Carotenoid Presence Is Associated with the *Or* Gene in Domesticated Carrot

Shelby L. Ellison,*[,1] Claire H. Luby,[†,1] Keo E. Corak,[†,1] Kevin M. Coe,[†] Douglas Senalik,* Massimo Iorizzo,[‡] Irwin L. Goldman,[†] Philipp W. Simon,* and Julie C. Dawson[†,2]

*Agricultural Research Service, United States Department of Agriculture, Madison and [†]Department of Horticulture, University of Wisconsin–Madison, Wisconsin 53706, and [‡]Department of Horticultural Science, North Carolina State University, Kannapolis, North Carolina 28081

ORCID IDs: 0000-0002-0790-4286 (S.L.E.); 0000-0002-4954-0468 (C.H.L.); 0000-0003-0766-5198 (K.M.C.); 0000-0001-8526-0554 (D.S.); 0000-0001-6978-6062 (P.W.S.); 0000-0002-9907-8611 (J.C.D.)

ABSTRACT Carrots are among the richest sources of provitamin A carotenes in the human diet, but genetic variation in the carotenoid pathway does not fully explain the high levels of carotenoids in carrot roots. Using a diverse collection of modern and historic domesticated varieties, and wild carrot accessions, an association analysis for orange pigmentation revealed a significant genomic region that contains the *Or* gene, advancing it as a candidate for carotenoid presence in carrot. Analysis of sequence variation at the *Or* locus revealed a nonsynonymous mutation cosegregating with carotenoid content. This mutation was absent in all wild carrot samples and nearly fixed in all orange domesticated samples. *Or* has been found to control carotenoid presence in other crops but has not previously been described in carrot. Our analysis also allowed us to more completely characterize the genetic structure of carrot, showing that the Western domesticated carrot largely forms one genetic group, despite dramatic phenotypic differences among market classes. Eastern domesticated and wild accessions form a second group, which reflects the recent cultivation history of carrots in Central Asia. Other wild accessions form distinct geographic groups, particularly on the Iberian peninsula and in Northern Africa. Using genome-wide F_{st}, nucleotide diversity, and the cross-population composite likelihood ratio, we analyzed the genome for regions putatively under selection during domestication and identified 12 regions that were significant for all three methods of detection, one of which includes the *Or* gene. The *Or* domestication allele appears to have been selected after the initial domestication of yellow carrots in the East, near the proposed center of domestication in Central Asia. The rapid fixation of the *Or* domestication allele in almost all orange and nonorange carrots in the West may explain why it has not been found with less genetically diverse mapping populations.

KEYWORDS *Daucus carota*; GWAS; population structure; carotenoids; domestication; selective sweep

CARROT domestication and modern breeding have been driven by selection for large roots containing abundant carotenoids, which are responsible for orange pigmentation in the taproot. The presence of carotenoids in root tissues is unlikely to confer an advantage for natural selection, but is meaningful in a domesticated context (Iorizzo *et al.* 2016) due to their visual appeal and the role of dietary provitamin A compounds in human health (Arscott and Tanumihardjo 2010). Carrots are among the richest sources of provitamin A carotenes in the human diet (Simon *et al.* 2009) and significant breeding effort has focused on increasing root carotenoid levels (Simon 2000; Simon and Goldman 2007; Simon *et al.* 2008). Although carotenoid biosynthetic genes have been mapped (Just *et al.* 2007), they do not fully explain the presence of high levels of carotenoids in carrot roots, leaving much of that mechanism unknown (Iorizzo *et al.* 2016; Ellison *et al.* 2017).

While carrot is known as a bright orange root crop, the original carrots domesticated in Central Asia ca. 900 CE were purple and yellow (Banga 1963) (Figure 1, A and B). There is some evidence for orange carrots earlier in history (Stolarczyk and Janick 2011), but it was not until six centuries after domestication that orange roots appeared

doi: https://doi.org/10.1534/genetics.118.301299
Manuscript received June 26, 2018; accepted for publication October 15, 2018; published Early Online October 23, 2018.
[1]These authors contributed equally to this work.
[2]Corresponding author: Department of Horticulture, University of Wisconsin-Madison, 1575 Linden Dr., Madison, WI 53706. E-mail: dawson@hort.wisc.edu

consistently in the historical record. Wild carrot is indigenous to Europe, North Africa, and Western Asia, with its center of diversity in present day Afghanistan (Vavilov and Dorofeev 1992). Based on most historical records, the first evidence of carrot being cultivated as a food crop was in the Iranian Plateau and Persia in the 10th century (Banga 1957a,b, 1963; Brothwell and Brothwell 1969), and molecular evidence supports a Central Asian origin of domesticated carrot (Iorizzo *et al.* 2013). Carrot cultivation spread westward to North Africa and Europe, and eastward to Asia. Orange roots appeared in Spain and Germany in the 16th century (Stolarczyk and Janick 2011), and quickly became the predominant color (Figure 1, C and D).

Carotenoid levels have doubled due to plant breeding over the past 60 years (Simon 1990). Hence, there has been substantial effort to understand the mechanisms of carotenoid accumulation and regulation. Allelic variation at two genes, *Y* and Y_2, accounts for most of the distinctive color and carotenoid accumulation differences observed in orange, yellow, and white carrot roots (Buishand and Gabelman 1979). However, carotenoid biosynthesis genes in carrot do not map near enough to *Y* or Y_2 to be responsible for these differences (Just *et al.* 2007). The popularity of orange carrot likely fixed many of the alleles responsible for carotenoid presence in roots in domesticated populations. Researchers have therefore looked outside the biosynthetic pathway to regulatory and other modifying genes for explanation. Iorizzo *et al.* (2016) used two mapping populations and the newly assembled carrot genome to identify a candidate outside of the carotenoid biosynthetic pathway for the *Y* gene, *DCAR_032551*, that regulates photosystem development and conditions a portion of carotenoid presence in carrot roots.

In cauliflower, the *Orange* (*Or*) gene accounts for elevated levels of carotenoids (Li *et al.* 2001). The *Or* gene is responsible for both the biogenesis of chromoplasts, where carotenoids are stored, and post-transcriptional regulation of Phytoene Synthase (PSY), an enzyme necessary for carotenoid biosynthesis (Lu *et al.* 2006; Yuan *et al.* 2015; Zhou *et al.* 2015). Mutations in the *Or* gene have been associated with the presence of carotenoids in nonleaf tissue through the differentiation of noncolored plastids into chromoplasts in *Arabidopsis*, cauliflower, and sweet potato (Yuan *et al.* 2015). Maass *et al.* (2009) noted that the presence of large amounts of β-carotene in the form of crystals in carrot is strikingly similar to that found in the cauliflower *Or* mutant (Maass *et al.* 2009). The *Or* gene has not previously been associated with carotenoid presence in carrot. Previous carotenoid studies have focused either on biparental populations derived from crosses among domesticated carrot (Buishand and Gabelman 1979), or on crosses between wild carrot from North America and domesticated carrot (Santos and Simon 2002; Just *et al.* 2007; Ellison *et al.* 2017). Previous studies were also limited in their ability to detect significant associations by population size and marker density (Iorizzo *et al.* 2013).

In this study, we use 674 globally distributed domesticated and wild carrot accessions to conduct a genome-wide association study (GWAS) for carrot root pigmentation. We also analyze the population structure that developed during carrot dispersal and domestication. We sampled germplasm from all major global regions where carrot originated or was domesticated. Previous studies have identified three major genetic groups—Western, Eastern, and wild—but with limited numbers of accessions and low marker density (Iorizzo *et al.* 2013). Our analysis enabled the identification of both new and previously characterized regions of the carrot genome that were likely involved in selective sweeps during domestication, and we present the first indication of the *Or* gene playing a role in carotenoid presence in carrot.

Materials and Methods

Plant materials and phenotypic evaluation

Included were 154 wild and 520 domesticated carrot (*Daucus carota* L.) samples. Samples 1–144 were sown on certified organic land at Tipi Organic Produce in Evansville, WI and Elderberry Hill Farm in Waunakee, WI in 2013 and 2014. Samples 43XXX and 53XXX were grown at the University of Wisconsin–Madison West Madison Agricultural Research Station (Verona, WI) in 2014 and 2015 (X stands for any digit 0–9). Samples 30XXX and 32XXX were grown at the University of Wisconsin–Madison Hancock Agricultural Research Station (Hancock, WI) in 2013, and samples labeled GHXXXX, DH, and 493 were grown at the Walnut Street Greenhouse (Madison, WI) in 2013. Two samples of *Daucus syrticus* (Ames 29096 and Ames 29108) were used as an outgroup species (Arbizu *et al.* 2014). Passport data for the 674 accessions can be found in Supplemental Material, Table S1 (available at Figshare: 10.6084/m9.figshare.6177110).

