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A low-density genetic map of onion reveals a role for tandem duplication in the evolution of an extremely large diploid genome

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Abstract The bulb onion, *Allium cepa* L., is a diploid ($2n = 2x = 16$) plant with a huge nuclear genome. Previous genetic and cytogenetic analyses have not supported a polyploid origin for onion. We developed a low-density genetic map of morphological markers, randomly amplified polymorphic DNAs (RAPD), and restriction fragment length polymorphisms (RFLP) as a tool for onion improvement and to study the genome organization of onion. A mapping population of 58 F_3 families was produced from a single F_1 plant from the cross of two partially inbred lines (Brigham Yellow Globe 15-23 and Alisa Craig 43). Segregations were established for restoration of male fertility in sterile cytoplasm, complementary light-red bulb color, 14 RAPDs, 110 RFLPs revealed by 90 anonymous cDNA clones, and 2 RFLPs revealed by a cDNA clone of alliinase, the enzyme responsible for the characteristic *Allium* flavors. Duplicated RFLP loci were detected by 21% of the clones, of which 53% were unlinked (> 30 cM), 5% loosely linked (10–30 cM), and 42% tightly linked (< 10 cM). This duplication frequency is less than that reported for paleopolyploids but higher than for diploid species. We observed 40% dominant RFLPs, the highest yet reported among plants. Among duplicated RFLP loci, 19% segregated as two loci each with two codominant alleles, 52% segregated as one locus with codominant alleles and one locus with only a dominant fragment, and 29% segregated as two loci with only dominant fragments. We sequenced cDNAs detecting duplicated RFLPs; 63% showed homology to known gene families (e.g., chlorophyll binding proteins, ubiquitin, or RuBISCO), and 37% were unique clones showing significant homology to known genes of low-copy number or no homology to database sequences. Duplicated RFLPs showing linkage could be due to retroviral-like sequences in adjacent coding regions or intrachromosomal, as opposed to whole genome, duplications. Previous cytological analyses and this genetic map support intrachromosomal duplication as a mechanism contributing to the huge onion genome.

Key words Map · Onion · *Allium* · Duplication

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Introduction

Genetic maps provide unique insights about contemporary genome structure and often reveal landmark events in genome evolution. Evolutionary insights derived from genetic maps include evidence supporting the previously suggested paleopolyploid origins of maize (Helentjaris et al. 1988), soybean (Keim et al. 1990; Funke et al. 1993; Shoemaker et al. 1996), and *Brassica oleracea* (Kianian and Quiros 1992), and syntenic linkage relationships among genomes within the Solanaceae (Bonierbale et al. 1988; Tanksley et al. 1988; Gebhardt et al. 1991; Tanksley et al. 1992) and Poaceae (Ahn et al. 1993; Devos et al. 1994; Dunford et al. 1995). Sequence and linkage conservation within the Poaceae is extensive enough that clones are commonly hybridized to other grass species, and linkage segments from the relatively small rice genome can be pieced together to reconstruct the larger genomes of wheat, maize, sorghum, millet, and sugarcane (Bennetzen and Freeling 1993; Moore 1995). These studies demonstrate

that speciation may be associated with chromosome rearrangements that shift blocks of linked loci (Bonierbale et al. 1988; Tanksley et al. 1988).

Detailed genetic maps have been developed for many species of gymnosperms and angiosperms (O'Brien 1993). However, among the monocotyledonous angiosperms, research has focused on the Poaceae, and the only genetic map published for a monocot species outside the Poaceae is for *Asparagus officinalis* L. (Restivo et al. 1995; Lewis and Sink 1996; Jiang et al. 1997). This under-represented group includes economically important species such as bulb onion (*Allium cepa* L.).

Few genetic analyses have been reported for onion, likely due to a restricted genetic background (Bark and Havey 1995), biennial generation time, and severe inbreeding depression (Jones and Davis 1944). Only 17 morphological or disease-resistance loci have been described, including the colors of anthers (Jones et al. 1944), bulbs (Clarke et al. 1944; Jones and Peterson 1952; El-Shafie and Davis 1967), foliage (Jones et al. 1944; Berninger and Buret 1967), and seed coat (Davis 1966); dwarf scape (Horobin 1986); male-fertility restoration in cytoplasmic male-sterile plants (Jones and Clarke 1943; Schweisguth 1973); and disease resistances (Nichols et al. 1965; Engle and Gabelman 1966). Of these 17 loci 4 were deleterious chlorophyll mutants (Jones et al. 1944). Little isozyme variability was observed among cultivated onion populations (Peffley and Orozco-Castillo 1987). The only genetic linkage reported in onion is between chlorotic seedlings and glossy foliage (Jones et al. 1944).

