Supplemental Materials & Methods for: **Monitoring rapid evolution of plant populations at scale with Pool-Sequencing**

Plant growth protocol

Experiment 1

The 231 ecotypes were bulked in growth chambers at 20°C (long-day conditions) in three locations. In the Max Planck Institute for Developmental Biology, Germany, the growth chambers at the University of Tübingen's Institute of Evolutionary Ecology, Germany, and the CNRS Centre for Functional and Evolutionary Ecology in Montpellier, France.

Experiment 2

Two distinct ecotypes part of the 231 set were grown in growth chambers at 20°C (long-day conditions)

Experiment 3

We took one tube containing 0.1 g of the founder seed mix (~5,000 seeds), bleach-sterilized it, washed it (20 min; 500ml solution, 10% bleach, 20% SDS) and submerged the seeds in 1% agar solution for 5 days at 4 °C in the dark. Seeds were planted in trays with soil (CL-P, Einheitserde Werkverband e.V., Sinntal-Altengronau Germany) in 25 trays, 1,000 pots, and germinants were thinned to one plant per pot. We watered abundantly, growing the plants at 16 °C for 13 days (long day conditions) before a 60 day vernalization at 4 °C (short day conditions). The vernalization approach aimed to avoid flowering time differences among our diverse genotypes. Subsequently, the trays were transferred to 20 °C long days (16h light / 8 dark) for flowering. Two weeks later, 50 pots were randomly chosen to sample leaves and flowers whenever the plants bloomed

Experiment 4

The genotypes planted are 451 natural accessions (**Dataset S1**), all mixed together, across three plots (about 2 seeds/cm^2 in $1m^2$ plots). 20 siliques from two different parental individuals of all genotypes were pooled and sowed in three batches: one in November 2014, one in February 2015, one in March 2015. After one year, plants were sampled in Spring 2016, where flowers from many individuals were harvested and pooled for Pool-seq whole genome sequencing. On March 2 2016, before flowering, bulk soil was taken to germinate seeds in the growth chamber and collect flowers for sequencing to avoid any selection of genotypes. Then on April 1, April 22, and May 6, 50-100 flowers, 80-200 flowers, and 60-300 flowers respectively for each timepoint and per plot (**Table S5**) and used for sequencing.

DNA extraction

Experiments 1, 3, and 4

The GrENE-net founder seed mix, containing 231 natural accessions (**Dataset S1**), was aliquoted into eight replicates according to the tissue input amount recommended by the Qiagen DNeasy Plant Mini kit (Hilden, Germany) (**Table S1**). Seed aliquots were suspended in 0.1% agar and kept at 4°C in the dark for 9 to 11 days to initiate germination. Then, seed aliquots were centrifuged and the supernatant was removed. 0.5 mL of rock and 800 µL of lysis buffer AP1 from the DNeasy kit were added to the seed tubes. Tissue homogenization was carried out using the Quickprep adapter in a FastPrep-24 (MP Biomedicals, Irvine, CA, USA) with the following setting: 6.0 m/sec for 40 seconds. Each tube was homogenized for a total of 3 rounds. 8 µL of RNase (100 mg/mL) from the DNeasy kit was added to the seed homogenate. After a short vortex and a quick spin, the seed homogenate was incubated at 65°C for 10 minutes. After the incubation, 185 µL of buffer P3 from the DNeasy kit

was added to the seed lysate. The tube was inverted and incubated on ice for 5 minutes. The rest of the extraction followed the standard Qiagen DNeasy Plant Mini protocol. DNA was eluted in 100 µL of AE buffer.

The leaf subsamples and flower subsamples were extracted similarly with the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) with modifications to the grinding step. Tissue samples and 5 ceramic beads were placed in a screw-cap tube and froze with liquid nitrogen. The homogenization was again carried out using FastPrep-24 (MP Biomedicals, Irvine, CA, USA) with a different setting: 4.0 m/sec for 15 seconds. Each tube was homogenized for a total of 2 rounds. The rest of the extraction followed the standard DNeasy protocol.

The Tübingen pilot samples were …?

Experiment 2

Due to the high cost of commercial kits such as the Qiagen DNeasy Plant Mini kit ($4.46 per isolation at listed price), we set up a cheaper plate-based DNA extraction protocol based on the widely used 2x CTAB protocol (Doyle and Doyle, 1987). All GrENE-net DNA extracts were isolated using this custom protocol from pooled flower samples collected from the 45 field sites. We partially replicated the leaf and flower comparison (see Experiment 3 in the main text) using our CTAB/chloroform protocol. In the case of flowers, two flowers of similar size were collected in the same tube prior to DNA extraction (n = 3). In the case of leaves, a leaf was independently extracted, but leaf extracts from two distinct ecotypes, Col-0 and RUM-20, were combined at similar DNA mass prior to library preparation (n = 3) (see Experiment 2 in the main text).

2-mercaptoethanol was added to the 2x CTAB buffer (1.4 M NaCl, 100 mM Tris pH 8.0, 20 mM EDTA pH 8.0, 2% w/v CTAB, 1% w/v PVP, ddH_2O) to a final concentration (v/v) of 0.3%. The buffer was warmed at 65°C for at least 30 minutes. Using a TissueLyser II (Qiagen, Hilden, Germany), frozen plant tissues were pulverized with 3.2 mm steel beads in 2.0 mL tubes on chilled adapter sets 2 x 24. Homogenization was carried out at 22/s for 35 sec and repeated until the frozen tissues attained the appearance of greenish white powders. 500 µL of pre-warmed 2x CTAB buffer was added to each tube to thoroughly resuspend the pulverized tissue. Samples were incubated at 65°C for 50 minutes and inverted every 10 to 15 minutes to resuspend the precipitates. After incubation, the lysate was transferred to a new 2.0 mL tube. When the lysate was cooled to room temperature, 500 µL of chloroform:isoamyl alcohol (24:1) was added to the lysate. The tube was vigorously shaken until the lysate and chloroform appeared well-mixed. The sample was centrifuged at 20,000 rcf for 14 minutes or until the upper aqueous layer appeared clear. 300 µL of the aqueous layer was transferred to a new tube or a 96-well deep well plate if doing high-throughput processing. 225 µL (0.75 vol) of isopropanol was added to the supernatant and mixed well by pipetting. The sample was incubated at 4°C for at least 30 minutes or at -20°C overnight. After incubation, the sample was centrifuged at max speed for 15 minutes in a tube. Alternatively, the 96-well plate was centrifuged at 6,100 rcf for 45 minutes. After discarding the supernatant, freshly prepared 70% ethanol was added to wash the DNA pellet. The sample was centrifuged at max speed for 5 minutes in a tube. Alternatively, the 96-well plate was centrifuged at 6,100 rcf for 30 minutes. The ethanol was removed and the pellet was left to air dry for 10 to 15 minutes. The DNA pellet was eluted in Tris buffer containing RNase A (10 mM Tris-HCl pH9.0, ddH_2O, 20 µg/mL RNase A). The eluate was incubated at 37°C for 30 minutes. After a pulse spin, the DNA extract was stored at -20°C.

Library preparation

Experiment 1

Because the total number of samples was small and we had large amounts of DNA (**Table S1**), we conducted library preparations with Illumina's TruSeq PCR library kit (Ilumina, San Diego, California).

Experiment 2

Because there were only 11 samples, tube-based quantification was performed using the Qubit dsDNA HS assay. The readings for the input DNA concentration are documented in Table SX. The library preparation protocol was based on (Baym et al., 2015) with some modifications. 2 µL of DNA sample was mixed with 2.75 µL TD buffer (Tagment DNA Buffer) and 0.25 µL TD enzyme (Mira Loma, California, USA). The tagmentation reaction mixture was mixed well by gentle pipetting. After a flash spin, the sample was incubated at 55°C for 10 minutes and held at 10°C.

Once equilibrated to room temperature or lower, the samples were flash spinned. Then, the tagmented DNA was mixed with 8 µL 2x KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Boston, MA, USA), 1.5 µL 10 µM P5 indexing primers (final concentration 0.75 µM), 1.5 µL 10 µM P7 indexing primers (equimolar to P5), and 4 µL Tris-Cl buffer (pH 8.0). The PCR reaction mixture was mixed well by gentle pipetting and the liquid was spinned down. The DNA was amplified using the following thermal cycling program:

1. 72°C for 3 minutes
2. 95°C for 3 minutes
3. 98°C for 20 seconds
4. 63°C for 30 seconds
5. 72°C for 30 seconds
6. Repeat from step 3 for 11 additional cycles (i.e. a total of 12 cycles)
7. 72°C for 5 minutes
8. 10°C hold

For post-amplification cleanup and size selection, the 11 libraries were multiplexed in a 1.5 mL tube by mixing 10 µL of each library. The library volume was estimated by aspirating with a P200 pipette (V_{lib}). 0.45 volume (i.e. 0.45 x V_{lib}) of homemade SPRI beads was added to the 11-plex library. The tube was incubated for 5 minutes on a regular rack and then incubated for 5 minutes on a magnet stand until a bead pellet forms. This bead pellet represents the first elution fraction. The supernatant excluding the first pellet was transferred to a new 1.5 mL tube on a regular rack. 0.6 volume (i.e. [0.6 - 0.45] x V_{lib}) of homemade SPRI beads was added to the supernatant. The tube was incubated for 5 minutes on a regular rack and then incubated for 5 minutes on a magnet stand until a bead pellet forms. This bead pellet represents the second elution fraction. The supernatant excluding the second pellet was removed. Each magnetic bead pellet was washed by gently adding 700 µL 70% ethanol and was incubated for 30 seconds before removing the ethanol. The ethanol wash was repeated once. The bead pellet was air dried until they lost the shine and began showing tiny cracks. The tube containing the bead pellet was taken off the magnet and resuspended in 36 µL of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0). After incubating on a regular rack for 3 minutes, the tube was put on magnet stands for 5 minutes until the bead pellet formed. 34 µL of the eluate fraction was transferred to a new 1.5 mL tube. Both eluted fractions were quantified with Qubit and analyzed

on a TapeStation 4150 (Memphis, Tennessee, USA) using a D1000 ScreenTape (Cedar Creek, Texas, USA). The second fraction was sequenced on a HiSeq 2 x 150 lane (**Fig. S6**).

