Extent of digestion affects the success of amplifying human DNA from blood meals of *Anopheles gambiae* (Diptera: Culicidae)

W.R. Mukabana[1,2,5]*, W. Takken[2], P. Seda[1], G.F. Killeen[1,3], W.A. Hawley[4] and B.G.J. Knols[1]

[1]International Centre of Insect Physiology and Ecology (ICIPE), PO Box 30772, Nairobi, Kenya: [2]Laboratory of Entomology, Wageningen University and Research Center, PO Box 8031, 6700 EH Wageningen, The Netherlands: [3]Department of Tropical Medicine, Tulane University Health Sciences Center, New Orleans, LA 70112, USA: [4]Division of Parasitic Diseases, Centers for Disease Control and Prevention (CDC), MF22, GA 30333, USA: [5]Department of Zoology, University of Nairobi, PO Box 30197, Nairobi, Kenya

Abstract

The success of distinguishing blood meal sources of *Anopheles gambiae* Giles through deoxyribonucleic acid (DNA) profiling was investigated by polymerase chain reaction (PCR) amplification at the TC-11 and VWA human short tandem repeats (STR) loci. Blood meal size and locus had no significant effect on the success of amplifying human DNA from blood meals digested for 0, 8, 16, 24 and 32 h ($P = 0.85$ and 0.26 respectively). However, logistic regression found a significant negative relationship between time since ingestion and the success probability of obtaining positive PCR products among meals digested for between 8 and 32 h ($P = 0.001$). Approximately 80% of fresh blood meals were successfully profiled. After 8 h, the proportion of blood meals that could be successfully profiled decreased slowly with time after ingestion, dropping to below 50% after approximately 15 h. There was no significant difference in the success of amplifying human DNA from blood meals of mosquitoes killed at time 0 and 8 h after ingestion ($P = 0.272$).

Introduction

Arthropod blood meal sources have traditionally been identified by immunological methods (Tempelis, 1975; Washino & Tempelis, 1983; Pant *et al.*, 1987). These methods mainly detect species-specific immunological targets and so are not suitable for distinguishing between meals obtained from members of the same, or closely related, host species. Immunological analyses based on ABO blood grouping and identification of serum haptoglobins in mosquito blood meals can, however, differentiate human individuals upon whom mosquitoes have fed (Boreham & Lenahan, 1976; Bryan & Smalley, 1978). Blood markers are less polymorphic and may lead to false identification especially when used to characterize blood meals of indoor- or outdoor-fed mosquitoes collected outside or inside houses respectively, such mosquitoes may have fed on hosts outside the target population whose phenotypes are usually identified for comparative purposes. The ability to characterize insect blood meal sources can be improved tremendously using molecular genetic methods such as hybridization with sequence-specific probes (Sato *et al.*, 1992) and DNA fingerprinting or profiling (Coulson *et al.*, 1990; Gokool *et*

*Fax: +254 385 22190
E-mail: rmukabana@mbita.mimcom.net

al., 1993; Kreiker & Kampfer, 1999; Chow-Shaffer *et al*., 2000).

DNA fingerprinting and/or profiling can generate species- or individual-distinctive DNA patterns, e.g. through detection of length variations in segments of the genome containing repetitive DNA sequences. The polymorphic fragments may be detected with (Jeffreys *et al*., 1988) or without (Jeffreys *et al*., 1985) amplification by the polymerase chain reaction (PCR). These methods have been used successfully to characterize individual host DNA present in blood meals of *Anopheles gambiae* Giles *sensu lato* (Diptera: Culicidae) (Coulson *et al*., 1990) and *Glossina* species (Diptera: Glossinidae) (Torr *et al*., 2001) and blood meals plus excreta of the human crab louse *Pthirus pubis* (Linnaeus) (Phthiraptera: Phthiridae) (Replogle *et al*., 1994). The capacity of these methods to identify blood meal sources of wild-collected mosquitoes has also been demonstrated (Gokool *et al*., 1993; Koella *et al*., 1998; Chow-Shaffer *et al*., 2000), albeit with varying levels of success.

