Dissertationes Forestales 73

The decline of northern malaria and population dynamics of *Plasmodium vivax*

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Academic dissertation
To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Lecture Hall 3, B-building, Latokartanonkaari 7, Helsinki, on October 24th 2008 at 10 o´clock.

Title of dissertation:
The decline of northern malaria and population dynamics of *Plasmodium vivax*

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ISSN 1795-7389
ISBN 978-951-651-234-4 (PDF)

(2008)

Publishers:
Finnish Society of Forest Science
Finnish Forest Research Institute
Faculty of Agriculture and Forestry of the University of Helsinki
Faculty of Forest Sciences of the University of Joensuu

Editorial Office:
Finnish Society of Forest Science
P. O. Box 18, 01301 Vantaa, Finland
http://www.metla.fi/dissertationes

Huldén, L. 2008. The decline of northern malaria and population dynamics of *Plasmodium vivax*. Dissertationes Forestales 73. 28 p.
Available at http://www.metla.fi/dissertationes/df73.htm

Europe was declared malaria free in 1975. The disappearance of malaria has traditionally been attributed to numerous deliberate actions like vector control, the screening of houses, more efficient medication etc. Malaria, however, disappeared from many countries like Finland before any counter measures had even started. The aim of this thesis is to study the population ecology of *P. vivax* and its interaction with the human host and the vector. By finding the factors that attributed to the extinction of *vivax* malaria it might be possible to improve the modern strategy against *P. vivax*. The parasite was studied with data from Finland, which provides the longest time series (1749-2008) of malaria statistics in the world. The malaria vectors, *Anopheles messeae* and *A. beklemishevi* are still common species in the country.

The eradication of vivax malaria is difficult because the parasite has a dormant stage that can cause a relapse long after a primary infection. It was now shown that *P. vivax* is able to detect the presence of a potential vector. A dormant stage is triggered even from a bite of an uninfected *Anopheles* mosquito. This optimizes the chances for the *Plasmodium* to reach a mosquito vector for sexual reproduction. The longevity of the dormant stage could be shown to be at least nine years.

The parasite spends several years in its human host and the behaviour of the human carrier had a profound impact on the decline of the disease in Finland. Malaria spring epidemics could be explained by a previous warm summer. Neither annual nor summer mean temperature had any impact on the long term malaria trend. Malaria disappeared slowly from Finland without mosquito control. The sociological change from extended families to nuclear families led to decreased household size. The decreased household size correlated strongly with the decline of malaria. That led to an increased isolation of the subpopulations of *P. vivax*. Their habitat consisted of the bedrooms in which human carriers slept together with the overwintering vectors. The isolation of the parasite ultimately led to the extinction of vivax malaria. Metapopulation models adapted to local conditions should therefore be implemented as a tool for settlement planning and socio-economic development and become an integrated part of the fight against malaria.

Keywords: malaria, *Plasmodium vivax*, Finland, relapse, household size, *Anopheles messeae, Anopheles beklemishevi,* vectorborne disease

ACKNOWLEDGEMENTS

Malaria research has almost 200 year long roots in Finland. Johan Haartman (1725–1788) was the first malariologist at the University of Helsinki (then in Turku). He conducted empirical research in the SW archipelago and gave useful advice for fighting against the disease. Since those days malaria has ceased to be a problem in Finland. My own interest in malaria started as a historical problem. Very soon I realized that I have left history and was analyzing the ecology of *Plasmodium vivax.*

During this work the most important person has been my husband and colleague Larry, whose advice and support has been invaluable. Kari Heliövaara has been an important friend and supervisor. Thanks for letting me join your team. Marika Tandefelt has patiently listened to endless discussions about housing and vector borne diseases.

Collecting mosquitoes and other insects together with Kauri Mikkola and Leena Saraste both at the shores of the Arctic Sea and the Baltic have been memorable. The visits to Edesbacka have been a relaxing change to the paper work. Heikki Hokkanen and Ingeborg Menzler-Hokkanen have showed both friendship and professional help.

