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1 **The genetic basis of natural variation in seed size and seed number and**  
2 **their trade-off using *Arabidopsis thaliana* MAGIC lines**

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17 **Short Title:** Mapping variation in seed size and number

18

19 **Keywords:** QTL mapping, Multi Parent Genetic InterCross, Life-history, Fecundity, Yield,  
20 Plants.

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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 Genetic Inter Cross (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 non-overlapping 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.

1 **Short summary:** Offspring number and size are central to fitness and yield.  
2 Understanding the genetic basis of these traits, and trade-offs between them, is  
3 important from an evolutionary and applied perspective. We investigated the genetic  
4 basis of natural variation in seed size and number using a set of *Arabidopsis thaliana*  
5 MAGIC lines; and whether life-history traits affect them and their trade-off. We found  
6 that both seed size and number are affected by a large number of non-overlapping QTL.  
7 The trade-off between them is dependent on life-history traits, and explains little  
8 phenotypic variance. Seed size can evolve independently and be a target for  
9 improvement.

## INTRODUCTION

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 (KESAVAN *et al.* 2013; SADRAS 2007; VAN DAELE *et al.* 2012). 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 (ADAMSKI *et al.* 2009; FANG *et al.* 2012; GARCIA *et al.* 2003; TZAFRIR *et al.* 2004). 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 (CHOU *et al.* 2011; TONSOR *et al.* 2005), 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

1 must occur (VENABLE 1992). Although the seed size/number trade-off is well accepted on  
2 theoretical grounds, empirical evidence for its existence is still contentious, and  
3 dependent upon the context under which it is evaluated (HOUSE *et al.* 2010; PAUL-VICTOR  
4 and TURNBULL 2009; SADRAS 2007; VENABLE 1992). One possible explanation for context  
5 dependency in trade-offs is that the resources available for reproduction are not discrete  
6 from the whole plant budget. With many competing allocations within the organism,  
7 trade-offs may arise not just between seed size and seed number, but also with other  
8 competing sources such as defense against biotic and abiotic stress (BAZZAZ *et al.* 1987;  
9 ZÜST *et al.* 2011; WITUSZYŃSKA *et al.* 2013). Alternatively, the expression of the trade-off  
10 might be dependent on the level of resources available (BENNETT *et al.* 2012; NOORDWIJK  
11 and JONG 1986; VENABLE 1992). Variation in life-history is common within populations; and  
12 typically, later flowering plants are larger and have more resources to invest in  
13 reproduction, reducing the expression of trade-offs (AARSEN and CLAUSS 1992; CLAUSS and  
14 AARSEN 1994; JAKOBSSON and ERIKSSON 2000; MENDEZ-VIGO *et al.* 2013). Thus, variation in  
15 seed size might be due to genetic factors with direct effects on seed size, or genetic  
16 factors with indirect effects - such as through resource uptake or life-history  
17 determinants, as well as non-genetic variation. A better understanding of natural  
18 variation in seed size therefore requires simultaneous consideration of genetic variation,  
19 and life-history strategies.

20

21 It is important to determine the existence and mechanism behind trade-offs,  
22 because environmentally-caused trade-offs can be modified by selection or genetic  
23 manipulation. However, for trade-offs that result from genetic pleiotropy or linkage  
24 disequilibrium, responses to selection will be constrained (LANDE and ARNOLD 1983; LATTA

1 and GARDNER 2009; ROFF and FAIRBAIRN 2007). In addition, the assumption of a trade-off  
2 between seed size and seed number has shaped breeding practices (SADRAS 2007; EGLI  
3 1998): Seed number has been the main target for crop improvement because it is more  
4 variable than seed size (HARPER *et al.* 1970; SADRAS 2007; SADRAS and EGLI 2008; VENABLE  
5 1992). However, if seed size shows less environmental variation and higher heritability  
6 than seed number, seed size might be a useful target for genetic crop improvement  
7 (SADRAS and SLAFER 2012); but only if the trade-off can be teased apart.

