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Article *in* Molecular Plant · April 2016

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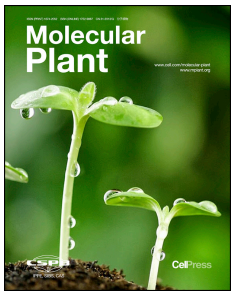

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PII: S1674-2052(16)30032-6
DOI: 10.1016/j.molp.2016.04.010
Reference: MOLP 286

To appear in: *MOLECULAR PLANT*
Accepted Date: 17 April 2016

Please cite this article as: **Kwong Q.B., Teh C.K., Ong A.L., Heng H.Y., Lee H.L., Mohamed M., Low J.Z.-B., Sukganah A., Chew F.T., Mayes S., Kulaveerasingam H., Tammi M., and Appleton D.R.** (2016). Development and Validation of A High Density SNP Genotyping Array for African Oil Palm. Mol. Plant. doi: 10.1016/j.molp.2016.04.010.

Development and Validation of A High Density SNP Genotyping Array for African Oil Palm

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Running Title: High-density Oil Palm SNP Genotyping Array

Abstract

High-density single nucleotide polymorphism (SNP) genotyping arrays are powerful tools that can measure the level of genetic polymorphism within a population. To develop this whole-genome SNP array, SNP discovery was performed using deep resequencing of eight libraries derived from 132 *Elaeis guineensis* and *Elaeis oleifera* palms belonging to 59 origins, resulting in the discovery of >3 million putative SNPs. After SNP filtering, the Illumina OP200K custom array was built with 170,860 successful probes. Phenetic clustering analysis revealed that the array could distinguish between palms of different origins in a way consistent with pedigree records. Genome-wide linkage disequilibrium (LD) declined more slowly for the commercial populations (ranging from 120Kb at r^2=0.43 to 146Kb at r^2=0.50) when compared with the semi-wild populations (19.5Kb at r^2=0.22). Genetic fixation mapping comparing the semi-wild and commercial population identified 321 selective sweeps. A genome-wide association study (GWAS) detected a significant peak on Chromosome 2 associated with the polygenic component of the shell thickness trait (based on the trait *shell-to-fruit*; S/F %) in *tenera* palms. Testing of a genomic selection model on the same trait resulted in good prediction accuracy (r=0.65) with 42% of the S/F % variation explained. The first high-density SNP genotyping array for oil palm has been developed and shown to be robust for use in genetic studies and with potential for developing early trait prediction to shorten the oil palm breeding cycle.

Keywords: genotypes, whole-genome SNP array, genome-wide association study, genomic selection, genetic fixation, shell thickness, oil palm, linkage disequilibrium, phenetic clustering, whole-genome variation, genomic prediction, genome-wide linkage disequilibrium, polygenic trait

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a monocot of the *Arecaceae* family that originated from tropical Guinea's rainforest in West Africa (Hartley, 1967c). This plant is the most efficient oil crop in the world, yielding commercially an average of 4 tons oil hectare^{-1} year^{-1}, equivalent to ten times the average oil yield hectare^{-1} year^{-1} of other oil crops; palm oil currently supplies 30% of the global edible oil demand (US Department of Agriculture, 2015). In order to address issues of climate change and land and labor shortage, the most sustainable strategy to meet future demand is to increase yields on the same planting area through oil palm breeding. For this purpose, oil palm breeding will need to improve multiple traits affected by biotic, abiotic, management and processing regimes simultaneously (Davidson, 1991; Meunier et al., 1979; Soh, 1981).

Genetic diversity is always the key resource for improvement of any agronomic trait. Comprehensive germplasm prospections of *E. guineensis* have been carried out in Africa since the first commercial cultivation of the crop in the early 20th century (Rajanaidu, 1986b). Prospection has been extended to the African oil palm's close species relative, *E. oleifera* H.B.K. Cortes, present in Central and South America (Rajanaidu, 1986a). The South American oil palm is superior in terms of commercially desirable oil quality (high level of unsaturated oils), has low height increment and carries wilt resistance (Hardon et al., 1985; Hartley, 1988; Mohd et al., 2002). However, the South American palm yields poorly, producing on average 0.5 tons oil hectare^{-1} year^{-1} (Wahid et al., 2004). Attempts to introduce these beneficial traits from prospective germplasm into the commercial *E. guineensis* through interspecific crossing programs have met with varying degrees of success. Molecular markers, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) were first used to assess genetic diversity of the collected and available germplasm (Barcelos et al., 2002; Shah et al., 1994). Attention then shifted to multi-allelic marker systems, particularly simple sequence repeats (SSRs), which detect a high degree of allelic variation even among closely related individuals. Hence, SSR markers for *E. guineensis* have been derived from genomic libraries and expressed sequence tag (EST) databases (Billotte et al., 2001; Ting et al., 2010). The aim of the molecular marker development was to identify new allelic variation in unexploited origins that could be used to

widen the genetic base of breeding programs (reducing potential inbreeding depression and introducing novel trait alleles) through introgression. Genetic improvement in oil palm through conventional breeding, however, is slow due to long selection cycles (typically 12 years) and the lack of genetic homozygosity in current advanced parental breeding materials (Mayes et al., 2000). To shorten the selection cycle, molecular markers can be deployed to identify quantitative trait loci (QTL) underlying traits of interests and to perform genome-wide screening of oil palm (Wong and Bernardo, 2008). The individual palms that possess desired allelic combinations of QTL can be identified in immature plants, even at the nursery stage, rather than relying on 7-10 years of phenotypic evaluation in the field, also facilitating rapid backcross introgression of novel traits into elite material.

Yield traits (e.g. oil yield and bunch production) in oil palm are mostly quantitatively inherited and controlled by multiple genes. Controlled cross-based linkage analysis and GWAS are the main methods that have been exploited to discover and locate genes/QTL for these complex traits. Multi-allelic markers such as RFLP and SSR used so far normally only provide low to medium mapping and genome coverage resolutions. In contrast, SNPs have a high level of abundance and distribution uniformity throughout a genome and are now widely deployed for developing high-density genome-wide scans in humans, and have been used more recently in plants (Huang et al., 2010; Wang et al., 1998). The publication of the 1.535 Gb oil palm genome and the independent assembly of Sime Darby's oil palm genome, together with the cost reduction in high-throughput sequencing provide an opportunity for large scale and high density SNP detection in oil palm (Singh et al., 2013b).