The coffee breaks discussions during these years, first at the department of Applied Biology and then at the department of Forest Ecology have been demanding, fascinating and great fun. Thanks to Jani Pellikka, Reetta Kettunen, Veli-Matti Väänänen, Petri Nummi and Esa Pienmunne for letting me join.

This work was funded by Svenska Litteratursällskapet i Finland and Otto A. Malms donationsfond, which I would also thank for their support.

Vantaa, September 2008, Lena Huldén

LIST OF ORIGINAL ARTICLES

This thesis consists of an introductory review followed by four research papers. The papers in the review are referred to by their Roman numerals.

I. **Huldén, Le.**, Huldén, La. & Heliövaara, K. 2005. Endemic malaria: an 'indoor' disease in northern Europe: Historical data analyzed. *Malaria Journal,* 4:19, 1–13. doi:10.1186/1475-2875-4-19

II. **Huldén, Le.**, Huldén, La. & Heliövaara, K. 2008**.** Natural relapses in *vivax* malaria induced by *Anopheles* mosquitoes. *Malaria Journal,* 7:64, 1–29. doi:10.1186/1475-2875-7-64

III. **Huldén, Le.** & Huldén, La. 2008. Dynamics of positional warfare malaria: Finland and Korea compared. *Malaria Journal*, **7**:171, 1–13 doi:10.1186/1475-2875-7-171

IV. **Huldén, Le.** & Huldén, La. 2008. Decline of malaria in Finland – the impact of the vector and social variables. Manuscript.

AUTHOR'S CONTRIBUTION

Lena Huldén is responsible for the summary of this thesis. She is the main author of all papers and developed the principle ideas of these papers. She drafted the manuscript of paper **I**. She collected and interpreted the historical data. Larry Huldén added and/or removed various sections and participated in the design of the study. Kari Heliövaara participated in its design. She drafted the manuscript of paper **II** and is responsible for the development of the method used for the correlation analysis. Larry Huldén and Kari Heliövaara participated in the design of the study. In paper **III** Lena Huldén drafted the manuscript and collected the data. Larry Huldén and Lena Huldén both made the correlation analysis. In paper **IV** Lena Huldén collected the data and drafted the manuscript. Larry Huldén added and/or removed various sections and participated in the design of the study.

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INTRODUCTION

The concept of species eradication, as a means of assuring the elimination of malaria, was basically wrong, that is, based on the wrong premises and assumption (de Zulueta 1990).

Background

Malaria is the most common vector borne disease in the world. All plasmodia that are human or simian parasites use *Anopheles* mosquitoes as vectors. Five *Plasmodium* species cause malaria in humans: *P. falciparum*, *P. vivax*, *P. malariae, P. ovale* and *P. knowlesi* (Cox-Singh et al. 2008, White 2008). The first two species are responsible for most of the burden of human malaria.

Today *vivax* malaria is often considered a tropical disease. It has, however, been indigenous in the countries such as Canada, Russia, Sweden and Finland (Bruce-Chwatt & de Zulueta 1980). During the malaria transmission season in Yakutia the temperature dropped to –71.2 ºC in 1926, which is the cold record of the northern hemisphere. In 1933 it was –67.7 ºC. In these years there were 819 and 12,758 malaria cases, respectively. Of the Yakutian population up to 4 % suffered from the disease. Malaria disappeared from Yakutia in 1960 (Lysenko & Kondraschin 1999). *P. vivax* has been the most common species in Europe (Lysenko & Semashko 1968). It is flexible and can survive long periods without the vector as dormant hypnozoites in the human liver. It adapts to local conditions and displays regionally different relapse patterns that optimize the transmission of the parasite (Price et al. 2007).

