The Genetic Basis of Natural Variation in Seed Size and Seed Number and Their Trade-Off Using *Arabidopsis thaliana* MAGIC Lines

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ABSTRACT Offspring number and size are key traits determining an individual's fitness and a crop's yield. Yet, extensive natural variation within species is observed for these traits. Such variation is typically explained by trade-offs between fecundity and quality, for which an optimal solution is environmentally dependent. Understanding the genetic basis of seed size and number, as well as any possible genetic constraints preventing the maximization of both, is crucial from both an evolutionary and applied perspective. We investigated the genetic basis of natural variation in seed size and number using a set of *Arabidopsis thaliana* multiparent advanced generation intercross (MAGIC) lines. We also tested whether life history affects seed size, number, and their trade-off. We found that both seed size and seed number are affected by a large number of mostly nonoverlapping QTL, suggesting that seed size and seed number can evolve independently. The allele that increases seed size at most identified QTL is from the same natural accession, indicating past occurrence of directional selection for seed size. Although a significant trade-off between seed size and number is observed, its expression depends on life-history characteristics, and generally explains little variance. We conclude that the trade-off between seed size and number might have a minor role in explaining the maintenance of variation in seed size and number, and that seed size could be a valid target for selection.

THE reproductive output of an organism is a critical life-history trait defining its fitness and is the result of both offspring number and quality. In the case of cereal crops, the number and size of seeds are also the main constituents of yield. Thus, understanding the genetic architecture of seed size and number, and any possible genetic constraints to maximizing them, is crucial from both an evolutionary and applied perspective (Sadras 2007; Van Daele *et al.* 2012; Kesavan *et al.* 2013). Despite its importance, the genetic basis of natural variation in seed size and number and their interaction with life-history traits remain poorly understood.

Previous studies on the genetic basis of seed traits have predominantly used mutant screens and identified genes in key pathways involved in seed development (Garcia *et al.* 2003; Tzafrir *et al.* 2004; Adamski *et al.* 2009; Fang *et al.* 2012). However, since this approach only allows for the comparison of phenotypic effects of genes that are "on" or "off" (Koornneef *et al.* 2004), genes' contribution to natural continuous variation in seed size or seed number remain largely uncharacterized. Because the effects of mutants are often dependent on the genetic background (Tonsor *et al.* 2005; Chou *et al.* 2011), a QTL mapping approach using multiple parents is ideal to identify genetic factors that can contribute to natural variation in these traits in a heterogeneous genetic background.

Identification of genetic factors affecting seed traits is further complicated by potential trade-offs between them. Life-history theory suggests that if there are finite resources to be invested in reproduction, a trade-off between seed size and seed number must occur (Venable 1992). Although the seed size/number trade-off is well accepted on theoretical grounds, empirical evidence for its existence is still contentious and dependent upon the context under which it is evaluated (Venable 1992; Sadras 2007; Paul-Victor and Turnbull 2009; House *et al.* 2010). One possible explanation for context dependency in trade-offs is that the resources available for reproduction are not discrete from the whole plant budget. With many competing allocations within the organism,

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trade-offs may arise not just between seed size and seed number, but also with other competing sources such as defense against biotic and abiotic stress (Bazzaz *et al.* 1987; Züst *et al.* 2011; Wituszyńska *et al.* 2013). Alternatively, the expression of the trade-off might be dependent on the level of resources available (Noordwijk and Jong 1986; Venable 1992; Bennett *et al.* 2012). Variation in life-history is common within populations; and typically, later flowering plants are larger and have more resources to invest in reproduction, reducing the expression of trade-offs (Aarssen and Clauss 1992; Clauss and Aarssen 1994; Jakobsson and Eriksson 2000; Mendez-Vigo *et al.* 2013). Thus, variation in seed size might be due to genetic factors with direct effects on seed size, or genetic factors with indirect effects, such as through resource uptake or life-history determinants, as well as nongenetic variation. A better understanding of natural variation in seed size therefore requires simultaneous consideration of genetic variation and life-history strategies.

It is important to determine the existence and mechanism behind trade-offs, because environmentally caused trade-offs can be modified by selection or genetic manipulation. However, for trade-offs that result from genetic pleiotropy or linkage disequilibrium, responses to selection will be constrained (Lande and Arnold 1983; Roff and Fairbairn 2007; Latta and Gardner 2009). In addition, the assumption of a trade-off between seed size and seed number has shaped breeding practices (Egli 1998; Sadras 2007): Seed number has been the main target for crop improvement because it is more variable than seed size (Harper *et al.* 1970; Venable 1992; Sadras 2007; Sadras and Egli 2008). However, if seed size shows less environmental variation and higher heritability than seed number, seed size might be a useful target for genetic crop improvement (Sadras and Slafer 2012), but only if the trade-off can be teased apart.