Whole-genome SNP arrays have been developed for many species including rice, corn, cattle, horse and more recently salmon (Chen et al., 2014; Ganal et al., 2011; Houston et al., 2014; McCue et al., 2012; Yu et al., 2014). To date, the only SNP genotyping array available for oil palm is a 4.5K custom Illumina SNP array, which has been proven to be useful in construction and comparison of linkage maps (Ting et al., 2014). However, for genome-wide scans, including GWAS and LD analysis, a higher density genotyping array is required.

This manuscript summarizes the overall properties of the first whole-genome SNP array for oil palm. In addition, the analyses of 312 *tenera* palms from different backgrounds using the genotyping array demonstrate a wide range of applications, including phenetic analysis, LD

decay analysis, selective sweep mapping and GWAS. Oil palm breeders and researchers can use this genetic information as the basis for crop improvement programs, through applied marker assisted selection and/or direct genomic selection approaches.

Results

Genome resequencing, SNP discovery and array design

The pooled resequencing generated approximately 73.76 Gb of data – approximately a 40x coverage of the oil palm genome. In total, 1,015,758,056 reads were generated. After filtering, 870,235,918 high quality reads were identified for mapping against the published oil palm genome and Sime Darby's in-house scaffolds independently. The sequencing data produced had a mean sequence quality Phred score of 39 (Figure S1). There were no over-represented sequences and the average GC content was within the expected range of between 40-45%.

More than seven million SNPs were called from the resequencing data. The SNPs which were unique for the *E. oleifera* genome (1,085,204 SNPs), those with mapping coverage less than x17 or more than x53 (669,950 SNPs), genotyping quality (GQ) scores of less than 8 (5,089 SNPs) and minor allele frequency (MAF) of < 0.05 (71,503 SNPs) were removed as part of the quality control procedure. Based on the technical requirements of Illumina, 5,174,660 SNPs that have secondary SNPs located within 60bp upstream or downstream were removed by filtering. In addition, other criteria for the removal of candidates included SNPs that were indels (56,357 SNPs), non-biallelic (408 SNPs) and SNP types that required two bead types on the array to be detected, i.e. A/T and C/G types (88,348 SNPs). Ambiguous bases within the 121bp probe region were also identified and removed (10,992) (Table S1). From the 593,888 high quality SNPs identified *in silico*, 200,000 SNPs were finally selected according to LD with a r^2 cut-off set at 0.3 and uniform spacing across the assembled genome to populate the OP200K array.

SNP genotyping

Out of the 200,000 designed SNPs, 170,860 markers passed bead representation and decoding quality metrics. By testing the array on 312 palms representing some of the 59 diverse oil palm origins, 161,073 SNPs generated reproducible scores and resulted in the basal data set used for the subsequent analysis. An example of the genotyping result is provided in Figure S2. Overall, only 0.65% of the entire genotyping data set was missing data.

A total of 70,804 genotyped SNPs could be located on the published oil palm physical map. The density of these SNPs on the chromosomes is illustrated in Figure 1. Using the published physical map as a reference, each chromosome has an average of 4,425 SNP loci. Chromosome 10 has the highest marker density (1 SNP every 8,344bp), whereas Chromosome 6 has the lowest (1 SNP every 10,254bp). Overall, the density of the array is 1 SNP per 11Kb of the oil palm physical map, inclusive of chromosomes and scaffolds. This resolution is comparable with other published large-scale whole genome SNP genotyping arrays for plant species (Chen et al., 2014; Ganal et al., 2011).

Figure 1. SNP distribution and density per chromosome in the oil palm genome assembly. The SNP distribution per chromosome is represented in the histogram. The mean genomic distance between markers on every chromosome is indicated as a line plot. The average SNP density of the OP200K array is 1 SNP per 11Kb, based on the oil palm physical map.

Phenetic analyses

To reveal the genetic relationship between all 312 *tenera* palms, a full neighbor-joining dendrogram was built for the 312 individuals genotyped using the full 161,073 SNP dataset (Figure S3). For illustration purposes, a representative dendrogram was also built using only 45 individuals (Figure 2). The bootstrap values from the representative dendrogram ranged from 80 – 100, indicating good reproducibility. Both results identified five main clusters from the major nodes, I. *Ulu Remis* (UR) x AVROS; II. *Johore Labis* (JL) x AVROS; III. Nigerian x AVROS; IV. *Gunung Melayu* (GM) x *Dumpy* AVROS (DA); and V. JL x DA. This finding was consistent with the different *dura* (maternal parent) and *pisifera* (paternal parent) used in breeding and with

pedigree records. The commercial planting materials (I and II) were grouped separately from the semi-wild (III) and breeding populations (IV and V).

Figure 2. Genetic relationships between representative *tenera* palms derived from different backgrounds. A neighbor-joining dendrogram was constructed for 45 representative *tenera* palms based on pairwise genetic distances. Five main clusters were identified, including I. UR x AVROS (red), II. JL x AVROS (purple), III. Nigerian x AVROS (green), IV. GM x DA (yellow) and V. JL x DA (blue).

Genome-wide LD decay

The average decay of LD with physical distance between SNPs was 146Kb (r^2 = 0.50) and 120Kb (r^2 = 0.43) for the UR x AVROS cluster and the JL x AVROS cluster. Average LD decayed faster at 87.7Kb (r^2 = 0.22) and 54.7Kb (r^2 = 0.21) for the JL x DA cluster and the GM x DA cluster, followed by 19.5Kb (r^2 = 0.22) for the Nigerian x AVROS cluster (Figure 3). By using the minimal LD decay of 19.5Kb as a base line reference and compared with Chromosome 6 with the lowest marker density (1 SNP every 10,254bp), this array provides sufficient genomic resolution to detect association signals in any oil palm population.

Figure 3. Decay of LD in the five *tenera* clusters from different backgrounds. Each cluster has a different LD decay pattern, expressed in r^2, which is probably due to different selection and breeding histories.

Mapping of selective sweeps

A total of 321 selective sweeps were detected in the genome of the commercial populations after comparison with the semi-wild population, with significant regions identified

on every chromosome in the oil palm genome. 38% of the selection events were observed on Chromosome 1, 2 and 3 (Figure 4).

Figure 4. Genome-wide mapping of selective sweeps. A total of 321 selective sweeps were identified in commercial populations by comparing them against the semi-wild Nigerian x AVROS population. Selective sweeps were defined as the top 1% of the score windows. The selective sweeps were highlighted in black.