P. vivax became extinct in Europe and the continent was declared malaria free in 1975. It still remains a major human health problem globally and the economic impact and severity of the disease has been neglected (Guerra et al. 2006, Price et al. 2007, Joshi et al. 2008). Even the number of annual cases has been discussed. The 1999 World Health Report by WHO estimated it between 72 and 80 millions. The statistics has been questioned and recent studies have made new estimates of 132 – 391 million annual cases of *P. vivax*, which then would account for 25 – 40 % of the global malaria burden (Snow & Hay 2006, Carter & Mendis 2006, Price et al. 2007).

A growing level of drug resistance is among the big problems concerning global human health today. Several studies have been carried out especially on *P. falciparum* (Hastings & Watkins 2005). The recent emergence of chloroquine resistant strains and the growing social and economic loss make more comprehensive research of *P. vivax* urgent. Chloroquine resistant strains are now established in Papua New Guinea and eastern Indonesia (Price et al. 2007). It is also reported from South America (Soto et al. 2001). The resistance against primaquine, which affects the hypnozoites, is widespread in Southeast Asia and Oceania (Baird & Hoffman 2004). Primaquine failures are also reported from sub-Saharan Africa and from Central and South America (Schwartz et al. 2000). Recombination is among the main factors affecting the theoretical models on drug resistance (MacKinnon 2005). In this thesis the possibility for rapid recombination of *P. vivax* is discussed.

The discovery of the *Plasmodium* in human blood by Alphonse Laveran in 1880 and the *Anopheles* vector in 1898 by Sir Ronald Ross changed the prerequisites for malaria research. The European malaria problem was discussed intensively at the beginning of the 20th century. Many early scientists, as the Italian malariologist Angelo Celli realized the connection between a decline of malaria and general economic development (Harrison 1978, Snowden 2006). After World War I, the Health Organization of the League of

Nations established the Malaria Commission to control and conduct research in malaria. In 1924, it considered drug treatment, the screening of houses and individual protection of primary importance while anti-larval measurements only were occasionally recommended (Bruce-Chwatt & de Zulueta 1980). There was a deep split between the malariologists. Some believed that malaria eradication could be contrived only by destroying larvae and adult mosquitoes. This view was represented by the Rockefeller Foundation, which had effectively used insecticides in Puerto Rico in the 1920's (Stapleton 2004). The "mitigaters", who usually represented the Europeans, especially the Italian malariologists, emphasized that malaria primarily was a social disease, which declined with better housing and a higher standard of living. They usually also considered prophylactic treatment by quinine of the inhabitants in malarious areas of great importance (Evans 1989). They remarked that there were areas with abundant *Anopheles* population but without the disease. The question of *anophelism without malaria* was intensively discussed in 1928 (League of Nations 1928), but still American experts like Lewis Hackett favoured mosquito-based antimalarial work (Hackett 1937).

The manufacturing of DDT started in Switzerland in 1939 (Becker et al. 2003). It offered a simple solution during the difficult situation during and after World War II when malaria again increased in Europe. The allied occupation of Italy gave the American entomologists an opportunity to apply the insecticide in large quantities. The most spectacular event was the powdering all internal walls in the houses in the swampy area along the SW coast of Italy, south from the river of Volturno with DDT between December 1944 and September 1945 (Bruce-Chwatt & de Zulueta 1980). The international health policy reflected the political situation after the World War II. Eradication with the use of DDT became predominant and that seemed like an easy and cheap solution. Not much has changed in the battle against malaria. DDT was reintroduced in 2006. Many projects still focus on the eradication of the vector species. Malaria is often seen as a problem, which is focused on the mosquito.

The decline of malaria in Europe has been explained in many different ways (Bruce-Chwatt & de Zulueta 1980). Most of the research is based on data from the beginning of the 20th century. Almost no effort has been made to quantitatively compare different variables with the malaria statistics. Finally, in 1994 de Zulueta stated that the decline was a consequence of improved rural housing and better social and economic conditions in general in the industrialized countries (de Zulueta 1994). Nevertheless why these factors affected the parasite has not been satisfactorily explained.