Here, we investigate the genetic basis of natural variation in seed size and its correlation with seed number using a set of recombinant inbred lines of *A. thaliana*, derived from MAGIC lines (Kover *et al.* 2009b). *A. thaliana* is an ideal model organism for the study of natural variation in seed size and number, because there is extensive variation among worldwide accessions for both of these traits and for many life-history traits (Krannitz *et al.* 1991; Alonso-Blanco *et al.* 1999; Kover *et al.* 2009b). Few studies have addressed the issue of QTL for seed size and number, taking into account other life-history traits (Alonso-Blanco *et al.* 1999; Van Daele *et al.* 2012), and these only used mapping lines created from two parents. Multiparental lines are better for addressing genetic correlations and possible trade-offs than traditional mapping lines, due to the larger number of alleles and recombination events. This allows mapping to smaller intervals (Kover *et al.* 2009b), reducing overlap in positions due to large confidence intervals. In addition, the larger number of alleles improves our ability to determine whether the distributions of allelic effects are compatible with pleiotropy. With only two alleles present, the same allele may increase the value of any two traits 50% of the time by chance alone. With multiple alleles, a significant correlation in allelic effects provides stronger evidence of a common genetic mechanism. Specifically, the following questions are addressed: (i) What is the genetic architecture underlying seed size and seed number per fruit? (ii) Is there evidence for a genetically determined seed size/number trade-off? (iii) How are seed traits and the seed size/number trade-off affected by life-history traits?

Materials and Methods

Plant material and growth conditions

The set of MAGIC lines used here was produced by advanced intermating of 19 parental accessions of *A. thaliana* for four generations, followed by seven generations of inbreeding (Kover *et al.* 2009b). These lines have been genotyped for 1260 single nucleotide polymorphisms (SNPs), distributed throughout the five chromosomes (Chr) at a spacing of ~96 kb apart, using an Illumina Golden Gate assay (Kover *et al.* 2009b). We have previously shown that these lines allow for QTL mapping with high resolution to chromosomal intervals smaller than 1 Mb (Kover *et al.* 2009b). The genotypes for all MAGIC lines are listed in Supporting Information, File S1.

Three replicates of each of 700 MAGIC lines were grown in the autumn of 2009 at the University of Bath greenhouse. The greenhouse was set at 21° day/18° night and 16/8 hr of light/dark. Seeds from each line were placed in three separate 5.5-cm diameter pots filled with F2+Sand Soil (Levington, The Scotts Company) and randomly allocated to trays that held 24 pots. Trays were rotated around the greenhouse at regular intervals to ensure uniform growth conditions and mitigate positional effects.

Phenotyping

All pots were monitored daily, and seed germination and flowering (appearance of flowering buds) day were recorded. Plants were grown until senescence, when the total number of branches expanded was counted and the inflorescence height recorded. Seed number per fruit was estimated by fruit length and seed counts. Seed size was estimated by digital collection of seed area and by weight. Three fruits (between the 6th and 10th fruit on the main stem) were collected per plant, after senescence. Fruits were dissected under a microscope, and images of fruits and seeds were captured with a Nikon Digital Sight DS-U1 camera. Fruit length (in millimeters) and seed area (in square millimeters) were estimated from these images using the "measure" function in ImageJ v.1.44p (National Institutes of Health; http://rsbweb.nih.gov/ij/). Seed number was counted from the images captured, using Windows Paint software and a hand tally. Average seed weight (in micrograms) was determined by weighing all the nonaborted seeds from all three fruits on a Mettler UMT2 Ultra Microbalance, and dividing by total number of seeds weighed. The number of aborted seeds (*i.e.*, seeds that can be seen but were not completely filled) per fruit were recorded as observed. All phenotypic data are listed in File S2.

Statistical analyses

Broad sense heritability (H^2) for each trait was estimated as the ratio of the variance among lines to the total variance. To determine genetic correlations between traits, pairwise Pearson correlations between line means were calculated. To determine whether flowering time affects the trade-off between seed size and seed number, we calculated the correlation between seed size and number separately for the 100 and 200 earliest and latest MAGIC lines. To determine the proportion of variation in seed weight or in seed number explained by each trait measured after model selection, we tested the following multiple linear regression model using either the data for average seed weight or seed number per fruit: seed weight (or number) = $\beta_{\text{intercept}} + \beta_{\text{seed weight (or number)}} + \beta_{\text{height}} + \beta_{\text{aborted}} + \beta_{\text{nodes}} + \beta_{\text{total branches}} + \beta_{\text{flowering}} + \varepsilon$. The bootStepAIC package for R was run in both directions for model selection ($\alpha = 0.05$; bootstrap resampling $\times 1000$).

We also estimated genetic (V_g) and environmental variances (V_e) for seed weight and total number of seeds per fruit by running a one-way ANOVA with MAGIC line as a random factor, using the mixed procedure in SAS (which uses REML to fit the model). These variances were used to calculate the genetic and environmental coefficient of variation (CV_g and CV_e), allowing for the comparison of the genetic and environmental variances across traits by scaling the variances to the mean ($CV_{g \text{ or } e} = 100\ \sqrt{V_g \text{ or } V_e}$ /trait mean).

QTL analyses were performed using the R software package HAPPY as described in Kover *et al.* (2009b). Briefly, this approach uses a hidden Markov model to make a multipoint probabilistic reconstruction of the genome of each MAGIC line as a mosaic of the founder haplotypes. Thus, at each marker, a probability of being derived from each of the parental accessions is assigned for each line, and the hypothesis that there is no QTL is evaluated by fitting a fixed-effect linear model with up to 18 degrees of freedom (d.f.). We performed QTL analysis for the line average of seed weight (in micrograms), and seed number per fruit and fruit length (in milligrams). Overlapping QTL (*i.e.*, QTL located <1 Mb away) for seed size and number would suggest that the trade-off is due to genetic pleiotropy. Concordance of allelic values was tested with Spearman rank correlation.