In this study, 679 genes were present in the detected selective sweeps (Table S2). These genes could be annotated into 1097 Gene Ontology (GO) classes and 120 Enzyme Classes (EC). Statistically, 162 GOs and ECs were observed to be significantly over-represented (at Chi-square $p<0.05$) in the selective sweep regions for the commercial populations, as compared to the semi-wild population (Table S3).

GWAS & Genomic selection

To evaluate the usefulness of this array in GWAS, we selected the polygenic S/F % of *tenera* palms as the trait of interest. While it has been shown that fruit type identity is controlled by a single gene (Singh et al., 2013a), once this is taken into account there is still significant variation remaining in breeding and commercial populations for S/F %. Here, all palms evaluated were *tenera*, which effectively removes the contribution of the *SHELL* identity gene, *per se*. In this analysis, population structure was controlled using a kinship matrix while environmental factors were initially tested as if unimportant. Genomic inflation was calculated to be 1.0, indicating that inflation was successfully controlled by this approach. From the GWAS result, we observed a number of significantly associated SNPs across a genomic region 1,378,847bp in length, located on Chromosome 2 from position 2,526,459bp to 3,905,306bp based on the genome-wide significance cut-off, $-\log_{10}$ *p-value* = 5.0 (Figure 5). Some of the genes that fall in this region include *La-related protein 6B* (*LARP6B*), *SHELL/Seedstick* (*STK*), *Cytochrome c oxidase* (*COX*), *Transcription factor CPC* (*CPC*) and *Inositol-tetrakisphosphate 1-kinase* (*ITPK1*). Two singleton markers were determined to be significantly associated at

position 5,708,516bp and 13,250,110bp of Chromosome 2. In addition, a few other signals were located at Chromosome 5 and 10 (Table 1). A full GWAS SNP list is presented in Table S4.

Figure 5. Genome-wide association study (GWAS) for polygenic shell thickness (S/F %) in *tenera* palms. A Manhattan plot of the compressed Mixed Linear Model (MLM) for 312 *tenera* palms. Negative $\log_{10}$ transformed *p-value*s from a genome-wide scan are plotted against position on each of the 16 chromosomes. The horizontal line indicates the genome-wide significance cut-off, $-\log_{10}(p\text{-}value)= 5.0$.

Table 1. SNPs in the main S/F %-associated peaks

SNP ID	Chromosome	Position (bp)	$-\log_{10}(p\text{-}value)$	SNP effect	R-sq* (%)	Gene
SD_SNP_000044630	2	2,526,459	5.73	0.82	3.27	*LARP6B, STK, COX, CPC, ITPK1*
SD_SNP_000044629	2	2,528,936	5.25	3.54	3.05	
SD_SNP_000054651	2	2,531,607	5.30	-3.71	2.92	
SD_SNP_000042701	2	3,516,726	5.88	-2.01	15.10	
SD_SNP_000022202	2	3,905,306	6.19	0.05	1.04	
SD_SNP_000008871	2	5,708,516	7.07	-1.46	2.30	*GONST1*
SD_SNP_000017557	2	13,250,110	5.83	1.31	2.09	*GBF4*
SD_SNP_000022069	5	16,977,016	5.04	1.58	14.16	*FAD3C*
SD_SNP_000034242	5	17,308,442	5.20	0.14	15.64	
SD_SNP_000026705	10	25,688,314	5.07	1.47	18.04	*NUD17*

*R-sq represents the amount of variance explained

A 5-fold cross-validation, as described in the Methods section, was carried out. The correlation between Genomic Estimated Breeding Value (GEBV) and realized S/F % trait acquired was 0.65 (Figure 6) based on the criteria given in Methods section. The prediction model built suggested that 42% of the trait variation could be explained in the validation population.

Figure 6. Representative regression plot of GEBV and true shell thickness for the validation population set. The GEBV was obtained by Bayes A in the final model. The correlation coefficient between GEBV and true trait was 0.65 with 42% S/F variation explained.

Discussion

The OP200K reported here is the first large scale oil palm SNP array. Since oil palm is a naturally outcrossing and monoecious species, each oil palm genome can be highly heterozygous (Hartley, 1967a). The SNP array is therefore designed to be applicable to a wide range of oil palm origins. As such, SNPs were discovered by resequencing palms of diverse origins including all fruit forms (*dura*, *tenera* and *pisifera*) and the major breeding origins available at Sime Darby Plantations. The different fruit forms are distinguished by the thickness of the shell, together with the presence/absence of a fiber ring: *dura* (thick-shelled; without fiber ring), *tenera* (thin-shelled; with fiber ring), and *pisifera* (shell-less; female-sterile; with fiber ring). Shell thickness is inversely correlated with the mesocarp percentage in each fruitlet, and therefore also to commercial crude palm oil yield; because *tenera*, a hybrid between *dura* and *pisifera*, has the highest mesocarp oil yield, it is the fruit form exploited commercially.

The analyzed OP200K array data clearly revealed the genetic stratification of the 312 *tenera* palms from a number of important origins in a way concordant with pedigree records. The five main clusters identified are I (UR x AVROS); II (JL x AVROS); III (Nigerian x AVROS); IV (GM x DA); and V (JL x DA). Clusters I and II include the commercial populations, whereas cluster III represents the semi-wild population. Cluster IV and V represent other breeding populations at Sime Darby Plantations. In Southeast Asia, oil palm breeding improvement is almost exclusively focused on the Deli *dura* origin on the maternal side, which is believed to be derived from the four palms planted at the Bogor Botanic Garden, Indonesia, in 1848. The subsequent distribution to several breeding programs stressing different selection criteria over a number of generations has led to differentiation between subpopulations, leading to pools of 'dissimilar' germplasm which have been termed 'breeding populations of restricted origins (BPROs)' (Rosenquist, 1986). The Deli *dura* BPROs, including UR, JL and GM, were selected

for different yield-related traits. The UR origin was chosen for high bunch number and high sex ratio (defined as the ratio of female inflorescences to total inflorescences) in Marihat Baris, Sumatra (Hartley, 1967b). Instead of bunch number, Socfindo in Sumatra selected the JL origin for bigger bunches (high bunch weight) and thinner shell. Another further interested origin, GM was planted by a Swiss company in the year 1919 and selections were made for low height increment and high oil/mesocarp (Corley and Tinker, 2003).