Experiment 3

To compare seeds with flowers and leaf extracts without library preparation differences, we conducted library preparations with Illumina's TruSeq PCR library kit (Ilumina, San Diego, California) as in Experiment 1.

Experiment 4

The library preparation procedure was similar to what was described in Rowan et al. 2019 *Genetics* with minor volume adjustments. Specifically, the twelve amplified libraries were multiplexed together by mixing 5 µL of each library. The total volume was brought up to 100 µL with 10 mM Tris-Cl (pH 8.5). The rest of the size selection was performed as written in Rowan et al. 2019. The fragment length distribution of bead fraction 3 was verified with a Bioanalyzer before being sent for sequencing on an Illumina HiSeq 3000.

References

1001 Genomes Consortium. 2016. 1,135 Genomes Reveal the Global Pattern of Polymorphism in Arabidopsis thaliana. *Cell* **166**:481–491.

Baym M, Kryazhimskiy S, Lieberman TD, Chung H, Desai MM, Kishony R. 2015. Inexpensive multiplexed library preparation for megabase-sized genomes. *PLoS One* **10**:e0128036.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue (No. RESEARCH). worldveg.tind.io.

Supplemental Datasets and Tables

Dataset S1 | Ecotype IDs used for outdoor experiment and GrENE-net seed mixture

Metadata of the 451 and 231 ecotypes lists.

<google drive link>

Table S1 | GrENE-net founder seed mix DNA extraction replicates

Sample identifier	Tissue amount (mg)	Approximate number of individuals	DNA concentration (ng/µL)
GrENE-net 231 founder seed mix #1	100	5000	7.98
#2	100	5000	6.48
#3	100	5000	8.56
#4	17.4	870	21.8
#5	18	900	24.8
#6	18.3	915	23.6
#7	20	1000	24.4
#8	21.6	1080	25

Table S2 | Input DNA concentration library preparation for additional validation of frequency inference

Sample identifier	Input concentration (ng/μL)
GrENE-net founder seed mix #1	1.67
GrENE-net founder seed mix #4	1.5
GrENE-net founder seed mix #8	1.49
Col-0 leaf extract	2.17
RUM-20 leaf extract	2.04
Pooled flower extract #1	1.84
Pooled flower extract #3	1.97
Pooled flower extract #5	1.98
Col-RUM leaf extract pool #1	2 (predicted, 2 μL of 8.33 ng/μL diluted in 6.33 μL Tris buffer)
Col-RUM leaf extract pool #2	2 (predicted, 2 μL of 8.53 ng/μL diluted in 6.53 μL Tris buffer)
Col-RUM leaf extract pool #3	2 (predicted, 2 μL of 8.71 ng/μL diluted in 6.71 μL Tris buffer)

*due to the flexibility (i.e. ≤3 ng/μL) in the acceptable input range for this protocol, the three leaf extract pools were not quantified after dilution

Table S3 | Sampling of 50 leaves

sample id	tray	pos	DNA (ng/µl)	Total DNA ng
1	1	a2	12.4	620
2	1	c3	23.2	1160
3	1	c7	23.2	1160
4	1	b7	13.5	675
5	1	b8	11.8	590
6	2	b2	17.3	865
7	2	c2	10.7	535
8	2	a4	17.4	870
9	2	a5	12.6	630
10	2	e7	14	700
11	3	c2	10.4	520
12	3	e3	7.54	377
13	3	a4	10.4	520
14	3	b7	18.9	945
15	3	c8	8.3	415
16	4	e2	12.1	605
17	4	d3	19.3	965
18	4	a5	17	850
19	4	e7	6.38	319
20	4	e6	7.06	353
21	4	b8	21.2	1060
22	5	e2	14.4	720
23	5	c3	21.4	1070
24	5	a5	19.1	955
25	5	e6	12.8	640
26	5	e8	10.2	510
27	6	b2	7.24	362
28	6	e3	15.5	775
29	6	d2	12.3	615
30	6	a5	7.3	365
31	6	e4	9.82	491
32	6	a6	8.2	410
33	6	e5	12	600
34	6	c5	9.9	495
35	6	e6	7.48	374
36	6	b7	12.3	615
37	7	d4	9.42	471
38	7	e2	9.42	471
39	7	b4	13.5	675

40	7	c3	7.84	392
41	7	d7	7.2	360
42	7	c7	11.7	585
43	8	e1	10.5	525
44	8	e2	7.22	361
45	8	d4	9.96	498
46	8	a4	16.1	805
47	8	d5	11.6	580
48	8	c6	7.02	351
49	8	b7	9.86	493
50	8	a8	15.7	785

Table S4 | Combinatorics of flower and leaf pooling

The 50 randomly selected plants were sampled for the 50 and 100 samples as well as for nested samples of smaller sets of plants. A graphical scheme of this sampling is in **Fig. S3**.

sample id	tray	pos	x100	x50a	x50b	x25a	x25b	x10b1	x10b2	x5
1	1	a2	yes	yes	yes		yes			yes
2	1	c3	yes	yes	yes		yes	yes		
3	1	c7	yes	yes	yes	yes				
4	1	b7	yes	yes	yes		yes		yes	
5	1	b8	yes	yes	yes	yes				
6	2	b2	yes	yes	yes		yes	yes		
7	2	c2	yes	yes	yes		yes	yes		
8	2	a4	yes	yes	yes		yes	yes		
9	2	a5	yes	yes	yes	yes				
10	2	e7	yes	yes	yes		yes		yes	
11	3	c2	yes	yes	yes		yes	yes		
12	3	e3	yes	yes	yes		yes			yes
13	3	a4	yes	yes	yes		yes	yes		
14	3	b7	yes	yes	yes	yes				
15	3	c8	yes	yes	yes		yes	yes		
16	4	e2	yes	yes	yes	yes				
17	4	d3	yes	yes	yes	yes				
18	4	a5	yes	yes	yes		yes		yes	
19	4	e7	yes	yes	yes	yes				
20	4	e6	yes	yes	yes		yes		yes	
21	4	b8	yes	yes	yes	yes				
22	5	e2	yes	yes	yes		yes	yes		
23	5	c3	yes	yes	yes	yes				
24	5	a5	yes	yes	yes		yes		yes	
25	5	e6	yes	yes	yes	yes				
26	5	e8	yes	yes	yes	yes				
27	6	b2	yes	yes	yes	yes				
28	6	e3	yes	yes	yes	yes				
29	6	d2	yes	yes	yes		yes			yes
30	6	a5	yes	yes	yes	yes				
31	6	e4	yes	yes	yes	yes				
32	6	a6	yes	yes	yes	yes				
33	6	e5	yes	yes	yes		yes			yes
34	6	c5	yes	yes	yes	yes				
35	6	e6	yes	yes	yes	yes				
36	6	b7	yes	yes	yes	yes				
37	7	d4	yes	yes	yes	yes				

38	7	e2	yes	yes	yes		yes		yes	
39	7	b4	yes	yes	yes		yes	yes		
40	7	c3	yes	yes	yes		yes		yes	
41	7	d7	yes	yes	yes		yes		yes	
42	7	c7	yes	yes	yes	yes				
43	8	e1	yes	yes	yes		yes		yes	
44	8	e2	yes	yes	yes	yes				
45	8	d4	yes	yes	yes		yes		yes	
46	8	a4	yes	yes	yes	yes				
47	8	d5	yes	yes	yes	yes				
48	8	c6	yes	yes	yes	yes				
49	8	b7	yes	yes	yes		yes	yes		
50	8	a8	yes	yes	yes		yes			yes

Table S5 | Sampling of pilot field experiment

A total of 12 samples were sequenced either from seed banks or flowers of surviving plants in the outdoor field. Sequencing metrics are provided for each sample and the final working in silico pool.