8

9         Here, we investigate the genetic basis of natural variation in seed size and its  
10 correlation with seed number using a set of recombinant inbred lines of *A. thaliana*,  
11 derived from a Multiparental Advanced Genetic InterCross population, known as MAGIC  
12 lines (KOVER *et al.* 2009b). *Arabidopsis thaliana* is an ideal model organism for the study of  
13 natural variation in seed size and number, because there is extensive variation among  
14 world-wide accessions for both of these traits and for many life-history traits (ALONSO-  
15 BLANCO *et al.* 1999b; KOVER *et al.* 2009b; KRANNITZ *et al.* 1991). Few studies have addressed  
16 the issue of QTL for seed size and number, taking into account other life-history traits  
17 (ALONSO-BLANCO *et al.* 1999a; VAN DAELE *et al.* 2012 ), and these only used mapping lines  
18 created from two parents. Multiparental lines are better for addressing genetic  
19 correlations and possible trade-offs than traditional mapping lines due to the larger  
20 number of alleles and recombination events. This allows mapping to smaller intervals  
21 (KOVER *et al.* 2009b), reducing overlap in positions due to large confidence intervals. In  
22 addition, the larger number of alleles improves our ability to determine whether the  
23 distributions of allelic effects are compatible with pleiotropy. With only two alleles  
24 present, the same allele may increase the value of any two traits 50% of the time by

- 1 chance alone. With multiple alleles, a significant correlation in allelic effects provides
- 2 stronger evidence of a common genetic mechanism. Specifically, the following questions
- 3 are addressed: (i) What is the genetic architecture underlying seed size and seed number
- 4 per fruit? (ii) Is there evidence for a genetically determined seed size/number trade-off?
- 5 (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 Multiparent Advanced Genetic Inter Cross (MAGIC) lines used here were produced by advanced intermating of 19 parental accessions of *A. thaliana* for 4 generations, followed by 7 generations of inbreeding (KOVER *et al.* 2009b). These lines have been genotyped for 1260 single nucleotide polymorphisms (SNPs), distributed throughout the 5 chromosomes at a spacing of approximately 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 1Mb (KOVER *et al.* 2009b). The genotypes for all MAGIC lines are listed on Supplementary 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°C day/18°C night and 16/8 hours of light/dark. Seeds from each line were placed in 3 separate 5.5 cm diameter pots filled with F2 + Sand soil (Levington®, The Scotts Company, UK) and randomly allocated to trays that held 24 pots. Trays were rotated around the greenhouse at regular intervals to ensure uniform growth conditions and to 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

1 by digital collection of seed area and by weight. Three fruits (between the 6th and 10th  
2 fruit on the main stem) were collected per plant, after senescence. Fruits were dissected  
3 under a microscope, and images of fruits and seeds were captured with a Nikon Digital  
4 Sight DS-U1 camera. Fruit length (mm) and seed area (mm<sup>2</sup>) were estimated from these  
5 images using the “Measure” function in ImageJ v.1.44p (NIH, USA;  
6 <http://rsbweb.nih.gov/ij/>). Seed number was counted from the images captured, using  
7 Windows Paint software and a hand tally. Average seed weight (µg) was determined by  
8 weighing all the non-aborted seeds from all 3 fruits on a Mettler UMT2 Ultra  
9 Microbalance, and dividing by total number seeds weighed. The number of aborted seeds  
10 (i.e. seeds that can be seen but were not completely filled) per fruit were recorded as  
11 observed. All phenotypic data are listed on File S2.