Molecular analyses of onion are hampered by an enormous nuclear genome of 17.9 pg (Labani and Elkington 1987) or 15,290 megabase-pairs (Arumuganathan and Earle 1991) per 1C nucleus, making it one of the largest among cultivated plants (6-, 16-, and 107-times greater than maize, tomato, and *Arabidopsis thaliana*, respectively). On average, each onion chromosome contains an amount of DNA equal to 75% of the 1C content of the maize nuclear genome. Molecular studies have revealed important characteristics of this extremely large genome. The GC content of onion DNA is only 32%, the lowest known for any angiosperm (Kirk et al. 1970; Stack and Comings 1979). CsCl- and Cs_2SO_4-Ag^+-density gradient centrifugation resulted in no significant satellite DNA, except a 375-bp telomeric sequence representing 4% of the genome (Barnes et al. 1988). Three repetitive fractions in the bulb-onion genome were detected by C_0t reassociation kinetics (Stack and Comings 1979). Fraction 1 represents 41% of the genome and is repeated approximately 21,600 times, fraction 2 comprises 36% of the genome and is repeated approximately 225 times, and fraction 3 comprises 6% of the genome and consists of single-copy DNA. Approximately 10% of the DNA was undetectable using reassociation kinetics. Overall, the onion genome consists of middle-repetitive sequences occurring in short-period interspersions among single-copy regions (Stack and Comings 1979).

There are significant differences in chromosome sizes and nuclear DNA content among related *Allium* species. A close phylogenetic relationship between bulb onion and *A. fistulosum* L. is supported by karyotype and heterochromatic banding (Vosa 1976; Narayan 1988), crossability (van Raamsdonk et al. 1992), and shared mutations in the chloroplast and nuclear 45s ribosomal DNAs (Havey 1992). However bulb onion has 30% more nuclear DNA than *A. fistulosum* (Labani and Elkington 1987; Jones and Rees 1968). This difference of 5.4 pg per 1C nucleus is approximately equal to the total 1C DNA content of barley (*Hordeum vulgare*), pepper (*Capsicum annuum*), or radish (*Raphanus sativus*) (Bennett and Smith 1976). This increase cannot be attributed to duplication of one or a few chromosomes. Jones and Rees (1968) and Narayan (1988) studied interspecific hybrids between *A. cepa* and *A. fistulosum* and observed that all eight bivalents were asymmetric, indicating that DNA differences were spread across all eight chromosomes. However, size differences among individual bivalents varied from a maximum of 60% to a minimum of 20% (Jones and Rees 1968). Pairing at pachytene revealed loops and overlaps, providing evidence for the accumulation of repetitive sequences or tandem duplication of chromosome segments (Jones and Rees 1968). Multiple loops per bivalent were not observed, eliminating intrachromosomal transposition as the mechanism.

Because onion is diploid ($2n = 2x = 16$), Jones and Rees (1968) and Ranjekar et al. (1978) proposed that intrachromosomal duplications contributed to increased chromosome sizes. This model of genome evolution, termed cryptopolyploidy by Sparrow and Nauman (1976), differs from whole genome or chromosome polyploidization in that increases occur in chromosome sizes, not numbers. Polyploid histories are well supported for many contemporary diploid plant species based on conserved linkage relationships among duplicated genome regions (Helentjaris et al. 1988; Keim et al. 1990; Landry et al. 1991; McGrath and Quiros 1991; Slocum et al. 1990; Whitkus et al. 1992; Pereira et al. 1994) and the existence of putative progenitors with lower base chromosome numbers (Anderson 1945; Celarier 1956). The duplication of specific genes or chromosome segments has been documented for many loci, e.g., the *R* (Robbins et al. 1991), *Rp1* (Hulbert and Bennetzen 1991), and *Kn1* (Veit et al. 1990) loci of maize. The origins of these duplications have been explained by unequal crossing over at meiosis (Smith 1976); however, the mechanisms controlling genome-wide increases or decreases in chromosome sizes, such as between *A. fistulosum* and *A. cepa*, are unknown.

We developed a low-density genetic map of onion based primarily on restriction fragment length polymorphisms (RFLPs). In addition to being a useful plant

breeding tool (Havey et al. 1997), this map provided insights into the contemporary structure of the onion genome. Duplicated RFLP loci were detected at a frequency lower than that found in paleopolyploid species but higher than in diploids. The distribution of duplicated loci suggests that intrachromosomal tandem duplications contributed to the huge nuclear genome of onion.

Materials and methods

Plant material

We reported previously the origin and generation of a segregating family from the cross of the partially inbred lines Brigham Yellow Globe (BYG) 15-23 and Alisa Craig (AC) 43 (Bradeen and Havey 1995). BYG15-23 and AC43 were caged individually with the cytoplasmic-male-sterile (S) F_1 lines (B1731A × MSU5785B) or (MSU611-1A × MSU611B) and pollinated by bees. Individual F_2 plants from BYG15-23 × AC43 were self-pollinated and testcrossed to S lines B1828A or (MSU5718A × MSU8155B) using house flies (Pike 1986) to produce 58 F_3 and testcross families. Testcrosses with F_2 plants and parental inbreds were scored for light-red bulb color controlled by the complementary interaction of two loci (Jones and Peterson 1952). For all of the tester lines (B1731A, B1828A, MSU5718A, MSU611-1A, MSU611B, MSU5785B, and MSU8155B), we have never observed light-red bulbs in testcrosses with diverse inbreds selected from US germplasm (M.J. Havey unpublished), and we assumed that these inbreds were homozygous recessive at one of the complementary loci. Testcross bulbs were vernalized, flowered, and scored for male-fertility restoration in S-cytoplasm (Jones and Clarke 1943). Genotypes of F_2 plants from BYG15-23 × AC43 were determined from the segregation of male-fertility restoration among testcross progenies.

