Variation and Selection at the CAULIFLOWER Floral Homeotic Gene Accompanying the Evolution of Domesticated Brassica oleracea

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ABSTRACT

The evolution of plant morphologies during domestication events provides clues to the origin of crop species and the evolutionary genetics of structural diversification. The CAULIFLOWER gene, a floral regulatory locus, has been implicated in the cauliflower phenotype in both Arabidopsis thaliana and Brassica oleracea. Molecular population genetic analysis indicates that alleles carrying a nonsense mutation in exon 5 of the B. oleracea CAULIFLOWER (BoCAL) gene are segregating in both wild and domesticated B. oleracea subspecies. Alleles carrying this nonsense mutation are nearly fixed in B. oleracea ssp. botrytis (domestic cauliflower) and B. oleracea ssp. italic (broccoli, both of which show evolutionary modifications of inflorescence structures. Tests for selection indicate that the pattern of variation at this locus is consistent with positive selection at BoCAL in these two subspecies. This nonsense polymorphism, however, is also present in both B. oleracea ssp. oca (cauliflower) and B. oleracea ssp. oleracea (wild cabbage). These results indicate that specific alleles of BoCAL were selected by early farmers during the domestication of modified inflorescence structures in B. oleracea.

DOMESTICATED plant species provide excellent models to study and test hypotheses on the genetics and evolution of morphological diversification (Doebley 1992, 1993; Doebley et al. 1997; Doebley and Lukens 1998). The domestication of crop species is invariably accompanied by evolutionary changes in suites of structural traits that differentiate cultivated species from their wild relatives, or even between various crop subspecies (Schwanitz 1967; Doebley 1993). Crop species have thus been widely regarded as providing some of the best and most dramatic examples of the degree to which plant morphologies evolve under selection pressures (Gottlieb 1984; Doebley 1992).

One approach to understanding the evolutionary dynamics of morphological change focuses on identifying genes that underlie trait differences between domesticated and wild species and exploring the population genetics of these domestication loci. A molecular population genetic approach provides a particularly powerful framework for assessing how evolutionary forces act to shape variation at genetic loci and delineate mechanisms that may accompany evolutionary diversification during crop domestication. Indeed, studies on molecular diversity at the sequence level have begun to be utilized in inferring both the general population structure and history of crop domestication events (Eyre-Walker et al. 1998; Hilton and Gaut 1998; Olsen and Schaal 1999) and the selective forces acting at specific genes that underlie domestication and divergence in early agricultural crops (Hanson et al. 1996; Wang et al. 1999).

The distinct morphologies exhibited by Brassica oleracea subspecies represent one of the most spectacular illustrations of structural evolution in plants under domestication. B. oleracea is a perennial herb found largely in Europe and the Mediterranean (Tsunoda et al. 1980; Song et al. 1988; Kallo and Bergh 1993) and is an extremely polymorphic species that includes at least six cultivated and one wild subspecies. Wild, perennial forms of B. oleracea, designated subspecies oleracea (wild cabbage, grow in coastal rocky cliffs of the Mediterranean, northern Spain, western France, and southern and southwestern Britain (Tsunoda et al. 1980). Selection for different characteristics during domestication, however, has resulted in extreme morphological divergence among cultivated subspecies. Of the six domesticated taxa, two subspecies, B. oleracea ssp. botrytis (cauliflower) and B. oleracea ssp. italic (broccoli), are characterized by the evolutionary modification of the inflorescence into large dense structures. The precociously large, undifferentiated inflorescence, termed the curd, is the defining characteristic of B. oleracea ssp. botrytis. The cauliflower curd consists of a dense mass of arrested inflorescence meristems, only ~10% of which will later develop into floral primordia and normal flowers (Sadik 1962).

The cauliflower phenotype characteristic of B. oleracea ssp. botrytis has been observed in mutants of the related crucifer Arabidopsis thaliana (Bowman et al. 1993; Weigel 1995; Yanofsky 1995). In Arabidopsis, the early
acting floral meristem identity genes are a class of flower developmental regulatory loci that specify the identity of the floral meristem (as opposed to the inflorescence meristem) in developing reproductive primordia. Members of this class include the genes APETALA1 (AP1; Mandel et al. 1992; Gustafson-Brown et al. 1994) and CAULIFLOWER (CAL; Kempin et al. 1995). Both the APETALA1 and CAULIFLOWER loci have also been shown to control the specification of floral meristem identity. Arabidopsis individuals that are mutant for both AP1 and CAL are arrested in development at the inflorescence meristem stage (Kempin et al. 1995). In these plants, a dense mass of inflorescence meristems develops, similar to the B. oleracea ssp. botrytis curd.

