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Human DNA profiles from mosquito blood meal for forensic application in tropical country

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ABSTRACT: In Thailand, mosquitoes are prevalent in every location and season. Thus, mosquitoes can be used as evidence in forensic investigations. A number of crime scenes can be solved by using STR markers to amplify DNA extracted from the mosquito abdomen. Nevertheless, there are several restrictions to their usage that may be of concern. One of them is that DNA testing more than two days after mosquitoes have eaten blood becomes problematic. Additionally, mosquito species may be implicated in blood degradation. The aim of the current study was to identify human DNA profiles from human blood obtained by three mosquito species, namely *Culex quinquefasciatus*, *Aedes aegypti*, and *Aedes albopictus*, at various post-feeding hours. DNA from unfed and fed mosquitoes at time points was extracted. DNA quality and quantity were determined using multiplex PCR and qPCR techniques with three sets of *CADM1* primers. Human DNA profiles were identified by STR genotyping for 16 STR loci. Three species of mosquitoes with high-quality and quantity DNA were detected from 0–24 h post-feeding and decreased at 36 h. Complete DNA profiling was able to be achieved at 0–36 h post-feeding. Human DNA profiles could not be detected at 48–60 h post-feeding. Of note, the present study demonstrated human DNA profiles from *C. quiquefasciatus* blood meal mixture between two individuals at 0–36 h post-feeding. The findings of the present study may prove to be useful for the identification of human DNA profiles from mosquito blood meal for forensic application.

KEYWORDS: human DNA, mosquito gut, post-feeding time, forensic application, tropical countries, Thailand

INTRODUCTION

As a tropical country, Thailand is infested with mosquitoes throughout the year and in every region. The mosquito is an invertebrate, which is an essential vector for parasitic diseases [1]. Parasitic diseases result from pathogen ingestion while female mosquitoes bite the hosts and transmit the pathogen to other hosts. Although mosquitoes can be troublesome for humans, they are helpful in forensic work [2, 3]. In response to their biting, the blood of the host was drawn into their intestines. Consequently, the mosquito intestines will contain a reservoir of the host DNA. Because there are mosquitoes carrying the host DNA everywhere, the host DNA could be crucial to solving a crime in tropical countries including Thailand. A number of crime scenes can be solved by mosquitoes [4–7]. An example, of this, involves a murder case that occurred on a beach in Sicily. The suspect was a businessman whose car was noted in the area of the crime scene. At the crime scene, there was no evidence available which could lead to the arrest of the culprit. The police raided the suspect's room. There was also no evidence apart from a mosquito blood meal stain on a wall. Finally, the police solved the case from this blood by using 15 STR loci for DNA profiling [4]. Another example of such a case occurred in 2008, involving a case of car theft in Finland. The police trapped the suspect with the mosquito found in the stolen car. The mosquito was sent to the laboratory for human DNA testing [7].

DNA profiling can identify a suspect who is in the police register. Although mosquitoes can provide evidence for unraveling a crime, there are some limitations to their use, which may be of some concern. Female mosquitoes bite the host, and the host blood is digested within 1–2 days [8, 9]. Blood digestion results in the degradation of the host DNA. Therefore, there is a limited time period in which DNA profiling can be performed. The study by Hiroshige et al demonstrated the estimation of the post-feeding time from a mosquito blood meal. Human blood from 2 species of mosquitoes, *C. pipiens pallens* and *A. albopictus* (AAL), were identified using 16 STR markers. The results revealed that the majority of alleles were detected in *C. pipiens pallens* blood from 0 to 24 h post-feeding, while at 0 to 18 h post-feeding in AAL blood. The number of alleles gradually decreased, and no alleles were detected at 72 h post-feeding in both *C. pipiens*

pallens and AAL blood [10]. Hence, DNA profiling derived from mosquito blood meal cannot only be used to identify criminals but may also indicate the time the incident occurred. However, various mosquito species have different post-feeding times for complete DNA profiling.

