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IDENTIFICATION OF THE PEOPLE FROM WHOM ENGORGED *AEDES AEGYPTI* TOOK BLOOD MEALS IN FLORIDA, PUERTO RICO, USING POLYMERASE CHAIN REACTION–BASED DNA PROFILING

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Abstract. We used polymerase chain reaction–based DNA profiling to construct allelic profiles for residents and visitors of 22 houses in Florida, Puerto Rico, and human DNA from blood meals in *Aedes aegypti* that were collected in those homes. Complete profiles were obtained for ≤ 2 days after blood ingestion. Eighteen percent of the meals came from two different people. There was no evidence of meals from ≥ 2 people. Eighty percent of the meal sources were identified, > 70% were taken from residents of the collection house, and > 90% were from residents of the study community. Across the community, feeding was non-random with a bias towards young adults and males. Three people accounted for 56% of the meals. Our results confirm that multiple feeding on different people is an important component in the role of *Ae. aegypti* in dengue virus transmission and help explain the spatial distribution of dengue cases in a previous epidemic in Florida, Puerto Rico.

INTRODUCTION

Medical entomologists have long recognized the fundamental importance of the interaction between arthropod vectors and their vertebrate hosts.[1–3] Consequently, for the last three decades they have tried to develop techniques to identify the specific person who was the source of mosquito blood meals. Initially, analyses of ABO blood groups[4] and serum protein haptoglobins[5,6] were used to examine the frequency of feeding on people who were selected *a priori* for marker differences. Because low variability limits the number of unique combinations of blood groups and serum proteins, those markers are not useful for cross-sectional, community-wide studies or for determining feeding patterns on populations of closely related people. During the early 1990s polymerase chain reaction (PCR)–based human DNA identification technology improved the discriminatory power of mosquito blood meal analysis (reviewed by Chow-Shaffer and others[7]). Recently, Chow-Shaffer and others[7] evaluated various protocols for PCR-based forensic DNA profiling and identified a procedure for studying the details and epidemiologic significance of *Aedes aegypti* (L.) blood-feeding behavior under field conditions.

The blood-feeding behavior of *Ae. aegypti* is both scientifically interesting and epidemiologically important because it is unusual compared with most other mosquito species and because *Ae. aegypti* is the principal vector of dengue virus, the most important arboviral infection of humans.[8] Unlike most other mosquito species, female *Ae. aegypti* tend to forego feeding on plant carbohydrates,[9–11] instead they feed frequently (approximately 0.6–0.8 blood meals per day) and preferentially on humans.[12–15] There is selective pressure to restrict their diet to frequent meals of human blood. Female fitness (a function of survival and reproduction) is greater when they feed only on human blood than on human blood and sugar or on blood from a non-human host.[16–21] The low concentration of isoleucine in human blood is believed to allow *Ae. aegypti* to use blood efficiently for vitellogenesis and synthesis of energy reserves.[21] Infected *Ae. aegypti* can transmit dengue virus by probing their mouth parts into a vertebrate ≤ 20 consecutive times or by imbibing a blood meal.[22] Once infective, a single *Ae. aegypti* can inoculate many people with virus in a short period of time. Multiple feeding, therefore, increases the probability that this species will contract and then transmit a dengue virus infection.

In this study, we used DNA profiling of four polymorphic human loci to examine three aspects of the interaction between *Ae. aegypti* and their human hosts in a suburban Puerto Rican community where dengue is endemic. First, we determined whether multiple feeding involved different people. Scott and others[12,15] used a histologic technique to estimate the number of times *Ae. aegypti* imbibed blood during each egg laying cycle. A limitation to this approach is that histologically one can not identify exactly which person or persons were bitten. If mosquitoes took multiple blood meals from the same person or from a non-human vertebrate rather than multiple blood meals from different people, the epidemiologic significance of multiple feeding would need to be reduced. For example, two meals from the same person or one meal from a person and one from dog would constitute multiple feeding, but from an epidemiologic perspective it would be equivalent to feeding on a single person. Second, we tested the hypothesis that mosquito bites are not uniformly distributed across the human host population.[23–25] Heterogeneity of human biting frequencies has been associated with differences in host permissiveness (i.e., defensive behavior) and attractiveness, both of which are affected by race, age, size, and health. Heterogeneity in human exposure to mosquito bites can have significant effects on the dynamics of pathogen transmission. Lastly, we established the extent to which household visitors are bitten by resident mosquitoes. Evidence that engorged *Ae. aegypti* contained blood from non-residents would be consistent with the hypothesis that the rapid spread of dengue cases within and among communities during an epidemic is due more to the movement of virus-infected people than to the flight of infected adult mosquitoes.[15,26]

MATERIALS AND METHODS

Study site. Our study site was comprised of the 25 southernmost houses in the Yanes III urbanization of Florida,

Puerto Rico.[26–28] The neighborhood is bounded on the south, east, and west by densely vegetated, steep, limestone escarpments, locally referred to as mogotes. We assumed that the mogotes were barriers to dispersal, so that the *Ae. aegypti* we studied were confined to the study site unless they dispersed to the north where there was more housing.

Mosquito collections. Mosquitoes were collected in 22 of the 25 houses. The other three houses were excluded because no one was home during sampling hours or the residents did not wish to participate in the study. Most homes lacked air conditioning, so windows were usually left open for ventilation. Most windows were not screened.

Mosquito collection teams typically consisted of two members of our field crew. Mosquitoes were collected daily in each of the 22 houses from January 28 to February 6, 1996 and from July 21–30, 1996, using Modified Centers for Disease Control (CDC) Backpack Aspirators (John W. Hock Co., Gainesville, FL). The collections were only made when a resident allowed entry, so collection times varied but were always during daylight hours. Out of respect for the privacy of the occupants and because it was beyond the scope of our approved human subjects protocol, no records were kept on who was in the houses or when they were home.