Results

Phenotypic variation

Extensive phenotypic variation was observed for all traits measured among the MAGIC lines (Table 1), including an approximately threefold variation in both mean seed size and seed number. While the coefficient of genetic variation (CV_g) is slightly larger for seed number than weight (0.13 *vs.* 0.15, respectively), the coefficient of environmental variation CV_e is much larger for seed number than weight (0.18 *vs.* 0.09, respectively).

Seed weight and seed area, our two estimates of seed size, are strongly correlated ($r = 0.838$; $P < 0.0005$); and, since seed weight has higher heritability (Table 1), we use seed weight as a proxy for seed size henceforth. The proportion of aborted seeds per fruit showed relatively low heritability and the majority of fruits contained very few aborted seeds ($<1\%$ of the total seeds, Table 1). Thus, total seed number per fruit and the number of healthy seeds per fruit displayed very similar variation, and only total seed number is used henceforth.

Genetic correlations: A number of significant pairwise correlations among the traits measured were observed (Table 2). While, a significant negative correlation was observed between seed size and number this is not the largest correlation among all traits measured, suggesting it is just one of many trade-offs. In addition, the low r^2 value of the correlation between seed size and number ($r^2 = 0.06$) indicates that variation in one trait explains a very small proportion of the variation in the other.

Given the extensive correlation among all traits, we used a multiple linear regression model to simultaneously consider the effect of the different life-history traits recorded on seed weight and seed number. The best fit model for seed weight ($F = 21.95$, $P < 0.0005$, d.f. = 494, $r^2 = 0.182$) included: plant height, seed number per fruit, percentage of aborted seeds, and the total number of branches. In a model with all these variables included, they explain 7.89, 7.08, 1.74, and 1.08% of the variation in seed weight, respectively (according with their partial r^2 estimates). Similarly, seed weight, flowering time, percentage of aborted seeds, plant height, and the total number of branches explain 7.01, 6.97, 6.02, 1.86, and 1.13% of the variation in seed number per fruit, respectively ($F = 29.51$, $P < 0.0005$, d.f. = 494, $r^2 = 0.230$). Thus, life-history traits can explain some of the variation in seed size and weight, but the variance explained is smaller than the heritability.

Flowering time correlates with seed number per fruit (with late flowering plants producing fewer seeds per fruit than early flowering lines), but not seed weight (Table 2). To determine if the trade-off between seed size and number is affected by time to flowering, we calculated the correlation between seed size and number separately for the first 100 and 200 lines to flower as well as the last 200 and 100 lines (Table 3). This comparison indicates that the trade-off is only significant among the early flowering lines. These results suggest that the seed size/number trade-off is enhanced by the limited resources caused by earlier reproduction.

QTL mapping: The QTL analysis for seed weight identified 8 QTL located on chromosomes 1, 3, 4, and 5 (Table 4). The largest QTL for seed size is located on chromosome 1 ($\sim$21.6 Mb) and explains 15% of the variation. For the average seed number per fruit, nine QTL were observed, also distributed across chromosomes 1, 3, 4, and 5. The most significant QTL were located at the top of chromosome 4 ($\sim$0.24 Mb) and the bottom of chromosome 5 ($\sim$21.0 Mb), explaining 9 and 8% of the phenotypic variation in this trait, respectively (Table 4). The results for the QTL analysis for average healthy seed number can be seen in Table S1, which shows qualitatively the same results.

Table 1 Phenotypic variation among MAGIC lines for all traits measured

Trait	Min	Max	Mean ± SD	H^2
Days to flowering	12.3	117.0	23.5 ± 9.9	0.92
Total no. of branches	1.5	12.7	5.8 ± 1.6	0.42
Inflorescence height	9.0	68.8	41.8 ± 9.2	0.62
Seed weight (μg)	11.8	37.7	22.2 ± 3.1	0.63
Seed area (mm^2)	0.4	1.1	0.74 ± 0.7	0.47
Total seeds per fruit	27.0	79.2	52.5 ± 9.5	0.43
% seeds aborted	0	37	0.9 ± 2.7	0.18
Fruit length (mm)	8.8	20.0	15.0 ± 1.8	0.22

Minimum (Min), maximum (Max) phenotypic values for each trait, as well as the phenotypic mean plus or minus their standard deviation (SD) and their broad-sense heritability (H^2) are shown.

Table 2 Pairwise Pearson's correlations between traits measured

Trait	Flowering	Branches	Height	Seed weight	Fruit length
Branches	0.198**				
Height	−0.304**	−0.138*			
Seed weight	0.018	−0.058	0.265**		
Fruit length	−0.102	−0.094	0.365 **	0.041	
Seed number	−0.311**	−0.143**	0.152**	−0.251**	0.506**

Using average MAGIC line values (correlation is significant at *P = 0.003 level, which is the Bonferroni corrected level equivalent to P = 0.05; and correlation is significant at the **P = 0.001 level).

