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TECHNICAL NOTE

PATHOLOGY/BIOLOGY

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A Test for Carrion Fly Full Siblings: A Tool for Detecting Postmortem Relocation of a Corpse*,†,‡

ABSTRACT: We propose a genetic test for full sibship for a pair of carrion flies that could reveal the postmortem relocation of a corpse. A carrion fly larva is sometimes left behind when a corpse is moved. The discovery of full sibling larvae of approximately the same developmental stage at two locations would strongly suggest that a corpse was moved between those two sites. Distributions of pairwise comparisons of relatedness (R) coefficients were generated using amplified fragment length polymorphism profiles for nine samples of laboratory-generated full siblings as well as for a reference sample of nonfull sibling *Phormia regina* (Diptera: Calliphoridae). The mean relative R coefficient, a pairwise measure of the proportion of shared alleles, was 0.479 (±0.289 SD) for full siblings, close to the theoretical expectation of 0.5. A likelihood ratio (LR) test was based on observed distributions of R. R >0.55 corresponded to an LR >1000 favoring full sibship for that pair of individuals.

KEYWORDS: forensic science, forensic entomology, *Phormia regina*, postmortem movement, likelihood ratio test, amplified fragment length polymorphisms

Transportation of a decomposed corpse may result in a carrion maggot left at the first location (1). The ability to associate such a "stray" larva with a corpse at a separate site would suggest that the corpse had been moved between the two sites. We have been involved in several cases in which maggots were found in the car or home of a person suspected of killing a victim discovered at a separate location. Maggot gut content analysis, in which a human genotype from the maggot gut is compared with the victim genotype, could serve this function (1–3). However, we have found it to be of limited utility because the gut contents are so quickly digested after a larva is removed from a food source (1).

Our recent population genetic survey of North American blow flies *Phormia regina* and *Lucilia sericata* (4,5) suggested alternative approaches. The first, multilocus assignment, is described elsewhere (5). Here, we propose another strategy: a genetic test for carrion full sibship. A female carrion fly will typically deposit her entire complement of mature eggs on a single corpse within a short period (6). To produce a subsequent batch of eggs ready to be laid, it has been shown in the blow fly *L. sericata* that a minimum of 2 days are needed (at 23°C) (7). Because of this, two carrion fly full siblings of about the same developmental stage almost certainly were deposited as eggs on the same corpse (or similar food item) at the same time. The discovery that a stray larva, found in the absence of a corpse at one location, was a full sibling of a larva of about the same stage of development in a corpse would indicate that the corpse had been relocated from where the stray larva was found. A test for this based on the insects' DNA rather than ephemeral gut contents should be more widely applicable than the latter approach.

Our blow fly population genetic methods rely on amplified fragment length polymorphism (AFLP) profiles (8). AFLP yields a dominant genotype (9), so standard kinship calculations based on allele frequencies are not possible. Therefore, we have adopted an empirical approach, using observed probability distributions of a relative relatedness (R) coefficient, for full siblings and unrelated *P. regina*.

Methods

Specimen Collection—Nonfull Siblings

P. regina adults were collected from the locations shown in Table 1 and were preserved in 95% ethanol until further use. A pair of individuals was considered to be unrelated if they were from separate locations, and only a single individual from each location was used.

Specimen Collection—Full siblings

Nine virgin male—female pairs of *P. regina* were isolated and allowed to mate, and progeny from each set were reared to adults. Originating locations of the mating flies are outlined in Table 2. All flies were preserved in 95% ethanol until further use.

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TABLE 1—*List of fly specimens used to obtain a frequency distribution for individuals which are assumed to be unrelated (=not full siblings) if collected at a different geographic location and/or time.*

Sample Name	Location, State (County)	Date Collected
IAPr01*	Winterset, IA (Madison)	4 July 2004
LGPr01*	Lyle Grove, WA (Whitman)	9 October 2006
MNPr01*	Orr, MN (St. Louis)	15 May 2001
PAPr01*	Bradford, PA (Bradford)	25 August 2006
PRPr01*	Prosser, WA (Benton)	10 October 2007
TCPr01*	Tucannon River, WA (Columbia)	9 October 2006
VAPr01*	Quantico, VA (Prince William)	18 May 2001
WAPr01*	Lyle Grove, WA (Whitman)	18 July 1999
WVPr01*	Morgantown, WV (Monongalia)	1 May 2005
WVPr50*	Morgantown, WV (Monongalia)	20 May 2006
WVPr60*	Morgantown, WV (Monongalia)	27 May 2005
WVPr70*	Morgantown, WV (Monongalia)	9 August 2006
WVPr80*	Coopers Rock, WV (Preston)	21 August 2006
WVPr90*	Bruceton Mills, WV (Monongalia)	17 August 2006
AL1Pr01	Birmingham, AL (Jefferson)	19 May 2008
AL2Pr02	Tuscaloosa, AL (Tuscaloosa)	20 May 2008
CA1Pr01	Clarksburg, CA (Yolo)	27 May 2008
CT1Pr01	West Haven, CT (New Haven)	8 June 2008
FL1Pr01	Quincy, FL (Gadsden)	19 May 2008
ID1Pr01	Riggins, ID (Idaho)	30 May 2008
ID2Pr01	Mountain Home, ID (Elmore)	30 May 2008
ID3Pr01	Blackfoot, ID (Bingham)	31 May 2008
ID4Pr01	Boise, ID (Ada)	30 May 2008
MA1Pr01	Otis, MA (Berkshire)	7 June 2008
NC1Pr01	Mt. Airy, NC (Surry)	17 May 2008
NY1Pr01	Severance, NY (Essex)	6 June 2008
OH1Pr01	North Olmstead, OH (Cuyahoga)	5 June 2008
PA1Pr01	West Springfield, PA (Erie)	6 June 2008
SD1Pr01	Tilford, SD (Meade)	2 June 2008
WA1Pr01	Pullman, WA (Whitman)	29 May 2008
WA2Pr01	Tucannon River, WA (Columbia)	29 May 2008
WV1Pr01	New River Gorge, WV (Fayette)	17 May 2008
WY1Pr01	Buffalo, WY (Johnson)	1 June 2008
WY2Pr01	Greybull, WY (Big Horn)	1 June 2008
WY3Pr01	Shell, WY (Big Horn)	1 June 2008