Captured mosquitoes were processed as described by Chow-Shaffer and others.[7] Briefly, mosquitoes were anesthetized with CO_2 and sorted to species. In addition to various species of indigenous mosquitoes, some collections included laboratory-reared *Ae. aegypti* that were used in mark-recapture studies (Edman JD, Scott TW, unpublished data). All wild *Ae. aegypti* females that contained any amount of blood and some of the recaptured laboratory-reared *Ae. aegypti* females were set aside for blood meal analyses. A unique collection number was assigned to each mosquito. The degree of engorgement was estimated and recorded, and the abdomens were then removed and squashed onto filter paper in the field laboratory. After drying, pieces of filter paper with the dried bloodstains were excised, individually placed into 1.5 mL screw-capped plastic tubes, and held at ambient temperature until taken to the University of Maryland (UM) or the University of California, Davis (UCD), where they were refrigerated until processed. Collection records were kept on a spreadsheet listing mosquito collection numbers, engorgement estimates, color of the squashed blood meal, collection dates, and the addresses of the house in which the mosquitoes were collected.

Human oral swabs. There were 84 residents in the 22 mosquito collection houses, 1–8 per house. Human DNA sampling was conducted after informed consent was obtained from all human adult participants and from parents or legal guardians of minors in adherence to human subjects protocol 01XM, assurance of compliance number MI-362, granted by the UM Institutional Review Board, and protocol 96-802, assurance of compliance number M-1325, granted by the UCD Institutional Review Board. Except for two occupants who chose not to participate in the study, oral swabs of each resident were taken for DNA isolation and typing during January or February 1996 using the procedure described by Chow-Shaffer and others.[7] Most residents were re-sampled in July 1996 and/or February 1997 to get more complete coverage and to confirm allelic profiles. Members of the field crew and any other consenting household visitors on mosquito collection days were also swabbed. For anonymity, swab numbers were assigned to residents and visitors as each was sampled. The individuals' ages, addresses, and relationships to other household members were associated with the swab number on a separate list. Over the three sampling periods, one or more oral swabs were obtained from 82 residents of the study site, 19 members of the field crew, and 5 visitors. Residents and local visitors were Hispanic whites; the field crew consisted of nine non-Hispanic whites, eight Hispanics, and two Asians.

DNA fingerprinting. DNA from the oral swabs and dried blood meals was extracted and isolated at UM or UCD between April 1996 and July 1997, following the procedures described or cited by Chow-Shaffer and others[7] and summarized later in this report. To improve DNA yields and purity, the extraction protocol was modified slightly as the samples were processed. Immersion of the blood meals in lysis buffer was lengthened to a minimum of seven days and Phase Lock Gel™ Light tubes (Eppendorf Scientific, Inc., Westbury, NY) were used for extraction and isolation of DNA. All the July 1996 mosquito blood meals and February 1997 oral swabs and some of the earlier samples were processed in this way.

Human DNA from the oral swabs and blood meals was extracted and isolated using phenol/chloroform and quantified using a slot blot manifold and ACES™ Human DNA Quantitation Probe Plus kits (Gibco-BRL Life Technologies, Gaithersburg, MD). Attempts were made to profile every oral swab regardless of DNA content and all but 10 of the 329 blood meals from which ≥ 3 ng of human DNA was recovered. After finding that only three of 95 samples with < 3 ng of human DNA could be completely profiled, profiling of the remaining 316 blood meals with < 3 ng of human DNA was not attempted.

Human DNA from oral swabs and mosquito blood meals was profiled first at the D1S80 VNTR locus using the AmpFlP™ D1S80 amplification kit (PE Biosystems, Foster City, CA). If D1S80 amplification was successful and sufficient sample remained, DNA was typed at the CSF1PO, TPO1, and THOX STR loci using the GenePrint™ STR Multiplex kit for CTT (Promega Corp., Madison, WI). Amplified allelic DNA was separated by gel electrophoresis and visualized by silver staining. If necessary, this process was repeated to confirm the presence of faint bands. If no alleles were visualized, amplification of the failed sample was repeated until the profile was complete or the sample was exhausted.

BloodID, version 1.01, software (Southern Stars Software, Saratoga, CA) was used to assist in matching DNA profiles of blood meals to those of the residents and visitors who were profiled. Conservative matching criteria were adopted *a priori,* as follows. 1) Every visualized band would be considered real and indicative of the presence of a human allele in the blood meal or oral swab. 2) There would be no presumption that alleles were present but not visualized at any locus where alleles were visualized. 3) Loci where no alleles were visualized would be ignored in seeking matches. 4) If the entire allelic profile of a blood meal were to match a person and also a pair of people, the meal would be presumed to contain the blood of one person rather than two. 5) If there were multiple matches, the mosquito would be considered to have been more likely to have taken blood from residents of the house in which the mosquito was collected than from neighbors and more likely to have bitten neighbors than someone from outside the study area. *A posteriori*, it was

necessary to modify matching criterion 1 in a few cases. Justification for this and the effects of these criteria on interpretation of the results are discussed later in this report.

The human source of a blood meal was categorized according to where the mosquito was collected and where the person bitten lived, as follows: Resident (R) alleles in the blood meal matched those of a profiled person who lived in the house where the mosquito was collected; a neighbor (N) was a profiled person who lived in the study area, but not in the house where the mosquito was collected; a visitor (V) was a person who was profiled and lived outside of the study area; and unidentified (U) indicated no match. An unidentified source could be someone from outside the study area or a neighborhood resident who was not profiled. Another potential explanation for unidentified sources was failure to match a profiled person because alleles in the blood meal were not visualized. Adherence to matching criterion 2 required categorization of such meal sources as U.

