Taylor & Francis
Taylor & Francis Group

Mitochondrial DNA
The Journal of DNA Mapping, Sequencing, and Analysis

ISSN: 1940-1736 (Print) 1940-1744 (Online) Journal homepage: http://www.tandfonline.com/loi/imdn20

Complete mitochondrial genome sequences of thirteen globally sourced strains of fruit fly (Drosophila melanogaster) form a powerful model for mitochondrial research

Jonci N. Wolff, M. Florencia Camus, David J. Clancy & Damian K. Dowling

To cite this article: Jonci N. Wolff, M. Florencia Camus, David J. Clancy & Damian K. Dowling (2015): Complete mitochondrial genome sequences of thirteen globally sourced strains of fruit fly (Drosophila melanogaster) form a powerful model for mitochondrial research, Mitochondrial DNA, DOI: 10.3109/19401736.2015.1106496

To link to this article: http://dx.doi.org/10.3109/19401736.2015.1106496


Published online: 28 Dec 2015.


Submit your article to this journal


Article views: 88


View related articles


View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=imdn20

Download by: [Monash University Library] **Date:** 14 August 2016, At: 23:35

Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA, Early Online: 1–3
© 2015 Taylor & Francis. DOI: 10.3109/19401736.2015.1106496

Taylor & Francis
Taylor & Francis Group

MITOGENOME ANNOUNCEMENT

Complete mitochondrial genome sequences of thirteen globally sourced strains of fruit fly (*Drosophila melanogaster*) form a powerful model for mitochondrial research

Jonci N. Wolff[1]*, M. Florencia Camus[1]*, David J. Clancy[2], and Damian K. Dowling[1]

[1]*School of Biological Sciences, Monash University, Clayton, Australia and* [2]*Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, UK*

Abstract

The complete mitogenomes of 13 strains of the fruit fly *Drosophila melanogaster* were sequenced. Haplotypes varied between 19 532 and 19 537 bp in length, and followed standard dipteran mitogenome content and organization. We detected a total of 354 variable sites between all thirteen haplotypes, while single pairs of haplotypes were separated by an average of 123 variable sites. The sequenced fly strains form a powerful model for mitochondrial research, when it comes to elucidating the links between the mitochondrial genotype and the phenotype.

Keywords

Drosophila melanogaster, fruit fly, mitochondrial model

History

Received 15 September 2015
Revised 1 October 2015
Accepted 7 October 2015
Published online 22 December 2015

Introduction

We sequenced 13 mitochondrial haplotypes sourced from around the globe (Alstonville, Australia; Barcelona, Spain; Brownsville, USA; Dahomey, Benin; Madang, Papua New Guinea; Mysore, India; Hawai'i, USA; Israel; Japan; Oregon, USA; Puerto Montt, Chile; Sweden; Zimbabwe; for geographical distribution map see: Wolff & Gemmell, 2013). These 13 haplotypes were placed, by forced chromosome replacement, alongside a single isogenic nuclear background (w^{1118}, Bloomington stock #3605; Clancy, 2008), and have since been frequently harnessed as a model for mitochondrial genetics research e.g. (Camus et al., 2012; Yee et al., 2013, Clancy, 2008; Clancy et al., 2011; Innocenti et al., 2011). Despite their frequent use in mitochondrial research, sequencing efforts on each of these haplotypes was previously limited to the protein-coding region. In light of an increasing appreciation for the non-coding region in mitochondrial research in recent years (Horan et al., 2013; Rackham et al., 2011), we have sequenced the complete mitogenomes of these haplotypes.

To warrant accuracy and highest-possible resolution, samples were enriched for mitochondria prior to DNA extraction (Pallotti & Lenaz, 2007). This strategy increased sequencing coverage per mitochondrial haplotype (on an average of 1000×), and served as a pre-emptive strategy to reduce the presence of nuclear-encoded mitochondrial pseudogenes (numts). The genome of *D. melanogaster* harbors four numts spanning 838 bp of partial sequences of CoxI - CoxIII, and three tRNAs (tRNA-W, tRNA-C, tRNA-Y)

Table 1. Mitogenome annotation for the consensus sequence of 13 *D. melanogaster* haplotypes.

