleotides were boiled for 3 min, transferred to plastic containers containing the filters and a minimal volume of the formamide solution, and incubated in a shaker at 42 °C overnight. The filters were first rinsed at room temperature in 2 × SSC, then for 15 min at 42 °C in 0.1 × SSC and 0.1% SDS, and finally for 5 min at 60 °C in fresh 0.1 × SSC and 0.1% SDS. For autoradiography, X-ray film (Kodak XAR-5) was placed on the filters with MCI-optonix high-speed intensifying screens at −80 °C for 4 days. Twenty-six cDNA probes were hybridized to *Eco*RI and *Hin*dIII digests and 78 cDNAs to *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII digests.

The presence or absence of major polymorphic fragments was scored. Because segregations were not established, the genetic bases of polymorphic fragments were not known and the data represent a molecular phenotype. Similarities were estimated as the proportion of polymorphic fragments (F) shared by two populations (Nei and Li 1979), $F = 2n_{XY}/(n_X + n_Y)$, where n_X and n_Y are the numbers of polymorphic fragments in populations *X* and *Y*, respectively, and n_{XY} is the number of polymorphic fragments shared by the two populations for polymorphic clone-enzyme combinations. More than one probe-enzyme combination was used only when profiles were different across populations. Relationships among OP populations were estimated using Phylogenetic Analysis Using Parsimony (PAUP version 3.0r, D.L. Swofford, Illionis Natural History Survey, Champaign, Ill. USA), assuming that (1) the identity-in-state of the fragments reflected homology because the majority of fragments were shared by most populations and (2) polymorphic fragments were from the loss or gain of a restriction-enzyme site as evidenced by the few polymorphic fragments for each probe-enzyme combination and RFLPs rarely being observed across all four restriction enzymes. *Allium fistulosum* and shallot were used as outgroups to root the tree. *Allium fistulosum* was placed close to bulb onion in a maternal phylogeny (Havey 1992); shallot was originally classified as *A. ascalonicum* auct. non Strand, but in now recongnized as a botanical variety of the bulb onion. Multivariate analysis using principal components (SAS Institute, Cary, N.C., USA) was used and the first and second components plotted to visualize associations among populations. Cluster analysis using the unweighted-pair-group method (UPGMA) was completed on the similarity matrix with NTSYS version 1.7 (Rohlf 1992). However, phylogenetic estimates using UPGMA assume equal rates of molecular divergence, likely violated by the populations in this study. For example, "BYG15-23' and 'Alisa Craig 43' ('AC') were derived from the self-pollination of single bulbs and 'NuMex Sunlite' from pollination among five bulbs (Corgan 1988); some of the older, not widely cultivated, OP populations ('Red Creole' or 'Yellow Bermuda') have undergone less recent selection relative to currently grown OP populations ('Rijnsburger' or 'Dorata di Parma'). We used UPGMA to visualize relationships among the OP populations as they presently exist and not to estimate phylogenies.

Results and discussion

RFLP variation in the bulb onion

The detection of nuclear RFLPs in onion using cDNA clones was technically difficult and required optimal conditions at every step, likely due to the fact that onion has one of the largest genomes (33.5 pg per 2C nucleus) among cultivated species (Bennett and Smith 1976). We observed that blots could be used approximately five times before high backgrounds became problematic. We found that decamer primers, rather than hexamers, more consistently generated probes with specific activities at approximately 1×10^9 dpm/μg. We also limited high-temperature (60 °C) low-salt (0.1 × SSC) washes to 5 min.

We calculated the frequency of RFLPs detected by cDNAs hybridized to four restriction-enzyme digests. Between the LD inbreds 'AC43' and 'BYG15-23', 35%, 50%, 58%, and 67% of 78 cDNAs detected RFLPs for one, two, three, and four restriction enzymes, respectively. The 35% value for one enzyme is lower than the 80% reported among maize inbred lines (Helentjaris et al. 1985) and 70% among subspecies of *Brassica* (Figdore et al. 1988). However, it is higher than the 20% found among common wheat species (Liu et al. 1990) and lines of tomato (Helentjaris et al. 1985). We also observed differences among restriction enzymes. *Dra*I detected the fewest polymorphisms (19%) and showed, in most cases, the shortest fragments among the four enzymes. *Eco*RV identified the most polymorphisms (49%). Different restriction enzymes detected similar levels of polymorphisms among maize inbreds (Helentjaris et al. 1985) and *Brassica* (Figdore et al. 1988) and different levels among alfalfa (Brummer et al. 1991) and rice (McCouch et al. 1988) cultivars. To identify RFLPs in onion, we concluded that using numerous restriction enzymes was economically more efficient and less laborious than increasing the number of hybridizations. For probes detecting RFLPs, 50% were visualized with one enzyme, 29% with combinations of any two enzymes, 16% with three, and 6% with all four enzymes. This indicates that RFLPs among onion OP populations may be due either to point mutations at, or small structural changes involving, restriction-enzyme sites.

