Pleiotropic effects of the wheat domestication gene *Q* on yield and grain morphology

Quan Xie[1], Na Li[1], Yang Yang[1], Yulong Lv[1], Hongni Yao[1], Rong Wei[1], Debbie L. Sparkes[2], Zhengqiang Ma[1]*

[1]The Applied Plant Genomics Laboratory, Crop Genomics and Bioinformatics Center, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

[2]Division of Plant and Crop Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK

***Corresponding author:**
E-mail: zqm2@njau.edu.cn
Tel: +86 25 8439 6029
Fax: +86 25 8439 6707

Main conclusion: Transformation from *q* to *Q* during wheat domestication functioned outside the boundary of threshability to increase yield, grains m^{-2}, grain weight and roundness, but to reduce grains per spike/spikelet.

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Abstract

Mutation of the *Q* gene, well known affecting wheat spike structure, represents a key domestication step in the formation of today′s free-threshing, economically important wheats. In a previous study, multiple yield components and spike characteristics were associated with the *Q* gene interval in the bread wheat 'Forno' × European spelt 'Oberkulmer' recombinant inbred line population. Here we reported that this interval was also associated with grain yield, grains m^{-2}, grain morphology and spike dry weight at anthesis. To clarify the roles of *Q* in agronomic trait performance, a functional marker for the *Q* gene was developed. Analysis of allelic effects showed that the bread wheat *Q* allele conferred free-threshing habit, soft glumes, and short and compact spikes compared with *q*. In addition, the *Q* allele contributed to higher grain yield, more grains m^{-2} and higher thousand grain weight, whereas *q* contributed to more grains per spike/spikelet likely resulting from increased preanthesis spike growth. For grain morphology, the *Q* allele was associated with reduced ratio of grain length to height, indicating a rounder grain. These results are supported by analysis of four *Q* mutant lines in the Chinese Spring background. Therefore, the transition from *q* to *Q* during wheat domestication had profound effects on grain yield and grain shape evolution as well, being a consequence of pleiotropy.

Keywords: Grain shape, Mutation, Spelt, Spike, Threshability, Yield components

Abbreviations

AP2	APETALA2
CS	Chinese Spring
LOD	Likelihood of odd
QTL	Quantitative trait locus
RIL	Recombinant inbred line
SNP	Single nucleotide polymorphism
TGW	Thousand grain weight

Introduction

Wheat (*Triticum* spp.) was one of the Neolithic founder crops, and played an important role in the transition from hunter-gathering to a sedentary agrarian society in the Near-Eastern Fertile Crescent approximately 10,000 years ago (Lev-Yadun et al. 2000). Today′s bread (*T. aestivum* L.) and durum wheats (*T. durum* Desf.) are still economically important, providing about 20% of the calories and proteins consumed by humans worldwide (Braun et al. 2010).

Domestication contributes fundamentally to the formation of modern wheats. This process has altered a number of key plant characteristics such as rachis fragility and grain threshability for efficient harvesting, as well as other agronomic traits (Faris 2014). Wild einkorn (*T. boeoticum* Boiss.) and emmer (*T. dicoccoides* Körn.) have brittle spikes that are disarticulated into spikelets and drop to the ground at maturity. Transformation of a brittle into non-brittle spike gave rise to domesticated einkorn (*T. monococcum* L.) and emmer (*T. dicoccum* Schübl.), which became the staple crops of early civilization for several thousand years (Salamini et al. 2002; Gill et al. 2007). In einkorn, this trait is controlled by two recessive genes mapped on chromosomes $5A^m$ and $7A^m$ (Sharma and Waines 1980; Gill et al. 2007), while in emmer it is conditioned by three recessive alleles on chromosomes 2AL, 3AS and 3BS (Watanabe et al. 2002; Peng et al. 2003; Peleg et al. 2011). With the help of the wild emmer reference genome sequence, those on 3AS and 3BS were identified to be orthologous to the *Btr1* and *Btr2* genes controlling brittle rachis in barley (Avni et al. 2017). Loss-of-function mutations by a 2-bp deletion and a 4-kb insertion in the wild emmer *TtBtr1-A* and *TtBtr1-B*, respectively, result into rough abscission scars of spikelets and non-brittle phenotype.

Modern wheats possess not only fully tough rachis but also free-threshing habit. It is believed that the bread wheat acquired its non-brittle rachis feature from the domesticated emmer or durum wheat during the amphiploidization event between the tetraploid wheat (genome AABB) and diploid goat grass (*Ae. tauschii* Coss., genome DD) (Faris 2014). The free-threshing character is mainly derived from a soft glume that allows seed to be easily separated from the spikelet. Einkorn and emmer usually have tenacious glumes while the bread and durum wheats have soft ones. Glume tenacity is under control of the genes in homoeologous group 2: *Sog* on $2A^mS$ in diploid, *Tg2* on 2BS in tetraploid and *Tg1* on 2DS in hexaploid wheats (Kerber and Rowland 1974; Simonetti et al. 1999; Taenzler et al. 2002; Jantasuriyarat et al. 2004). Moreover, the *Q* gene on chromosome 5A has major effects on glume tenacity and grain threshability. In hexaploid wheat, *Tg1* derived from *Ae. tauschii* is epistatic to *Q*, and both the *tg1* and *Q* alleles are required to confer the free-threshing habit. In addition to threshability, the

Q gene has been found to pleiotropically affect plant height, spike emergence time, and spike traits such as spike fragility, spike shape (square or speltoid), spike length and spike compactness (Muramatsu 1963; Jantasuriyarat et al. 2004; Simons et al. 2006). The *Q* locus encodes a member of APETALA2 (AP2) transcription factor family (Faris et al. 2003; Simons et al. 2006). The wild and domesticated einkorn and emmer, *T. urartu* Thum. ex Gandil. (donor of A genome for hexaploid wheat) and European spelt (*T. spelta* L.) all possess the primitive *q* allele, while the bread and durum wheats have the *Q* allele. Comparison between the *q* and *Q* sequences reveals a conserved single nucleotide polymorphism (SNP) at position 2123 that leads to amino acid substitution from valine to isoleucine, and a synonymous SNP in the microRNA172 target site of exon 10 (Simons et al. 2006; Zhang et al. 2011). The *Q* effects are dosage dependent (Muramatsu 1963; Simons et al. 2006), and the microRNA target site variation makes the *Q* allele expression higher (Debernardi et al. 2017; Greenwood et al. 2017; Liu et al. 2017).