		Position					
Gene	Direction	From	To	Size	Anticodon	Start codon	Stop codon
tRNA-I	F	1	65	65	GAT		
tRNA-Q	R	97	165	69	TTG		
tRNA-M	F	171	239	69	CAT		
ND2	F	240	1265	1026		ATT (M)	TAA
tRNA-W	F	1264	1329	66	TCA		
tRNA-C	R	1322	1383	62	GCA		
tRNA-Y	R	1403	1468	66	GTA		
CoI	F	1474	3009	1535		TCG (S)	TAA
tRNA-L	F	3012	3077	66	TAA		
CoII	F	3083	3767	685		ATG (M)	T—
tRNA-K	F	3768	3838	71	CTT		
tRNA-D	F	3840	3906	67	GTC		
ATP8	F	3907	4068	162		ATT (M)	TAA
ATP6	F	4062	4736	675		ATG (M)	TAA
CoIII	F	4736	5524	789		ATG (M)	TAA
tRNA-G	F	5543	5607	65	TCC		
ND3	F	5608	5961	354		ATT (M)	TAA
tRNA-A	F	5979	6043	65	TGC		
tRNA-R	F	6053	6116	64	TCG		
tRNA-N	F	6117	6181	65	GTT		
tRNA-S	F	6182	6249	68	TCT		
tRNA-F	F	6250	6316	67	TTC		
tRNA-E	R	6335	6399	65	GAA		
ND5	R	6399	8116	1718		ATT (M)	TA–
tRNA-H	R	8132	8197	66	GTG		
ND4	R	8197	9536	1340		ATG (M)	TA–
ND4L	R	9536	9826	291		ATG (M)	TAA
tRNA-T	F	9829	9894	66	TGT		
tRNA-P	R	9895	9955	61	TGG		

(*continued*)

*These authors contributed equally to this work.

Correspondence: Jonci Wolff, School of Biological Sciences, Monash University, Clayton 3800, Australia. E-mail: jonci.wolff@gmail.com
Damian K. Dowling, School of Biological Sciences, Monash University, Clayton 3800, Australia. E-mail: damian.dowling@monash.edu

Table 1. Continued.

Gene	Direction	Position From	To	Size	Anticodon	Start codon	Stop codon
ND6	F	9962	10 486	525		ATT (M)	TAA
Cyt-b	F	10 490	11 626	1137		ATG (M)	TAA
tRNA-S	F	11 629	11 694	66	TGA		
ND1	R	11 712	12 650	939		ATA (M)	TAG
tRNA-L	R	12 661	12 725	65	TAG		
lrRNA	R	12 726	14 049	1324			
tRNA-V	R	14 049	14 121	73	TAC		
srRNA	R	14 122	14 907	786			
Control region	–	14 908	19 532	4625			
Repeat I-A	F	15 559	15 937	379			
Repeat I-B1	F	15 938	16 276	339			
Repeat I-C/A	F	16 277	16 623	347			
Repeat I-B2	F	16 624	16 964	341			
Repeat I-C	F	16 965	17 311	347			
Repeat II-A2 (partial)	F	17 435	17 571	137			
Repeat II-A1	F	17 572	18 038	467			
Repeat II-B1	F	18 039	18 504	466			
Repeat II-B2	F	18 505	18 969	465			
Repeat II-C	F	18 970	19 435	466			

(Rogers & Griffiths-Jones, 2012). All four numts harbor several additional polymorphisms and deletions, and are thus unambiguously distinguishable from their *bona fide* counterparts. Sequencing was conducted using the *Illumina MiSeq* platform (San Diego, CA). All sequence and phylogenetic analyses were conducted using *Geneious 2* (Kearse et al., 2012).