This investigation focused on 3 mosquito species that are primarily prevalent in Thailand [11, 12]: *C. quinguefasciatus* (CQ), *A. aegypti* (AG), and *A. albopictus* (AA) to investigate the post-feeding times for complete DNA profiling. In addition, multiple human DNA profiles from CQ mosquito blood meal were also detected [3]. Furthermore, investigations involving qualification, quantification, and STR genotyping were conducted utilizing a non-commercial reagent. In general, commercial kits are utilized in forensic work such as A StepOnePlusTM Real-Time PCR System (Life Technologies, Massachusetts, USA) for quantification or the mpFlSTR1 Identifiler1 Plus PCR Amplification Kit (IDPlus) (Life Technologies) for STR genotyping. Here, we attempted to design our own primers for quantification, qualification, and set of STR primers. Due to the high cost of the commercial reagents, if we are able to design our own primer sets, it will be advantageous for general laboratories to use in forensics. Taken together, the findings of the present study may prove to be useful for the police and even general laboratories to identify offenders in both crimes and non-violent cases.

MATERIALS AND METHODS

Mosquitoes and food source

Three species of female mosquitoes: CQ, AA, and AG were received from the Department of Medical Science, Ministry of Public Health, Bangkok, Thailand. All mosquitoes were mature, non-pathogenic and had never fed on blood. Each species ($n = 100$) was maintained in a separate cage. They were grown at room temperature and fed with 5% sucrose with multivitamin as a food source. Prior to blood feeding for 1 h, the food was removed from the cage.

Blood-feeding experiments

The blood used for feeding all mosquitoes was donated by 4 volunteers: 2 males and 2 females, after obtaining consent forms. The present study was approved by the Ethics Committee of Chulalongkorn University, Bangkok, Thailand (COA no. 029/2563). The artificial feeding method was used in the present study [12]. Briefly, a parafilm was stretched thinly to form the membrane to contain the blood on the underside of the flask, which was placed in the cage. Blood (3 ml) was used to fill the membrane with a controlled temperature in the flask at 37 °C. All mosquitoes were grown at room temperature with 60–90% humidity under a 12-h light/dark cycle. There were 7 mosquito cages consisting of CQ with the 1st male blood, CQ with the 1st female blood, AG with the 1st male blood, AG with the 1st female blood, AA with the 1st male blood, AA with the 1st female blood, and CQ with a 1:1 mixture of the blood of the 2nd male and female. A total of 3 mosquitoes were collected before the blood feeding as a negative control. The experiment commenced by allowing the mosquitoes to suck the blood from the membrane for 30 min. Subsequently, 3 mosquitoes from each cage were randomly collected at 0, 12, 24, 36, 48, 60, and 72 h post-blood feeding. The selected mosquitoes were sacrificed by knocking down at −20 °C.

Stereomicroscopic examination

The structure of the sacrificed mosquitoes at each time point post-feeding was examined using a Stereo microscope by measuring the length of the body and obtaining images to observe the blood in their abdomens compared to a negative control.

Design of STR primer sets

The primers used were obtained from https://strbase.nist.gov/str. All 16 STR were divided into 5 sets according to each PCR product size for fluorescence labeling. Four fluorescence dyes, PET, NED, VIC, and 6-FAM (Integrated DNA Technologies, CA, USA) were used for SRT primer labeling. Each primer in a set was labeled with a different dye. Primer sets included as follows: Set 1 – D3s1358, D7s820, and D19s433; Set 2 – D21s11, D8s1179, and D16s539; Set 3 – CSF1PO, TH01, TPOX, and D13s317; Set 4 – FGA, D2s1338, and D5s818; and Set 5 – vWA, D18s51, and amelogenin. The sequences, PCR product, and fluorescence labeling of each primer are presented in Table S1.

DNA extraction

To collect the DNA from the abdomens of the mosquitoes, DNA was extracted using the QIAamp® DNA Micro kit (Qiagen GmbH, Hilden, Germany). Briefly, the gut of the mosquitoes was first broken up by placing in 100 µM ATL buffer and using the pipette tip. Subsequently, 10 µl proteinase K (USB) was added followed by incubation at 56 °C for 10 min. The supernatant was obtained following centrifugation at 6000 × *g* for 1 min, and 50 µl of 100% ethanol was then added and allowed to be set at room temperature for 3 min.

