Contents lists available at ScienceDirect





Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Verification of AFLP kinship methods of entomological evidence by sequencing



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ARTICLE INFO

Article history: Received 25 June 2014 Received in revised form 27 August 2014 Accepted 30 September 2014 Available online 13 October 2014

Keywords: Forensic entomology Amplified fragment length polymorphism Phormia regina Calliphoridae Cytochrome oxidase Kinship

ABSTRACT

Kinship analysis allows the determination of sibship based on the individuals' genetic profile. In a recent empirical study, amplified fragment length polymorphism (AFLP) analysis was proposed as a test to determine kinship between *Phormia regina* individuals useful in inferring postmortem transport of a corpse. In order to validate this technique, mitochondrial DNA gene cytochrome oxidase II was sequenced for all individuals used in the previous study. Then, the relatedness coefficient based on AFLP profiles was determine a conservative false positive error rate of this proposed test. A majority, 96%, of pair wise comparisons of individuals with different haplotypes had relatedness coefficients <0.41 supporting the conclusion that AFLP analysis for full sibship is a valid and robust technique and thus useful for the detection of postmortem movement of a corpse.

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The determination of kinship among pairs of individuals, or the probability that two individuals share alleles that are identical by descent, is usually based on microsatellite loci [1]. These types of applied kinship analyses [2,3] may be used for identifying a corpse in a mass fatality event [4], and for managing a captive breeding program in conservation efforts [5]. It is also possible to use these types of analyses to detect postmortem movement of a corpse using entomological specimens [6].

Dominant loci can be used to determine kinship among pairs of individuals [7], though the Hardy–Weinberg equilibrium cannot be tested and is therefore assumed [8]. Because a dominant genotype does not reveal individual alleles, it is used to calculate "conditional kinship" coefficients [7], defined as the difference in probabilities of identity-in-state (IIS) between homologous genes.

Since IIS is the only information available to use with dominant genetic data, this is the most powerful approach available to determine kinship between individuals. When a pairwise comparison is made between two individuals of unknown relationship, a value of approximately 0.5 indicates individuals are full siblings, 0.25 indicates half siblings, and 0 corresponds to unrelated

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http://dx.doi.org/10.1016/j.forsciint.2014.09.024 0379-0738/© 2014 Elsevier Ireland Ltd. All rights reserved. individuals. Blow fly parent-offspring matings are highly unlikely because females mate only once [9], and because the likelihood of mean survival of the adults is three to four weeks [10], thus, time to mate with the offspring is unlikely, so for the purpose of this project, this relationship is not considered.

In forensic entomology, kinship estimations can be used to link separate crime scenes in the event of postmortem movement of a corpse by determining the kinship of stray larvae with larvae present on the discovered corpse [6]. This analysis was done on *Phormia regina* (Diptera: Calliphoridae) using amplified fragment length polymorphism (AFLP) data of nuclear DNA and yielded an empirical probability distribution of relatedness coefficients for pairwise comparisons of full siblings and unrelated individuals. It was determined that it was $1000 \times$ more likely that a relatedness coefficient of 0.41 or greater would be observed if two individuals were full siblings.

Picard and Wells [6] generated relatedness values for known siblings through samples produced by breeding experiments, but non-sibling comparisons were assumed to be so because they involved wild flies collected at separate locations. It was the goal of this work to further validate the AFLP-based kinship test by comparison to an independent genetic test for non-sibship, mitochondrial DNA (mtDNA) haplotyping. In contrast to our earlier study [6], in which only single individuals from each geographic sample were analyzed so that almost certainly no two individuals were siblings, this study was based on pairwise

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comparisons within samples. Therefore full sibship was a realistic possibility. Although a shared haplotype is not strong evidence of sibship, different haplotypes is strong evidence of non-sibship. Therefore our purpose was to estimate the false positive rate of the AFLP kinship test by calculating AFLP relatedness values for known non-siblings from the same location.

1. Methods

1.1. Sample collection, extraction, and AFLP profiling

All DNA extracts were those previously used by Picard and Wells for an AFLP population survey of *P. regina* [6].

Table 1

Sequence polymorphisms (all silent mutations) of the 10 haplotype designations and abundance. The majority (*N*=109) of the specimens had a previously published haplotype (DQ315102.1). The nine new unique haplotypes are listed A–I. For each haplotype, the polymorphic site is indicated with the change in base at the position that was polymorphic.