After identifying meal sources, feeding activity was tested for randomness. If feeding was random, the number of meals per potential human host would be expected to follow a Poisson distribution and the meals would be distributed between the sexes and among age categories in proportion to the actual ages and sexes of potential human hosts. We used chi-square and t tests to determine whether differences between expected and observed distributions were significant.

RESULTS

Human population. Complete profiles (amplification and visualization of alleles at all four loci) were obtained for 70 of the 82 sampled residents, 15 of 19 members of the field crew, and 2 of 5 visitors. Partial profiles (identification of alleles at 1–3 of the 4 loci) were obtained for all but eight of the others. Two of the eight people for whom we obtained no genetic information declined to provide a cheek swab specimen, and we failed to visualize any alleles of the other six.

Allelic frequencies approximated those reported for Hispanics (Federal Bureau of Investigation, 1994. *PCR-Based Typing Protocols*. Quantico, VA: Federal Bureau of Investigation Laboratory and Technical Manual, 1998. *GenePrint STR Systems (Silver Stain Detection)*. Madison, WI: Promega Corp.) (Table 1). There were 17 different alleles detected at the D1S80 locus with 80% (78 of 98) heterozygosity. For the STR triplex, there were seven, six, and six alleles detected with 76% (66 of 87), 65% (58 of 89), and 80% (71 of 89) heterozygosity at the CSF1PO, TPOX, and THO1 loci, respectively.

Allelic profiles of individuals and of pairs of residents and visitors were compared to determine whether profiles were unique. Among the 87 completely profiled people, two had identical profiles, a 21-year-old woman who lived in a house near the middle of the study area and an unrelated 71-year-old man in a house at the northwest corner of the study site. Among pairs of the completely profiled people, the combined alleles of 2,801 of the 3,741 possible pairs (75%) differed from those of all other pairs or individuals. The combined alleles of 18 pairs (< 1%) were identical to those of a completely profiled individual, with 17 of those also identical to one or more pairs of people. The combined alleles of the remaining 922 pairs (25%) matched those of one or more other pairs. There

TABLE 1

Allelic frequencies of VNTR locus D1S80 and STR loci CSF1PO, TPOX, and THO1 among completely and partially profiled residents and visitors of Yanes III, Florida, Puerto Rico compared with published data for Hispanics

Locus	Allele	n	Allelic frequency		Locus	Allele	n	Allelic frequency	
			Puerto Rico	Hispanics*				Puerto Rico	Hispanics†
D1S80	15	0	0.000	0.000	CSF1PO	6	0	0.000	0.000
	16	0	0.000	0.004		7	1	0.006	0.002
	17	1	0.005	0.012		8	2	0.011	0.005
	18	60	0.306	0.225		9	1	0.006	0.025
	19	1	0.005	0.004		10	49	0.282	0.241
	20	6	0.031	0.010		11	42	0.241	0.296
	21	5	0.026	0.030		12	74	0.425	0.358
	22	7	0.036	0.028		13	5	0.029	0.060
	23	1	0.005	0.014		14	0	0.000	0.007
	24	60	0.306	0.316		15	0	0.000	0.007
	25	3	0.015	0.059					
	26	0	0.000	0.080	TPOX	6	1	0.006	0.005
	27	2	0.010	0.012		7	0	0.000	0.002
	28	3	0.015	0.081		8	92	0.517	0.502
	29	6	0.031	0.079		9	18	0.101	0.089
	30	8	0.041	0.018		10	15	0.084	0.052
	31	16	0.082	0.051		11	39	0.219	0.248
	32	3	0.015	0.006		12	13	0.073	0.102
	33	0	0.000	0.004		13	0	0.000	0.000
	34	4	0.020	0.008					
	35	0	0.000	0.000	THO1	5	0	0.000	0.000
	36	0	0.000	0.014		6	38	0.213	0.239
	37	0	0.000	0.006		7	56	0.315	0.309
	38	0	0.000	0.000		8	16	0.090	0.085
	39	0	0.000	0.002		9	32	0.180	0.139
	40	0	0.000	0.000		9.3	35	0.197	0.218
	41	0	0.000	0.000		10	1	0.006	0.009
	>41	1	0.005	0.008		11	0	0.000	0.000

* Federal Bureau of Investigation, 1994. *PCR-Based Typing Protocols.* Quantico, VA: Federal Bureau of Investigation Laboratory, 10–15.
† Technical Manual, 1998. *GenePrint STR Systems* (*Silver Stain Detection*). Madison, WI: Promega Corp., 49.

TABLE 2

Profiling success of human DNA from blood meals in wild *Aedes aegypti* categorized by color of the squashed blood meal and human DNA content

	<3 ng of DNA			≥3 ng of DNA		
	Red	Red+	Other	Red	Red+	Other
January						
Complete	2	0	1	14	21	0
Partial	0	0	0	13	18	2
Failed	33	16	38	24	26	6
Not attempted	52	42	54	1	1	0
July						
Complete	0	0	0	71	53	11
Partial	0	1	0	4	5	2
Failed	0	2	1	4	2	0
Not attempted	31	52	61	7	1	0

* Red = red squash; Red+ = red with another color; Other = no red in squash. Complete profiling was visualization of alleles at D1S80, CSF1PO, TPOX, and THO1 loci. Partial profiling was visualization at 1–3 loci.

were no unique profiles of any of the possible meals from ≥ 3 different people.

Because of differences in the certainty of meal source identities, blood meal analyses of completely and partially profiled meals are presented separately. Because the laboratory-reared mosquitoes were allowed to feed to repletion on specific members of the field crew immediately before release, data on meals of recaptured mosquitoes are excluded from the results, except when relevant to the goals of the study.