The aim of this thesis is to study the population ecology of *P. vivax* and its interaction with the human host and the vector. Today *anophelism without malaria* is prevailing in the Nordic countries. It was achieved without large investments in mosquito control. By finding the factors that attributed to the extinction it might be possible to improve the modern strategy against *P. vivax*. The parasite was studied with data from Finland, which provides the longest time series (1749–2008) of malaria statistics in the world (Paper IV). During the first hundred years, malaria was common and the situation equivalent of any highly malarious underdeveloped country today. The difference was that the Finnish peasants rarely used china-bark or quinine before 1850. The data are therefore not contaminated by medication.

The lifecycle of *Plasmodium vivax*

During the blood meal, an infected female of *Anopheles* mosquito injects an infective stage of the parasite, a sporozoite, into the human host (Fig. 1). A study of *P. falciparum* has shown that the process is not always successful: 50 % of the bites of infected mosquitoes fail to cause an infection (Rickham et al. 1990). How effective the process was for the Nordic strains of *P. vivax* is not known.

The number of injected sporozoites is low, only 10–20 (max 80) (Sinden & Billingsley 2001). There can be thousands of sporozoites in the salivary glands of the vector. Early experiments with *Plasmodium vivax* have shown that an infective mosquito bites 30–40 times (James & Shute 1926). Why such a mass of sporozoites is produced by the parasite is unknown. Yet it seems to be important and several explanations have been given (Paul et al. 2003). It probably changes the composition of the anticoagulants and vasodilators in the saliva.

The injected sporozoite has to pass through several cells in the skin before entering the blood stream (Paul et al. 2003). In the blood stream it must escape the human immune system and reach the target cell, the hepatocyte, within 30 minutes to survive (Kettle 1995). The proportion of sporozoites that survives the travel from the vector to the liver is not known. If *P. vivax* is comparable with *P. falciparum,* only 50 % of the mosquito bites