The ability to detect mosquito blood meal sources using DNA-based markers is affected by a variety of factors. Amount of DNA extracted from blood meals, conditions under which dead engorged females are stored and lengths of digestion time are important (Coulson *et al*., 1990; Chow-Shaffer *et al*., 2000). Although the efficacy of various storage conditions for preserving mosquitoes killed after different digestion time lengths has been studied (Coulson *et al*., 1990; Sato *et al*., 1992; Kreiker & Kampfer, 1999; Chow-Shaffer *et al*., 2000), maximum digestion time lengths at which positive reactions are expected are emphasized (Sato *et al*., 1992; Kreiker & Kampfer, 1999). Knowing how the extent of blood meal digestion affects the probability of obtaining positive amplification reactions could increase the potency of DNA-based methods for distinguishing mosquito blood meal sources in the field.

This study evaluated the effect of time since ingestion on the ability to identify mosquito blood meals through DNA profiling. Two tetrameric short tandem repeats (STR) loci were used as markers to differentiate known human individuals upon which *A. gambiae* sensu stricto mosquitoes were fed. The proportion of profiling tests that were successful was assessed as a function of post-feeding time intervals. The effect of blood meal size on success of profiling human DNA contained in the mosquito blood meals was also investigated.

Materials and methods

Mosquitoes

Experiments were carried out using a stock culture of *A. gambiae* s.s. mosquitoes supplied by Professor M. Coluzzi, Rome, originating from Suakoko, Liberia. The mosquitoes were reared under controlled conditions, $27 \pm 1°C$, $80 \pm 5\%$ relative humidity and 12L:12D photoperiod. Adult mosquitoes were maintained on a 6% glucose solution. Test mosquitoes were starved overnight and supplied only with water on cotton wicks. The mosquitoes had not taken a blood meal prior to the experiments.

The amount of blood taken by a proportion of the mosquitoes was estimated by gravimetric analysis. This method has the disadvantage that it could underestimate blood meal size (Redington & Hockmeyer, 1976) because it does not account for the changes in blood meal size that occur as a result of excretion of water and salts during (pre-

diuresis) and after feeding (diuresis). However, the method is simple and non-lethal and as the amount of fluid excreted is directly related to the amount of blood ingested (Nijhout & Carrow, 1978), the overall increase in weight resulting from blood feeding can be used as an indicator of blood meal size. Further, although weight increase by feeding does not take into account the weight lost as a result of diuresis and pre-diuresis, the genetic information of the blood host is contained in the cellular fraction of the blood meal which is retained in the midgut.

Test mosquitoes were immobilized through cold-treatment (4°C, 15 min) and weighed individually on a Cahn (C-33) microbalance and then kept in glass tubes. Mosquitoes that became active again were fed on human blood by placing the mesh-covered tubes against the forearm of one of three volunteer test persons designated X, Y and Z. Mosquitoes were removed from the arm after voluntary withdrawal of the mouthparts from the skin and feeding had ceased. Fed mosquitoes were reweighed and blood uptake by weight recorded after feeding-associated diuresis had ceased. The effect of meal size on profiling success was estimated after categorizing the blood meals as small or large according to whether they were less or greater than the median (1.48 mg) respectively. This helped to minimize the probable error that could occur as a result of estimating blood meal sizes using the gravimetric method.

A few mosquitoes were offered double meals by removing them mid-way during feeding from the arm of the first person and transferring them gently to that of a second where they were allowed to feed to repletion. Of these mosquitoes, only three were selected including one that was fed on persons X and Y, a second on Y and Z and a third on persons X and Z. It was not our goal to rigorously demonstrate the efficacy of profiling multiple feeds. Forty-four mosquitoes were freeze-killed (−80°C, 5 min) immediately after blood feeding and subdivided into five classes with respect to blood meal size (class interval = 0.5 mg). Other blood-fed mosquitoes were held under the rearing conditions ($27 \pm 1°C$, $80 \pm 5\%$ RH) and freeze-killed in sets of ten at 8, 16, 24 and 32 h after feeding. Blood meals of all experimental mosquitoes were processed following storage at −80°C for two weeks. Control mosquitoes were males (n = 5) as well as cow-fed (n = 5) and un-fed (n = 5) females.

Ethical clearance

Informed consent was obtained from all the three adult human participants. The project was approved by the Kenya National Ethical Review Committee at the Kenya Medical Research Institute.

Preparation of DNA samples

DNA was isolated from human cheek cells, male mosquitoes, human-fed, cow-fed and un-fed female mosquitoes. Isolation was by modification of a phenol extraction procedure in which DNA was precipitated with ethanol (Wetton *et al*., 1987). Extracted DNA pellets were air-dried and dissolved in 50 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA).