IAPr01 = Iowa *Phormia regina* sample #01, etc. Individual genotypes are listed in (8).

*Corresponds to individuals from a published study (4).

TABLE 2—*Collection location of the pairs of individuals used to produce a set of full siblings.*

Sample Name	Number of Full Siblings	Collection Location of Breeding Pairs
J2	15	AL (f) + WA (m)
J5	15	WA (f) + AL (m)
Pr1	10	WV (f) + WV (m)
Pr12	10	WV (f) + WV (m)
Pr20	10	WV (f) + WV (m)
Pr22	10	WV (f) + WV (m)
Pr25	10	WV (f) + WV (m)
Pr28	10	WV (f) + WV (m)
Pr29	10	WV (f) + WV (m)

The sample name is listed, along with the number of full siblings genotyped, and the collection locations. AL (f) refers to the female coming from a colony in which the flies originated from Birmingham, AL collected on May 19, 2008. WA (m) refers to a male used from a colony established using flies collected from in Pullman, WA on May 29, 2008. The WV samples were all from a well established lab colony in which flies originated from Morgantown, WV collected during the summer of 2008. Individual genotypes are listed in (8).

DNA Extractions

DNA from thoracic tissue (for unrelated samples, Table 1) (10) or fly heads (full siblings, Table 2) was extracted using a Qiagen DNeasy Kit (Qiagen, Valencia, CA) according to the manufacturers' protocol with a final elution volume of 100 or 150 μL, respectively. DNA samples were stored at −20°C until further use.

Amplified Fragment Length Polymorphism Genotyping

All primers and adaptors were purchased from Integrated DNA Technologies (Coralville, IA). The amplification procedures and locus selection criteria followed Picard and Wells (4). This resulted in the scoring of 433 polymorphic alleles from two primer combinations (PstI + AAC and PstI + ATC).

Relatedness Calculations

R calculations were performed using SPAGeDi (11) to produce pairwise statistics of genetic differentiation between individuals. An R coefficient is defined as the proportion of alleles shared between individuals relative to the average for all pairwise comparisons in the population. Therefore, R is dependent on reference allele frequencies that were calculated according to Hardy (12). R calculations were performed for all pairs of nonfull siblings and also for all pairs of full siblings and distributions of R coefficients were generated. A probability distribution function was fitted to each distribution to estimate the likelihood of observing R for each of the two types of relationship (see Fig. 1), and the likelihood ratio (LR) was used to determine the degree to which a given R value favored the hypothesis of full sibship compared with two individuals being nonfull siblings.

Results and Discussion

The distributions of full siblings and nonfull siblings are shown in Fig. 1*A*. The means of the distributions (± standard deviations) for full siblings and nonfull siblings were 0.479 (0.286) and −0.001 (0.142), respectively, matching theoretical expectations (13). In addition, the distributions overlapped similarly to microsatellites (14).

For all values of R under each hypothesis (full sibling or not), the log-likelihood ratio for each hypothesis was calculated (Fig. 1*B*). An R value >0.22 indicates a positive LR (Table 3). Choosing a meaningful LR value would be somewhat arbitrary, but we note that the U.S. legal system has established standards for an analogous statistic: the human paternity index (PI) (15). R >0.55 corresponds to LR >1000, a value near the high end of the range of U.S. state PI standards (15), so we suggest it as a similar threshold for this analysis. The proportion of nonfull sibling comparisons that had an R coefficient >0.55 was 0.1%, while the proportion of full siblings that exceed this threshold was 37%. In practice, a single larva from a corpse could be used (provided a reference data set was in hand). In the event that the corpse is infested with a large number of larvae, increasing the number of larvae sampled from the corpse would increase the probability of finding a full sibling, if one were present.