Marker analysis

Genomic DNA of BYG15-23, AC43, and F_3 families was CsCl-purified as described previously (Bradeen and Havey 1995). For RFLP analyses, 14 μg of DNA were digested singly with restriction endonucleases (listed below), size fractionated through 0.5% agarose gels in 1 × TBE for 200 mAmp-h, and blotted onto Zetaprobe GT nylon membranes (BioRad, Richmond, Calif.) as described previously (Bark and Havey 1995). Membranes were baked at 80°C for 1 h, washed for 1 h at 60°C in 0.1 × SSC and 0.1% SDS, and incubated at 60°C for 6 h in 6 × SSC, 5 × Denhardt's reagent (1 g per liter each of Ficoll, Polyvinyl-pyrrolidone, and Bovine Serum Albumin), 0.5% SDS, and 100 μg ml sheared boiled herring sperm DNA.

Probe and marker evaluation

Synthesis of onion cDNAs and cloning into λgt10 have been described (Bark and Havey 1995). Clones designated with the prefixes AOB, AJB, or AJK were synthesized in our laboratory; those with the prefix API were cDNAs cloned into a pUC vector by Native Plants, Inc (Salt Lake City, Utah) and provided by Pioneer Hi-Bred International (Johnston, Iowa). Prefixes were followed by clone numbers reflecting the order of isolation. A cDNA clone of the enzyme alliinase (cysteine sulphoxide lyase; EC 4.4.1.4) was the gift of E.J.M. van Damme (van Damme et al. 1992). This enzyme is the limiting step in the hydrolysis of cysteine sulphoxide precursors to produce the volatile compounds yielding flavors characteristic of *Allium* species (Schwimmer and Weston 1961). Probes were amplified by the polymerase chain reaction (PCR) using oligonucleotide primers flanking the cloning sites, 5′-CTTTTGAGCAAGTTCAGCCTGGTTA-3′ and 5′-GAGGTGGCTTATGAGTATTTCTTCC-3′ for λgt10 and 5′-GTCACGACGTTGTAAAACGA-3′ and 5′-GGCGAGTGTTAAGGTGTGTT-3′ for pUC, as previously described (Bark and Havey 1995). An aliquot of each reaction was run on a 1% agarose gel to confirm amplification, and the remainder was purified with the Wizard PCR Preps DNA Purification System (Promega, Madison, Wis.). Concentrations of DNA in samples were determined by measuring the fluorescence in ethidium bromide-containing gels relative to known concentrations of the bacteriophage λ. Probes were labeled by random priming (Feinberg and Vogelstein 1983) using 25 ng of template DNA, decamer primers (Ambion, Austin, Texas), and α-[^{32}P]-dCTP. Membranes and probes were incubated overnight at 60°C in the hybridization solution described above. Membranes were washed at room temperature in 2 × SSC for 5 min, 50°C in 0.1 × SSC and 0.1% SDS for 30 min, and 60°C in 0.1 × SSC and 0.1% SDS for 8–13 min. Membranes were rinsed in 2 × SSC, wrapped in plastic film, and placed on X-ray film at − 80°C for 2–10 days depending on signal intensity.

Polymorphisms were identified initially on parental blots containing DNA from BYG15-23 and AC43 digested with *Dra*I, *Eco*RI, *Eco*RV, or *Hin*dIII. The alliinase clone was hybridized to parental DNA digested with these enzymes plus *Bam*HI, *Bgl*II, *Sst*I, and *Xba*I. Segregations were established using F_3 family DNAs, and F_2 genotypes were assigned as homozygous parental or heterozygous. In many cases, more than two segregating bands were detected by a single probe. Codominant alleles at a locus were determined by first scanning F_3 families for individuals missing two segregating bands, implying nonallelism of these fragments. If putative alleles were unresolvable, all segregating fragments were scored for presence versus absence as dominant markers (3 : 1). If no recombination was detected between two repulsion-phase fragments (i.e., no dual absences were observed), they were scored as codominant alleles at a single locus. This approach may underestimate true map distances if the fragments represent closely linked loci. However, we view this as a more conservative approach since, within the resolution limits of our analysis, the fragments were functionally codominant alleles, and we have no evidence for distinct loci. Heterozygosity in BYG15-23 (S_2 family) and AC43 (S_1 family) produced situations in which polymorphisms based on heterozygosity in one parent and homozygosity for a shared allele in the second parent were monomorphic among F_3 families if both parents contributed the shared allele to the F_1. The complementary situation occurred frequently in which both parents were heterozygous and each contributed a different allele to the F_1, yielding segregation among F_3 families. In these cases, the most likely parental phases for segregating alleles were determined by entering two lines of data representing complementary parental phases. When segregation data for all markers were analyzed, one phase would typically be linked to several other phase-known loci. This phase was retained in the data set, and the unlinked phase was omitted. If both phases were either linked or unlinked, both were omitted from the data set.

Segregations of randomly amplified polymorphic DNAs (RAPDs) were described previously (Bradeen and Havey 1995).