There is little overlap between QTL for seed size and seed number (Table 4, Figure S2). All QTL for seed size are located >1 Mb away from a QTL for seed number, suggesting that these traits are determined by independent genetic factors. The one exception is the seed size QTL on chromosome 3 (~18.5 Mb), which is only 400 kb away from a seed number QTL (~18.9 Mb). Comparison of the distribution of allelic effects at this QTL (Table 5, Table S3) suggests a similar, but not identical distribution of effects. The Bur-0 allele at this location causes the largest seed and the smallest number of seed per fruit. In addition, there is a significant correlation in allelic effects (rho = −0.52, P = 0.02). Thus, it is possible that the same genetic factor is affecting both traits in a pleiotropic manner. However, this QTL does not explain much variation (5% of seed weight and 7% of fruit number).

The estimated value of each of the 19 haplotypes (Table 5) also shows that, for six of the eight seed size QTL identified, the allele conferring the largest seed size is from the Bur-0 accession. At the other two QTL, the Bur-0 allele leads to the second largest seed size. In contrast, the alleles causing the largest or smaller number of seeds per fruit are from a number of different accessions (*i.e.*, there is no clear pattern that the allele from Bur-0 causes the smallest number of seeds per fruit at most QTL; Table S3).

When all lines are included in the QTL analysis, there is a strong QTL for fruit length on chromosome 2 (~11 Mb), which is nonoverlapping with QTL for seed number. This QTL is likely due to the mutation *ERECTA*, which is known to affect fruit length and is due to the allele from the Ler accession. Reanalysis of QTL for fruit length after removal of the lines with the erecta phenotype, reveal five smaller QTL on chromosomes 1, 2, and 5 (Table S2). One of these overlaps with fruit number (Chr 4, 16.5 Mb). At this location, there is a significant correlation between the average allelic values of the 19 parental accessions for the two traits (rho = 0.613, P = 0.05), suggesting a common genetic basis to these traits at this locus.

Discussion

Our study finds that both seed number and seed weight are genetically variable among natural accessions of *A. thaliana*. Both traits are affected by a large number of QTL, but there is little evidence for overlap in their genetic architecture.

Only a few studies have previously performed QTL analysis specifically for seed size or seed number per fruit (Alonso-Blanco *et al.* 1999; Herridge *et al.* 2011; Van Daele *et al.* 2012; Moore *et al.* 2013). In these four studies, recombinant inbred lines (RILs) derived from two accessions were used, and typically each QTL explained <15% of the variation (as with our study). Alonso-Blanco *et al.* (1999) found 10 QTL for seed weight and 4 QTL for seed number/fruit using 162 RILs derived from the accessions Cvi and Ler. When Van Daele *et al.* (2012) and Moore *et al.* (2013) used the same lines, they found only 8 QTL for seed size, but they used seed area instead of seed weight. Herridge *et al.* (2011) used two set of RILs: one derived from Bur and Col where they found 4 QTL for seed size, and the other derived from Cvi and Col, for which they identified 5 QTL. It has been suggested that power issues reduce the number of QTL observable with multiparent mapping lines (Keurentjes *et al.* 2011). There were, for example, fewer QTL observed for flowering time using multiparent populations of *A. thaliana* (Kover *et al.* 2009b; Huang *et al.* 2011) than in studies that used mapping populations from intercrosses of two accessions. However, in this study the number of QTL identified (8 QTL for seed size and 9 QTL for seed number) is comparable to the other QTL studies. In only one mapping line, in one of the studies, a larger number of QTL was found for seed weight. This raises interesting questions about whether there is some implicit power reduction for detecting QTL in multiparent populations due to the large number of alleles, or whether this a trait-specific issue. While more multiparent mapping studies are needed before we can better determine if there is a problem, it is possible that traits where most of the genetic architecture is additive will not show a reduction in the number of QTL identified, while other traits that include many loci with genetic background-dependent effects will show a reduction in the number of QTL identified using multiparent lines. This may not necessarily be a disadvantageous feature of MAGIC populations if it allows the identification of QTL with consistent effects over a diverse set of complex backgrounds. It has also been suggested that with the increased number of recombinations, there could be a breakage of small QTL that were previously linked, reducing the ability to detect them (Huang *et al.* 2010). Such an effect has not been seen

Table 3 Mean for seed weight and seed number, as well as their correlation for MAGIC lines grouped by flowering time

Flowering lines	Seed weight	Seed number	Correlation
100 earliest flowering lines	21.1	50.1	−0.48***
200 earliest flowering lines	21.1	53.2	−0.35***
200 latest flowering lines	22.9	48.5	−0.13
100 latest flowering lines	21.8	45.7	−0.17

*** indicates significance with $p < 0.05$.

here, given that the number and the size of the effects are comparable to QTL identified using only two parental accessions. Nevertheless, it may explain the reduction in detected QTL for other traits not included in this study.

So far, only a few genes have been identified to be involved in determining seed size, and genes that explain natural variation on seed size or number remain unknown (Herridge *et al.* 2011; Van Daele *et al.* 2012; Moore *et al.* 2013). The QTL on the bottom of chromosome 1 is a good candidate for further fine mapping of a genetic factor that affects quantitative variation in seed size, since it explains a reasonable amount of variation (15%). At this QTL, the Bur-0 allele was found to be associated with larger seed size. Thus, to identify possible candidate genes, we searched for genes containing nonsynonymous SNPs unique to this accession. Based on the resenquencing and reannotation of the 19 parental accessions (Gan *et al.* 2011), we identified two strong candidate genes located <250 kb from the largest seed size QTL on chromosome 1: *AAP1* (AT1G58360) and *KLUH* (AT1G13710). Both of these genes contain nonsynonymous substitution unique to Bur-0 and have been previously identified through mutation studies to affect seed size (Adamski *et al.* 2009; Sanders *et al.* 2009). Candidate genes for the QTL on chromosomes 4 and 5 were identified by searching for genes previously identified to affect seed or ovule number. *JAGGED LATERAL ORGANS* (AT4G00220), *YABBY 3* (AT4G00180), and *BEL1* (AT5G41410) (Nole-Wilson and Krizek 2006; Borghi *et al.* 2007; Brambilla *et al.* 2007) are good candidates based on their close proximity (<250 kb away) to the identified QTL.

