Supplementary Information for

Oil palm genome sequence reveals divergence of interfertile species in old and new worlds

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Supplementary Figure 1. 454 Linker Library Production. The fragment sizes going into each 454 linker library type were retrospectively monitored by BLAST comparison of linker reads from each half-plate sequencing run to an initial assembly of the *pisifera* reference genome. 50C50 linker library reads (including at least 50 bases up- and downstream of the identified center adapter sequence) that mapped to a single contig or scaffold were identified. The DNA fragment size spanning the two paired linker read sequences was calculated based on the alignment to the initial reference sequence. The intended target insert sizes for each library type are represented by the solid blue line. The average calculated insert size for each half-plate run is indicated by a red box. Error bars represent +/- one standard deviation from the mean of all successfully measured insert sizes for the given half-plate 454 sequencing run. As shown in Supplementary Table 1, the intended 10 Kb target insert size linker libraries displayed a low 50C50 rate (27% overall). For this reason, too few reads generated successfully measured insert sizes to accurately calculate an actual average insert size, and therefore this library class is excluded from the graph.

Supplementary Figure 2. Average insert size and distribution sizes of Origen_1 BAC library. Methods for BAC library construction were performed essentially as previously described^{44,45}. The average insert size and size distribution of the library were determined by selecting random BAC clones and isolating BAC plasmid DNA followed by digestion with *Not*I restriction enzyme and separation on 1% agarose with pulse field gel electrophoresis (PFGE) (CHEF Mapper, BIO-RAD) at 1–50 sec linear ramp, 6 volts/cm, 14°C in 0.5X TBE buffer for 18–20 hours. The insert size of each clone was determined by comparison with molecular weight standards and averages and size distributions were calculated.

60,297 clones group into 11,457 contigs

Supplementary Figure 3. Incremental assembly of fingerprinted BACs. Fingerprinted BACs were assembled incrementally into a total of 17 builds with FPC software (incremental builds on x-axis). The final build of the reference *pisifera* physical map resulted in 63,989 (51%) BACs which remained as singletons and 60,297 (49%) which formed 11,457 contigs. Of the 11,457 contigs, 3,579 (31%) had two BACs, 6,359 (56%) had 3-9 BACs each, 1,463 (13%) had 10-24 BACs each, and 56 (0.5%) contigs comprised 25 or more BAC clones each.

Supplementary Figure 4. The genetic map of the selfed Nigerian palm T128, constructed using a combination of markers namely SNP, SSR and RFLP. SSR markers are in red, RFLP markers are labeled green, the *Shell* gene is in blue colour while SNP markers are denoted in black. The single asterisk shows markers skewed at $P < 0.1$; double asterisk: skewed marker at $P < 0.05$; three asterisks: skewed marker at $P < 0.01$; four asterisks: skewed marker at $P \le 0.005$; five asterisks: skewed marker at $P \le 0.001$; six asterisks: skewed marker at P<0.0005.

Supplementary Figure 5. Isotigs (unigene clusters) formed by assembly of transcriptome libraries. Thirty transcriptome libraries were constructed and sequenced by Roche/454 technology. In total, 15.4 million transcriptome sequences were generated comprising 5.2 Gb of sequence. Each of the 30 libraries was assembled, producing between 4,528 and 18,936 isotigs from fertile *pisifera* pollen (T124) and *pisifera* fruit, respectively.

Supplementary Figure 6. Repeat content. Sequences from the P4 build were screened against known repeat databases from TIGR and RepBase⁴⁶ as well as against the previously constructed PisiferaRepeat and OleiferaRepeat databases using TBLASTN $(Eval < 10^{-5}$, low complexity filters disabled). Regions of the P4 build matching these repeat databases were extracted and annotated based on the repeat class to which they most closely matched. This resulted in genomic regions totaling 282.3 Mb (14 bp to 5106 bp in size, mean 363 bp, median 197).

 Known repeats in oil palm are dominated by LTR (long terminal repeat) retrotransposons, especially the RIRE1-like subfamily which has undergone significant expansion, as have other *copia* elements. Among *copia* elements, RIRE1 was the most abundant subclass (20%), resembling certain species of rice in this respect⁴⁷, in agreement with the prevalence of *copia* elements in oil palm predicted by $FISH¹⁷$. There are very few non-LTR and DNA class transposons. Of the non-LTR retrotransposons, LINEs make up less than 1% of total repetitive elements, and SINEs are negligibly

observed. Other classes of repetitive elements such as Class II DNA transposons, hATlike transposons, MITEs and CACTA repeats make up relatively small proportions of the overall repeat content of the oil palm genome. About half of the repeats in the oil palm genome show no sequence similarity to other previously identified retro elements, although 7 percent are composed of a previously observed but uncharacterized dispersed repeat in *E. guineensis* DRepEG¹⁷.