Pigmentation analysis was conducted within 5 weeks of carrot harvest. Roots were sliced in cross section 5–10 cm from the root tip and root phloem color was classified as orange, purple, red, white, or yellow. Visual assessment data can be found in Table S2. Carotenoid presence/absence and root color classes are highly heritable, and not strongly influenced by environmental effects, with evidence of discrete inheritance of total carotenoids in crosses of white carrots with carrots of other colors (Buishand and Gabelman 1979; Fernandes Santos and Simon 2006). However, carotenoid concentration can be influenced by environmental conditions and so samples used for quantifying carotenoids were all taken from the Hancock Agricultural Research Station. Carotenoid content was quantified using lyophilized root tissue for HPLC analysis, as modified from Simon and Wolff (1987) and Simon *et al.* (1989). Briefly, 0.1 g of lyophilized carrot root tissue was crushed and then soaked in 2.0 ml of petroleum ether at 4°. After 12–16 hr, 300 μl of the petroleum ether extract was added to 700 μl of methanol, eluted through a Rainin Microsorb-MV column, and analyzed on a Millipore (Bedford, MA) Waters 712 WISP HPLC system. Synthetic β-carotene (Sigma [Sigma Chemical], St. Louis, MO) was used in each independent run as a reference

Figure 1 Carrot accessions illustrating the stages of carrot domestication and improvement. Carrots shown are fully mature (100 days). (A) Wild, (B) Eastern landrace, (C) Western historic open pollinated, and (D) modern hybrids (left: processing type and right: imperator type). Photo courtesy of Matthew Mirkes.

standard for calibration. Lutein, α-carotene, and β-carotene were quantified by absorbance at 450 nm. Concentrations are described in $\mu g\ g^{-1}$ dry weight. HPLC data can be found in Table S3.

Genotyping, SNP production, and filtering

Total genomic DNA of individual plants was isolated from ~0.1 g of lyophilized leaves of 4-week-old plants following the 10% CTAB protocol described by Murray and Thompson (1980), with modifications by Boiteux *et al.* (1999). All DNA was quantified using the Quantus PicoGreen dsDNA Kit (Life Technologies, Grand Island NY) and normalized to 10 ng/μl. Genotyping-by-sequencing (GBS), as described by Elshire *et al.* (2011), was carried out at the University of Wisconsin–Madison Biotechnology Center (WI) with minimal modification and half-sized reactions. Briefly, DNA samples were digested with *Ape*KI, barcoded, and pooled for sequencing, and 80–95 pooled samples were run per single Illumina (San Diego, CA) HiSeq 2000 lane, using 100-nt reads and v3 Sequencing by Synthesis reagents (Illumina). Images were analyzed using CASAVA 1.8.2. and bcl2fastq-1.8.4.

The TASSEL-GBS pipeline version 5.2.26 was used to call SNPs as described by Bradbury *et al.* (2007) and Glaubitz *et al.* (2014) using the carrot reference genome (GenBank accession LNRQ01000000.1; Iorizzo *et al.* 2016). SNPs were filtered into two data sets. D1 had < 30% missing data for genotype and marker, a 5% minor allele frequency, no more than two alleles, and at least 5× depth per marker. Markers were further filtered to set heterozygous markers with an allele ratio < 0.3 or > 0.7 to missing, leaving 39,710 SNPs in 674 samples. Missing genotype calls in D1 were imputed using Beagle 4.1 with n iterations = 10 (Browning and Browning 2016). The average SNP distribution across the carrot genome was ~54 SNPs per 500 kb bin or ~1 SNP per 10 kb (Figure S1) with an average 18× coverage per SNP. To reduce potential confounding effects of population structure and differentiation, we excluded 21 wild samples from Portugal (D1-noPT).

The same filtering parameters were used for data set D2 except that SNPs were filtered using 10% missing data and were not imputed. Additionally, two samples from the outgroup *D. syrticus* were included for a total of 676 individuals and 32,128 SNPs. The SNP distribution for D2 was 43 SNPs per 500 kb (Figure S2) and 20× coverage per SNP. Additional information about SNP filtering can be found in Figure S3. A subsample of D2 was created to exclude samples with > 30% admixture (D2-lowAd) as determined by STRUCTURE.

SNP density across chromosomes, using 500,000-nt bins for D1 and D2, can be found in Figures S1 and S2. Filtering parameters for each SNP data set can be found in Figure S3. SNP data sets are in Data D1 and Data D2.

Linkage disequilibrium

TASSEL 5 (Bradbury *et al.* 2007) was used to calculate linkage disequilibrium (LD) for the full matrix of SNPs for data set D1-noPT. Reported values of LD decay use an r^2 cutoff of 0.1 and 0.2 for filtered SNPs ($P < 0.01$) (Vos *et al.* 2017). The half-distance of LD decay was calculated as when the LD decay curve intersected with half the maximum LD value. Genome-wide sliding window analysis of LD was conducted for both wild and domesticated samples using VCFtools with the parameters –geno-r2 –ldwindow 100 (51). r^2 values with fewer than 95 SNPs per bin were removed. Sliding window analysis was visualized using qqman in R studio (Wickham 2009).

Population structure

We used data set D2 and conducted eight replications of the Bayesian clustering program STRUCTURE version 2.3.4 (Pritchard *et al.* 2000) with populations (K value) ranging from 1 to 14, with a burn-in length of 20,000 and 50,000 Monte Carlo iterations, respectively. An admixture model with no previous population information was included; all other parameters were set to default values. STRUCTURE results were processed in the software STRUCTURE HARVESTER 0.6.94 with parameter–evanno (Earl and vonHoldt 2012), to detect the most likely number of clusters by using the rate of change in the log probability between successive values of K (ΔK) (Evanno *et al.* 2005). Population structure was visualized using distruct software version 1.1 (Rosenberg 2004).

Principal component analysis

An eigenvalue decomposition of the SNP covariance matrix was performed using TASSEL 5 using default parameters for D2 and D2-lowAd. All individuals' loadings were plotted along the first and second principal components (PCs) using ggplot in R. Individuals were colored according to their STRUCTURE group identity.

Maximum-likelihood tree

Using data sets D2 and D2-lowAd, maximum likelihood analyses were conducted with the GTR+G nucleotide substitution model using RAxML version 8.2.9 (Stamatakis 2014). GATK HaplotypeCaller (McKenna *et al.* 2010) with parameters –genotyping_mode GENOTYPE_GIVEN_ALLELES was used to call SNPs for the two outgroup accessions, *D. syrticus* SRR2147152 and SRR2147153 (Arbizu *et al.* 2016). FigTree (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize phylogenetic trees.

Pairwise F_{st}

Weir and Cockerham's method for calculating pairwise F_{st} (Weir and Cockerham 1984) was implemented within the genet.dist function of the R package hierfstat (Goudet 2005). Pairwise values were calculated on all $K = 6$ subpopulations using data sets D2 and D2-lowAd. The data set was first converted to a genind object using the df2genind command of the R package adegenet using default parameters.

