bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Title: Assessment of population differentiation and linkage disequilibrium in *Solanum pimpinellifolium* using genome-wide high-density SNP markers

Authors: Ya-Ping Lin, Chu-Yin Liu, Kai-Yi Chen

Institutional affiliations:

Department of Agronomy, National Taiwan University, Taipei, Taiwan, 10617

Data available:

Short reads of DNA sequences: NCBI SRA BioProject Number PRJNA358110

Supplemental figures: https://doi.org/10.6084/m9.figshare.7010495.v1

Supplemental tables: https://doi.org/10.6084/m9.figshare.7010492.v1

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Short running title: RADseq in *S. pimpinellifolium*

Keywords: *Solanum pimpinellifolium*, population differentiation, linkage disequilibrium pattern, restriction site associated DNA sequencing.

Corresponding author: Kai-Yi Chen

Office mailing address: No.1, Sec. 4, Roosevelt Rd., Da'an Dist., Department of Agronomy, National Taiwan University, Taipei City, Taiwan, 10617

Phone number: +886 2 3366 4766

Email address: kaiychen@ntu.edu.tw

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

ABSTRACT

To mine new favorable alleles for tomato breeding, we investigated the feasibility of utilizing *Solanum pimpinellifolium* as a diverse panel of genome-wide association study through the restriction site-associated DNA sequencing technique. Previous attempts to conduct genome-wide association study using *S. pimpinellifolium* were impeded by an inability to correct for population stratification and by lack of high-density markers to address the issue of rapid linkage disequilibrium decay. In the current study, a set of 24,330 SNPs was identified using 99 *S. pimpinellifolium* accessions from the Tomato Genetic Resource Center. Approximately 84% *Pst*I site-associated DNA sequencing regions were located in the euchromatic regions, resulting in the tagging of most SNPs on or near genes. Our genotypic data suggested that the optimum number of *S. pimpinellifolium* ancestral subpopulations was three, and accessions were classified into seven groups. In contrast to the SolCAP SNP genotypic data of previous studies, our SNP genotypic data consistently confirmed the population differentiation, achieving a relatively uniform correction of population stratification. Moreover, as expected, rapid linkage disequilibrium decay was observed in *S. pimpinellifolium*, especially in euchromatic regions. Approximately two-thirds of the flanking SNP markers did not display linkage disequilibrium. Our result suggests that higher density of molecular markers and more accessions are required to conduct the genome-wide association study utilizing the *Solanum pimpinellifolium* collection.

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

INTRODUCTION

The wild tomato species *Solanum pimpinellifolium* is a native perennial shrub in Ecuador and Peru and is believed to have originated in northern Peru and then diversified into several subpopulations after it migrated to Ecuador and southern Peru (Rick *et al.* 1977; Zuriaga *et al.* 2009; Blanca *et al.* 2012, 2015). The accessions in northern Peru display higher genetic variation and a higher outcrossing rate than those in southern Peru (Rick *et al.* 1977; Caicedo and Schaal 2004). Recent studies suggested that *S. pimpinellifolium* can be divided into at least three subpopulations: one in Peru, one in northern Ecuador and one in the mountains of Ecuador (Zuriaga *et al.* 2009; Blanca *et al.* 2012, 2015). In addition, the major climatic parameters, such as temperature and precipitation, show unidirectional gradient changes from southern Peru towards Ecuador (Zuriaga *et al.* 2009). Because geographic distributions of distinct *S. pimpinellifolium* subpopulations also aligned from south to north, it was proposed that the genetic distances between subpopulations were correlated with climatic differences (Zuriaga *et al.* 2009; Blanca *et al.* 2012, 2015).

S. pimpinellifolium is an attractive resource for tomato breeding because it can freely cross with cultivated tomatoes and introduces novel alleles into the limited gene pool of cultivated tomatoes (Tanksley and Mccouch 1997; Spooner *et al.* 2005; Moyle 2008). *S. pimpinellifolium* has been used as a genetic resource for disease resistance and fruit quality traits in tomato breeding (Grandillo *et al.* 2011; Víquez-zamora *et al.* 2014; Capel *et al.* 2015). A core collection of *S. pimpinellifolium* was developed by AVRDC for the

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

purpose of preservation and utilization (Rao *et al.* 2012). This core collection has been used to mine novel alleles of salt tolerance via genome-wide association study (GWAS) (Rao *et al.* 2015).

GWAS utilizes linkage disequilibrium (LD), the non-random association between marker alleles and alleles conferring targeted phenotypes in a given population of germplasm, to map quantitative trait loci (QTLs) (Soto-Cerda and Cloutier 2012). The average ranges of LD decay in different collections of cultivated tomatoes varied from 6 to 13 cM (Sim *et al.* 2012a; Pascual *et al.* 2015; Bauchet *et al.* 2017). It is expected that the range of LD decay is smaller in *S. pimpinellifolium* populations because *S. pimpinellifolium* presents larger genetic variation than cultivated tomatoes (Blanca *et al.* 2012, 2015; Ranc *et al.* 2012; Bauchet *et al.* 2017). Indeed, the SolCAP array with 7,720 SNPs did not achieve full LD coverage across all chromosomal regions for GWAS using *S. pimpinellifolium* accessions (Sim *et al.* 2012a, 2012b; Bauchet *et al.* 2017). The restriction site-associated DNA sequencing (RADseq) technique might provide an inexpensive solution to address this challenge (Davey and Blaxter 2010). The RADseq technique limits sequencing resources at the vicinity of restriction enzyme cutting sites and therefore provides flexibility of experimental design in terms of the trade-off between cost-effectiveness and marker densities (Chen *et al.* 2014; Bhakta *et al.* 2015).

The objective of the current study was to develop genome-wide high-density SNP markers for a subset of *S. pimpinellifolium* collections from the Tomato Genetic Resource Center (TGRC) through the RADseq approach. The population differentiation and the range of LD decay were then assessed.

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

MATERIALS AND METHODS

Plant materials

All plant materials and their information were obtained from TGRC (Table S1; http://tgrc.ucdavis.edu/). In this study, 12 accessions from Ecuador and 87 accessions from Peru were utilized. According to their mating types, 43 accessions were facultative self-compatible (FSC) and 56 accessions were autogamous self-compatible (ASC). Seeds were propagated by self-pollination for two generations using the method of single-seed descent in a greenhouse. Young leaves collected from plants of these single-seed descendent seeds were used for DNA extraction.