DNA qualification and quantification

The extracted DNA was amplified using multiplex PCR and quantitative PCR (qPCR) with 3 primer sets for *CADM1*. The PCR product sizes were 121, 229, and 397 bp. The sequences of the primers are presented in Table S2. Multiplex PCR was performed for DNA qualification. The PCR mixture contained 1X buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.3 µM *CADM1* primers, 0.25 U HotStarTaq DNA polymerase (Qiagen GmbH),

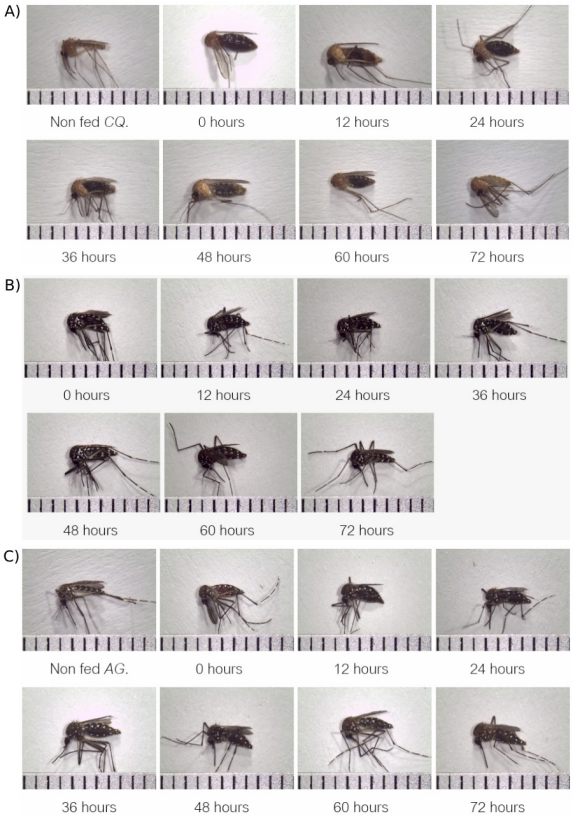

Fig. 1 The structure of 3 mosquito species after feeding at different post-feeding times by stereomicroscope. (A) CQ mosquitoes, (B) AA mosquitoes, and (C) AG mosquitoes.

50 ng/µl DNA (in the case with the concentration < 50 ng/ µl, DNA was used with that concentration). The DNA was initially denatured at 95 °C for 15 min, followed by 35 cycles of 1 min at 95 °C, 1 min at 62 °C and 1 min at 72 °C, with a final extension at 72 °C for 7 min. Three PCR product sizes were separated by 6% acrylamide gel electrophoresis. qPCR was performed for DNA quantification. The PCR mixture contained PowerUp™ SYBR®-Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc., USA), 2 µM *CADM1* and *GAPDH* primers, and 50 ng/µl DNA. The PCR condition was UDG activation at 50 °C for 2 min, Dual-Lock™ DNA polymerase at 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C, 1 min at 60 °C, and 30 s at 72 °C, with a final extension at 72 °C for 10 min. DNA quantification was analyzed by observing the Ct value of each PCR product. Qualification and quantification analyses were performed in triplicate.

RESULTS

Stereomicroscopic examination

All 3 species of female mosquito structures were subjected to a stereomicroscopic examination. The CQ mosquitoes had light brown bodies, while the AA and AG ones had dark brown bodies. Therefore, it was observed that the CQ translucent abdomen turned red after feeding, while this was difficult to see in the AA and AG mosquitoes. Their bellies were blown up and red between 0–12 h after the feeding of blood. After 12 h, the bellies became slightly whiter. The bellies were visible at 48 h (Fig. 1A–C).

DNA quantification

The amount of human blood DNA was quantified using qPCR. The Ct mean values produced by 3 sets of *CADM1* primers (121, 229, and 397 bp) were used for the analysis at each time point. The 1st male DNA obtained from CQ was detectable at 0 to 24 h. The amount of the 1st female DNA obtained from AG was detectable at 0–12 h. The amount of the 1st female DNA obtained from AA was detectable at 0 h. All Ct mean values were shown in Table 1.