12

### 13 ***Statistical analyses***

14 Broad sense heritability ( $H^2$ ) for each trait was estimated as the ratio of the  
15 variance among lines to the total variance. To determine genetic correlations between  
16 traits, pairwise Pearson correlations between line means were calculated. To determine  
17 whether flowering time affects the trade-off between seed size and seed number, we  
18 calculated the correlation between seed size and number separately for the 100 and 200  
19 earliest and latest MAGIC lines. To determine the proportion of variation in seed weight  
20 or in seed number explained by each trait measured after model selection, we tested the  
21 following multiple linear regression model using either the data for average seed weight  
22 or seed number per fruit: Seed weight (or number) =  $\beta_{\text{Intercept}} + \beta_{\text{Seed weight (or number)}} + \beta_{\text{Height}}$

1 +  $\beta_{\text{Aborted}}$  +  $\beta_{\text{Nodes}}$  +  $\beta_{\text{Total branches}}$  +  $\beta_{\text{flowering}}$  +  $\epsilon$ . The *bootStepAIC* package for R was run in  
2 both directions for model selection ( $\alpha=0.05$ ; bootstrap resampling 1000x).

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

10 QTL analyses were performed using the R software package HAPPY as described in  
11 Kover *et al.* (2009b). Briefly, this approach uses a Hidden Markov model to make a  
12 multipoint probabilistic reconstruction of the genome of each MAGIC line as a mosaic of  
13 the founder haplotypes. Thus, at each marker a probability of being derived from each of  
14 the parental accessions is assigned for each line; and the hypothesis that there is no QTL  
15 is evaluated by fitting a fixed-effect linear model with up to 18 degrees of freedom. We  
16 performed QTL analysis for the line average of seed weight ( $\mu\text{g}$ ), seed number per fruit  
17 and fruit length (mm). Overlapping QTL (i.e. QTL located  $<1$  Mb away) for seed size and  
18 number would suggest that the trade-off is due to genetic pleiotropy. Concordance of  
19 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 a ~ 3-fold 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.

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

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

21

## 22 **QTL mapping**

23           The QTL analysis for seed weight identified 8 QTL located on chromosomes 1, 3, 4,  
24 and 5 (Table 4). The largest QTL for seed size is located on chromosome 1 (~21,6 Mb), and

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

7  
8       There is little overlap between QTL for seed size and seed number (Table 4; Figure  
9 S2). All QTL for seed size are located > 1 Mb away from a QTL for seed number,  
10 suggesting that these traits are determined by independent genetic factors. The one  
11 exception is the seed size QTL on chromosome 3 (~18.5 Mb) which is only 400kb away  
12 from a seed number QTL (~18,9Mb). Comparison of the distribution of allelic effects at  
13 this QTL (Tables 5 and S3) suggests a similar, but not identical distribution of effects. The  
14 Bur-0 allele at this location causes the largest seed and the smallest number of seed per  
15 fruit. In addition, there is a significant correlation in allelic effects ( $\rho = -0.52$ ,  $p = 0.02$ ).  
16 Thus, it is possible that the same genetic factor is affecting both traits in a pleiotropic  
17 manner. However, this QTL does not explain much variation (5% of seed weight and 7%  
18 of fruit number).

19  
20       The estimated value of each of the 19 haplotypes (Table 5) also shows that, for 6  
21 of the 8 seed size QTL identified, the allele conferring the largest seed size is from the  
22 Bur-0 accession. At the other 2 QTL, the Bur-0 allele leads to the second largest seed size.  
23 In contrast, the alleles causing the largest or smaller number of seeds per fruit are from a

1 number of different accessions (i.e. there is no clear pattern that the allele from Bur-0  
2 cause the smallest number of seeds per fruit at most QTL, Table S3).

3

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

13

## 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 and MOORE et al 2013). In these four studies, recombinant inbred lines (RILs) derived from two accessions were used, and typically each QTL explained less than 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* (HUANG *et al.* 2011; KOVER *et al.* 2009b) 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



1 QTL in multiparent populations due to the large number of alleles; or whether this a trait  
2 specific issue. While more multiparent mapping studies are needed before we can better  
3 determine if there is a problem, it is possible that traits where most of the genetic  
4 architecture is additive will not show a reduction in the number of QTL identified, while  
5 other traits that include many loci with genetic background-dependent effects will show a  
6 reduction in the number of QTL identified using multiparent lines. This may not  
7 necessarily be a disadvantageous feature of MAGIC populations if it allows the  
8 identification of QTL with consistent effects over a diverse set of complex backgrounds. It  
9 has also been suggested that with the increased number of recombinations there could  
10 be a breakage of small QTL that were previously linked, reducing the ability to detect  
11 them (HUANG et al. 2010). Such an effect has not been seen here, given that the number  
12 and the size of the effects are comparable to QTL identified using only two parental  
13 accessions. Nevertheless, it may explain the reduction in detected QTL for other traits  
14 not included in this study.