Amplification of DNA

DNA extracts were PCR-amplified at the human tyrosine hydrolase (TC-11 or HUMTHO1) (Polymeropoulos *et al*.,

1991) and VWA (HUMVWFA31/A) (Kimpton *et al.,* 1992) genetic loci. Reactions were carried out in 15 µl volumes each containing 3.75 µl of template DNA, 0.36 µl 25 mM MgCl₂, 1.5 µl 10 × PCR buffer, 3.1 units *Taq* DNA polymerase, 1.2 µl each of 10 mM dNTPs and 1 pmole of each primer. Amplifications were done on a Techne Unit Progene thermal cycler. DNA was denatured at 95°C for 20 s and 94°C for 45 s for the VWA and TC-11 loci respectively. For each cycle oligonucleotide primers were annealed to opposite DNA strands at 59°C for 20 s (VWA) or 62°C for 20 s (TC-11), the annealed primers were extended at 72°C for 20 s (both loci). Thermal cycling was carried out for either 30 (VWA) or 27 cycles (TC-11) followed by further extension at 72°C for 5 min (both loci). Some attributes of the short tandem repeats loci investigated are shown in table 1.

Electrophoresis

PCR-amplified products were separated on high-resolution, horizontal-slab, polyacrylamide gels (Budowle *et al.,* 1993). The gels were about 480 µm thick, backed on Gel Bond™ (FMC Corp., Rockland, Maine, USA). A resolution of 2–4 base pairs (bp) can be achieved for fragments between 100 and 500 bp long (Allen *et al.,* 1989), alleles of loci used in this study are in the 154–178 (TC-11) and 126–166 (VWA) bp size range (Urquhart *et al.,* 1995). Amplified DNA was surface-loaded (in 5–8 µl volumes) on pieces of sample applicator tabs (Pharmacia-LKB) and separation carried out at constant temperature (15°C) on an E-C 1001 isothermally controlled apparatus (E-C, St Petersburg, Florida) set at 600 V, 25 mA and 20 W. A discontinuous buffer system using formate-borate (pH 9.0) as leading and trailing ions, respectively, was employed on 7% (TC-11) or 8% T (VWA) gels crosslinked with 5% C (N,N′-methylene-bisacrylamide). Tris-formate (pH 9.0), 0.12 M with respect to the formate ion, was the gel buffer. Tris-borate (pH 9.0) (0.28 M with respect to the borate ion) was contained in paper wicks (1 cm × 13 cm, Gibco Biotechnology Resource Laboratories, Gaithersburg, Maryland, USA) on the anode and cathode ends of the gel and served as the cathode buffer.

Silver staining

Size separated amplification products were visualized by silver staining (Cairns & Murray, 1994). Stained gels were air-dried overnight and attached to transparency film for storage and handling.

Genetic profiling of mosquito blood meals

Amplified fragment length products generated from mosquito blood meals and human cheek cells were matched to differentiate between the human individuals upon whom

mosquitoes fed. Genotypes of the human volunteers were determined using allelic ladders constructed by mixing DNA samples extracted from persons with variable alleles. These helped to distinguish mosquito blood meals obtained from the different human individuals. The approximate molecular weight of the amplification products was determined using a size marker from digested pBR322 DNA.

Statistical analysis

Data were analysed using the Statistical Analysis System (SAS, version 8E) and Statistical Products and Service Solutions (SPSS version 10). Effects of locus and time since ingestion on success of detecting human DNA in the mosquito blood meals were tested by logistic regression using a forward conditional stepwise selection procedure. The influence of blood meal size was analysed in the same way after categorizing into large and small blood meals according to whether they were less or greater than the median.

Results

A total of 84 blood meals from *A. gambiae* were PCR-amplified at the TC-11 and VWA short tandem repeats loci. The blood meals were obtained from one of three human volunteers. Only three mosquitoes of those that were offered double feeds were analysed. Although mosquitoes were given the chance to feed until feeding-associated diuresis had ended, a few did not feed to repletion. As the amount of fluid excreted is directly related to the amount of blood ingested (Nijhout & Carrow, 1978), blood meals were analysed regardless of whether mosquitoes had engorged fully or partially.