S	ID	Time	rep	# flowers	origin	Input DNA (ng/µL)	read count	bp	coverage	pool coverage
1	1_0	0	1	56	Seed bank	0.376	40214556	6032183400	48.3	
2	2_0	0	2	69	Seed bank	0.366	62882228	9432334200	75.5	199.6
3	3_0	0	3	101	Seed bank	0.424	63206340	9480951000	75.8	
4	1_1	1	1	80	Flowers from field	0.404	51991084	7798662600	62.4	
5	2_1	1	2	160	Flowers from field	0.474	65024068	9753610200	78	219.3
6	3_1	1	3	200	Flowers from field	0.454	65731096	9859664400	78.9	
7	1_2	2	1	65	Flowers from field	0.374	71196816	1.068E+10	85.4	
8	2_2	2	2	205	Flowers from field	0.452	47974450	7196167500	57.6	206.3
9	3_2	2	3	296	Flowers from field	0.33	52761222	7914183300	63.3	
10	1_3	3	1	19	Flowers from field	0.25	60332690	9049903500	72.4	
11	2_3	3	2	50	Flowers from field	0.434	102265250	15339787500	122.7	206.3
12	3_3	3	3	97	Flowers from field	0.452	64052368	9607855200	76.9	

Table S6| Pool extraction of flower combinatorics

Tube	ID mix	Total DNA yield
X5		
X10		
X10		
X25		
X25		
X50		
X50		
X100		

Supplemental Figures

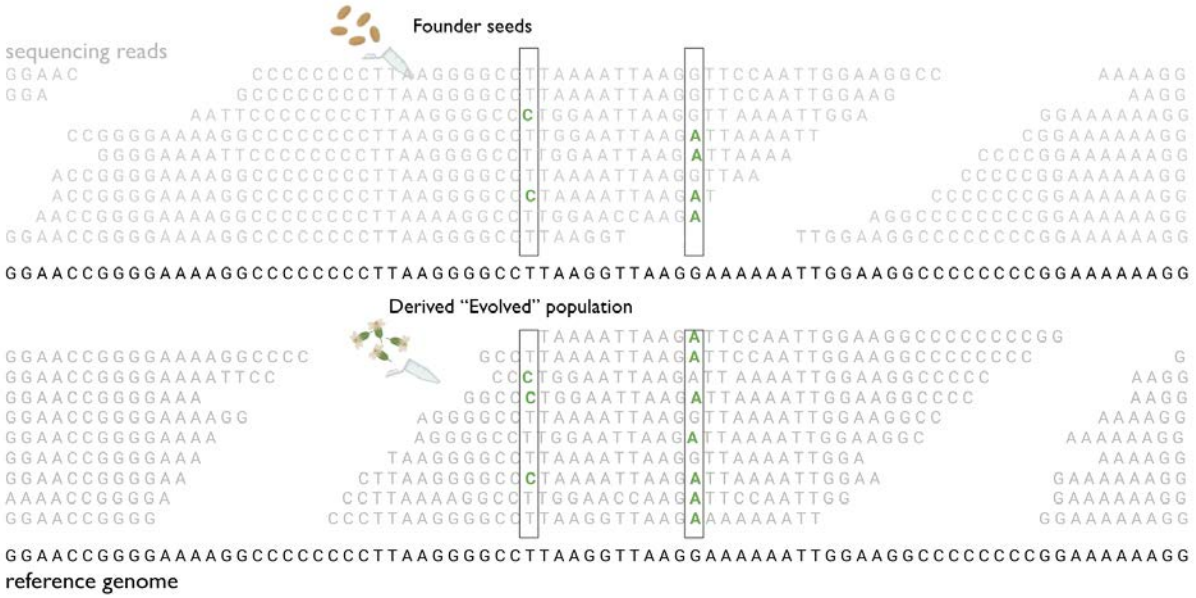


Fig. S1 | Cartoon of rationale of Pool-Seq

Reads from Illumina sequencing (typically ~150 bp, not at scale) "piled" against the region of the genome where they map to. The rationale is that if founder allele frequencies, or a reference sample that did not experience natural selection, we can extract meaningful evolutionary insights from comparing those with "evolved" populations, i.e. those that have grown in outdoor environments.

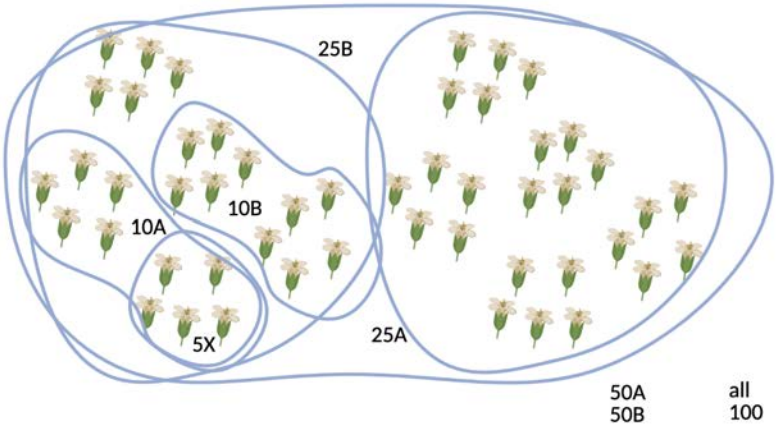


Fig. S2 | Hierarchical sampling of flowers for Pool-sequencing of different sizes
The selection of 50 individuals of Experiment 3 was conducted randomly from ~2,500 plants, but smaller sets of individuals were conducted in a nested fashion (e.g. the 25B samples were the same individuals as the 10A, 10B, and 5X sample. This may enable downstream allele frequency comparisons).

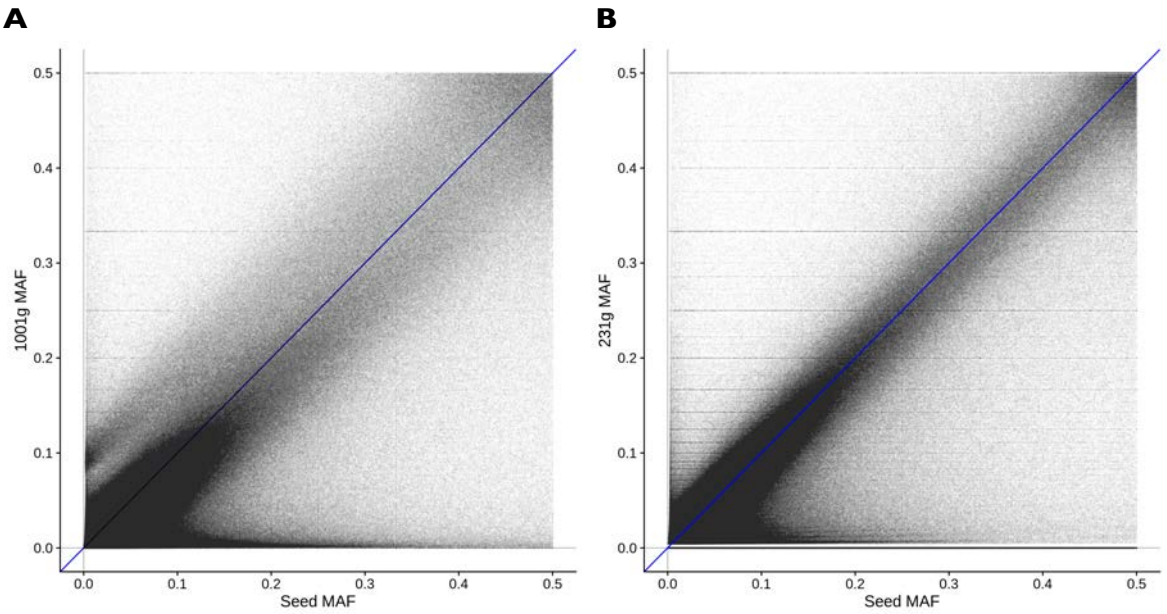


Fig. S3 | Relationship between the *in silico* quantified minor allele frequency of the 1001 Arabidopsis Genomes VCF file and the minor allele frequency of seed founders using Pool-Seq bam/pileup-based computation.

The x-axis is the folded seed founder frequency used to source outdoor experiments of GrENE-net.org, based on counting nucleotides at each locus in a bam/pileup file of the mapped reads (we converted bam to pileup for easier file parsing; same in all bam-based plots below). The y-axis is the folded frequency characterized from (A) the 1001 Genomes VCF and (B) the GrENE-net founder VCF.

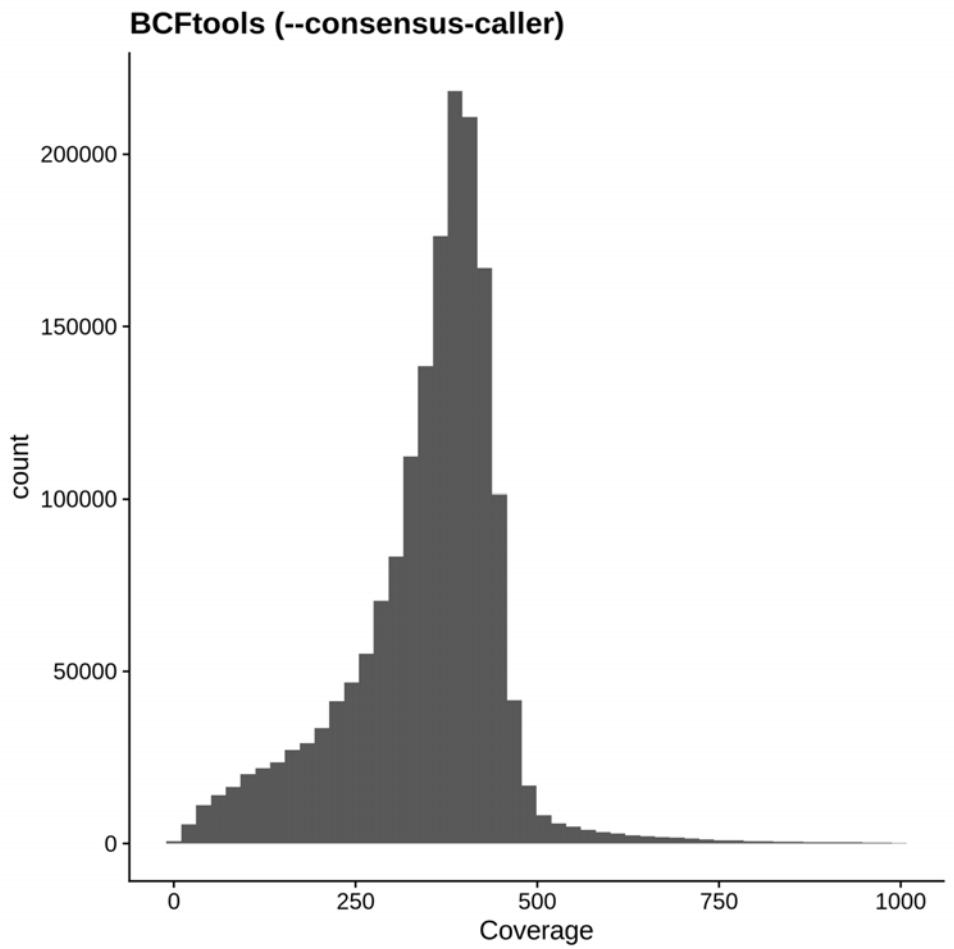


Fig. S4 | Coverage of the seed sequencing

Example of the coverage of the seeds as a *in silico* merge of 8 library samples (see **Table SX2**). The medium coverage is 400X.