15

16         So far, only a few genes have been identified to be involved in determining seed  
17 size, and genes that explain natural variation on seed size or number remain unknown  
18 (HERRIDGE et al. 2011; VAN DAELE et al. 2012; MOORE et al. 2013). The QTL on the bottom of  
19 chromosome 1 is a good candidate for further fine-mapping of a genetic factor that  
20 affects quantitative variation in seed size, since it explains a reasonable amount of  
21 variation (15%). At this QTL, the Bur-0 allele was found to be associated with larger seed  
22 size. Thus, to identify possible candidate genes we searched for genes containing non-  
23 synonymous SNPs unique to this accession. Based on the ressequencing and  
24 reannotation of the 19 parental accessions (GAN *et al.* 2011), we identified two strong

1 candidate genes located < 250 Kb from the largest seed size QTL on chromosome 1: *AAP1*  
2 (*AT1G58360*) and *KLUH* (*AT1G13710*). Both of these genes contain non-synonymous  
3 substitution unique to Bur-0, and have been previously identified through mutation  
4 studies to affect seed size (ADAMSKI *et al.* 2009; SANDERS *et al.* 2009). Candidate genes for  
5 the QTL on chromosomes 4 and 5 were identified by searching for genes previously  
6 identified to affect seed or ovule number. *JAGGED LATERAL ORGANS* (*AT4G00220*),  
7 *YABBY 3* (*AT4G00180*), and *BEL1* (*AT5G41410*) (BORGHI *et al.* 2007; BRAMBILLA *et al.* 2007;  
8 NOLE-WILSON and KRIZEK 2006), are good candidates based on their close proximity (less  
9 than 250 kb away) to the identified QTL.

10

11         The existence of such extensive, within-species, genetic variation in seed size is  
12 puzzling because life-history theory would predict selection for an optimal seed size that  
13 best resolve the trade-off between seed size and number (SMITH and FRETWELL 1974;  
14 HALPERN 2005). Given the complex genetic architecture of seed size, it is possible that  
15 balancing selection could maintain some of the genetic variation (TURELLI and BARTON  
16 2004). It is also possible that divergent environmental selection at sites where the  
17 parental accessions were originally collected may explain the observed variation (MACKAY  
18 1981). Orr (1998) suggested that selection could be inferred from the direction of QTL  
19 effects being non-randomly distributed between parental accessions. Although his sign  
20 test was proposed for QTL studies using intercross between two accessions, its logic can  
21 be equally applied to multiparent populations. We argue that there is a very small chance  
22 of observing that all alleles that produce large seeds come from the same accession (Bur),  
23 when there are 19 parental accessions, if seed size variation was neutral. Thus, we  
24 propose that the large seed size observed in the Bur accession is due to directional

1 selection, and that at least some of the variation in seed size within *A. thaliana* is due to  
2 adaptive processes.

3

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

19

20 While a significant negative correlation is observed between seed size and seed  
21 number, the overall variance explained by this correlation is relatively small. Relative to  
22 other life-history trade-offs, the seed size/number trade-off is not very strong. For  
23 example, plant height is as strongly correlated with seed size as it is with seed number,  
24 and there is a stronger negative correlation between seed number and flowering time