Sliding window analysis of nucleotide diversity, F_{st}, and cross-population composite likelihood ratio

Selective sweep detection analyses used data set D1-noPT. VCFtools was used to calculate genetic diversity (π) in 500-kb windows across the carrot genome (–window-pi 500000) for wild and domesticated carrot samples. Genomic bins were set at 500-kb regions. Each bin was analyzed for reduced nucleotide diversity, elevated F_{st}, and high cross-population composite likelihood ratio (XP-CLR) scores. Potential sweeps corresponded to each bin; however, the start and stop points of the sweep are very likely different from those of the bin. Bin sizes were selected to contain an average of 50 SNPs. Potential selective sweep regions were found by calculating the difference between wild and domesticated nucleotide diversity bins, and selecting bins in the top 5% of values ($P > 1.578$). The population differentiation statistic, F_{st}, was estimated between wild and domesticated samples in VCFtools in 500-kb windows with 100-kb steps (-weir -fst-pop -fst-window-size 500000-fst-window-step 100000) (Danecek *et al.* 2011). Potential sweep regions were defined as the top 5% of values ($F_{st} > 0.29$). A third method, XP-CLR, was implemented to test for selective sweeps (Chen *et al.* 2010). The XP-CLR software was run with parameters: -w1 0.005 50 100 1 -p1 0.9 for each chromosome. Sweeps were detected unidirectionally from wild to domesticated. The genetic distances between SNPs were interpolated according to their physical distances in a high-density genetic map (Iorizzo *et al.* 2016). Mean XP-CLR scores were tabulated in nonoverlapping 10-kb windows across the genome. Windows with the top 1% of XP-CLR values (11.93) were selected and placed in corresponding bins from the F_{st} and nucleotide diversity analyses. Genome-wide sliding window analyses were plotted using the R package qqman (Turner 2014). Overlapping genomic regions in the top 5% for nucleotide diversity and F_{st}, and top 1% for XP-CLR scores, were identified as the most likely selective sweeps. The physical positions of four previously identified Diversity Arrays Technology (DArT) markers (crPT9895272, crPT9891905, crPT9890532, and crPT9885102; Grzebelus *et al.* 2014), which flank previously identified domestication signatures, were located in the carrot genome (GenBank accession LNRQ01000000.1; Iorizzo *et al.* 2016) using BLASTn.

Genome-wide association analysis

A GWAS was performed for carrot root pigmentation using data set D1 by implementing the EGSCORE function in the GenABLE R package (Aulchenko *et al.* 2007). The following parameters were used: naxes = 2, times = 1, quiet = FALSE, bcast = 0, clamda = T, and propsPs = 1. No fixed effects were included as covariates. The kinship matrix was calculated using the ibs command in GenABLE with the weight parameter set to "freq." The diagonal of the kinship matrix was replaced with the default option of the variance of the average homozygosity within each individual to account for the level of inbreeding of an individual with itself. Manhattan and qqplots were drawn using the R package qqman (Turner 2014). While there may be some confounding of population structure and polymorphisms due to the orange phenotype being absent in wild carrot accessions but common in domesticated accessions, when accounting for kinship, the Q-Q plot does not show inflation of observed $-log_{10}(\mathrm{P})$-values below expected $-\log_{10}(\mathrm{P})$-values of at least 2.5 (Figure S4). This indicates that any potential confounding of the root pigmentation phenotype with the underlying population structure was adequately controlled in our model. The most significant SNP in our GWAS, located on chromosome 3 at position 5,224,824, was used to analyze allele frequency distribution according the groupings identified by STRUCTURE at $K = 6$, PC1 and PC2, domestication or wild status, and orange or nonorange status.

Observed heterozygosity, homozygosity, and gene diversity

Observed heterozygosity H_o, within population gene diversity (H_S), overall gene diversity (H_t), and overall F_{st} were calculated using the basic.stats function in the R package hierfstat (Goudet 2005) using data sets D1, D2, and D2 lowAd. Data sets were first converted to genind objects using the df2genind command of the R package adegenet using default parameters. Sample homozygosity was calculated using data set D1 by implementing the hom() function in the GenABLE R package (Aulchenko *et al.* 2007). Differences in mean homozygosity among STRUCTURE populations were

ascertained via pairwise Student's *t*-tests with pooled SDs and Bonferroni correction using the pairwise.t.test command in R.

Candidate gene sequence analysis

Thirteen previously resequenced carrot samples (Table S4) were surveyed for any sequence variation within the open reading frame of the *Or* gene (DCAR_009172). One SNP was identified between low- and high-carotenoid genotypes within exon 5. A transition of T to C at position 3350 resulted in a change of the codon TTG to TCG, causing a missense mutation of leucine to serine. This SNP is located on chromosome 3, position 5,197,361. To genotype carrot samples for T3350C, primers that flank the SNP were generated (Table S5). PCR-based sequencing was performed on 197 domesticated and 82 wild carrot samples. Sanger sequencing was performed on an ABI 3730xl DNA Analyzer by the University of Wisconsin Biotechnology Center DNA Sequence Facility. A gene model for *Or* was generated from the website http://wormweb.org/exonintron. Phenotypic differences for lutein, α-carotene, and β-carotene were analyzed for the three *Or* genotypic classes. For each trait, significance between different genotypic classes was determined by using the aov and TukeyHSD functions in R. A genotyping workflow can be found in Figure S5.

Data availability

Carrot sequences used for the *Or* gene alignment and *D. syrticus* samples used as an outgroup for phylogenetic analysis are available under the National Center for Biotechnology Information Bioproject accession PRJNA291976. All supplementary tables and data sets necessary to reproduce the analyses for this manuscript are available at Figshare: 10.6084/m9.figshare.6177110.

Results

Rapid decay of LD in carrot

LD analysis of wild carrot accessions demonstrated a very rapid genome-wide decay between ~100 bp ($r^2 = 0.2$) and ~1 kb ($r^2 = 0.1$), and a rapid decay of ~400 bp ($r^2 = 0.2$) and ~13 kb ($r^2 = 0.1$) in domesticated accessions. This rapid decay was further supported by estimates of wild and domesticated samples having an LD half-life of 67 and 6544 bp, respectively (Figure S6). Determination of LD decay distances does not have a consensus method in the literature, with both thresholds (0.1 and 0.2) and half-life methods used (Vos *et al.* 2017). Half-life methods may be more robust to differences in minor allele frequencies and have been used in a number of species (Kim *et al.* 2007; Lam *et al.* 2010; Branca *et al.* 2011; Zhao *et al.* 2011; Vos *et al.* 2017). This is the first report of genome-wide LD in carrot. LD decay rates appear slower in domesticated samples around regions putatively under selection, such as the Y region (Iorizzo *et al.* 2016) and the cult region (Macko-Podgorni *et al.* 2017), as well as several carotenoid biosynthesis genes (Clotault *et al.* 2010; Soufflet-Freslon *et al.* 2013).

Population structure dynamics among wild and domesticated carrot

An examination of population structure was carried out using STRUCTURE software with $K = 6$ as the number of groups strongly supported by the Evanno method (Evanno *et al.* 2005) (Figure 2A and Figures S7–S10). The support for $K = 6$ was slightly stronger than $K = 4$ or $K = 5$, and as we are interested in understanding population structure in carrot, we chose to work with the largest K value strongly supported by the data.

To maximize cluster separation, a low-admixture group (D2-LowAd) of 463 accessions was created by only including samples when the proportion of inferred ancestry was $> 70\,\%$ ($q \geq 70\%$) (Figure 2E and Table S1). Clustering with STRUCTURE indicated divisions between Western domesticated, Western wild, and all Eastern samples, as well as emergent subclusters corresponding to geographic origin, including samples from Tunisia, which were mostly wild with a few domesticated samples, and wild accessions from Portugal (Figure 2A). An additional cluster formed for Western Imperator hybrids (Western-HI) (Figure 1D and Figure 2A). Imperator carrots are one of the major commercial market classes in the US and cultivars are primarily F1 hybrids. This market class has been the focus of much of US carrot breeding efforts in recent decades (Simon 2000). The Q matrix of individual accessions is reported in Table S1. In the presentation and discussion of results, we use W to refer to wild accessions, D to refer to domesticated accessions, and HI to refer to Hybrid Imperator accessions.

The observed population substructure was supported by phylogenetic analysis, PC analysis (PCA), and pairwise F_{st}. Using *D. syrticus* as an outgroup (Arbizu *et al.* 2014), the maximum-likelihood analysis identified the same six strongly supported clades (bootstrap $> 97\%$): Portuguese-W, Western-W, Eastern-W/D, Tunisian-W, Western-D, and Western-HI carrots (Figure 2D). PCA revealed a clear separation between wild and domesticated carrots along the first PC (12.4% of variation explained), and between Eastern and Western samples along the second PC (4.6% of variation explained, Figure 2C). Pairwise F_{st} calculations further supported differentiation between the six subclusters (Table S6). The Portuguese-W samples were the most strongly divergent of all the STRUCTURE groups, forming a very distinct subpopulation separate from other wild carrot accessions. Observed heterozygosity (H_o) for accessions in data set D2-lowAd was 0.18 (Table S7). All analyses and results were also confirmed on data set D2, without removal of high-admixture samples (Figure S8, Table S6, and Table S7).

