Identification of a Death-scene Maggot using Standardized Molecular Methods: Sarcophagabullata Parker 1916 (Sarcophagidae) Out-numbers Blowflies (Calliphoridae) on an Urban Cadaver in Southeastern Texas

Rekha Raghavendra¹, Christopher P. Randle² and Sibyl Rae Bucheli²*

¹School of Medicine Case Western Reserve University, Cleveland OH, USA
²Department of Biological Sciences Sam Houston State University, Huntsville, TX, USA

Abstract

In forensic entomology, fly data including maggot age are frequently used to help estimate the time since death. Accurate identification of the maggot to species is critical for time since death estimations. However, within a family, maggots are notoriously difficult to identify to species. In this study, we employ phylogenetic data from the mtDNA genes COI and COII to identify an unknown maggot to species (member of the family Sarcophagidae) harvested from a cadaver in June 2009 in Harrison County, Texas. The most closely related species to our unknown maggot was Sarcophagabullata Parker 1916, a somewhat common carrion-feeding species in southeastern United States that is now gaining greater recognition as a forensically significant species.

Keywords: Forensic entomology; case study; Sarcophagidae; Sarcophagabullata Parker 1916

Introduction

Decomposition of a large mammalian carcass is greatly accelerated through the action of insects belonging to the order Diptera (flies). In southeastern Texas, initial colonizers include members of the families Calliphoridae (blow flies), Sarcophagidae (flesh flies), and Muscidae (house flies), with blow flies and flesh flies often arriving and laying eggs or giving birth to maggots (rather than laying eggs) within minutes of death (unpublished records from cadavers at the Southeastern Texas Forensic Science Facility at the Center for Biological Field Studies at Sam Houston State University). Maggots acquire biomass as a function of physiological time rather than calendar time and therefore develop at a predictable rate. Since flies arrive and lay eggs or maggots immediately, they are considered useful tools for estimation of the time that has elapsed since death, or the postmortem interval (PMI), by estimating the time since maggot colonization [1-7]. By recreating the conditions of the death scene in the laboratory and working backwards through time to determine the age of the oldest maggot, the forensic entomologist can correlate the age of the maggot to the PMI [1-7].

Identification of maggots to species remains a challenging aspect to forensic science even though maggots are frequently collected evidence during a death scene investigation. Identification keys are not currently available for all life stages are not currently available and maggots are difficult to identify particularly at early life stages because morphological features among maggots are similar, rendering them virtually undistinguishable beyond the family level [8-12]. Molecular data can aid in the identification of larvae where morphology is limited in utility [8-12]. In this study, we employ an established phylogenetic protocol by Wells et al. [10] using the mitochondrial DNA genes of COI and COII to identify an unknown maggot of the family Sarcophagidae harvested from a cadaver in Harrison County, Texas.

Materials and Methods

Specimens: The unknown maggot was recovered from a body discovered in June 2009 in Harris County, TX, and was the largest observed maggot and most abundant larval type; in fact, no other species were collected despite law enforcement agents reporting the remains to be in a state of fresh/bloated decomposition. The unknown maggots were identified as members of the family Sarcophagidae using standard morphological features of the spiracular complex but could not be further identified (Peterson 1960). Common species of Sarcophagidae which frequent cadavers in this area include Sarcophaga (Neobellieria) bullata Parker, 1916 and Sarcophaga (Bercaea) africa (Wiedemann 1824: 49) (= crucenta Meigen 1826; = haemorrhoidalis auct.) [14-16]. Proper species identification is critical to generate proper growth curves for age estimation; Wells et al. [10] demonstrate that these two species grow at rates disparate enough to create as much as a 24 hour discrepancy.

DNA extraction: Genomic DNA was extracted from the unknown maggots starting with tissue homogenization using a Disruptor Genie TM and followed by a standard Chelex DNA extraction method [17].

Amplification and Sequencing: PCR protocols were modified from Wells et al. [10] using their published primers for COI and COII in various combinations (Table 1) and carried out in 50 μl volumes including 1X PCR buffer (Promega, Madison WI), 0.4 μM forward and reverse primers, 0.2mMdNTPs, 2.5UGoTaq polymerase (Promega, Madison WI), with 3 μl of template DNA. PCR reaction conditions were as follows: 94°C for 2 min (initial denaturation), continued with 35 cycles of 94°C for 1 min (denaturation), 50°C for 1 min (primer annealing), 72°C for 2 min (extension), and 72°C for 10 min (final extension). PCR products were visualized on 1% agarose and purified.