The AP2 class of transcription factors that *Q* belongs to is characterized by a tandem repetition of two AP2 DNA binding domains (each consisting of *c*. 60 amino acid residues), and well known for their roles in the regulation of floral meristem, floral organ identity and flower development in *Arabidopsis* (Irish and Sussex 1990; Jofuku et al. 1994; Yant et al. 2010). The AP2 orthologs in other plants such as rice (*Oryza sativa* L.), maize (*Zea mays* L.) and petunia (*Petunia hybrida* L.), function similarly in inflorescence development (Maes et al. 2001; Lee et al. 2006; Chuck et al. 2008). In cereal crops, the inflorescence is the organ bearing seeds that ultimately form grain yield. Thus, modifications in inflorescence architecture may alter floral fertility, seed growth and consequently final yield. Evidence now shows that in addition to its functions for flower development, the *Arabidopsis AP2* genes also serve as a determinant of seed number, seed size and seed yield as well as the accumulation of seed oil and protein (Jofuku et al. 2005; Ohto et al. 2005). Given the conserved roles of *AP2* among plant species, their effects on agronomically relevant traits could be applied to crops like wheat.

In a previous study using the recombinant inbred line population derived from the cross of the bread wheat variety ‘Forno’ with the European spelt variety ‘Oberkulmer’, the *Q* gene interval on chromosome 5A was associated with thousand grain weight (TGW), grain set and spike characteristics including grain threshability, glume tenacity, spike length and spike compactness (Xie et al. 2015). The current study was conducted to clarify the roles of *Q* gene in wheat agronomic trait performance through allelic analysis and using mutant lines. We

concluded, based on our study, that the transformation from *q* to *Q* during wheat domestication increased grain yield, grains m^{-2}, TGW and grain roundness as well.

Materials and methods

Plant materials

An F_5 recombinant inbred line (RIL) population, derived from the cross between the Swiss winter bread wheat 'Forno' and Swiss winter spelt 'Oberkulmer' (Messmer et al. 1999), was used in this study. Forno is free-threshing, harboring the *Q* allele, whereas Oberkulmer is hulled and non-free-threshing, harboring the *q*. This mapping population included 226 RILs, and varied greatly in spike characteristics.

The *Q* allele mutants of Chinese Spring (CS), namely mq36, mq125 and mq194, were obtained by exposing the CS grains to 0.4% ethyl methanesulfonate (EMS), followed by screening for the speltoid phenotype in the M_2 families (Simons et al. 2006). The mq36 and mq125 mutants have point mutations in introns 7 and 2 of the *Q* gene, respectively, which cause alternate splicing. The mq194 mutant has a single nucleotide substitution in exon 5, which led to change of a cysteine to a tyrosine (Simons et al. 2006). The fourth mutant, fndel143, was produced by exposing the CS grains to fast neutrons (5 Gy), followed by screening the M_2–M_4 plants for spike traits. The resulting fndel143 has a segmental deletion spanning the *Q* locus (Faris et al. 2003). These four mutant lines were kindly provided by Dr Justin Faris (USDA-ARS Cereal Crops Research Unit).

Phenotyping for the field and glasshouse experiments

Two field experiments were carried out for the Forno × Oberkulmer mapping population, and described in Xie et al. (2015). Additionally, one glasshouse experiment was conducted in the 2013–2014 season at University of Nottingham Farm, Leicestershire, UK. The grains were sown on 17 December 2013, and then subjected to vernalization at 6 ^{o}C for nine weeks. The seedlings were transferred to 1-liter pots (one plant per pot) filled with the loam-based compost, and arranged according to a randomized complete block design with three replicates. Water, fertilizer (40 kg N ha^{-1} at early stem elongation) and fungicides were applied to maintain undisturbed plant growth. Evaluation of the CS and four *Q* mutant lines was conducted in a glasshouse in 2016–2017 at Nanjing Agricultural University Experimental Station, China, using a design similar to the Nottingham glasshouse experiment.

In the field experiments, measurements on spike characteristics, TGW, grains per spike/spikelet and spikelets per spike were described in Xie et al. (2015). All the plots were combined and recorded for the fresh grain weight (only partially threshed depending on genotypes) after measuring the actual harvesting areas. Total grain number of the collected sample from a plot was calculated from its fresh weight and the grain number of 10-g subsample taken for threshability analysis. Grains m^{-2} was the result of the total grain number divided by the harvested area. Grain yield was then obtained based on the plot grain number and TGW. Meanwhile, a yield-related trait, spike dry weight at anthesis, was analyzed by collecting five main spikes exactly at anthesis, followed by oven-drying at 85ºC for 48 h.

Grain length, width and height were quantified to analyze grain shape. Nine grains equally from the basal, central and apical parts of five spikes from a plot in 2012 were measured using an electronic caliper (OD-15CP, Mitutoyo, Andover, Hampshire, UK). In 2013, 20 representative grains from a plot were scanned by a desk scanner (Officejet 4500, HP), with the grain crease and lateral side downward in sequence. The images were processed for grain dimensions by ImageJ (NIH, USA; http://rsbweb.nih.gov/ij/). The grain dimensions were defined as: grain length, the horizontal distance between the embryo and the other end, along the ventral crease; grain width, the maximum horizontal distance between the two 'cheeks' beside the crease; grain height, the maximum vertical distance with the dorsal side upward and the crease downward. The ratios of grain length to width and grain length to height were used to represent grain shape.

For the field traits investigated, threshability, grain yield, grains m^{-2}, TGW and grain shape were evaluated for all 226 RILs, while the remaining ones evaluated only in the subsets including 72 RILs in 2012 and 110 RILs in 2013. The latter 110 RILs were also used for the 2013–2014 glasshouse experiment to quantify grains per spike/spikelet and TGW. As to the CS and four *Q* mutant lines, the spike characteristics, yield components and grain shape were measured at maturity.