Effect of blood meal size on profiling success

The number of blood meals present and successfully amplified were counted at six size classes with respect to blood meal weight (0.01–0.50, 0.51–1.00, 1.01–1.50, 1.51–2.00, 2.01–2.50 and 2.51–3.00 mg). A total of 44 blood meals were evaluated, all from mosquitoes killed immediately after blood feeding. Figure 1 is the relative frequency histogram showing the relationship between blood meal size and amplification success. The smallest and largest blood meals that were successfully amplified at both loci were 0.08 and 2.74 mg, respectively. Logistic regression found that meal size and locus did not significantly affect the success of amplifying human DNA from blood meals digested for 0, 8, 16, 24 and 32 h ($P = 0.85$ and 0.26 respectively). However, time since blood meal ingestion had a significant effect on ability to yield amplification products ($P < 0.001$).

Table 1. Chromosomal location and primer sequences of the two human loci amplified from blood meals of *Anopheles gambiae* sensu stricto.

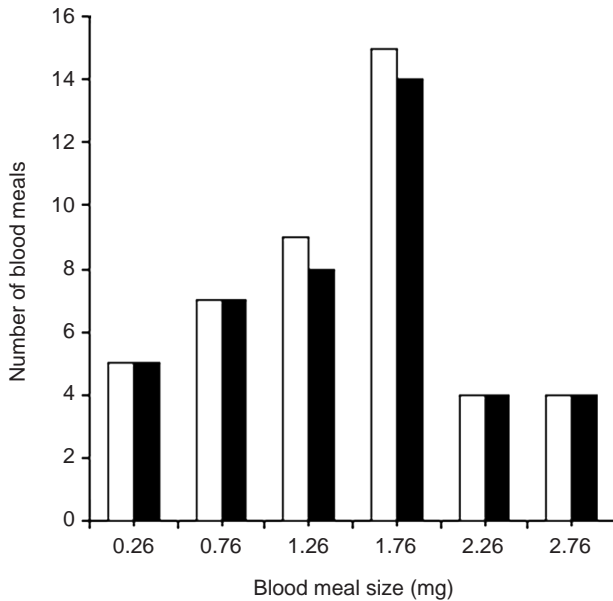| Locus	Chromosomal location	Repeat sequence	Primer sequence (5′–3′)	References
TC – 11	11p 15–15.5	AATG	GTGGGCTGAAAAGCTCCCGATTAT ATTCAAAGGGTATCTGGGCTCTGG	Edwards *et al.,* 1991
VWA	12p 12-pter	TCTA	CCCTAGTGGATGATAAGAATAATC GGACAGATGATAAATACATAGGATGGATGG	Kimpton *et al.,* 1992

Fig. 1. Frequency distribution of *Anopheles gambiae* blood meals present (□) and successfully amplified (■) per size class for mosquitoes killed immediately after ingestion.

Effect of extent of mosquito digestion on profiling success

Analysis by logistic regression of the dependence of profiling success upon time for blood meals between 8 and 32 h old found a significant negative relationship between time since ingestion and the success probability of obtaining PCR products ($P < 0.001$). At least half of the blood meals could be successfully profiled from mosquitoes killed within 15 h of feeding at both TC-11 and VWA human genetic loci. After 8 h the proportion of blood meals which could be successfully profiled decreased slowly with time after ingestion dropping below 50% after approximately 15 h. There was no significant difference in amplification success between meals of mosquitoes killed at 0 and 8 h after ingestion ($P = 0.27$). The number of blood meals that successfully amplified were 39/44 (89%), 9/10 (90%), 4/10 (40%), 1/10 (10%) and 0/10 (0%) (locus TC-11) and 32/44 (73%), 9/10 (90%), 4/10 (40%), 1/10 (10%) and 1/10 (10%) (locus VWA) for meals digested for 0, 8, 16, 24 and 32 h respectively (fig. 2). Although slightly more meals were successfully amplified at locus TC-11 (53/84, i.e. 63%) than VWA (45/84, i.e. 54%) multiple regression analysis did not find a significant relationship between locus and amplification success ($P = 0.35$).

