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TECHNICAL NOTE

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Isolation and characterization of human DNA from mosquitoes (*Culicidae*)

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Abstract Human DNA was prepared from mosquitoes (*Culicidae*) which were collected in a room shared by four human individuals. Several insects did not contain human blood and DNA preparation from them was not successful. However, high molecular weight human genomic DNA could be isolated from four insects. HLA-DQα and D1S80 analysis showed that the blood from one insect was a mixture from two persons, whereas the others contained blood from single individuals. Human DNA isolated 26 h after ingestion was still suitable for typing. These results showed that DNA isolated from mosquitoes is qualitatively and quantitatively sufficient for DNA typing and could be helpful to identify individuals involved in certain cases of body violence or captivity.

Key words Mosquito · Culicidae · PCR · Human DNA · DNA typing

Introduction

In forensic cases of body injury and sexual assaults the analysis of biological stains is of major importance for clarification of the circumstances of the crime and identification of the offender(s). In this respect, the significance of each stain has to be to considered individually, since DNA can be prepared from such specimens up to several years later. (Hochmeister 1991; Sparkes et al. 1996).

These considerations motivated us to look for a novel type of stain which may be more relevant in relation to the moment of the crime and to investigate whether mosquitoes present at the crime scene during the assault could be useful stain carriers. Human blood cells in the digestive tract of the living insect will be gradually degraded. Therefore, mosquitoes may be helpful for identification of the offender(s), if found at the location by the criminal investigator.

Here we present results which show that sufficient human DNA can be isolated from mosquitoes found in a hotel apartment at dawn or up to 26 h later to identify individuals present it during the previous night(s).

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Materials and methods

Mosquitoes in a room shared by four human individuals were killed at dawn and blood was dried on tissue paper. In another experiment insects were collected at dawn and kept at room temperature in 50 ml Falcon tubes until freezing 26 h later. DNA was prepared from the digestive fluid using standard methods and purified using Chroma spin-100 columns (Clontech). DNA isolated from saliva or blood from the probands was used without further purification. HLA-DQα and D1S80 analyses were performed as described by the manufacturer (Perkin Elmer/Roche) except that the number of D1S80 PCR cycles was increased to 32 to facilitate detection of the amplified products. The efficiency of PCR amplification was estimated by agarose gel electrophoresis of HLA-DQα PCR products in comparison to PCR assays with known amounts of genomic DNA from human blood. Agarose and GeneAmp Detection gels (Perkin Elmer/Roche) were stained by ethidium bromide and photographed under UV-light.

Results

We collected mosquitoes in a hotel room shared by four persons, isolated human DNA from the digestive fluid and compared it with DNA from saliva samples from the four probands. Several insects did not contain human DNA but from four of them variable amounts of human DNA could be isolated. The efficiency of PCR amplification was in correspondence to that using a DNA preparation containing 0.5 μg human genomic DNA (data not shown). This is in line with the observation that the area of digestive fluid from the insects on tissue paper is comparable to that of 2–10 μl of fresh blood. HLA-DQα

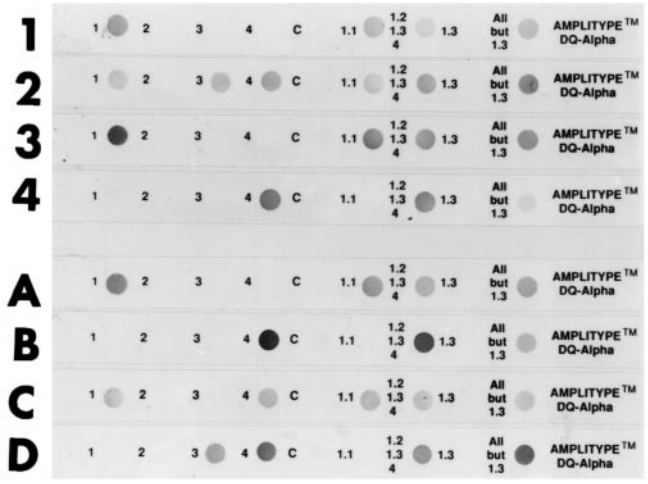

Fig. 1 HLA-DQα analysis of human DNA prepared from four mosquitoes killed at dawn (strips 1–4) compared to DNA isolated from saliva of four probands sharing the hotel room (strips A–D) in the preceding night