Quantitative trait locus (QTL) mapping

A functional marker of the *Q* gene on chromosome 5A was developed according to the conserved SNP between *q* and *Q* at position 2123 that causes amino acid change (I329V) (Simons et al. 2006). Primers for the cleaved amplified polymorphic sequence (CAPS) marker were designed by dCAPS Finder v2.0 (Neff et al. 2002) and Premier 5.0 (http://www.premierbiosoft.com/) (forward: CCAAGAGGGACAACATCATCG and reverse:

ATGTTTTAGGAGAGCAGGCGTG). Genomic DNA was extracted from the leaves of young seedlings, following Ma and Sorrells (1995). PCR was undertaken in a 10-µl volume consisting of 10 to 20 ng template, 2 pmol each of forward and reverse primers, 2 nmol each of deoxynucleotide triphosphates, 15 nmol $MgCl_2$, 0.1 U Taq DNA polymerase, and 1 × PCR buffer. PCR was run on a PE9600 thermal cycler (Perkin Elmer, Norwalk, CT, USA), and cycling conditions were the following: 94°C for 5 min, 36 cycles of 94 °C for 40 s, 58 °C for 40 s, 72 °C for 40 s, and a final extension step at 72 °C for 5 min. PCR products were digested with *Mbo*I, separated on a 8% acrylamide/bisacrylamide (29: 1) gel for 70 min, and then visualized by silver staining.

In addition to *Q*, the other 16 markers on chromosome 5A for the Forno × Oberkulmer population were obtained from the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml). All the markers were used to construct the genetic map of chromosome 5A using JoinMap v4 (Van Ooijen 2006). Interval mapping was performed using MapQTL v6 (Van Ooijen 2009). A QTL was declared when its likelihood of odd (LOD) was higher than 3. QTL nomenclature followed the Catalogue of Gene Symbols for Wheat (http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/).

Statistical analysis

Best linear unbiased estimates (BLUEs) were generated for each trait over replicates and years using TASSEL v5 (Bradbury et al. 2007). Analysis of variance (ANOVA) and multiple comparisons (Fisher′s unprotected LSD) were used to test for the significance of genotypic differences. Pearson correlations were performed to identify the relationships between different traits. These statistical analyses were carried out using Genstat v17 and GraphPad Prism v6.05.

Results

Phenotypic variation in the Forno × Oberkulmer RIL population

Xie et al. (2015) showed that the *Q* gene interval was associated with TGW, grains per spike/spikelet, and spike characteristics including grain threshability, glume tenacity, spike length and spike compactness. To examine the effects of the *Q* gene interval on other yield traits, grain yield, grains m^{-2}, grain shape and spike dry weight at anthesis were investigated. The bread wheat Forno displayed higher grain yield and grains m^{-2} than the spelt Oberkulmer (Fig. 1). The two parents also differed substantially in grain shape: Forno showed lower ratios of grain length to width and grain length to height than Oberkulmer did, indicating a rounder grain. Additionally, there was a significant difference between the two parents in spike dry weight at anthesis, and Forno had smaller spikes relative to Oberkulmer. Frequency distributions of these yield and yield-related traits in the RIL population were approximately normal (Fig. 1).

Associations of the *Q* interval with the spike and yield traits

To conduct QTL mapping of the *Q* interval for the spike and yield traits, the genetic map of chromosome 5A was reconstructed by combining the *Q* functional marker. The resulting map covered a total of 233.1 cM, with the *Q* at 217.2 cM (Fig. 2). Interval mapping was then performed with this new map, and confirmed the associations of the *Q* interval with TGW, grain set and spike characteristics (Fig. 2 and Table 1). In addition, the *Q* interval was associated with grain yield and grains m^{-2}, explaining 21.9% and 15.6% of their phenotypic variation, respectively (Fig. 2 and Table 1). For grain shape, this interval showed an association with grain length/height, but not with grain length/width. QTL mapping also identified a significant effect of the *Q* interval on spike dry weight at anthesis (Fig. 2 and Table 1).

Allelic analysis of *Q*

To determine the allelic effects of *Q*, all RILs were grouped according to their *Q* genotypes diagnosed by the functional marker (*q*/*Q*; Table 2). Results showed that the *q* and *Q* alleles made significant differences in all traits studied except for spikelets per spike and grain length/width. The *Q* allele coming from the bread wheat Forno conferred higher threshability, softer glumes, and shorter and denser spikes, while the *q* allele coming from the spelt Oberkulmer did the opposite. Interestingly, the *Q* allele was associated with increased grain yield, grains m^{-2} and TGW, but reduced grains per spike/spikelet. The *q*/*Q* also affected grain shape differently, with the *Q* allele resulting in lower grain length/height and consequently

rounder grains. Furthermore, smaller spikes at anthesis were observed for the *Q* allele, in contrast with *q*. Taken together, selection of the *q*/*Q* alleles had significant impacts on yield and yield-related traits apart from spike characteristics in the Forno × Oberkulmer mapping population.

The *Q* mutations altered the spike and yield traits

To determine whether *Q* affected the yield traits through pleiotropy or its linkage with other genes, the phenotypes of CS and the four *Q* mutant lines (mq36, mq125, mq194 and fndel143) were analyzed (Fig. 3). As expected, the four mutant lines all had tougher glumes, and longer and laxer spikes compared with CS. Importantly, the *Q* allele mutations generally resulted in more grains per spikelet, and the increases of mq125 and mq194 were highly significant ($P < 0.01$). TGW was greatly reduced in all mutant lines while grain length/height was boosted, indicating smaller and slimmer grains. These results confirmed the roles of *Q* in yield components and grain shape, and it functioned most likely through pleiotropy. In addition, the mutant lines differed among themselves in spike and yield traits, implying distinct maintenance of the *Q* functions, as seen for the *AP2* mutations in *Arabidopsis* (Ohto et al. 2005).