coverage-all

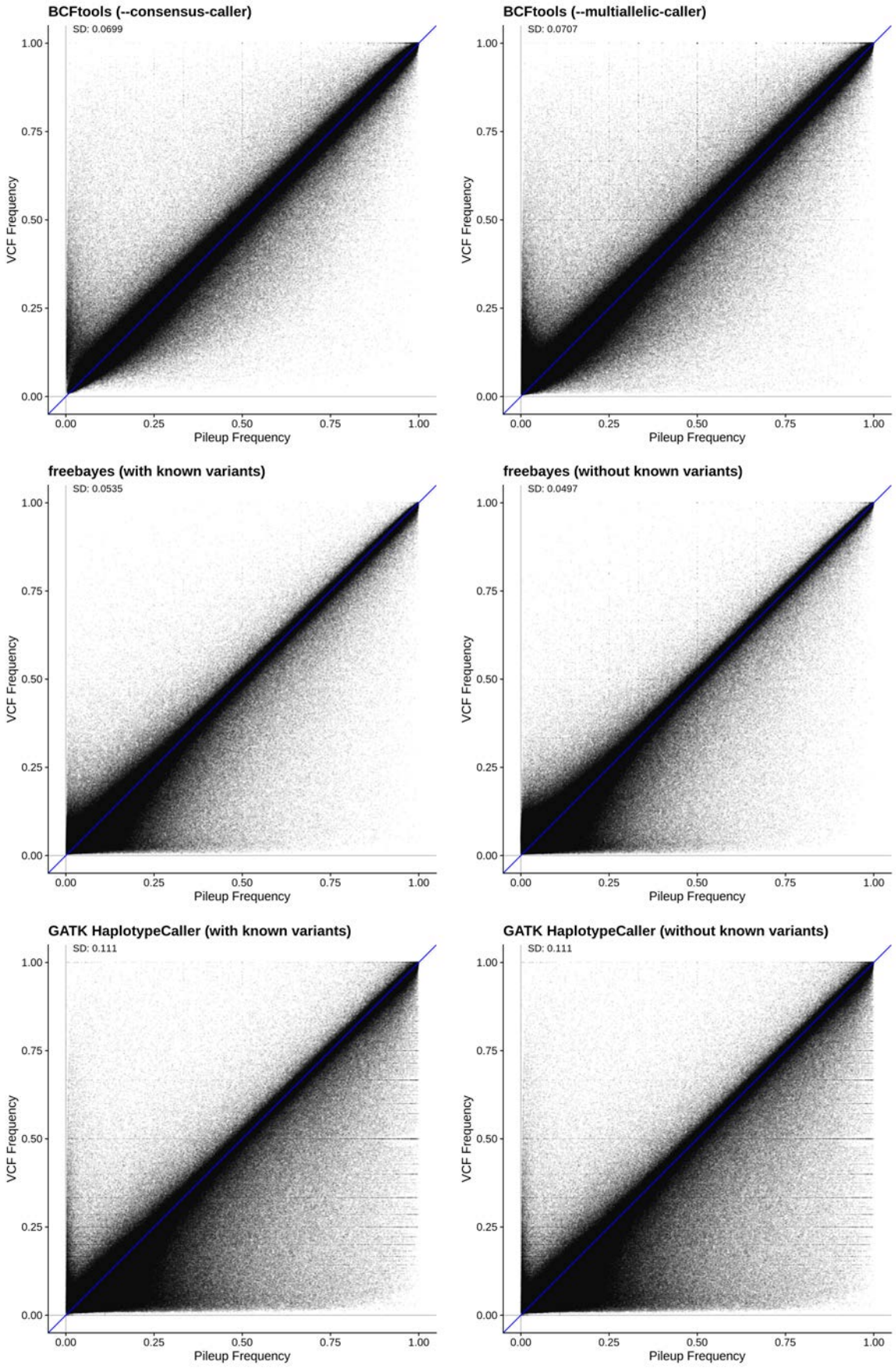
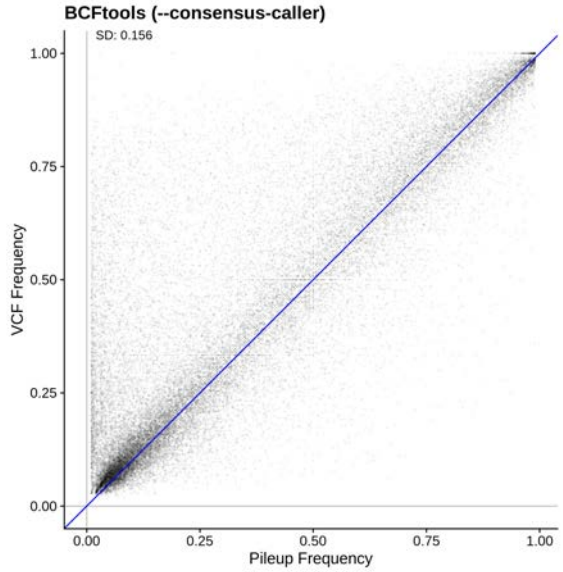
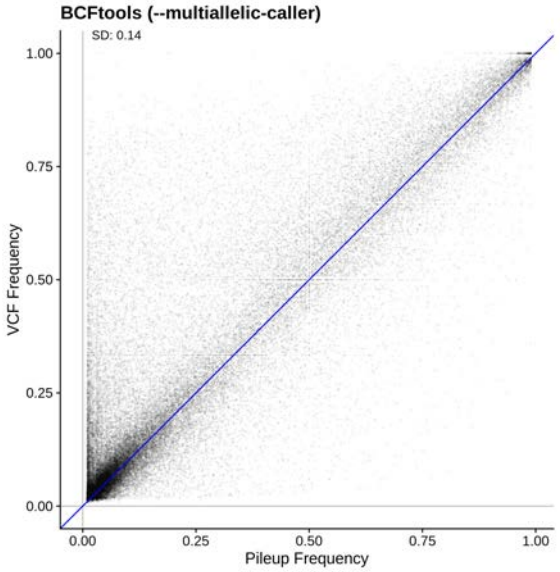
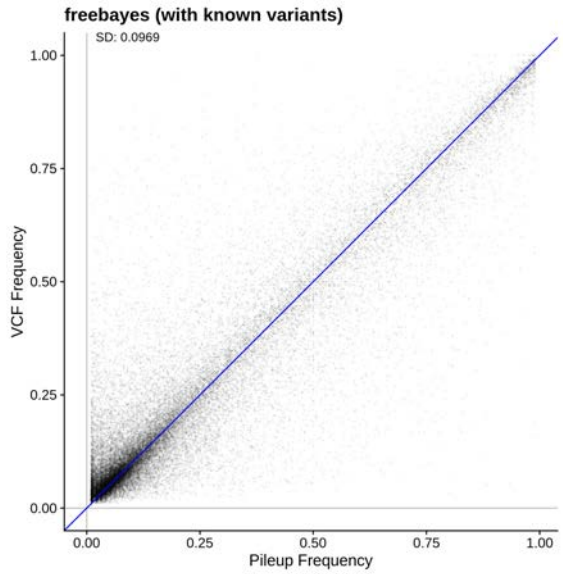


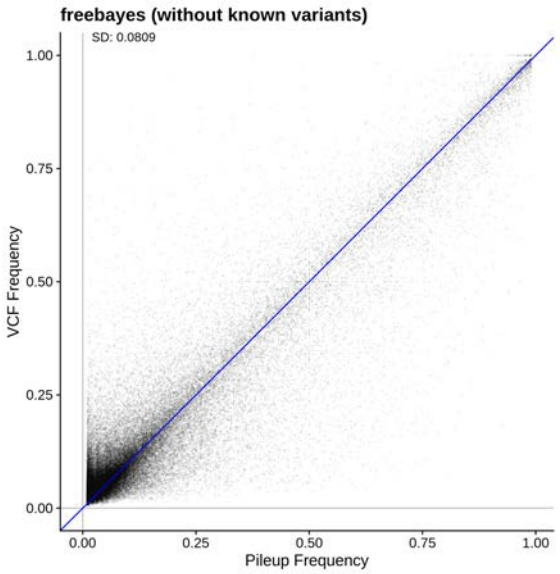
Fig. S5 | Correlation between raw frequencies computed with Pileup and frequencies estimated from different SNP callers's allelic depth field across all genomic positions regardless of coverage.