RFLPs in onion frequently showed simple banding patterns with numerous monomorphic fragments (Fig. 1); the average of 1.9 polymorphic fragments per polymorphic probe-enzyme combination observed among the OP populations was fewer than the 4.3 (Melchinger et al. 1991) found among field and the 4.1 found among sweet (Gerdes and Tracy 1994) maize

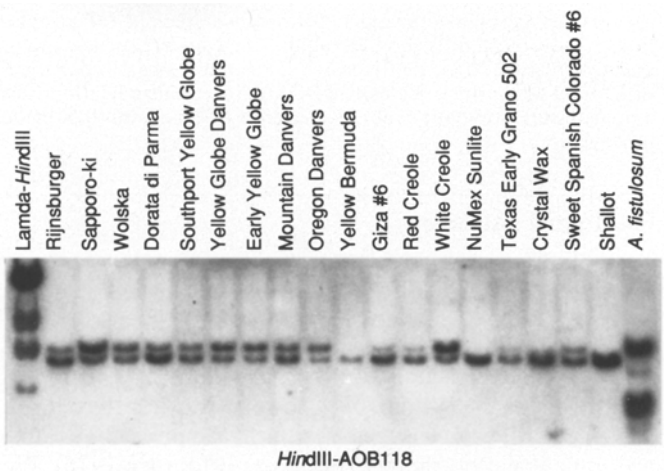


Fig. 1 Autoradiogram showing an RFLP among open-pollinated populations of onion (*Allium cepa*), shallot (*A. cepa* var. *ascalonicum*), and 'Ishikura' (*A. fistulosum*). DNAs were digested with *Hin*dIII and hybridized with random cDNA clone AOB118. Note varying intensities of bands that may represent different frequencies of putative alleles in open-pollinated populations

inbreds. We were surprised to observe relatively few polymorphic fragments among OP populations, as compared to maize, considering the phenotypic diversity, outcrossing nature, and severe inbreeding depression shown by onion. However, the frequency of nuclear RFLPs (approximately two-thirds of the cDNAs using four restriction enzymes) indicated that sufficient RFLPs exist among divergent OP populations to construct a genetic map of onion.

Similarities among OP populations of bulb onion

Out of 104 random cDNA clones with two to four restriction enzymes, 60 (58%) detected at least 1 polymorphism among the OP populations and shallot. The presence versus absence of 146 polymorphic fragments was scored and used to estimate similarities among OP populations. However, the varying intensities of bands within a single lane on the autoradiographs may reflect unequal frequencies of putative alleles in each OP population (Fig. 1), indicating that similarity estimates would be more accurately determined using DNA isolated from individual plants to calculate putative allelic frequencies. F values ranged from 0.53 to 0.95 (mean 0.79 ± 0.09) among LD onions from 0.56 to 0.83 (mean 0.67 ± 0.08) among SD onions and from 0.46 to 0.83 (mean 0.68 ± 0.07) between LD and SD onions (Table 2). When the mean similarities were compared by *t*-tests, the estimate between LD and SD onions was not significantly different from that among SD onions ($P > 0.1$) and the estimate among LD onions was significantly greater than that among SD onions or between LD and SD ($P < 0.01$) onions. This indicates that lower molecular similarities exist among SD onions than among the LD onions studied. However, these statistical tests may be compromised by covariance among means derived from pairwise combinations (Lynch 1991).