Discussion

Transition from the hulled to free-threshing spikes is a key step during wheat domestication (Salamini et al. 2002), and this process was mainly attributed to appearance of the *Q* allele mutated from the primitive *q*. In the current study, the roles of *Q* gene in shaping spike characteristics were confirmed in the bread wheat Forno × spelt Oberkulmer population and the CS *Q* mutant lines. Along with improved harvesting efficiency, we proposed that selection for the *Q* allele over *q* might also have modified yield and yield components. Utilization of the *Q* allele could improve grain yield by 18%, which resulted from both increased grains m^{-2} and TGW (Table 2). Grains m^{-2} is an outcome of spikes m^{-2} and grains per spike, and the latter was actually reduced for the *Q* allele; therefore, more grains m^{-2} might be a consequence of increased spikes m^{-2}. Another possible driver of increased grains m^{-2} may come from the semi-dwarfing effect of the *Q* allele on plant height (Kato et al. 2003; Zhang et al. 2011; Debernardi et al. 2017; Greenwood et al. 2017), just like the contribution of semi-dwarf cultivars introduced during the Green Revolution of the 1960s and 1970s (Foulkes et al. 2011). Due to unchanged spikelet number per spike, fewer grains per spike derived from the *Q* allele would be the product of fewer grains per spikelet that had already been observed here. There is evidence that spike/spikelet fertility is largely determined by floret survival before flowering, which is eventually modulated by the preanthesis spike growth (Fischer 2011; González et al. 2011). This concurs with the present study, where the spike dry weight at anthesis showed positive correlations with both grains per spike ($r = 0.63$, $P < 0.01$) and grain per spikelet ($r = 0.52$, $P < 0.01$). Genetically, all these traits were found to be associated with *Q*. The *q* allele contributed to larger spikes at anthesis and more grains per spike/spikelet. When it was substituted for the *Q* allele, smaller spikes and subsequently lower spike fertility were produced. On the contrary, higher TGW was observed for the *Q* allele. These results are in line with the previous reports. Kato et al. (2000) identified a QTL for grain weight at the *Q* gene interval using single-chromosome (5A) recombinant lines, and the larger grains were associated with the *Q* allele. When the *Q* allele was replaced with *q* through chromosome substitution between CS and wild emmer, grains per spike were significantly increased whereas grain weight was reduced (Zhang et al. 2011). More recently, it was found in tetraploid wheat that truncation mutations in *Q* resulted in more florets per spikelet (Debernardi et al. 2017). Likewise, overexpressing the gene of *miR172d* led to lower expression of *Q* and more florets, whereas reducing the activity of miR172 showed the opposite effects (Debernardi et al. 2017). In the hexaploid wheat background, the loss-of-function mutation of *Q′* (the *Q* allele with a novel SNP in the miR172

binding site), also produced the extra florets as the sham ramification trait (Greenwood et al. 2017). Many of the extra florets developed normally and had potential to produce additional grains, which provides another potential explanation for the increased grain number per spike/spikelet associated with *q*. In the present study, we cannot rule out the possibility that the effects of *Q* on grain yield, grains m^{-2} and spike dry weight at anthesis in the Forno × Oberkulmer population could stem from other gene(s) closely linked to *Q* as these traits were not measured in the *Q* mutant lines. It is believed, however, that this is unlikely in light of the known effects of *Q* on spike architecture as well as on TGW and grains per spike/spikelet, the major components of grain yield and grains m^{-2}.

Grain shape does not appear to be a key domestication trait of wheat, in contrast with other crops like rice (Kovach et al. 2007). It has recently been targeted in wheat breeding because spherical grains can improve milling performance and, in turn, increase their market value (Gegas et al. 2010). In this study, it is proposed that grain shape was also altered, from a slimmer to a rounder one, as a byproduct of selection for the *Q* allele during domestication. This change was mainly due to reduced grain length/height during the transformation from *q* to *Q*. The *q* plants have tenacious, hard glumes that may act as a direct physical constraint on grain shape. This is supported by a positive correlation between glume tenacity and grain length/height ($r = 0.46$, $P < 0.01$). During grain filling, the tenacious glume holds the young grain tightly, and forces it along the dorsal side, which would lead to a limit for grain thickening. Indeed, grain height, rather than grain length, showed an association with the *Q* gene interval, and the *q* allele from the spelt Oberkulmer contributed to a thinner grain (data not shown). With replacement of the allele *q* with *Q*, the soft glume would allow grain to thicken relatively freely, resulting in a thicker and rounder grain as it is today. The maternal physical constraint for grain morphology has been reported in rice. Examples include the *GW2* gene of rice that negatively regulates cell division, and loss of *GW2* function produces a larger spikelet hull and consequently enhanced grain width (Song et al. 2007). Moreover, *qSW5* gene affects grain width by influencing cell number in the outer glume of the rice flower (Shomura et al. 2008). During rice domestication, grain shape and glume size might have undergone artificial selection simultaneously (Shomura et al. 2008). In wheat, Gegas et al. (2010) hypothesized that domestication has transformed a long thin primitive grain existing in the ancestral wheat species to a rounder modern grain existing in today′s varieties. The present study provide evidence that selection for the domestication gene *Q* responsible for the free-threshing habit might have

driven this transformation, which led to co-evolution for grain shape and glume characteristics, concurring with the observation in rice.