Blood meal analyses. Alleles at all four loci were visualized for 13% (56 of 417) and 44% (137 of 308) for the January and July 1996 blood meals, respectively (data from laboratory-reared marked-released and recaptured mosquito meals and Table 2). When we excluded meals that were not subjected to the PCR, 62% (190 of 307) of the meals with ≥ 3 ng of human DNA were completely typed compared with only 3% (3 of 95) of those with < 3 ng (Table 2). Squashes of 93% (180 of 193) of the completely typed blood meals were categorized as red or red with another color (Table 2).

Evidence of two different sources of human blood in a single mosquito (i.e., three or four visualized alleles at one or more loci) was detected in seven (18%) of 38 mosquitoes collected during January and in 24 (18%) of 135 mosquitoes collected during July (Table 3). No mosquito had > 4 alleles at any locus in their blood meal.

For matching meals to specific human sources, D1S80 gels having a band in the region of alleles 20–22 required deviation from matching criterion 1. As previously reported,[7] some blood meals, but no oral swabs, have a D1S80 band within this region that apparently is an artifact. Since D1S80 alleles 20, 21, and 22 were found in DNA from the Puerto Rican cheek swabs (Table 1), it was necessary to consider that bands in this region from blood meals could either be an artifact or real. Two attempts, one including and the other excluding the D1S80 allele, were made to match blood from mosquitoes having this band to potential human sources. There were no instances of a meal matching a human source when allele 20, 21, or 22 was included and also matching a source when it was excluded, except for a few partially profiled meals. For completely profiled meals, the band was considered to be an allele when its inclusion resulted in an exact match to a human source and ignored as artifactual in all other cases. For partially profiled meals, matching criterion 5 was used to assign the source of a meal to a particular person and to decide whether the band was likely to be real. For example, if inclusion of the band identified the source as a neighbor or visitor and exclusion identified the source as a resident, the band was considered to be artifactual. Using these criteria, the band was considered to be artifactual in five meals in which it was the only band visualized and in 28 meals in which other bands were visualized. The meals in which no other bands were visualized were categorized as failed amplification (Table 2).

When blood meals could be matched to study participants, the matches were unique except when from two groups. Some completely profiled meals contained DNA matching that of the two individuals (53 and 88) with identical profiles. With three exceptions, individual 88 was selected as the source because the mosquito was collected in her home, the meal also contained her husband's or child's DNA, or the mosquito was collected in another house on the same day as mosquitoes containing DNA matching that of her child or husband. The three exceptions were assigned to individual 88, but the match was considered to be ambiguous (Tables 3 and 4). The other matches with individual 88 were categorized as unambiguous. The second group of non-unique matches occurred when the potential human source(s) and/or the meal were partially profiled. If partial profiles did not contain enough information to determine a unique match, matching criterion 5 was used to assign meals to a particular source and the source was considered to be ambiguous (Tables 3 and 4).

Because of potential differences in foraging behavior, we excluded findings from the laboratory-reared mosquitoes from the results mentioned earlier and those in Tables 2 and 3. Laboratory-reared, marked, and released mosquitoes were allowed to feed to repletion on specific members of the field

TABLE 3

Residency categorization of human sources of completely and partially profiled blood meals in wild *Aedes aegypti* collected from homes in Yanes III, Florida, Puerto Rico*

	Single source meals				Double source meals							
Month profile	R	N	V	U	R/R	R/N	R/V	R/U	N/N	N/V	N/U	V/U
January												
Complete	21	4	0	6	3	0	0	3	0	0	0	1
Partial	25	7	0	0	1	0	0	0	0	0	0	0
July												
Complete	82	14	1	14	6	2	0	13	2	0	1	0
Partial	8	1	0	2	0	0	0	0	0	0	1	0

* R = resident; N = neighbor; V = visitor; U = unidentified.
See Materials and Methods for definitions.

TABLE 4

Age (years), sex, and residence of identified human sources of blood meals of wild *Aedes aegypti**

Human source					January				July			
					At home		Visiting		At home		Visiting	
Swab no.	Age	Sex	House	Total	U	A	U	A	U	A	U	A
			Residents									
1	8	M	393	2	1	–	–	–	1	–	–	–
2	4	M	393	2	–	–	–	–	–	–	1	–
3	32	F	393	1	1	–	–	–	–	–	–	–
4	11	M	393	1	–	–	–	–	1	–	–	–
5	38	M	393	1	–	–	–	–	–	–	1	–
10	1	M	395	3	1	–	1	–	1	–	–	–
12	51	F	396	1	–	–	–	–	1	–	–	–
13	51	M	396	3	–	2	–	1	–	–	–	–
15	44	M	398	2	2	–	–	–	–	–	–	–
16	21	M	398	1	1	–	–	–	–	–	–	–
17	62	F	398	5	–	–	–	–	5	–	–	–
19	23	M	397	1	–	1	–	–	–	–	–	–
20	16	F	397	2	–	–	–	–	2	–	–	–
24	20	F	392	1	–	–	–	–	1	–	–	–
28	28	M	397	1	–	–	–	–	1	–	–	–
32	22	F	391	6	1	4	–	–	1	–	–	–
35	34	F	391	6	–	–	–	–	6	–	–	–
36	26	F	387	1	–	–	–	–	1	–	–	–
37	6	M	387	1	–	–	–	–	–	1	–	–
38	51	M	387	2	2	–	–	–	–	–	–	–
43	33	F	384	3	1	–	–	–	2	–	–	–
47	32	M	384	2	–	–	–	–	2	–	–	–
49	22	M	367	4	–	1	–	–	2	1	–	–
50	54	F	367	3	–	1	–	–	2	–	–	–
56	60	F	381	2	2	–	–	–	–	–	–	–
57	25	M	381	2	1	–	–	–	1	–	–	–
58	7	M	381	6	3	1	–	–	2	–	–	–
59	37	F	380	1	–	1	–	–	–	–	–	–
60	21	M	380	2	1	1	–	–	–	–	–	–
65	19	M	367	2	–	–	–	–	2	–	–	–
69	27	F	367	1	–	–	–	–	1	–	–	–
88	21	F	390	41	6	6	–	1	19	–	7	2
89	4	M	390	4	1	–	–	–	3	–	–	–
90	20	M	390	48	9	–	–	–	30	–	9	–
91	33	F	383	5	–	–	–	–	3	–	2	–
92	10	F	383	1	1	–	–	–	–	–	–	–
93	13	M	383	2	–	–	–	–	2	–	–	–
94	53	M	383	3	1	–	–	–	1	–	1	–
98	16	M	392	2	–	–	–	–	2	–	–	–
99	54	M	379	21	1	–	–	–	20	–	–	–
			Subtotal	198	36	18	2	2	115	2	21	2
			Visitors									
34	8†	M	391v‡	1	–	–	1	–	–	–	–	–
96	37	M	Crew§	1	–	–	–	–	–	–	1	–
			Subtotals	2	0	0	1	0	0	0	1	0
			Total	200	36	18	3	2	115	2	22	2