Genetic analyses in B. oleracea suggest the involvement of the B. oleracea CAL gene, referred to as BoCAL, in the formation of the altered inflorescence in B. oleracea ssp. botrytis (Kempin et al. 1995). It has been demonstrated already that the BoCAL allele in domesticated cauliflower has a premature termination codon at position 151 (E → stop; Kempin et al. 1995). This nonsense mutation appears to have arisen fairly recently within B. oleracea. In this article, we report that haplotypes carrying this polymorphism are fixed or nearly fixed in B. oleracea ssp. botrytis and B. oleracea ssp. italicata, the two subspecies that have undergone selection for altered patterns of inflorescence development. Our tests for selection suggest that the BoCAL gene in B. oleracea ssp. botrytis and B. oleracea ssp. italicata experienced a recent adaptive sweep, consistent with the evolution of the characteristic inflorescence structures in these subspecies. These results suggest that the floral regulatory gene BoCAL was one of the targets of selection during the evolutionary domestication of subspecies within the vegetable crop B. oleracea.

MATERIALS AND METHODS

Study species: The following B. oleracea subspecies were used in these analyses: B. oleracea ssp. oleracea (wild cabbage), B. oleracea ssp. acephala (kale), B. oleracea ssp. botrytis (cauliflower), and B. oleracea ssp. italicata (broccoli). The wild relative B. incana was also utilized in this study. Seeds from these subspecies were obtained from the HRl Genetic Resources Unit at Wellesbourne, UK, the Center for Genetics Resources in The Netherlands, and the USDA-ARS Plant Genetic Resources Unit at Geneva, NY.

Isolation and sequencing of BoCAL alleles: Genomic DNA from young B. oleracea leaves was isolated using the plant DNAeasy miniprep kit (QIAGEN, Chatsworth, CA). PCR was performed with an initial 10 cycles of 15 sec at 94°C, 30 sec at 48°C, and 2 min at 68°C followed by 25 cycles with an incremental increase of 20 sec cycle of the extension time. The error-correcting recombinant Pwo polymerase (Boehringer Mannheim, Mannheim, Germany) was used to minimize nucleotide misincorporation. The error rate for this polymerase, based on multiple amplification and resequencing of known genes, is similar to other error-correcting polymerases and is <1 in 7000 bp (Purugganan and Suddith 1999). We estimate that the nonsampling variance of nucleotide diversity due to PCR misincorporation, Var(π), is negligible [Var(π)/Var(π) ~ 0.14; J. I. Suddith and M. D. Purugganan, unpublished results]. The BoCAL-specific primers BoCALSF2 (5′-TAATCATAGCATTACTGGG-3′) and BoCALBR3 (for exons 8 reverse; 5′-TGCACTAATTGTTTCAAAGTC-3′) were used in PCR reactions to amplify alleles from most B. oleracea accessions. For two B. oleracea ssp. acephala and one B. incana allelic gene, the gene was isolated in two pieces: two additional internal primers (BoCALSR2 [5′-CAACCAAGAGTGTGGATGCTCTA-3′] and BoCALBR2 [5′-GATGCACTTGGTTACATAATGAAG-3′]) were constructed in addition to BoCALSF2 and BoCALBR3 to isolate these alleles. Amplified DNA was cloned into pCR2.1 using the TA cloning kit (Invitrogen, Carlsbad, CA). DNA sequencing for both genes was conducted with the ABI377 automated sequencer using a series of nested internal sense and antisense primers. All sequence polymorphisms were visually rechecked from chromatograms, with special attention to low-frequency polymorphisms (Hamblin and Aquadro 1997). The DNA sequences are available from GenBank (accession nos. AF241113–AF241150).