In *Arabidopsis*, *AP2* is essential for the establishment of floral meristem and organ identity, and the temporal and spatial regulation of floral homeotic gene expression (Bowman et al. 1989; Irish and Sussex 1990; Yant et al. 2010). The *ap2* loss-of-function mutations induce defects in inflorescence structure, giving rise to reduced flower fertility, fewer elongated siliques and fewer seeds per silique as compared with those of wild type (Jofuku et al. 1994; Ohto et al. 2005). On the other hand, the *ap2* mutants produce dramatically larger seeds, which are not solely due to the expense of seed number but a direct control by the mutation likely through its effects on sugar metabolism (Jofuku et al. 2005; Ohto et al. 2005). The larger seeds resulting from the *ap2* mutations are also rounder, though less regular, in shape relative to wild-type ones, possibly because of the change in seed coat that lacks 'epidermal plateau' or columellae (Jofuku et al. 1994; Ohto et al. 2005). Taken together, the loss-of-function *ap2* mutations lead to reduced flower fertility, and larger and rounder seeds. These phenotypic changes are similar to the hypermorphic mutation from the *q* to *Q* alleles in wheat as observed in the present study. Two additional *AP2* genes, *TaPARG-2A* and *TaPARG-2D*, were recently identified, and showed pleiotropic effects on plant architecture and yield-related traits (Li et al. 2016). In rice, the *shattering abortion1* gene was isolated as an AP2 transcription factor to control seed shattering, floral organ development, and seed set rate as well as seed size and shape (Zhou et al. 2012). These studies suggest that *AP2* genes have conserved roles between *Arabidopsis* and cereals in determining both flower characteristics and yield traits, and could be utilized for future crop improvement.

Author contribution statement

QX designed the experiments, performed phenotyping and data analysis, and wrote the paper. NL grew and phenotyped the *Q* mutants. YY developed the *Q* functional marker and genotyped the RILs. YL, HY and RW helped with the field trials and data collection. DLS and ZM contributed to the experimental design and paper writing.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

References

Avni R, Nave M, Barad O, Baruch K, Twardziok SO, Gundlach H, Hale I, Mascher M, Spannagl M, Wiebe K, Jordan KW, Golan G, Deek J, Ben-Zvi B, Ben-Zvi G, Himmelbach A, MacLachlan RP, Sharpe AG, Fritz A, Ben-David R, Budak H, Fahima T, Korol A, Faris JD, Hernandez A, Mikel MA, Levy AA, Steffenson B, Maccaferri M, Tuberosa R, Cattivelli L, Faccioli P, Ceriotti A, Kashkush K, Pourkheirandish M, Komatsuda T, Eilam T, Sela H, Sharon A, Ohad N, Chamovitz DA, Mayer KFX, Stein N, Ronen G, Peleg Z, Pozniak CJ, Akhunov ED, Distelfeld A (2017) Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science 357:93-97

Bowman JL, Smyth DR, Meyerowitz EM (1989) Genes directing flower development in *Arabidopsis*. Plant Cell 1:37-52

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635

Braun HJ, Atlin G, Payne T (2010) Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds MP (ed) Climate change and crop production. CABI, Wallingford, UK, pp 115-138

Chuck G, Meeley R, Hake S (2008) Floral meristem initiation and meristem cell fate are regulated by the maize *AP2* genes *ids1* and *sid1*. Development 135:3013-3019

Debernardi JM, Lin H, Faris JD, Dubcovsky J (2017) microRNA172 plays a critical role in wheat spike morphology and grain threshability. Development 144:1966-1975

Faris JD (2014) Wheat domestication: Key to agricultural revolutions past and future. In: Tuberosa R, Graner A, Frison E (eds) Genomics of plant genetic resources. Springer, Houten, The Netherlands, pp 439-464

Faris JD, Fellers JP, Brooks SA, Gill BS (2003) A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. Genetics 164:311-321

Fischer RA (2011) Wheat physiology: a review of recent developments. Crop Pasture Sci 62:95-114

Foulkes MJ, Slafer GA, Davies WJ, Berry PM, Sylvester-Bradley R, Martre P, Calderini DF, Griffiths S, Reynolds MP (2011) Raising yield potential of wheat. III. Optimizing

partitioning to grain while maintaining lodging resistance. J Exp Bot 62:469-486

Gegas VC, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan JH, Snape JW (2010) A genetic framework for grain size and shape variation in wheat. Plant Cell 22:1046-1056

Gill BS, Li W, Sood S, Kuraparthy V, Friebe BR, Simons KJ, Zhang Z, Faris JD (2007) Genetics and genomics of wheat domestication-driven evolution. Isr J Plant Sci 55:223-229

González FG, Miralles DJ, Slafer GA (2011) Wheat floret survival as related to pre-anthesis spike growth. J Exp Bot 62:4889-4901

Greenwood JR, Finnegan EJ, Watanabe N, Trevaskis B, Swain SM (2017) New alleles of the wheat domestication gene *Q* reveal multiple roles in growth and reproductive development. Development 144:1959-1965

Irish VF, Sussex IM (1990) Function of the *apetala-1* gene during *Arabidopsis* floral development. Plant Cell 2:741-753

Jantasuriyarat C, Vales M, Watson C, Riera-Lizarazu O (2004) Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). Theor Appl Genet 108:261-273

Jofuku KD, den Boer BGW, Van Montagu M, Okamuro JK (1994) Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. Plant Cell 6:1211-1225

Jofuku KD, Omidyar PK, Gee Z, Okamuro JK (2005) Control of seed mass and seed yield by the floral homeotic gene *APETALA2*. Proc Natl Acad Sci USA 102:3117-3122

Kato K, Miura H, Sawada S (2000) Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. Theor Appl Genet 101:1114-1121

Kato K, Sonokawa R, Miura H, Sawada S (2003) Dwarfing effect associated with the threshability gene *Q* on wheat chromosome 5A. Plant Breeding 122:489-492

Kerber ER, Rowland GG (1974) Origin of the free threshing character in hexaploid wheat. Can J Genet Cytol 16:145-154

Kovach MJ, Sweeney M, Mccouch SR (2007) New insights into the history of rice domestication. Trends Genet 23:578-587

Lee D, Lee J, Moon S, Park SY, An G (2006) The rice heterochronic gene *SUPERNUMERARY BRACT* regulates the transition from spikelet meristem to floral meristem. Plant J 49:64-

Lev-Yadun S, Gopher A, Abbo S (2000) The cradle of agriculture. Science 288:1602-1603

Li B, Li Q, Mao X, Li A, Wang J, Chang X, Hao C, Zhang X, Jing R (2016) Two novel AP2/EREBP transcription factor genes *TaPARG* have pleiotropic functions on plant architecture and yield-related traits in common wheat. Front Plant Sci 7:1191

