Available online at www.sciencedirect.com
SCIENCE DIRECT®
Acta Tropica 90 (2004) 181–185
ACTA TROPICA
www.elsevier.com/locate/actatropica

# Malaria dispersal among islands: human mediated *Plasmodium falciparum* gene flow in Vanuatu, Melanesia

J. Koji Lum [a,*], Akira Kaneko [a,c], Kazuyuki Tanabe [b], Nobuyuki Takahashi [a], Anders Björkman [c], Takatoshi Kobayakawa [a]

[a] *Department of International Affairs and Tropical Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan*
[b] *Laboratory of Biology, Osaka Institute of Technology, Ohmiya, Asahi-ku, Osaka 535-8585, Japan*
[c] *Unit of Infectious Diseases, Department of Medicine, Karolinska Institutet, 17176 Stockholm, Sweden*

Received 24 January 2003; received in revised form 26 September 2003; accepted 29 September 2003

**Abstract**

A comparison of human and *Plasmodium falciparum* gene flow patterns in the model island system of Vanuatu, the limit of malaria in the Pacific reveals that human movement is essential for long, but not short distance *P. falciparum* gene flow. This suggests that long distance movement of humans may accelerate the evolution and spread of drug resistance and therefore exacerbate the global malaria problem.


*Keywords:* *Plasmodium falciparum*; Gene flow; Human movement; Vanuatu; mtDNA control region; Remote Oceania

## 1. Introduction

Malaria is endemic throughout the old world tropics and the Pacific until Buxton's Line at the southernmost islands of Vanuatu. Anthropological studies suggest that humans colonized the Pacific in two distinct stages. New Guinea-Australia and the islands as far east as the northern Solomon Islands were settled during the Pleistocene, >29,000 years ago (Allen et al., 1988). The second phase began in the Holocene, <3500 years ago in the Bismarck Archipelago and led to the human occupation of all the remaining islands of the Pacific known as Remote Oceania (Spriggs and Anderson, 1993; Green, 1999). These two human colonization phases are also reflected in the distribution of malaria.

The principal vectors of malaria in the Pacific belong to the *Anopheles punctulatus* group that consists of 12 cryptic species with overlapping morphologies. The 12 genetically defined species are adapted to different ecological zones and vary substantially in their salt tolerance and dispersal abilities (Beebe and Cooper, 2002). Although *Anopheles farauti s.s.* has apparently dispersed several times among northern Australia and the Archipelagos of western Melanesia, there remains extensive geographic structure among

* Corresponding author. Present address: Department of Anthropology, Binghamton University, Binghamton, NY 13902-6000, USA. Tel.: +1-607-777-3106; fax: +1-607-777-2477.
*E-mail addresses:* klum@binghamton.edu, i8uni@yahoo.com (J.K. Lum).

0001-706X/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2003.09.022

populations indicating gene flow across water gaps has been infrequent even in this region of Pleistocene human habitation (Beebe et al., 2000). New Guinea and the northern Solomon Islands are home to all 12 *A. punctulatus* species, however, only the salt tolerant and widely dispersed *A. farauti s.s.* is found in Holocene settled Vanuatu, the sole malarious Archipelago of Remote Oceania (Cooper et al., 2002). In addition, the genetic diversity of *Plasmodium falciparum* in Vanuatu is more restricted than in southeast Asia (Sakihama et al., 2001) and Papua New Guinea (Paul et al., 1995). Although it remains unclear when and how malaria