Genetic profiling of mosquito blood meals

Analysis of blood meals of *A. gambiae* originating from the three test persons detected three distinct alleles at the TC-11 locus and five at locus VWA (table 2). Although most blood meals yielded PCR products on first trial some did so on second or third attempts when template DNA was either doubled or tripled. The TC-11 alleles are shown in fig. 3 (see also table 2). Persons X and Y were heterozygous (allelic genotypes 2,3 and 1,2 respectively) whilst person Z was homozygous (genotype 2,2). The homozygous allele of
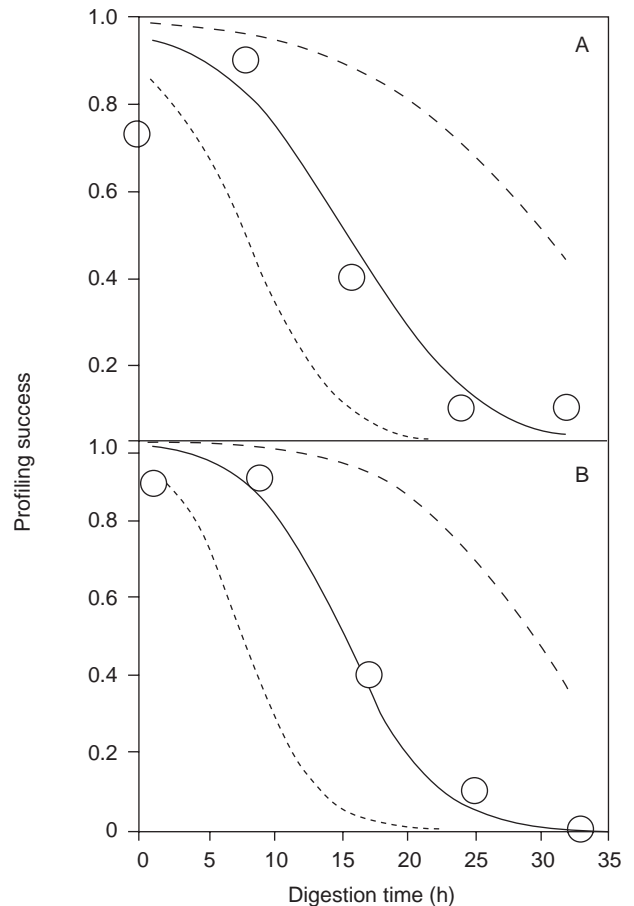
Fig. 2. Effect of extent of digestion on success of amplifying human DNA from blood meals of *Anopheles gambiae*. Panel A represents locus VWA and panel B locus TC-11. Dashed lines represent upper and lower confidence intervals defined by plus or minus one standard error of both β_0 and β_1. Solid lines describe the fitted logistic relationships between profiling success for each locus and time elapsed since feeding (Logit (profiling success) = $\beta_0 + \beta_1$ time). Open circles denote observed profiling successes at 0, 8, 16, 24 and 32 h of digestion.

person Z (allele 2) was also present in persons X and Y. In addition, persons X and Y had fast (allele 3) and slower migrating (allele 1) alleles respectively. Both single and multiple blood meals were successfully profiled (fig. 3). Blank and un-fed female, male and cow-fed mosquitoes (not shown) yielded no PCR product implying that only human, but not mosquito, DNA patterns were detected in the amplifying specimens.

Discussion

This study demonstrated that the extent of digestion but not blood meal size affects the success of amplifying human DNA from blood meals of *Anopheles gambiae* s.s. Since the relationship between blood uptake and mosquito body size was not investigated, it cannot be inferred from these results that the success rate of DNA profiling was associated with mosquito size. The lack of a positive relationship between blood meal size and success of generating PCR products

Table 2. TC-11 and VWA locus alleles detected in blood meals of *Anopheles gambiae* fed on persons X, Y and Z. Alleles are numbered from slowest (allele 1 at locus TC-11 and allele 2 at locus VWA) to the fastest migrating (allele 3 at locus TC-11 and allele 8 at locus VWA).

Person	Alleles detected (see also fig. 3)	
	Locus VWA	Locus TC-11
X	5,7	2,3
Y	5,8	1,2
Z	2,4	2,2

from the mosquito blood meals was not surprising because PCR amplification can be primed from single target molecules (Saiki *et al.*, 1988). Since 0.01 μl of human blood contains approximately 50 nucleated cells (Jeffreys *et al.*, 1988) and one nucleated human blood cell contains approximately 6 pg DNA (Rees & Jones, 1972) the smallest and largest blood meals which successfully amplified were estimated to contain 2 and 82 ng DNA respectively. The quantity of DNA could have even been higher because anopheline mosquitoes concentrate host blood cells, which contain the DNA, during feeding by pre-diuresis (Vaughan *et al.*, 1991; Clements, 1992).