Data analysis: Sequences used in this study were visually aligned. Phylogenetic analyses were conducted using PAUP* 4.0d54 (maximum parsimony; Swofford 1993). The heuristic search algorithm was utilized using the tree bisected-reconnection procedure, with the B. incana orthologue as the outgroup. Node support is assessed with 500 bootstrap replicates of the data. The polymorphism data were analyzed using the SITES (Hey and Wakeley 1997) and DNASP programs (Rozas and Rozas 1997). Levels of nucleotide diversity were estimated as mean pairwise differences (π) and number of segregating sites (S; Nei 1987). Identification of possible recombinants utilized the four-gamete test (Hudson and Kaplan 1985). The Tajima (1989) and Fu and Li (1993) tests for distribution of nucleotide polymorphisms were conducted without specifying an outgroup. These tests are known to have low power with small sample size; we thus pooled allelic data for subspecies B. oleracea ssp. botrytis and B. oleracea ssp. italicata, and for B. oleracea ssp. oleracea and B. oleracea ssp. acephala. The former group includes those subspecies that showed evolutionary alterations in inflorescence morphologies.

RESULTS AND DISCUSSION

Nucleotide variation at the B. oleracea CAULIFLOWER floral regulatory locus: We isolated alleles of the BoCAL gene from 37 worldwide accessions, representing four distinct subspecies. These include three domesticated subspecies that display differing reproductive or vegetative morphologies as a result of the selective pressures that accompanied domestication of this vegetable crop. Two subspecies, B. oleracea ssp. botrytis (cauliflower) and B. oleracea ssp. italicata (broccoli), display altered inflorescence morphologies as a result of evolutionary divergence in reproductive developmental programs. The other domesticated study subspecies, B. oleracea ssp. acephala, shows the curling of leaf edges characteristic of kale, but otherwise produces a stereotypical Brassica raceme. Finally, B. oleracea ssp. oleracea accessions were included to represent the closest wild relatives of the domesticated subspecies; plants in this subspecies display no apparent changes in either vegetative or reproductive form. Most of the accessions utilized were from the Mediterranean and Northern Europe, where this...
The BoCAL orthologue from the closely related congener B. incana was also isolated to provide an interspecific comparison of gene divergence.

Approximately 2.01 kb of the BoCAL gene was sequenced for each isolated allele; the sequenced region spans intron 2 to exon 8 (Figure 1) and includes the coding region for the moderately conserved K-domain of the BoCAL MADS-box transcriptional activator. The K-domain is believed to serve as a dimerization interface among MADS-box proteins. The sequenced region also encodes the C-terminal region as well as a portion of the linker I-region; the former is believed to contain the transcriptional activation domain of MADS-box proteins (Riechmann and Meyerowitz 1997).

Molecular analyses reveal a large amount of variation at the BoCAL locus (Figure 2 and Table 1). A total of 87 variant sites are present in these sampled alleles, of which 35 are nucleotide polymorphisms and 52 are from insertion/deletion (indel) changes of 1–12 bp in length. All of the indels are in introns. Of the 35 nucleotide polymorphisms found in BoCAL, 25 are located within introns while 10 are in coding regions. The coding region polymorphisms include 7 replacement and 3 silent site variants. The estimate of species-wide nucleotide diversity, \( \pi \), for BoCAL is 0.0030, which is about half the value observed for the Arabidopsis CAL gene (Purugganan and Suddith 1998). The levels of nucleotide diversity at the BoCAL gene differ between B. oleracea subspecies (Table 1). Nucleotide diversity estimates within B. oleracea range from 0.0003 in B. oleracea ssp. botrytis to 0.0053 in the wild B. oleracea ssp. oleracea.

A total of 17 distinct BoCAL haplotypes are evident within B. oleracea. One of these haplotypes predominates in the sample and accounts for 16 of the 37 alleles (43%). Of the other haplotypes, 12 are singletons, while 3 are found twice and 1 haplotype is observed three times in the data. The genealogy of these alleles is shown in Figure 3. The phylogeny is the result of 500 bootstrap replicates under maximum parsimony, and a tree based on neighbor-joining analysis gives the same topology. There appear to be two major BoCAL allele classes within the sample: (i) class I alleles, which are found in one B. oleracea ssp. italica and two B. oleracea ssp. oleracea accessions; and (ii) class II alleles, which account for the majority of the observed alleles (92%). The two classes are differentiated by 28 fixed nucleotide differences (Figure 2), including three replacement changes. The two allele classes do not originate from different BoCAL genes. We have isolated all three BoCAL genes in the B. oleracea genome (A. L. Boyles, S. Halldorsdottir and M. D. Purugganan, unpublished results), and the alleles in this study originate from the one locus previously identified as responsible for the Brassica cauliflower phenotype (Kempin et al. 1995). One allele from B. oleracea ssp. acephala (accession HRI7556 from Ireland) appears to be the product of the recombination between class I and II alleles. Overall, the method of Hudson and Kaplan (1985) detects a total of two recombination events among alleles in the sampled B. oleracea accessions.

