Development of a Large SNP Genotyping Array and Generation of High-Density Genetic Maps in Tomato

Sung-Chur Sim1, Gregor Durstewitz2, Jörg Plieske2, Ralf Wieseke2, Martin W. Ganal2, Allen Van Deynze3, John P. Hamilton4, C. Robin Buell4, Mathilde Causse5, Saranga Wijeratne6, David M. Francis1*

1 Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, United States of America, 2 TraitGenetics GmbH, Gatersleben, Germany, 3 Seed Biotechnology Center, University of California Davis, Davis, California, United States of America, 4 Department of Plant Biology, Michigan State University, East Lansing, Michigan, United States of America, 5 Institut National de la Recherche Agronomique, INRA, Unite´ de Ge´ne´tique et d’Amelioration des Fruits et Legumes, Montfavet, France, 6 Molecular Cellular and Imaging Center, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, United States of America

Abstract

The concurrent development of high-throughput genotyping platforms and next generation sequencing (NGS) has increased the number and density of genetic markers, the efficiency of constructing detailed linkage maps, and our ability to overlay recombination and physical maps of the genome. We developed an array for tomato with 8,784 Single Nucleotide Polymorphisms (SNPs) mainly discovered based on NGS-derived transcriptome sequences. Of the SNPs, 7,720 (88%) passed manufacturing quality control and could be scored in tomato germplasm. The array was used to generate high-density linkage maps for three interspecific F2 populations: EXPEN 2000 (Solanum lycopersicum LA0925 x S. pennellii LA0716, 79 individuals), EXPEN 2012 (S. lycopersicum Moneymaker x S. pennellii LA0716, 160 individuals), and EXPIM 2012 (S. lycopersicum Moneymaker x S. pimpinellifolium LA0121, 183 individuals). The EXPEN 2000-SNP and EXPEN 2012 maps consisted of 3,503 and 3,687 markers representing 1,076 and 1,229 unique map positions (genetic bins), respectively. The EXPEN 2000-SNP map had an average marker bin interval of 1.6 cM, while the EXPEN 2012 map had an average bin interval of 0.9 cM. The EXPIM 2012 map was constructed with 4,491 markers (1,358 bins) and an average bin interval of 0.8 cM. All three linkage maps revealed an uneven distribution of markers across the genome. The dense EXPEN 2012 and EXPIM 2012 maps showed high levels of colinearity across all 12 chromosomes, and also revealed evidence of small inversions between LA0716 and LA0121. Physical positions of 7,666 SNPs were identified relative to the tomato genome sequence. The genetic and physical positions were mostly consistent. Exceptions were observed for chromosomes 3, 10 and 12. Comparing genetic positions relative to physical positions revealed that genomic regions with high recombination rates were consistent with the known distribution of euchromatin across the 12 chromosomes, while very low recombination rates were observed in the heterochromatic regions.

Introduction

Tomato (Solanum lycopersicum L.) has been a model species for basic studies in plant biology. The strength of genetic resources anchored to high-density maps has permitted the map-based cloning of genes involved in disease resistance [1–4], plant and fruit development [5,6], and regulation of biochemical processes [7]. The first high-density genetic map for tomato consisted of over 1,000 restriction fragment length polymorphism (RFLP) markers segregating in an interspecific F2 population derived from a wide cross between S. lycopersicum and S. pennellii [8]. More recently, mapping studies have focused on polymerase chain reaction (PCR)-based markers with genetic maps of cultivated tomato developed using 344 Simple Sequence Repeat (SSR) and 793 Singe Nucleotide Polymorphism (SNP) markers [9] and integrated S. lycopersicum x S. pimpinellifolium maps based on 434 PCR-based markers [10].

The genomic resources available for tomato are rapidly expanding due to the increased throughput of next generation sequencing (NGS) technologies that have significantly reduced the cost and time of sequencing relative to the Sanger method and facilitated whole-genome sequencing, transcriptome profiling, and discovery of variation across genomes [11–13]. NGS has permitted genome-wide SNP discovery in many crop species including rice.
Table 1. Number of SNP markers and coverage in cM of each chromosome in three linkage maps.