Data analysis

Segregations were tested for goodness-of-fit to 1 : 2 : 1 or 3 : 1 ratios with chi-square analysis. A genetic map was developed using MAPMAKER/EXP version 3.0 (Lander et al. 1987). Linkage groups were resolved with two-point analysis at a minimum LOD = 3.5 and maximum $\theta = 0.30$ using the *group* command. Map distances (in centiMorgans) were calculated using the Kosambi mapping function (Kosambi 1944). Multipoint analysis was used to order markers within linkage groups based initially on a framework order

of the most informative markers with the subsequent placement of other markers in the linkage group using the *try* command. Ambiguous orders were resolved with multipoint analyses of subsets of six or fewer closely linked markers using the *compare* command, or by evaluating single marker placements within sequential windows of five markers using the *ripple* command.

Sequence analysis

We sequenced the 19 cDNA clones detecting duplicated RFLPs. API clones were purified using Qiagen (Chatsworth, Calif.) plasmid purification kits and concentrations determined spectrophotometrically. Inserts in the pUC and λgt10 vectors were PCR amplified by 2 min at 96°C and 30 cycles of 96°C for 30 s, 50°C for 15 s, and 60°C for 2 min. PCR products were analyzed on 1.0% agarose gels to insure that single, well-defined fragments were present. We sequenced twice in both directions using cycle sequencing reactions with dye terminators (Perkin-Elmer, Foster City, Calif.), on an ABI377 (Applied Biosystems) automatic sequencing machine. Base ambiguities were edited using the SEQUENCHER 3.0 software (Gene Codes, Ann Arbor, Mich.) by alignment and comparison with the sequence generated in the opposite direction. Vector sequences were deleted and comparisons with the National Center for Biotechnology Information sequence databases were carried out using BLASTX (ver. 1.4.9MP, March 26, 1996).

Results

A total of 349 cDNA clones produced scorable bands for at least one restriction-enzyme digest, of which 214 (61%) detected polymorphisms between BYG15-23 and AC43. Among probes scorable across all four enzymes, 59% detected polymorphisms with more than one digest suggesting that, in addition to losses or gains of restriction-enzyme sites (Bark and Havey 1995), genomic rearrangements contributed significantly to polymorphisms between BYG15-23 and AC43. The detection of many fragments (mean 5.9 ± 2.6) was common, but only a subset of these was polymorphic (mean 2.5 ± 1.4). Polymorphic fragments revealed by most clones were relatively large [mean 9.6 kilobases (kb)]; polymorphic fragments smaller than 2.0 kb were rare.

One hundred and fifty-seven cDNA clones detecting polymorphisms between BYG15-23 and AC43 were hybridized to F_3 family DNAs. Segregating fragments were scored for 91 cDNA clones yielding 112 loci (Fig. 1). Eleven clones were redundant based on banding patterns and segregations, while 39 clones revealed polymorphisms that did not segregate among F_3 families, likely due to heterozygosity in the parents. The remaining 16 clones yielded poor hybridization signals and were not scored.

Segregation analyses identified 44 dominant (39%) and 68 codominant (61%) RFLPs (Fig. 1). Distorted segregation ratios ($P < 0.01$) occurred for 19 (17%) RFLPs. Among markers showing distortion, 79% favored BYG15-23 alleles, 5% favored heterozygotes, and 16% favored AC43 alleles. Segregation was distorted in favor of the BYG15-23 alleles for many of the markers on linkage group E (Fig. 1) and may be the result of better storage ability of BYG15-23 bulbs as compared to AC43 (M. J. Havey, unpublished). Based on the segregation of 128 loci [112 RFLPs, 14 RAPDs (Bradeen and Havey 1995), and two morphological markers], we developed a low-density genetic map of onion (Fig. 1). The map contains 114 loci distributed on 11 linkage groups and one linked pair, covering 1,064 centiMorgans (cM) with an average of 9.2 cM between loci. Twelve markers remained unlinked (Fig. 1). Among the 112 segregating RFLP markers, 17 were monomorphic between BYG15-23 and AC43 and were analyzed as parental phase-unknown markers as described in the Materials and methods. For 15 of these markers, one phase was linked to phase-known markers in a linkage group, whereas the other phase was unlinked and therefore deleted from the data set. For 2 markers, both phases were unlinked and omitted from the analysis.

Segregation of one of the complementary loci conditioning light-red bulb color (*Crb-1* for *C*omplementary *r*ed *b*ulb) fit a 1:2:1 ratio ($P = 0.531$). All F_2 progenies and F_3 families from BYG15-23 × AC43 produced only yellow bulbs. All testcross bulbs from BYG15-23 were yellow; those from AC43 segregated 55 yellow to 45 light red, fitting the expected ratio of 1:1 ($P = 0.317$). Therefore, BYG15-23 is likely homozygous recessive at both of the complementary loci conditioning light-red bulbs; AC43 must be segregating at one of the complementary loci and homozygous recessive at the second (Jones and Peterson 1952). Linkage with *Crb-1* was established on group H between RFLP markers API94 (16.1 cM) and API76 (15.9 cM) (Fig. 1).

For onion plants possessing S-cytoplasm, male fertility is restored by a dominant allele at the nuclear male-fertility restoration locus *Ms* (Jones and Clarke 1943). Segregation of male-fertility restoration was scored among testcross progenies and fit a 1:2:1 ratio ($P = 0.120$). Linkage with *Ms* was established on group B between RFLP markers AOB210 (14 cM) and API65 (15 cM) (Fig. 1).