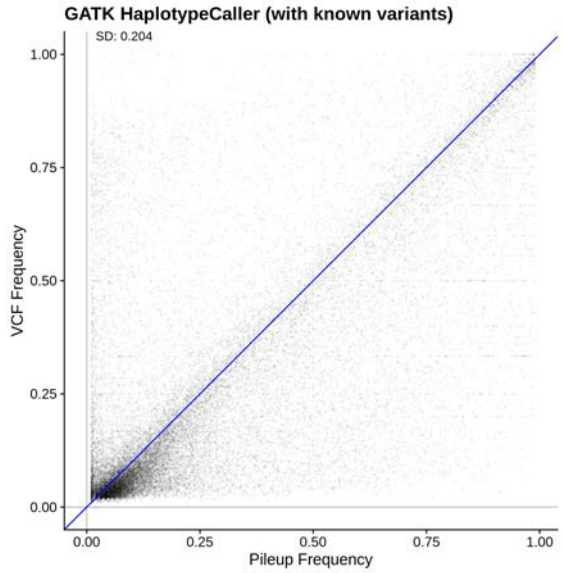
Coverage 50-100X











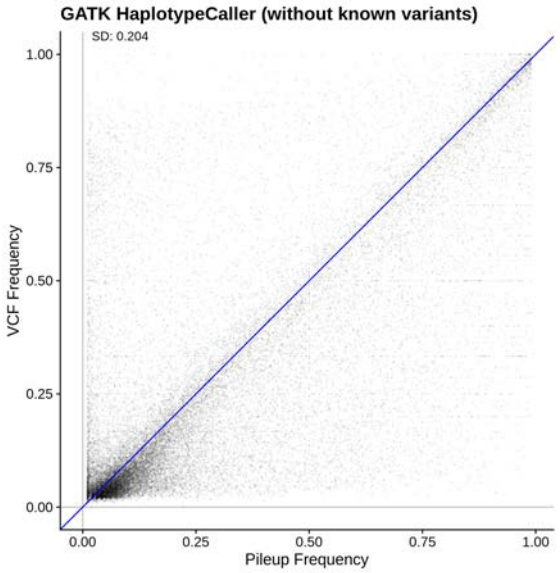


Fig. S6 | Correlation between raw frequencies computed with Pileup and frequencies estimated from different SNP callers's allelic depth field across genomic positions with 50x to 100x coverage

coverage-100-250

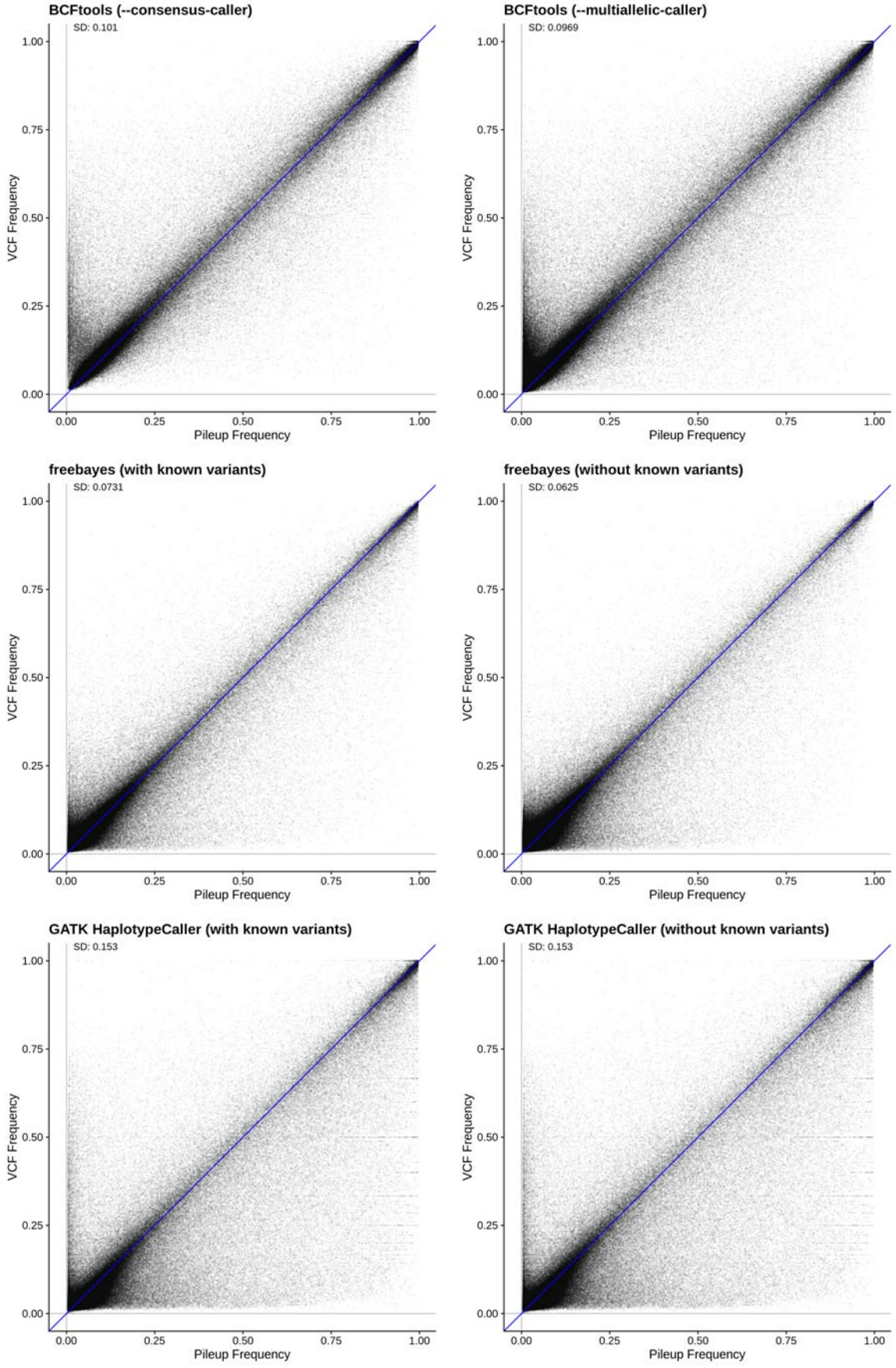


Fig. S7 | Correlation between raw frequencies computed with Pileup and frequencies estimated from different SNP callers's allelic depth field across genomic positions with 100x to 250x coverage

Coverage-250-500

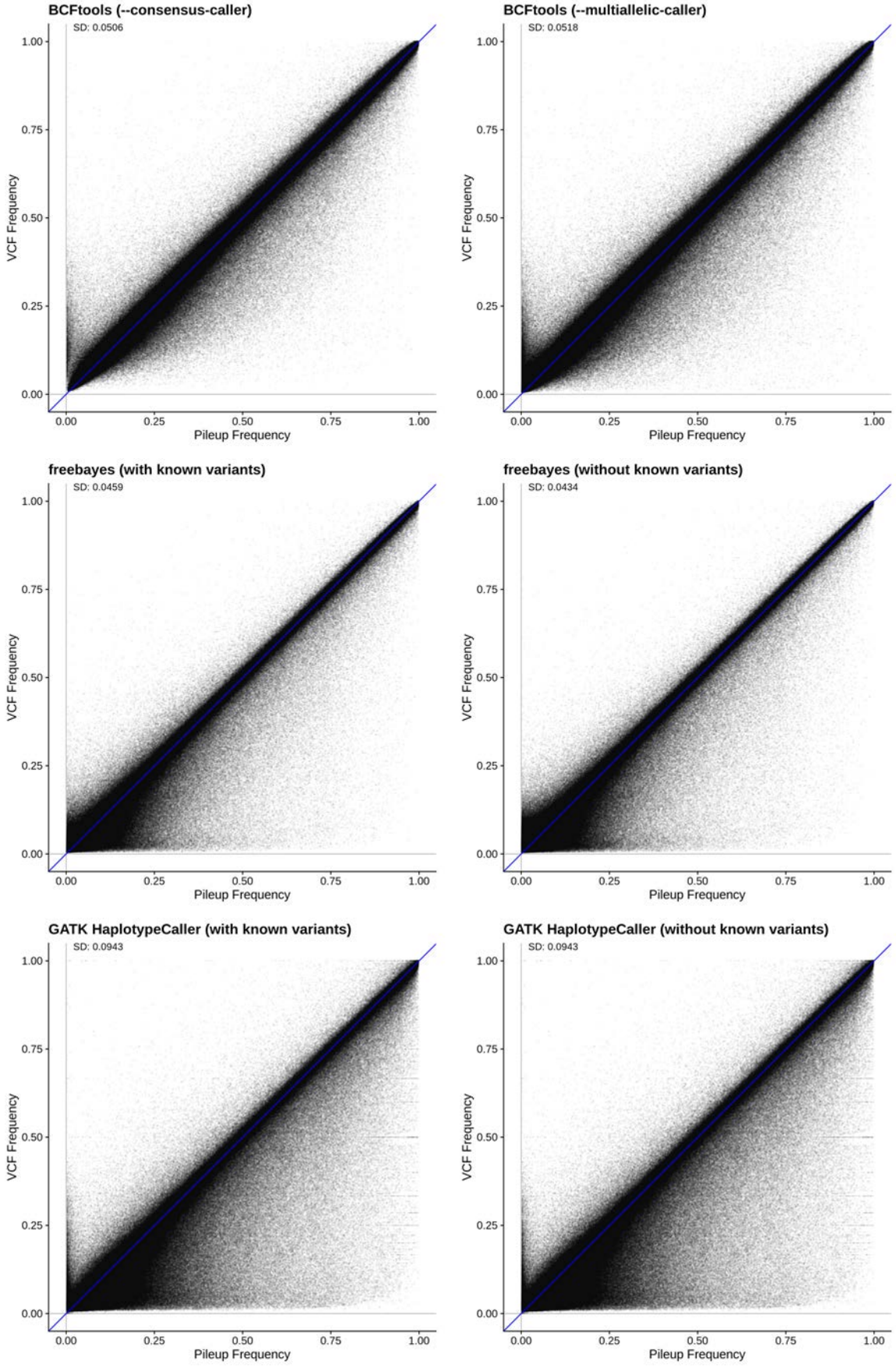


Fig. S8 | Correlation between raw frequencies computed with Pileup and frequencies estimated from different SNP callers's allelic depth field across genomic positions with 250x to 500x coverage

