Medical and Veterinary Entomology (2008) **22**, 89–91

Mitochondrial genomes of the sheep blowfly, *Lucilia sericata*, and the secondary blowfly, *Chrysomya megacephala*

J. R. STEVENS[1], H. WEST[1] and R. WALL[2]

[1]School of Biosciences, University of Exeter, Exeter, U.K. and [2]School of Biological Sciences, University of Bristol, Bristol, U.K.

Abstract. This paper presents complete mitochondrial genomes for the sheep blowfly, *Lucilia sericata* (Meigen), and the secondary blowfly, *Chrysomya megacephala* (Fabricius). Both *L. sericata* and *C. megacephala* had standard dipteran-type mitochondrial genome architectures and lengths of 15 945 bp and 15 831 bp, respectively. Additionally, *C. megacephala* possessed a tRNA duplication either side of the D-loop, as previously reported in another *Chrysomya* species, *C. putoria*; this duplication appears to be synapomorphic for the genus *Chrysomya.* As in other insect mitochondrial genomes, base compositions had a high AT content, with both genomes more than 76% AT-rich.

Key words. *Chrysomya megacephala*, *Lucilia sericata*, *Phaenicia*, blowfly, forensic entomology, mitochondrial genome, myiasis, screwworm fly.

Introduction

Mitochondrial genes are among the most widely used markers for phylogenetic studies of animals (Avise, 2004). Within insects, in terms of the number of mitochondrial genomes characterized, Diptera are among the best studied of all orders (Cameron *et al.*, 2007; Shao & Barker, 2007). However, to date, evolutionary and systematic studies on one economically important agent of myiasis, the sheep blowfly, *Lucilia sericata* (Meigen), have been limited by lack of a complete mitochondrial genome sequence (Stevens, 2003). Accordingly, this paper presents a complete mitochondrial genome for the sheep blowfly, *L. sericata*, together with that of the secondary blowfly, *Chrysomya megacephala* (Fabricius).

Materials and methods

Template DNA

Complete mitochondrial genomes were sequenced for two species of blowfly: *L. sericata* (AJ422212) and *C. megacephala* (AJ42604). The flies used for this were reared in laboratory colonies maintained at the University of Bristol. The *C. megacephala* fly colony originated from larvae that infested drying fish in Calicut, Kerala State, India in 1998; the *L. sericata* fly colony originated from larvae collected from sheep-strikes in the Bristol area of southwest England in 1990 (Stevens & Wall, 1995).

Total DNA was obtained from thoracic flight muscle tissue of freshly killed adult flies by phenol-chloroform extraction (Sambrook *et al.*, 1989). To obtain sufficient DNA for sequencing (3 μg per mitochondrial genome), DNA was extracted from 10 male *C. megacephala* and 30 male *L. sericata*. Template DNA was then sequenced by Lark Technologies, Inc. (Saffron Walden, U.K.).

Mitochondrial genome sequencing

The *L. sericata* mitochondrial genome was amplified in a series of fragments using universal mitochondrial polymerase chain reaction (PCR) primers (Simon *et al.*, 1994) modified as required to the *Drosophila melanogaster* mitochondrial genome. The products from successful PCR reactions were cloned into a pCR4 TOPO vector. The pCR4 TOPO clones were sequenced initially from vector primers (M13 forward and M13 reverse), then from primers designed to the sequence data generated from the vector primers. This strategy did not cover the entire mitochondrial genome and so data generated were used to design more PCR primers; successful PCR products were cloned and sequenced as before. Finally, a

Correspondence: Dr J. R. Stevens, Hatherly Laboratories, School of Biosciences, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, U.K. Tel.: + 44 1392 263775; Fax: + 44 1392 263700; E-mail: j.r.stevens@ex.ac.uk

further round of PCR with the new primers was used to amplify any regions where additional sequence was needed. Products from these reactions were sequenced directly and a consensus sequence was built up from 94 overlapping partial sequences.

For *C. megacephala*, the PCR primers originally used for the mitochondrial genome of *L. sericata* were used to amplify a series of fragments; additionally, primers developed by Lessinger & Azeredo-Espin (2000) to amplify the control region were used. Successful PCR reactions were sequenced directly with their PCR primers, followed by primers designed to sequences generated by these primers. However, this strategy did not cover the entire mitochondrial genome and additional PCR primers were designed to the published *Chrysomya chloropyga* mitochondrial genome to amplify regions where DNA sequence was lacking. These PCR products were sequenced directly and a consensus sequence was built up from 41 overlapping partial sequences.

All DNA sequencing was performed using BigDye™ (Applied Biosystems, Foster City, CA) terminator cycle sequencing reactions. Analyses of sequencing reactions were carried out on 5.75% and 4.75% Long Ranger™ (Lonza, Wokingham, U.K.) gels for ABI 373 and ABI 377. Full details of the primers tested and used are available on request from JRS; see http://www.projects.ex.ac.uk/meeg/.