An immediate 30% increment of oil yield per hectare was gained after the introduction of the Deli *dura* x BM119 AVROS *pisifera* program to the Southeast Asia region (Corley and Tinker, 2003; Hardon et al., 1987). The AVROS *pisifera* paternal parent line confers superiority in uniformity of growth, general combining ability, precocity and high mesocarp oil content in the commercial Deli x AVROS (*tenera*) hybrid progeny. However, the narrow genetic base of both Deli and AVROS - theoretically descended from four and two palms, respectively - may hinder future breeding progress (Rosenquist, 1990). Consistent with this, it was observed that the LD decayed slowest at 120Kb and 146Kb in both commercial JL x AVROS and UR x AVROS clusters, respectively, compared with 19.5Kb in the semi-wild Nigerian x AVROS cluster, indicating the lower genetic diversity/narrower genetic base and potentially the effects of higher selection pressure in these materials.

Alternative genetic resources, such as semi-wild Nigerian *dura* and the DA *pisifera* breeding materials are being exploited by the breeders to broaden the genetic variability. Direct improvement in oil yield per palm is the highest priority for oil palm breeding. However, due to an acute labor shortage for fresh fruit bunch (FFB) collection, palm structural traits such as height are also of potential interest. One approach to mitigate this problem has led to a shift in the oil palm breeder's attention to DA, GM and JL populations for development of materials with lower annual height increment, to facilitate harvesting. The DA originated from the well-known *Dumpy* E206 self-pollinated cross, the parental palm that had an unusually large girth and low height increment (Jagoe, 1952). The DA *pisifera* lines have been crossed with the short-stemmed GM *dura* and JL *dura* to reduce trunk height increment in their resulting *tenera* progeny (Corley and Tinker, 2003). The introduction of new genetic material into elite breeding programs has indeed introduced more recombination into these *tenera* clusters, breaking the long-range LDs. Hence, the LD in both the breeding populations (at 87.7Kb for JL x DA cluster;

at 54.7Kb for UR x DA cluster) and the semi-wild Nigerian x AVROS cluster (at 19.5 Kb) decayed at least two-fold faster when compared to the commercial populations. Using the most rapid rate of LD as the reference (i.e. 19.5Kb), the OP200K array provides sufficient mapping resolution for whole genome linkage analysis and GWAS in any of the materials examined to date.

The long-range LD observed in the commercial populations can potentially be explained further by breeding selection. The selected alleles will rise in frequency towards fixation in a population and nearby linked alleles on the chromosome will be co-selected through linkage drag with the selected locus to high frequency. This process is termed a 'selective sweep'. A total of 321 potential selective sweeps were observed in the oil palm genome based on the available physical map. In these selective sweeps, the over-represented gene classes (based on Chi-square tests) can be classified into different groups, including floral and fruit development (GO:0009733 response to auxin, GO:0048574 long-day photoperiodism, flowering, GO:0048575, short-day photoperiodism, flowering, GO:0010483 pollen tube reception and GO:0010183 pollen tube guidance) and lipid biosynthesis (GO:0019216 regulation of lipid metabolic process, GO:0000038 very long-chain fatty acid metabolic process, GO:0042304 regulation of fatty acid biosynthetic process, GO:0006636 unsaturated fatty acid biosynthetic process, GO:0045300 acyl-[acyl-carrier-protein] desaturase activity , EC:2.3.1.41 beta-ketoacyl-[acyl-carrier-protein] synthase I). The selection of these gene classes might be associated with the main breeding objective in oil palm: maximizing oil yield per unit area for plantation profitability (Corley and Tinker, 2003). As a direct comparison, in another oil crop, soybean, the main gene classes over-represented in the cultivated population were flowering time/floral development, fatty acid biosynthesis and plant structure (height and pubescence) (Zhou et al., 2015). In rice, however, the main gene classes were flowering time/floral development, plant growth and morphology (Xu et al., 2012). In all three species, we observed flowering or floral development to be a key trait of importance for breeding programs. For the oil species, as expected, the fatty acid related classes were crucial. In the longest selective sweep of Chromosome 2, we have identified the *MADS-box transcription factor 16* gene, which is known to be responsible for floral identity control and development (Moon et al., 1999). In oil palm, female inflorescence development determines bunch yield components (Breure and Menendez, 1990). Even though allelic frequency can reflect artificial selection pressure on the genes, it is

also possible that some of these genes might be inherited through linkage drag with the oil yield-related genes selected under breeding. Also, given that the population base of the modern elite breeding materials is narrow, it is possible that there might be a number of false positives. The true causality can only be concluded through functional analysis, which is laborious. To narrow the scope of functional studies, GWAS can be used in conjunction with selective sweep analysis as additional evidence to identify QTL. Knowing that fruit and floral development is one of the key gene classes found in the selective sweeps, identification of the important QTL in one of the fruit components, S/F %, was selected to illustrate the applicability of this array in GWAS.

In oil palm, the fruit forms are determined as thick-shelled *dura* (*Shell*$^{+}$ *Shell*$^{+}$) , shell-less *pisifera* (*Shell*$^{-}$ *Shell*$^{-}$) and their hybrid, thin-shelled *tenera* (*Shell*$^{+}$ *Shell*$^{-}$), in a co-dominant Mendelian inheritance pattern, first reported in the 1940s (Beirnaert and Vanderweyen, 1941). The gene responsible for fruit form, determined by the presence or absence of shell and fiber ring, has been reported to be *SHELL*, a gene homologous to the Arabidopsis *SEEDSTICK* (*STK*) gene (Singh et al., 2013a). This gene is located at position 3,058,076bp of Chromosome 2. However, within the *tenera* fruit form, varying degrees of thickness of the shell exists. Presumably controlled by minor genes, it is known that there is polygenic variation in shell thickness, superimposed onto the major *SHELL* effect (Corley and Tinker, 2003). The *tenera* palms with a low S/F % trait have more mesocarp per fruit, leading to more oil production. Given the importance of this trait in the *tenera* population, GWAS was carried out to study key loci responsible for the variation in this trait that is not explained by the *SHELL* gene. Due to the relatively small sample size of the populations, all of the 312 palms were treated as a single group before performing association mapping. The kinship and population substructure of the set were controlled for by using a kinship matrix. The S/F %-associated GWAS peak at Chromosome 2 position 2,526,459bp to 3,905,306bp contains the published *SHELL* gene. This could argue for allelic effects of different sources of *SHELL* and the surrounding regions. This peak overlaps with the selective sweeps detected in Chromosome 2. It should be noted, however, that two independent signals on Chromosome 2 were also found farther away at 5,708,516bp and 13,250,110bp, and a few other signals were identified on other chromosomes. These additional signals might indicate that there are other minor factors influencing S/F % in *tenera* palms. By having a larger sample set and through fine mapping, these minor factors can be identified and the markers combined to define a genetic ideotype for marker assisted breeding of shell-

thickness. The new OP200K genotyping array has also been proven to be robust in detecting significant associations for a polygenic complex trait, which is high mesocarp oil content in oil palm (Teh et al., 2016).