Mitogenomes (Genbank accession nos. KP843842–KP843854) were 19 532–19 537 bp in length, and followed typical insect mitogenome content and organization: 13 protein-coding, 2 ribosomal RNA, and 22 transfer RNA genes (Table 1). The coding regions were highly similar to other closely related insect genomes, with all but one (CoxI) protein-coding genes utilizing the ATN motif as start codon and TAA (or a truncated variation thereof) as stop codon. Typical for Diptera, the start codon for *CoxI* was TCG. As expected, structural RNAs were also highly similar to those of other insect mitogenomes, and polymorphisms did not indicate any changes to anticodon sites or secondary structures. Noteworthy, however, was the significantly increased length of the genome ($\geq$19 532 bp) relative to other insect genomes, and typical for this species (Rand, 1993). This increase is due to the presence of two large macrorepeat regions within the control region. At a considerable length of 4.6 kb, the A/T-rich region contains several homopolymer and microsatellite-type repeats, and two macrorepeat regions, each composed of four repeat motifs (A–C). In addition to these motifs, repeat region I

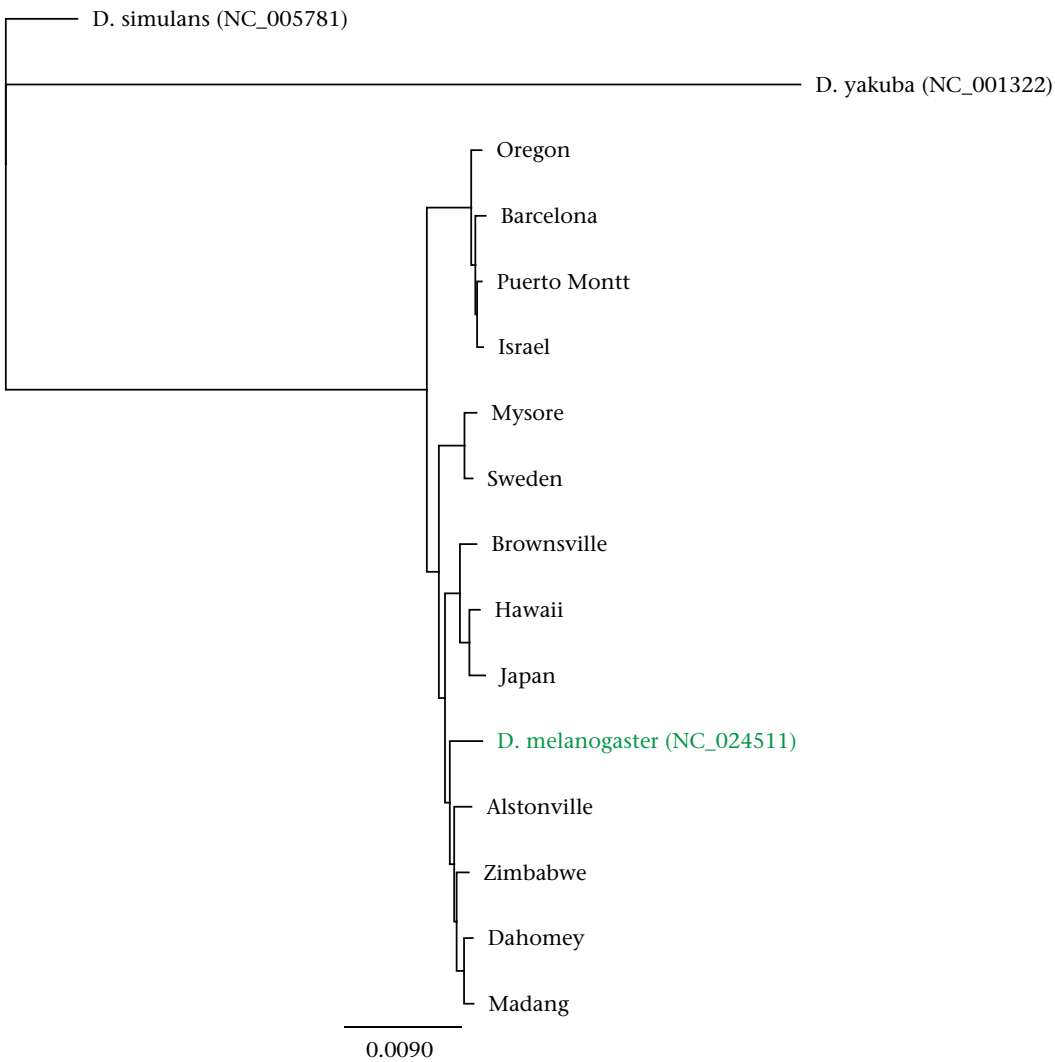

Figure 1. HKY neighbor-joining tree of 13 *Drosophila melanogaster* mitochondrial genomes, and the mitochondrial reference genomes of *D. melanogaster* (NC_024511), *D. simulans* (NC_005781), and *D. yakuba* (NC_001322). Scale bar: substitutions per site.

contains a chimera of motifs A and C, while repeat region II contains a partial repeat of motif A.