DNA qualification

The quality of DNA was examined using multiplex PCR using 3 sets of *CADM1* primers. The quality of DNA was counted by the number of PCR product bands. Male DNA obtained from CQ at the time points of 0–24 h and from AA and AG at the time points of 0–36 h was of good quality. Female DNA obtained from CQ and AG at the time points of 0–24 h and from AA at the time points of 0–12 h was also of good quality. The male and female mixture DNA obtained from CQ at the time points of 0–24 h was of good quality.

STR genotyping DNA from males and females separately

Each DNA sample was amplified by using 16 STR primers. All 16 STR markers were detected in DNA samples from the 1st male obtained by CQ mosquitoes at 0–24 h, from AG and AA mosquitoes at 0–36 h, from the 1st female obtained by AA mosquitoes at 0–12 h, and from CQ and AG mosquitoes at 0–24 h. A total of 8 STR markers obtained from the 1st male DNA from CQ mosquitoes at 36 h were detected. In addition, 3 STR markers from the 1st female and 7 STR markers from the 1st male DNA from CQ mosquitoes at 48 h were identified. Furthermore, 3 STR markers from the 1st female DNA from AG mosquitoes at 48 h were observed (Table 2).

DNA from a mixture of the blood of the 2nd male and female obtained from CQ mosquitoes

DNA samples from the 2nd male and female obtained by CQ mosquitoes at 0–48 h were amplified using 16 STR primers. All 16 STR markers were detected from the 2nd male DNA, whereas 15 STR markers were detected from the 2nd female DNA (vWA marker was not detectable in even the control). STR genotyping obtained from CQ mosquitoes at 0–48 h were shown