*Corresponding author: Sibyl Rae Bucheli, Department of Biological Sciences, Sam Houston State University, Huntsville, TX, USA, Tel: 936-294-1550; Fax: 936-294-3940; E-mail: bucheli@shsu.edu

Received August 02, 2011; Accepted August 25, 2011; Published September 03, 2011

Citation: Raghavendra R, Randle CP, Bucheli SR (2011) Identification of a Death-scene Maggot using Standardized Molecular Methods: Sarcophagabullata Parker 1916 (Sarcophagidae) Out-numbers Blowflies (Calliphoridae) on an Urban Cadaver in Southeastern Texas. J Forensic Res 2:133. doi:10.4172/2157-7145.1000133

Copyright: © 2011 Raghavendra R, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
using a QIAquick PCR Purification Kit (QIAGEN INC., Valencia, CA). The COI and COII regions were sequenced on a Beckman-Coulter 8000 CEQ Genetic Analyzer using the GenomeLab DTCs Quick Start Kit method. Contig assembly was performed using Geneious [18].

Selection of Sequences for comparison: NCBI nucleotide MEGABLAST was used to identify 100 closest GenBank sequences to the unidentified maggot sequence. These were downloaded and aligned with the unknown sequence using the MUSCLE [19] algorithm in Geneious [18]. Of these, 81 sequences were chosen that had sufficient overlap with each other and the unknown sequence to allow unambiguous alignment. Multiple accessions for species were retained when available, including two sequences each from Sarcophaga africa and S. bullata. Additionally, a sequence obtained from Eucalliphora latifrons (Calliphoridae) was selected as an outgroup. The reduced matrix was realigned using the MUSCLE [19] algorithm for phylogenetic analysis.

Phylogenetic Analysis: Maximum parsimony searches were conducted using WinClada [20] as a shell program. The parsimony ratchet [21] was implemented with 200 iterations (10% of the matrix sampled; one tree held per iteration). The tree generated via the ratchet search was the starting tree for a more thorough analysis conducted in NONA ver. 2.0 [22] using the commands “rs 0; hold 1000; mult*” Parsimony jackknife percentiles (23) were calculated in NONA ver. 2.0 using the commands “rs 0; hold 1000; mult*”.

Selection of Sequences for comparison: NCBI nucleotide MEGABLAST was used to identify 100 closest GenBank sequences to the unidentified maggot sequence. These were downloaded and aligned with the unknown sequence using the MUSCLE [19] algorithm in Geneious [18]. Of these, 81 sequences were chosen that had sufficient overlap with each other and the unknown sequence to allow unambiguous alignment. Multiple accessions for species were retained when available, including two sequences each from Sarcophaga africa and S. bullata. Additionally, a sequence obtained from Eucalliphora latifrons (Calliphoridae) was selected as an outgroup. The reduced matrix was realigned using the MUSCLE [19] algorithm for phylogenetic analysis.

Phylogenetic Analysis: Maximum parsimony searches were conducted using WinClada [20] as a shell program. The parsimony ratchet [21] was implemented with 200 iterations (10% of the matrix sampled; one tree held per iteration). The tree generated via the ratchet search was the starting tree for a more thorough analysis conducted in NONA ver. 2.0 [22] using the commands “rs 0; hold 1000; mult*” Parsimony jackknife percentiles (23) were calculated in NONA ver. 2.0 [22] with 1000 replications (200 search steps; one starting tree per replication; rs 0). For maximum likelihood (ML) analysis, the most appropriate stationary model of evolution was inferred using the Akaike Information Criterion [24] in jMODELTEST [25,26]. ML searches were performed using GARLI 1.0 (27) using the default configuration. One thousand non-parametric bootstrap replicates were analyzed with two search replicates each to obtain clade support. Phyutility [28] was used to generate the majority rule consensus of 1,000 bootstrap trees.