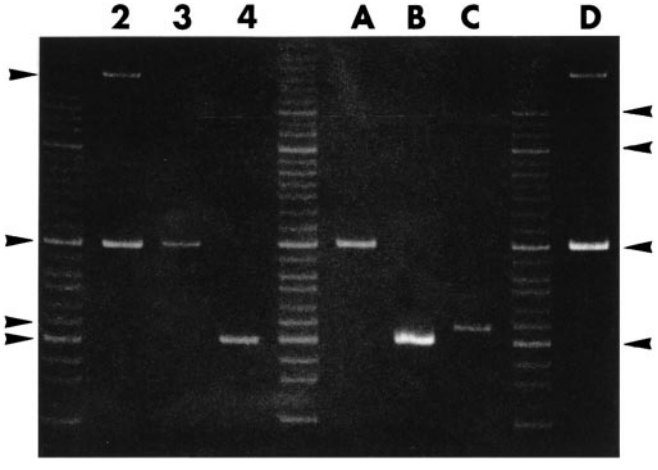

Fig. 2 D1S80 analysis of human DNA isolated from three mosquitoes (#2, #3 and #4) killed at dawn (lane 2, 3 and 4, respectively) compared to DNA isolated from saliva of four individuals sharing the hotel room (lanes A–D) in the preceding night. Of the 27 alleles present in the D1S80 allelic ladder (T18, T24, T31 and T34, from bottom to top) 4 are indicated by arrowheads at the right. D1S80 alleles (T18, T19, T24 and T37, from bottom to top) from insects and probands are indicated by arrowheads at the left

analysis of DNA isolated from the four insects (#1–#4) and from saliva samples of the four participants (A–D) is shown in Fig. 1. Insects #1 and #3 apparently fed on person (A), insect #4 on person (B), whereas insect #2 contained blood from persons (A) and (D), or from persons (C) and (D). The D1S80 analysis showed that insect #2 ingested blood from person (A) and (D) (Fig. 2). It showed the alleles T24 and T37 also present in DNA from persons A and D (Fig. 2, lane 2) but not allele T19 from person C. The results of these analyses are summarized in Table 1.

In order to investigate how rapidly human blood is digested in the living insect we collected six mosquitoes from a probands bedroom, killed two of them at dawn and kept the others in a 50 ml Falcon tube for 26 h at room temperature. This preliminary experiment showed that even after a 26 h survival period human DNA was still present in the insect in quantitatively and qualitatively suitable amounts for successful amplification of D1S80 products (data not shown). More exact determination of the rate of degradation of DNA in the insect will be necessary.

Table 1 Summary of HLA-DQα and D1S80 analyses from Figs. 1 and 2. The asterisk indicates that allele (1.2) cannot be resolved in the actual allele combination of this mixed specimen

Insect/Person	HLA-DQα	D1S80	Blood from
Insect #1	1.1 / 1.2	n.d.	A
Insect #2	1.1 / 1.2* / 3 / 4	T24 / T37	A + D
Insect #3	1.1 / 1.2	T24 / T24	A
Insect #4	4 / 4	T18 / T18	B
Person A	1.1 / 1.2	T24 / T24	–
Person B	4 / 4	T18 / T18	–
Person C	1.1 / 4	T19 / T19	–
Person D	3 / 4	T24 / T37	–

n.d. not done

Discussion

The HLA-DQα and D1S80 systems are reliable PCR systems in the analysis of forensic stains in cases of body violence or sexual assaults (Saiki et al. 1989; Kasai et al. 1990).

When stains on the clothing of a victim or offender or found at the crime scene are kept under appropriate conditions, DNA can be prepared from them even after several years. The question whether stain material is a result of the criminal event itself or not, must be considered carefully for each individual specimen (Hochmeister 1991). In this respect the age of a stain is crucial to estimate its significance. Methods to determine the age of bloodstains have been described (Kind et al. 1972; Andrasko 1997).

We have used an alternative strategy. In a search for novel stain carriers which may have been deposited at the scene of the crime during the offence, we showed that the widely distributed mosquito (*Culicidae*) is a suitable organism for the isolation of human blood. Mosquitoes of the family *Culicidae* have a world-wide distribution and are absent only in Antarctica. They transmit illnesses such as malaria, yellow fever and several viruses (Boreham 1975; Service 1980). Only female insects have fully developed piercing mouthparts and can take blood meals needed for development and maturation of the eggs. The speed of digestion of the blood takes 2–3 days in tropical species and longer in a more moderate climate. Only after oviposition is a new blood meal taken (Service 1980). After the blood meal the insects rest on walls, ceiling or curtains and can easily be captured for subsequent DNA

preparation. Using hybridization to radioactive probes human DNA was detectable in mosquitoes 20 h after ingestion (Coulson et al. 1990) and using non-radioactive dot blot hybridization up to 100 h after ingestion (Sato et al. 1993). Our preliminary result that human DNA is suitable for typing also after 26 h after a blood meal is in line with these observations. Also human crab louse excreta have been reported as source of human DNA. Several insects were placed on the skin of human volunteers and from the collected excrements up to 10 ng of human DNA could be isolated (Replogle et al. 1994). Alu-PCR with DNA from single mosquitoes has also been described (Coulson et al. 1990) but this method was not yet suitable for highly discriminative DNA typing. In this report we showed that from single mosquitoes sufficient DNA can easily be isolated for this purpose using modern PCR-techniques.

Several criminal cases of prolonged confinement, sexual torture and body violence, where offender and victim shared a room over longer periods of time, have been reported in our district, as well as several cases of robbery by groups of persons who spent several days together in a hotel appartment. When the doors and windows are closed and mosquitoes cannot easily enter or leave the room and when the room is investigated shortly after the person(s) of interest has/have left, human DNA from mosquitoes may be an interesting item of evidence to identify the culprits in such cases, in addition to the other stain material or when other stain material is not available.

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