Supplementary Figure 7 continued on next page.

Supplementary Figure 7. Segmental duplications in *E. guineensis***.** MUMmer plots generated by the PROmer program are presented for observed segmental duplications within oil palm. Dots represent windows of matching protein translations on the two chromosomes, matches in the same direction are shown in red, opposite strand in blue. Chromosome names are based on the nomenclature of the linkage groups in the T128 genetic map (Supplementary Table 7).

a E.oleifera.

 07 sc00067->

Scale: one character equals 157449 bases

 ${\tt Chr3_SegDup1}$ |……………………………………………………… à, \cdots $\ddot{}$ an
Alban
Alban \cdots \mathbb{Z}^2 ji. aan i $\mathcal{L}_{\mathbf{A}}$ \mathbf{r} \ldots تسب 07 sc00002->
07 sc00048<-
07 sc00204<-
07 sc000204<-
07 sc000205<-. . . . \bar{a} $\ddot{}$ Scale: one character equals 400822 bases

07_sc00032-> ~ 100 Scale: one character equals 68955 bases

Supplementary Figure 8a continued on next page.

Supplementary Figure 8a continued on next page.

b *P.dactylifer*

Supplementary Figure 8b continued on next page.

Scale: one character equals 188810 bases

Scale: one character equals 114020 bases

Supplementary Figure 8. **Segmental duplications. a,** Validating segmental duplications in *E.oleifera*. For each segmental duplication in *E. guineensis*, we verified the unique representation of the two corresponding EG-5 linkage groups in the O7 Build in *E.oleifera* using comparative genomics. The Segmental Duplication (SegDup) is plotted as a line of $+$ signs each representing a number of bp (indicated below each plot). O7 scaffolds on the y-axis) correspond to each duplicated EG-5 region (x-axis). Plots for the two halves of the duplication in EG-5 are presented side-by-side. Note that some smaller overlapping scaffolds have been removed for clarity. **b,** Validating segmental duplications in *P.dactylifera*. For each EG-5 linkage group involved in a segmental duplication, we verified the unique representation of that region in the *P. dactylifera* scaffold assembly as above.

Supplementary Figure 9. Synteny between banana and oil palm. Two mummer plots showing synteny between *E. guineensis* chromosomes 4 and 11 with *M. acuminata* chromosomes 11 and 8. Plots show protein level conservation, and imply that several ancient internal rearrangements have occurred. Matches in the same direction are shown in red, opposite strand in blue.

Supplementary Tables.

		All Reads			Useful Reads	
Target Lib (Kb)	Plates	Reads (M)	Seq Cvg	50C50 Rate	Reads (M)	Seq Cvg
Fragments	41.0	53.5	11.6		99.6	20.0
0.75	4.0	5.4	1.0	63%	3.4	0.6
1.5	8.0	9.1	1.6	60%	5.4	1.0
3	3.0	2.5	0.5	54%	1.4	0.3
5	21.5	23.5	4.5	53%	12.5	2.4
8	8.5	11.8	2.3	51%	6.0	1.2
10	10.0	11.5	1.9	27%	3.1	0.6
13	8.0	10.1	1.9	46%	4.6	0.9
20	11.0	15.2	2.7	43%	6.6	1.2
Total	115.0	142.6	28.1		142.6	28.1

Supplementary Table 1. *Pisifera* **genome fragment and linker library statistics**

For fragment (non-linker) and linker libraries of the indicated insert sizes (Target Lib (Kb)), the number of 454 full plate runs (Plates), number of reads in millions (Reads), and fold sequence coverage generated (Seq Cvg) are provided. All Reads heading refers to total reads and raw sequence coverage. Useful Reads heading refers to linker library reads in which the center adapter, as well as at least 50 bases of sequence before and after the center adapter, were sequenced (50C50). Those reads which included genome sequence, but were not 50C50, were removed from linker library read groups and added to the fragment library read group. Total raw sequence fold coverage reported in Supplementary Table 1 is greater than that reported in the main text. Raw sequence coverage reported above (28.1x) is based on the total base pairs of sequence generated. Raw sequence coverage reported in the main text (26x) is based on quality filter passed reads used by Newbler in the genome assembly.