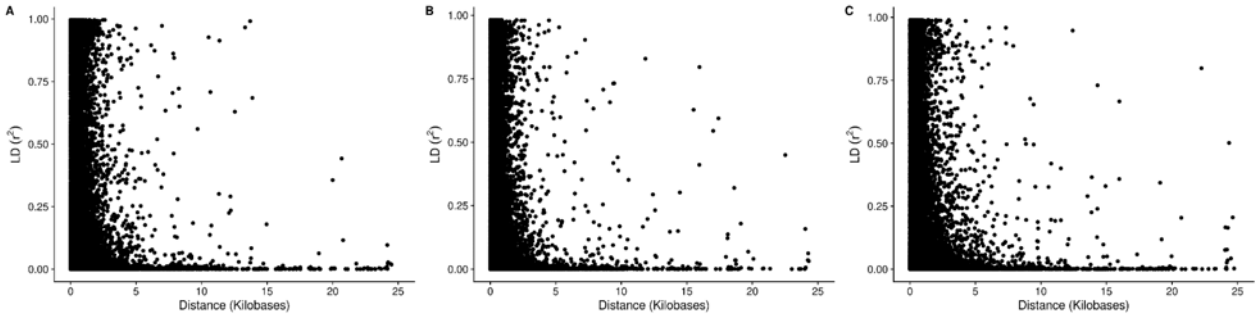


Fig. S9 | LD decay in the 1001G, the 470 and 231 and accessions sets

Linkage Disequilibrium LD decay using r^2 for the genome collection of the (**A**) 1001 Genomes Project, (**B**) a subset of 231 used in Experiment 1-3, and (**C**) a subset of 451 of the 1001 Genomes used in Experiment 4.

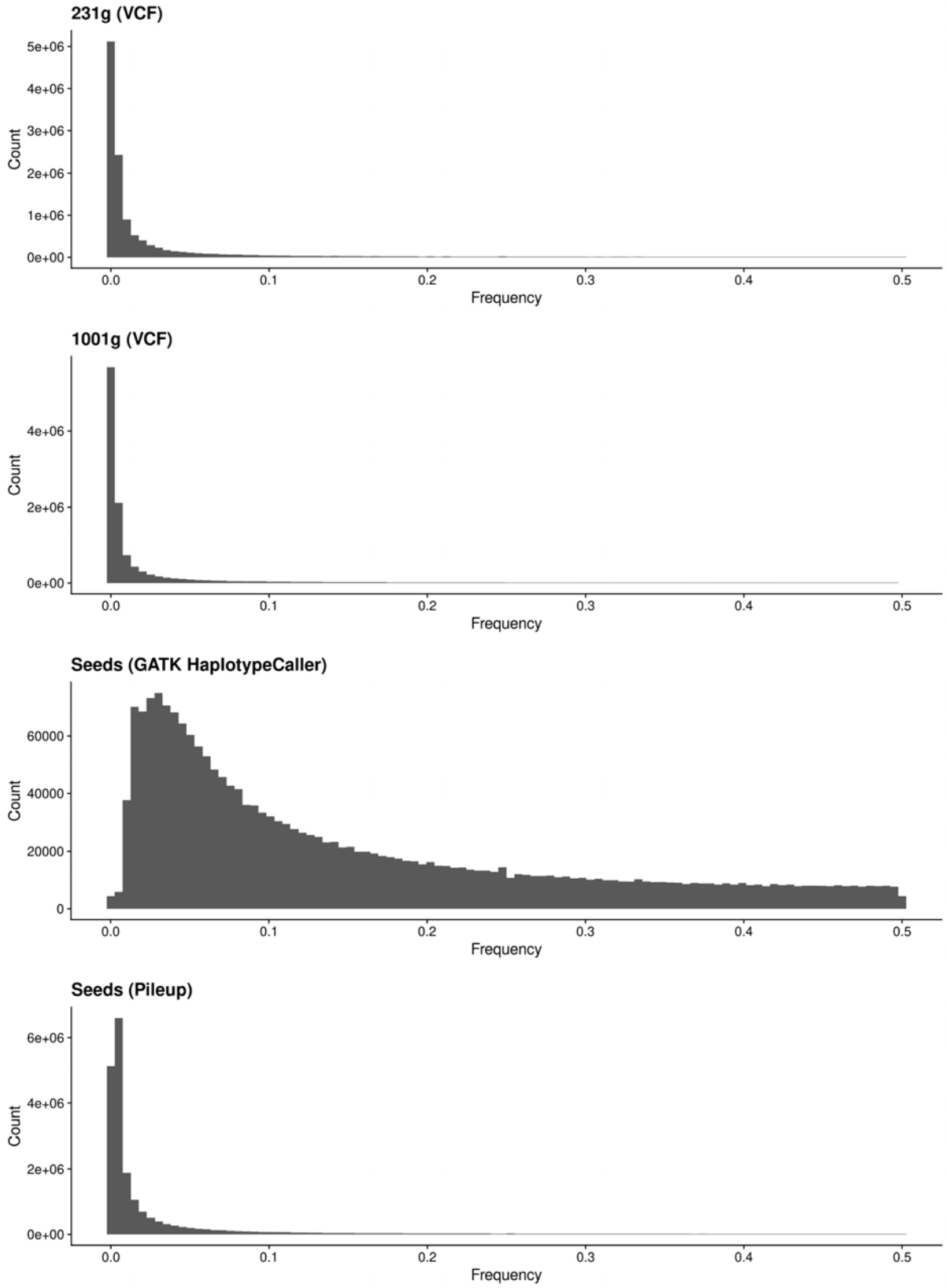


Fig. S10 | The problem of SNP calling for Pool-Seq using diploid callers

We can show the frequency of variants in the 1001 genomes or the 231 genomes subset is negative exponential, as expected from the Site Frequency Spectrum. The frequency calling of seeds, with high coverage, appears to be biased for intermediate frequencies in GATK HaplotypeCaller but retains the exponential decay using ratios of bases based on pileup using g.enedalf.

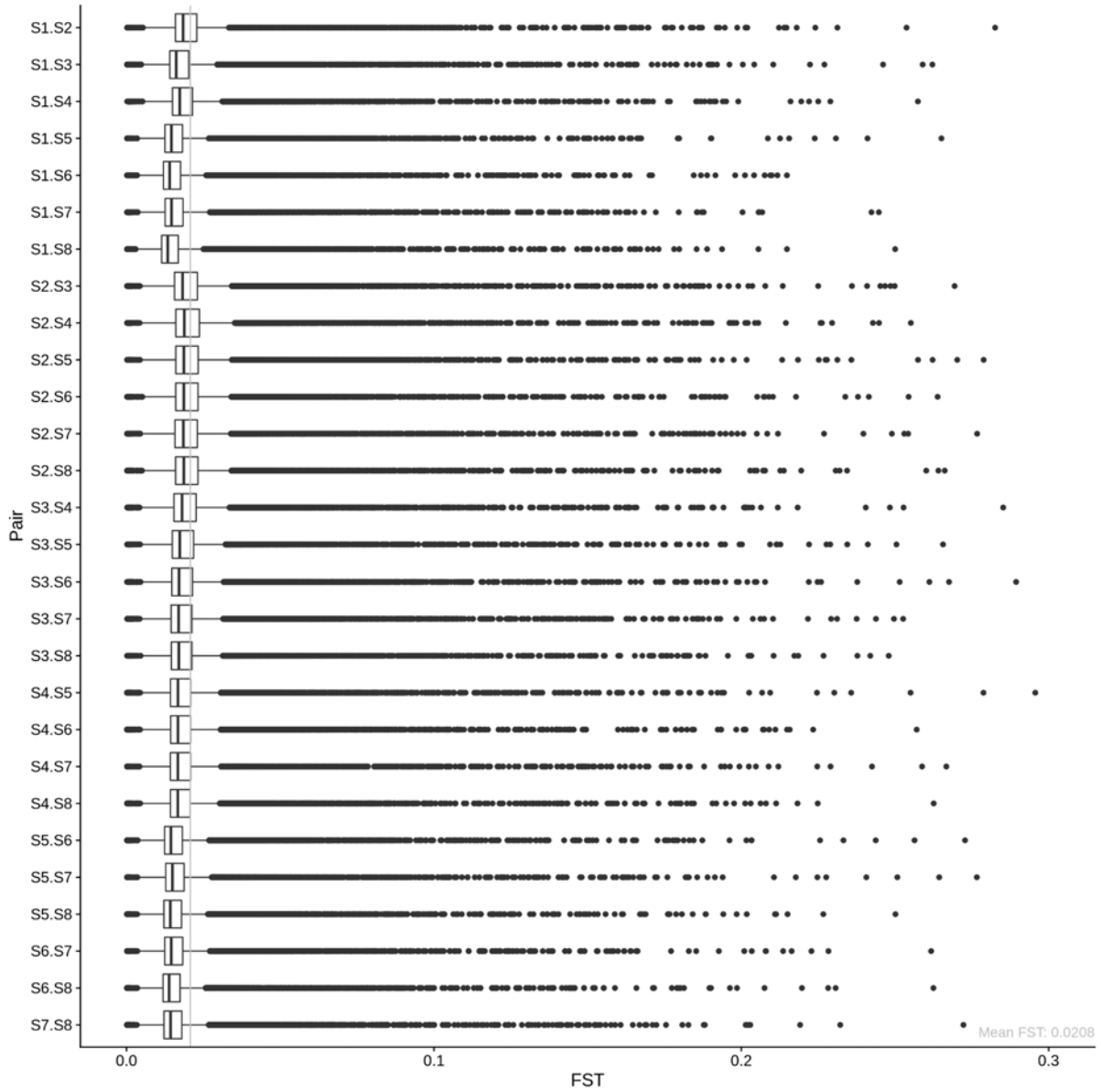


Fig. S11 | Genome-wide *Fst* in windows of 10k base pairs, between all pairs of founder seeds.