Results

NCBI nucleotide MEGABLAST returned a COI sequence from Sarcophaga bullata as the best sequence match with 97% sequence identity, while S. africa obtained 92% identity and the outgroup, Eucalliphora latifrons obtained an identity of 86%. Sequence alignment resulted in a matrix of 2,305 characters. Parsimony searches resulted in a single most parsimonious trees (L=3921;CI=0.30; RI=0.65). AIC identified GTR+I as the best fitting model of evolution. The maximum likelihood tree obtained anln likelihood score = -21,146.499. MP and ML trees were largely congruent, differing only in the resolution of clades that were poorly supported and inconsequential in the identification of the known sequence. While overall clade support was low, the two most probable species matches, Sarcophaga africa and S. bullata were separated by several strongly supported nodes (jackknife and bootstrap >80%; Figure 1 (included as supplementary data)). Both parsimony and likelihood identified the unknown sequence as sister to the two sequences of Sarcophaga bullata (jackknife =100%; bootstrap = 67%). Sarcophaga polistensis (jackknife =85%; bootstrap = 89%) is sister to this clade, and S. cooley is sister to the clade including S. polistensis-S. bullata (jackknife =95%; bootstrap = 97%). Sarcophaga polistensis occurs in Texas, but is not known to feed on carrion [14]. Sarcophaga cooley is not known to occur in Texas. Therefore, evidence best supports the hypothesis that the unknown maggot is S. bullata.

Discussion

Many modern forensic techniques that employ DNA profiling to make associations between individuals and individuals, individuals and locations, and/or individuals and events (such as RFLP analysis, PCR analysis, STR analysis, AmpFLP, DNA family relationship analysis, Y-chromosome analysis, mitochondrial analysis) [29] are sound due to the process of evolution acting on marker loci. Marker similarity is interpreted as evidence for shared ancestry [30]. Overall, the process leads to situations where more closely related organisms share in common more regions of their DNA. In most situations, DNA profiling analyses are based in principles of phylogenetics (the study of evolutionary relatedness among groups of organisms) and population genetics (the study of the effects of evolutionary processes on allele frequencies in populations) [30,31]. In a growing number of situations, it has been useful to extend methods commonly employed in human DNA analyses to non-human organisms (for a discussion see 29). For species identification of unknown organisms, modern methods of

| Location on the mtDNA | Primer Sequence | Paired combination of primers used in this study |
|-----------------------|----------------|-----------------------------------------------|
| 1 TY-J-1460           | TACAAATTATGCCCAAATCTTCAGCC | 2, 4 |
| 2 C1-N-1687           | CAATTTTCAAATTCTCCAATTAT | 1 |
| 3 C1-J-1751           | GGATCCCTGTATAGCCATTCCC | 6, 8 |
| 4 C1-N-1840           | AGGAGGATCAAAGGTCAC/TCC | 1 |
| 5 C1-J-2183           | CAACATTATTTTGATTTTTG | 11 |
| 6 C1-N-2191           | CCCGTATTAATATATATAACTTC | 3 |
| 7 C1-J-2319           | TAGCAATTGGAATTTAGATTAGG | 10, 13 |
| 8 C1-N-2293           | AGTAACCGTGTTACGTTAGTACC | 3 |
| 9 C1-J-2495           | CAGCTACTTTATAGCATTTCGG | 13, 14 |
| 10 C1-N-2514          | AAACACCTTATACCTTCTATC | 7 |
| 11 C1-N-2659          | GCTAACCGTGTAATTAGGG | 5 |
| 12 C1-J-2792          | ATACCGTGACGTTATCCAGA | 16 |
| 13 C1-N-2800          | CATTTCCAAGCTGTAAGAGACTC | 16 |
| 14 TL2-N-3014         | TCCAAATGCATATCTGCCCATATA | 9 |
| 15 C2-J-3138          | AGAGCCTCTCCTTTAATAGAA | 18 |
| 16 C2-N-3389          | CTGATAGCCATGATAGTG | 12 |
| 17 C2-J-3408          | CATGATAGCTGAGATATAAGA | 18 |
| 18 TK-N-3775          | GAGGACCATTCTGCCACAATCTC | 15, 17 |