The existence of such extensive, within-species, genetic variation in seed size is puzzling because life-history theory would predict selection for an optimal seed size that best resolves the trade-off between seed size and number (Smith and Fretwell 1974; Halpern 2005). Given the complex genetic architecture of seed size, it is possible that balancing selection could maintain some of the genetic variation (Turelli and Barton 2004). It is also possible that divergent environmental selection at sites where the parental accessions were originally collected may explain the observed variation (Mackay 1981). Orr (1998) suggested that selection could be inferred from the direction of QTL effects being nonrandomly distributed between parental accessions. Although his sign test was proposed for QTL studies using intercross between two accessions, its logic can be equally applied to multiparent populations. We argue that there is a very small chance of observing that all alleles that produce large seeds come from the same accession (Bur), when there are 19 parental accessions, if seed size variation was neutral.

Table 4 Significant QTL detected for average seed weight, total number of seeds per fruit, and fruit length

Chr	Peak (kb)	−log*P*	Genome-wide *P*	r^2
Seed weight (μg)				
1	4,569	5.97	<0.01	0.09
1	21,669	13.05	<0.01	0.15
3	18,903	4.42	0.02	0.05
4	10,777	9.29	<0.01	0.09
4	16,702	5.92	<0.01	0.07
5	4,149	5.29	<0.01	0.08
5	20,022	4.31	0.02	0.06
5	26,708	5.48	<0.01	0.03
Seed number/fruit				
1	20,175	4.48	0.01	0.06
1	24,795	3.89	0.02	0.04
3	15,233	4.41	0.01	0.05
3	18,512	4.76	0.01	0.07
4	269	5.53	<0.01	0.09
4	5,290	4.34	0.01	0.06
4	7,177	5.44	<0.01	0.07
5	16,446	4.07	0.02	0.03
5	21,039	5.37	<0.01	0.08
Fruit length (mm)				
2	11,207	23.5	<0.01	0.21
5	17,597	3.8	0.03	0.06
5	21,039	5.1	0.01	0.07

"Chr" indicates the chromosome location, and "Peak (kb)" the position in the chromosome of the QTL peak in kilobases. Statistical significance of each QTL is indicated by log*P* and Genome-wide *P*. r^2 indicates the amount of variation explained by the QTL.

Thus, we propose that the large seed size observed in the Bur accession is due to directional selection, and that at least some of the variation in seed size within *A. thaliana* is due to adaptive processes.

Fruit length is sometimes used as a proxy for seed number and for estimates of fitness in *A. thaliana* (*e.g.*, Brachi *et al.* 2012). Here, we find that although there is a significant correlation between fruit length and seed number (Table 2), it is far from perfect. Although there is overlap for one QTL for fruit length and seed number, this is not a particularly strong QTL. It is possible that larger fruits are due to more seeds or larger seeds. Thus, caution must be exercised when using fruit length as a proxy for seed number. This is particularly inappropriate when the study includes the accession Ler, which contains the mutation *ERECTA* (Torii *et al.* 1996). This mutation shortens the fruit length and the plant height, reducing the correlation between fruit length and seed number, as seen when comparing Table 2 to Table S2 (which shows the genetic correlations for nonerecta lines). Recent studies suggest that seed area can be used as a proxy for seed size to automate phenotyping (Herridge *et al.* 2011; Van Daele *et al.* 2012). While we find that actual seed weight shows higher heritability than seed area, the correlation is high enough to make a suitable substitute, since pictures make the phenotyping significantly more efficient.

While a significant negative correlation is observed between seed size and seed number, the overall variance explained by this correlation is relatively small. Relative to other life-history

Table 5 Estimated value for each of the 19 parental alleles on seed size (μg), seed number per fruit, and fruit length at each detected QTL