Here, we computed Fst between all pairs of seeds (Experiment 1) in windows of 10k base pairs across all five chromosomes, based on frequencies from the mapped data (bam/pileup files). The window size was chosen to roughly fit the expected LD decay in *A. thaliana*. The boxes show the 25th, 50th, and 75th percentiles (i.e., quartiles), with whiskers extending to 1.5 times the interquartile range (distance between the first and third quartiles). Data points outside this range are plotted as individual points. The overall average Fst between all pairs is 0.0208, shown here as a gray vertical line, which represents the biological and statistical noise in population structure between replicates, and hence is the lower bound and baseline that we expect in other comparisons of Fst, as shown below.

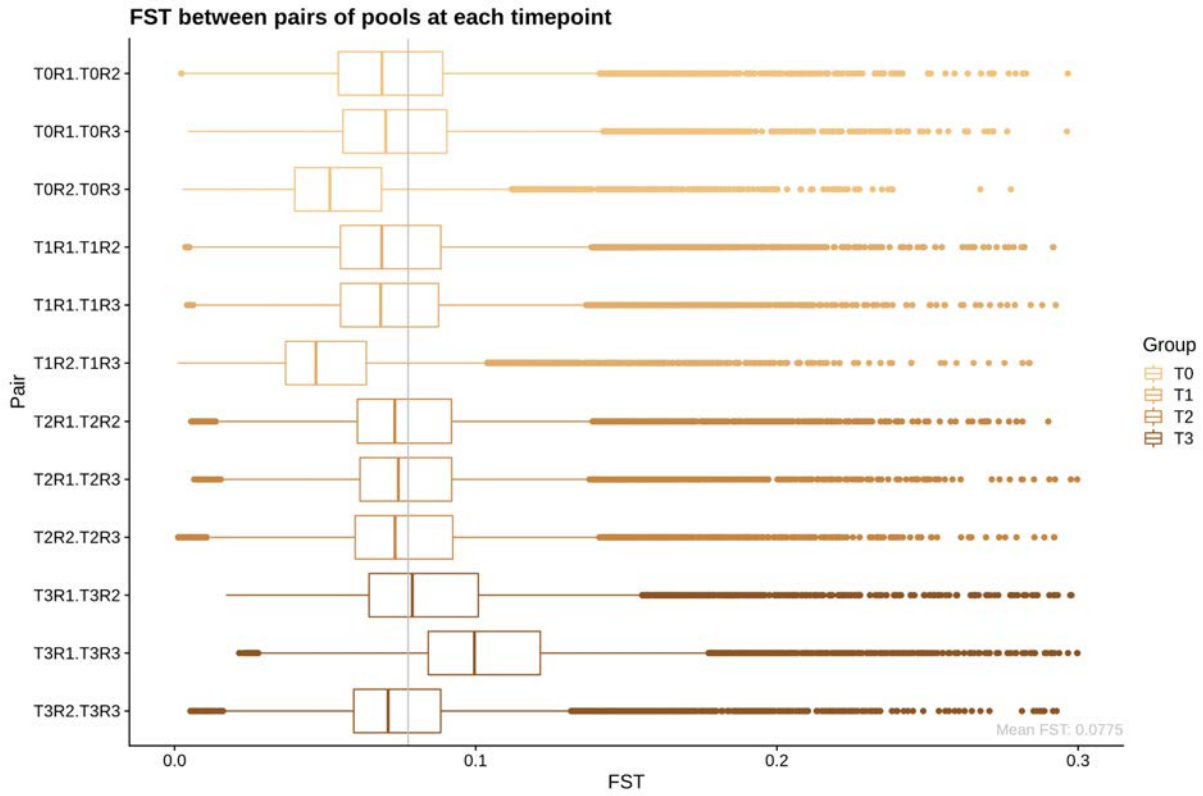


Fig. S12 | Genome-wide *Fst* in windows of 10k base pairs, between all technical replicates of one generation of our common garden experiment at the same time point. Here, we used the data from Experiment 4, which are three technical replicates (R1-R3) grown from the same seed mix (here encoded as "time point" T0), where flowers were collected at three different time points (T1-T3) during flowering time in the spring of 2016. We here show Fst between all pairs of replicates, across all time points, in windows of 10k base pairs across all five chromosomes. The window size was again chosen to fit the expected LD decay in *A. thaliana*. Properties of the box plots are as above in Figure SX. The mean Fst, again represented as a gray vertical line here, is 0.0775, which is more than three times the value of the seed baseline of 0.0208 in Figure X. Note that the Fst of pairs that involve Replicate 1 is higher than that of the R2 vs R3 pairs. This is likely because R1 suffered a disturbance in the soil that could have created a bottleneck in this population and hence increased differentiation.

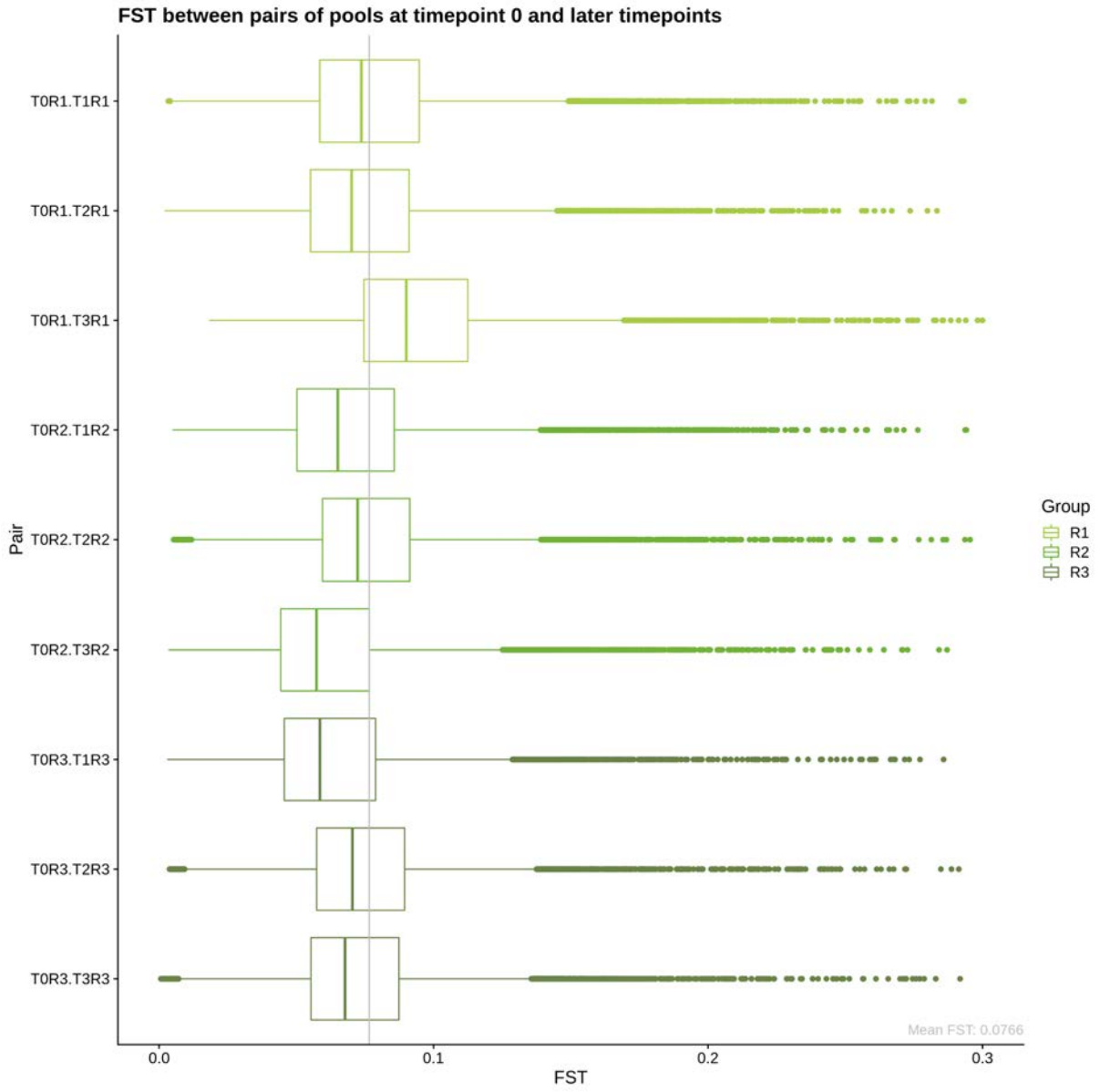


Fig. S13 | Genome-wide *Fst* in windows of 10k base pairs, between founder seeds and technical replicates of one generation of our common garden experiment.
Here, we used the data from Experiment 4, which are three technical replicates (R1-R3) grown from the same seed mix (here encoded as "time point" T0), where flowers were collected at three different time points (T1-T3) during flowering time in the spring of 2016. We here show Fst between the seeds and the flowering time points for each replicate, in windows of 10k base pairs across all five chromosomes. The window size was again chosen to fit the expected LD decay in *A. thaliana*. Properties of the box plots are as above in Figure X. The mean Fst across all replicates and timepoints, again represented as a gray vertical line here, is 0.0766, which is more than three times the value of the seed baseline of 0.0208 in Figure X. This indicates that even within one generation (from seeds to flowers), there is some differentiation happening, which suggests that rapid adaptation to the local environment of the field site has taken place.