The alliinase clone revealed four to ten fragments (depending on enzyme digest) on parental DNA blots, most of which were monomorphic. Polymorphic fragments between BYG15-23 and AC43 generated with *Bam*HI (two fragments) and *Eco*RV (one fragment) segregated; all other fragments generated by these digests (five and nine, respectively) were monomorphic between parents and did not segregate. Mapping revealed 2 closely linked (6.9 cM on group A) RFLPs hybridizing with alliinase (Fig. 1).

Duplicated and triplicated loci were detected by 20% and 1%, respectively, of the cDNA clones. Signal intensities were approximately equal for fragments representing multiple loci. The distributions of multiple loci detected by single clones were 42% tightly linked (< 10 cM), 5% loosely linked (10–30 cM), and 53% unlinked (> 30 cM) (Fig. 1). Several linkage groups

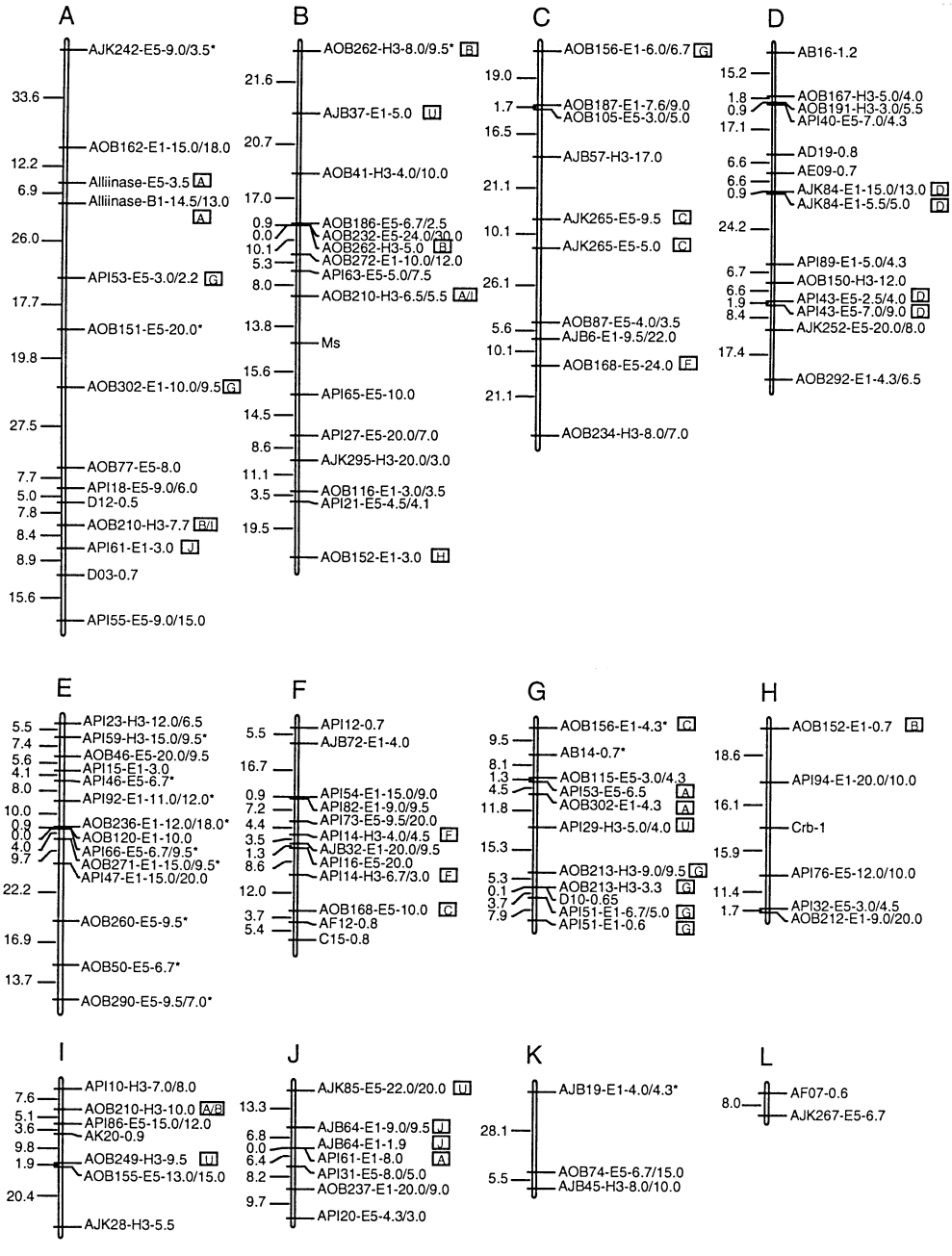

Fig. 1 Linkage groups in the genetic map of *Allium cepa*. Map distances (in cM) are to the *left*, and locus designations are to the *right* of each group. RFLP loci are indicated by clone designations of *AOB*, *AJB*, *AJK*, or *API*. *Prefixes* are followed by *numbers*, reflecting the order of clone isolation, then by an abbreviation for the restriction enzyme-generating polymorphic fragments detected by the clone (*E1*, *E5*, *H3*, and *B1* for *Eco*RI, *Eco*RV, *Hin*dIII, and *Bam*HI, respectively), then by estimated sizes (in kb) of polymorphic fragments as AC43 fragment/BYG15-23 fragment. RAPD loci are indicated by the primer followed by *sizes* in kilobases of the mapped polymorphic PCR products (Bradeen and Havey 1995). *Asterisks* indicate distorted segregation at $P < 0.01$. Location of duplicated loci are indicated by *boxed letters* corresponding to linkage groups (U = unlinked). Two RAPD (AB20 and AG19) and 10 RFLP (AJB37-E1-5.0, AJK85-E5-10.0, AJK248-E5-5.0, AOB107-E5-10.0, AOB114-E1-7.0, AOB117-E5-6.7, AOB200-E5-7.0/4.0, AOB249-H3-4.3, API29-H3-9.0/9.3, and API81-H3-6.7) markers remained unlinked