Liu P, Liu J, Dong H, Sun J (2017) Functional regulation of Q by microRNA172 and transcriptional co-repressor TOPLESS in controlling bread wheat spike density. Plant Biotechnol J doi: 10.1111/pbi.12790

Ma ZQ, Sorrells ME (1995) Genetic analysis of fertility restoration in wheat using restriction fragment length polymorphisms. Crop Sci 35:1137-1143

Maes T, Van de Steene N, Zethof J, Karimi M, D'Hauw M, Mares G, Van Montagu M, Gerats T (2001) Petunia *Ap2*-like genes and their role in flower and seed development. Plant Cell 13:229-244

Messmer MM, Keller M, Zanetti S, Keller B (1999) Genetic linkage map of a wheat × spelt cross. Theor Appl Genet 98:1163-1170

Muramatsu M (1963) Dosage effect of the spelta gene *q* of hexaploid wheat. Genetics 48:469-482

Neff MM, Turk E, Kalishman M (2002) Web-based primer design for single nucleotide polymorphism analysis. Trends Genet 18:613-615

Ohto M, Fischer RL, Goldberg RB, Nakamura K, Harada JJ (2005) Control of seed mass by *APETALA2*. Proc Natl Acad Sci USA 102:3123-3128

Peleg Z, Fahima T, Korol AB, Abbo S, Saranga Y (2011) Genetic analysis of wheat domestication and evolution under domestication. J Exp Bot 62:5051-5061

Peng J, Ronin Y, Fahima T, Röder MS, Li Y, Nevo E, Korol A (2003) Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. Proc Natl Acad Sci USA 100:2489-2494

Salamini F, Özkan H, Brandolini A, Schäfer-Pregl R, Martin W (2002) Genetics and geography of wild cereal domestication in the Near East. Nat Rev Genet 3:429-441

Sharma HC, Waines JG (1980) Inheritance of tough rachis in crosses of *Triticum monococcum* and *T. boeoticum*. J Hered 71:214-216

Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yano M (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat Genet 40:1023-1028

Simonetti MC, Bellomo MP, Laghetti G, Perrino P, Simeone R, Blanco A (1999) Quantitative trait loci influencing free-threshing habit in tetraploid wheats. Genet Resour Crop Evol 46:267-271

Simons KJ, Fellers JP, Trick HN, Zhang Z, Tai YS, Gill BS, Faris JD (2006) Molecular characterization of the major wheat domestication gene *Q*. Genetics 172:547-555

Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39:623-630

Taenzler B, Esposti RF, Vaccino P, Brandolini A, Effgen S, Heun M, SchÄFer-Pregl R, Borghi B, Salamini F (2002) Molecular linkage map of Einkorn wheat: mapping of storage-protein and soft-glume genes and bread-making quality QTLs. Genet Res 80:131-143

Van Ooijen JW (2006) JoinMap® 4, software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen

Van Ooijen JW (2009) MapQTL® 6, software for the mapping of quantitative traits loci in experimental populations of diploid species. Kyazma BV, Wageningen

Watanabe N, Sugiyama K, Yamagishi Y, Sakata Y (2002) Comparative telosomic mapping of homoeologous genes for brittle rachis in tetraploid and hexaploid wheats. Hereditas 137:180-185

Xie Q, Mayes S, Sparkes DL (2015) Spelt as a genetic resource for yield component improvement in bread wheat. Crop Sci 55:2753-2765

Yant L, Mathieu J, Dinh TT, Ott F, Lanz C, Wollmann H, Chen X, Schmid M (2010) Orchestration of the floral transition and floral development in *Arabidopsis* by the bifunctional transcription factor APETALA2. Plant Cell 22:2156-2170

Zhang Z, Belcram H, Gornicki P, Charles M, Just J, Huneau C, Magdelenat G, Couloux A, Samain S, Gill BS, Rasmussen JB, Barbe V, Faris JD, Chalhoub B (2011) Duplication and partitioning in evolution and function of homoeologous *Q* loci governing domestication characters in polyploid wheat. Proc Natl Acad Sci USA 108:18737-18742

Zhou Y, Lu D, Li C, Luo J, Zhu B, Zhu J, Shangguan Y, Wang Z, Sang T, Zhou B, Han B (2012)

Genetic control of seed shattering in rice by the APETALA2 transcription factor *SHATTERING ABORTION1*. Plant Cell 24:1034-1048

Figure captions

Fig. 1 Frequency distributions of the yield and associated traits measured in the recombinant inbred line population of Forno (F) × Oberkulmer (O). For each trait, the data is the best linear unbiased estimate over replicates and environments

Fig. 2 The genetic map of chromosome 5A and QTL mapping for the spike and yield traits measured in the recombinant inbred line population of Forno × Oberkulmer. The hollow bar represents the chromosome 5A, with the molecular markers on the right and their locations on the left (cM). The filled vertical bars on the right of the chromosome indicate the 2–LOD support intervals of the significant QTLs, followed by their names (see Table 1 for details)

Fig. 3 Spike and yield trait performance of the CS and its *Q* mutant lines (mq36, mq125, mq194 and fndel143). The values represent means ± SD, and the asterisks indicate significant differences between the CS and mutant lines (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$)