Assembly and annotation

Sequence assembly was performed using the GAP4 suite of programs in the Staden Package (Staden *et al.*, 2000). The mitochondrial genomes of *L. sericata* and *C. megacephala* were annotated in accordance with the genomes of *Drosophila yakuba* (NC_001322; Clary & Wolstenholme, 1985), *Cochliomyia hominivorax* (AF260826; Lessinger *et al.*, 2000) and *C. chloropyga* (AF352790; Junqueira *et al.*, 2004); sequences were aligned using CLUSTAL X (Chenna *et al.*, 2003). Gene identities were verified by comparing nucleotide sequences against previously described genes and proteins by BLAST searching against GENBANK. All features noted within the genomes (Table 1) are based on sequence similarity and have not been determined experimentally; additional details are available in the corresponding European Molecular Biology Laboratory (EMBL) entry.

Table 1. Mitochondrial genome architecture of *Lucilia sericata* and *Chrysomya megacephala*.

	Base position	
Feature	*L. sericata*	*C. megacephala*
trnI	1125–1186	20–85
trnQ	1187–1259	90–158
trnM	1263–1331	167–235
nad2	1132–2348	236–1252
trnW	2347–2415	1251–1318
trnC	2402–2482	1305–1372
trnY	2496–2537	1382–1446
cox1	2539–4077	1447–2985
trnL	4074–4137	2980–3047
cox2	4145–4832	3052–3739
trnK	4833–5903	3740–3810
trnD	5904–5969	3811–3876
atp8	4970–5134	3877–4041
atp6	5128–5805	4035–4712
cox3	5805–6593	4717–5505
trnG	6601–6665	5512–5576
nad3	6666–7019	5577–5930
trnA	7022–7085	5933–5996
trnR	7086–7148	5997–5660
trnN	7149–7214	6068–6133
trnS	7215–7282	6134–6201
trnE	7285–7351	6202–6269
trnF	7370–7946	6288–6354
nad5	7437–9156	6355–8074
trnH	9127–9236	8090–8154
nad4	9237–10 575	8155–9493
nad4L	10 569–10 865	9487–9783
trnT	10 868–10 932	9786–9850
trnP	10 933–10 994	9851–9912
nad6	11 001–11 525	9919–10 433
cob	11 525–12 661	10 433–11 579
trnS	12 662–12 729	11 580–11 647
nad1	12 744–13 682	11 662–12 600
trnL	13 693–13 757	12 611–12 175
rrnL	13 760–15 086	12 679–14 005
trnV	15 089–15 158	14 008–14 077
rrnS	15 158–15 943	14 077–14 861
trnI	–	14 951–15 016

Results and discussion

Mitochondrial genome architectures

The mitochondrial genome of *C. megacephala* (AJ42604) was 15 831 bp in length and that of *L. sericata* (AJ422212) was 15 945 bp. Each mitochondrial genome was divided into 13 protein-coding genes, two ribosomal RNAs and 22 transfer RNAs (Table 1), with *C. megacephala* showing the same duplication for one transfer RNA, *trnI*, as first seen in another *Chrysomya* species, *C. chloropyga* (Junqueira *et al.*, 2004). The codon positions of each of these genes are shown in Table 1. As in other insect mitochondrial DNA, typical base compositions with a high AT content were observed: 76.5%, *C. megacephala*; 77.6%, *L. sericata*.

The new mitochondrial genomes had variants of the standard dipteran genome architecture (Shao & Barker, 2007), which in turn is similar to the inferred ancestral mitochondrial DNA arrangement of arthropods (Staton *et al.*, 1997). As in all other Diptera sequenced to date (Shao & Barker, 2007), the mitochondrial DNA genome arrangement in *C. megacephala* and *L. sericata* differed from that in the ancestral architecture in the placement of the $trnL_2$ gene, which was located between *cox1* and *cox2*. *Chrysomya megacephala* also exhibited a *trnI* duplication, as reported either side of the D-loop (or 'large non-coding region') in *Chrysomya putoria* (Junqueira *et al.*, 2004). This duplication appears to be synapomorphic for the genus *Chrysomya*.

cox1 *initiation codon*

In both *C. megacephala* and *L. sericata*, the *cox1* start codon encodes methionine as TCG, which does not conform to the

standard invertebrate mitochondrial code (TCG normally encodes serine). However, such a result is not unusual in Diptera and this non-standard initiation codon has previously been reported in a range of Calliphoridae species (Sperling *et al.*, 1994; Wells & Sperling, 1999; Lessinger *et al.*, 2000), *Anopheles* species (Beard *et al.*, 1993; Mitchell *et al.*, 1993) and in the fruitfly, *Ceratitis capitata* (Spanos *et al.*, 2000).