There is no discernible structuring of alleles along subspecific boundaries for B. oleracea ssp. oleracea and B. oleracea ssp. acephala. The gene genealogy indicates that alleles isolated from these two subspecies are interspersed in the genealogy, for example, some B. oleracea ssp. acephala alleles are more closely related to either B. oleracea ssp. botrytis or B. oleracea ssp. italica than they are to those found in other kales (Figure 3). The genealogy does indicate a close relationship, however, between B. oleracea ssp. botrytis and B. oleracea ssp. italica. Except for one B. oleracea ssp. italica class I allele, the alleles in these two groups are all found in the same clade in the reconstructed genealogy.

A nonsense polymorphism is segregating in B. oleracea populations: Among the 10 coding region polymorphisms observed in B. oleracea, 6 result in amino acid replacements in the BoCAL protein encoded by specific alleles. Three of these replacements are singletons found in class II alleles, while three others differentiate the two allele classes observed in this species. A G → T transversion in exon 5 results in a replacement polymorphism (GAG → TAG) that changes a glutamic acid to a premature termination codon in position 151 of the encoded protein (Figure 2); this previously identified nonsense mutation results in a truncated protein that includes the DNA-binding MADS-box, the I-region, and a portion of the K-domain.

This nonsense polymorphism is present at moderate frequencies in the sampled alleles. Of the 37 B. oleracea alleles, 23 contain this premature stop codon (62%); all nonsense alleles are found in the class II haplotypes.
The nonsense mutation that gave rise to this polymorphism appears to be of fairly recent origin; all alleles that contain this substitution differ from each other by fewer than two nucleotide substitutions.

The nonsense polymorphism in BoCAL predomi-

nates, and indeed is close to fixation, in subspecies that
have evolved altered inflorescence structures under do-
mestication. All B. oleraceassp. botrytis alleles contain this
premature termination codon, and it is also found in 8
of the 9 alleles sampled from B. oleracea ssp. italic

<table>
<thead>
<tr>
<th>Group</th>
<th>Common name</th>
<th>Modification</th>
<th>n</th>
<th>S</th>
<th>π</th>
<th>Θ</th>
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<tbody>
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<td>37</td>
<td>35</td>
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</tr>
<tr>
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<td>31</td>
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<tr>
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<td>11</td>
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<td>0.0023</td>
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<td>3</td>
<td>0.0003</td>
<td>0.0006</td>
</tr>
<tr>
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<td>Inflorescence</td>
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<td>0.0059</td>
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<td>Inflorescence</td>
<td>19</td>
<td>33</td>
<td>0.0018</td>
<td>0.0049</td>
</tr>
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</table>

NA, not applicable.
a Number of sampled accessions.
b Number of segregating sites.
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Figure 3.—Gene genealogy of BoCAL alleles. All nodes with <50% bootstrap support are collapsed; the other bootstrap values are indicated next to relevant nodes. Class I and II alleles for BoCAL are indicated. The arrow shows the probable branch associated with the origin of the nonsense mutation found in many BoCAL alleles. The total tree length is 101 steps, and the consistency index is 0.8713.

(95%). This nonsense polymorphism, however, is not confined to taxa that display altered inflorescence morphologies. This mutation is also found in 3 of the 7 B. oleracea ssp. acephala (43%) and 2 of the 11 B. oleracea ssp. oleracea alleles (18%; Figure 2). The widespread distribution of this polymorphism in B. oleracea subspecies suggests either that (i) it arose prior to the origin of cauliflower and broccoli or that (ii) it originated in B. oleracea ssp. botrytis and/or B. oleracea ssp. italica, but spread to other groups via hybridization. The frequency of this allele also suggests that there is no strong negative selection for this mutation in these subspecies.