RAD sequencing

Total genomic DNA was extracted from young leaves using a modified CTAB method (Fulton *et al.* 1995) and purified with a DNeasy Blood & Tissue Kit (QIAGEN, Venlo, Netherland) following the manufacturer's instructions. *Pst*I-digested DNA libraries were prepared following the protocol of Etter *et al*. (Etter *et al.* 2011). Four RADseq libraries were constructed, and each was sequenced in one lane of an Illumina HiSeq2000 flow cell (100 bp single-end reads) (Illumina Inc., San Diego, CA, USA).

SNP calling

Reads were analyzed with Stacks version 1.37 (Catchen *et al.* 2013) and with CLC Genomics Workbench software version 6.5.1 (QIAGEN, Venlo, Netherlands) ("CLC Genomics Workbench 6.5.1"). First, the *process_radtags* command in Stacks filtered out low-quality reads with Q scores less than 20.

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

The remaining reads were mapped to the tomato reference genome SL2.50 (Fernandez-Pozo *et al.* 2015) using the "Map Reads to Reference" tool in the CLC Genomics Workbench software. Considering that genetic variation between the tomato reference genome *S. lycopersicum* and *S. pimpinellifolium* is larger than genetic variation within *S. lycopersicum*, mapping parameters were set as 0.5 for the length fraction and 0.9 for the similarity fraction. The reads of the same individual in different lanes were merged together. In the subsequent analyses using Stacks, the *ref_map.pl* command set the parameter –m (minimum read depth to create a stack) as 10, and the *populations* command set the parameter –p (minimum number of populations a locus must be present) as 75. SNPs with a minor allele frequency of less than 0.05 were further excluded, and a set of 24,330 SNP markers was obtained. ITAG2.4 gene model from SGN was used as the reference gene annotation.

Identification of insertion/deletion (InDel) and simple sequence repeat (SSR) markers

InDels were identified from the Sequence Alignment Map (SAM) files of all *S. pimpinellifolium* accessions with a read depth of no less than two using the "InDels and Structural Variants" tool and the "Compare variants" tool provided in the CLC Genomics Workbench software ("CLC Genomics Workbench 6.5.1"). InDel markers in the form of tandem repeated sequences were classified as SSR markers.

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Population differentiation

To avoid redundant SNP markers used in the subsequent analyses, only one SNP that showed complete LD (r^2 = 1) in the same sequencing block around a *Pst*I site was kept whenever more than one SNP existed in the sequencing block. This process resulted in a total of 19,993 SNP markers extracted from the set of 24,330 SNPs noted above. Principle component analysis (PCA) was performed using TASSEL5.0 (Bradbury *et al.* 2007). Population differentiation was investigated via ADMIXTURE (Alexander *et al.* 2009). Calculation of pairwise F_{st} (Weir and Cockerham 1984) and analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) were conducted in the R packages hierfstat (Goudet and Jombart 2015) and StAMPP (Pembleton *et al.* 2013), respectively.

Estimate of genetic variation and LD

The genotypes of the 24,330 SNP markers were used to estimate genetic variation and LD in this *S. pimpinellifolium* population. Genetic variation within overall accessions and each of seven groups was assessed based on observed heterozygosity and the within-population gene diversity (expected heterozygosity) using the R package hierfstat (Goudet and Jombart 2015). Pairwise r^2 values between SNP markers were calculated to assess overall extent of LD via plink1.9 within a 1-Mb window (Gaunt *et al.* 2007) and fit by non-linear regression (Remington *et al.* 2001). The baseline of the r^2 value was set at 0.1 (Bauchet *et al.* 2017). To assess the local LD along each chromosome, we defined the basic unit for local LD as the sequencing region surrounding a *Pst*I site, usually 186 bp long, which has at least one SNP with a

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

minor allele frequency greater than 0.05 in the *S. pimpinellifolium* population. For each pair of consecutive basic units, the average r^2 was calculated between two SNPs in different basic units and plotted along the left *Pst*I cutting site based on the physical position. The heterochromatin regions were marked according to the genetic map of EXPIM 2012 and the physical map of the tomato reference genome (Sim *et al.* 2012b).

Analysis of SolCAP array data of *S. pimpinellifolium*

The SolCAP data of 214 samples of *S. pimpinellifolium* were downloaded from previous studies (Blanca *et al.* 2012, 2015; Sim *et al.* 2012a). A set of 4,326 bi-allelic SNPs was first extracted and filtered with the criteria that minor allele frequency is greater than 0.05 and the proportion of missing genotypes is less than 25%. These SNP-filtering criteria are the same as the criteria applied to the SNP dataset generated in this study. This procedure resulted in 2,817 SNPs. Subsequently, population differentiation was investigated by ADMIXTURE (Alexander *et al.* 2009). Because some accessions appeared in different SolCAP genotyping studies and their genotypes were not completely matched, different suffixes—"_2012S," "_2012B," and "_2015B,"—were added to the accession name to indicate their origins from references Sim *et al.* 2012a, Blanca *et al.* 2012, and Blanca *et al.* 2015, respectively. In addition, the percentage of identical SNP genotypes between accessions with the same name was calculated based on the 4,326 SNP genotypes and excluding missing values.

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Data availibility

All the sequences of RADseq are available at the NCBI SRA database, and the BioProject Number is PRJNA358110. Supplemental files available at FigShare: https://doi.org/10.6084/m9.figshare.7010495.v1 for supplemental figures; https://doi.org/10.6084/m9.figshare.7010492.v1 for supplemental tables.

RESULTS

Identification of 24,330 SNPs from *Pst*I-digested DNA libraries

A total of 655,973,270 short DNA reads were obtained from four lanes of the Illumina HiSeq2000 flow cell and were divided into 99 parts according to barcode sequences. Each part was derived from the DNA of a *S. pimpinellifolium* accession and contained at least 3.7 million DNA reads, except for LA2647 (Table S1). To ensure the accuracy of SNP calling and genotype calling, two criteria were set: one was that the read depth aligning to the reference sequences was equal to or greater than 10, and the other was that at least 75% of the accessions showed genotypes associated with a defined SNP marker.