| Chr | EXPEN 2000 (LA0925 x LA0716) | EXPEN 2012 (Moneymaker x LA0716) | EXPIM 2012 (Moneymaker x LA0121) |
|-----|-------------------------------|----------------------------------|----------------------------------|
|     | No. Unique Coverage Marker Interval (cM) | No. Unique Coverage Marker Interval (cM) | No. Unique Coverage Marker Interval (cM) |
|     | Bin | (cM) | Maximum | Average | Bin | (cM) | Maximum | Average | Bin | (cM) | Maximum | Average |
| 1   | 252 | 113  | 201.8   | 8.5     | 1.8  | 266 | 110  | 117.2   | 8.9     | 1.1  | 332 | 158  | 127.5   | 6.0    | 0.8 |
| 2   | 416 | 125  | 165.5   | 6.2     | 1.3  | 434 | 145  | 110.2   | 3.5     | 0.8  | 507 | 123  | 80.2    | 5.7    | 0.7 |
| 3   | 286 | 81   | 121.7   | 5.6     | 1.5  | 299 | 97   | 105.4   | 7.0     | 1.1  | 339 | 139  | 108.2   | 5.7    | 0.8 |
| 4   | 385 | 113  | 159.5   | 5.0     | 1.4  | 427 | 123  | 108.1   | 6.5     | 0.9  | 574 | 135  | 93.0    | 3.6    | 0.7 |
| 5   | 363 | 99   | 154.3   | 6.1     | 1.6  | 381 | 118  | 95.5    | 5.2     | 0.8  | 494 | 129  | 88.9    | 4.2    | 0.7 |
| 6   | 374 | 78   | 111.3   | 5.6     | 1.4  | 384 | 89   | 87.7    | 7.2     | 1.0  | 306 | 94   | 66.8    | 4.3    | 0.7 |
| 7   | 224 | 70   | 108.2   | 7.8     | 1.5  | 237 | 71   | 74.8    | 4.2     | 1.1  | 290 | 111  | 83.2    | 3.6    | 0.7 |
| 8   | 189 | 75   | 124.4   | 8.1     | 1.7  | 198 | 87   | 76.9    | 3.8     | 0.9  | 258 | 95   | 77.4    | 4.2    | 0.8 |
| 9   | 218 | 84   | 144.2   | 8.7     | 1.7  | 234 | 100  | 96.7    | 4.5     | 1.0  | 228 | 83   | 72.4    | 5.1    | 0.9 |
| 10  | 167 | 80   | 122.8   | 5.5     | 1.5  | 178 | 79   | 84.5    | 6.8     | 1.1  | 270 | 87   | 75.5    | 3.1    | 0.9 |
| 11  | 466 | 82   | 114.4   | 9.0     | 1.4  | 484 | 126  | 98.8    | 4.8     | 0.8  | 691 | 115  | 92.1    | 8.7    | 0.8 |
| 12  | 163 | 76   | 141.9   | 9.7     | 1.9  | 165 | 84   | 99.1    | 8.9     | 1.2  | 202 | 89   | 84.1    | 8.3    | 0.9 |
| Total| 3,503 | 1,076 | 1,669.9 | 1.6 | 3,687 | 1,229 | 1,154.6 | 0.9 | 4,491 | 1,358 | 1,049.2 | 0.8 |

1 Unique map positions covered by SNP markers.
2 Average marker interval (cM) = coverage/number of unique bins.
3 Map length recalculated based on subsets of markers that were separated by at least 5 cM interval.

doi:10.1371/journal.pone.0040563.t001
[14,15], maize [16], durum wheat [17], sugarcane [18], soybean [19,20], and potato [21]. In tomato, NGS of the transcriptome produced 17 Gb of sequence for six accessions and led to the identification of 62,576 non-redundant SNPs [22].

High-throughput SNP discovery has been paralleled by the development of genotyping platforms that permit cost-effective scoring of many thousands of SNPs in a highly parallel fashion [25,24] facilitating high-density genetic map construction. For maize, an array consisting of 49,583 SNPs was used to develop two linkage maps with 20,912 and 14,524 markers, respectively [25]. In the age of incomplete genome sequences and partial physical maps, high resolution genetic maps remain an essential resource. Such maps help to improve genome assemblies, provide estimates of recombination relative to physical distance, and remain an essential feature for the dissection of complex traits. The information provides an essential guide to genomic assisted crop improvement, where recombination remains a constraint.

In order to facilitate genetic analysis and breeding, we developed the first large scale SNP genotyping array for tomato using 8,784 SNPs mainly discovered based on NGS-derived transcriptome sequences for six accessions [22]. Three high-resolution linkage maps were constructed using interspecific F2 populations to provide details of genetic order, recombination, and their position relative to the draft assembly of the tomato reference genome sequence. The SNP array and high-density linkage maps will be useful for population level analysis, trait discovery, and selection for cultivar improvement in tomato.

Results

SNP Array

We developed a genotyping array on the Illumina Infinium platform (Illumina Inc., San Diego, CA, USA) based on 8,784 SNPs. These SNPs represented a highly filtered and selected set, optimized for polymorphism detection among cultivated germplasm and spread throughout the genome. Of these, 7,720 SNPs (88%) passed manufacturing quality control (Table S1). A failure rate of 12% was considered normal and acceptable (less than 15% is expected according to the manufacturer). The scorable SNPs included 501 from candidate genes and 1,307 that were cross-validated with community data sets from TraitGenetics (Gate- rsleben, Germany), the French National Institute for Agricultural Research (Institut National de la Recherche Agronomique, INRA), and previously published SNPs [22,26,27] (Table S1).

Genetic Map Construction

The widely used tomato reference population EXPEN 2000 was used to develop a SNP map (EXPEN 2000-SNP) based on 79 F2 individuals from a cross between LA0925 (S. lycopersicum) and LA0716. Of 3,770 polymorphic SNPs between the parental lines, 4,491 markers were mapped as codominant loci representing 1,358 genetic bins with an average marker bin interval of 0.8 cM and the largest gap of 8.7 cM on chromosome 11 (Table 1 and Table S4). The distribution of the SNP markers across all chromosomes was again similar to the other linkage maps (Table 1). The map of chromosome 1 consisted of 332 SNP markers covering 127.5 cM and 158 unique bins while the map of chromosome 11 consisted of 691 SNP markers covering 92.1 cM and 115 unique bins.

Genetic Map Length

The total genetic distance of the EXPEN 2000-SNP map was estimated as 1,669.9 cM, or approximately 45% larger than the EXPEN 2012 map (1,546.4 cM) and 59% larger than the EXPIM 2012 map (1,049.2 cM) (Table 1). Although our estimate of genetic length for the EXPEN 2000-SNP map was marginally larger than expected based on previous estimates of genetic map length for this population (1,503 cM) [9], we were concerned about discrepancies in size between the three populations. One possible explanation for the observed increase in the amount of recombination in the EXPEN 2000-SNP map could be selection at gametophytic and post-zygotic stages, leading to distorted segregation and inflated estimates of recombination in that specific population. To address this possibility, we investigated whether there was an excess of chromosomes with distorted makers. Chromosomes 1, 10 and 11 contained a high proportion of distorted markers. A test for correlations between map expansion and distorted segregation did not support a positive relationship (m = -0.7; R² = 0.19; P = 0.146) suggesting that distorted segregation was not responsible for the expanded map.