Selective sweep at the BoCAL gene in B. oleracea ssp. botrytis and B. oleracea ssp. italica is associated with fixation of nonsense haplotypes: The extent and patterning of nucleotide variation along the BoCAL locus suggests that this regulatory gene is evolving in a nonneutral fashion. Specifically, it appears that alleles containing the nonsense polymorphism in exon 5 have been one of the targets of selection in subspecies displaying altered inflorescence development. Allelic variation is expected to be reduced in a gene under selection (Aquadro 1997). Indeed, levels of molecular variation for this floral meristem identity gene are markedly reduced in those subspecies that show evolutionary alterations in inflorescence development. The value of π for the combined data from B. oleracea ssp. botrytis and B. oleracea ssp. italica alleles is less than
half of that estimated for B. oleracea ssp. acephala and B. oleracea ssp. olareca (τ = 0.0018 vs. 0.0040). B. oleracea ssp. botrytis BoCAL alleles are nearly identical to one another, with only four polymorphic nucleotide sites within the cultivated group. In B. oleracea ssp. italica, all of the variation is contributed by the presence in the sample of one class I allele; all the other alleles in this subspecies are identical to one another and all contain the nonsense mutation. The reduction in polymorphism at BoCAL within these two subspecies does not appear to be due to a population bottleneck during domestication. Both randomly amplified polymorphic DNA and isozyme studies indicate a significant level of polymorphism within these two subspecies at other molecular markers (Hu and Quiros 1991; Simonsen and Heneen 1995).

The action of historical adaptive sweeps in genes can also be detected by a number of tests for selection. Two tests, proposed by Tajima (1989) and Fu and Li (1993), compare the nucleotide diversity with the distribution of segregating sites expected under the neutral model of molecular evolution (Simonsen et al. 1995). Both tests reveal that the BoCAL gene is not evolving according to the predictions of the neutral model, and the pattern of nucleotide variation in this regulatory locus is consistent with a hypothesis of positive selection within some B. oleracea subspecies. Specifically, there is evidence of selection in subspecies that display altered inflorescence morphologies. In the combined alleles of BoCAL from B. oleracea ssp. botrytis and B. oleracea ssp. italica, the skewness in the frequency distribution of polymorphisms is significant in both tests. The Tajima test statistic $D$ is $-2.4418$ ($P < 0.001$) for the combined alleles in these two subspecies; the negative value of the $D$ statistic indicates that sampled alleles have an excess of low-frequency nucleotide polymorphisms over that expected in a neutrally evolving population. The excess of rare polymorphisms in these subspecies is due primarily to the presence of the single, divergent class I allele in B. oleracea ssp. italica. The Fu and Li test statistic $D^*$ is also significantly negative for this gene ($D^* = -3.56997$, $P < 0.02$). In contrast, results of both the Tajima ($D = -0.7070$, $P > 0.10$) and Fu and Li tests ($D^* = 1.1645$, $P > 0.10$) with the combined alleles in both B. oleracea ssp. acephala and B. oleracea ssp. oleracea reveal that these genes are evolving according to the predictions of the equilibrium-neutral theory.

**Molecular population genetics of regulatory genes associated with evolution under crop domestication:** A comprehensive understanding of the process by which plant morphologies evolve under domestication requires us to (i) isolate genes that were the targets of selection by early agriculturalists and (ii) dissect the evolution of these domestication loci. Understanding the molecular genetics of a developmental system allows us to identify candidate genes and gene-gene interactions that may be the focus of selection during the process of morphological diversification. Subsequent studies on the molecular population genetics of morphological loci can then provide us with crucial information on the origin, history, and evolutionary forces that underlie the transformation in plant morphologies that accompany crop domestication events.

We have focused our attention on the genes that underlie the transformation in inflorescence morphologies observed in some subspecies within B. oleracea. Specifically, it has been suggested that the presence of a nonsense mutation at position 151 of the BoCAL floral regulatory locus is responsible for part of the evolution of the cauliflower curd in B. oleracea ssp. botrytis (Kempin et al. 1995). Alleles containing this nonsense mutation are expected to produce proteins of 150 amino acids in length (as compared to the 254-amino-acid full-length protein), which are truncated in the middle of the K-domain of the encoded MADS-box transcriptional activator.