Among the 82,814 *Pst*I sites in the tomato reference sequence SL2.50, only 23,988 *Pst*I sites were around the sequenced DNA reads (Table S2). The sequenced regions included 0.54% of the SL2.50 reference sequences and 12,790 annotated genes (Table 1). Interestingly, approximately 84% of the sequenced *Pst*I sites were located in the euchromatic regions (Table S2). Nevertheless, no significant difference was observed in the proportion of

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

sequencing regions for SNP discovery between the euchromatic regions (68.85%) and the heterochromatic regions (60.59%) (Table S2). A total of 67,804 SNPs were identified in the sequenced regions of 99 *S. pimpinellifolium* accessions, and 24,330 of them had a minor allele frequency greater than 0.05.

In the genotypic dataset of 24,330 SNP markers (Table S3), the missing proportion of each accession ranged from 0.72% to 15.92%, except for LA2647, for which the value was 65.68% due to a low number of sequencing reads (Table S1). Regarding the features of these 24,330 SNPs, 16,365 SNPs were located in 7,383 annotated genes (Table 1) and the remaining SNPs were located in the intergenic regions. In addition, 3,068 InDels (Table S4) and 107 SSR markers (Table S5) were obtained. In the subsequent analyses, only SNP markers were utilized, and the genotypic data of the LA0411 accession was dropped because the observed heterozygosity of LA0411 was inconsistent with its mating type (Table S1).

Genetic differentiation of *S. pimpinellifolium* corresponded to the geographic area

The collection of 98 *S. pimpinellifolium* accessions in this study was divided into seven groups corresponding to three ancestral populations using the ADMIXTURE software (Figure 1A and Figure S1). The seven groups included three groups with pure ancestry, three groups with an admixture of two different ancestries, and one group with an admixture of three ancestries. As expected, accessions in each group were clustered together in the PCA

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

plot, in which principal component 1 (PC1), PC2, PC3, PC4 and PC5 explained 16.04%, 8.00%, 3.94%, 3.12% and 2.54% of the variation, respectively (Figure 1B). Interestingly, most accessions in the same group were in the same vicinity in terms of their collection sites (Figure 1C). In addition, different ancestral groups were spread in somewhat distinct geographic areas along the coastline from Ecuador to southern Peru (Figure 1C). The geographic distribution of these groups appeared in the following order from north to south: the pure red ancestral group, the admixture group with red-blue ancestries, the pure blue ancestral group, the admixture group with blue-green ancestries, and the pure green ancestral group (Figure 1C). This geographic distribution showed a trend in which the admixture groups were located between their corresponding pure ancestral groups.

To compare genetic variation within pure ancestral groups or within admixture groups, the within-population gene diversity of each group was calculated. The blue group and the red-blue group showed the highest genetic variation among the pure ancestral groups and the admixture groups, respectively (Table 2). Both groups were in northern Peru, which indicated that northern Peru is the origin of *S. pimpinellifolium*. Pairwise F_{st} confirmed the population differentiation (Table S6), and AMOVA revealed that the variation between groups was 41.96% (p-value < 0.001).

The differentiation of two mating types, FSC and ASC, was expected because non-random mating would disrupt the Hardy-Weinberg equilibrium, leading to population structure (Weir and Cockerham 1984; Holsinger and Weir 2009). In this collection, most FSC accessions were clustered in northern Peru, while ASC accessions were scattered in Ecuador and central and

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

235 southern Peru, along the western side of the Andes Mountains to the coast
236 (Figure S2). The pairwise F_{st} (0.0029) of FSC and ASC was significant
237 (p-value < 0.001). However, PCA presented unclear clusters between FSC
238 and ASC (Figure S3). In addition, the variation between FSC and ASC was
239 only 5.91% despite the significance of AMOVA (p-value < 0.001).

240 **Rapid LD decay**

241 Overall LD decay was estimated for the mapping resolution in GWAS. In
242 this population, the non-linear regression curve dropped very quickly (Figure
243 S4). Following the non-linear regression curve, the overall LD decay was
244 within 18 Kb when the baseline of the r^2 value was set at 0.1 (Table 3 and
245 Figure 2A). The fastest LD decay was within 10 Kb on chromosome 9 while the
246 slowest decay was within 30 Kb on chromosome 4 (Table 3 and Figure S5).
247

248 **Heterogeneity of genetic recombination within each chromosome**

249 LD decay has often been estimated for each chromosome (Sim *et al.*
250 2012a; Bauchet *et al.* 2017). However, the LD decay per chromosome was
251 insufficient to capture the local variations of historically accumulated
252 recombination events because the tomato genome comprises more than 75%
253 heterochromatin, which usually suppresses recombination events (Sim *et al.*
254 2012a). The local LD profile of individual chromosomes was assessed based
255 on the average r^2 value of flanking sequencing units that contained at least
256 one SNP marker. Two major trends were observed (Figure 2B and Figure S6).
257 Marker density in the heterochromatic regions was lower than that in the
258 euchromatic regions, and approximately two-thirds of the r^2 values were less

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

than 0.1 (Table 3). The latter observation indicated that these flanking SNP markers were not in a state of linkage disequilibrium.

DISCUSSION

A similar distribution between genes and SNPs was identified in the vicinity of *Pst*I cutting site throughout the genome

The observation that 67.26% (16,365 to 24,330) of the SNPs were located in the annotated gene regions (Table 1) implied a correlation between the distribution of the identified SNPs in the current study and the distribution of the annotated genes. Additional observations in the current study indicated a preference for genomic DNA digestion by the *Pst*I restriction enzyme in the euchromatic regions: only 28.97% (23,988 to 82,814) of *Pst*I sites were found in the deep sequencing regions, and 83.55% (20,043 to 23,988) of the deep sequencing regions were located in the euchromatic region (Table S2). It is worth noting that the current RADseq protocol did produce low coverage of sequencing reads in certain *Pst*I sites (with a read depth less than 10), and these *Pst*I sites were filtered by the criteria of SNP and genotype calling; therefore, the deep sequencing regions indicated that their read depths were no less than 10. Incidentally, because SNPs can be identified only in the sequenced regions, it is a reasonable deduction that most SNPs found in the current study are located in the euchromatic regions. Plotting the annotated genes, the expected *Pst*I sites, the *Pst*I sites in the deep sequencing regions, and the 24,330 SNPs identified in the current study (Figure 3A, 3B, 3C, and 3D,

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

282 respectively), shows clearly that the annotated tomato genes, the *Pst*I sites in
283 the deep sequencing regions, and identified SNPs are mainly located in the
284 euchromatic regions.