As demonstrated, the association peaks, which is representative of the QTL, can be detected through GWAS. In order to predict the total genetic breeding value of the genome relative to a trait, instead of using only specific previously identified loci, the genetic effects of the entire genome were evaluated. The approach adopted is known as genomic selection, where all QTL are expected to be in LD with at least one marker locus (Goddard and Hayes, 2007). As previously shown, marker density for the current array is likely to fulfill this requirement, potentially ensuring high prediction accuracy in breeding selection (Meuwissen et al., 2001b). S/F % trait was again selected to test the applicability of genomic selection using this array. Even without addressing environmental variation between palms and populations, within population substructures and with a limited sample size for the training population, a prediction accuracy of about 0.65 was still achieved, thereby illustrating the viability of genomic selection with this array for S/F %. The method used here for genomic selection is Bayes A (Meuwissen et al., 2001a). There are other methods that might give higher prediction accuracy and reduce processing time for larger sample sizes, which will be the focus for future studies (Resende et al., 2012).

The applications of the OP200K genotyping array in genetic diversity assessment, GWAS and genomic selection have proven to be robust and consistent using existing data for palms in these important origins of oil palm. This SNP array has allowed the current mapping resolution to be improved significantly and the array has become the densest genotyping array for oil palm. Hence, the array can be referred to as the first whole genome genotyping tool for oil palm breeding programs. To optimize the genotyping cost, the array can further be customized for the different BPROs developed in different breeding selection programs. From our experience, at this time, the array-based approach is still far cheaper than genotyping by sequencing for high-density marker analysis. The marker density can be reduced according to the BPRO-specific LD decay analysis as shown in this study to ensure good genome coverage with there likely to be at least one representative SNP in every LD block. Overall, the new OP200K should facilitate genome-wide genetic discovery for the oil palm research community.

Methods

Whole genome resequencing

A total of 132 palms from 59 diverse origins were selected for resequencing using Illumina GAIIx, as reported previously (Teh et al., 2016). Genomic DNA (gDNA) for each palm was isolated from 100 milligram of dried leaf tissue using the DNAeasy Plant Mini Kit (Qiagen). The resulting gDNA samples were pooled, with equal amounts of gDNA from all samples. The DNA quantity and quality were then assessed using the NanoDrop ND-1000 Spectrophotometer. A total of eight paired-end (75bp each end) libraries with insert size of 100bp were constructed from the pool according to Illumina's standard protocol for whole genome shotgun sequencing.

SNP discovery and array design

The raw reads acquired from resequencing were trimmed to remove poor quality base calls (Phred<30) and mapped against both the published oil palm genome and in-house scaffolds using the BWA software (sampe and aln) with default parameters (Li and Durbin, 2010). SNP calls were made using samtools programs with parameters of -d 1000, -C 50, -D -S -g -u -q 20 -m 3 (Li et al., 2009). The SNPs were filtered using the vcfutils script with parameters -Q25, -w 2, -W 2, -D 1000, -d 3. From the resulting vcf files, SNPs that had a mapping coverage between 17 and 53, a GQ score of >7, and a MAF of >0.05 were kept.

Only SNPs that had no secondary SNPs 60bp upstream and downstream were used. Other criteria for removal of candidates included SNPs that were indels, were non-biallelic and SNP types that required two bead types on the array, i.e. A/T and C/G types. Ambiguous bases within the 121bp probe region were also identified and removed. The probes were then sent to Illumina to score for their designability. In-house scripts were used to select 200,000 SNPs with the best Illumina designability, LD with r^2 cut-off was set at 0.3 and the set chosen to give a uniform distribution across the entire genome, based on the published and in-house genome. The OP200K arrays were manufactured by Illumina in a 12-sample per array format.

403

404 **SNP genotyping**

405 The OP200K arrays were used to genotype a panel of 312 accessions from commercial
406 oil palm populations of UR x AVROS and JL x AVROS (66 and 33 individuals), a semi-wild
407 population of Nigerian x AVROS (101 individuals) and breeding populations of GM x DA and
408 JL x DA (13 and 99 individuals).

409 For each of the accessions, gDNA was extracted using the same method as described
410 under the resequencing step. Prior to hybridization to the bead arrays, DNA was diluted to
411 25ng/ul and DNA quantification was obtained with Hoechst (33258 Pentahydrate (Invitrogen),
412 using the FLUOstar Omega (BMG Labtech)). DNA quality was assessed on a 0.8% agarose
413 gel. The genotyping was carried out using the designed array on the Infinium iScan platform
414 (Illumina Inc, San Diego, CA) according to the recommendations of the manufacturer.

415 The raw intensity SNP data was analyzed using GenomeStudio version 20011.1 by
416 Illumina with genotyping module version 1.8.4. Using a GenCall score cutoff of 0.15, auto-
417 cluster of the SNPs was done. Cluster refining of the SNP clusters was done manually by visual
418 inspection so that identifiable and scorable clusters were generated. The SNP calls were exported
419 into the PLINK program for MAF and call rate filtering (Purcell et al., 2007). A minimal call rate
420 of 90% and a MAF filter of 0.01 were set as baseline cut-offs.

421

422 **Phenetic analysis**

423 The SNP genotypes of 312 *tenera* palms were compiled. Missing SNP genotypes were
424 imputed using the mean value for each of the populations in this study. The genotypes were first
425 recoded into 0 (AA), 1 (AB) and 2 (BB). Pairwise genetic distance was estimated using the
426 dist.dna function of the APE package in R (Paradis et al., 2004). The dendrogram was generated
427 using the njs function, which implements the neighbor-joining clustering method (Saitou and
428 Nei, 1987). The bootstrap consensus tree was inferred from 1,000 replicates and the other
429 parameters were used as default. Illustration of the tree was done using FigTree version 1.4.2,
430 acquired from http://tree.bio.ed.ac.uk/software/figtree/.