		Parental accession																		
Chr	Mb	Bur-0	Can-0	Col-0	Ct-1	Edi-0	Hi-0	Kn-0	Ler-0	Mt-0	No-0	Oy-0	Po-0	Rsch-4	Sf-2	Tsu-0	Wil-2	Ws-0	Wu-0	Zu-0
Seed size																				
1	4.5	24.7	22.3	20.8	21.2	22.7	21.5	22.5	22.4	22.4	22	22.7	22.6	22.1	21.9	22.2	21.7	**20.4**	22.4	23.2
	21.7	24.7	22.5	22.6	23.4	24.3	22.1	21.4	20.5	22.4	21.9	21.8	23.3	22.1	21.4	22.6	22.5	**19.5**	21.7	22.9
3	18.9	23.4	**20.5**	21.7	22.6	22.2	22.5	21.6	21.7	22.6	21.2	22.9	22.7	22.6	21.9	21.9	21.7	22.5	22.2	22.7
4	10.8	25.4	22.8	22	22.2	21.9	22	**20.5**	22	22.3	22.1	22.6	22.5	21.4	21.6	22.2	21.4	22.2	21.9	22.2
	16.7	25.1	22.4	22.9	22.2	22.4	22.1	**21.1**	21.9	21.7	21.8	21.9	22.1	21.7	21.5	22.3	21.6	22.4	21.3	22.3
5	4.1	23.7	21.7	22.4	23.1	22.2	21.9	21.8	21.9	22.2	21.3	22.2	24.5	21.8	23.5	**21.2**	22.1	21.4	21.6	21.9
	20.0	23.2	22.4	21.8	22.1	22.7	21.2	21.8	21.8	22.5	21.1	**21**	23.5	23	23	22.6	22	21.2	21.7	22.5
	26.7	23.6	22.5	21.7	22.3	22.6	21.6	21.5	21.9	21.8	21.4	22.5	22.5	22.4	22.1	22	21.7	**21.4**	22.2	22.1
Seed number/fruit																				
1	20.1	**46.8**	51.3	53.8	54.6	49.8	53.7	55.4	55.1	51.0	52.4	51.0	51.3	53.6	53.4	51.6	52.5	49.7	54.4	48.4
1	24.8	52.4	**49.1**	51.4	52.7	52.3	52.9	54.9	55.9	50.4	53.6	53.0	50.9	53.8	52.0	51.7	51.0	49.4	53.7	49.6
3	15.2	50.5	49.9	55.6	51.4	52.0	53.7	54.1	53.1	53.3	55.8	50.8	50.7	50.9	55.4	49.1	53.3	49.1	52.8	**48.4**
3	18.5	**47.1**	51.1	55.8	52.2	52.0	54.6	53.1	53.3	52.7	57.9	51.5	51.3	51.0	54.4	51.1	51.6	48.7	53.7	48.2
4	0.3	48.0	46.5	53.1	54.4	49.0	53.0	52.4	53.7	53.7	52.3	51.2	52.7	56.1	50.8	54.9	51.6	**46.1**	52.7	48.0
4	5.3	50.6	48.8	55.5	54.0	48.8	52.2	53.5	54.2	54.1	53.5	50.3	49.5	54.1	52.7	54.8	50.3	50.5	51.0	**48.3**
4	7.2	**46.5**	50.1	55.4	54.6	48.2	52.1	53.2	57.2	53.4	51.5	51.9	50.8	54.2	52.7	53.4	51.1	50.9	51.1	48.0
5	16.5	51.8	51.4	52.6	52.6	51.1	52.9	53.4	55.9	51.5	53.8	50.8	52.4	53.3	49.5	53.5	50.5	**49.0**	50.4	51.1
5	21.0	50.7	51.3	55.4	56.0	48.8	52.6	54.7	52.5	52.0	55.0	53.1	52.9	53.3	**46.3**	54.8	48.1	48.5	52.7	51.3
Fruit length																				
2	11.2	15.05	13.92	15.35	15.20	14.45	15.53	15.23	**12.40**	15.17	14.71	15.48	15.36	15.20	15.07	14.76	15.35	14.75	15.49	15.27
5	17.6	**14.29**	15.05	15.24	15.16	14.71	14.77	14.69	15.66	14.84	14.98	14.95	14.51	15.83	14.42	15.48	14.43	14.38	14.76	15.11
5	21.0	14.17	14.62	15.56	15.28	14.85	14.67	14.98	15.36	14.78	14.71	14.72	14.95	15.79	**14.11**	15.48	14.31	14.38	15.01	15.18

Alleles having the largest effect in increasing and decreasing the trait are underlined and in boldface type, respectively.

trade-offs, the seed size/number trade-off is not very strong. For example, plant height is as strongly correlated with seed size as it is with seed number, and there is a stronger negative correlation between seed number and flowering time (Table 2). In addition, there is little evidence for common genetic regulation for both of these traits. Kover *et al.* (2009a) estimated that causal polymorphism for traits mapped with these MAGIC lines should lie within 200 kb of the peak of the identified QTL. Thus, QTL identified for seed weight and seed number do not overlap (except for one QTL on chromosome 3) and distinct allelic effects are observed at QTL for the two traits (Table 5, Table S3). In light of previous conflicting evidence regarding the presence of the seed size/number trade-off, our data suggest that although significant for this population, the trade-off may not be as important to explain variation in these traits as theoretically predicted. The two other studies that simultaneously mapped seed number and size in *A. thaliana* (Alonso-Blanco *et al.* 1999; Van Daele *et al.* 2012) concluded that both of these traits map to similar locations and could be pleiotropic. However, their confidence intervals were quite large (sometimes encompassing the whole half of a chromosome) and thus difficult to compare with our results.