Relationships among OP populations of bulb onion

Out of 146 polymorphic fragments, 136 (93%) were shared by more than 2 populations, and were informa-

Table 2 Pair-wise similarities (F × 100) among open-pollinated populations of onion (*Allium cepa* L.) and shallot (*A. cepa* var. *ascalonicum*)

	RIJ[a]	SKI	WOL	DdP	SYG	YGD	EYG	MD	OD	YB	G6	RC	WC	NMS	TEG	CW	SSC	SLT	BYG	AC
RIJ																				
SKI	89																			
WOL	86	89																		
DdP	83	85	90																	
SYG	74	74	76	72																
YGD	81	84	79	77	77															
EYG	86	91	86	85	80	87														
MD	85	88	84	83	78	84	93													
OD	85	89	84	84	79	83	95	94												
YB	58	58	58	61	46	57	60	61	58											
G6	74	72	75	77	67	75	78	79	77	62										
RC	75	71	72	77	67	71	76	77	74	58	74									
WC	73	70	70	70	67	68	73	75	71	58	70	83								
NMS	75	73	69	73	60	69	73	73	73	57	69	63	63							
TEG	71	68	67	71	68	73	73	77	73	56	71	70	66	80						
CW	61	59	55	61	59	59	66	67	66	67	64	61	58	65	70					
SSC	75	70	70	71	71	73	74	77	73	61	74	70	70	73	84	67				
SLT	66	67	67	68	64	65	72	70	68	60	69	67	65	61	65	63	67			
BYG	72	75	71	69	70	73	74	74	76	55	66	60	60	61	57	53	63	59		
AC	67	64	70	71	63	61	69	70	67	55	68	69	71	63	68	61	70	61	53	

[a] Abbreviations are listed in Table 1

tive for parsimony analysis. With shallot as the outgroup, 21 most-parsimonious trees of 450 steps were generated by branch swapping in the heuristic mode of PAUP. A homoplasy of 0.70 was consistent with other studies of approximately 20 taxa (Sanderson and Donoghue 1989). The strict consensus of these 21 trees showed a well-supported clade (indicated by arrow) for all but 1 LD onion with unresolved polychotomous relationships to the SD OP populations (Fig. 2). The branch lengths of this tree are proportional to unit distances of characters and reflect the degree of divergence of the populations from their closest node on the tree. The relatively few most-parsimonious trees and low homoplasy may be the result of little-to-no hybridization and transfer of chromosome regions among OP populations. Due to their specific day-length requirement for bulbing and distinct phenotypes, OP populations of onion have likely been introduced and selected for production in specific environments, as opposed to crossing among phenotypically diverse populations, to develop new populations.

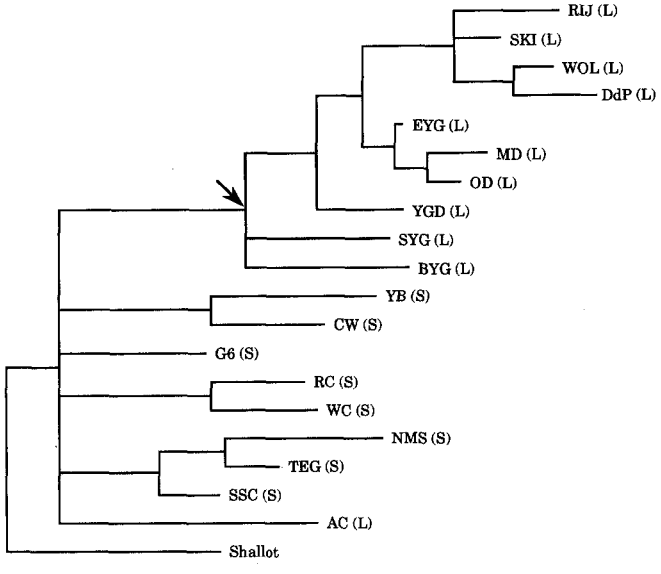
The consensus of the 21 most-parsimonious trees reflected known relationships among some OP populations. For LD yellow storage onions, 'BYG15-23' is an inbred selected out of 'Brigham Yellow Globe' ('BYG'), a well-storing strain of 'Southport Yellow Globe' ('SYG'). 'SYG' has been grown in the northeastern USA at least since 1882 (Magruder et al. 1941). These two related populations were the most important LD yellow storage onions in the USA (Magruder et al. 1941). 'Early Yellow Globe' ('EYG'), 'Mountain Danvers' ('MD'), and 'Oregon Danvers' ('OD') are selections out of 'Yellow Globe Danvers' ('YGD') an OP population originally grown in Massachusetts, USA, at least since 1850 (Magruder et al. 1941). The Japanese cultivar 'Sapporo-ki' ('SKI') was likely derived from an introduction of a 'Danvers'-type onion into 'Hokkaido' in 1878 (Nagai 1983). Importantly, 'YGD' was placed at a node before 'EYG', 'MD', and 'SKI', which are most likely selections of 'YGD' as cultivation spread westward. 'BYG', 'EYG', 'MD', 'OD', 'SYG', 'SKI', and 'YGD' are closely related to 'Wolska' ('WOL'), 'Rijnsburger' ('RIJ'), and 'Dorata di Parma' ('DdP'), which are LD yellow storage OP populations grown in Europe (Fig. 2).