Genome-wide LD decay

Genome-wide LD was estimated by calculating pairwise correlation coefficient (r^2) values between all pairs of SNPs in a 1-Kb window size within each chromosome across the five clusters of oil palm. An inter-SNP distance of more than 10Mb was ignored. The LD decay rate was defined as the chromosomal distance at which the average r^2 dropped to half of its maximum value. For this step, the R package of SNPRelate was used, with the LD estimation based on the composite method (Zheng et al., 2012).

Mapping of selective sweeps

This step was carried out using XP-CLR software (Chen et al., 2010). The window size of 0.1 cM and a grid size of 20000 kb was set for this step. Selective sweeps identified side by side were combined. Top 1% of the XP-CLR scores was selected as the threshold to identify selective sweep (Kim et al., 2015). The result was illustrated in chromosomal format using MapChart version 2.30, with the density of the chromosomal map spaced by 0.5Mb (Voorrips, 2002).

Identification of genes that fall into these regions was determined using published genome information (Singh et al., 2013b). These genes were clustered based on GO by using BLAST2GO (Conesa and Gotz, 2008). The Chi-square method implemented in R was used to test for the enrichment of putative artificially selected genes in all the GOs/ECs identified. With a *p-value* cut-off set at 0.05, a GO/EC class was considered to be significantly enriched if the number of putative selected genes belonging to that class were significantly more than the expected number calculated for that particular class across the significance region from the total number of genes.

GWAS & Genomic selection

The polygenic shell thickness of 312 *tenera* palms was measured according to weight difference between shell and the total fruit as S/F %. The individual palms were phenotyped to generate a reliable mean S/F % value for analysis as per industry standards with modifications (Blaak et al., 1963; Rao et al., 1983). GWAS was then carried out using the GenABEL package in R, which implements a mixed linear model to detect association signals (Aulchenko et al., 2007). To control for cryptic relatedness in the population, a kinship matrix was generated by the ibs function in the package, which computes the identity by state for the markers data. The default weight parameter for the kinship matrix construction was used. The association score was calculated using the mmscore function, with all the parameters set to default. Population inflation was estimated using the estlambda function implemented in the package. A threshold $-\log_{10}(p\text{-}value)$ of 5.0 was set to identify an association peak.

The genotype data used for GWAS was also used for genomic selection. A 5-fold cross validation was carried out to separate the data into training set and validation sets. Genomic selection was done using Bayes A under the BGLR package in R (Perez and de los Campos, 2014). The number of burn-in iterations was set to 10,000 and the Gibbs sampler iteration was set to 20,000. Accuracy was measured through the correlation between the GEBV versus the realized S/F % trait value.

Data availability

All the raw data used in this study are publicly available. The sequencing raw reads have been deposited in SRA under Bioproject PRJNA288621 and Accession ID of SRR2132870, SRR2132879, SRR2132880, SRR2132872, SRR2132874, SRR2132884, SRR2132881, SRR3110655, whereas the SNPs used in the array have been deposited in dbSNP under the handle of SDTC_BB with NCBI submitted SNP (ss) accession numbers of 1810069240-1810592638.

Author Contributions

Conceptualization, Q.B.K., C.K.T., A.L.O. and F.T.C.; Methodology, Q.B.K., C.K.T., A.L.O.; Software, Q.B.K., A.L.O. and H.Y.H.; Formal Analysis, Q.B.K., C.K.T. and A.L.O., Investigation Q.B.K., C.K.T.., A.L.O., H.Y.H. and H.L.L., Resources, M.M., A.S.; Data Curation, Q.B.K, A.L.O. and J.Z.L.; Writing – Original Draft, Q.B.K, C.K.T and A.L.O.; Writing – Review & Editing, H.Y.H, A.S., S.M., F.T.C, H.K., M.T. and D.R.A.; Visualization, Q.B.K., A.L.O. and H.Y.H; Supervision, H.K., M.T. and D.R.A.; Project Administration, Q.B.K., C.K.T., A.L.O.; Funding Acquisition, D.R.A. and H.K.

Acknowledgement

We will like to thank Breeding and Molecular Breeding Units of Sime Darby Plantation R&D for their assistance in sample collection and DNA preparation, and the IT team for High Performance Computing-related support. We will also like to thank DNALandmarks Inc., Canada for their sequencing and genotyping service. In addition, we will like to thank Prof. Jennifer Ann Harikrishna from Institute of Biological Sciences, Faculty of Science, Universiti Malaya for reviewing this manuscript.

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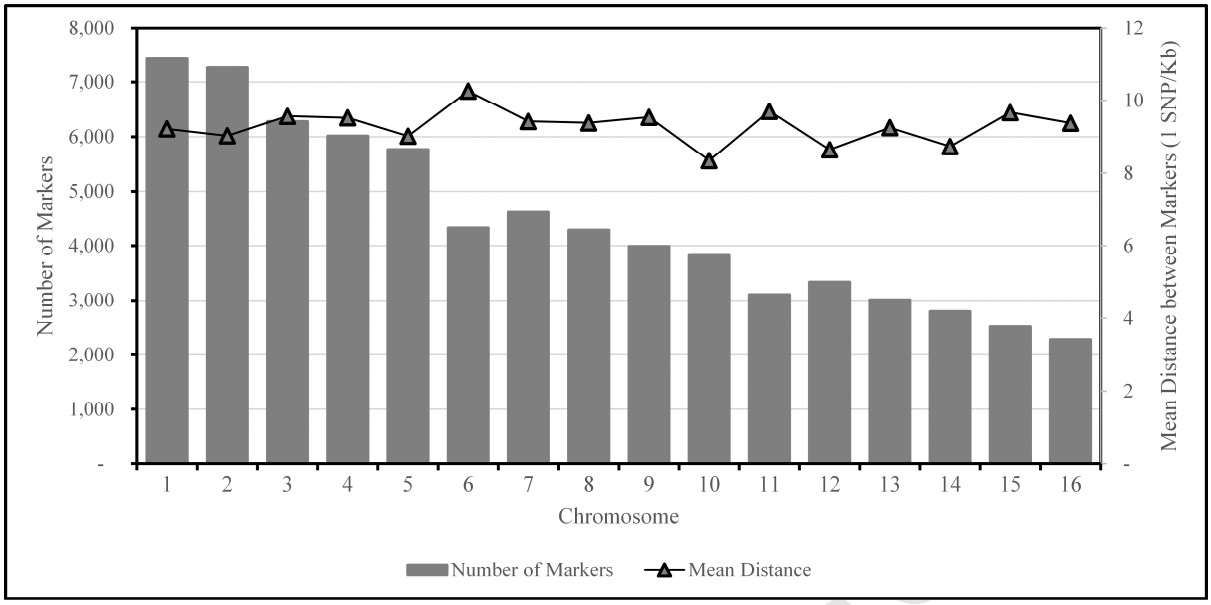