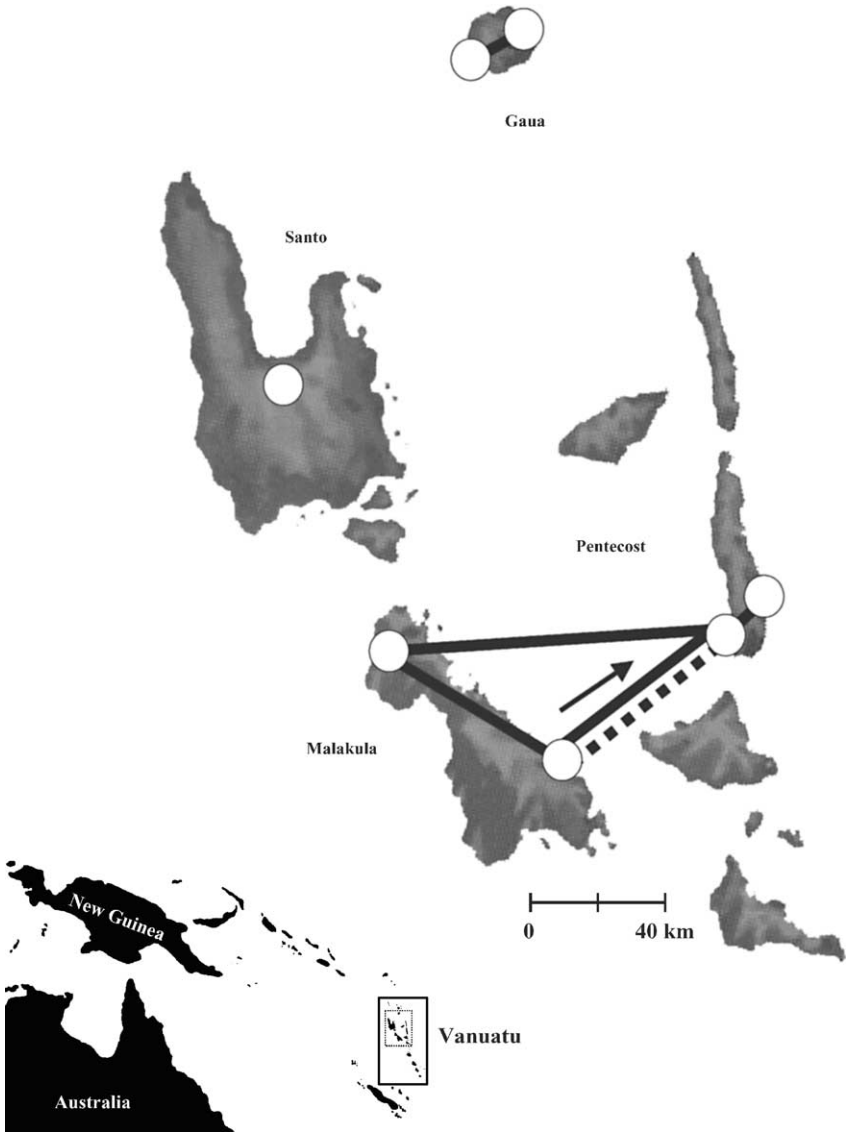


Fig. 1. Schematic of gene flow among the seven villages of Gaua, Santo, Malakula, and Pentecost sampled. The lines connect populations (open circles) which are not significantly different ($P > 0.05$). The solid lines reflect *P. falciparum* and the dashed line *H. sapiens* gene flow. The *P. falciparum* haplotypes on Pentecost are a subset of those on Malakula and thus, are inferred to be introduced from there via human mediated gene flow (arrow). The inset shows the location of the Vanuatu Archipelago (solid box) and the region sampled (dashed box) within the western Pacific.

became established in Melanesia, these distributions suggest that the length of human habitation (Pleistocene versus Holocene) and cumulative human movement is proportional to the extent of anopheline and malaria distributions and diversities.

Human populations of Vanuatu have limited linguistic diversity relative to New Guinea (Grimes and Grimes, 1996), but extensive linguistic and genetic diversity relative to other Remote Oceanic Archipelagos (Grimes and Grimes, 1996; Lum et al., 2002). Each island of Vanuatu is inhabited by small, subsistence populations often speaking multiple languages indicating cultural isolation even among villages on the same island that are separated by short distances. We studied human mtDNA sequences representing seven villages on four islands of north-central Vanuatu and compared them to merozoite surface protein-1 genetic diversities of *P. falciparum* infections obtained during malariometric surveys of the same populations (Sakihama et al., 2001). We wanted to explore the influence of human gene flow on the distribution of malaria haplotypes over short (intra-island) and long (inter-island) distances. In our sample only the two populations from Pentecost speak the same language (Fig. 1).

## 2. Materials and methods

Human and *P. falciparum* DNA was extracted from human blood collected during malariometric surveys of seven villages on four islands of Vanuatu (Gaua: 2, Santo: 1, Malakula: 2, and Pentecost: 2) (Fig. 1). PCR primers for and amplification of the non-coding control region, the most variable segment of human mtDNA were as previously described (Lum et al., 1998). PCR products were purified using the ExoSAP-IT procedure (USB) to remove unincorporated dNTPs and primers. The purified PCR products were sequenced in both directions using the DYEnamic ET Dye Terminator cycle sequencing kit and the MegaBACE 1000 automated sequencer (Amersham Pharmacia Biotech).

Genetic distances ($F_{ST}$) among the seven human ($n = 159$) and *P. falciparum* ($n = 142$) populations and the proportions of genetic variance within populations, within islands, and among islands were estimated using Arlequin 2.0 (Schneider et al., 2000). Human populations from Remote Oceania are composed of multiple clades of mtDNA control region sequences that reflect contributions from both the pleistocene and holocene colonizations and thus, predate the settlement of the region. Genetic distances between populations are therefore based on the shared frequencies of mtDNA haplotypes defined by sequence variants as described previously (Lum and Cann, 2000). Genetic distances between *P. falciparum* populations were estimated from frequencies of previously published shared compound haplotypes incorporating both 5′ haplotype and 3′ sequence variation (Sakihama et al., 2001). The statistical significance of a pairwise $F_{ST}$ value was assessed by randomly permuting individuals between populations 10,000 times and determining the proportion of the data sets that yielded an $F_{ST}$ greater than that of the original data set (Schneider et al., 2000). The human and *P. falciparum* pairwise $F_{ST}$ genetic distances and their estimated *P* values are shown in Table 1. Congruence between genetic distances among populations of the two species was evaluated with the Mantel permutation method (Mantel, 1967) using matrix correlation analysis version 1.0 (Long, 1996).