285 *Pst*I is a methylation-sensitive restriction enzyme and recognizes the
286 sequences “CTGCAG” (Dobritsa and Dobritsa 1980). The study of the
287 genome-wide methylation pattern in tomato leaves and immature fruits
288 revealed that the gene-rich euchromatic regions at the distal ends of
289 chromosomes were characterized as the regions with low levels of cytosine
290 methylation at the “CG”, “CTG”, and “CAG” sequences, and the
291 pericentromeric heterochromatin regions were the regions with high levels of
292 cytosine methylation (Zhong *et al.* 2013). Because the young tomato leaves
293 were used as the DNA source to construct the RADseq libraries, it is
294 reasonable to infer that the *Pst*I-digested RADseq-targeted chromosomal
295 regions were concentrated in the gene-rich euchromatic regions. Therefore,
296 one can emphasize the sequencing resources on euchromatic regions via *Pst*I
297 RADseq when preparing candidate gene research for tomatoes.
298

299 **The discrepancy in inferences of population differentiation of *S.***
300 ***pimpinellifolium***

301 The estimation of the best subpopulation number (K) is very important in
302 GWAS because population structure is integrated as a correction to eliminate
303 the inflated significance due to confounding effects (Korte and Farlow 2013). If
304 the best K of a certain population could not be confirmed, the results of GWAS
305 would be unreliable. However, several previous studies did not achieve the

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

306 same best K of *S. pimpinellifolium*: 10 SSR markers for 248 individuals
307 obtained an unclear K (Zuriaga *et al.* 2009); 48 SSR markers for 190
308 individuals revealed a best K = 2 with admixtures following a K = 5 (Rao *et al.*
309 2012); finally, the SolCAP array for two collections of 63 and 112 individuals
310 obtained the same best K = 3 with admixtures (Blanca *et al.* 2012, 2015). Our
311 study obtained the best K = 3, but our ancestral and admixture groups were
312 different from the latter studies of the SolCAP array (Blanca *et al.* 2012, 2015).
313 These previous studies suggested that the *S. pimpinellifolium* population was
314 differentiated into three ancestral groups: one in the northern Ecuador; another
315 in the mountainous area from southern Ecuador extending to northern Peru,
316 and the third in the low-altitude areas of Peru, along with certain admixtures
317 (Blanca *et al.* 2012, 2015). In contrast, our study showed that the
318 *S. pimpinellifolium* accessions were clustered into three pure ancestral groups,
319 with one in Ecuador (the red group), another in northern Peru (the blue group),
320 and the third in southern Peru (the green group), as well as three clearly
321 identified admixture groups (Figure 1C).
322 To investigate the potential reasons for the inconsistent conclusions
323 between the current study and the previous studies based on SNP markers,
324 genotypic data of the *S. pimpinellifolium* accessions made from the SolCAP
325 array in three previous studies were obtained from internet (Blanca *et al.* 2012,
326 2015; Sim *et al.* 2012a) and a meta-analysis was conducted using our
327 workflow (please see details in the “Materials and Methods” section) (Table
328 S7). A total of 214 samples representing 126 accessions were divided into 11
329 groups via ADMIXTURE using filtered genotypes of 2,817 SNP markers.
330 However, the results of this meta-analysis pose two problems. First, the

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

cross-validation error did not confirm that K = 11 was the optimal grouping method (Figure S7 and Figure S8). This condition can be explained as a low population structure in a population with high genetic diversity, which may have resulted from frequent gene flow (Gevaert *et al.* 2013). Overrepresentation of common SNPs within *S. pimpinellifolium* accessions on the SolCAP genotyping array could be the other reason given that the SolCAP array was originally created to explore the genetic variation within cultivated tomatoes and to map genes (Hamilton *et al.* 2012; Sim *et al.* 2012a, 2012b). The second problem was that certain samples belonging to the same accession were not clustered in the same group. For example, two samples of the BGV007104 accession, which shared 93.23% genotypic identity in this SNP set (Table S8) and were labeled as BGV007104_2012B and BGV007104_2015B, were assigned to different groups (Figure S8). The wrong grouping for the samples of the same accession may result from an insufficient number of SNP markers that were unable to capture similarity within the same accession when the sample size increases. In an empirical study of *Arabidopsis halleri*, a few thousand SNP markers were required to estimate the genetic diversity among populations with different genetic variation (Fischer *et al.* 2017). The latter problem prevented meaningful comparisons between the inference of population differentiation in the current study and that of the meta-analysis. Regardless of the inconsistent best K among these studies, the results of pairwise F_{st} and AMOVA statistically supported the subpopulations in this study, suggesting that this set of high-density SNPs could stably estimate the best K.

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

356 **A group of individuals would be a better representative of an accession**

357 An accession should be represented by a group of samples rather than
358 only a single individual since an accession in its natural habitat is composed of
359 a group of individuals, especially when gathering accessions with high
360 diversity. However, under circumstances with limited resources, we instead
361 prepared a collection representing a population rather than only several
362 accessions because our final goal was to apply *S. pimpinellifolium* in GWAS.
363 In addition, the mating system and the propagation method of *S.*
364 *pimpinellifolium* made the variations between accessions greater than that
365 within accessions. Therefore, our only option was to involve as much diversity
366 as possible to enhance the efficiency of GWAS.

367

368 **High genetic variation leads to rapid LD decay**

369 The observed and expected heterozygosity of this population were
370 0.0761 and 0.2786, respectively, slightly higher than in previous research
371 (Blanca *et al.* 2012, 2015). Since *S. pimpinellifolium* was detected with up to a
372 40% outcrossing rate (Rick *et al.* 1977) and demonstrated high genetic
373 variation, it is expected to cause rapid LD decay. In this study, LD decay was
374 within 18 Kb throughout the genome, which was much shorter than in
375 cultivated tomatoes (Sim *et al.* 2012a; Bauchet *et al.* 2017). However, such
376 high genetic variation requires much more markers to enable the
377 comprehensive detection in GWAS. The 900-Mb tomato genome requires at
378 least 50,000 markers to cover the entire genome evenly. Therefore, acquiring
379 more SNPs using different methods is essential to conduct a GWAS in the