An alternative explanation for the map expansion observed for the EXPEN 2000-SNP map compared to the EXPEN2012 map relates to the large number of makers scored and the small population size. The accuracy of the calculations for genetic distance is influenced by population size since a falsly scored or incorrectly ordered marker has a larger effect in a smaller population. The EXPEN 2000-SNP map length may be overestimated as a result of population size which limits accurate estimation of marker order and genetic distances. To address this
hypothesis, we repeated the EXPEN 2000-SNP map construction by selecting 307–325 markers that were separated by at least 5 cM interval and recalculated the genetic map. This resampling analysis led to estimates of map length that were reduced by an average of 25% (range 22–27%) relative to the EXPEN 2000-SNP map length based on all markers (Table 1). This reduction was not observed when the same approach was used in the EXPEN 2012 and EXPIM 2012 populations (Table 1). These results suggest that the small population size of the EXPEN 2000-SNP reference map limited the ability to accurately determine marker distance based on recombination when marker density was high.

The approach of creating a series of resampled maps allowed us to compare map length between the EXPEN 2012 and EXPIM 2012 populations. The 10% difference between the two maps was significant based on over 100 iterations. The EXPEN 2012 map was significantly ($P<0.001$) longer for chromosomes 2, 4, 5, 6, 9, 10, 11 and 12. The EXPIM 2012 map was significantly ($P<0.001$) longer for chromosome 1 and 7. No differences were detected for average distances on chromosome 3 and 8, though there may be differences in recombination length between the two maps for the arms of chromosome 8.

**Chromosome Assignment and Colinearity between Genetic Maps**

The genetic positions of 5,621 SNP markers across 12 chromosomes could be determined with 3,149 markers in common between the EXPEN 2000-SNP and EXPEN 2012 maps; 2,509 markers in common between EXPEN 2000-SNP and EXPIM 2012 maps; and 2,841 markers in common between EXPEN 2012 and EXPIM 2012 maps (Table 2 and Tables S5, S6, S7). All of the shared markers showed highly conserved chromosome assignments. As with the individual maps, the number of markers in common for each chromosome varied and ranged from 106 on chromosome 12 to 413 on chromosome 11 (Table 2). In order to assess levels of colinearity between the linkage maps, the common markers were ranked based on their chromosome positions and their rank orders were used for regression analysis. High levels of colinearity (0.96–1.00 regression coefficients) were observed across 12 chromosomes between both EXPEN maps (Table 2). The EXPIM 2012 map showed coefficients of colinearity ranging between 0.85–0.99 for the EXPEN 2000-SNP comparison and 0.98–1.00 for the EXPEN 2012 comparison again indicating that the larger EXPEN 2012 map is most likely more accurate. Due to map quality, further comparative analysis was conducted only between the EXPEN 2012 and EXPIM 2012 maps which were of comparable population size (160 vs. 183 individuals). Plotting the common markers on rank order revealed several regions with inverse marker orders, characterized by a strong linear correlation with a negative slope over short distances, between these linkage maps. Specifically, patterns on chromosome 1 (coordinates 20, 20), chromosome 3 (coordinates 40, 40), chromosome 6 (coordinates 20, 20), chromosome 7 (coordinates 5, 5 and 140, 140), and chromosome 9 (coordinates 20, 20) are consistent with inversions between the Solanum pimpinellifolium LA0121 and S. pennellii LA0716 parents (Figure 1). Regions on chromosome 1 (coordinates 100, 100) and chromosome 2 (coordinates 60, 60) highlight where marker order diverges, but evidence for a simple inversion based on a strong negative correlation is less robust (Figure 1).

**Comparison between Genetic and Physical Positions**

In addition to the genetic map position, the physical positions of 7,666 SNPs were determined relative to the tomato reference genome sequence [30] (Table S1) and available through the Solanaceae Genome Network (SGN; http://solgenomics.net). A total of 738 Mb of the tomato genome was covered by the SNP markers on the array with an average distance between markers of 0.12 Mb (Table 3). Chromosome 1 showed the largest physical gap with no markers (7.36 Mb) followed by a region on chromosome 12 (4.73 Mb). The most markers (1,059 SNPs) were mapped on chromosome 11, which is cytologically one of the smallest tomato chromosomes [29].

Among the 7,666 SNPs with physical positions, 5,296 SNP markers were mapped on one or both of the EXPEN 2012 and EXPIM 2012 genetic linkage maps (Table S8). These markers were used for comparative analysis of genetic and physical positions. We found that the vast majority (99.7%) of the SNPs in the linkage maps showed conserved chromosome assignments with the corresponding physical positions. Sixteen non-syntenic markers were not genetically mapped to the assigned physical chromosomes (Table S8). Among the 16 non-syntenic markers, there were eight markers mapped on both linkage maps with consistent chromosome assignments. For example, two markers were mapped genetically on chromosome 2, while they were physically placed on chromosomes 1 and 3. Further comparative analysis was conducted to determine colinearity within chromosomes. The two linkage maps revealed conserved marker order with the physical map for most regions of the genome, with chromosomes 4, 5, 8, and 11 having a very high level of colinearity (Figure 2 and Figure 3). A number of markers assigned to chromosomes 3, 10 and 12 in both linkage maps were not colinear with the physical map.