Fig. 1

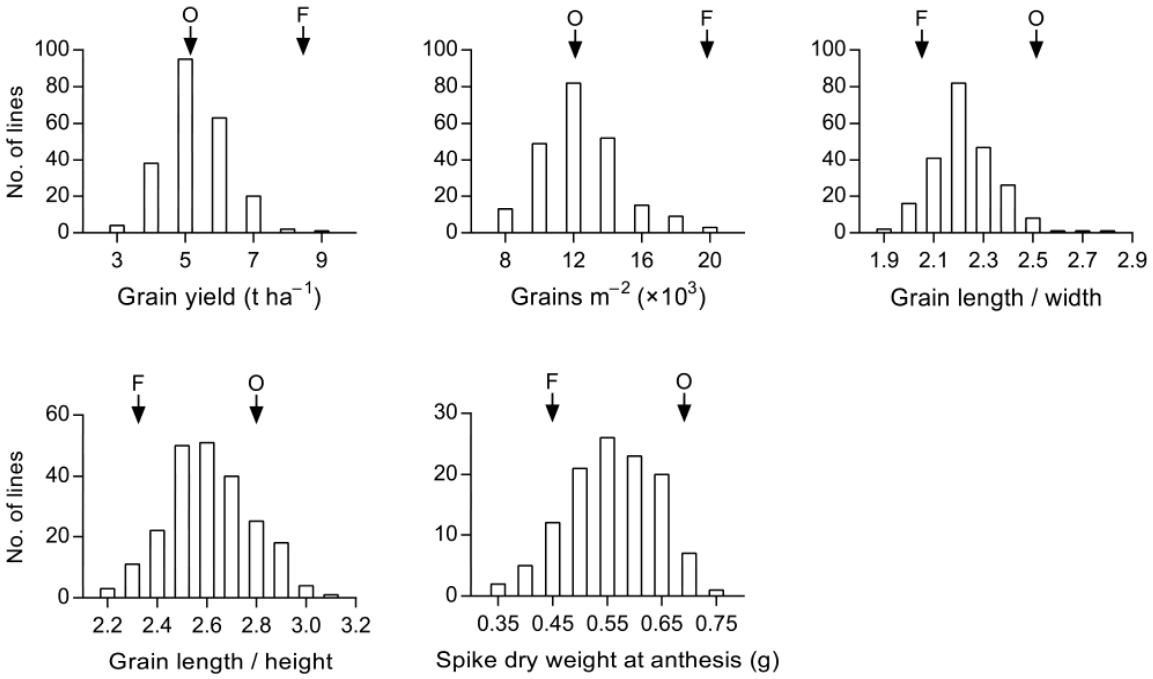

No. of lines
Grain yield (t ha^{-1})
Grains m^{-2} (×10^3)
Grain length / width
Grain length / height
Spike dry weight at anthesis (g)
O
F

Fig. 2

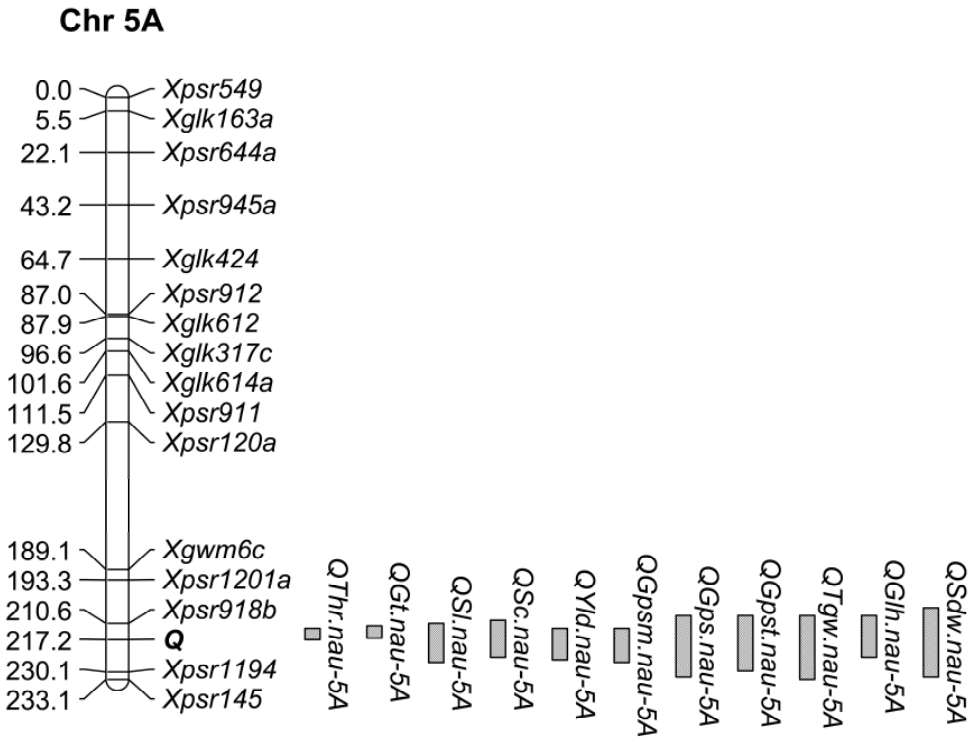
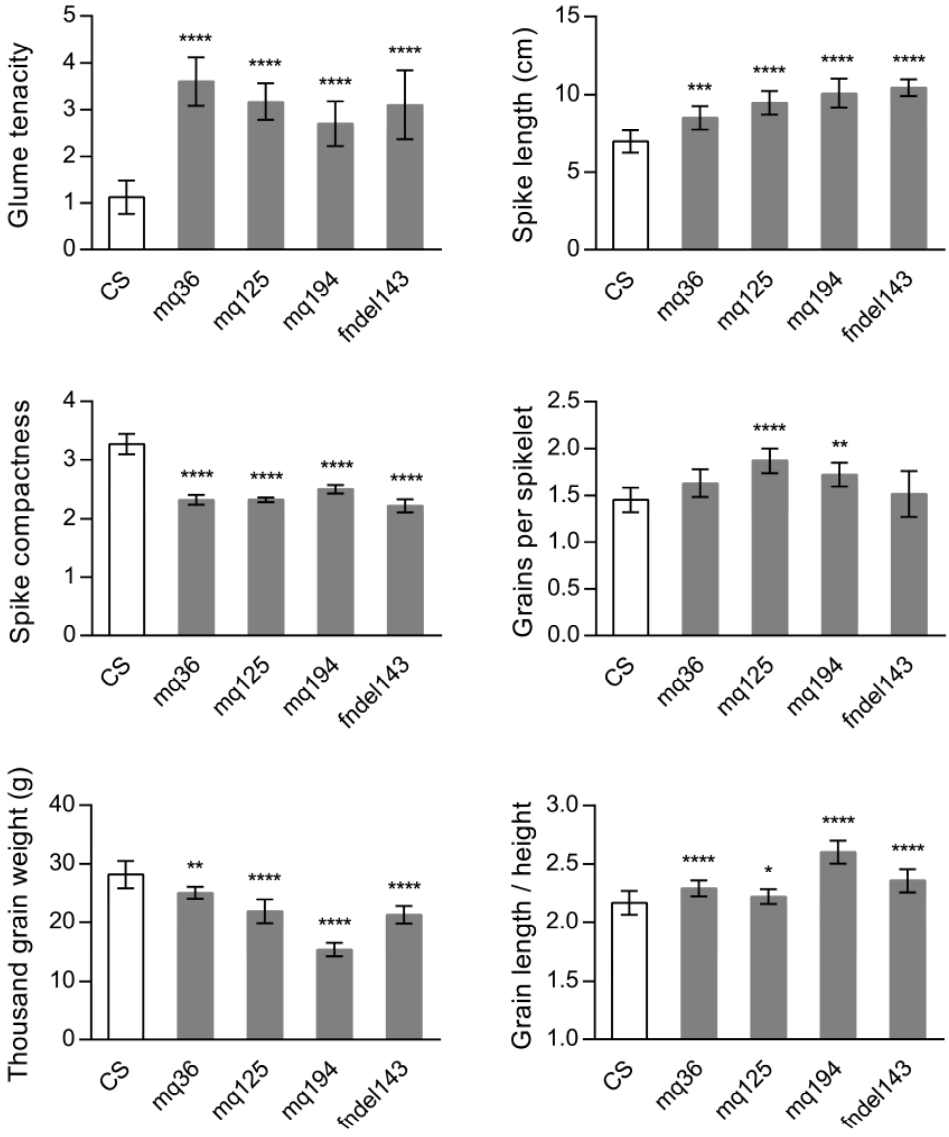