This kinship test is universal in that AFLP data can be applied to any sexually reproducing species with no need for preliminary genomic knowledge. It does, however, require a population genetic survey to measure and define expected relatedness between randomly selected individuals (currently available for two calliphorid species: *P. regina* and *L. sericata* [4,5]). Full siblings at about the same stage of larval or pupal development, and found at different locations, could strongly suggest transportation of a corpse between those locations. This is particularly important if the crop contents of the maggot have been digested and it is no longer possible to obtain a human genotype from the maggot (1). A potential

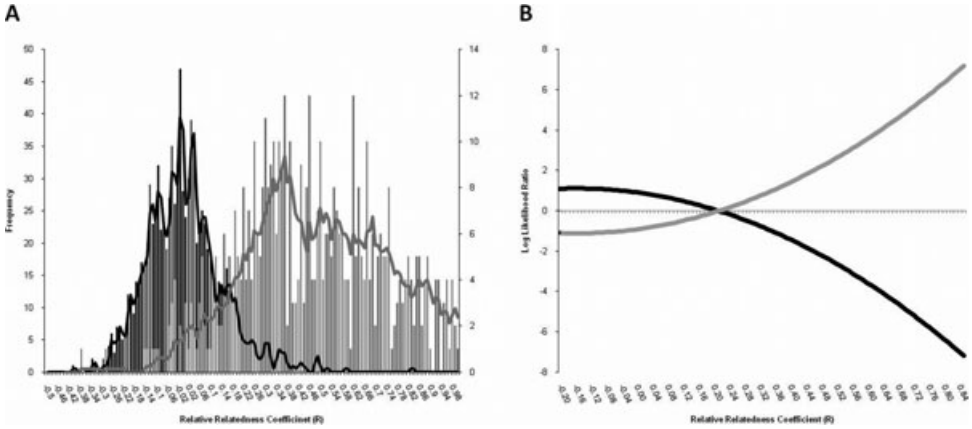

FIG. 1—*Distributions of pair wise comparisons and log-likelihood ratio test for full sibship. (A) Frequency distribution of relatedness coefficients for both unrelated (black bars, Table 1) and full siblings (gray bars, Table 2). The trend lines were created in Excel and are a moving average. The means (standard deviation) of the unrelated and full sibling distributions are −0.001 (±0.142) and 0.479 (±0.286), respectively. (B) Graph representing the log likelihoods under each possible hypothesis for the determination of full sibship. The black line represents the likelihood ratio under the hypothesis of two individuals are not full siblings, and the gray line represents the hypothesis of two individuals if they are full siblings.*

TABLE 3—*Relatedness coefficients and proportions of full siblings, half siblings and nonfull siblings exceeding commonly used likelihood ratio (LR) thresholds. LRs were chosen based on state standards for paternity testing (ranging from 20–1000X).*

Hypothesis	LR Threshold	Relatedness Coefficient	Full Sibs (%)	Half Sibs (%)	Non-Full Siblings (%)
Full Siblings (vs. nonfull sibs)	>1	0.22	82	n/a	6.4
	20	0.38	58	n/a	1.4
	1000	0.55	37	n/a	0.1
Full Siblings (vs. simulation—half sibs)	>1	0.42	54	11	n/a
	20	0.63	29	1.3	n/a
	1000	0.82	11	0.1	n/a

disadvantage of this approach compared with gut content analysis is that a large larval population in a corpse may make it difficult to find a stray larva's siblings, even if they are present. The logistical implications of this are not clear to us. We are aware of only anecdotal data on corpse larval population size. Depending on the case it ranges from a few, probably the offspring of a single female, to at least hundreds of thousands representing several species. Further research is needed to clarify the sampling effort needed make this analysis a practical forensic tool.

We defined the competing hypotheses as a pair of *P. regina* individuals being either full siblings (therefore originating from the same corpse) or not close relatives (possibly originating from different corpses). This raises the question of whether or not these are the only relevant genetic alternatives. Could individuals that originated in separate corpses be closely related rather than unrelated? The greatest violation of our assumption would be the presence of half siblings of approximately equal age on separate corpses. These are likely to be rare in nature. A female blow fly typically mates with only one male, storing sperm for use throughout her reproductive life (16). Therefore, half siblings would be the result of the same father and separate mothers (17). Male blow flies can mate with more than one female, as demonstrated in *Lucilia cuprina*, but there is a log decline of success with each subsequent insemination, owing to a decline in accessory gland material transferred by the male (18).

Nevertheless, to explore this "worst case scenario" of a half sibling stray larva and a larva from a corpse, we simulated the distribution of R for half siblings by adding 0.25 to each R coefficient of the nonfull sibling pool. For the alternative hypotheses of full versus half siblings, the R coefficient necessary to yield a positive LR favoring full sibship was 0.42 (with a proportion of half sibs with an R coefficient >0.42 equal to 11% Table 3). To obtain a likelihood >20 and 1000 (lowest and highest state standards for human paternity), the R coefficient must be >0.63 and 0.82, respectively. The proportion of half sibs that would have these R coefficients were 1.3% for LR = 20 and 0.1% for LR = 1000. The proportion of full sibs that exceed these thresholds are 29% and 11%, respectively.

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