The meiotic recombination rate within each chromosome was estimated based on the 5,280 SNP markers with conserved chromosome assignments between genetic and physical maps. High recombination was found on the distal regions across all 12 chromosomes in both linkage maps, while recombination was suppressed in large regions that are most likely pericentromeric (Figure 3). The linkage maps also revealed similar patterns of variation in recombination rate between chromosomes. However, recombination rates appeared to be higher in the EXPEN 2012 map relative to the EXPIM 2012 map on chromosomes 2, 4, 5, 6, 9, 10, and 12, while the EXPIM 2012 population showed higher levels of recombination on chromosomes 1 and 7 (Figure 3). On chromosome 8, the overall rate of recombination appears similar, though the rate within each arm appears to differ between the two populations. These results are consistent with the results of the iterative mapping, described above. In addition, there was suppression of recombination specific to the EXPEN 2012 map on chromosome 1 (70–75 Mb), chromosome 6 (36–38 Mb), chromosome 7 (0–2 Mb and 58–60 Mb), and chromosome 8 (0–2 Mb) (Figure 3). A recombination suppression specific to the EXPIM 2012 map was found on the region spanning 0–4 Mb on chromosome 9.

**Discussion**

The array with 7,720 scorable SNPs provides a valuable tool for high-throughput and cost-effective genotyping and mapping in tomato. The SNPs used for the array were derived from a computational pipeline based on cDNA sequences from six accessions including four representatives of large-fruited cultivated tomato, a cherry tomato and a closely related wild relative [22]. The array was optimized based on polymorphic SNP markers within cultivated lineages, allele frequency and genome coverage. In addition, 501 functional SNPs on the array were derived from candidate genes for traits such as disease resistance and carotenoid biosynthesis.
Given the physical length of chromosome 1 (largest chromosome), the number of markers is lower than expected while the number of markers on chromosome 11 is higher than expected. This distribution is not due to a lack of or excess of genes on these chromosomes but is likely due to the process of SNP marker selection. Alternatively, the distribution may reflect the introgression of highly polymorphic regions (e.g. containing disease resistance loci such as the I2 Fusarium resistance gene or the Rx-4 and Xe3 bacterial spot resistance genes on chromosome 11) that have created an ascertainment bias.

Despite the SNP selection for cultivated populations and the observed over- and under-representation, the SNP array provides a powerful resource for genetic map construction in interspecific populations. The EXPEN 2000 population has been used in the last ten years as a reference mapping population in tomato and 2,506 markers have been previously mapped [http://solgenomics.net] [31,32]. With the SNP array, we mapped 3,503 SNP markers to this population. We also generated the EXPEN 2012 map for the S. lycopersicum Moneymaker x S. pennellii LA0716 population with 3,687 markers and the EXPIM 2012 map for the S. lycopersicum Moneymaker x S. pimpinellifolium LA0121 population with 4,491 markers. In total, we genetically positioned 5,621 SNP markers including common sets of 2,509–3,149 markers between EXPEN 2000 vs. EXPEN 2012 EXPEN 2000 vs. EXPIM 2012 EXPEN 2012 vs. EXPIM 2012

| Chr | No. Common Marker | Coefficient of Colinearity | No. Common Marker | Coefficient of Colinearity | No. Common Marker | Coefficient of Colinearity |
|-----|------------------|----------------------------|------------------|----------------------------|------------------|----------------------------|
| 1   | 216              | 1.00                       | 184              | 0.99                       | 226              | 0.99                       |
| 2   | 377              | 1.00                       | 308              | 0.99                       | 328              | 0.99                       |
| 3   | 280              | 0.96                       | 213              | 0.85                       | 227              | 0.99                       |
| 4   | 341              | 1.00                       | 306              | 0.99                       | 361              | 1.00                       |
| 5   | 349              | 1.00                       | 287              | 0.99                       | 313              | 1.00                       |
| 6   | 340              | 1.00                       | 196              | 0.98                       | 222              | 0.99                       |
| 7   | 203              | 0.98                       | 168              | 0.95                       | 194              | 0.99                       |
| 8   | 163              | 1.00                       | 135              | 0.99                       | 165              | 0.99                       |
| 9   | 184              | 0.97                       | 120              | 0.91                       | 138              | 0.98                       |
| 10  | 153              | 0.99                       | 125              | 0.98                       | 147              | 1.00                       |
| 11  | 413              | 0.97                       | 361              | 0.92                       | 387              | 1.00                       |
| 12  | 130              | 0.99                       | 106              | 0.97                       | 133              | 0.99                       |
| Total| 3,149            | 0.99                       | 2,509            | 0.96                       | 2,841            | 1.00                       |

1Colinearity within each chromosome was assessed using common markers. The markers were ranked based on their map positions and the rank order was used for regression analysis, and expressed as R².