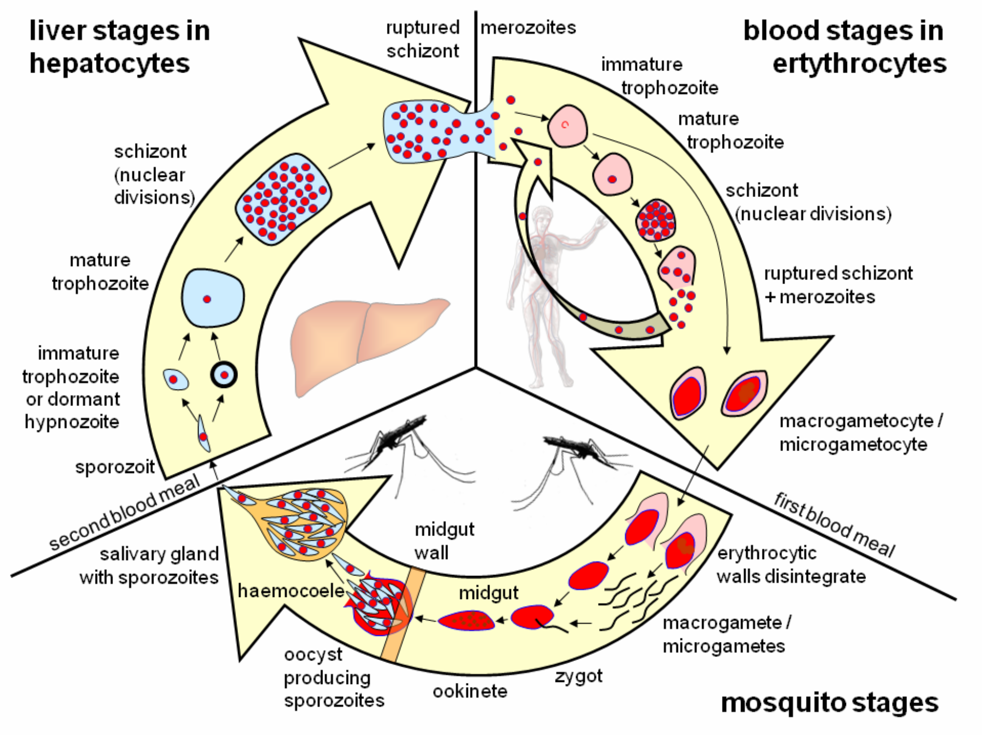


Figure 1. The life-cycle of *P. vivax.*

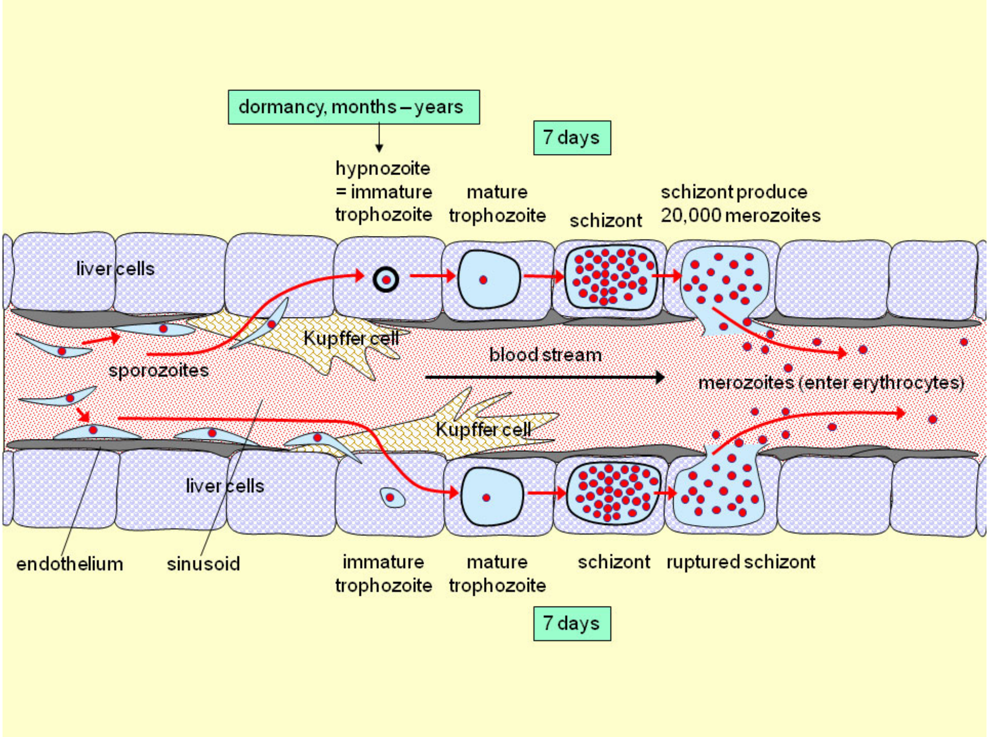


Figure 2. The transformation of the sporozoite of *Plasmodium vivax* in the hepatocyte.

causes an infection and 10–20 sporozoites are typically injected. A crude estimate of 5–10 % survival rate may be assumed.

When the sporozoite reaches the liver, the blood stream brings it through the narrow liver sinusoids (Fig. 2). Within the sinusoid the sporozoite first enters a Kupffer cell and passes through it into a hepatocyte (Barnwell 2001). The sporozoite cannot pass through the endothelium of the sinusoid except where there are Kupffer cells.

The Kupffer cell is specialized in engulfing foreign bacteria and to recycle essential components from dead blood cells. The sporozoite is somehow able to neutralize the primary function of that cell and continue to move into the hepatocytes. The sporozoite's ability to pass through it is inadequately understood (Pradel et al. 2004). The sporozoite recognizes the chemical compounds of the surface of both the Kupffer cell and the hepatocyte.