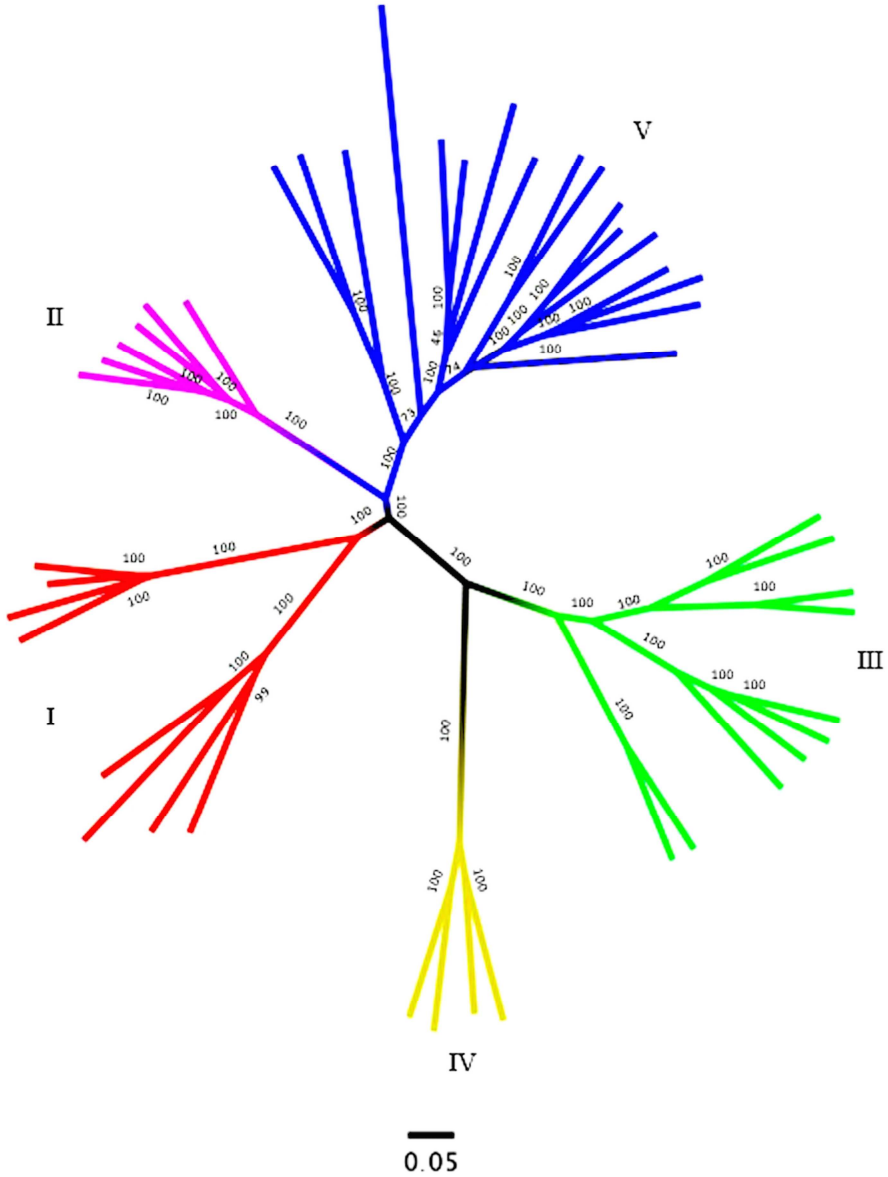

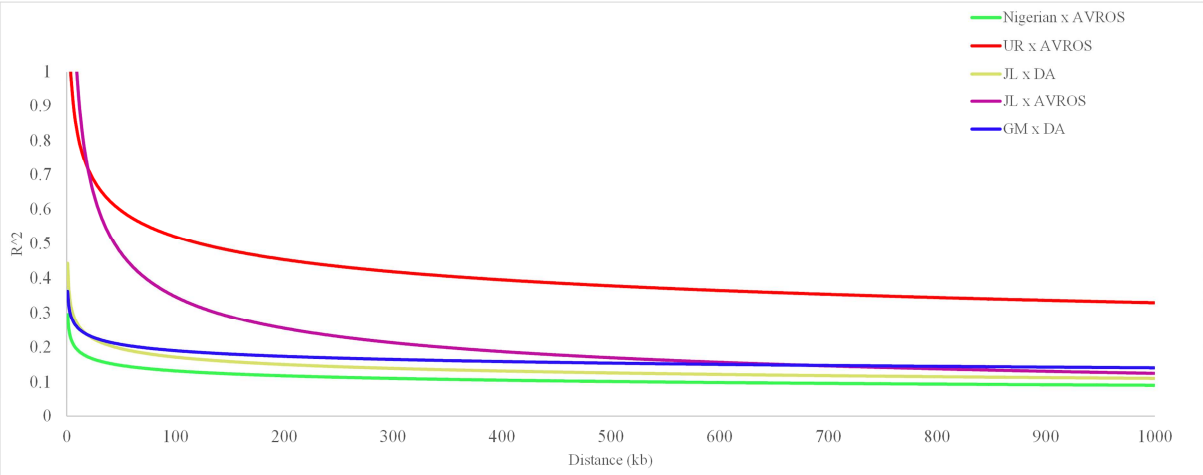

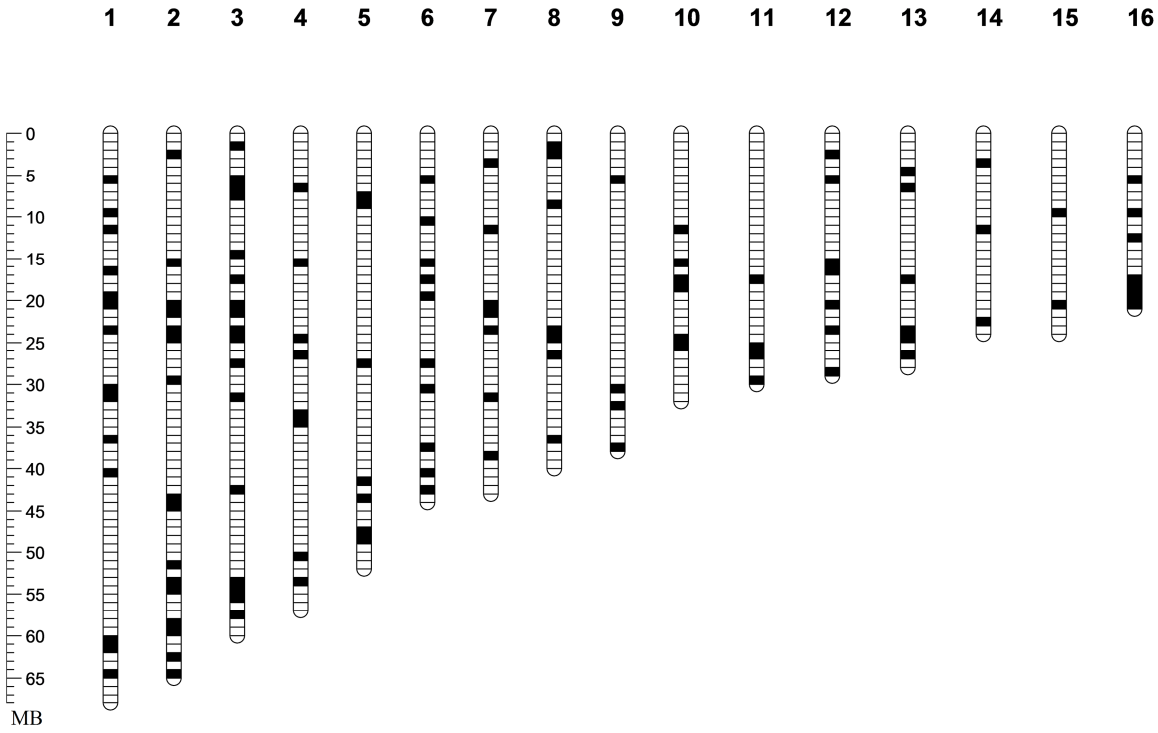