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

380 *S. pimpinellifolium* population. One possible approach is to increase the
381 sample size evenly for each subpopulation (Brachi *et al.* 2011). Since
382 approximately 64% of alleles were rare in this population, the augmentation of
383 the subpopulation size may adjust rare alleles to common alleles, potentially
384 increasing the SNPs without extending coverage. Another possible strategy is
385 exome sequencing, a selective genome sequencing technology that selects
386 desired sequencing regions by the hybridization of designed probes (Kaur and
387 Gaikwad 2017). Based on tomato genome sequence information, such as the
388 gene model or EST database, one could design different sets of probes to limit
389 sequencing regions (Ruggieri *et al.* 2017). Given the approximately 110 Mb
390 total gene length in the ITAG2.4 gene model, the potential coverage could
391 reach 12% and all target the gene region. This exome sequencing strategy
392 may be able to increase SNPs without increasing the population size.
393

394 **A reproductive strategy would reduce the genetic diversity of *S.***
395 ***pimpinellifolium***

396 These accessions were propagated using single-seed descent for two
397 generations. Therefore, the heterozygosity would be reduced compared to the
398 original specimens, especially for FSC accessions. Here, we revealed that an
399 ASC accession, LA0411, presented 40.25% heterozygosity, which highlighted
400 the contradiction of self-fertilization consequence. Lacking the same accession
401 as a reference in previous studies and considering the 0 to 22% heterozygosity
402 of other accessions in the original published research (Rick *et al.* 1977), we
403 could remove only LA0411 from our analyses based on the fact that its

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

heterozygosity was too high for an ASC accession.

ACKNOWLEDGMENTS

We thank the C.M. Rick Tomato Genetics Resource Center for generously providing plant materials to conduct this experiment and Dr. Tze-Tze Liu in the Genome Research Center at the National Yang-Ming University, Taiwan, for providing help and advice of the massive parallel DNA sequencing task. This work was supported by grants from the Ministry of Science and Technology, Taiwan R.O.C. (Project Nos. NSC 96-2313-B-002-034-MY3 and NSC 101-2313-B-002-006-MY3) to KYC and from National Taiwan University (Project Nos. 103R7858 and 104R7858) to KYC. This work represents the partial fulfillment of requirements for YPL's Ph.D. degree.

LITERATURE CITED

Alexander, D. H., J. Novembre, and K. Lange, 2009 Fast Model-Based Estimation of Ancestry in Unrelated Individuals. Genome Res. 1655–1664.

Bauchet, G., S. Grenier, N. Samson, J. Bonnet, L. Grivet *et al.*, 2017 Use of modern tomato breeding germplasm for deciphering the genetic control of agronomical traits by Genome Wide Association study. Theor. Appl. Genet. 130: 875–889.

Bhakta, M. S., V. A. Jones, and C. E. Vallejos, 2015 Punctuated distribution of recombination hotspots and demarcation of pericentromeric regions in *Phaseolus vulgaris* L. PLoS One 10(1): e0116822.

Blanca, J., J. Cañizares, L. Cordero, L. Pascual, M. J. Diez *et al.*, 2012

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

426 Variation Revealed by SNP Genotyping and Morphology Provides Insight
427 into the Origin of the Tomato. PLoS One 7(10): e48198.
428 Blanca, J., J. Montero-Pau, C. Sauvage, G. Bauchet, E. Illa *et al.*, 2015
429 Genomic variation in tomato, from wild ancestors to contemporary
430 breeding accessions. BMC Genomics 16: 257.
431 Brachi, B., G. P. Morris, and J. O. Borevitz, 2011 Genome-wide association
432 studies in plants: the missing heritability is in the field. Genome Biol. 12:
433 232.
434 Bradbury, P. J., Z. Zhang, D. E. Kroon, T. M. Casstevens, Y. Ramdoss *et al.*,
435 2007 TASSEL: Software for association mapping of complex traits in
436 diverse samples. Bioinformatics 23: 2633–2635.
437 Caicedo, A. L., and B. A. Schaal, 2004 Population structure and
438 phylogeography of *Solanum pimpinellifolium* inferred from a nuclear gene.
439 Mol. Ecol. 13: 1871–1882.
440 Capel, C., A. Fernández, J. Manuel, V. Lima, S. Francesc *et al.*, 2015
441 Wide-genome QTL mapping of fruit quality traits in a tomato RIL population
442 derived from the wild-relative species *Solanum pimpinellifolium* L. Theor.
443 Appl. Genet. 128: 2019–2035.
444 Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko, 2013
445 Stacks: An analysis tool set for population genomics. Mol. Ecol. 22: 3124–
446 3140.
447 Chen, A. L., C. Y. Liu, C. H. Chen, J. F. Wang, Y. C. Liao *et al.*, 2014
448 Reassessment of QTLs for late blight resistance in the tomato accession
449 L3708 using a restriction site associated DNA (RAD) linkage map and
450 highly aggressive isolates of *Phytophthora infestans*. PLoS One 9(5):

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

e96417.

CLC Genomics Workbench 6.5.1.

Davey, J. L., and M. W. Blaxter, 2010 RADseq: Next-generation population genetics. Brief. Funct. Genomics 9: 416–423.

Dobritsa, A. P., and S. V. Dobritsa, 1980 DNA protection with the DNA methylase M · *Bbv*I from *Bacillus brevis* var. GB against cleavage by the restriction endonucleases *Pst*I and *Pvu*II. Gene 10: 105–112.

Etter, P. D., S. Bassham, P. A. Hohenlohe, E. A. Johnson, and W. A. Cresko, 2011 SNP Discovery and Genotyping for Evolutionary Genetics Using RAD Sequencing. Mol. Methods Evol. Genet. 772: 1–19.

Excoffier, L., P. E. Smouse, and J. M. Quattro, 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Fernandez-Pozo, N., N. Menda, J. D. Edwards, S. Saha, I. Y. Tecle *et al.*, 2015 The Sol Genomics Network (SGN)-from genotype to phenotype to breeding. Nucleic Acids Res. 43: D1036–D1041.

Fischer, M. C., C. Rellstab, M. Leuzinger, M. Roumet, F. Gugerli *et al.*, 2017 Estimating genomic diversity and population differentiation- an empirical comparison of microsatellite and SNP variation in *Arabidopsis halleri*. BMC Genomics 18: 1–15.

Fulton, T. M., J. Chunwongse, and S. D. Tanksley, 1995 Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol. Biol. Report. 13: 207–209.