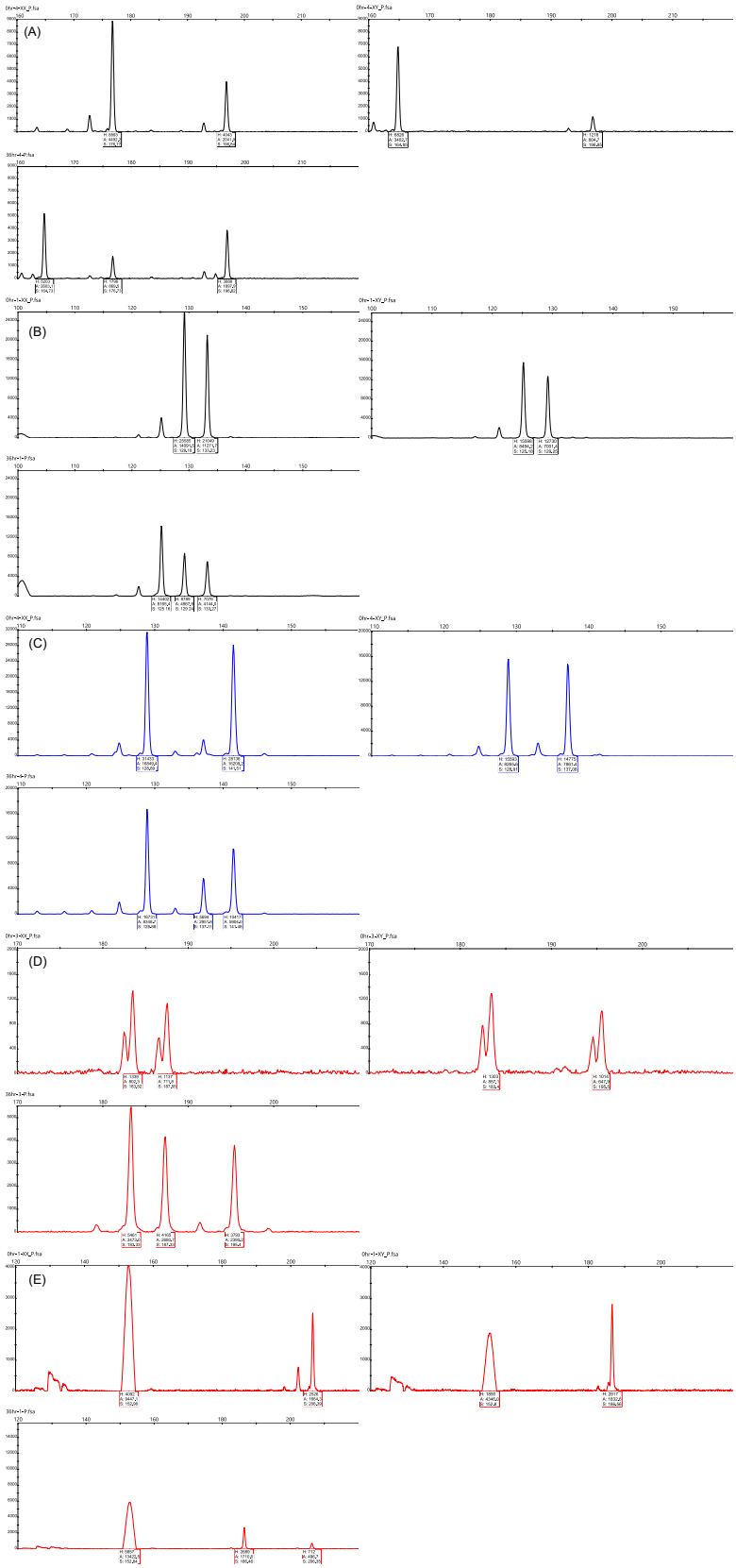

Fig. 2 STR Genotyping at different loci from a mixture between the 2nd male and female blood obtained from CQ mosquitoes. (A) D2S1338, (B) D3S1358, (C) D5S818, (D) D13S317, and (E) D19S433.

Table 1 Ct mean value derived from qPCR using 3 primer sets for *CADM1* in quantification analysis among 3 species of mosquitoes and post-feeding times.

Mosquito species		*C. quinquefasciatus*			*A. eagytis*			*A. albopictus*		
Collected hour	Prod. size	*CADM1* 121 bp	*CADM1* 229 bp	*CADM1* 397 bp	*CADM1* 121 bp	*CADM1* 229 bp	*CADM1* 397 bp	*CADM1* 121 bp	*CADM1* 229 bp	*CADM1* 397 bp
0	Mean	24.513	26.947	26.250	29.405	28.677	28.942	26.006	23.615	25.362
	SD	0.03	0.05	0.41	0.45	0.02	0.10	0.04	0.04	0.03
12	Mean	26.433	28.485	27.559	28.468	27.447	27.732	45.527	43.501	42.359
	SD	0.63	0.11	0.03	0.26	0.12	0.22	3.15	4.58	3.13
24	Mean	26.386	27.398	28.455	–	–	36.357	46.397	49.349	43.092
	SD	0.11	1.70	0.86	–	–	–	–	–	–
36	Mean	–	48.627	–	46.461	48.620	44.147	–	46.954	40.689
	SD	–	–	–	–	1.66	0.68	–	2.02	1.35
48	Mean	–	–	39.675	41.028	–	39.824	44.227	43.304	40.690
	SD	–	–	4.34	6.33	–	–	–	–	–
60	Mean	36.853	–	40.843	–	44.851	39.924	48.679	42.511	41.144
	SD	2.04	–	–	–	0.62	2.10	0.97	5.78	2.52
72	Mean	41.929	46.911	42.095	–	41.399	35.800	44.278	47.296	39.136
	SD	–	–	1.80	–	–	–	2.23	2.53	1.02
Non Fed	Mean	45.325	44.024	47.439	29.730	28.667	28.783	29.281	26.838	27.690
	SD	–	–	3.19	0.45	0.51	0.14	0.08	0.07	0.09
Neg Ctrl	Mean	-	44.288	–	–	43.088	–	–	–	–
	SD	–	–	–	–	–	–	–	–	–
Pos Ctrl	Mean	20.284	22.137	21.679	24.322	22.498	25.789	23.275	20.872	21.933
	SD	0.07	0.25	1.08	0.06	–	0.23	0.08	0.04	0.32

Table 2 STR genotyping of DNA obtained from mosquitoes at different post-feeding times, 3 species of mosquitoes, and distinct individuals.