The decline in profiling success over time greatly resembles the time profile observed in the disappearance of blood meal protein from midguts of *Anopheles stephensi* (Liston) (Diptera: Culicidae) (Billingsley & Hecker, 1991) and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) (Briegel & Lea, 1975) which were held at 27°C as done in this study. Billingsley & Hecker (1991) observed a reduction in protein content first at 9 h after feeding, the decline was rapid between 12 and 24 h with the midgut protein content reaching less than 20% by 36 h after feeding. The time profile associated with the degradation of host DNA in the mosquito midgut may thus be similar to that of protein digestion because DNA is contained within cells and packaged with chromatin proteins such as histones. Indeed, the decline in amplification success over time coincides with

the production pattern of late trypsin and the onset of the most rapid phase of blood meal digestion (Briegel & Lea, 1975; Noriega & Wells, 1999).

Although it was possible to profile blood meals digested for up to 32 h after feeding, for maximizing the proportion of meals that can be successfully profiled it is essential to time mosquito collection exercises such that fed mosquitoes are collected within 8 h of feeding. This emphasizes the need for selecting suitable field-sampling methods that would allow quick and effective collection of blood-fed mosquitoes. For example, the use of exit or bed net traps may not only facilitate quick and effective collections but would also allow all-night collections in which trapped blood-fed females are aspirated at selected time intervals and preserved before blood meals become adversely degraded (Service, 1977). Besides, if sampling cannot be done at night it is important to collect (and appropriately store) wild-caught mosquitoes at daybreak so that blood meals of mosquitoes that begin to host-seek and subsequently blood-feed in the early evening hours have a higher chance of being profiled successfully.

Even if blood-fed mosquitoes are collected early enough and stored appropriately the probability of obtaining positive amplification reactions can still be limited by the potential effect of ambient temperature on the kinetics of blood meal digestion. Under natural conditions mosquitoes are often exposed to wide variations in environmental temperature, which can affect their metabolism. For example, it was demonstrated that *A. aegypti* mosquitoes digested their blood meals twice as fast when kept at 32°C as compared to 22°C (Briegel & Lea, 1975). Conversely, when night-time temperatures in the field are substantially cooler or warmer than the 27°C used in this study, the 8 h period of maximal amplification success may be lengthened or reduced respectively. Further studies describing the efficacy of typing blood meals of mosquitoes with different abdominal appearances (e.g. fully fed, half gravid and sub gravid) are necessary in order to know beforehand what likelihood a field collected mosquito has of successful analysis.

Despite the recent rapid advances in molecular genetic technology, the usefulness of molecular biological tools for
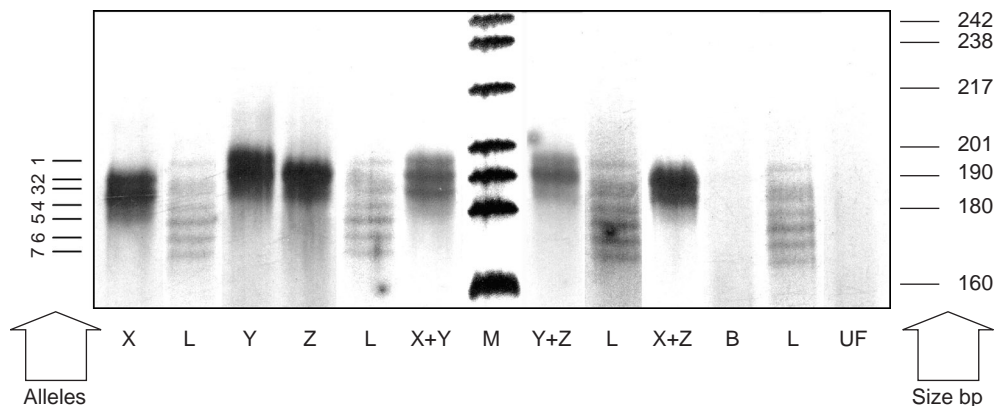