## 3. Results

Human and *P. falciparum* genetic distances among the seven populations were significantly correlated ($r = 0.62$, $P < 0.02$) implicating long-term human contacts in the dispersal of malaria. The human and parasite genetic patterns within islands were however, very different. The *P. falciparum* populations on a single island are relatively homogenous containing only 5.7% of the variance of populations on different islands. In contrast, human populations on the same island had 77.6% of the variance of populations on separate islands, consistent with their distinct languages and cultural isolation. The only two human populations that were not statistically distinct ($P > 0.09$) (Table 1) were from separate islands (Malakula and Pentecost) and speak distinct languages. *P. falciparum* populations from these two islands were likewise not significantly distinct ($P > 0.14$) (Table 1, Fig. 1).

These analyses suggest that genetically interacting human populations are essential for malarial gene flow between islands. In contrast, human populations on the same island, despite little apparent human gene flow

Table 1
$F_{ST}$ genetic distances (upper triangle) and $P$ values[a] (lower triangle) between populations

| | East Malakula | Northwest Malakula | East Pentecost | West Pentecost | Santo | East Gaua | West Gaua |
|---|---|---|---|---|---|---|---|
| Plasmodium falciparum[b] | | | | | | | |
| East Malakula | – | 0.026 | 0.359 | 0.121 | 0.277 | 0.115 | 0.309 |
| Northwest Malakula | 0.194 | – | 0.302 | 0.049 | 0.423 | 0.154 | 0.386 |
| East Pentecost | 0.003 | 0.002 | – | 0.000 | 0.393 | 0.351 | 0.417 |
| West Pentecost | 0.141 | 0.289 | 0.617 | – | 0.318 | 0.154 | 0.282 |
| Santo | 0.001 | 0.000 | 0.000 | 0.000 | – | 0.307 | 0.270 |
| East Gaua | 0.031 | 0.003 | 0.000 | 0.047 | 0.000 | – | 0.044 |
| West Gaua | 0.000 | 0.000 | 0.000 | 0.010 | 0.000 | 0.241 | – |
| Human[c] | | | | | | | |
| East Malakula | – | 0.034 | 0.076 | 0.021 | 0.072 | 0.107 | 0.113 |
| Northwest Malakula | 0.027 | – | 0.092 | 0.050 | 0.079 | 0.132 | 0.150 |
| East Pentecost | 0.000 | 0.000 | – | 0.033 | 0.150 | 0.148 | 0.188 |
| West Pentecost | 0.096 | 0.006 | 0.049 | – | 0.082 | 0.112 | 0.121 |
| Santo | 0.004 | 0.005 | 0.000 | 0.003 | – | 0.171 | 0.155 |
| East Gaua | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | – | 0.088 |
| West Gaua | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.012 | – |

[a] $P$ values are the proportion of permuted data sets that yielded $F_{ST}$ values greater than the actual value with significant values in bold.
[b] Plasmodium falciparum genetic distances based on MSP-1 variation.
[c] Human genetic distances are from mtDNA control region haplotype frequency variation.

share a common parasite population indicating a major contribution of vector movement in malaria dispersal over short distances. Malaria dispersal via human movement within islands is also expected, but may be minor among these genetically and linguistically isolated populations.

## 4. Discussion

During the approximately 3300 years of traditional human movement into and within Remote Oceania, anophelines became established only as far East as Vanuatu. Analyses of Remote Oceanic human populations suggest substantial gene flow (Lum et al., 2002), but this did not result in the transfer of anophelines beyond Vanuatu. Modern transportation and globalization however, have introduced vector species to previously virgin island populations making them vulnerable to malaria and other vector-borne diseases. Hence 17 non-indigenous mosquito species including five anopheline species have become established on Guam and a number of mosquito species have also invaded Hawai'i ($n = 5$), New Zealand ($n = 4$), and Australia ($n = 4$) (Lounibos, 2002). Global human movement of returning vacationers or those seeking asylum (Holloway, 2001) that harbour parasites potentially spread diseases to islands with recently introduced vectors (e.g. dengue fever epidemics in Hawai'i). For example, in Queensland, Australia during October 2002 five people from three separate camping groups contracted *Plasmodium vivax* malaria traced to an individual who had contracted malaria in Indonesia during 2001 and had stayed in the same area a month earlier (Hanna, personal communication). These data highlight the potential risks of disease epidemics resulting from human movement and/or the passive transport of insect vectors. On Aneityum, the southernmost island of Vanuatu we demonstrated that malaria could be eradicated and maintained free of transmission for over a decade by chemotherapeutic and integrated vector control measures coupled with strong community commitment and importantly, malaria surveillance and treatment of all arriving visitors to prevent reintroduction of parasites (Kaneko et al., 2000).