(A, B, C, D, G, J) contained many duplicated loci whereas other groups (E, F, H, I, K) contained few or no duplications (Fig. 1). Among duplicated loci detected by single clones, 19% segregated as 2 loci each with two codominant alleles, 52% segregated as 1 locus with codominant alleles and 1 locus with only a dominant fragment, and 29% segregated as 2 loci with only dominant fragments.

Of the cDNAs detecting duplicated loci, 12 (67%) showed homology to known gene families that often show linkage in other plants (Table 1). Six (33%) cDNA clones had no matches in the sequence databases, of which 2 and 4 detected linked (< 30 cM) and unlinked (> 30 cM) duplicated RFLPs, respectively (Table 1). Clone AOB156 showed significant homology with glutathione S-transferase but possessed an internal poly A tract and may be chimeric.

Discussion

Our low-density genetic map of onion is composed of 116 markers on 12 linkage groups encompassing

Table 1 Sequence homology of onion cDNA clones detecting duplicated restriction fragment length polymorphisms

cDNA	Linkage[a]	BLASTX score[b]	Organism	Product
AJB064	Linked	711	Shallot	Mannose-specific lectin
AJK084	Linked	81	Potato	Ubiquinol-cytochrome C reductase
AJK265	Linked	686	Cotton	Chlorophyll A/B binding protein
AOB213	Linked	775	Tobacco	Chlorophyll A/B binding protein
API14	Linked	No match		
API43	Linked	No match		
API51	Linked	416	*Arabidopsis*	Chlorophyll A/B binding protein
AJB037	Unlinked	No match		
AJK085	Unlinked	No match		
AOB152	Unlinked	311	Rice	Phospholipase D
AOB156	Unlinked	342	Tobacco	Glutathione S-transferase
AOB168	Unlinked	297	Yeast	Protein phosphatase
AOB210	Unlinked	351	*Arabidopsis*	RuBISCO small subunit
AOB249	Unlinked	573	Onion	Alliinase
AOB262	Unlinked	643	*Arabidopsis*	Ubiquitin
AOB302	Unlinked	248	Apple	Elongation factor
API29	Unlinked	246	Pea	High mobility group protein
API53	Unlinked	No match		
API61	Unlinked	347	Tobacco	RuBISCO small subunit

[a] Linkage relationships shown in Fig. 1
[b] BLASTX searches completed on databases of the National Center for Biotechnology Information (ver. 1.4.9MP, March 26, 1996)

10.6 Morgans. Although we estimated the genetic map length of onion to be approximately 10 Morgans [based on an average of 19 chiasmata per meiosis (Albini and Jones 1988)], we have not yet resolved the 8 linkage groups of onion. The average marker spacing on the map is 9 cM, and the largest gap between markers is 33.6 cM (linkage group A).

With this map, we have significantly expanded the single previously reported linkage in onion (Jones et al. 1944) and established linkages with two morphological loci (*Crb*-1 and *Ms*) conditioning traits of economic importance. RFLPs API94 and API76 flanking *Crb-1* on linkage group H (Fig. 1) may be useful to select against dominant alleles that produce undesirable light-red bulbs when crossing between North American and European or South American germplasms (Jones and Peterson 1952). RFLPs revealed by clones AOB210 and API65 flank the *Ms* locus. Onion is a biennial, and 4–8 years are required to establish the cytoplasm and genotype at the *Ms* locus of individual onion plants (Havey 1995). Molecular markers flanking the *Ms* locus will provide a significantly more efficient method to establish genotypes at *Ms*, and we are currently working to identify markers linked closer to *Ms*.

Alliinase plays an pivotal role in the development of onion flavor and pungency (Schwimmer and Weston 1961). We hybridized an alliinase cDNA clone (van Damme et al. 1992) and revealed tandemly duplicated loci (Fig. 1). One anonymous clone, AOB249, showed significant homology to alliinase and detected two unlinked RFLPs. Because van Damme et al. (1992) detected only one alliinase transcript in onion and garlic shoots, the duplicated loci may produce transcripts identical to the gene identified by van Damme et al. (1992), may be expressed in tissues other than shoots, or may be pseudogenes. Future experiments will determine if these chromosome regions explain a significant component of the phenotypic variability for onion pungency.