		All Reads			Useful Reads	
Target Lib (Kb)	Plates	Reads (M)	Seq Cvg	50C50 Rate	Reads (M)	Seq Cvg
Fragments	34.0	49.8	10.0		88.6	17.1
0.75	4.0	4.1	0.7	58%	2.4	0.4
1.5	4.5	5.3	0.9	63%	3.3	0.6
3	1.0	1.1	0.2	56%	0.6	0.1
5	27.5	31.3	5.5	57%	17.9	3.2
8	7.5	7.9	1.4	47%	3.7	0.7
10	14.0	17.3	3.3	42%	7.3	1.4
13	1.0	1.5	0.3	49%	0.7	0.1
20	8.5	12.1	2.4	48%	5.8	1.1
Total	102.0	130.3	24.7		130.3	24.7

Supplementary Table 2. *Oleifera* **genome fragment and linker library statistics**

Plate runs, reads and sequence coverage are provided as described in Supplementary Table 1.

		All Reads			Useable Reads	
Target Lib (Kb)	Plates	Reads (M)	Seq Cvg	50C50 Rate	Reads (M)	Seq Cvg
Fragments	4.0	3.6	0.6		8.1	1.5
1.5	0.5	0.6	0.1	49%	0.3	0.1
3	4.5	4.2	0.8	47%	2.0	0.4
5	0.5	0.7	0.1	49%	0.4	0.1
8	1.5	2.0	0.4	47%	1.0	0.2
10	0.5	0.9	0.2	45%	0.4	0.1
Total	11.5	12.2	2.2		12.2	2.2

Supplementary Table 3. BAC Pool Fragment and Linker Library Statistics

Plate runs, reads and sequence coverage are provided as described in Supplementary Table 1.

Supplementary Table 4. The oil palm genome sequence

a Characteristics of the *E. guineensis* genome assembly.

^bMany partial, degraded and unknown retroelements included

c Characteristics of gene predictions for the *E. guineensis* genome.

^dGene predictions matching genes in RefSeq database version 35.

^eGene predictions supported by transcriptome sequencing, but without RefSeq match.

^fGene predictions matching elements in RepBase and/or TIGR Gramineae repeat database.

 The assembly resulted in 1,309,411 contigs and 40,360 scaffolds. Scaffold sizes range from 2.0Kb to 22.1Mb. The scaffold N50 (minimal scaffold length of the smallest set of scaffolds that are able to span 50% of the genome) is 1.045Mb, and the combined total length of the assembly (P5 Build) is 1.535Gb, representing 85% of the estimated 1.8Gb genome size (Supplementary Table 5). Comparison of the P5 Build to genetic linkage maps (Supplementary Fig. 4, Methods) resulted in unambiguous positioning of 366 molecular markers within 304 of the longest P5 Build scaffolds, forming a new assembly, "EG-5 Linked". This new assembly includes 16 genetic scaffolds (one scaffold for each of the 16 oil palm chromosomes) and 658Mb of sequence. EG-5 Linked scaffold sizes range from 21.3Mb to 68.4Mb, and the EG-5 Linked assembly has an N50 of

44.3Mb. Including the EG-5 Linked and P5 Build (unlinked) together, the combined assembly contains 40,072 scaffolds with an N50 of 1.26Mb.

 Gene predictions from the P4 build were generated using Glimmer and SNAP resulting in 151,252 predictions from Glimmer and 158,897 predictions from SNAP. The two were screened for overlap in the P4 build, and overlapping predictions from SNAP were removed from the set resulting in 158,946 predicted transcripts in the P4 build. Gene predictions were then classified by BLAST searching (Eval $< 10^{-5}$) to reference genomes of *A. thaliana*, *O. sativa*, curated RefSeq35 (with retro-elements removed), and *E. guineensis* transcriptome libraries. In addition, gene predictions were screened against the TIGR Gramineae repeat database, as well as *pisifera* and *oleifera* repetitive sequence databases (see Repetitive sequence detection). Predictions were then grouped as follows: RefSeq support: 34,802 genes; retroelements: 67,169; Transcriptome only: 15,311; Gene predictions with no supporting evidence: 41,664.

Supplementary Table 5. Genome size as determined by flow cytometry

The genome sizes of *E. guineensis* (*dura* and *pisifera*) and *E. oleifera* were determined by flow cytometry. Four independent measurements were made for each genome (personal communication, Dr. K. Arumuganathan, Virginia Mason Research Center).