Fig. 3. Human TC-11 locus profiles generated from blood meals of *Anopheles gambiae*. Samples include single (X, Y and Z) and multiple meals (X+Y, Y+Z and X+Z) as well as allelic ladders (L) plus blank (B) and un-fed female (UF) controls. Lengths (base pairs) of size-separated pBR322 marker DNA are shown (lane marked M). Alleles in the ladder are numbered from slowest (allele 1) to the fastest migrating (allele 7).

insect blood meal analysis remains underutilized. The application of these tools for mosquito blood meal analysis needs to be thoroughly evaluated and harnessed for routine field application. A large number of loci suitable for identifying mosquito blood meal sources need to be tested so that closely related individuals within houses or from kin networks within villages can be distinguished with increased precision. Meanwhile, DNA profiling methods have been useful to verify the occurrence of multiple feeding (Ansell *et al.*, 2000; Chow-Shaffer *et al.*, 2000) in malaria-infected mosquitoes (Koella *et al.*, 1998) and to quantify the amount of protection provided by impregnated bednets (Gokool *et al.*, 1992, 1993). These methods could be further refined so that the extraction of DNA for mosquito species identification, parasite detection and host identification can be combined. By correctly selecting mosquito sampling time it should be possible to increase the reproducibility of DNA-based tests for mosquito blood meal identification as a tool for field entomology and epidemiology.

Acknowledgements

References

Allen, R.C., Graves, G. & Budowle, B. (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. *BioTechniques* **7**, 736–744.

Billingsley, P.F. & Hecker, H. (1991) Blood digestion in the mosquito *Anopheles stephensi* Liston (Diptera: Culicidae): activity and distribution of trypsin, aminopeptidase and α-glucosidase in the midgut. *Journal of Medical Entomology* **28**, 865–871.

Boreham, P.F.L. & Lenahan, J.K. (1976) Methods for detecting multiple blood meals in mosquitoes (Diptera: Culicidae). *Bulletin of Entomological Research* **66**, 671–679.

Briegel, H. & Lea, A.O. (1975) Relationship between protein and proteolytic activity in the midgut of mosquitoes. *Journal of Insect Physiology* **21**, 1597–1604.

Bryan, J.H. & Smalley, M.E. (1978) The use of ABO blood groups as markers for mosquito biting studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **72**, 357–360.

Budowle, B., Comey, C.T. & Baechtel, F.S. (1993) Forensic analysis. pp. 617–640 *in* Keller, G.H. & Manak, M.M. (*Eds*) *DNA probes: background, applications and procedures*. New York, Stockton Press.

Cairns, M.J. & Murray, V. (1994) Rapid silver staining and

recovery of PCR products separated on polyacrylamide gels. *Biotechniques* **17**, 915–919.

Chow-Shaffer, E.C., Sina, B., Hawley, W.A., Benedicts, J.D. & Scott, T.W. (2000) Laboratory and field evaluation of polymerase chain reaction-based DNA profiling for use in identification of human blood meal sources of *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* **37**, 492–502.

Clements, A.N. (1992) Adult food and feeding mechanisms. pp. 220–250 in *The biology of mosquitoes*, Vol 1. London, Chapman & Hall.

Coulson, R.M.R., Curtis, C.F., Ready, P.D., Hill, N. & Smith, D. (1990) Amplification and analysis of human DNA present in mosquito blood meals. *Medical and Veterinary Entomology* **4**, 357–366.

Edwards, A., Civitello, A., Hammond, H.A. & Caskey, C.T. (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics* **49**, 746–756.

Gokool, S., Smith, D.F. & Curtis, C.F. (1992) The use of PCR to help quantify the protection provided by impregnated bednets. *Parasitology Today* **8**, 347–350.

Gokool, S., Curtis, C.F. & Smith, D.F. (1993) Analysis of mosquito blood meals by DNA profiling. *Medical and Veterinary Entomology* **7**, 208–215.

Jeffreys, A.J., Wilson, V. & Thein, S.L. (1985) Hypervariable 'minisatellite' regions in human DNA. *Nature* **314**, 67–73.

Jeffreys, A.J., Wilson, V., Neumann, R. & Keyte, J. (1988) Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells. *Nucleic Acids Research* **16**, 10953–10971.

Koella, J.C., Sørensen, F.L. & Anderson, R.A. (1998) The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. *Proceedings of the Royal Society of London B* **265**, 763–768.