STR 16 loci		*CQ*	*AG*	*AA*
1st female	0 h	16/16	16/16	16/16
	12 h	16/16	16/16	16/16
	24 h	16/16	15/16	0/16
	36 h	16/16	0/16	0/16
	48 h	3/16	3/16	0/16
	60 h	0/16	0/16	0/16
1st male	0 h	16/16	16/16	16/16
	12 h	16/16	16/16	16/16
	24 h	16/16	16/16	16/16
	36 h	8/16	16/16	16/16
	48 h	7/16	0/16	0/16

in Table 3. Example pictures of the STR genotyping at 36 h were shown in Fig. 2A–E.

DISCUSSION

A mosquito is one of the insects that may be able to provide crucial evidence from a crime scene [14]. However, there are some limitations to the use of mosquitoes that police need to be aware of. The post-feeding time is one of these limitations. If a criminal event occurred several days prior, the police would not be able to capture the villain using blood from mosquitoes. If DNA profiling can be detected, the police can estimate when an individual was present at the crime scene. If complete STR profiling is detected, this indicates that the owner of the DNA should have been present at the crime scene 0–36 h prior. In addition, if incomplete STR profiling is detected, the DNA owner should have been present at the crime scene 36–48 h prior [6, 10]. Another limitation is that the person who owns the DNA profiling may be someone other than the person at the crime scene, because mosquitoes may fly large distances and hence bite a lot of people other than those engaged in the crime. The present study focused on post-feeding hours and species of mosquitoes. It was found that the mosquito species used in the study, comprising CQ, AA, and AG, did not significantly affect the complete STR genotyping, as the post-feeding hours were the key factor. The 36-h post-feeding time point was the maximum time point that a complete STR could be obtained. This finding was supported by the study of Okuda et al in 2002, in which it was found that the maximum activity of serine proteases for CQ was reached at 36 h post-feeding. Moreover, the study by O'gower revealed a 50% rate of digestion for AA at 38 h post-feeding [8].

In addition, the post-feeding hours at which complete STR profiles can be detected varied from person to person in the present study. Herein, complete STR profiles could be detected from the 1st male blood from CQ mosquitoes at 0–24 h post-feeding, while for the 2nd male, complete STR profiles could be

Table 3 STR genotyping of DNA obtained from a mixture between the 2nd male and female blood from CQ mosquitoes at different post-feeding times.

Hour	STR loci	Female DNA allele (bp)	Male DNA allele (bp)	Fed Mosquito allele (bp)	Hour	STR loci	Female DNA allele (bp)	Male DNA allele (bp)	Fed Mosquitoes allele (bp)
0	D3S1358	129, 133	125, 129	125, 129, 133	0	D13S317	183, 187	183, 195	183, 187, 195
12		129, 133	125, 129	–	12		183, 187	183, 195	183, 187, 195
24		129, 133	125, 129	–	24		183, 187	183, 195	183, 187, 195
36		129, 133	125, 129	125, 129, 133	36		183, 187	183, 195	183, 187, 195
48		129, 133	125, 129	129, 133	48		–	–	–
0	D19S433	152, 205	152, 186	152, 186, 205	0	TH01	252	248, 252	248, 252
12		152, 205	152, 186	152, 186, 205	12		252	248, 252	248, 252
24		152, 205	152, 186	152, 186, 205	24		252	248, 252	248, 252
36		152, 205	152, 186	152, 186, 205	36		252	248, 252	248, 252
48		152, 205	152, 186	152	48		–	–	–
0	D7S820	231, 234	231, 234	231, 234	0	FGA	349	337, 341	337, 341, 349
12		231, 234	231, 234	231, 234	12		349	337, 341	337, 341, 349
24		231, 234	231, 234	231, 234	24		349	337, 341	337, 341, 349
36		231, 234	231, 234	231, 234	36		349	337, 341	337, 341, 349
48		234	231, 234	231	48		349	337, 341	349
0	D21S11	224	238, 246	224, 238, 246	0	D5S818	128, 141	128, 137	128, 137, 141
12		224	238, 246	224, 238, 246	12		128, 141	128, 137	128, 137, 141
24		224	238, 246	224, 238, 246	24		128, 141	128, 137	128, 137, 141
36		224	238, 246	224, 238	36		128, 141	128, 137	128, 137, 141
48		–	–	–	48		128, 141	128, 137	–
0	D16S539	289, 292	277, 289	277, 289, 292	0	D2S1338	177, 196	164, 196	164, 177, 196
12		289, 292	277, 289	277, 289, 292	12		177, 196	164, 196	164, 177, 196
24		289, 292	277, 289	277, 289, 292	24		177, 196	164, 196	164, 177, 196
36		289, 292	277, 289	289	36		177, 196	164, 196	164, 177, 196
48		–	–	–	48		176, 196	164	–
0	D8S1179	230, 238	234, 238	230, 234, 238	0	Amelogenin	106	106, 112	106, 112
12		230, 238	234, 238	230, 234, 238	12		106	106, 112	106, 112
24		230, 238	234, 238	230, 234, 238	24		106	106, 112	106, 112
36		230, 238	234, 238	230, 234, 238	36		106	106, 112	106
48		–	–	–	48		106	106, 112	106, 112
0	CSF1PO	315, 319	315	315, 319	0	vWA	–	137, 155	137, 155
12		315, 319	315	315, 319	12		–	137, 155	137, 155
24		315, 319	315	315, 319	24		–	137, 155	137, 155
36		315, 319	315	315, 319	36		–	137, 155	137, 155
48		–	–	–	48		–	137, 155	–
0	TPOX	115, 126	115	115, 126					
12		115, 126	115	115, 126					
24		115, 126	115	115, 126					
36		115, 126	115	115, 126					
48		–	–	–					