The estimated genome size of *E. guineensis* is similar to previous flow cytometry studies^{48,49}. Interestingly, the genome size of *E. oleifera* is similar to that of *E. guineensis*. However, the genome size of *E. oleifera* reported here is almost twice of that previously reported for an E . *oleifera* palm from Surinam⁴⁹. The fibrous and lignified nature of the Surinam *E. oleifera* leaf samples most likely contributed to poor isolation of nuclei, and the subsequent lower genome size estimate for *E. oleifera*49

Supplementary Table 6. Transcriptome library sequencing and assembly details

Assembly Group

Tissue sources for each of the 30 RNA sequencing libraries is presented. Tissues and trees were selected to maximize library complexity for genome annotation purposes as well as to measure interesting expression variation for high value breeding traits such as oil production and clonal stability. Libraries were individually assembled using the Newbler program from Roche 454, resulting in transcriptome sets ranging from 4,258 to 18,936 isotigs per library. In addition to individual assemblies, group assemblies were generated for *dura* (A), *E. oleifera* (B), *pisifera* (C), and *tenera* tissue libraries (D), as well as normal clonal (E), abnormal (mantled) clonal (F), and all clonal tissues (G) derived from crosses of *dura* and *pisifera*. Validation of gene models for *E. guineensis* was based on homology to isotigs from all combined *E. guineensis* libraries (H).

Chromosome	T128_codominant	P2/Billotte	Size (bp)
CHR ₁	LG1	lg_8	68,432,966
CHR ₂	LG7	$\lg 4$	65,556,141
CHR ₃	LG10	$lg_1 1$	60,058,032
CHR ₄	LG ₆	$lg_1 11$	57,248,047
CHR ₅	LG ₁₂	$lg_1 12$	51,953,839
CHR ₆	LG ₅	lg ₁₀	44,354,769
CHR ₇	LG8	lg_6	43,453,266
CHR ₈	LG9	$lg_2 2$	40,192,799
CHR ₉	LG4	\lg 7	38,054,796
CHR 10	LG13	\lg 15	31,889,635
CHR 11	LG11	\lg 14	30,067,610
CHR 12	LG14	\lg 13	28,799,275
CHR 13	LG15	lg_9	27,816,170
CHR 14	LG ₂	lg_3	24,378,543
CHR 15	LG16	$lg_1 16$	24,313,565
CHR 16	LG ₃	$lg_{-}5$	21,370,583

Supplementary Table 7. Chromosomes and linkage groups in oil palm

Linkage groups in the T-128 Nigerian population⁵⁰ as well as the DxP (P2) pseudotestcross population which followed the nomenclature of Billotte et al. $(2005)^{51}$, were aligned using common SNP and SSR markers. Cytogenetically, individual oil palm chromosomes are difficult to distinguish but fall into 4 groups, comprising (I) the largest chromosome (which hybridizes to 5SrDNA), (II) 8 medium chromosomes; (III) 6 small chromosomes and (IV) a small acrocentric chromosome, which carried the 18S-25S $rDNA^{17, 52}$. Attempts were made to identify these chromosomes by FISH using RFLP clones from early genetic maps, but the results were often ambiguous. We have renumbered the chromosomes according to the size of sequence scaffolds in the EG5 linked build. The chromosomes correspond to Linkage Groups in each mapping population as shown.

Supplementary Table 8. Comparative genomics of oil palm

Rows (Gene) represent previously reported gene models for *Arabidopsis* (*A. thaliana*), banana (*M. acuminata*) and date palm (*P. dactylifera*), as well as oil palm (*E. guineensis*) gene models reported here. Columns (Genome) represent the complete genome sequences for each species. The percentage of query genes matched to the target genome is shown for each pairwise comparison of gene models to genome.

Supplementary Table 9. Transcriptome sequencing of oil palm mesocarp and kernel at time points prior to and at the peak of oil accumulation.

For each functional classification group of genes, the average transcripts per million tags sequenced is presented in the header for each group. For individual genes, expression levels are presented as transcripts per million tags sequenced. Data is presented for transcriptomes from mesocarp (15 and 20 WAA) and kernel (10 and 15 WAA).

Supplementary Methods

Repetitive sequence detection

 To construct *Elaeis* specific repeat databases, repetitive sequences of *pisifera* and *oleifera* were determined by a genome wide self-self comparison based on 60mer windowed 15mer word frequencies. Each genome was decomposed into 60mer words with 40 base overlap, and scored for uniqueness using a SSAHA³¹ based pipeline. The uniqueness score for an individual 60mer was calculated as: 60mer Uniqueness Score = Sum (i=1 to 45) [$log(freq(15mer_i))$]. Perfectly unique 60mers receiving a score of 0, and highly repetitive 60mers scoring as high as 727 in the *pisifera* genome were calculated. Regions of each genome with uniqueness scores in the highest (most repetitive) 20% were collected and clustered using the PCAP algorithm to create the PisiferaRepeat and OleiferaRepeat databases with 5,426 and 440 sequences, respectively.