In our map, 21% of cDNA probes detected more than 1 segregating RFLP. This level of duplication is greater than that reported for other diploids, including *Arabidopsis thaliana* (Chang et al. 1988), barley (Kleinhofs et al. 1993), rice (McCouch et al. 1988), celery (Yang and Quiros 1995), bean (Nodari et al. 1993), cucumber (Kennard et al. 1994), tomato (Helentjaris et al. 1986), and asparagus (Restivo et al. 1995). However, some of these studies used genomic clones selected for low-copy number and may have reduced the proportion of clones detecting duplicated regions (Nodari et al. 1993; Restivo et al. 1995). The level of duplication in onion was less than that reported for maize (Helentjaris et al. 1988; Whitkus et al. 1992), soybean (Keim et al. 1990; Funke et al. 1993; Shoemaker et al. 1996), *Brassica* spp. (McGrath and Quiros 1991; Song et al. 1991), or loblolly pine (Neale et al. 1994). However unlike these species, approximately one-half of the duplicated bulb-onion RFLPs were linked at less than 30 cM (Fig. 1). We believe this level of duplication in the onion genome is a minimal estimate because we employed high-stringency hybridization and wash conditions, our probes commonly detected numerous monomorphic restriction fragments, and we scored duplications only when recombination was detected among 58 segregating progenies. Under these conditions, divergent duplications would be undetected and tightly linked duplicated loci would be scored as a single locus.

We observed 40% dominant RFLP loci, the highest reported for any plant including the diploid species barley (Kleinhofs et al. 1993), bean (Nodari et al. 1993), *Arabidopsis thaliana* (Chang et al. 1988), cucumber (Kennard et al. 1994), lentil (Havey and Muehlbauer 1989), tomato (Helentjaris et al. 1986), or asparagus (Restivo et al. 1995). Xu et al. (1994) reported 14% dominant RFLP loci in sorghum using *Pst*I-genomic clones. Dominant RFLPs could be due to hemizygous duplications (present in only one parent of the mapping population) or the comigration of duplicated fragments. The probabilities of both scenarios increase in genomes with substantial sequence reiteration. In cotton, a paleopolyploid species, segregation of dominant RFLPs was attributed to comigrating duplicated fragments arising from polyploidy (Reinisch et al. 1994). In onion, recent tandem duplication and retropositional events (discussed below) could increase the frequency of dominant RFLPs.

The evolution of large genomes can be attributed to sequence multiplication and divergence (Kimura 1961). Leipoldt and Schmidtke (1982) proposed that polyploidy is widespread in prokaryotic and eukaryotic evolution and that all eukaryotes are diploidized polyploids. Polyploidy can arise from the duplication of entire genomes through somatic doubling, restitution gametes, or wide hybridization. Many contemporary genomes in which mapping revealed frequent duplications are polyploids or diploidized products of ancient polyploids, e.g., soybean (Keim et al. 1990; Funke et al. 1993; Shoemaker et al. 1996), *Brassica* spp. (McGrath and Quiros 1991; Song et al. 1991), and maize (Helentjaris et al. 1988). In the evolution of paleopolyploid genomes, chromosome rearrangements often shift linkage relationships among blocks of markers, yielding duplicated regions with conserved linkage orders shuffled throughout the genome (Helentjaris et al. 1988; Bonierbale et al. 1988; Tanksley et al. 1988; Whitkus et al. 1992). Increased genome sizes could also result from the duplication of individual or sets of chromosomes followed by centric fusions to produce larger metacentric chromosomes (Ohno 1970), transposition events including the movement of DNA fragments and RNA-mediated retroposition (Vanin 1985), and tandem duplications through infidelity of meiotic recombination (Smith 1976). In general, mechanisms associated with paleopolyploidy increase chromosome numbers, whereas these latter mechanisms increase chromosome sizes (Sparrow and Nauman 1976).

Contemporary frequencies and distributions of duplicated loci provide criteria for distinguishing the origins of duplications during genome evolution. However, comparative models of genome evolution based on frequencies of duplications detected in mapping experiments are subject to misinterpretation due to differences in experimental methods, including criteria for selecting mapping clones (i.e., genomic versus cDNA clones and choice of probes based on signal intensities or numbers of fragments), degrees of polymorphism and genetic distance between parents of mapping populations, and methods of calculating percent duplication. For example, Dubcovsky et al. (1996) reported 27–34% multiple loci in *Triticum monococcum*, which has a genome three-fold smaller in size than onion. Their values were calculated as the percentage of total loci that were part of multiple locus sets detected by single clones. Our 23% duplication rate in onion was calculated as the percentage of cDNA clones detecting more than 1 segregating locus. When this method is applied to the data reported for *T. monococcum*, about 16% of clones detect multiple loci, and when our data are analyzed as in Dubcovsky et al. (1996), 39% of the total loci were part of multiple locus sets. While the percentage of clones detecting multiple loci appears to be a more common criterion for reporting duplications (Helentjaris et al. 1988; Keim et al. 1990; Gill et al. 1991; McGrath et al. 1993; Chittenden et al. 1994; Neale et al. 1994; Reinisch et al. 1994; Whitkus et al. 1994; Gentzbittel et al. 1995), both of these approaches may produce reasonable estimates of genome duplication. However, without standardized experimental procedures, simply comparing estimates of genome duplication across species may be confusing.