Pair-wise comparison between all 13 mitogenomes revealed the presence of 354 single nucleotide polymorphisms (SNPs; 140 transitions; 214 transversions). Of these, 81 SNPs affected the protein-coding region (62 synonymous and 19 non-synonymous), two SNPs were located within intergenic regions, one SNP in tRNA-E, seven SNPs in small and large ribosomal RNAs, and 263 within the A + T-rich control region. On an average, mitogenomes were separated by 123 SNPs. Variable sites were evenly distributed across coding regions, and occurred at higher frequency within the hyper-variable control region. Phylogenetic analysis revealed that the 13 mitochondrial haplotypes subdivide into four main clades (Figure 1). Oregon, Barcelona, Puerto Montt, and Israel cluster together, and form the most distantly related clade to all other haplotypes and clades. The clade composed of Alstonville, Zimbabwe, Dahomey, and Madang groups together with the *D. melanogaster* mitochondrial reference genome NC_024511, and shows increasing genetic distance to the clade containing Brownsville, Hawaii, and Japan, and the clade comprising of Mysore and Sweden. Together, insight into the model's underlying genetic structure, as well as into complete sequences, SNP distribution, SNP location, and nature of polymorphisms are important extensions to the existing fly model, and will help further our understanding of the genetic processes that mediate the link between the mitochondrial genotype and the phenotype.

Acknowledgements

The authors thank Winston Yee for his help with fly husbandry.

Declaration of interest

Full sequencing of mitogenomes was funded by the Australian Research Council Discovery Project Grant DP1092897 to D. K. D. Original sequencing of protein-coding regions was funded by DP0773672 to D. J. C. The authors report no conflict of interests. The authors alone are responsible for the content and writing of the article.

References

Camus MF, Clancy DJ, Dowling DK. (2012). Mitochondria, maternal inheritance, and male aging. Curr Biol 22:5–1.

Clancy DJ. (2008). Variation in mitochondrial genotype has substantial lifespan effects which may be modulated by nuclear background. Aging Cell 7:795–804.

Clancy DJ, Hime GR, Shirras AD. (2011). Cytoplasmic male sterility in *Drosophila melanogaster* associated with a mitochondrial CYTB variant. Heredity 107:374–6.

Horan MP, Gemmell NJ, Wolff JN. (2013). From evolutionary bystander to master manipulator: The emerging roles for the mitochondrial genome as a modulator of nuclear gene expression. Eur J Human Genet 21:1335–7.

Innocenti P, Morrow EH, Dowling DK. (2011). Experimental evidence supports a sex-specific selective sieve in mitochondrial genome evolution. Science 332:845–8.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, et al. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–9.

Pallotti F, Lenaz G. (2007). Isolation and subfractionation of mitochondria from animal cells and tissue culture lines. Methods Cell Biol 80: 3–44.

Rackham O, Shearwood A-MJ, Mercer TR, Davies SMK, Mattick JS, Filipovska A. (2011). Long noncoding RNAs are generated from the mitochondrial genome and regulated by nuclear-encoded proteins. RNA 17:2085–93.

Rand DM. (1993). Endotherms, ectotherms, and mitochondrial genome-size variation. J Mol Evol 37:281–95.

Rogers HH, Griffiths-Jones S. (2012). Mitochondrial pseudogenes in the nuclear genomes of *Drosophila*. PLoS One 7:e32593.

Wolff JN, Gemmell NJ. (2013). Mitochondria, maternal inheritance, and asymmetric fitness: Why males die younger. BioEssays 35: 93–9.

Yee WK, Sutton KL, Dowling DK. (2013). *In vivo* male fertility is affected by naturally occurring mitochondrial haplotypes. Curr Biol 23: R55–6.