Table 1: PCR primers* used in this study.* Primers were taken from Wells et al. [10]. N-forward primer; J-reverse primer.
Sarcophaga bullata utility of so to draw attention to the fact that very little is known regarding the species in forensic applications exist; an entry made on the open-access GenBank database is a sensible tool to reveal identity of an unknown sequence. (Figure 1 (included as supplementary data)) shows all species included in the analysis and their GenBank accession numbers. In the analysis, Sarcophagidae forms a monophyletic group. Analysis of reference sequences downloaded from GenBank database shows little variation between species with different accession numbers. This suggests that the protocol developed by Wells et al. [10] for the use of reference sequences available in the GenBank database is a sensible tool to reveal identity of unknown specimen. Phylogenetic analysis using these reference sequences was able to determine the species of the flesh fly collected from a cadaver and hence may be used to provide supporting information to aid in the estimation of the time since insect colonization.

The occurrence of Sarcophaga bullata as the largest and most abundant species of larval fly recovered from the corpse is note-worthy. Despite the remains being reported by law enforcement as fresh/bloated, this species outnumbered members of the family Calliphoridae (no larvae of Calliphoridae were recovered). While many published accounts of necrophagous species biodiversity of a corpse note the presence of S. bullata, no published accounts rely primarily on data provided by this species as the largest and most abundant member of the community for applied aspects of the science. Ancodatal accounts of the utility of this species in forensic applications exist; an entry made on the open-access on-line Encyclopedia Wikipedia discusses their forensic importance. Their abundance in this situation may be explained by the location of the corpse and time of death in terms of season. In June, southeastern Texas (Houston and surrounding cities) experiences average daytime high temperatures above 90°F/32°C, nighttime lows around 70°F/21°C, and relative humidity levels that fluctuate widely between 50% at noon and 90% at midnight (average minimum and maximum when not raining) [37]. This generally results in dehydration of tissues of the corpse at an accelerated rate (personal observations made of human decomposition at STAFS at CBFS at SHSU, Bucheli and Lindgren) when compared to published descriptions of cadavers at other forensic anthropology stations through out the United States [2; 3; 5; 6; 38; 39]. Furthermore, extensive areas of Montgomery County are urbanized. Unpublished photos of crime scenes from various Houston, TX, urban and rural locations reveal corpses with few to no observed species of Calliphoridae and much greater numbers of Sarcophagidae (personal observations, Bucheli). Reasons for the absence of Calliphoridae may include the lack of a constant supply of large, fresh mammalian corpses due to urbanization in certain areas to sustain populations of significant size. The authors recognize this discussion as largely speculative but do so to draw attention to the fact that very little is known regarding the utility of Sarcophaga bullata in forensic situations.

Acknowledgements

The authors would like to thank the following individuals for their help in the research: Natalie Lindgren, Alan Archambeault, Karen Walters, Jeffery Kelley, and Angela Hawkins.