GWAS analysis identifies Or as a candidate gene for carotenoid presence in carrot

To identify genomic regions potentially related to carotenoid presence, we performed a GWAS for orange pigmentation in

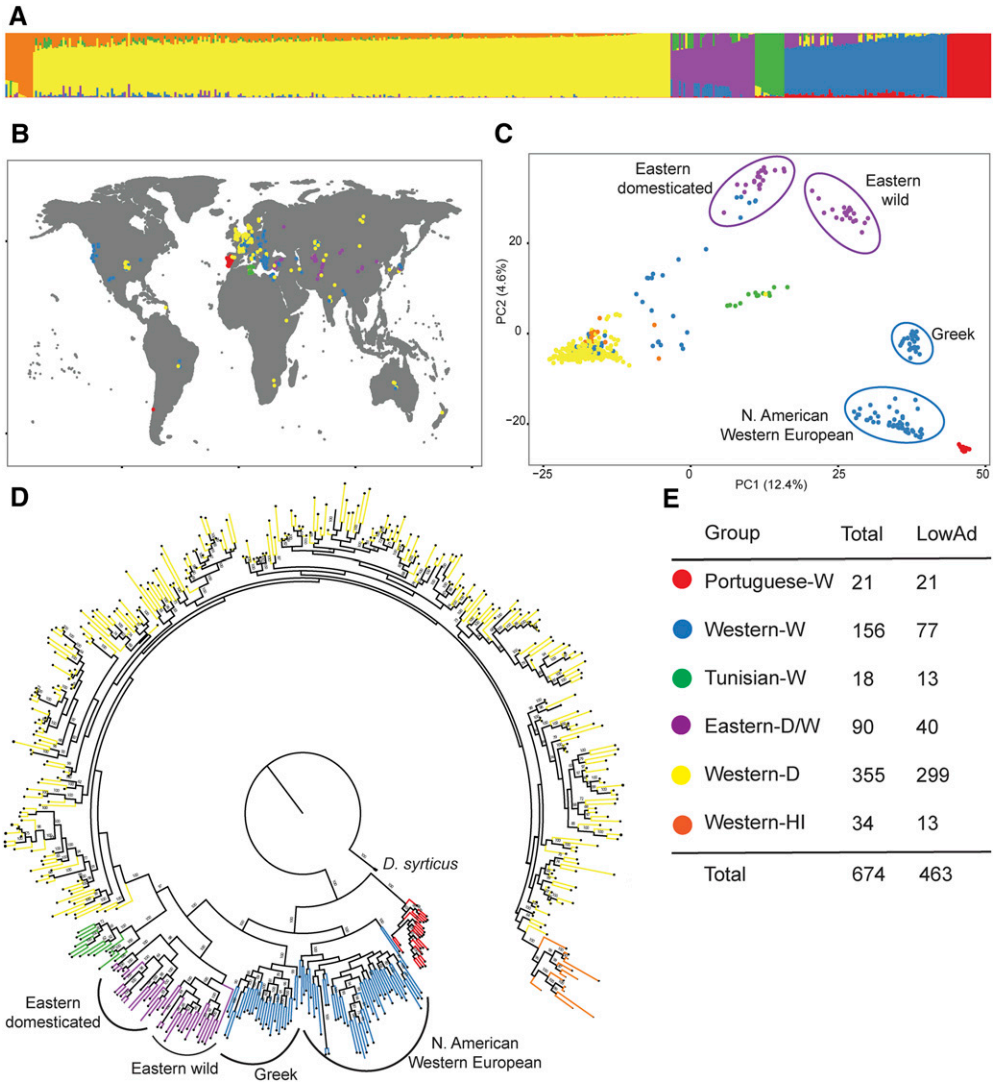


Figure 2 Population structure of 463 carrot accessions with < 30% admixture (D2-LowAd). (A) STRUCTURE groups. Percentage of membership (q) for each group identified at K=6. (B) Geographic distribution of accessions, each represented by a point on the map colored according to STRUCTURE group. Current commercial varieties not shown. (C) Principal component (PC) analysis plot of the first two principal components. PC1 and PC2 account for 12.4 and 4.6% of the total variation, respectively. (D) Maximum-likelihood tree of carrot accessions. Numbers on the branches indicate bootstrap support. Black branch represents outgroup *D. syrticus*. (E) Color key. Total number of accessions in each STRUCTURE Group. LowAd, low admixture.

carrot root using data set D1 (Figure S3). We found a previously unidentified significant 143-kb GWAS signal on chromosome 3 containing 17 annotated genes (Figure 3A and Table S8). *Or*, a gene associated with carotenoid biosynthesis regulation and chromoplast formation (Lu *et al.* 2006; Li *et al.* 2012; Zhou *et al.* 2015), is in the middle of the 143-kb region encompassing the most significant SNPs in our GWAS analysis. No other genes in the 143-kb region are known to be associated with carotenoid presence. We selected the most significant GWAS SNP, located on chromosome 3 at position 5,224,824, to serve as a proxy to analyze the *Or* allele frequency distribution with respect to PC1 and PC2 of our PCA (Figure 3B), STRUCTURE populations (Figure 3C), domestication status (wild and domesticated), and pigmentation status (orange and nonorange) (Figure 3D). The wild-type allele (A) appears nearly fixed in all wild STRUCTURE groups as well as the eastern domesticated nonorange samples. The domestication allele (T) was found at high frequency in the eastern domesticated orange samples as well as western domesticated nonorange samples, and is fixed in all western domesticated orange samples. Further, we observe the domestication genotype (TT) to be absent in all wild samples and the wild genotype (AA) to be absent in orange samples.

To better characterize the association of carotenoid presence and the *Or* gene, we looked for mutations cosegregating between five high- and eight low-carotenoid accessions that had been previously resequenced (Iorizzo *et al.* 2016), and found a nonsynonymous mutation at position 3350 in exon 5, causing a serine to leucine amino acid change (Figure 3B). An additional 198 domesticated and 81 wild samples were phenotyped for total carotenoid content ($\mu g/g$ dry weight) using HPLC and genotyped at *Or*. Those samples with the T/T genotype had significantly higher amounts of total carotenoid content than those that were heterozygous (C/T) or homozygous wild-type (C/C) (Figure 3F and Table S3). This is the first report of an association between *Or* and carotenoid presence in carrot.

Identification of selection signatures during carrot domestication

During crop domestication, highly favorable alleles undergo intensive selection and reach fixation rapidly, resulting in reduced variation in neighboring genomic regions and thereby creating a signature of a selective sweep. We used three measures to analyze sweeps: reduced nucleotide diversity (π) (Nei and Li 1979) in domesticated samples as compared to wild ones, high population differentiation (F_{st}) (Wright 1951) between wild and domesticated samples, and allele frequency differentiation between populations (XP-CLR) (Chen *et al.* 2010). To reduce potential confounding effects of population structure and differentiation, we removed the 21 Portuguese-W samples from the selective sweep analyses (data set D1-noPT, Figure S3).

Overall, we found little reduction in genetic diversity in all domesticated carrot (3.13×10^{-5}) compared to all wild carrot (3.25×10^{-5}), averaged across the whole genome level. Differences in nucleotide diversity between wild and domesticated samples were estimated for 500-kb bins across the carrot genome. The average difference between groups was 1.080 with 37 potential selective sweep regions detected using the top 5% of calculated values (1.578) (Figure 4A and Table S9).

The genome-wide average F_{st} between domesticated and wild carrot was 0.14. We detected 38 genomic regions with F_{st} values above the 95% percentile (> 0.29), differentiating wild and domesticated accessions (Figure 4A and Table S9). These regions with high levels of differentiation likely experienced selective sweeps during domestication or improvement (Wright 1951). The recently identified *Y* gene (Iorizzo *et al.* 2016), a candidate for carotenoid presence in carrot taproot, is located within one of these regions of high differentiation between wild and domesticated carrots (24.5–25.0 Mb on chromosome 5). The carotene hydroxylase *DcCYP97A3* gene associated with increased α-carotene (Arango *et al.* 2014) is in a 500-kb bin (6.0–6.5 Mb) that has high F_{st} and is adjacent to a 500-kb bin (6.5–7 Mb) that is one of the 12 regions identified in all tests (Table S9).