Public database sources for comparative genome sequences and gene models

Genome source:

A. thaliana:

ftp://ftp.arabidopsis.org/home/tair/Sequences/whole_chromosomes/TAIR10

O. sativa:

ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v8.0/Osativa/assembly/Osativa_193.fa.gz *P. dactylifera*:

http://qatar-

weill.cornell.edu/research/datepalmGenome/edition3/PdactyKAsm30_r20101206.fasta.gz

Gene model source:

A. thaliana:

ftp://ftp.arabidopsis.org/home/tair/Sequences/blast_datasets/TAIR10_blastsets/TAIR10_p ep_20110103_representative_gene_model_updated

O. sativa:

ftp://ftp.jgi-

psf.org/pub/JGI_data/phytozome/v8.0/Osativa/annotation/Osativa_193_peptide.fa.gz

P. dactylifera:

http://qatar-weill.cornell.edu/research/datepalmGenome/edition3/PDK30-pep.fsa.gz

Supplementary Notes

Triacylglycerol biosynthesis

 TAG usually accumulates in oil bodies. Seeds (including oil palm kernel) maintain oil bodies as small individual units $(0.5 \text{ to } 2 \mu \text{m})$ preventing their coalescence during seed desiccation and providing high surface area to volume ratio to facilitate access by lipases during germination. In contrast, the oleaginous fruits, oil palm, olive and avocado have large oil bodies (up to $20 \mu m$) in the mesocarp owing to the lack of oleosins, structural proteins that stabilize and prevent the coalescence of the oil bodies. Unlike the date genome, oleosins are represented in the oil palm. Consistently, the transcriptome profile showed very high oleosin expression in the kernel and very low expression in the mesocarp (Figure 3c, Supplementary Table 9).

Fruit ripening and abscission

 Interestingly the oil palm has two-fold more ripening and abscission genes than date palm (Fig. 2c). The oil palm has more *AMINOCYCLOPROPANE CARBOXYLATE OXIDASE* (*ACO*) genes responsible for the burst of ethylene and increased respiration on ripening. The oil palm genome is also enriched in *MAPKK* genes which are involved in ethylene signal transduction. *BRASSINOSTEROID INSENSITIVE1* Leucine-Rich Repeat (LRR) genes and *BLADE ON PETIOLE2* (*BOP2*) genes are similarly more highly represented in the oil palm genome. *BRI1* regulates transduction of steroid signals across membranes while *BOP2* controls abscission zone formation.

References

- 44. Luo, M. & Wing, R. A. An improved method for plant BAC library construction. *Methods Mol Biol* **236**, 3-20 (2003).
- 45. Ammiraju, J. S. et al. The Oryza bacterial artificial chromosome library resource: construction and analysis of 12 deep-coverage large-insert BAC libraries that represent the 10 genome types of the genus Oryza. *Genome Res* **16**, 140-7 (2006).
- 46. Jurka, J. et al. Repbase Update, a database of eukaryotic repetitive elements. *Cytogenet Genome Res* **110**, 462-7 (2005).
- 47. Piegu, B. et al. Doubling genome size without polyploidization: dynamics of retrotransposition-driven genomic expansions in Oryza australiensis, a wild relative of rice. *Genome Res* **16**, 1262-9 (2006).
- 48. Rival, A. et al. Comparative flow cytometric estimation of nuclear DNA content in oil palm (Elaeis guineensis, Jacq.) tissue cultures and seed derived plants. . *Plant Cell Reports* **16**, 884-887 (1997).
- 49. Madon, M., Phoon, L. Q., Clyde, M. M. & Mohd Din, A. Application of flow cytometry for estimation of nuclear DNA content in Elaeis. *Journal of Oil Palm Research* **20**, 447-452 (2008).
- 50. Rajanaidu, N., Rao, V., Abdul Halim, H. & A.S.H., O. Genetic resources: New developments in Oil Palm breeding. *Elaeis* **1**, 1-10 (1989).
- 51. Billotte, N. et al. Microsatellite-based high density linkage map in oil palm (Elaeis guineensis Jacq.). *Theor Appl Genet* **110**, 754-65 (2005).
- 52. Madon, M., Clyde, M. M. & Cheah, S. C. Cytological analysis of Elaeis guineensis (tenera) chromosomes. . *Elaeis* **7**, 122-134 (1995).