1 (Table 2). In addition, there is little evidence for common genetic regulation for both of  
2 these traits. Kover *et al.* (2009) estimated that causal polymorphism for traits mapped  
3 with these MAGIC lines should lie within 200kb of the peak of the identified QTL. Thus,  
4 QTL identified for seed weight and seed number do not overlap (except for one a QTL on  
5 chromosome 3) and distinct allelic effects are observed at QTL for the two traits (Tables 5  
6 and S3). In light of previous conflicting evidence regarding the presence of the seed  
7 size/number trade-off, our data suggests that although significant for this population, the  
8 trade-off may not be as important to explain variation in these traits as theoretically  
9 predicted. The two other studies that simultaneously mapped seed number and size in  
10 *A. thaliana* (ALONSO-BLANCO *et al.* 1999a; VAN DAELE *et al.* 2012), concluded that both of  
11 these traits map to similar locations and could be pleiotropic. However, their confidence  
12 intervals were quite large (sometimes encompassing the whole half of a chromosome),  
13 and thus difficult to compare with our results.

14

15 Previous work has shown that parental resource status (NOORDWIJK and JONG 1986;  
16 PAUL-VICTOR and TURNBULL 2009; VENABLE 1992), plant size (JAKOBSSON and ERIKSSON 2000)  
17 and age (CLAUSS and AARSEN 1994) can affect or even mask the trade-off between  
18 offspring size and number. Here, we found that flowering time alter the seed size/number  
19 trade-off in *A. thaliana*, with later flowering lines showing no significant trade-off. The link  
20 between age at reproduction and the seed size/number trade-off is supported by a  
21 similar effect of flowering on seed set in a northern temperate flora (BOLMGREN and  
22 COWAN 2008). In terms of life-history theory, this result makes intuitive sense as early  
23 flowering plants should have smaller rosettes and thus reduced resources to invest into  
24 reproduction (COLAUTTI and BARRETT 2010; MÉNDEZ-VIGO *et al.* 2010; MITCHELL-OLDS 1996).

1 Hence, it is likely that the observed modest trade-offs are a consequence of restricted  
2 resources and not genetic pleiotropy. However, it is puzzling that later flowering plants  
3 also show reduced number of seeds per fruit, given that previous studies have also shown  
4 that they also produce fewer fruits (KOVER *et al.* 2009a; SPRINGATE and KOVER 2014). If  
5 flowering later allows for the accumulation of more resources for reproduction, releasing  
6 maternal plants from the trade-off, fecundity should be maintained. Thus, it is possible  
7 that the reduction in the trade-off represents a change in allocation pattern due to  
8 developmental processes and is not simply a function of more resources due to a later  
9 transition to reproduction.

10

11 Independent genetic regulation of seed size and seed number could be valuable  
12 because it means that improvement in one trait can be accomplished without a  
13 corresponding decrease in the other, so that overall yield can be increased. Here, we find  
14 that the genetic factors affecting seed size variation are at least partly independent of the  
15 genetic factors affecting seed number variation. In agreement with our finding, a recent  
16 study in Maize show that lines selected for increased kernel size did lead to larger plants,  
17 with kernels double the size of lines selected for smaller kernels, but only 20% fewer rows  
18 per cob (SEKHON *et al.* 2014). Also, in rice a Receptor-like kinase (*RLK1*) cloned from a yield  
19 QTL was transformed to determine the specific gene action, and found to significantly  
20 increase yield through a ~30% increase in seed number/panicles, with only a 5%  
21 reduction in seed weight (ZHA *et al.* 2009). We also found that that seed size was found to  
22 display higher heritability and a reduced plastic response to flowering time (Table 2,  
23 Figure S1) than seed number. Similar conclusion was reached by Sadras and Slafer (2012)  
24 in their meta analysis of cereals. The combination of genetic independence of seed size

1 from seed number, and the higher heritability and plasticity of weed size, suggest that  
2 seed size might be a better target for yield and fitness improvement than seed number.

3

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1 **Table 1.** Phenotypic variation among MAGIC lines for all traits measured. Minimum  
 2 (Min.), maximum (Max.) phenotypic values for each trait, as wells as the phenotypic mean  
 3 plus or minus their standard deviation (SD) and their broad-sense heritability ( $H^2$ ) are  
 4 shown.