Lastly we used the XP-CLR method to compare the wild and domesticated accessions in 10-kb bins across the genome (Chen *et al.* 2010). The top 1% of XP-CLR values (> 11.94) identified 78 potential sweep bins (Figure 4A and Table S9). A candidate domestication gene associated with root thickening, *DcAHLc1* (Macko-Podgorni *et al.* 2017), is located at 41.8 Mb on chromosome 2, near one of the regions with the highest XP-CLR scores (42.0–42.5 Mb). Another region, 33.5–34.0 Mb on chromosome 7, overlaps with the recently fine-mapped QTL, *Y2*, a gene associated with carotenoid accumulation (Ellison *et al.* 2017). Two carrot DArT markers, crPT9892544 and crPT894175, which were identified in Grzebelus *et al.* (2014) to show signatures of selection mapped between positions 21,070,220 and 30,397,440 on chromosome 2, and position 18,823,084 and 28,211,752 on chromosome 6, respectively. Both of these regions align with selective sweep bins identified in this study (Figure 4B).

To identify the most supported potential selective sweeps during domestication, we considered regions that were significant for all three methods of detection. Using this approach, 12 regions were identified (Figure 4, A and B and Figures S11–S13). The candidate carotenoid presence gene *Or*, which was identified in our GWAS, falls in 1 of these 12 regions. A genome-wide sliding window analysis of LD also identified the same region on chromosome 3 to have the slowest LD decay in domesticated carrots (Figure 4C) but not wild carrots (Figure S14). These results strongly suggest that selection pressures acted on the *Or* locus during carrot domestication. It is possible that high-carotene alleles at the *Or* locus have been fixed in most Western domesticated carrots, which may explain why it was not identified until a globally distributed data set of wild and domesticated carrots was used.

Discussion

In this study, we genotyped a large and diverse collection of carrot accessions to determine the global structure of LD in the genome. Genome-wide coverage was ~1 SNP per 10 kb, dense enough to give an initial assessment of the pattern of LD in carrot. The pattern of LD in a genome is a powerful signal of the population genetic processes that are structuring it, and similar LD decay rates have been found in other highly heterozygous outcrossing species, such as maize and grape (Yan *et al.* 2009; Myles *et al.* 2011). We find LD decays very rapidly in both wild and domesticated accessions, with a half-life of 67 and 6544 bp, respectively (Figure S6), and we also demonstrate that LD decline is variable across the nine chromosomes as well as between wild and domesticated accessions (Figure 4C and Figure S14). The observed rapid decay suggests that GWAS should be very useful for identifying candidate genes in carrot as long as SNP density and coverage is comprehensive. To date, only two GWAS studies have been conducted in carrot. Prior to the availability of the carrot reference genome, Jourdan *et al.* (2015) conducted a candidate gene association study in 380 carrots using 109 SNPs spread across 17 carotenoid biosynthesis genes. The strongest associations with pigmentation were the carotenoid genes *ZEP* and *CRISTO*. More recently, Keilwagen *et al.* (2017) used 85 carrot cultivars and 168,000 SNPs to identify 30 QTL for 15 terpenoid volatile organic compounds, which were further localized to four genomic regions on three different carrot chromosomes containing candidate terpene synthase genes. Future GWAS and LD projects in carrot will benefit from improved genotyping techniques, such as resequencing or two-enzyme GBS (Poland *et al.* 2012), to increase SNP density. There were likely some regions of the genome that did not have adequate SNP coverage in this study due to rapid linkage decay.

The primary divisions of population structure across our diverse carrot accessions are geographic distribution, west to east, and intensity of breeding effort, wild to domesticated. Previous studies have also demonstrated that wild and domesticated carrots are genetically distinct (Shim and

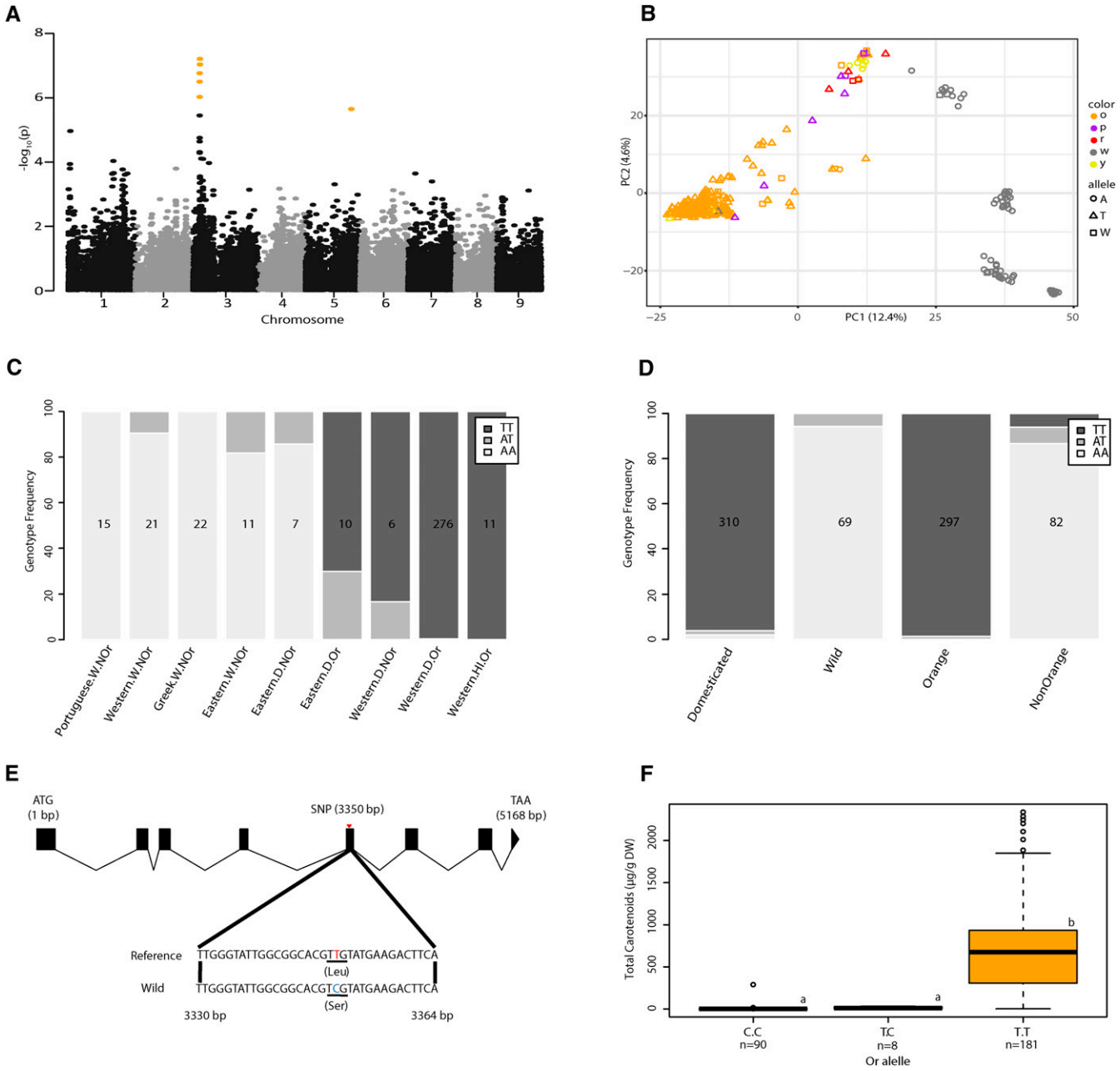
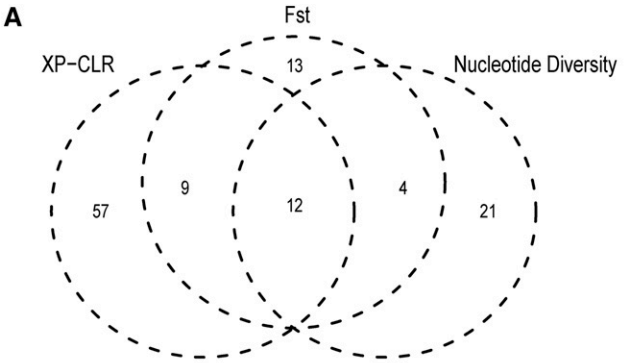


Figure 3 Genome-wide association analysis of orange pigmentation and identification of the candidate gene *Or* on chromosome 3. (A) Manhattan plot for orange carrot root color. SNPs with empirically-adjusted *P*-values < 0.05, were defined as significant and are colored orange. (B) Allele frequency of SNP S3_5228434 and pigmentation classification superimposed on the principal component analysis from Figure 2C. o, orange; p, purple; r, red; w, white; y, yellow. (C) SNP S3_5228434 genotype frequency separated by STRUCTURE classification. Or and NOr indicates orange or nonorange root pigmentation. W, D, or HI is wild, domesticated, or Hybrid Imperator, respectively. (D) SNP S3_5228434 genotype frequency separated by root pigmentation and domestication status. Quantities indicate the number of samples in each group. (E) Open reading frame of *Or* and the nonsynonymous mutation in exon 5 at position 3350 (T3350C). (F) Box plots for total carotenoids for the three *Or* genotypes (C/C, T/C, and TT) at position 3350. Center line = median, box limits = upper and lower quartiles, whiskers = 1.5× the interquartile range, dots = outliers. Different letters indicate significant differences between genotypes ($P < 0.05$, Tukey's honest significant difference). Reported values are in $\mu g/g$ dry weight.