Table 2. Colinearity between common markers for the three linkage maps.

doi:10.1371/journal.pone.0040563.t002

High-resolution genetic mapping with a large number of markers has helped to improve genome sequence assemblies in plants [25]. Comparison of genetic positions with physical positions provides an independent validation of reference genome sequence assembly. Most regions of the EXPEN 2012 and EXPIM 2012 linkage maps were fully colinear with the current assembly of the tomato reference sequence, suggesting a very good quality of the assembly. Sixteen markers with inconsistent chromosome assignment between genetic and physical maps were observed. Among them, eight markers had consistent chromosome assignments between the EXPEN 2012 and EXPIM 2012 maps, suggesting that the physical position may be incorrect or that the sequences are duplicated in the genome. Thus, the high-density genetic maps provide a guide to improve the assembly of genome expansions. Through the iterative analysis of marker subsets, we showed that the difference in genetic length between the two EXPEN maps was most likely due to the effect of scoring or ordering mistakes being amplified due to the small size the EXPEN 2000 population. Nevertheless both EXPEN maps are larger in terms of cM than the map from the EXPIM (S. lycopersicum x S. pimpinellifolium) population, and these differences were significant based on iterative estimates of map length. We expected that a genetic map generated from two more closely related parents would display a generally higher level of recombination. Our observation of greater map distance in the EXPEN populations is even more surprising given the likely existence of several small inversions between S. lycopersicum and S. pennellii which suppress recombination in these regions. Comparing the EXPEN 2012 and EXPIM 2012 maps suggests that there could be regions on chromosomes 1, 3, 6, 7, and 9 where small inversions differentiate LA0716 and LA0121. A paracentric inversion on the distal end of chromosome 7 was previously reported in S. pennellii LA0716 relative to S. pimpinellifolium LA1589 [34]. Further, cytogenetic analysis revealed that interspecific crosses between S. lycopersicum and S. pennellii can lead to changes in chromosome structure presumably due to inversions and translocations [35].

High-resolution genetic mapping with a large number of markers has helped to improve genome sequence assemblies in plants [25]. Comparison of genetic positions with physical positions provides an independent validation of reference genome sequence assembly. Most regions of the EXPEN 2012 and EXPIM 2012 linkage maps were fully colinear with the current assembly of the tomato reference sequence, suggesting a very good quality of the assembly. Sixteen markers with inconsistent chromosome assignment between genetic and physical maps were observed. Among them, eight markers had consistent chromosome assignments between the EXPEN 2012 and EXPIM 2012 maps, suggesting that the physical position may be incorrect or that the sequences are duplicated in the genome. Thus, the high-density genetic maps provide a guide to improve the assembly of genome expansions. Through the iterative analysis of marker subsets, we showed that the difference in genetic length between the two EXPEN maps was most likely due to the effect of scoring or ordering mistakes being amplified due to the small size the EXPEN 2000 population. Nevertheless both EXPEN maps are larger in terms of cM than the map from the EXPIM (S. lycopersicum x S. pimpinellifolium) population, and these differences were significant based on iterative estimates of map length. We expected that a genetic map generated from two more closely related parents would display a generally higher level of recombination. Our observation of greater map distance in the EXPEN populations is even more surprising given the likely existence of several small inversions between S. lycopersicum and S. pennellii which suppress recombination in these regions. Comparing the EXPEN 2012 and EXPIM 2012 maps suggests that there could be regions on chromosomes 1, 3, 6, 7, and 9 where small inversions differentiate LA0716 and LA0121. A paracentric inversion on the distal end of chromosome 7 was previously reported in S. pennellii LA0716 relative to S. pimpinellifolium LA1589 [34]. Further, cytogenetic analysis revealed that interspecific crosses between S. lycopersicum and S. pennellii can lead to changes in chromosome structure presumably due to inversions and translocations [35].
The comparisons between genetic and physical distances with several thousand markers reveal that there are similar patterns of variation in recombination rates along the tomato chromosomes. Strong recombination suppression occurs in the large pericentric regions within each chromosome. These regions represent repeat-rich and gene-poor heterochromatin encompassing 77% of the tomato genome [28,36]. Such recombination suppression has been noted before for tomato and is also found in many other plant species [25,37,38] albeit often not as pronounced as in tomato.

With the availability of complete genome sequences, there is a tendency for genetic mapping to be relegated to a position of secondary importance. However, trait discovery, functional characterization, and crop improvement are largely dependent on recombination. Therefore, the construction of genetic maps which maximize the amount of recombination remains an essential tool in plant biology and plant breeding for precise and cost-efficient localization of traits and the generation of specific recombination events adjacent to interesting genes. Our data suggest that different crosses could reveal different general and location-specific levels of recombination, and that these differences are not necessarily related to the genetic distance between parents.

The SNP array and high-density genetic maps developed in this study will be useful in population level analysis of germplasm collections representing different market classes of cultivated tomato, regionally adapted populations and wild relatives. Other applications of the resource include genome-wide association mapping with high resolution and marker-assisted selection (MAS) for tomato breeding. For association mapping, accounting for population structure and/or familial relatedness is often necessary to avoid spurious marker-trait associations [39]. Large sets of genome-wide SNP markers will help to precisely estimate the relatedness and capture effects of quantitative trait loci (QTL). Association mapping has the potential to increase the efficiency of MAS by identifying markers tightly linked to traits of interest in germplasm panels that are directly relevant to plant breeders. In addition, the SNP array may facilitate genomic selection (GS) for plant breeding. As first suggested in animal improvement, GS seeks to predict the breeding value of individuals using markers distributed across the genome [40]. With the advent of high-throughput and cost-effective genotyping methods, GS is showing promise for improving complex traits in plant populations [41–43]. In summary, the SNP array provides a survey tool for the tomato research community and creates new opportunities for innovative strategies in both basic research and applied breeding.

Materials and Methods

Plant Material

For genetic mapping, we used 79 F2 progeny from the EXPEN 2000 population S. lycopersicum (LA0925) x S. pennellii (LA0716) which was previously published [31,32]. To distinguish the new SNP map from the EXPEN 2000 reference map, we referred to the map described here as EXPEN 2000-SNP. The two other mapping populations were generated by TraitGenetics with the EXPEN 2012 consisting of 160 F2 progeny from a S. lycopersicum Moneymaker x S. pennellii (LA0716) cross and the EXPIM 2012 population of 183 F2 progeny derived from Moneymaker x S. pimpinellifolium (LA0121) [44]. The available S. pennellii introgression lines in the MB2 background [29] were also used to compare marker assignment with the EXPEN SNP maps.