* Source is categorized as at home if the mosquito was collected at the source's residence. Except in the case of individual #88, identification is categorized as unambiguous (U) when the DNA profile of the meal matched only one person. Ambiguous (A) matches were assigned to a single source based upon matching criterion 5. See Materials and Methods for definitions.

† 8 months.

‡ Visitor to house 391.

§ A member of the mosquito collecting crew.

crew immediately before release, enabling us to determine how long the person fed on could be identified under field conditions. Complete or partial profiles of 27 blood meals were obtained from marked, released, and recaptured mosquitoes, all but seven of which were recaptured on the first sampling date, two days after release. The source of 65% (13 of 20) of the meals in mosquitoes recaptured on the first collection date was a member of our field crew who had allowed mosquitoes to feed upon him just prior to release. The sources of the other seven meals were an individual resident (R, n = 5), two residents (R/R, n = 1), or a visitor (V, n = 1). Except for an ambiguous match of a field crew member to a partially profiled meal in a mosquito recaptured three days after release, none of the seven meals in mosquitoes recaptured > 2 days after release matched anyone in our field crew. Additional evidence that blood meal sources can be identified for no more than two days after feeding came from blood meals of three mosquitoes, one wild and two recaptured, containing DNA matching visitors to the same collection house on January 28, 29, or 30. Two non-resident visitors, who had been orally swabbed while visiting the house on January 28, were sources of the meals. The visitors were not identified as a source of blood for any of the mosquitoes collected after January 30.

Identities and classification of meal sources are summarized in Tables 3–5. Of the 217 completely or partially profiled meals from wild mosquitoes, at least one source was categorized as R (n = 174) in 164 meals (76%), N (n = 34) in 32 meals (15%), V (n = 2) in 2 meals (1%), and U (n = 41) in 41 meals (19%) (Table 3); totals exceed 100% due to two-source meals. When we used our ambiguity criterion for individual 88 and counted all U as ambiguous sources, 85% (147 of 173) of the completely profiled and 39% (17 of 44) of the partially profiled blood sources were considered unambiguous. Only 5% (2 of 44) of the partially profiled meals appeared to be from more than one human source, compared with 18% (31 of 173) of the completely profiled meals. For single-source meals, classification frequencies of partially profiled meals (74% R, 15% N, 0.0% V, 10% U) approximated that of completely profiled meals (71% R, 15% N, 1% V, 14% U).

We could identify a specific individual as the source of 200 (80%) of the 250 completely and partially profiled blood meals from wild mosquitoes (Table 4). In addition to 41 unknown sources, nine sources of partially profiled meals that could not be limited to a single person when applying matching criterion 5 are excluded from the table. One hundred seventy-six of the 200 identified meal sources were classified as unambiguous. The ambiguous sources include 21 non-unique matches that were assigned to a source based upon matching criterion 5 and three exact matches to individual 88 from mosquitoes collected outside her home. Two of the 200 identified sources were non-resident visitors.

If feeding were random, it would be expected that the 198 meals taken by *Ae. aegypti* on the 75 profiled and partially profiled Yanes III residents would have a Poisson distribution with $\lambda = 2.64$ (Table 5), and the meals would be taken in proportion to the distribution of the ages and sexes of the

TABLE 5

Observed and expected (if random) frequencies at which blood from a person living in Yanes III, Florida, Puerto Rico was detected in engorged *Aedes aegypti**

Frequency	Number of times a person's blood was detected in mosquitoes						
	0	1	2	3	4	5	≥6
Observed	35	13	12	5	2	2	6
Expected	5	14	19	16	11	6	4

* If random, meals per person would be expected to follow a Poisson distribution. The distribution among sexes and age groups would be in proportion to the ages and sexes of people living in the community. $\chi^2 = 185.4$, degrees of freedom = 6, $P = 2.42 \times 10^{-17}$.

residents (Table 6). Observed frequencies indicate that feeding was not random ($\chi^2 = 185.4$, degrees of freedom [df] = 6, $P = 2.42 \times 10^{-17}$), the meals were not taken in proportion to the age groups of Rodriguez-Figueroa and others[29] ($\chi^2 = 74.9$, df = 3, $P = 3.81 \times 10^{-16}$) of the 75 Yanes III residents, and there was a tendency to feed more frequently on males than females ($P = 0.013$). This non-random pattern was due to three people who accounted for 56% (110 of 198) of the blood meals. These were the two adults living in house 390, individuals 88 (n = 41 meals) and 90 (n = 48 meals), and the sole male resident of house 379 (n = 21 meals) (Table 4). No other person was identified as the source of blood in more than six mosquitoes.