Chr 5A
0.0 Xpsr549
5.5 Xglk163a
22.1 Xpsr644a
43.2 Xpsr945a
64.7 Xglk424
87.0 Xpsr912
87.9 Xglk612
96.6 Xglk317c
101.6 Xglk614a
111.5 Xpsr911
129.8 Xpsr120a
189.1 Xgwm6c
193.3 Xpsr1201a
210.6 Xpsr918b
217.2 Q
230.1 Xpsr1194
233.1 Xpsr145
QThr.nau-5A
QGt.nau-5A
QSl.nau-5A
QSc.nau-5A
QYld.nau-5A
QGpsm.nau-5A
QGps.nau-5A
QGpst.nau-5A
QTgw.nau-5A
QGlh.nau-5A
QSdw.nau-5A

Fig. 3


Glume tenacity
Spike length (cm)
Spike compactness
Grains per spikelet
Thousand grain weight (g)
Grain length / height
CS
mq36
mq125
mq194
fndel143

Tables

Table 1 QTL mapping for the *Q* gene interval in the Forno × Oberkulmer RIL population

Trait / QTL	LOD	Support interval (cM) [a]	Flanking marker	R^2 (%) [b]	Additive effect [c]
Threshability (%)					
QThr.nau-5A	40.9	212.6–217.3	*Xpsr918b–Q*	61.0	19.5
Glume tenacity (score: 1–5)					
QGt.nau-5A	34.9	211.6–217.3	*Xpsr918b–Q*	75.9	–1.0
Spike length (cm)					
QSl.nau-5A	16.3	210.6–226.2	*Xpsr918b–Q*	48.5	–1.8
Spike compactness (spikelets cm^{-1})					
QSc.nau-5A	19.3	209.3–224.2	*Xpsr918b–Q*	54.4	0.3
Grain yield (t ha^{-1})					
QYld.nau-5A	10.7	212.6–225.2	*Q–Xpsr1194*	21.9	0.50
Grains m^{-2}					
QGpsm.nau-5A	7.4	212.6–226.2	*Q–Xpsr1194*	15.6	1035
Grains per spike					
QGps.nau-5A	4.6	207.3–232.1	*Xpsr918b–Q*	17.0	–2.7
Grains per spikelet					
QGpst.nau-5A	6.4	207.3–229.6	*Xpsr918b–Q*	22.8	–0.14
Thousand grain weight (g)					
QTgw.nau-5A	4.1	207.3–233.1	*Q–Xpsr1194*	9.1	1.28
Grain length / height					
QGlh.nau-5A	5.3	207.3–224.2	*Xpsr918b–Q*	11.5	–0.07
Spike dry weight at anthesis (g)					
QSdw.nau-5A	4.2	204.3–232.1	*Xpsr918b–Q*	15.6	–0.04

[a]QTL support interval is indicated by the 2–LOD (the QTL maximum LOD score minus 2).

[b]Phenotypic variation explained by the QTL.

[c]Positive and negative effects indicate that the alleles from Forno and Oberkulmer increased the values of the traits, respectively.

Table 2 Allelic effects of the *Q* gene on the spike and yield traits in the Forno × Oberkulmer mapping population

Trait [a]	*q* allele		*Q* allele		Difference	*P* value
	No. of lines	Mean	No. of lines	Mean		
Threshability (%)	126	54.5	60	91.4	36.9	< 0.001
Glume tenacity (score: 1–5)	65	3.6	31	1.8	–1.8	< 0.001
Spike length (cm)	65	14.2	31	10.7	–3.5	< 0.001
Spike compactness (spikelets cm^{-1})	65	1.6	31	2.1	0.5	< 0.001
Grain yield (t ha^{-1})	126	5.49	60	6.50	1.01	< 0.001
Grains m^{-2}	126	12767	60	15194	2427	< 0.001
Grains per spike	65	39.6	31	36.0	–3.6	< 0.01
Grains per spikelet	65	1.85	31	1.67	–0.18	< 0.001
Thousand grain weight (g)	117	44.9	50	46.8	1.9	< 0.001
Grain length / height	127	2.67	60	2.52	–0.15	< 0.001
Spike dry weight at anthesis (g)	65	0.60	31	0.53	–0.07	< 0.001

[a] Part of the RILs were excluded for analysis because of failure to amplification for *Q* or the high degree of heterozygous/non-parental bands at other loci in genome (Messmer et al. 1999).