Jorgensen 2000; Clotault *et al.* 2010; Baranski *et al.* 2012; Iorizzo *et al.* 2013; Rong *et al.* 2014), and that they separate into geographically discrete Eastern and Western groups (Baranski *et al.* 2012; Iorizzo *et al.* 2013, 2016; Grzebelus *et al.* 2014). However, there is evidence of continued gene flow where populations overlap geographically, such as in Western-W accessions, which are present in areas where domesticated carrot is grown. It also appears that there is significant overlap in wild and domesticated samples from the Eastern group. This may be attributed to either recent admixture or to domesticated carrots sharing many of the same alleles with wild carrots from the region. While STRUCTURE



B

Chromosome	Start	End
1	21000001	21500000
2	21500001	22000000
3*	5000001	5500000
3	13500001	14000000
3	21500001	22000000
5	29500001	30000000
5	34000001	34500000
6	27500001	28000000
7	6500001	7000000
9	31000001	31500000
9	31500001	32000000
9	33000001	33500000

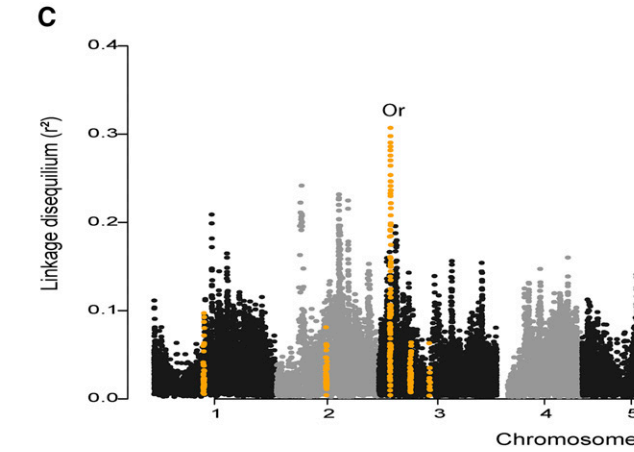


Figure 4 Regions of the carrot genome that likely underwent a selective sweep during domestication. (A) Venn diagram represents the overlapping of 500-kb regions tested for selection signatures: top 5% of F_{st} and nucleotide diversity difference between wild and domesticated carrot accessions, and top 1% of cross-population composite likelihood ratio (XP-CLR) values. (B) Genomic location of potential selective sweeps identified by F_{st}, nucleotide diversity, and XP-CLR. The asterisk signifies the genome region carrying the candidate orange pigmentation gene *Or*. (C) Genome-wide linkage disequilibrium averaged across sliding windows of 100 SNPs in domesticated carrots. Regions identified as significant in (A) and (B) are highlighted in orange. The region containing the *Or* candidate gene for orange pigmentation in carrot is marked "Or."

failed to identify a distinction between Eastern wild and Eastern domesticated carrots, these do appear as sister clades in the phylogeny with wild Western carrots at the root of both clades (Figure 2D), supporting recent findings that domesticated carrots are genetically closer to Eastern wild carrots than to Western wild carrots (Vavilov and Dorofeev 1992; Iorizzo *et al.* 2013).

Carrots from Northern Africa, Tunisian-W, form a distinct group but show the least differentiation from all other groups (Table S6). Previously, North African samples clustered closer to wild samples from the West and Middle East (Iorizzo *et al.* 2013) but here, using a much larger data set and number of SNPs, Tunisian-W samples appear at the base of all domesticated western carrots (Figure 2D), suggesting that carrots from this region of the world may have been important for the improvement of domesticated carrots. Future field sampling efforts and population dynamics analysis should include more representation from North Africa, to better understand carrot domestication and diversity. Finally, we observe that Portuguese-W samples are highly diverged from other accessions. Gene flow in and out of the Iberian peninsula region is likely limited because of the Pyrenees mountain range. However, crosses with Western domesticated carrot have been successful and therefore Portuguese-W samples may provide a novel source of alleles for abiotic stresses.

The analysis of an extensive and representative sample of modern domesticated, historic domesticated, and wild accessions allowed us to identify genomic regions putatively under selection. False positives can be exacerbated by large genomic data sets, so we used a conservative approach to only consider regions identified by all three detection tests (decreased nucleotide diversity, high F_{st}, and elevated XP-CLR scores) and identified 12 putative genomic regions under selection during domestication (Figure 4, A and B). One selective sweep located on chromosome 3 overlapped with the most significant SNPs in our GWAS analysis for carotenoid presence and contained the candidate gene *Or*. Analysis of the *Or* sequence between samples with varying carotenoid content found a nonsynonymous mutation in exon 5 that associates with the presence of α- and β-carotene, and to a lesser extent lutein. Single amino acid substitutions in the Or homologs in melon and *Arabidopsis* have led to increased carotenoid presence (Tzuri *et al.* 2015; Yuan *et al.* 2015). Two markers that had been previously identified to show domestication signatures (Grzebelus *et al.* 2014) overlapped with 2 of the 12 bins identified in this study. Interestingly, chromosome 2 was previously shown to carry *Vrn1*, implicated in flowering habit (Alessandro *et al.* 2013), which was likely a target to favor biennial growth habit during the course of carrot domestication.

Or is important for chromoplast development, a necessary precursor to the accumulation of carotenoids (Lu *et al.* 2006). *Or* differentiates noncolored plastids into chromoplasts, which provide the deposition sink for carotenoid accumulation (Lu *et al.* 2006). *Or* also post-transcriptionally regulates PSY, the most important regulatory enzyme in the carotenoid pathway (Li *et al.* 2012; Zhou *et al.* 2015; Park *et al.* 2016).

This post-transcriptional effect may be why *Or* has not been identified in previous carrot studies that have looked at carotenoid accumulation mechanisms at the transcription level (Simpson *et al.* 2016). Mutations in the *Or* gene are associated with increased chromoplast formation, thereby providing more storage capability for carotenoids (Yuan *et al.* 2015). In the closest significant GBS SNP to the *Or* gene, we find that the wild-type allele (A) appears nearly fixed in all wild STRUCTURE groups as well as the eastern domesticated nonorange samples. The domestication allele (T) was found at high frequency in the eastern domesticated orange samples as well as western domesticated nonorange samples, and is fixed in all western domesticated orange samples. The allele frequency distribution of *Or* across STRUCTURE groups aligns well with the historical record that the first domesticated samples were yellow and only later did orange carrot roots become common (Banga 1963; Stolarczyk and Janick 2011). We hypothesize that a mutation in *Or* enhanced carotenoid sequestration by optimizing chromoplast formation. It was likely selected after the initial domestication of carrot in conjunction with y_2 to increase carotenoid formation and storage in the taproot. The resulting orange phenotype was then rapidly selected in Western domesticated carrots.

This study brings us one step closer to understanding the presence of carotenoids in carrots. Future work should analyze *Or* expression at the transcript and protein levels, and verify the effect of disrupting its functionality on carotenoid presence. Additionally, the 11 other genomic regions showing consistent signatures of selection (Figure 4, A and B) should be explored for candidate domestication genes, and be considered in tandem with GWAS and mapping studies. Understanding the genetic consequences of domestication and selection on carrot can inform future plant breeding efforts, and allow us to achieve greater gains from selection.