DISCUSSION

Using PCR-based technology to study mosquito blood feeding behavior is a marked improvement over previous methodologies and is best applied to mosquitoes that contain only one or two sources of blood. For mosquito species that are likely to imbibe a single blood meal in each gonotrophic cycle, identification of the meal source using DNA profiling only requires selecting a set of loci for which the probability of two people having the same alleles is very small. When multiple feeding in a single gonotrophic cycle is common, as it is for *Ae. aegypti*,[15] identification of the human meal sources becomes more complicated. When there are as many as three sources in a blood meal, there are always instances when all hosts cannot be specifically identified using DNA profiling (e.g., the presence of a child's blood cannot be detected in a meal containing the blood of both biologic parents). Thus, to study multiple feeding mosquito species using DNA profiling, ideally the mosquito must feed at a frequency that is in synchrony with its rate of blood meal digestion so that there is never DNA from more than two sources in sufficient quantity or of sufficient quality to be amplified by PCR. There must also be a set of markers that produces unique allelic profiles for a great proportion of the individuals and potential pairs of sources. Such was the case with our methodology.

Our analyses of meals from marked, released, and recaptured laboratory-reared mosquitoes that were fed human blood just prior to release indicate a two-day limitation for allele detection under natural field conditions. Because *Ae. aegypti* take 0.6–0.8 blood meals per day,[15] we expected that engorged females of this species would rarely, if ever, take more than two meals in 48 hours. Were *Ae. aegypti* to feed more often or if meals could be detected after more than two days, we would expect some mosquitoes to contain blood from three or more people. There was no evidence in the blood meals we profiled that this happened.

Relatively minor changes in our methodology (i.e., longer lysis buffer immersion times and use of the Phase Lock gel tubes for extraction of DNA) increased complete profiling success from 13% for the January collections to 43% in July. If several January samples that were contaminated with CTT allelic ladder aerosol are excluded, the percentage of January samples that were completely profiled is 17%, approximately the same (20%) as Chow-Shafer and others[7] achieved analyzing *Ae. aegypti* meals from Thailand. The proportion of blood meals completely profiled was likely a function of when mosquitoes were collected during the process of blood meal digestion and template DNA degradation. Consequently, we do not recommend comparison among different fingerprinting methodologies based solely on the proportion of blood meals completely profiled. Because *Ae. aegypti* blood feed and rest in the houses where we captured them,[14,15] it is not surprising that we collected specimens that had digested their blood meals beyond the point where they could be completely profiled. Reports of an inverse relationship between blood meal size and ambiguous PCR results[30] may represent a decreasing ability to amplify alleles in blood meals that are being digested and decreasing in size. Improved DNA preservation and extraction techniques may make it possible to amplify DNA for a longer time post-ingestion. This would be detrimental, by complicating the interpretation of the allelic profile, if multiple feeding from more than two different human sources is commonly detected.

Accuracy in identifying the human sources of *Ae. aegypti* blood meals is greatest when complete genetic fingerprints are known for all of the people living in and visiting the study community. We lacked information on eight residents and some visitors to our study area, and we had only partial profiles of several others. In addition, when we used our choice of markers, two people had identical profiles and the profiles of only 75% of all possible pairs of the Yanes III residents and visitors that we analyzed were unique. Other STR multiplex systems and sex-specific markers are currently available that could be used to help differentiate people with identical profiles. In our study, for example, a sex-specific locus such as amelogenin would have differentiated a man and woman with identical profiles. Substituting a second STR triplex system for D1S80 might similarly improve discriminatory power without requiring more template DNA. These modifications require evaluation because they could increase the frequency of missing data, leading to mismatches or matching failures.

Despite having partial and non-unique profiles for several people, we could identify 80% of the blood meals sources (200 of 250) to a single person. Identification of 69% (172 of 250) of the sources was unambiguous. Consequently, we were able to reconstruct *Ae. aegypti* blood feeding behavior in its natural environment with far greater detail than was possible with previous technology.

Blood from more than one person was detected in 18% (41 of 173) of the completely profiled mosquito meals. We did not detect seasonal variation of multiple feeding frequency between January and July. The absence of seasonal variation agrees with estimates derived from histologic examination of *Ae. aegypti* collected in San Juan (34%, n = 1,156),[15] but the percentage of PCR-detected multiple feeding in Florida, Puerto Rico was lower than the histology-based estimates.

TABLE 6

Distributions of blood meals in wild *Aedes aegypti* from residents of Yanes III, Florida, Puerto Rico categorized by the age groups of Rodriguez-Figueroa and sex*

	Age (years)				Sex	
	<10	10–19	20–49	≥50	Male	Female
Observed	18	10	130	40	118	80
Expected	53	34	79	32	106	92

* Numbers of expected meals are proportional to the comparison of the 75 profiled or partially profiled human residents. $\chi^2 = 74.9$, degrees of freedom = 3, $P = 3.81 \times 10^{-16}$. † $P = 0.013$

We eliminated two potential explanations for the difference between the Puerto Rican sites by using only the completely profiled meals. None of the completely profiled meals (but several of the partially profiled meals) matched a single individual and also two people. There remain at least four other explanations for differences between fingerprinting and histology results. First, there may be differences in the blood-feeding behavior of mosquitoes at the two locations. Second, the histologic procedure identifies multiple meals from different people, from the same person, and from non-human hosts, whereas PCR-based detection identifies only multiple meals from different human sources. If rates of multiple feeding were similar in San Juan and Florida, our fingerprinting results indicate that approximately half of the multiple meals were taken from the same person or non-human hosts. Third, digested or very small blood meals that can be detected histologically may contain DNA that is too degraded or of insufficient quantity to be amplified by the PCR. Fourth, because a greater percentage of the engorged females were successfully analyzed using histology than the PCR, the difference in results was a sampling error. Evidence to refute the last of these hypotheses is the seasonally consistent PCR-based estimates for Florida, despite a much larger percentage of completely profiled meals in July (43%) than in January (13%).