Previous work has shown that parental resource status (Noordwijk and Jong 1986; Venable 1992; Paul-Victor and Turnbull 2009), plant size (Jakobsson and Eriksson 2000), and age (Clauss and Aarssen 1994) can affect or even mask the trade-off between offspring size and number. Here, we found that flowering time alters the seed size/number trade-off in *A. thaliana*, with later flowering lines showing no significant trade-off. The link between age at reproduction and the seed size/number trade-off is supported by a similar effect of flowering on seed set in a northern temperate flora (Bolmgren and Cowan 2008). In terms of life-history theory, this result makes intuitive sense as early flowering plants should have smaller rosettes and thus reduced resources to invest into reproduction (Mitchell-Olds 1996; Colautti and Barrett 2010; Méndez-Vigo *et al.* 2010). Hence, it is likely that the observed modest trade-offs are a consequence of restricted resources and not genetic pleiotropy. However, it is puzzling that later flowering plants also show reduced number of seeds per fruit, given that previous studies have also shown that they also produce fewer fruits (Kover *et al.* 2009a; Springate and Kover 2014). If flowering later allows for the accumulation of more resources for reproduction, releasing maternal plants from the trade-off, fecundity should be maintained. Thus, it is possible that the reduction in the trade-off represents a change in allocation pattern due to developmental processes and is not simply a function of more resources due to a later transition to reproduction.

Independent genetic regulation of seed size and seed number could be valuable because it means that improvement in one trait can be accomplished without a corresponding decrease in the other, so that overall yield can be increased. Here, we find that the genetic factors affecting seed size variation are at least partly independent of the genetic factors affecting seed number variation. In agreement with our finding, a recent study in maize shows that lines selected for increased kernel size did lead to larger plants, with kernels double the size of lines selected for smaller kernels, but only 20% fewer rows per cob (Sekhon *et al.* 2014). Also, in rice a receptor-like kinase

(*RLK1*) cloned from a yield QTL was transformed to determine the specific gene action and was found to significantly increase yield through a ~30% increase in seed number/panicles, with only a 5% reduction in seed weight (Zha *et al.* 2009). We also found that that seed size was found to display higher heritability and a reduced plastic response to flowering time (Table 2, Figure S1) than seed number. A similar conclusion was reached by Sadras and Slafer (2012) in their metaanalysis of cereals. The combination of genetic independence of seed size from seed number, and the higher heritability and plasticity of weed size, suggest that seed size might be a better target for yield and fitness improvement than seed number.

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The Genetic Basis of Natural Variation in Seed Size and Seed Number and Their Trade-Off Using *Arabidopsis thaliana* MAGIC Lines

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Table S1 Significant QTLs detected for total number of healthy seeds per fruit. "Chr." indicates the chromosome location, and "Peak (Kb)" the position in the chromosome of the QTL peak in kilobases. Statistical significance of each QTL is indicated by LogP and Genome wide P. r^2 indicates the amount of variation explained by the QTL. Positions highlighted in bolt are the same as when QTL for total seed number was mapped (see Table 1 in the main text).

Chr.	Peak (kb)	-logP	Genome-wide P	r^2
1	**20,175**	4.691553	0.006	0.06
1	**24,795**	4.287234	0.01	0.04
3	**15,233**	4.885016	0.005	0.05
3	**18,512**	4.951098	0.004	0.07
4	**269**	5.615617	0.002	0.09
4	2,588	4.288144	0.01	0.03
4	**5,290**	4.408708	0.009	0.06
4	**7,177**	5.41221	0.002	0.07
4	9,198	3.70522	0.032	0.02
4	13,180	3.822907	0.026	0.03
4	14,787	3.556139	0.043	0.03
5	3,696	3.497428	0.048	0.03
5	**16,446**	3.918824	0.024	0.03
5	**21,039**	5.1142	0.003	0.08

Table S2 Significant QTLs detected for fruit length when the 39 lines with the erecta phenotype were removed from the analysis. "Chr" indicates the chromosome location, and "Peak Kb" the position in the chromosome of the QTL peak. Significance of QTL is indicated by LogP and Genome wide P. r^2 indicates the amount of variation explained by the QTL.

Chr.	Peak bp	Marker	-logP	Genome-wide P	r^2
1	9516363	MASC00393	3.95	0.02	0.04
1	16874541	MN1_16874540	4.07	0.02	0.02
2	16893586	MASC06022	3.80	0.03	0.06
5	16446291	NMSNP5_16446291	7.22	0.00	0.04
5	23815655	MN5_23815654	3.88	0.03	0.05

Table S3 The estimated values of 19 parent alleles on fruit length (mm) for each significant QTL detected by QTL mapping. Alleles having the largest effect in increasing and decreasing the trait are underlined and bolded, respectively.

		Parental accession																	
Chr.	**Marker**	**Bur-0**	**Can-0**	**Col-0**	**Ct-1**	**Edi-0**	**Hi-0**	**Kn-0**	**Ler-0**	**Mt-0**	**No-0**	**Oy-0**	**Po-0**	**Rsch-4**	**Sf-2**	**Tsu-0**	**Wil-2**	**Ws-0**	**Wu-0**	**Zu-0**
1	MASC00393	14.8	15.6	15.3	15.7	**14.3**	15	14.9	15.3	15.1	15.4	15.2	15.3	15.6	15.2	15	15.1	15.3	14.5	15.6
	MN1_16874540	15	15.2	14.9	15.8	14.8	15.2	15.1	15.1	15	15	15.1	15.3	15.5	**14.8**	15	15	15	15.1	15.4
2	MASC06022	15.3	14.9	15.3	15	15.1	16.5	15.4	14.7	15.2	15.2	15.4	15.8	14.8	14.9	14.8	15.1	15.1	14.8	**14.6**
5	NMSNP5_14661352	14.9	15	15.6	15.1	**14.4**	14.9	14.9	15.7	15.1	15.6	15.1	14.9	15.6	14.5	15.7	14.9	14.8	15.1	15.4
	NMSNP5_16446291	14.7	15.4	15.4	15.2	14.9	14.9	14.9	15.9	15.2	15.1	15.1	15.2	15.6	**14.6**	15.6	14.6	14.7	15	15.1
	MN5_23815654	**14.2**	15.2	15.3	15.9	15.3	15	15.1	15.3	15.2	14.9	15.2	15.2	15.5	14.3	15.8	14.8	15	15	15.3