References

1. Byrd JH, Cashner JL (2000) The utility of arthropods in legal investigations. Forensic Entomology CRC Press LLC.
2. Early M, Goff ML (1986) Arthropod succession patterns in exposed carrion on the island of O‘ahu, Hawaiian Islands, USA. Journal of Medical Entomology 5: 520-531.
3. Tullis K, Goff ML (1987) Arthropod succession in exposed carrion in a tropical rainforest on O‘ahu island, Hawai‘i. Journal of Medical Entomology 24: 520-529.
4. Catts PE, Haskell NH (1990) Entomology & death: a procedural guide. Clemson, S.C.: Joyce’s Print Shop, Inc.
5. Goff ML (1993) Estimation of postmortem interval using arthropod development and successional patterns. Forensic Science Review 5:81-94.
6. Richards EN, Goff ML (1997) Arthropod succession on exposed carrion in three contrasting tropical habitats on Hawaiian Island, Hawaii. Journal of Medical Entomology 34: 520-531.
7. Haskell NH, Williams RE (2008). Entomology & death: a procedural guide, 2nd Edition. Clemson, S.C: East Park Printing.
8. Sperling FAH, Anderson GS, Hickey DA (1994) A DNA-based approach to the identification of insect species used for postmortem interval estimation. Journal of Forensic Science39:418-427.
9. Wells JD, Sperling FAH (1989) Molecular phylogeny of Chrysomya albiceps and Chrysomya rufifacies (Diptera: Calliphoridae). Journal of Medical Entomology 36: 222-26.
10. Wells JD, Pape T, Sperling FAH (2001) DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). Journal of Forensic Science 5:1098-102.
11. Wells JD, Wall R, Stevens JR (2007) Phylogenetic analysis of forensically important Lucilia flies based on cytochrome oxidase I sequence: a cautionary tale for forensic species determination. International Journal of Legal Medicine 121: 229-233.
12. Wells JD, Williams D (2007) Validation of a DNA-based method for identifying Chrysomyinae (Diptera: Calliphoridae) used in a death investigation. International Journal of Legal Medicine 1: 1-8.
13. Peterson A (1960) Larvae of insects; an introduction to Nearctic species. Forth Edition. Columbus, Ohio.
14. Giroux M, Wheeler TA (2009) Systematics and Phylogeny of the subgenus Sarcophaga (Neobellieria) (Diptera: Sarcophagidae). Annals of the Entomological Society of America 4: 567–587.
15. Systema Dipterorum (2010) Version 1.0. Web Service available online at http://www.diptera.org/, accessed on 16 March 2011.
16. Fauna Europaea (2011) Fauna Europaea, version 2.4. Web Service available online at http://www.faunaeur.org, accessed on 16 March 2011.
17. Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques10: 506-513.
18. Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, et al. (2011) Geneious v5.4. Available from http://www.geneious.com.
19. Edgar RC(2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792-1797.
20. Nixon KC (1999) WinClada ver. 1.00.08. Published by the author, Ithaca, NY.
21. Scaduto et al. [32] demonstrate the source of transmission of HIV strains by standard and rigorous phylogenetic analysis (using maximum likelihood and Bayesian estimators). Such methods are frequently employed in insect identification (for a forensic focus on Calliphoridae and Sarcophagidae only see: [8-12,33-36]).
Citation: Raghavendra R, Randle CP, Bucheli SR (2011) Identification of a Death-scene Maggot using Standardized Molecular Methods: Sarcophagabullata Parker 1916 (Sarcophagidae) Out-numbers Blowflies (Calliphoridae) on an Urban Cadaver in Southeastern Texas. J Forensic Res 2:133. doi:10.4172/2157-7145.1000133

24. Akaike H (1974) A new look at the statistical model identification. IEEE Transactions on Automatic Control 19: 716-723.
25. Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696-704.
26. Posada D (2008) ModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25: 1253-1256.
27. Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin.
28. Smith SA, Dunn CW (2008) Phyutility: a phyloinformatics tool for trees, alignments, and molecular data. Bioinformatics 24: 715-716.
29. Jobling MA, Gill P (2004) Encoded evidence: DNA in forensic analysis. Nature Reviews Genetics 5: 739-751.
30. Ridley ME (2007) Evolution. New Delhi: Oxford University Press.
31. National Research Council (U.S.) (1996) The evaluation of forensic DNA evidence. Washington, D.C.: National Academy Press.
32. Scaduto DI, Brown JM, Haaland WC, Zwickl DJ, Hillis DM et al. (2010) Source identification in two criminal cases using phylogenetic analysis of HIV-1 DNA sequences. Proceedings of the National Academy of Sciences of the United States of America 50: 21242-7.
33. Singh B, Kurahashi H, Wells JD (2011) Molecular phylogeny of the blowfly genus Chrysomya. Medical and Veterinary Entomology 25: 126-34.
34. Wells JD, Infrona F, Di Vella G, Campobasso CP, Hayes J, Sperling FAH (2001) Human and insect mitochondrial DNA analysis from maggots. Journal of Forensic Sciences 3: 685-7.
35. Wells JD, Stevens JR (2008) Application of DNA-based methods in forensic entomology. Annual Review of Entomology 53: 103-20.
36. Wells JD, Sperling FAH (2001) DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). Forensic Science International 120: 110-115.
37. National Climatic Data Center Land-Based Data. (Report generated May 2011). National Oceanic and Atmospheric Administration.
38. Goff ML (1991) Determination of postmortem interval by arthropod succession: A case study from the Hawaiian Islands. Journal of Forensic Sciences 2: 220-225.
39. Bass WM (1996) Chapter 12. Outdoor Decomposition Rates in Tennessee. Forensic taphonomy: The postmortem fate of human remains. Haglund WD and Sorg MH (eds.) Boca Raton: CRC Press.