SNP Array-Based Genetic Mapping in Tomato

SNPs for the array were selected based on a multi-tier strategy that was optimized for polymorphisms within and among cultivated types. Briefly, SNP discovery was based on the Genome Analyzer II-derived transcriptome sequences of four cultivated tomato accessions (NC84173, Fla.7600, OH08-6405, and OH9242), an S. lycopersicum var. cerasiforme accession (PI 114490), and an S. pimpinellifolium accession (PI 128216) [22]. SNPs were filtered such that any SNP within 50 bp of an intron/exon junction was removed and SNPs within 50 bp of a second polymorphism were excluded. The frequency of SNP occurrence among the six sequenced accessions was then assessed, with SNPs preferentially chosen based on their occurrence in multiple accessions. Genome coverage was assessed, and additional SNPs were selected to improve spacing across the genome. The research community provided a set of candidate genes of interest and 567 SNPs in the high confidence SNP set were located in these genes. Finally, SNPs were cross-validated with data sets from TraitGenetics, INRA, and previously published SNPs [26,27]. We included 1,470 validated SNPs from these data sets on the array. A total of 8,784 SNPs detected with 10,000 probes were used to design the array (Table S1).

Genotyping

Genomic DNA was isolated from fresh, young leaf tissue using a modified CTAB method [45]. Original DNA for the 75 F2 individuals of the EXPEN 2000 population was provided by

Table 3. Physical coverage of 7,666 SNP markers.

| Chr | No. Marker | Coverage (Mbp) | Marker Interval (Mbp) |
|-----|------------|----------------|-----------------------|
|     |            | Maximum       | Average               |
| 1   | 554        | 90.13         | 7.36                  | 0.17                  |
| 2   | 871        | 49.48         | 3.83                  | 0.06                  |
| 3   | 679        | 64.70         | 4.38                  | 0.10                  |
| 4   | 861        | 64.01         | 2.03                  | 0.08                  |
| 5   | 783        | 64.91         | 2.70                  | 0.09                  |
| 6   | 748        | 45.88         | 2.66                  | 0.08                  |
| 7   | 443        | 64.98         | 3.93                  | 0.15                  |
| 8   | 396        | 62.97         | 2.95                  | 0.16                  |
| 9   | 473        | 67.60         | 4.52                  | 0.15                  |
| 10  | 405        | 64.74         | 3.17                  | 0.16                  |
| 11  | 1,059      | 53.28         | 2.37                  | 0.05                  |
| 12  | 394        | 65.32         | 4.73                  | 0.17                  |
| Total | 7,666          | 758.00         | 0.12                  |

Flanking sequences of SNPs were used for the automatic batch BLAST against the Tomato WGS chromosome database (v SL2.40; http://solgenomics.net/organism/Solanum_lycopersicum/genome). The actual SNP positions relative to the Tomato genome sequence were identified using a custom Python script.

doi:10.1371/journal.pone.0040563.t003
Steven Tanksley (Cornell University, Ithaca, New York, USA) We also obtained DNA from the *S. pennellii* introgression lines in the M82 background from Dani Zamir (Hebrew University, Rehovot, Israel). Genotyping with the array was performed according to the manufacturer’s instructions for Illumina Infinium assay. The resulting intensity data was processed using the genotyping module v1.7.4 of the GenomeStudio software (Illumina Inc., San Diego, CA, USA) for SNP calling. In order to determine SNP genotype, a cluster file developed by TraitGenetics based on 92 hybrids facilitated allele calling in the Genome Studio software.

**Genetic and Physical Mapping**

Three different software packages were used for mapping of the markers: JoinMap 4.0 [46], Map Manager QT Xb20 [47], and MapChart 2.2 [48]. First, the genotyping data were transformed into the respective mapping data format (“ABH”, A = genotype parent 1, B = genotype parent 2, H = heterozygous). Subsequently, the JoinMap 4.0 program was used for verification of the segregation patterns, the formation of linkage groups and the preliminary positioning of the markers on chromosomes using the default grouping settings and the maximum likelihood mapping algorithm.

The final map position of the markers and the genetic distances between the markers were further optimized manually with respect to the number of crossovers (as low as possible) and the length of the linkage group (as short as possible) using the ABH mapping data file in Excel and MapManager QT X (settings: linkage evaluation F2 intercross, search linkage criterion *P* = 0.05, map function Kosambi, cross type line cross). The final map was drawn using MapChart 2.2.

In order to compare maps, an iterative approach was used in which at least 60 independent maps were created for each of the three populations. For each iteration, 217–325 markers were chosen based on a filter for 5 cM separation (determined by initial mapping). Map construction followed the steps described above, and comparisons between total map length and individual chromosome lengths were based on Analysis of Variance.

We determined the physical map position of the SNPs based on the flanking sequences used to develop the high-density Infinium array. These sequences were oriented relative to the genome sequence using the automated batch BLAST feature to search the tomato reference genome sequence (v SL2.40; http://solgenomics.net/organism/Solanum_lycopersicum/genome).