A survey of nucleotide variation at BoCAL in four subspecies of B. oleracea indicates that this nonsense polymorphism appears to have originated once in this species and that alleles containing this mutation are close to fixation in groups that display alterations in inflorescence morphology. In B. oleracea ssp. botrytis, all alleles isolated contain this nonsense polymorphism, while only one B. oleracea ssp. italica allele did not have this premature stop codon. Based on tests of selection, the near fixation of nonsense haplotypes in B. oleracea ssp. botrytis and B. oleracea ssp. italica is consistent with a selective sweep that, based on genetic studies in A. thaliana, is likely associated with the evolution of the altered inflorescence morphologies in these cultivated groups.

Although previous studies in A. thaliana strongly implicate mutations at the BoCAL gene in the cauliflower phenotype, it is possible that mutations at this locus may play a role in the evolution of broccoli as well. Indeed, at least one naturally occurring allele in the A. thaliana CAL gene appears to produce a high density of floral meristems reminiscent of that seen in domestic B. oleracea ssp. italica (Purugganan and Suddith 1998). The presence of a highly divergent haplotype in B. oleracea ssp. italica that does not contain the nonsense polymorphism does suggest, however, that this mutation is not necessary for the formation of the broccoli phenotype. This could suggest that other mutations at this or related genes may also be associated with the evolution of the distinct inflorescence phenotype of B. oleracea ssp. italica (Kempin et al. 1995; Lowman and Purugganan 1999). Moreover, there is a wide range of variation to the density, size, and degree of floral differentiation between different cultivars of B. oleracea ssp. italica, and this may reflect the greater variation observed at the BoCAL locus within this subspecies.

It is also clear from our analyses that the nonsense mutation at the BoCAL locus is not sufficient to condi-
tion the cauliflower phenotype in *B. oleracea*. This non-sense polymorphism is also found at moderate frequencies in both *B. oleracea* ssp. *oleracea* and *B. oleracea* ssp. *acephala*, two groups that produce normal inflorescences. Genetic studies in *A. thaliana* indicate that mutations at both the CAL and AP1 floral meristem identity genes are necessary to produce the cauliflower phenotype in this model plant (Bowman et al. 1993; Kempin et al. 1995). The AP1 orthologues in *B. oleracea* have been identified and exist in at least two copies (BoAP1-A and -B), and no mutation in either copy is clearly associated with either *B. oleracea* ssp. *botrytis* or *B. oleracea* ssp. *italica* (Carr and Irish 1997; Lowman and Purugganan 1999). Although inheritance in *B. oleracea* is digenic, comparative gene mapping studies indicate that this species is a polyploid with two to three copies of each genetic locus (Bohuon et al. 1998). It may be that mutations at another, as yet unidentified BoAP1 gene, may act in concert with the nonsense mutation at BoCAL to condition the altered inflorescence development observed in *B. oleracea* ssp. *botrytis*. It is also possible that polymorphisms at other BoCAL gene copies may be involved in the cauliflower phenotype. Indeed, we have identified two other BoCAL copies that appear to have arisen as a result of the ancient polyploidization event that led to the present-day *B. oleracea* genome (A. C. Lowman, S. Hallidordottir and M. D. Purugganan, unpublished results). Moreover, the presence of this non-sense polymorphism at moderate frequencies in *B. oleracea* ssp. *oleracea* and *B. oleracea* ssp. *acephala* suggests that negative selection is not acting strongly at this locus and that these BoCAL copies may be genetically redundant to one another.

The molecular population genetics of developmental loci that control morphological traits are poorly understood, particularly within crop species groups that exhibit marked morphological divergence as a result of domestication. There has been recent work on the population genetics of loci such as teosintebranched1 (tb1; Wang et al. 1999) and C1 (Hanson et al. 1996), both of which control traits that differ between *Zea* subspecies. It has been suggested that variation at promoter regions in these and other developmental control genes may be responsible for the evolution of plant developmental patterns (Doebley and Lukens 1998), and there is indeed evidence that selection at the tba1 promoter may have been associated with maize evolution (Wang et al. 1999). Our study suggests, however, that evolutionary changes in regulatory proteins themselves may also permit the diversification of plant morphologies. Although the altered inflorescences of subspecies within *B. oleracea* may be extreme, they nonetheless illustrate the possible role that regulatory proteins may play during crop domestication. The study of the molecular population genetics of these loci provides insights into the extent to which variation at these key regulatory loci accomplish morphological divergence in crop plant species.

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