detected at 0–36 h post-feeding. Any evidence does not conclusively support the answer to this finding. The probable reason may be that the amount of blood from the 1st male obtained by CQ mosquitoes was low at 36 h post-feeding, whereas the amount of blood from the 2nd male obtained by CQ mosquitoes at 36 h post-feeding was high. The post-feeding hours which were able to yield a complete STR profile of the 1st male and female obtained by AA mosquitoes differed. Complete STR profiles were detected in the DNA sample from the 1st male and female at 36 h and 12 h post-feeding, respectively. No PCR product detection was achievable from 24 and 36 h post-feeding for the female DNA samples for DNA qualification analysis. Therefore, STR genotyping was not performed from 24 and 36 h post-feeding for the female DNA samples. Furthermore, this study demonstrated that STR markers can distinguish DNA from a mixture of the male and female blood obtained from CQ mosquitoes at 0–36 h post-feeding. Our result was similar to the study of Ahmed et al which revealed that human DNA can be obtained from mosquito blood meals, even if it is mixed with non-human blood, for up to 36 h post-feeding [15]. However, in both our and Ahmed's investigations, 2 human or human/mouse blood samples were mixed in a 1:1 ratio, allowing the complete DNA profile to be detected after 36 h. In reality, mosquitoes may not consume each person's blood in the same proportion or at the

same time, resulting in the incomplete DNA profiles of some individuals. This is one of the limitations of using mosquitoes to investigate crimes. A point of interest in the present study was that all PCR reactions were not amplified using commercial kits. All primers for DNA qualification and quantification were designed by the author [16, 17]. *CADM1* was used for human DNA quality and quantity measurement as it was specific for humans. Each STR primer set was grouped manually using the information from the STR database. Of note, the PCR-positive results were shown from the primers designed by the authors. However, *CADM1* primers were designed at its promoter, as it is quite difficult for amplification. For DNA qualification using multiplex PCR for the 1st male and female DNA, HotStarTaq DNA polymerase was used for the PCR reaction. For the mixture of DNA from the 2nd male and female, Taq polymerase (Thermo Fisher Scientific, Inc.) was used instead of HotStarTaq. There was no PCR product amplified by *CADM1*. It is thus recommended that HotStarTaq be used for this PCR reaction, along with primer sets developed for *CADM1*. As a result, avoiding a promoter region should be enhanced when creating *CADM1* primers for further studies. Despite the fact that the *CADM1* primer in this study was difficult to amplify, it can create the product and aid in the selection of DNA for STR genotyping. Hence, the present study may be helpful for researchers in other laboratories that cannot afford commercial kits in order to provide proficient forensic work without investing in large costs.