Kimpton, C.P., Walton, A. & Gill, P. (1992) A further tetranucleotide repeat polymorphism in the VWF gene. *Human Molecular Genetics* **1**, 287.

Kreiker, S. & Kampfer, S. (1999) Isolation and characterization of human DNA from mosquitoes (Culicidae). *International Journal of Legal Medicine* **112**, 380–382.

Nijhout, H.F., & Carrow, G.M. (1978) Diuresis after a blood meal in female *Anopheles freeborni*. *Journal of Insect Physiology* **24**, 293–298.

Noriega, F.G. & Wells, M.A. (1999) A molecular view of trypsin synthesis in the midgut of *Aedes aegypti*. *Journal of Insect Physiology* **45**, 613–620.

Pant, C.P., Houba, V. & Engers, H.D. (1987) Blood meal identification in vectors. *Parasitology Today* **3**, 324–326.

Polymeropoulos, M.H., Xiao, H., Rath, D.S. & Merril, C.R. (1991) Tetranucleotide repeat polymorphism at the human tyrosine hydrolase gene (TH). *Nucleic Acids Research* **19**, 3753.

Redington, B.C. & Hockmeyer, W.T. (1976) A method for estimating blood meal volume in *Aedes aegypti* using a radioisotope. *Journal of Insect Physiology* **22**, 961–966.

Rees, H. & Jones, R.N. (1972) The origin of the wide species variation in nuclear DNA content. *International Review of Cytology* **32**, 53–92.

Replogle, J., Lord, W.D., Budowle, B., Meinking, T.L. & Taplin, D. (1994) Identification of host DNA by amplified fragment length polymorphism analysis: preliminary analysis of human crab louse (Anoplura: Pediculidae)

excreta. *Journal of Medical Entomology* **31**, 686–690.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. & Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.

Sato, C., Furuya, Y., Harada, M. & Suguri, S. (1992) Identification of human blood in mosquitoes (Diptera: Culicidae) using nonradioactive DNA dot blot hybridization. *Journal of Medical Entomology* **29**, 1045–1048.

Service, M.W. (1977) A critical review of procedures for sampling populations of adult mosquitoes. *Bulletin of Entomological Research* **67**, 343–382.

Tempelis, C.H. (1975) Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. *Journal of Medical Entomology* **11**, 635–653.

Torr, S.J., Wilson, P.J., Schofield, S., Mangwiro, T.N.C., Akber, S. & White, B.N. (2001) Application of DNA markers to identify the individual-specific hosts of tsetse feeding on cattle. *Medical and Veterinary Entomology* **15**, 78–86.

Urquhart, A., Oldroyd, N.J., Kimpton, C.P. & Gill, P. (1995) Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. *BioTechniques* **18**, 116–121.

Vaughan, J.A., Noden, B.H. & Beier, J.C. (1991) Concentration of human erythrocytes by anopheline mosquitoes (Diptera: Culicidae) during feeding. *Journal of Medical Entomology* **28**, 780–786.

Washino, R.K. & Tempelis, C.H. (1983) Mosquito host blood meal identification. *Annual Review of Entomology* **28**, 179–201.

Wetton, J.H., Carter, R.E., Parkin, D.T. & Walters, D. (1987) Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* **327**, 147–149.

Insect Movement: Mechanisms and Consequences

Edited by **I P Woiwod**, *IACR-Rothamsted,* **D R Reynolds**, *NRI, University of Greenwich,* and **C D Thomas**, *University of Leeds, UK*

Readership: Entomology, animal physiology, behaviour and ecology.

Knowledge of insect movement, particularly of flight, is crucial to our understanding of the great ecological and evolutionary success of insects. The last 20 years have seen many advances in this subject area. New fields have arisen, such as metapopulation theory, and dramatic developments have taken place in methods of studying movement, as a result of new techniques in molecular biology and radar monitoring. There have also been advances in our knowledge of flight-related physiology and behaviour. This book, which is based on the main papers presented at the Royal Entomological Society's 20th Symposium held in September 1999, brings us up to date with these developments.

It contains chapters on:
• flight mechanisms
• foraging movements
• migration
• the evolution of movement strategies
• the interactions between dispersal rates, population structure and gene flow
• the effects of climate change on geographical distribution
It is essential reading for entomologists, and of interest to those researching animal behaviour, physiology, ecology and genetics.

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