Gaunt, T. R., S. Rodríguez, and I. N. Day, 2007 Cubic exact solutions for the

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

476 estimation of pairwise haplotype frequencies: implications for linkage
477 disequilibrium analyses and a web tool “CubeX”. BMC Bioinformatics 8:
478 428.
479 Gevaert, S. D., J. R. Mandel, J. M. Burke, and L. A. Donovan, 2013 High
480 genetic diversity and low population structure in Porter’s sunflower
481 (*Helianthus porteri*). J. Hered. 104: 407–415.
482 Goudet, J., and T. Jombart, 2015 hierfstat: Estimation and Tests of Hierarchical
483 F-Statistics.
484 Grandillo, S., R. Chetelat, S. Knapp, D. Spooner, I. Peralta *et al.*, 2011 *Wild*
485 *Crop Relatives: Genomic and Breeding Resources Vegetables* (C. Kole,
486 Ed.). Springer Heidelberg Dordrecht London New York.
487 Hamilton, J. P., S. Sim, K. Stoffel, A. Van Deynze, C. R. Buell *et al.*, 2012 Single
488 Nucleotide Polymorphism Discovery in Cultivated Tomato via Sequencing
489 by Synthesis. Plant Genome 5: 17–29.
490 Holsinger, K. E., and B. S. Weir, 2009 Genetics in geographically structured
491 populations: defining, estimating and interpreting F_{ST}. Nat. Rev. Genet. 10:
492 639–650.
493 Kaur, P., and K. Gaikwad, 2017 From Genomes to GENE-omes: Exome
494 Sequencing Concept and Applications in Crop Improvement. Front. Plant
495 Sci. 8: 1–7.
496 Korte, A., and A. Farlow, 2013 The advantages and limitations of trait analysis
497 with GWAS: a review. Plant Methods 9: 29.
498 Moyle, L. C., 2008 Ecological and evolutionary genomics in the wild tomatoes
499 (Solanum Sect. Lycopersicon). Evolution. 62: 2995–3013.
500 Pascual, L., N. Desplat, B. E. Huang, A. Desgroux, L. Bruguier *et al.*, 2015

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Potential of a tomato MAGIC population to decipher the genetic control of quantitative traits and detect causal variants in the resequencing era. Plant Biotechnol. J. 13: 565–577.

Pembleton, L. W., N. O. I. Cogan, and J. W. Forster, 2013 StAMPP: An R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Mol. Ecol. Resour. 13: 946–952.

Ranc, N., S. Munos, J. Xu, M. C. Le Paslier, A. Chauveau *et al.*, 2012 Genome-wide association mapping in tomato (*Solanum lycopersicum*) is possible using genome admixture of *Solanum lycopersicum* var. *cerasiforme*. G3 2: 853–864.

Rao, E. S., P. Kadirvel, R. C. Symonds, S. Geethanjali, and A. W. Ebert, 2012 Using SSR markers to map genetic diversity and population structure of *Solanum pimpinellifolium* for development of a core collection. Plant Genet. Resour. 10: 38–48.

Rao, E. S., P. Kadirvel, R. C. Symonds, S. Geethanjali, R. N. Thontadarya *et al.*, 2015 Variations in *DREB1A* and *VP1.1* genes show association with salt tolerance traits in wild tomato (*Solanum pimpinellifolium*). PLoS One 10: 1–19.

Remington, D. L., J. M. Thornsberry, Y. Matsuoka, L. M. Wilson, S. R. Whitt *et al.*, 2001 Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proc. Natl. Acad. Sci. U. S. A. 98: 11479–11484.

Rick, C., J. Fobes, and M. Holle, 1977 Genetic variation in *Lycopersicon pimpinellifolium*: Evidence of evolutionary change in mating systems. Plant Syst. Evol. 127: 139–170.

Ruggieri, V., I. Anzar, A. Paytuvi, R. Calafiore, R. A. Cigliano *et al.*, 2017

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

526 Exploiting the great potential of Sequence Capture data by a new tool,
527 SUPER-CAP. DNA Res. 24: 81–91.
528 Sim, S. C., A. van Deynze, K. Stoffel, D. S. Douches, D. Zarka *et al.*, 2012a
529 High-density SNP genotyping of tomato (*Solanum lycopersicum* L.)
530 reveals patterns of genetic variation due to breeding. PLoS One 7(9):
531 e45520.
532 Sim, S. C., G. Durstewitz, J. Plieske, R. Wieseke, M. W. Ganal *et al.*, 2012b
533 Development of a large SNP genotyping array and generation of
534 high-density genetic maps in tomato. PLoS One 7: e40563.
535 Soto-Cerda, B. J., and S. Cloutier, 2012 Association mapping in plant genomes,
536 in *Genetic Diversity in Plants*, edited by C. Mahmut. InTech, Rijeka.
537 Spooner, D. M., I. E. Peralta, and S. Knapp, 2005 Comparison of AFLPs with
538 other markers for phylogenetic inference in wild tomatoes [Solanum L.
539 section Lycopersicon (Mill.) Wettst.]. Taxon 54: 43–61.
540 Tanksley, S. D., and S. R. Mccouch, 1997 Seed Banks and Molecular Maps :
541 Unlocking Genetic Potential from the Wild. Science. 277: 1063–1066.
542 Víquez-zamora, M., M. Caro, R. Finkers, Y. Tikunov, A. Bovy *et al.*, 2014
543 Mapping in the era of sequencing : high density genotyping and its
544 application for mapping TYLCV resistance in *Solanum pimpinellifolium*.
545 BMC Genomics 15: 1–10.
546 Weir, B. S., and C. C. Cockerham, 1984 Estimating F-Statistics for the Analysis
547 of Population Structure. Evolution. 38: 1358–1370.
548 Zhong, S., Z. Fei, Y. R. Chen, Y. Zheng, M. Huang *et al.*, 2013 Single-base
549 resolution methylomes of tomato fruit development reveal epigenome
550 modifications associated with ripening. Nat. Biotechnol. 31: 154–159.

bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

551 Zuriaga, E., J. M. Blanca, L. Cordero, A. Sifres, W. G. Blas-Cerdán *et al.*, 2009
552 Genetic and bioclimatic variation in *Solanum pimpinellifolium*. Genet.
553 Resour. Crop Evol. 56: 39–51.

554

555 **Figure legend**

556 **Figure 1**. Ancestry and geographic distribution of 98 *Solanum pimpinellifolium*
557 accessions from the Tomato Genetics Resource Center. A) Model-based
558 ancestry for each accession. B) Principle component analysis of the
559 *S. pimpinellifolium* population. C) Geographical distribution of the 98
560 *S. pimpinellifolium* accessions. Symbol and color codes are as follows: square
561 symbols with red, blue and green colors were used to indicate three pure
562 ancestry groups corresponding to the same colors in the ancestry plot; triangle
563 symbols with goldenrod, purple and aquamarine colors were used to present
564 the three admixture groups with red-green, red-blue and blue-green mixing
565 ancestries, respectively; black circle symbols were used for the group with
566 admixture of three ancestries.