![Figure 2. Comparative analysis of the EXPEN 2012 and EXPIM 2012 genetic maps relative to the draft assembly (v SL2.40; http://solgenomics.net/organism/Solanum_lycopersicum/genome) of the tomato reference genome sequence.](http://solgenomics.net/organism/Solanum_lycopersicum/genome)

doi:10.1371/journal.pone.0040563.g002
Tomato WGS chromosome (v SL2.40; http://solgenomics.net/organism/Solanum_lycopersicum/genome) [30]. For a SNP with multiple BLAST hits, the best match was used to infer a map position. A custom Python script was then used to identify the actual SNP positions relative to the SL 2.40 genome sequence. We first calculated the 3' flanking sequence length for each SNP. The script determined sequence orientation based on start and end positional information, and the SNP position was determined by adding or subtracting, depending on sequence orientation, the length of the flanking sequence to the corresponding subject start position. The accuracy of SNP positions was manually verified using a subset of data.

Supporting Information

Table S1 8,784 SNPs used for array development in this study.

Table S2 3,503 SNP markers in the EXPEN 2000 (LA9025 x LA7016) linkage map and their assignment on the introgression line population of S. pennelli (IL).

Table S3 3,687 SNP markers in the EXPEN 2012 (Moneymaker x LA0716) linkage map.

Table S4 4,491 SNP markers in the EXPIM 2012 (Moneymaker x LA0121) linkage map.

Table S5 3,149 SNP markers mapped on both the EXPEN 2000 and EXPEN 2012 linkage maps.

Table S6 2,509 SNP markers mapped on both the EXPEN 2000 and EXPIM 2012 linkage maps.

Table S7 2,841 SNP markers mapped on both the EXPEN 2012 and EXPIM 2012 linkage maps.

Table S8 5,295 SNP markers with both genetic and physical positions.

Acknowledgments

We would like to thank Cindey Lawley of Illumina Inc. for her coordination of the Tomato SNP array Consortium. We also thank internal reviewers at The Ohio State University, OARDC for comments and helpful suggestions on the manuscript. TraitGenetics acknowledges the excellent technical assistance of Sandra Reis and Steffie Wehle. For the INRA SNPs, Gautier Sarah and Jean Paul Bouchet are acknowledged for bioinformatic analyses and Stéphane Munos, Nicolas Ranc, Sylvain Santoni and Laure Senté for production of sequences.

Author Contributions

Conceived and designed the experiments: SCS MWG AVD DMF. Performed the experiments: SCS GD JP RW JPH DMF. Analyzed the data: SCS. Contributed reagents/materials/analysis tools: MC GD JP RW JPH. Performed the experiments: SCS GD JP RW JPH. Analyzed the data: SCS.