The analyses of our model island system indicate that both vector and human movement are important for malaria gene flow at different levels of geographic isolation. On continents where human populations are contiguous, malaria gene flow likely occurs between adjacent populations via both human and vector movement. Modern global human movement is expected to

increase the risk and rate of evolving drug resistance by transporting diverse genetic strains among continents. Further studies to explore the contribution of human and vector movement on malaria dispersal including data from additional plasmodium loci and also anopheles vector gene flow are currently underway.

## Acknowledgements

We thank the people who donated blood samples for this study. This study was supported in part by grants-in-aid for Research on International Medical Cooperation from the Ministry of Health, Labour, and Welfare of Japan, Tokyo, Japan and by grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, Tokyo, Japan (11670254, 13576030, 13770127, and 14021125).

## References

Allen, J., Gosden, C., Jones, R., White, J.P., 1988. Pleistocene dates for the human occupation of New Ireland, northern Melanesia. Nature 331, 707–709.

Beebe, N.W., Cooper, R.D., 2002. Distribution and evolution of the *Anopheles punctulatus* group (Diptera: Culicidae) in Australia and Papua New Guinea. Int. J. Parasitol. 32, 563–574.

Beebe, N.W., Cooper, R.D., Foley, D.H., Ellis, J.T., 2000. Populations of the south-west pacific malaria vector *Anopheles farauti s.s.* revealed by ribosomal DNA transcribed spacer polymorphisms. Heredity 84, 244–253.

Cooper, R.D., Waterson, D.G.E., Frances, S.P., Beebe, N.W., Sweeney, A.W., 2002. Speciation and distribution of the members of the *Anopheles punctulatus* (Diptera: Culicidae) group in Papua New Guinea. J. Med. Entomol. 39, 16–27.

Green, R.C., 1999. Integrating historical linguistics with archaeology: insights from research in Remote Oceania. Indo-Pacific Prehistory Assoc. Bull. 18, 3–16.

Grimes, J.E., Grimes, B. (Eds.), 1996. Ethnologue: Languages of the World, 13th ed. Summer Institute of Linguistics Inc., Dallas, TX.

Hanna, J., personal communication. http://abc.net.au/news/newsitems/s716958.htm (web reports are archived and can be obtained from JKL).

Holloway, G., 2001. http://www.cnn.com/2001/WORLD/asiapcf/auspac/09/19/nauru.asylumseekers/index.html (web reports are archived and can be obtained from JKL).

Kaneko, A., Taleo, G., Kalkoa, M., Yamar, S., Kobayakawa, T., Björkman, A., 2000. Malaria eradication on islands. Lancet 356, 1560–1564.

Long, J.C., 1996. Matrix Correlation Analysis, version 1.0. Distributed by author. Section on Population Genetics and Linkage, Laboratory of Neurogenetics, NIAAA/NIH, Rockville, MD.

Lounibos, L.P., 2002. Invasions by insect vectors of human disease. Annu. Rev. Entomol. 47, 233–266.

Lum, J.K., Cann, R.L., 2000. mtDNA lineage analyses: origins and migrations of Micronesians and Polynesians. Am. J. Phys. Anthropol. 113, 151–168.

Lum, J.K., Cann, R.L., Martinson, J.J., Jorde, L.B., 1998. Mitochondrial and nuclear genetic relationships among Pacific Island and Asian populations. Am. J. Hum. Genet. 63, 613–624.

Lum, J.K., Jorde, L.B., Schiefenhovel, W., 2002. Affinities among Melanesians, Micronesians, and Polynesians: a neutral, biparental genetic perspective. Hum. Biol. 74, 413–430.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.

Paul, R.E.L., Packer, M.J., Walmsley, M., Lagog, M., Ranford-Cartwright, L.C., Paru, R., Day, K.P., 1995. Mating patterns in malaria parasite populations of Papua New Guinea. Science 269, 1709–1711.

Sakihama, N., Kaneko, A., Hattori, T., Tanabe, K., 2001. Limited recombination events in merozoite surface protein-1 alleles of *Plasmodium falciparum* on islands. Gene 279, 41–48.

Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin version 2.000: Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Spriggs, M., Anderson, A., 1993. Late colonization of East Polynesia. Antiquity 67, 200–217.