Our PCR-based estimate of the frequency that *Ae. aegypti* bites different people during a 48-hour period is best considered a conservative estimate. Some captured mosquitoes might have taken additional meals had they not been collected. There may be individual variation in digestive rates, as was indicated by blood digestion studies using laboratory mosquitoes.[7] Our methodology cannot detect interactions when a mosquito probes a person with its mouthparts without imbibing blood. If the mosquito was infectious, probing could result in pathogen transmission and, therefore, would be epidemiologically important.

Although it may underestimate the frequency of *Ae. aegypti* feeding behavior, 18% of meals taken during a 48-hour period coming from two different human sources demonstrates that multiple feeding is a significant factor in the epidemiology of dengue virus transmission. Because there is a nonlinear relationship between the biting rate of a mosquito vector and the potential for the spread of a vector-borne pathogen, small increases in the rate of human host contact are expected to result in relatively large increase in the number of new human infections.[31] Caveats to this are that heterogeneity in vector-host interactions may influence patterns of pathogen transmission and if increased host contact is the result of interrupted meals, enough virus must be ingested to produce a vector infection.[24] Once an *Ae. aegypti* is infective, complete engorgement, a partial meal, or simply probing without imbibing blood can result in dengue virus transmission.[22]

There was no evidence that at our level of detection *Ae. aegypti* fed from more than two human sources; we never detected > 4 alleles at a locus. During our histologic studies of mosquitoes from San Juan, we rarely detected three meals (2%, n = 1,675) and when we did, the oldest meal always appeared well-digested,[15] perhaps degraded beyond what can be amplified by the PCR.

Geographic variation in the frequency at which *Ae. aegypti* bite different people deserves more thorough study. Differences in feeding frequency could be an underlying component of spatial variation in the force of dengue virus transmission.[15] Considerably more sampling needs to be done in a variety of locations to determine if regional differences are real and epidemiologically significant.

Our data indicate that wild *Ae. aegypti* females did not feed randomly on members of the resident human population (Table 5) nor among age categories or between sexes (Table 6). Although these data suggest that for undefined reasons *Ae. aegypti* prefer young adults and males, an alternative explanation is that this is an opportunistic species, feeding on whomever is most often present in the house in which and when they seek a meal. This notion is supported by our finding that > 70% of blood meals were taken from residents of the collection house (Table 3). Admittedly, the application of matching criterion 5 introduces a bias towards categorization as resident, but blood meal sources remain > 70% resident even if individuals identified using criterion 5 are not considered. This indicates that the appropriate spatial scale at which to study *Ae. aegypti*-human interactions is individual households. In other words, within-house feeding patterns (Table 7) are more likely to be indicative of mosquito-human interactions than are community-wide analyses (Tables 5 and 6).

With the exception of house 390, there were either not enough mosquitoes collected or not enough household residents for meaningful statistical analyses by household, but the trend seems to be that older residents were fed upon more often than younger ones (Table 7). Three of the 70 people completely profiled accounted for 110 the 200 identified blood meal sources (Table 4). The most frequent meal

TABLE 7

Distribution of blood meals taken from residents and visitors of selected houses in Yanes III, Florida, Puerto Rico, January and July 1996*

House number		Residents						Non-Residents		
367	Age/Sex	54/F	27/F	22/M	6/M	5/M	3/M	N	V	U
	Meals (n)	3	1	4	0	2	0	1	1	3
379	Age/Sex	54/M								U
	Meals (n)	21								3
384	Age/Sex	33/F	32/M	14/M	12/F	11/M	10/M	N		U
	Meals (n)	2	2	0	0	†	0	2		1
390	Age/Sex	21/F	20/M	4/M						U
	Meals (n)	31	39	4						12
391	Age/Sex	34/F	15/F	1/F				N		U
	Meals (n)	6	2	0				3		2

* Only houses and their residents from which DNA in at least five *Aedes aegypti* blood meals matched a household resident are listed. Non-residents are listed as Yanes III neighbors (N), as visitors from outside the community (V), or an unidentified (U). Age is in years.
† DNA profile was not obtained for this person.

sources were three of the four residents of the two houses (379 and 390) in which the most mosquitoes were collected. The strongest case for differential feeding is that two of these, an adult male and female in house 390, were identified as sources of 48 and 41 meals, respectively, yet their young son was identified as a source of only four meals (Table 4). Moreover, those two adults were also the source of nine meals in mosquitoes collected in neighbors' homes. No other person was identified in more than two meals of mosquitoes collected outside of their home. The sole resident of house 379, the third most frequent blood meal source (n = 21), was never identified in meals of mosquitoes collected in any neighbor's home. Because we did not track the movements or anti-mosquito behavior of anyone in the study site and we did not investigate the use of mosquito netting, repellents, and other mosquito avoidance practices, we cannot determine whether the observed feeding patterns are indicative of host preference or a combination of variation among human hosts, human defensive behavior, and opportunistic feeding by mosquitoes.

At most, 77 of the 250 meal sources (31%) were visitors to the house where the mosquito was collected. Two non-residents and 34 Yanes III residents were identified as sources of blood meals of mosquitoes collected in another person's home (Tables 3 and 5). An additional 41 other meals came from unidentified sources.

The exact percentage is likely lower. Twenty of the 41 unidentified sources were from mosquitoes collected in homes where one or more residents were not profiled. Nineteen were in two-source meals, where partial profiling increases the possibility of mismatching. Another consideration is that our presence increased the number of non-resident visitors in the community, since one of the two non-resident visitors bitten was a member of our field crew. If 25–50% of the unidentified sources were non-residents and if the effects of flight activity are relatively unimportant, as discussed later in this report, then 74–82% of the meals were taken from household residents and > 90% of the feeding was on residents of the Yanes III community.