Acknowledgments

The authors thank Rob Kane (deceased), Paul Miller, Brian Emerson, Thomas Hickey, Steve Pincus, Beth Kazmar, Eric Elderbrock, and Holly Ruess for field assistance, Terri Theisen for phenotyping historic accessions, David Spooner for technical comments on the manuscript, the University of Wisconsin Biotechnology Center DNA Sequencing Facility for providing genotyping-by-sequencing library preparation and sequencing services, and the many seed companies and plant breeders that provided seed for this project. S.L.E. was supported by the National Science Foundation under grant 1202666 and the National Institute of Food and Agriculture, U. S. Department of Agriculture (NIFA-USDA), under award number 2016-51181-25400. M.I. was supported by NIFA-USDA Hatch project 1008691, and K.E.C. was supported by NIFA-USDA Hatch project 1002863 and NIFA-USDA award number 2017-67013-26187. C.H.L. was supported by The Clif Bar Family Foundation Seed Matters Fellowship and NIFA-USDA North Central Region-SARE graduate student grant (GNC13-175). K.M.C. was supported by a University of Wisconsin–Madison Science and Medicine Graduate Research Scholars Fellowship.

Literature Cited

Alessandro, M. S., C. R. Galmarini, M. Iorizzo, and P. Simon, 2013 Molecular mapping of vernalization requirement and fertility restoration genes in carrot. Theor. Appl. Genet. 126: 415–423. https://doi.org/10.1007/s00122-012-1989-1

Arango, J., M. Jourdan, E. Geoffriau, P. Beyer, and R. Welsch, 2014 Carotene hydroxylase activity determines the levels of both alpha-carotene and total carotenoids in orange carrots. Plant Cell 26: 2223–2233. https://doi.org/10.1105/tpc.113.122127

Arbizu, C., H. Ruess, D. Senalik, P. Simon, and D. Spooner, 2014 Phylogenomics of the carrot genus (*Daucus*, Apiaceae). Am. J. Bot. 101: 1666–1685. https://doi.org/10.3732/ajb.1400106

Arbizu, C., S. Ellison, D. Senalik, P. Simon, and D. Spooner, 2016 Genotyping-by-sequencing provides the discriminating power to investigate the subspecies of *Daucus carota* (Apiaceae). BMC Evol. Biol. 16: 234. https://doi.org/10.1186/s12862-016-0806-x

Arscott, S., and S. Tanumihardjo, 2010 Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. Compr. Rev. Food Sci. Food Saf. 9: 223–239. https://doi.org/10.1111/j.1541-4337.2009.00103.x

Aulchenko, Y. S., S. Ripke, A. Isaacs, and C. M. van Duijn, 2007 GenABEL: an R library for genome-wide association analysis. Bioinformatics 23: 1294–1296. https://doi.org/10.1093/bioinformatics/btm108

Banga, O., 1957a The development of the original European carrot material. Euphytica 6: 64–76.

Banga, O., 1957b Origin of the European cultivated carrot. Euphytica 6: 54–63.

Banga, O., 1963 *Main Types of the Western Carotene Carrot and Their Origin*. W. E. J. Tjeenk Willink, Zwolle, The Netherlands.

Baranski, R., A. Maksylewicz-Kaul, T. Nothnagel, P. F. Cavagnaro, P. W. Simon *et al.*, 2012 Genetic diversity of carrot (*Daucus carota* L.) cultivars revealed by analysis of SSR loci. Genet. Resour. Crop Evol. 59: 163–170. https://doi.org/10.1007/s10722-011-9777-3

Boiteux, L., M. Fonseca, and P. Simon, 1999 Effects of plant tissue and DNA purification method on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. J. Am. Soc. Hortic. Sci. 124: 32–38.

Bradbury, P., Z. Zhang, D. Kroon, T. Casstevens, and Y. Ramdoss, 2007 TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23: 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Branca, A., T. Paape, P. Zhou, R. Briskine, A. Farmer *et al.*, 2011 Whole-genome nucleotide diversity, recombination, and linkage disequilibrium in the model legume Medicago truncatula. Proc. Natl. Acad. Sci. USA 108: E864–E870. https://doi.org/10.1073/pnas.1104032108

Brothwell, D., and P. Brothwell, 1969 *Food in Antiquity: A Survey of the Diet of Early Peoples*. Thames & Hudson, London, UK.

Browning, B., and S. Browning, 2016 Genotype imputation with millions of reference samples. Am. J. Hum. Genet. 98: 116–126. https://doi.org/10.1016/j.ajhg.2015.11.020

Buishand, J., and W. Gabelman, 1979 Investigations on the inheritance of color and carotenoid content in phloem and xylem of carrot roots (*Daucus carota* L.). Euphytica 28: 611–632. https://doi.org/10.1007/BF00038928

Chen, H., N. Patterson, and D. Reich, 2010 Population differentiation as a test for selective sweeps. Genome Res. 20: 393–402. https://doi.org/10.1101/gr.100545.109

Clotault, J., E. Geoffriau, E. Lionneton, M. Briard, and D. Peltier, 2010 Carotenoid biosynthesis genes provide evidence of geographical subdivision and extensive linkage disequilibrium in the carrot. Theor. Appl. Genet. 121: 659–672. https://doi.org/10.1007/s00122-010-1338-1

Danecek, P., A. Auton, G. Abecasis, C. Albers, E. Banks *et al.*, 2011 The variant call format and VCFtools. Bioinformatics 27: 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Earl, D. A., and B. M. vonHoldt, 2012 STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4: 359–361. https://doi.org/10.1007/s12686-011-9548-7

Ellison, S., D. Senalik, H. Bostan, M. Iorizzo, and P. Simon, 2017 Fine mapping, transcriptome analysis, and marker development for Y_2, the gene that conditions β-carotene accumulation in carrot (*Daucus carota* L). G3 (Bethesda) 7: 2665–2675. https://doi.org/10.1534/g3.117.043067

Elshire, R., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto *et al.*, 2011 A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6: e19379. https://doi.org/10.1371/journal.pone.0019379

Evanno, G., S. Regnaut, and J. Gouder, 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fernandes Santos, C. A., and P. W. Simon, 2006 Heritabilities and minimum gene number estimates of carrot carotenoids. Euphytica 151: 79–86. https://doi.org/10.1007/s10681-006-9130-7

Glaubitz, J., T. Casstevens, F. Lu, J. Harriman, R. Elshire *et al.*, 2014 TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. PLoS One 9: e90346. https://doi.org/10.1371/journal.pone.0090346

Goudet, J., 2005 Hierfstat, a package for r to compute and test hierarchical f-statistics. Mol. Ecol. Notes 5: 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Grzebelus, D., M. Iorizzo, D. Senalik, S. Ellison, P. Cavagnaro *et al.*, 2014 Diversity, genetic mapping, and signatures of domestication in the carrot (*Daucus carota* L.) genome, as revealed by Diversity Arrays Technology (DArT) markers. Mol. Breed. 33: 625–637. https://doi.org/10.1007/s11032-013-9979-9

Iorizzo, M., D. Senalik, S. Ellison, D. Grzebelus, P. Cavagnaro *et al.*, 2013 Genetic structure and domestication of carrot (*Daucus carota* subsp. *sativus*) (Apiaceae). Am. J. Bot. 100: 930–938. https://doi.org/10.3732/ajb.1300055

Iorizzo, M., S. Ellison, D. Senalik, P. Zeng, P. Satapoomin *et al.*, 2016 A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. Nat. Genet. 48: 657–666. https://doi.org/10.1038/ng.3565

Jourdan, M., S. Gagné, C. Dubois-Laurent, M. Maghraoui, S. Huet *et al.*, 2015 Carotenoid content and root color of cultivated carrot: a candidate-gene association study using an original broad unstructured population. PLoS One 10: e0116674. https://doi.org/10.1371/journal.pone.0116674

Just, B. J., C. A. Santos, M. E. Fonseca, L. S. Boiteux, B. B. Oloizia *et al.*, 2007 Carotenoid biosynthesis structural genes in carrot (*Daucus carota*): isolation, sequence-characterization, single nucleotide polymorphism (SNP) markers and genome mapping. Theor. Appl. Genet. 114: 693–704. https://doi.org/10.1007/s00122-006-0469-x