Extensive duplication might suggest paleopolyploidy and subsequent diploidization in the evolution of onion. However, there is no cytogenetic evidence for a polyploid origin of the bulb onion (Narayan 1988), and the linked nature of many dupicated loci, prevalence of dominant RFLPs, and absence of conserved, duplicated linkage blocks in onion differ from most paleopolyploids. Transposition of DNA (Pichersky 1990) and RNA-mediated retroposition (Vanin 1985) are known to duplicate coding (Matters and Goodenough 1992; Kvarnheden et al. 1995) and noncoding (Smith 1976; SanMiguel et al. 1996) regions of genomes. In loblolly pine, Devey et al. (1994) observed a level of duplication similar to onion, and many pine cDNAs detected unlinked duplicated regions concentrated on five linkage groups. The authors concluded that this distribution of duplications was not due to paleopolyploidy and proposed retrotransposition as the mechanism of gene amplification. Retrotransposons are abundant in the intergenic regions of maize and may account for at least 50% of the nuclear genome (SanMiguel et al. 1996). Retroposition tends to insert sequences randomly into genomes (Vanin 1985) and could account for the unlinked duplications in the onion genome. Hybridization of *del2,* an abundant non-LTR retrotransposon from *Lilium speciosum*, to *Bam*HI-digested onion DNA detected a prominent band at 6.6 kb, suggesting that retrotransposon-like sequences are present in the onion genome (Leeton and Smyth 1993). The *del2*-like sequence in onion may contribute mainly to an expansion of intergenic regions and, to a smaller degree, duplication of coding regions (as detected by cDNA probes). The detection of mul-

tiple loci and numerous restriction fragments with our cDNA probes could be revealing retropseudogenes in many gene families.

Tandem duplication of DNA is attributed most commonly to unequal crossing over during meiotic recombination (Smith 1976). This process, proposed by Jones and Rees (1968) and Ranjekar et al. (1978) to explain uniform size increases of onion chromosomes, would increase DNA content without increasing chromosome number, produce closely linked duplicated loci, and account for the loops observed during pachytene in interspecific hybrids between *A. cepa* and *A. fistulosum* (Jones and Rees 1968). Meiotic pairing and unequal crossing-over (Anderson 1987; Tartof 1988) at homologous middle-repetitive regions flanking single-copy sequences (Stack and Comings 1979) could duplicate the single-copy regions. This would produce gametes with tandemly duplicated and deficient regions. The union of a wild-type gamete with the gamete carrying the tandemly duplicated region would produce a viable progeny with linked codominant and dominant loci. Presumably, the deficient gamete would be detrimental and selected against. Continued unequal crossing-over within the middle-repetitive region could separate the tandemly duplicated single-copy regions, allowing for the occasional recombinants observed in our mapping population.

The majority of clones detecting duplicated RFLPs in onion showed significant homology with gene families encoding abundant transcripts (Table 1). These gene families often show linkage in other plants, e.g., ubiquitin (Callis et al. 1995), RuBISCO (Sugita et al. 1987; Krebbers et al. 1988), chlorophyll AB-binding proteins (Pichersky et al. 1985; Leutwiler et al. 1986, Pichersky et al. 1987), lectins (van Damme et al. 1993), elongation factors (Fukuda et al. 1992), protein phosphatases (Shiozaki et al. 1994), and DNA binding proteins (Grasser et al. 1993). Pichersky (1990) observed that genes encoding nonabundant proteins are seldom duplicated and, when they are, the two or more copies are almost always unlinked. If tandem duplication of chromosomal segments were important in the evolution of the huge nuclear genome of onion, the mapping of relatively low-copy cDNAs should reveal tandemly duplicated RFLPs. Clones API14 and API43 showed no significant sequence homology with the major sequence databases (Table 1) and revealed duplicated RFLPs linked at 13.4 and 1.9 cM, respectively (Fig. 1). Clones AJB037, AJK085, AOB249 and API53 detected unlinked duplicated RFLPs (Fig. 1) and had no significant homology with previously characterized sequences (Table 1). Clone AOB152 showed significant homology to phospholipase D, a likely low-copy gene (Ueki et al. 1995), and revealed unlinked duplicated RFLPs.

Our low-density genetic map of onion revealed insights about the structure and organization of one of the largest diploid genomes among cultivated plants. The relatively high frequency of linked duplicated and dominant RFLPs was unique among plant species. The distribution of duplicated RFLPs was more similar to that observed in loblolly pine (Neale et al. 1994) than paleopolyploid angiosperms (Helentjaris et al. 1988; Whitkus et al. 1992; Shoemaker et al. 1996). Our data do not support paleopolyploidy in onion, instead they indicate that tandem, transpositional, or retropositional duplication of specific chromosomal regions and possibly subsequent rearrangements have occurred. This model accounts for linked and unlinked duplications, the prevalence of dominantly inherited RFLP loci, as well as the very large genome with a low base chromosome number. However, the possibility remains that comigration of duplicated restriction fragments could produce the dominant RFLPs and, in an extensively rearranged paleopolyploid genome, synteny among duplications would not be detected.

Tandem duplication of chromosomal segments may be an important factor in the evolution of large-genome species. Although unequal crossing over has been well characterized at individual loci (Veit et al. 1990; Robbins et al. 1991), the extent to which it contributes to gene duplication and genome expansion is unknown. In *Allium*, there is cytological (Jones and Rees 1968; Ranjekar et al. 1978; Narayan 1988) and now genetic (Fig. 1, Table 1) evidence for tandem duplication of chromosomal regions. Over the long term, a thorough understanding of the forces that control duplication and expression of duplicated regions of the genome will provide insights about this potentially important mechanism of genome evolution.

Disclaimer Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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