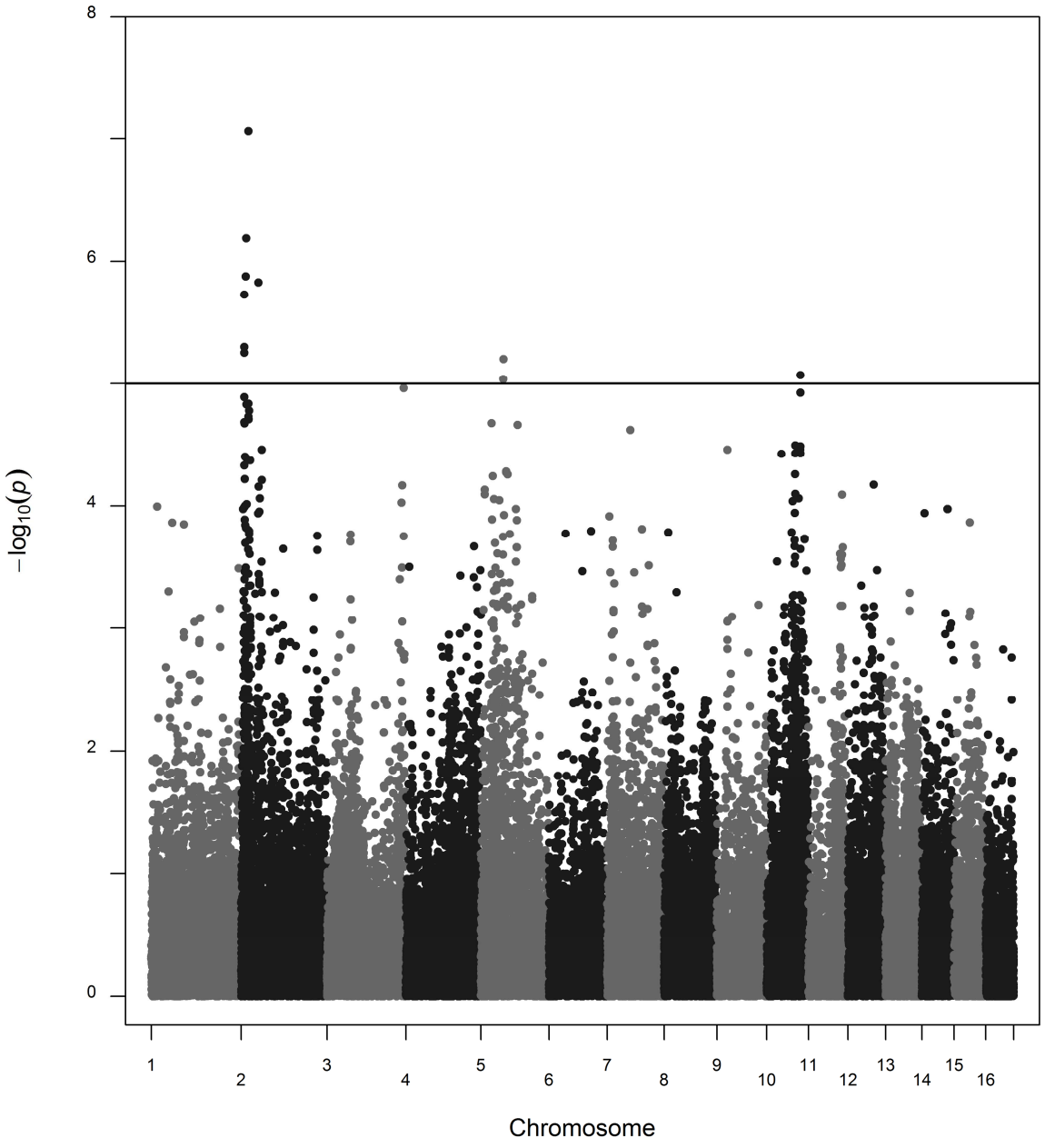
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