Although 'AC43' is a LD onion, it was placed close to SD OP populations and shallot. 'AC43' may have been derived from a SD OP population by selection for production in northern Europe. Its bulbs are soft, poor storing, and tall, markedly different from LD storage onions. However, the positions of 'AC43' and 'BYG15-23' may have been affected by a loss of polymorphic fragments during inbreeding.

'Red' ('RC') and 'White' ('WC') 'Creoles' are SD populations that differ for bulb color; both are highly pungent, flat, and store relatively well under hot humid conditions. The Creoles were grouped together, as expected, and are likely to be the same populations fixed for different bulb colors. 'Giza#6' ('G6') is a relatively well-storing Egyptian onion grown primarily for export (Jones and Mann 1963, p 143). 'Yellow Bermuda' ('YB') and 'Crystal Wax' ('CW') were placed together as expected; both have poor-storing flattened bulbs, originated from the Canary Islands (Jones and Mann 1963, p 100), and are likely the same population differing in bulb color (Magruder et al. 1941). 'NuMex Sunlite' ('NMS') was selected out of 'Texas Early Grano ('TEG') 502 PRR' (Corgan 1988) and was placed together with 'TEG502', a parent of 'TEG502 PRR'. The longer branch length of NMS might reflect artificial selection on NMS or that TEG502 PRR may have been generated from a cross between 'YB' and 'TEG502' (Havey and Bark 1994). 'Sweet Spanish Colorado #6' ('SSC') was selected out of an introduction from Valencia, Spain and released in 1936 (Allen 1931; Anonymous 1935). It was placed close to the SD population 'TEG502', which was derived from an introduction in 1925 of 'Valencia Grano' from Spain. This close relationship indicates that 'SSC' and 'TEG502' may have been derived from related Spanish populations with selection for production under different daylengths (38th and 26th parallels, respectively).

Trees of 451 and 452 steps (longer than the 21 most-parsimonious tress of 450 steps) were generated and the strict consensus of 547 trees examined. The relationships among the LD yellow storage onions collapsed to one unresolved polychotomy (Fig. 3). 'CW' with 'YB' and 'RC' with 'WC', each pair differing for bulb color, remained as resolved clades. The relationships among these 3 groups and the other OP populations were not resolved.

Fig. 2 Strict consensus of 21 most-parsimmonious trees of 17 open-pollinated populations and 2 inbreds of onion (AC and BYG) and rooted using shallot. *Arrow* indicates a putative common ancestor of long-day storage onions. Tree length = 450, consistency index = 0.302. Branch lengths are proportional to the distances calculated using polymorphic fragments as unit characters. *L*, *S* long- or short-day population, respectively. Abbreviations are listed in Table 1



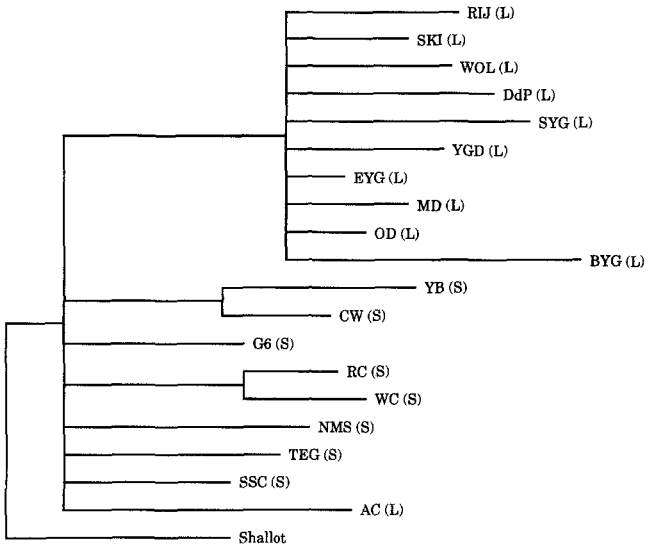


Fig. 3 The strict consensus tree of 547 possible trees of 450, 451, and 452 steps, 2 steps longer than the tree in Figure 2. *L, S* long- or short-day population, respectively. Abbreviations are listed in Table 1