567

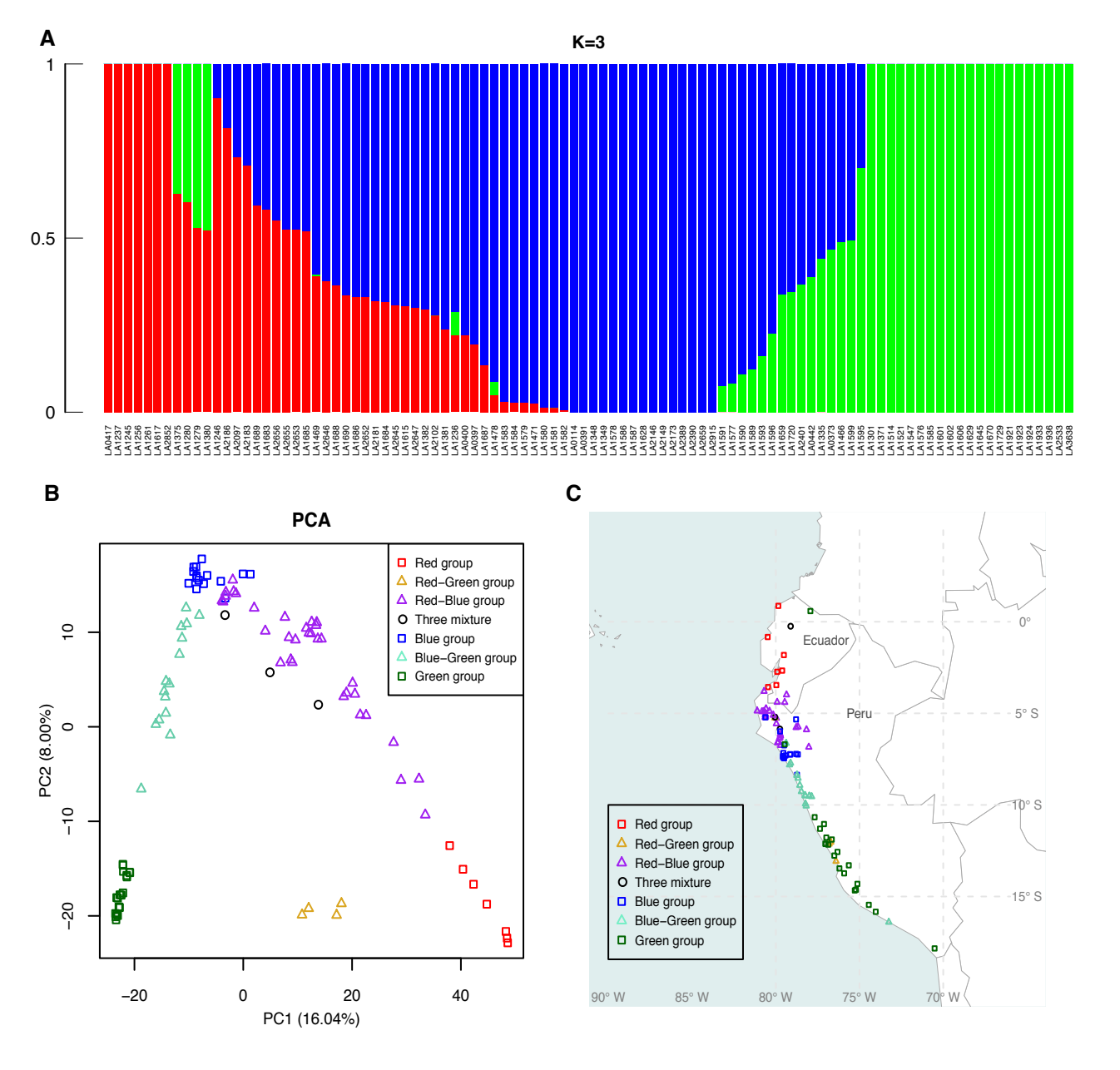
568 **Figure 2**. Visualization for LD. A) The 50 Kb interval of overall LD decay. The
569 red curve indicates non-linear regression, and the black dotted line indicates
570 the baseline of r^2 at 0.1. B) The local LD of chromosome 1. The red dotted line
571 indicates the baseline of r^2, and the orange line indicated the heterochromatic
572 region.