Keilwagen, J., H. Lehnert, T. Berner, H. Budahn, T. Nothnagel *et al.*, 2017 The terpene synthase gene family of carrot (Daucus carota L.): identification of QTLs and candidate genes associated with terpenoid volatile compounds. Front. Plant Sci. 8: 1930. https://doi.org/10.3389/fpls.2017.01930

Kim, S., V. Plagnol, T. Hu, C. Toomajian, R. Clark *et al.*, 2007 Recombination and linkage disequilibrium in Arabidopsis thaliana. Nat. Genet. 39: 1151–1155. https://doi.org/10.1038/ng2115

Lam, H., X. Xu, X. Liu, G. Chen, W. Yang *et al.*, 2010 Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. Nat. Genet. 42: 1053–1059. https://doi.org/10.1038/ng.715

Li, L., D. Paolillo, M. Parthasarathy, E. Dimuzio, and D. Garvin, 2001 A novel gene mutation that confers abnormal patterns of beta-carotene accumulation in cauliflower (*Brassica oleracea* var. *Botrytis*). Plant J. 26: 59–67. https://doi.org/10.1046/j.1365-313x.2001.01008.x

Li, L., Y. Yang, Q. Xu, K. Owsiany, R. Welsch *et al.*, 2012 The *Or* gene enhances carotenoid accumulation and stability during post-harvest storage of potato tubers. Mol. Plant 5: 339–352. https://doi.org/10.1093/mp/ssr099

Lu, S., J. Van Eck, X. Zhou, A. Lopez, D. O'Halloran *et al.*, 2006 The cauliflower Or gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of beta-carotene accumulation. Plant Cell 18: 3594–3605. https://doi.org/10.1105/tpc.106.046417

Maass, D., J. Arango, F. Wüst, P. Beyer, and R. Welsch, 2009 Carotenoid crystal formation in Arabidopsis and carrot roots caused by increased phytoene synthase protein levels. PLoS One 4: e6373. https://doi.org/10.1371/journal.pone.0006373

Macko-Podgórni, A., G. Machaj, K. Stelmach, D. Senalik, E. Grzebelus *et al.*, 2017 Characterization of a genomic region under selection in cultivated carrot (*Daucus carota* subsp. *sativus*) reveals a candidate domestication gene. Front. Plant Sci. 8: 12. https://doi.org/10.3389/fpls.2017.00012

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis *et al.*, 2010 The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20: 1297–1303. https://doi.org/10.1101/gr.107524.110

Murray, M., and W. Thompson, 1980 Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321–4326. https://doi.org/10.1093/nar/8.19.4321

Myles, S., A. Boyko, C. Owens, P. Brown, F. Grassi *et al.*, 2011 Genetic structure and domestication history of the grape. Proc. Natl. Acad. Sci. USA 108: 3530–3535. https://doi.org/10.1073/pnas.1009363108

Nei, M., and W. Li, 1979 Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269–5273. https://doi.org/10.1073/pnas.76.10.5269

Park, S., H. Kim, Y. Jung, S. Kim, C. Ji *et al.*, 2016 Orange protein has a role in phytoene synthase stabilization in sweet potato. Sci. Rep. 6: 33563. https://doi.org/10.1038/srep33563

Poland, J., P. Brown, M. Sorrells, and J. Jannink, 2012 Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7: e32253. https://doi.org/10.1371/journal.pone.0032253

Pritchard, J., M. Stephens, and P. Donnelly, 2000 Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Rong, J., Y. Lammers, J. Strasburg, N. Schidlo, Y. Ariyurek *et al.*, 2014 New insights into domestication of carrot from root transcriptome analyses. BMC Genomics 15: 895. https://doi.org/10.1186/1471-2164-15-895

Rosenberg, N., 2004 DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4: 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Santos, C. A., and P. W. Simon, 2002 QTL analyses reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrot roots. Mol. Genet. Genomics 268: 122–129. https://doi.org/10.1007/s00438-002-0735-9

Shim, S., and R. Jorgensen, 2000 Genetic structure in cultivated and wild carrots (*Daucus carota* L.) revealed by AFLP analysis. Theor. Appl. Genet. 101: 227–233. https://doi.org/10.1007/s001220051473

Simon, P., 1990 Carrots and other horticultural crops as a source of provitamin a carotenes. HortScience 25: 1495–1499.

Simon, P., 2000 Domestication, historical development, and modern breeding of carrot. Plant Breed. Rev. 19: 157–190.

Simon, P., and I. Goldman, 2007 Carrot, pp. 497–517 in *Genetic Resources, Chromosome Engineering and Crop Improvement: Vegetable Crops*, edited by R. J. Singh. CRC Press, Boca Raton, FL.

Simon, P., and X. Wolff, 1987 Carotenes in typical and dark orange carrots. J. Agric. Food Chem. 35: 1017–1022. https://doi.org/10.1021/jf00078a038

Simon, P., X. Wolff, C. Peterson, and D. Kammerlohr, 1989 High carotene mass carrot population. HortScience 24: 174–175.

Simon, P., R. Freeman, J. Vieira, L. Boiteux, M. Briard *et al.*, 2008 Carrot, pp. 327–357 in *Handbook of Crop Breeding*, Vol. 1, Vegetable Breeding. Springer-Verlag, Heidelberg, Germany.

Simon, P., L. Pollak, B. Clevidence, J. Holden, and D. Haytowitz, 2009 Plant breeding for human nutrition. Plant Breed. Rev. 31: 325–392.

Simpson, K., A. Cerda, and C. Stange, 2016 Carotenoid biosynthesis in Daucus carota. Subcell. Biochem. 79: 199–217.

Soufflet-Freslon, V., M. Jourdan, J. Clotault, S. Huet, M. Briard *et al.*, 2013 Functional gene polymorphism to reveal species history: the case of the CRTISO gene in cultivated carrots. PLoS One 8: e70801. https://doi.org/10.1371/journal.pone.0070801

Stamatakis, A., 2014 RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stolarczyk, J., and J. Janick, 2011 Carrot: history and iconography. Chron. Hortic. 51: 13–18.

Turner, S., 2014 qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv. Available at: https://www.biorxiv.org/content/early/2014/05/14/005165. https://doi.org/10.1101/005165

Tzuri, G., X. Zhou, N. Chayut, H. Yuan, V. Portnoy *et al.*, 2015 A 'golden' SNP in CmOr governs the fruit flesh color of melon (*Cucumis Melo*). Plant J. 82: 267–279. https://doi.org/10.1111/tpj.12814

Vavilov, N., and V. Dorofeev, 1992 *Origin and Geography of Cultivated Plants*. Cambridge University Press, Cambridge [England]; New York.

Vos, P. G., M. J. Paulo, R. G. Voorrips, R. G. Visser, H. J. van Eck *et al.*, 2017 Evaluation of LD decay and various LD-decay estimators in simulated and SNP-array data of tetraploid potato. Theor. Appl. Genet. 130: 123–135. https://doi.org/10.1007/s00122-016-2798-8

Weir, B., and C. Cockerham, 1984 Estimating f-statistics for the analysis of population structure. Evolution 38: 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Wickham, H., 2009 *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York.

Wright, S., 1951 The genetical structure of populations. Ann. Eugen. 15: 323–354. https://doi.org/10.1111/j.1469-1809.1949.tb02451.x

Yan, J., T. Shah, M. Warburton, E. Buckler, M. McMullen *et al.*, 2009 Genetic characterization and linkage disequilibrium estimation of a global maize collection using SNP markers. PLoS One 4: e8451. https://doi.org/10.1371/journal.pone.0008451

Yuan, H., J. Zhang, D. Nageswaran, and L. Li, 2015 Carotenoid metabolism and regulation in horticultural crops. Hortic. Res. 2: 01536.

Zhao, K., C. Tung, G. Eizenga, M. Wright, M. Ali *et al.*, 2011 Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nat. Commun. 2: 467. https://doi.org/10.1038/ncomms1467

Zhou, X., R. Welsch, Y. Yang, D. Álvarez, M. Riediger *et al.*, 2015 Arabidopsis OR proteins are the major posttranscriptional regulators of phytoene synthase in controlling carotenoid biosynthesis. Proc. Natl. Acad. Sci. USA 112: 3558–3563. https://doi.org/10.1073/pnas.1420831112

Communicating editor: A. Charcosset