Haplotype (# specimens)	Accession #	3145	3256	3301	3355	3391	3493	3544	3604	3622	3667	3685
(109)	DQ345102.1	Т	А	G	Т	А	С	G	Т	Т	А	A
A (1)	KF926691	•	G	•	С	•	•	•	•	•	•	•
B (7)	KF926692	•	•	•	•	•	Т	•	•	•	•	•
C (2)	KF926693	•	•	А	•	•	•	•	•	•	•	•
D (1)	KF926694	•	•	•	•	•	•	Α	•	•	•	•
E (1)	KF926695	•	••	•	•	•	•	•	•	С	•	•
F (2)	KF926696	•	•	•	•	•	•	•	С	•	•	•
G (1)	KF926697	•	•	•	•	•	•	•	•	•	G	•
H (1)	KF926698	•	•	•	•	•	•	•	•	•	•	G
I (1)	KF926699	•	•	•	•	G	•	•	•	•	•	•

Table 2

Haplotype comparisons between two samples with different haplotypes and known relatedness coefficients, pairs of individuals in bold had relatedness coefficients greater than 0.41. Each relatedness coefficient was obtained from analysis from [1].

Geographic location (U.S.A.)	Individual 1	Haplotype 1	Individual 2	Haplotype 2	Relatedness coefficient
Tuscaloosa, AL	AL2-Pr1	DQ345102.1	AL2-Pr9	В	0.051268
	AL2-Pr2	DQ345102.1	AL2-Pr9	В	0.096201
	AL2-Pr3	DQ345102.1	AL2-Pr9	В	0.048697
	AL2-Pr4	DQ345102.1	AL2-Pr9	В	0.111162
	AL2-Pr5	DQ345102.1	AL2-Pr9	В	0.165364
	AL2-Pr7	DQ345102.1	AL2-Pr9	В	0.201681
	AL2-Pr8	DQ345102.1	AL2-Pr9	В	0.209443
	AL2-Pr10	DQ345102.1	AL2-Pr9	В	0.098729
West Haven, CT	CT1-Pr1	DQ345102.1	CT1-Pr5	В	0.064307
	CT1-Pr2	DQ345102.1	CT1-Pr5	В	0.091174
	CT1-Pr3	DQ345102.1	CT1-Pr5	В	0.027823
	CT1-Pr4	DQ345102.1	CT1-Pr5	В	0.033078
	CT1-Pr6	DQ345102.1	CT1-Pr5	В	-0.053402
	CT1-Pr7	DQ345102.1	CT1-Pr5	В	0.022829
	CT1-Pr8	DQ345102.1	CT1-Pr5	В	0.068293
	CT1-Pr9	DQ345102.1	CT1-Pr5	В	0.026631
	CT1-Pr10	DQ345102.1	CT1-Pr5	В	0.099067
Riggins, ID	ID1-Pr6	DQ345102.1	ID1-Pr7	В	0.197518
Mountain Home, ID	ID2-Pr2	DQ345102.1	ID2-Pr1	I	0.060036
	ID2-Pr3	DQ345102.1	ID2-Pr1	Ι	-0.003846
	ID2-Pr4	DQ345102.1	ID2-Pr1	Ι	0.060559
	ID2-Pr5	DQ345102.1	ID2-Pr1	Ι	0.012776
	ID2-Pr6	c	ID2-Pr1	I	0.023589
	ID2-Pr7	DQ345102.1	ID2-Pr1	I	0.14301
	ID2-Pr8	DQ345102.1	ID2-Pr1	I	-0.094031
	ID2-Pr9	c	ID2-Pr1	I	0.013672
	ID2-Pr10	DQ345102.1	ID2-Pr1	I	-0.169358
	ID2-Pr2	DQ345102.1	ID2-Pr6	С	-0.038109
	ID2-Pr3	DQ345102.1	ID2-Pr6	С	0.100603
	ID2-Pr4	DQ345102.1	ID2-Pr6	С	0.136066
	ID2-Pr5	DQ345102.1	ID2-Pr6	C	0.059341
	ID2-Pr7	DQ345102.1	ID2-Pr6	C	-0.041961
	ID2-Pr8	DQ345102.1	ID2-Pr6	C	-0.047466
	ID2-Pr10	DQ345102.1	ID2-Pr6	c	-0.007025
	ID2-Pr2	DQ345102.1	ID2-Pr9	C	-0.10591
	ID2-Pr3	DQ345102.1	ID2-Pr9	C	-0.111908
	ID2-Pr4	DQ345102.1	ID2-Pr9	C	0.039323
	ID2-Pr5	DQ345102.1	ID2-Pr9	C	0.078366
	ID2-Pr7	DQ345102.1	ID2-Pr9	C	-0.109762
	ID2-Pr8	DQ345102.1	ID2-Pr9	C	0.116269
	ID2-Pr10	DQ345102.1	ID2-Pr9	C	0.128599
	102-1110	DQ343102.1	102-115	c	0.120333

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