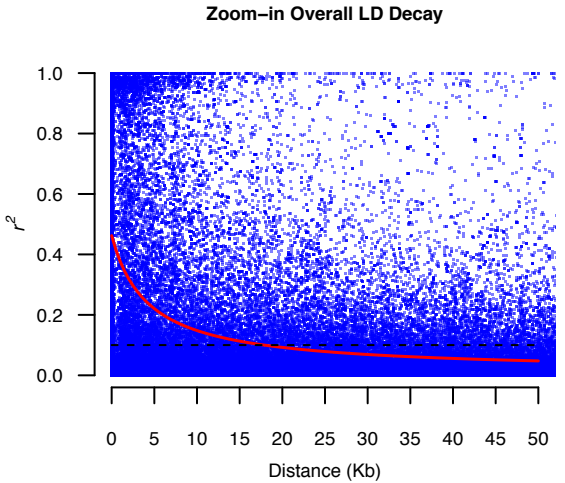
573

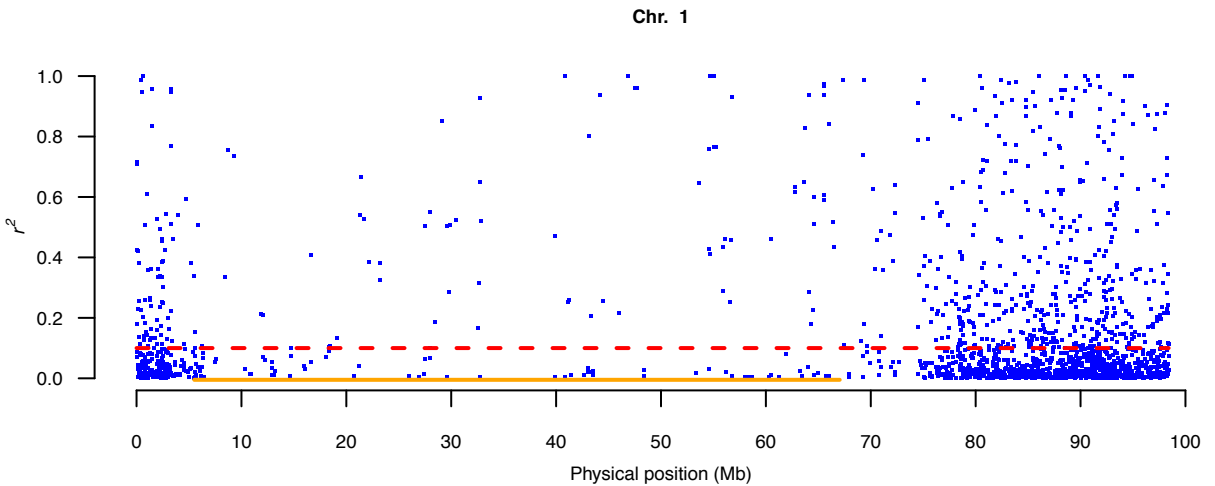
574 **Figure 3.** The distributions of ITAG2.4 gene model, *Pst*I cutting sites and
575 SNPs throughout the genome. Each section indicates one chromosome, with

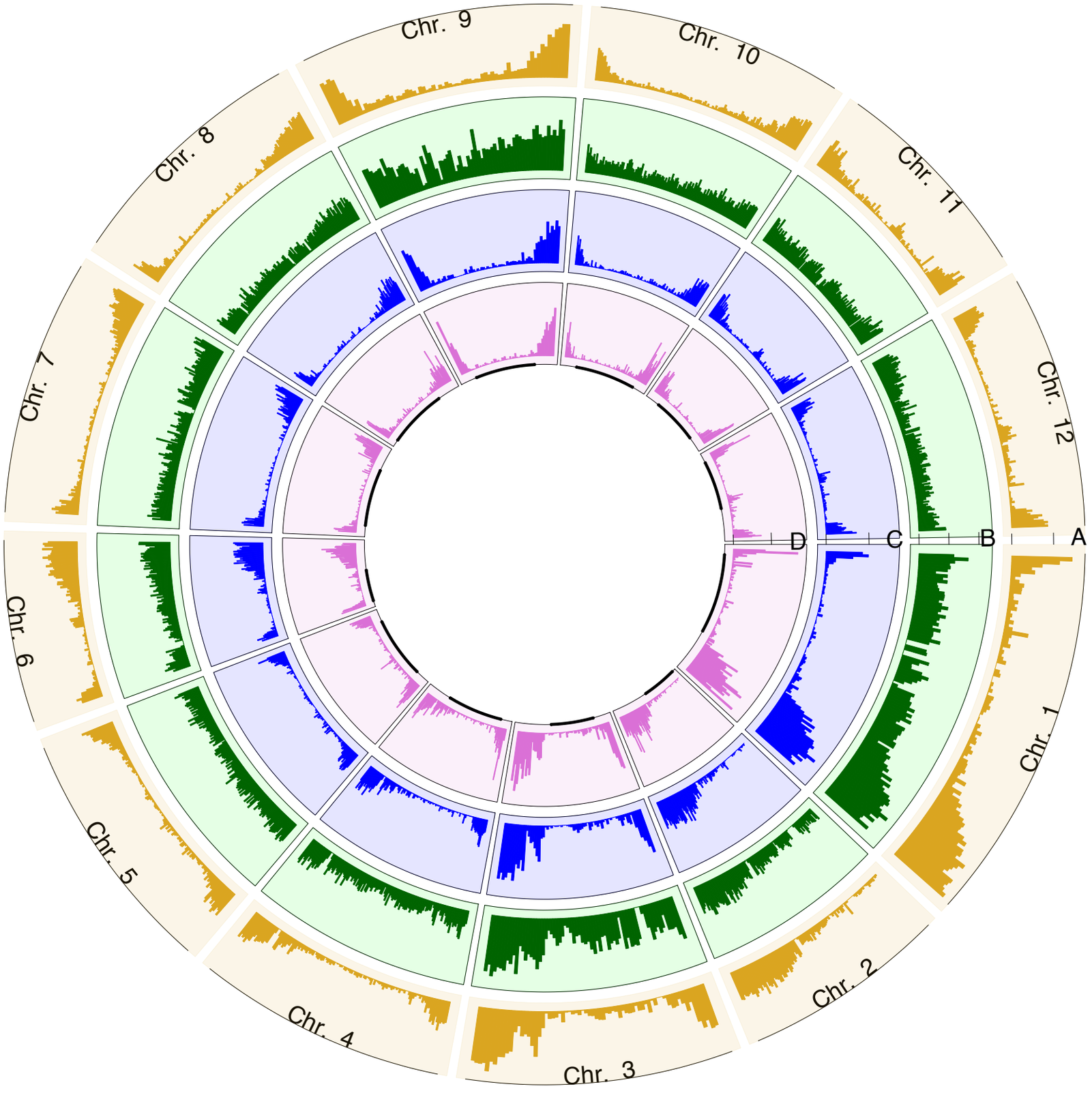
bioRxiv preprint doi: https://doi.org/10.1101/402420; this version posted August 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

576 labeling on the circumference. Circles A, B, C, and D indicate the distribution of
577 ITAG2.4 genes, expected *Pst*I cutting sites, *Pst*I cutting sites in the deep
578 sequencing regions and RADseq SNPs, respectively. The black lines in the
579 inner D layer indicate the heterochromatic regions.
580


A
K=3
1
0.5
0
B
PCA
Red group
Red–Green group
Red–Blue group
Three mixture
Blue group
Blue–Green group
Green group
PC2 (8.00%)
PC1 (16.04%)
C
Ecuador
Peru
0°
5° S
10° S
15° S
90° W
85° W
80° W
75° W
70° W


Zoom−in Overall LD Decay
1.0
0.8
0.6
0.4
0.2
0.0
r^2
0
5
10
15
20
25
30
35
40
45
50
Distance (Kb)


Chr. 1
1.0
0.8
0.6
0.4
0.2
0.0
r^2
0
10
20
30
40
50
60
70
80
90
100
Physical position (Mb)


Chr. 1
Chr. 2
Chr. 3
Chr. 4
Chr. 5
Chr. 6
Chr. 7
Chr. 8
Chr. 9
Chr. 10
Chr. 11
Chr. 12
A
B
C
D