References

1. Martin GB, Brommeleneker HL, Chunwongse J, Frary A, Ganal MW, et al. (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262: 1432–1436.
2. Jones DA, Thomas CM, Hammoodiosasske KE, Balkinikar PJ, Jones JDG (1994) Isolation of the tomato Cf-9 gene for resistance to Cladosporium fulvum by transposon tagging. Science 260: 789–793.
3. Kawchuk LM, Hachey J, Lynch DR, Kulesar F, van Rooijen G, et al. (2001) Tomato Fv disease resistance genes encode cell-surface-like receptors. Proc Natl Acad Sci U S A 98: 6511–6515.
4. Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, et al. (1998) The root knot nematode resistance gene Ms from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10: 1307–1319.
5. Puel L, CarmelGoren L, Hareven D, Gunfletter T, Alvarez J, et al. (1998) The SELF-PRC/NPG gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEV and TFL1. Development 125: 1979–1989.
6. Xiao H, Jiang N, Schaffner E, Stockinger EJ, van der Knaap E (2006) A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. Science 319: 1527–1530.
7. Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to beta-carotene formation in plant chromoplasts developed by map-based cloning of Beta and old-gold color mutations in tomato fruit. Science 319: 1527–1530.
8. Tanksley SD, Ganal MW, Prince JP, Devicente MC, Bonierbale MW, et al. (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141–1160.
9. Shirasawa K, Isobe S, Hirakawa H, Asamizu E, Fukuoka H, et al. (2010) Genome-wide SNP discovery and linkage Map Construction in Cultivated Tomato. DNA Research 17: 381–391.
10. Robbins MD, San S, Yang W, Van Deyne A, van der Knaap E, et al. (2011) Mapping and linkage disequilibrium analysis with a genome-wide collection of SNPs that detect polymorphism in cultivated tomato. Journal of experimental botany 62: 1831–1845.
11. Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotechnol 26: 1135–1145.
12. Deschamps S, Campbell MA (2010) Utilization of next-generation sequencing platforms in plant genomics and genetic variant discovery. Mol Breed 25: 553–570.
13. Davey JW, Hohenhohe PA, Eiter PD, Boone JQ, Catchen JM, et al. (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics 12: 499–510.
14. McNally KL, Childs KL, Bohmer R, Davidson RM, Zhao K, et al. (2009) Genomewide SNP variation reveals relationships among landraces and modern varieties of rice. Proc Natl Acad Sci U S A 106: 12273–12278.
15. Yamamoto T, Nogasaki H, Yonemaru J, Ehana K, Nakajima M, et al. (2010) Fine definition of the pedigree haplotypes of closely related rice cultivars by means of genome-wide discovery of single-nucleotide polymorphisms. BMC Genomics 11: 267.
16. Barbazuk WB, Ensmich SJ, Chen HD, Li L, Schnable PS (2007) SNP discovery via 454 transcription sequencing. Plant J 51: 910–918.
17. Trebbi D, Maccaferri M, de Heer P, Sorensen A, Giuliani S, et al. (2011) High-throughput SNP discovery and genotyping in durum wheat (Triticum durum Desf.). Theor Appl Genet 123: 535–569.
18. Bundock PC, Elston FG, Allen G, Benson AD, Casu RE, et al. (2009) Targeted single nucleotide polymorphism (SNP) discovery in a highly diploid plant species using 454 sequencing. Plant Biotechnol J 7: 547–534.
19. Hyten DL, Cannon SB, Song QJ, Weeks N, Fickus EW, et al. (2010) High-throughput SNP discovery through deep sequencing of a reduced representation library to anchor and orient scaffolds in the soybean whole genome sequence. BMC Genomics 11: 38.
20. Kim MY, Lee S, Van K, Kim TH, Jeong SC, et al. (2010) Whole-genome sequencing and intensive analysis of the undomesticated soybean (Glycine soja Sieb and Zucc.) genome. Proc Natl Acad Sci U S A 107: 22032–22037.
21. Hamilton JP, Hansey CN, Whitty BR, Stoffel K, Masana A, et al. (2011) Single nucleotide polymorphism discovery in elite north american potato germplasm. BMC Genomics 12: 12.
22. Harashin JP, San S, Stoffel K, Van Deyne A, Buell CR, et al. (2012) Single nucleotide polymorphism discovery in cultivated tomato via sequencing by synthesis. The Plant Genome 5: 17–29.
23. Steenwyk EJ, Chang WH, Lee G, Barker DL, Shen R, et al. (2006) Whole-genome genotyping with the single-base extension assay. Nat Methods 3: 51–53.
24. Gupta PK, Rustgi S, Mie RR (2008) Array-based high-throughput DNA markers for crop improvement. Heredity 101: 5–18.
25. Ganal MW, Durstewitz G, Polley A, Berard A, Buckler ES, et al. (2011) A large maize (Zea mays L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. PLoS ONE 6: e23374.
26. Van Deun A, Stoffel K, Buell CR, Koiz A, Liu J, et al. (2007) Diversity in conserved genes in tomato. BMC Genomics 8: 163.
27. Sim SC, Robbins MD, Chilcott C, Zhu T, Francis DM (2009) Oligonucleotide array discovery of polymorphisms in cultivated tomato (Solanum lycopersicum L.) reveals patterns of SNP variation associated with breeding. BMC Genomics 10: 10.
28. Sherman JD, Stack SM (1992) Two-dimensional spreads of synaptonemal complexes from solanaceous plants.5. Tomato (Lycopersicon esculentum) karyotype and idiogram. Genome 35: 354–359.
29. Eshed Y, Zamir D (1995) An introgression line population of Lycopersicon pennellii in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141: 1147–1162.
30. The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485: 635–641.
31. Fulton TM, Van der Hoeven R, Eannetta NT, Tanksley SD (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell 14: 1457–1467.
32. Frary A, Xu YM, Liu JP, Mitchell S, Tedeschi E, et al. (2005) Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. Theor Appl Genet 111: 291–312.
33. Chetelat RT, Deverna JW (1994) Expression of unilateral incompatibility in pollen of Lycopersicon pennellii is determined by major loci on chromosomes 1, 6 and 10. Theor Appl Genet 82: 704–712.
34. van der Knaap E, Sanyal A, Jackson SA, Tanksley SD (2004) High-resolution fine mapping and fluorescence in situ hybridization analysis of sun, a locus controlling tomato fruit shape, reveals a region of the tomato genome prone to DNA rearrangements. Genetics 168: 2127–2140.
35. Anderson LK, Covey PA, Larsen LR, Bedinger P, Stack SM (2010) Structural differences in chromosomes distinguish species in the tomato clade. Cytogenet Genome Res 129: 24–34.
36. Stack SM, Royer SM, Shearer LA, Chang SB, Giovannoni JJ, et al. (2009) Role of Fluorescence in situ Hybridization in Sequencing the Tomato Genome. Cytogenet Genome Res 124: 339–350.
37. Frary A, Presting GG, Tanksley SD (1996) Molecular mapping of the centromeres of tomato chromosomes 2 and 9. Mol Gen Genet 250: 295–304.
38. Wenz P, Li HB, Carling J, Zhou MX, Ramam H, et al. (2006) A high-density consensus marker of barley linking DAR/T markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7: 206.
39. Yu J, Pressoir G, Briggs WH, Vroh Bi I, Yamasaki M, et al. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38: 205–208.
40. Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819–1829.
41. Aoso FG, Newell MA, Beavis WD, Scott MP, Jannink JL (2011) Accuracy and Training Population Design for Genomic Selection on Quantitative Traits in Elite North American Oats. The Plant Genome 4: 132–144.
42. Hefner EL, Jannink JL, Iwata H, Souza E, Sorrells ME (2011) Genomic Selection Accuracy for Grain Quality Traits in Biparental Wheat Populations. Crop Sci 51: 2597–2606.
43. Zhao Y, Gowda M, Liu W, Wunschum T, Maurer HP, et al. (2012) Accuracy of genomic selection in European maize elite breeding populations. Theor Appl Genet 124: 769–776.
44. Ernst K, Kumar A, Kriseleti D, Kloos DU, Phillips MS, et al. (2002) The broad-spectrum potato cyst nematode resistance gene (Hero) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. Plant J 31: 127–136.
45. Kabelka E, Franchino B, Francis DM (2002) Two loci from Lycopersicon hirsutum L4A07 confer resistance to strains of Clavibacter michiganensis subsp. michiganensis. Phytopathology 92: 504–510.
46. Van Ooijen JW (2006) JoinMap® 4.0, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands.
47. Manly KF, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamman Genome 12: 930–932.
48. Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93: 77–78.