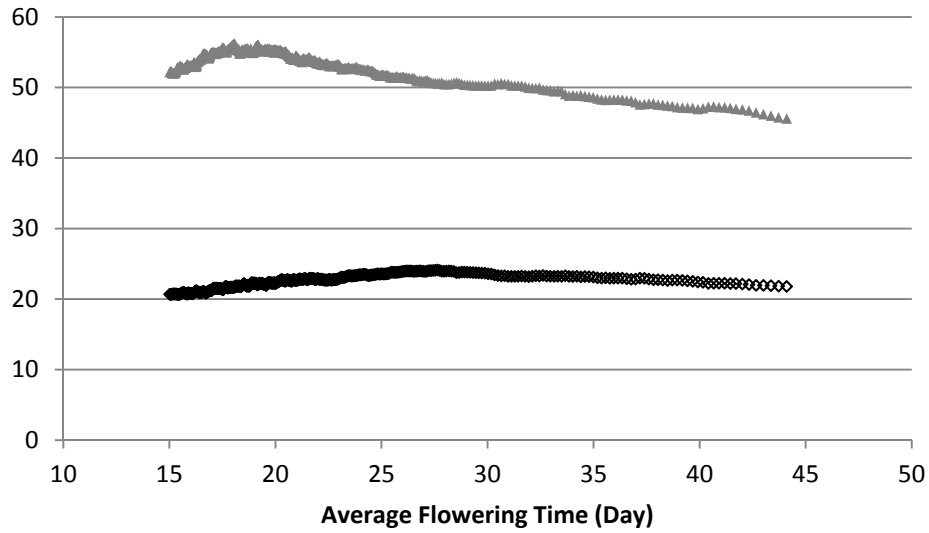

Figure S1 Changes in average of seed weight and seed number across flowering time. Average seed weight and seed number were calculated for 100 lines in a sliding window across increasing flowering time. The open black diamonds represent the seed weight averages in µg, and the grey triangles represent the average seed number/fruit for each window; plotted against the average flowering time for the same window.

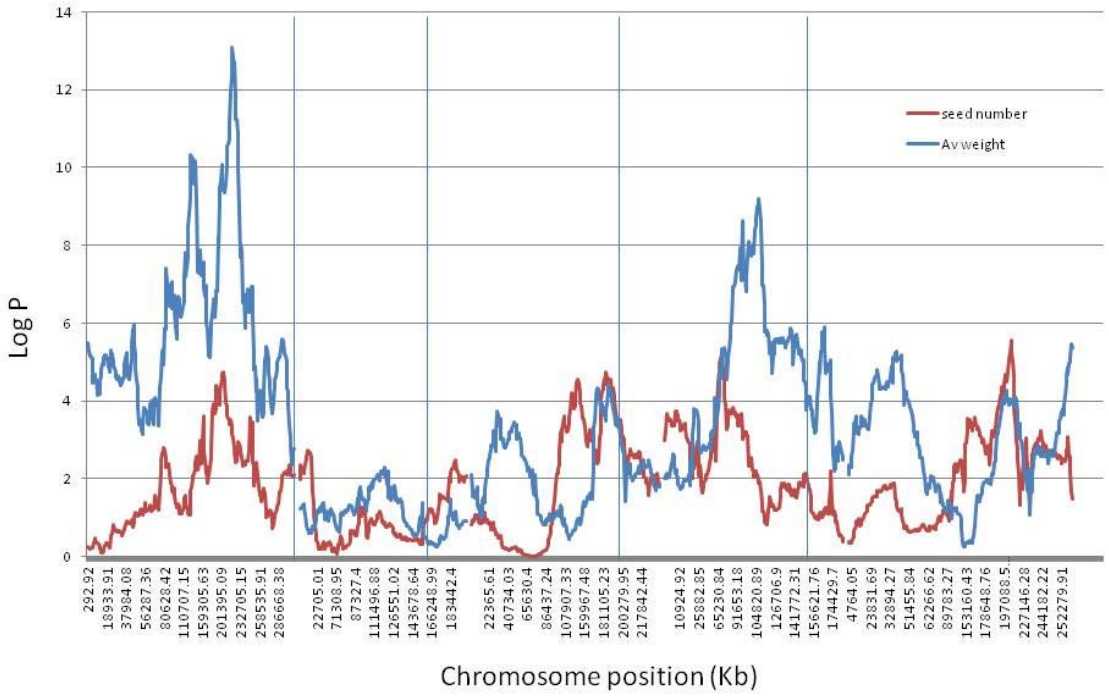

Figure S2 QTL scan for seed number per fruit (red) and seed weight (blue) for MAGIC lines (-LogP of 3.51 corresponds to a genome-wide p-value <0.05).

Files S1-S2

Available for download as Excel files at
http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.170746/-/DC1

File S1 Genotypes for all MAGIC lines

File S2 All phenotypic data