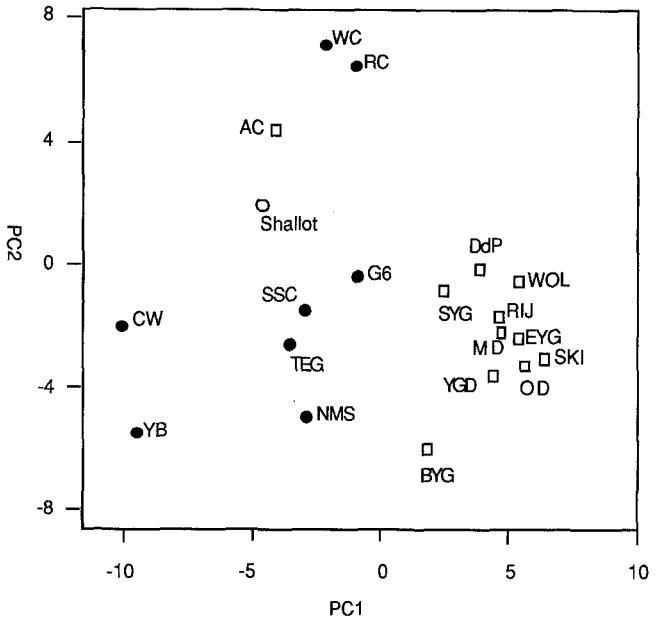


Fig. 4 Plot of first and second principle components for 19 populations of onion and shallot. □, ● long- and short-day onion, respectively, ○ shallot. Abbreviations are listed in Table 1

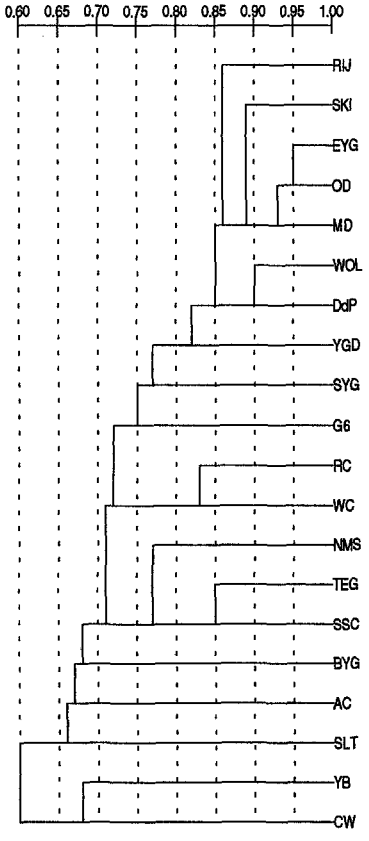
Unrooted trees without an outgroup were generated to examine if the relationships among OP populations changed. The strict consensus tree showed exactly the same relationships as the tree with shallot as the outgroup (Fig. 3), except that the SD OP populations, AC43, and shallot formed an inner unresolved polychotomy and the LD storage onions moved to an outer trichotomous basal node (tree not shown). Importantly, shallot was placed closer to SD than LD onions.

Allium fistulosum showed distinct patterns from *A. cepa*; 21 (14%) fragments were identical-in-state between *A. fistulosum* and at least 1 of the bulb-onion populations. Although the paucity of shared fragments indicates that *A. fistulosum* may not be a proper outgroup, we nevertheless generated trees. Six LD storage OP populations ('EYG', 'MD', 'OD', 'RIJ', 'SKI', and 'WOL') were placed together, and the relationships between this group and the other OP populations were not resolved (tree not shown).

PCA closely grouped all the LD storage onions except the inbred 'BYG15-23'; as observed in the cladistic analysis, 'AC43' (not a storage onion) and the SD OP populations were dispersed outside this group (Fig. 4). This first two principle components accounted for only 16.8% and 10.3% of the variability, respectively. This low level of explained variability was likely due to the relatively few unique fragments observed among the OP populations.

The results of clustering using UPGMA (Fig. 5) generally agreed with parsimony and principle-component analysis in that all LD storage OP populations were placed together. The positioning of inbred 'BYG15-23' was likely affected by the loss of polymorphic fragments during inbreeding. As in the principle-component analy-

Fig. 5 Cluster analysis using UPGMA of similarities (Table 2) among long- and short-day populations of onion and shallot. Abbreviations are listed in Table 1



sis 'G6', a SD storage onion from Egypt, was placed closer to the LD storage populations. The only major difference was that shallot was placed internally and the related SD populations 'YB' and 'CW' placed more distantly. Because of its internal placement, shallot may not be an appropriate outgroup for parsimony analyses. When 'YB' or 'CW' were used as outgroups, strict consensus of the most-parsimonious trees were the same, i.e., only the clades containing the LD storage OP populations and the related OP populations 'RC' and 'WC' remained resolved for strict consensus of the most-parsimonious trees (tree not shown).