In conclusion, mosquitoes are applicable for use to obtain critical evidence for forensic work with certain limitations. Apart from mosquito blood, authorities also need to have other evidence which will lead to the identification of the perpetrator.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2024.090. The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

Table S1 STR primer sets for genotyping.

STR marker (size)	labelled	Primer sequence (5′ → 3′)	
		Forward	Reverse
Set 1 PCR			
D3S1358 (99–147 bp)	NED	5′-ACT GCA GTC CAA TCT GGG T-3′	5′-ATG AAA TCA ACA GAG GCT TG-3′
D19S433 (199–221bp)	PET	5′-CCT GGG CAA CAG AAT AAG AT-3′	5′-TAG GTT TTT AAG GAA CAG GTG G-3′
D7S820 (211–251 bp)	6-FAM	5′-ATG TTG GTC AGG CTG ACT ATG-3′	5′-GAT TCC ACA TTT ATC CTC ATT GAC-3′
Set 2 PCR			
D21S11 (154–272 bp)	VIC	5′-ATA TGT GAG TCA ATT CCC CAA G-3′	5′-TGT ATT AGT CAA TGT TCT CCA G-3′
D8S1179 (203–255 bp)	6-FAM	5′-ATT GCA ACT TAT ATG TAT TTT TGT ATT TCA TG-3′	5′-ACC AAA TTG TGT TCA TGA GTA TAG TTT C-3′
D16S539 (260–308 bp)	NED	5′-GGG GGT CTA AGA GCT TGT AAA AAG-3′	5′-GTT TGT GTG TGC ATC TGT AAG CAT GTA TC-3′
Set 3 PCR			
TPOX (105–112 bp)	PET	5′-CAC TAG CAC CCA GAA CCG TC-3′	5′-CCT TGT CAG CGT TTA TTT GCC-3′
D13S317 (157–205 bp)	PET	5′-ATT ACA GAA GTC TGG GAT GTG GAG GA-3′	5′-GGC AGC CCA AAA AGA CAG A-3′
TH01 (230–274 bp)	VIC	5′-GCT TCC GAG TGC AGG TCA CA-3′	5′-CAG CTG CCC TAG TCA GCA C-3′
CSF1PO (287–331 bp)	VIC	5′-AAC CTG AGT CTG CCA AGG ACT AGC-3′	5′-TTC CAC ACA CCA CTG GCC ATC TTC-3′
Set 4 PCR			
D5S818 (115–163 bp)	6-FAM	5′-GGT GAT TTT CCT CTT TGG TAT CC-3′	5′-AGC CAC AGT TTA CAA CAT TTG TAT CT-3′
D2S1338 (165–205 bp)	NED	5′-CCA GTG GAT TTG GAA ACA GA-3′	5′-ACC TAG CAT GGT ACC TGC AG-3′
FGA (308-464 bp)	PET	5′-GGCTGCAGGGCATAACATTA-3′	5′-ATT CTA TGA CTT TGC GCT TCA GGA-3′
Set 5 PCR			
Amelogenin (105–112 bp)	VIC	5′-CCC TGG GCT CTG TAA AGA ATA GTG-3′	5′-ATC AGA GCT TAA ACT GGG AAG CTG-3′
VWA (122–182 bp)	NED	5′-CCC TAG TGG ATG ATA AGA ATA ATC AGT ATG-3′	5′-GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG-3′
D18S51 (262–349 bp)	PET	5′-ACC AAA TTG TGT TCA TGA GTA TAG TTT C-3′	5′-ATT GCA ACT TAT ATG TAT TTT TGT ATT TCA TG-3′

Table S2 *CADM1* primer for quantification and qualification.

Primer name	Primer sequence (5′ → 3′)
Forward *CADM1* primer	ACT CCG CCT CCA GCG CAT GT
Reverse *CADM1* primer 121 bp product	TCC GCT CGG CAG CAC TAC ACT
Reverse *CADM1* primer 229 bp product	CCC ACA CCT ACC TGT GGG GAT
Reverse *CADM1* primer 397 bp product	GGC TCA CAG ATG CCC TCA GC
Forward *GAPDH1* primer	TGG AAG GAC TCA TGA CCA CAG
Reverse *GAPDH1* primer	TTC AGC TCA GGG ATG ACC TT