Although *Ae. aegypti* has the capacity to fly ≥ 1 km, the general conclusion from a long list of studies is that female *Ae. aegypti* tend not to fly far compared with other mosquito species; typically ≤ 100 meters as reviewed, for example, by Christophers,[32] Kuno,[33,34] and Monath.[35] It has been suggested that among contiguous houses in urban habitats the mean dispersal distance may increase to ~280 meters over a three-day period.[36] Even though this still represents a limited tendency to disperse, results from that study require confirmation. In two mark-recapture experiments carried out during 1996 and 1997 at our Florida study site, ≥ 77% of the recaptured mosquitoes were collected in the house where they were released. The maximum dispersal distances detected were 65 meters[27] and 79 meters.[28] Such short-range dispersal (50–300 m) is not consistent with the spatial and temporal patterns of a dengue epidemic that took place in Florida in 1991–1992.[26] Published information on *Ae. aegypti* dispersal, patterns of human infection, and our finding that engorged mosquitoes took recent blood meals from visitors to the homes in which the mosquitoes were caught support the conclusion that it is the movement of infected people rather than flying, infected, adult mosquitoes that is the primary means of virus movement within and among communities. Our data suggest that between-community visitation can be responsible for spatial patterns of human infection observed in Florida during a dengue epidemic.

Our results support the hypothesis that multiple feeding is an important factor in the transmission of dengue virus by *Ae. aegypti*. We conservatively estimate that a significant portion (18%) of the engorged mosquitoes we collected imbibed blood from two different people during single gonotrophic cycles. They fed on different people at different rates and on household visitors. These patterns of mosquito-human interactions are expected to enhance virus transmission[23–25] and are consistent with results from spatial and temporal analyses of a dengue epidemic in Florida, Puerto Rico in 1991–1992. Morrison and others[26] determined that over periods of ≤ 3 days, dengue cases in the Florida epidemic were clustered within short distances (individual houses). Over longer time intervals (several weeks), cases spread rapidly throughout the municipality. Clustering of cases within a house could be the result of a single or very few infected mosquitoes biting different household members during a short period of time, perhaps within a single gonotrophic cycle. Over longer distances and time spans, the explosive spread of dengue through the community is likely the result of virus being transported in people who were bitten by *Ae. aegypti* when they visited other people's homes. Flight of infected mosquitoes might contribute to virus dispersal over short distances (≤ 100 meters), but based on the knowledge that *Ae. aegypt* does not tend to fly far[27,28,33–35] and the low dengue virus infection rates for this species,[34] we conclude that mosquito flight is less significant than human movement in community-wide spread of dengue.

The epidemiologic significance of heterogeneity in the rates at which different people are bitten has been explored theoretically. Its relevance to natural field conditions is not as well resolved. For example, we established that *Ae. aegypti* bites were not randomly distributed among all members of the Florida study community. Young adults and males were bitten more often than children or females. However, during the 1991–1992 Florida dengue outbreak, a cross-sectional serologic survey revealed that disease rate was unaffected by age or sex.[29] Although not always the case, several other investigators have reported results similar to ours, i.e., that mosquitoes are attracted to or feed on adult hosts (human and non-human) more often than children or immatures.[24,37,38] Interestingly, Dye and Hasibeder[23] suggest that in small communities, like the study area in Yanes III, age may explain most of the variability in vector-host contact. In larger populations, they propose that spatial variation is likely important.

It is generally accepted, however, that when certain hosts are bitten more often than others, the basic reproductive rate (R_o) of a vector-borne pathogen, and thus the spread of disease, increases.[23–25] Prevalence of host infection is expected to increase or decrease depending on the extent of non-random host contacts and the distribution of infected individuals in the host subpopulation(s) that are bitten most often. For example, as transmission accelerates fewer susceptible individuals will remain in the preferred group making it progressively more difficult for infected mosquitoes to locate and bite a susceptible host. The probability that immune hosts will be bitten increases and the incidence rate correspondingly decreases. An application of this argument is that control directed at the individuals who are most likely to be bitten will reduce R_o of the pathogen by reducing the vari-

ance and mean of the biting rate.[24] Failure to prevent infection in the most frequently bitten individuals will result in a less effective disease prevention program.[25] The precision of human host identification using PCR-based methodology provides a tool that can be used to test assumptions of this approach for dengue. Can generalizations be made regarding the people who are likely to be bitten most often? If so, can those individuals be protected from infection (i.e., targeted vaccination, vector control or housing construction) in an economically feasible way?

DNA profiling of *Ae. aegypti* blood meals provides, for the first time, the detail necessary to identify community-wide dengue risk groups based on their exposure to vector mosquito bites and their susceptibility to infection. A sample of engorged *Ae. aegypti* larger than that in this study and information on the immune status of human residents is necessary to define risk groups accurately and then to determine whether cost-effective means of preventing infection in high risk portions of the population can be developed and deployed. Specific, quantitative, and tested targets for vector control are important public health issues because there is no licensed vaccine or clinical cure for dengue. Currently, the only means of dengue control is mosquito vector control.[39]

The high frequency at which *Ae. aegypti* imbibes blood[15] and patterns of feeding on different humans, are important reasons why *Ae. aegypti* is such an efficient vector of viral pathogens and why so few adult females are sufficient to maintain dengue virus transmission.[34,40] Because of frequent human-host contact by this species, we expect that entomologic thresholds for sustaining dengue virus transmission will be low,[15] and that dengue control based on reduction of *Ae. aegypti* population densities will need to be thorough to reduce virus transmission and to eliminate or minimize disease.

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