No distinct groupings exclusively comprised of LD and SD onions were observed from parsimony analyses, cluster analysis using UPGMA, and plots of the first and second principal components. All three analyses grouped LD storage OP populations (Figs. 3–5). However, this delineation may result from a relatively narrow genetic background for LD storage OP populations, as evidenced by the significantly higher mean similarity (0.79), and not be evidence of a divergent genetic background. The relationships between this LD group and the rest of the OP populations, including SD OP populations, shallot, and LD 'AC43', were not resolved. The paucity of unique fragments and relatively few RFLPs among OP populations, as compared to maize or *Brassica*, indicate that phenotypic variations (e.g., day-length response, bulb color, or storage ability) do not reflect diverse sources of onion germ plasm.

Based on historical records and the relationships estimated by shared fragments, we can speculate on the geographic distribution of the bulb onion towards the western hemisphere. From the primary center of origin [Iran, Afghanistan, Pakistan, and the central Asian republics of the former Soviet Union (Jones and Mann 1963, p 98)], the bulb onion may have been distributed along three routes towards the New World. The first may be through northern Europe to northeastern USA. These OP populations were selected for bulbing under longer days and stored prior to marketing. OP populations ('SYG' and/or 'YGD') of this phenotypic class were initially cultivated in the northeastern USA, and cultivation continually moved westward across the northern USA ('BYG', 'EYG', 'MD', and 'OD') with further introduction into Japan ('SKI') and eastern Asia. The close relationships among LD yellow storage onions from northern Europe ('WOL', 'RIJ', and 'DdP') and USA ('BYG', 'EYG', 'MD', 'OD', 'SYG', 'YGD') could also have resulted from a reintroduction of germ plasm from the USA to northern Europe.

A second route may have been from central Asia through the Mediterranean region. Vavilov (cited by McCollum 1976) recognized the great diversity of onions from Mediterranean countries and proposed this area as a secondary center of origin. Onion populations introduced into the USA from Spain ('Valencia Grano') and the Canary Islands ('YB'), may have been derived from this second route and gave rise to the SD OP populations 'CW', 'SSC', and 'TEG502'. 'Alisa Craig' (from which 'AC43' was selected) is a phenotypically diverse LD OP population that may have been selected from a SD Mediterranean OP population.

The Creole onions ('RC' and 'WC') were originally grown in Louisiana, USA, and possess high solids, allowing them to be stored under hot humid conditions. Currah and Proctor (1990) have suggested that these onions were introduced into the Caribbean from western Africa and maybe originally from the Indian subcontinent, potentially a third route of introduction.

Importantly, the mean similarity among SD OP populations (0.67) is less than that among LD OP populations (0.79) and not significantly different from the similarity among both LD and SD onions (0.68). This would indicate that greater genetic variability exists among SD OP populations than among LD storage germ plasm. We offer two possible explanations for this observation. Firstly, onion is thought to have evolved in Central Asia [above the 30th parallel (Jones and Mann 1963, p 98)]. As a result, SD OP populations may have been distributed throughout the lower latitudes without requiring selection for bulbing under longer days. Secondly, the bulk of the world's onions are grown under short-day regimes and, because greater populations exist in the tropical and subtropical regions, more OP populations may have been separately maintained. The fact that shallot was placed among the SD OP populations further supports our hypothesis that SD OP populations are the progenitor form and that LD OP populations represent a derived state selected for northern production environments. Shallot has been selected for vegetative propagation and represents a distinct form of onion (Hanelt 1990). We assumed that fragments shared between shallot and OP populations represent the progenitor state.

We conclude that because of a relatively narrow genetic background, LD storage onions may benefit from crossing with divergent types to broaden their genetic base and develop new populations. Crossing distantly related LD onions, e.g., 'AC43', or SD types grown under relatively longer days, e.g., 'SSC', with storage types would minimize problems with day-length adaptation. However, over the longer term, crosses with SD onions should increase the genetic variability within LD storage germ plasm. Finally, because of the lower similarities among SD populations, their acquisition and maintenance in germ plasm collections is important for the preservation of genetic diversity in onion.

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