	Min.	Max.	Mean $\pm$ SD	$H^2$
Days to flowering	12.3	117.0	23.5 $\pm$ 9.9	0.92
Total # of branches	1.5	12.7	5.8 $\pm$ 1.6	0.42
Inflorescence height	9.0	68.8	41.8 $\pm$ 9.2	0.62
Seed weight ( $\mu$ g)	11.8	37.7	22.2 $\pm$ 3.1	0.63
Seed area (mm <sup>2</sup> )	0.4	1.1	0.74 $\pm$ 0.7	0.47
Total seeds per fruit	27.0	79.2	52.5 $\pm$ 9.5	0.43
% seeds aborted	0	37	0.9 $\pm$ 2.7	0.18
Fruit length (mm)	8.8	20.0	15.0 $\pm$ 1.8	0.22

6

7

**Table 2.** Pairwise Pearson's correlations between traits measured, 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 0.001 level).

	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**



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

	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

**Table 4.** Significant QTLs detected for average seed weight, total number of seeds per fruit and fruit length. “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.

Chr.	Peak (kb)	-logP	Genome-wide P	$r^2$
<b>Seed weight (<math>\mu\text{g}</math>)</b>				
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
<b>Seed number/fruit</b>				
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
<b>Fruit length (mm)</b>				
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

**Table 5.** Estimated value for each of the 19 parental alleles on seed size ( $\mu\text{g}$ ), seed number per fruit, and fruit length at each detected QTL. Alleles having the largest effect in increasing and decreasing the trait are underlined and bolded, respectively.

Chr	Mb	Parental accession																			
		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	
<b>Seed size</b>																					
1	4.5	<u>24.7</u>	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	<b>20.4</b>	22.4	23.2	
		21.7	<u>24.7</u>	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	<b>19.5</b>	21.7	22.9
3	18.9	<u>23.4</u>	<b>20.5</b>	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	<u>25.4</u>	22.8	22	22.2	21.9	22	<b>20.5</b>	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	<u>25.1</u>	22.4	22.9	22.2	22.4	22.1	<b>21.1</b>	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	<b>21.2</b>	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	<b>21</b>	23.5	23	23	22.6	22	21.2	21.7	22.5
		26.7	<u>23.6</u>	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	<b>21.4</b>	22.2	22.1
<b>Seed number/fruit</b>																					
1	20.1	<b>46.8</b>	51.3	53.8	54.6	49.8	53.7	<u>55.4</u>	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	<b>49.1</b>	51.4	52.7	52.3	52.9	54.9	<u>55.9</u>	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	<u>55.8</u>	50.8	50.7	50.9	55.4	49.1	53.3	49.1	52.8	<b>48.4</b>	
3	18.5	<b>47.1</b>	51.1	55.8	52.2	52.0	54.6	53.1	53.3	52.7	<u>57.9</u>	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	<u>56.1</u>	50.8	54.9	51.6	<b>46.1</b>	52.7	48.0	
4	5,3	50.6	48.8	<u>55.5</u>	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	<b>48.3</b>	
4	7,2	<b>46.5</b>	50.1	55.4	54.6	48.2	52.1	53.2	<u>57.2</u>	53.4	51.5	51.9	50.8	54.2	52.7	53.4	51.1	50.9	51.1	48.0	

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5	16,5	51.8	51.4	52.6	52.6	51.1	52.9	53.4	<u>55.9</u>	51.5	53.8	50.8	52.4	53.3	49.5	53.5	50.5	<b>49.0</b>	50.4	51.1
5	21,0	50.7	51.3	55.4	<u>56.0</u>	48.8	52.6	54.7	52.5	52.0	55.0	53.1	52.9	53.3	<b>46.3</b>	54.8	48.1	48.5	52.7	51.3
<b>Fruit Length</b>																				
2	11.2	15.05	13.92	15.35	15.20	14.45	<u>15.53</u>	15.23	<b>12.40</b>	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	<b>14.29</b>	15.05	15.24	15.16	14.71	14.77	14.69	15.66	14.84	14.98	14.95	14.51	<u>15.83</u>	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	<u>15.79</u>	<b>14.11</b>	15.48	14.31	14.38	15.01	15.18

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