Acknowledgements

We thank Stephen Foster and David Buck, Lark Technologies, Inc. (U.K.) for co-ordinating the sequencing, and Scott Federhen (National Center for Biotechnology Information, Bethesda, MD) and Gertraud Burger (University of Montreal, Canada) of the GenBank Taxonomy Project for confirming the status of the *cox1* start codon. We also thank Patrick Hamilton (University of Exeter, U.K.) for comments on the manuscript and Lucie Evans (University of Exeter, U.K.) for technical support. This work was funded by the award of a Wellcome Trust Biodiversity Fellowship (050808/Z/97) and a Natural Environment Research Council New Investigator's Award (NER/M/S/2003/00081) to JRS.

References

Avise, J.C. (2004) *Molecular Markers, Natural History, and Evolution*, 2nd edn, p. 684. Sinauer Associates, Sunderland, MA.

Beard, C.B., Hamm, D.M. & Collins, F.H. (1993) The mitochondrial genome of the mosquito *Anopheles gambiae*: DNA sequence, genome organization and comparisons with mitochondrial sequence of other insects. *Insect Molecular Biology*, **2**, 103–124.

Cameron, S.L., Lambkin, C.L., Barker, S.C. & Whiting, M.F. (2007) A mitochondrial genome phylogeny of Diptera: whole genome sequence data accurately resolve relationships over broad timescales with high precision. *Systematic Entomology*, **32**, 40–59.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G. & Thompson, J.D. (2003) Multiple sequence alignment with the clustal series of programs. *Nucleic Acids Research*, **31**, 3497–3500.

Clary, D.O. & Wolstenholme, D.R. (1985) The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization and genetic code. *Journal of Molecular Evolution*, **22**, 252–271.

Junqueira, A.C.M., Lessinger, A.C., Torres, T.T., Da Silva, F.R., Vettore, A.L., Arruda, P. & Azeredo-Espin, A.M.L. (2004) The mitochondrial genome of the blowfly *Chrysomya chloropyga* (Diptera: Calliphoridae). *Gene*, **339**, 7–15.

Lessinger, A.C. & Azeredo-Espin, A.M.L. (2000) Evolution and structural organization of mitochondrial DNA control region of myiasis-causing flies. *Medical and Veterinary Entomology*, **14**, 71–80.

Lessinger, A.C., Martins Junqueira, A.C., Lemos, T.A. *et al*. (2000) The mitochondrial genome of the primary screwworm fly *Cochliomyia hominivorax* (Diptera: Calliphoridae). *Insect Molecular Biology*, **9**, 521–529.

Mitchell, S.E., Cockburn, A.F. & Seawright, J.A. (1993) The mitochondrial genome of *Anopheles quadrimaculatus* species. A: complete nucleotide sequence and gene organization. *Genome*, **36**, 1058–1073.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*, 2nd edn, pp. 6.4–6.11. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shao, R. & Barker, S.C. (2007) Mitochondrial genomes of parasitic arthropods: implications for studies of population genetics and evolution. *Parasitology*, **134**, 153–167.

Simon, C., Frati, F., Beckenback, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Spanos, L., Koutroumbas, G., Kotsyfakis, M. & Louis, C. (2000) The mitochondrial genome of the Mediterranean fruitfly, *Ceratitis capitata*. *Insect Molecular Biology*, **9**, 139–144.

Sperling, F.A.H., Anderson, G.S. & Hickey, D.A. (1994) A DNA-based approach to the identification of insect species used for postmortem interval estimation. *Journal of Forensic Science*, **39**, 418–427.

Staden, R., Beal, K.F. & Bonfield, J.K. (2000) The Staden Package, 1998. *Methods in Molecular Biology*, **132**, 115–130.

Staton, J.L., Daehler, L.L. & Brown, W.M. (1997) Mitochondrial gene arrangement of the horseshoe crab *Limulus polyphemus* L: conservation of major features among arthropod classes. *Molecular Biology and Evolution*, **14**, 867–874.

Stevens, J.R. (2003) The evolution of ectoparasitism in Calliphorid blowflies. *International Journal for Parasitology*, **33**, 1105–1113.

Stevens, J. & Wall, R. (1995) The use of random amplified polymorphic DNA (RAPD) analysis for studies of genetic variation in populations of the blowfly *Lucilia sericata* in southern England. *Bulletin of Entomological Research*, **85**, 549–555.

Wells, J.D. & Sperling, F.A.H. (1999) Molecular phylogeny of *Chrysomya albiceps* and *C. rufifacies* (Diptera: Calliphoridae). *Journal of Medical Entomology*, **36**, 222–226.

Accepted 10 September 2007