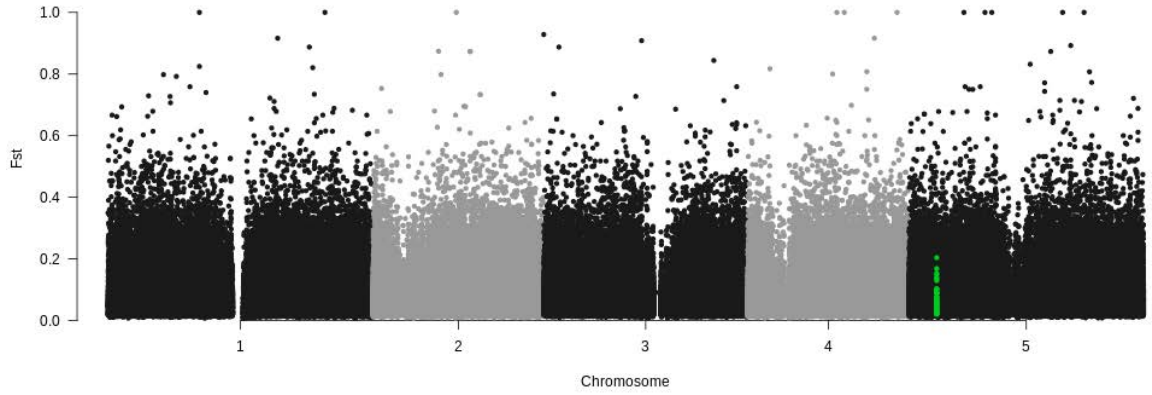


Fig. S14 | Example genome-wide F_{ST} for outdoor replicate 1 from baseline "without selection" and the flower samples of timepoint T3.

Genome-wide F_{ST} calculated using g$_r$enedalf

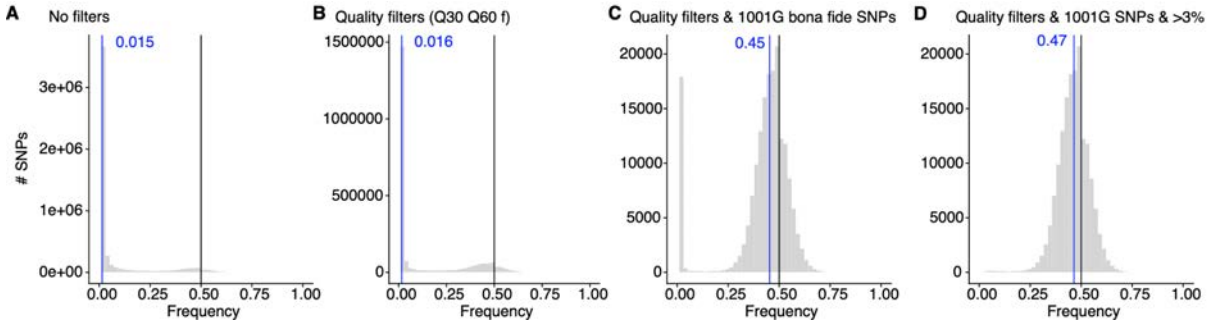


Fig. S15 | Allele frequencies in the 2 ecotype equal mass DNA pool from leaf tissue using different quality filters

Histograms of allele frequencies from a pooled library of 2 distinct ecotypes and the expectation of 50% (black line). (**A**) Allele frequencies without any filter show the great majority of alleles must be artifacts, as there is a high point mass close to 0 frequency. (**B**) Reduction of likely artifacts, yet still high noise, using quality filters of bases with PHRED score above 30 (Q30) and from reads with mapping quality over 60 (q60) and for reads where forward and reverse map to the same region (f). (**C**) Subsetting allele frequencies to only those SNP found in the 1001 Genomes (1001 Genomes Consortium, 2016) mostly removes all the noise signal with the exception of some rare variants. (**D**) Final removal of SNPs with only 1 or 2 bases supporting the alternative allele (minimum allele count >2) finally leaves a clean Normal distribution of allele frequencies (owed to limited coverage) around the expected 50%.

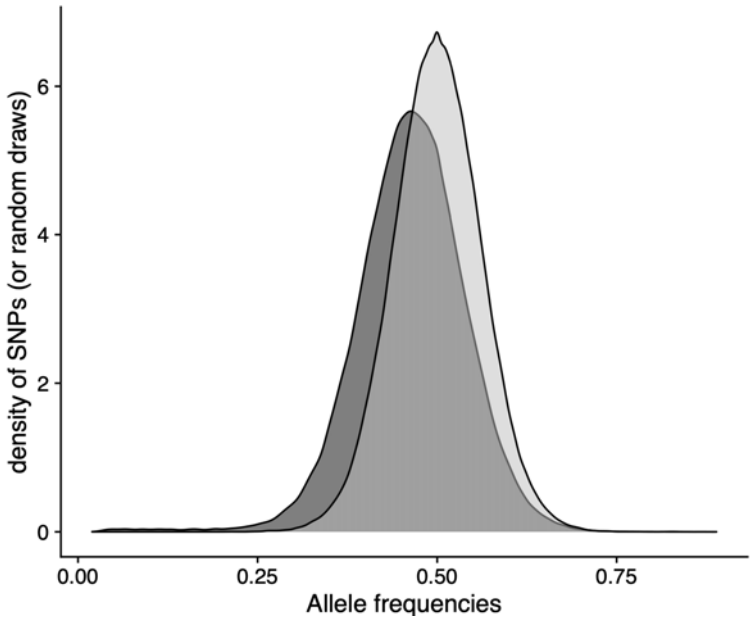


Fig. S16 | Example random Binomial draws and recovered allele frequencies

The distribution from **Fig. S15D** (black) compared to a random Binomial distribution with expected average frequency 50% (grey) and the same coverage distribution as the empirical sample.

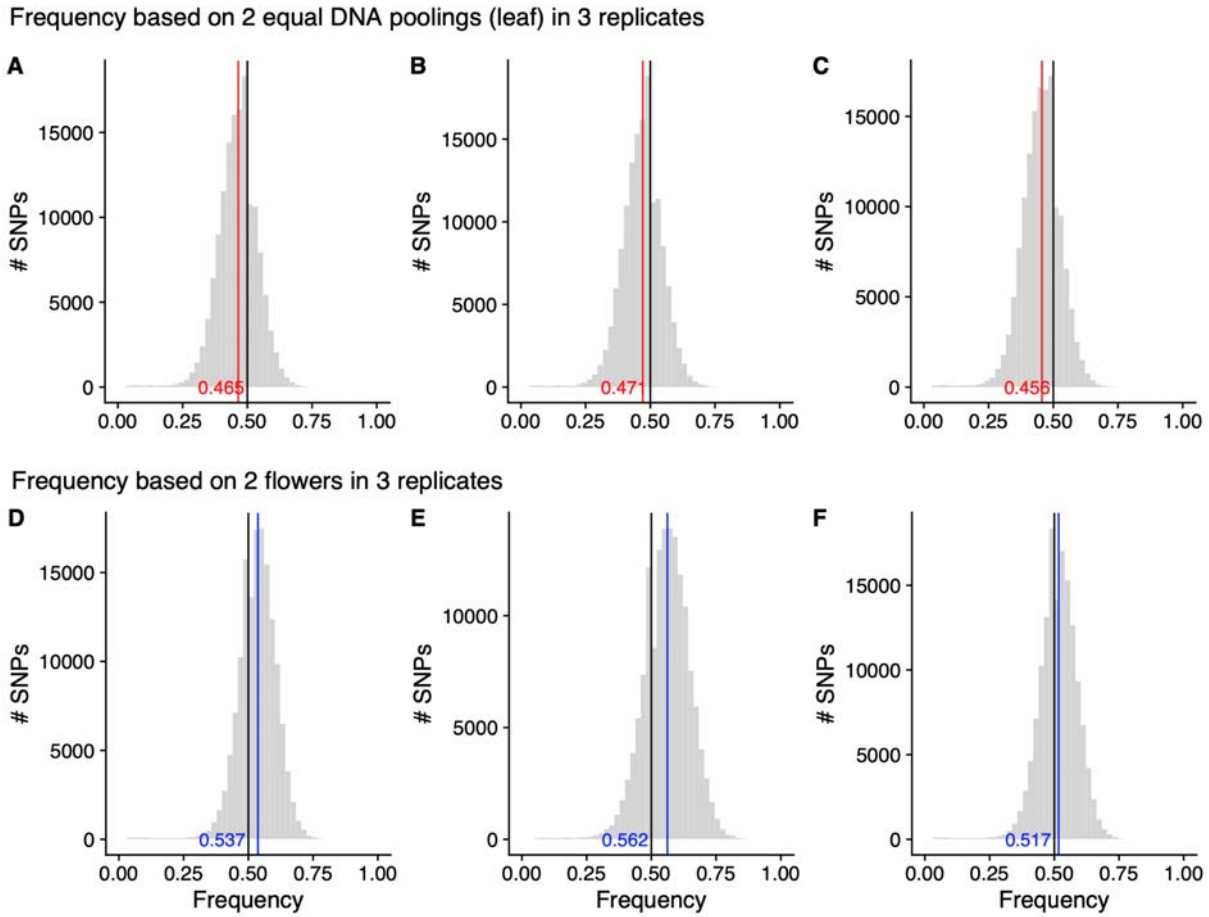


Fig. S16 | Fraction of DNA contribution to Pool-seq for a 2-flower pool and a 2-leaf pool Dispersal of allele frequencies from the expected 50% (black vertical lines) for a pool of 2 DNA sources at equal concentration (red, **A-C**) and two flower pools (**D-F**). Both replicated three times.