After passing the Kupffer cell, the sporozoite moves through one or several hepatocytes, which will recover or sometimes die (Mota et al. 2001). Finally, it settles in one hepatocyte and becomes an immature trophozoite. Principally the immature trophozoite grows and becomes rounded until it enters the schizont stage. The schizont undergoes extensive nuclear divisions until 10,000–20,000 merozoites are generated. The pre-erythrocytic phase takes about 7 days (Paul et al. 2003).

Not all of the sporozoites of *P. vivax* undergo mitosis immediately. The immature trophozoite can also remain in a dormant stage called a hypnozoite. How the immature trophozoite delays its further development and becomes dormant is not understood. The sporozoites are not genetically identical and the tendency to be dormant has been suggested to be a polymorphic character (Adak et al. 2001). Conditions with strong vector seasonality favour strains with more hypnozoites or longer incubation time (Lysenko & Semashko 1968).

The development of the *Plasmodium* within the liver has until very recently incorrectly been called an "exo-erythrocytic cycle" instead of pre-erythrocytic phase. A hepatic schizont can develop exclusively from a sporozoite during a primary infection. The merozoite from a hepatic schizont can only continue its development in erythrocytes and accordingly there is no cycle in the liver.

As the mature schizont ruptures and releases merozoites into the blood vessels the erythrocytic cycle starts. The merozoite of *P. vivax* invades a young erythrocyte, a reticulocyte, where it develops into a trophozoite. As a trophozoite it uses the resources of the erythrocyte and grows until it fills up the space of the erythrocyte. During the schizont stage nuclear divisions generate 16 new merozoites. These burst back into the blood stream. To survive the merozoite has to find a suitable blood cell in a few minutes. The erythrocytic cycle duration is 48 hours (Paul et al. 2003). During each cycle, 1–2 % of the merozoites develop into male or female gametocytes. The gametocytes remain within the erythrocytes and do not develop further in the human host. They mature in 4 days in *P. vivax* (Kettle 1995). It is not known why *P. vivax* needs several rounds of asexual reproduction with a huge number of merozoites and only a small number of gametocytes. Several species of *Plasmodium* can produce gametocytes directly and they do not undergo extensive asexual reproduction in the host (Paul et al. 2003). Only the sexual stages are transmitted from the human host to the midgut of the *Anopheles* female.

During a blood meal, the *Anopheles* female may ingest tens of thousands of gametocytes. The gametocytes are transferred into the midgut of the mosquito where they shed the remains of the erythrocyte. The female macrogametocyte becomes a macrogamete. The male microgametocyte undergoes exflagellation. In 10–15 min., it produces eight microgametes, which are mobile and move to find macrogametes. They fuse and form a motile ookinete. Only 1 % of the gametes succeed in the transformation. Normally just 50–100 ookinetes are produced (Sinden & Billingsley 2001). The ookinete passes between the cells of the midgut and forms an oocyst. The sporozoites develop in the oocyst, invade the haemocoele and finally penetrate the salivary glands (Kettle 1995).

Plasmodium vivax spends a limited time in the vector. To secure its survival the parasite manipulates the behaviour of the vector. During the development of the oocyst the vector feeds less often. The fecundity is reduced. When the sporozoites are mature, they invade the salivary glands (Koella 1999). Then the feeding behaviour of the mosquito changes, it probes longer and feeds more often (Paul et al. 2003, Rossignol et al. 1986). British experiments with *P. vivax* in the 1920's showed that infected mosquitoes bit 30 to 40 times (James & Shute 1926). It has also been shown that *P. falciparum* alters the feeding behaviour of *Anopheles gambiae* in two ways. It increases the probing time of the vector and the vector tends to bite several people per night (Koella et al. 1998). It has also been demonstrated that *P. chabaudi*, which causes rodent malaria, stimulates the vectors' appetite for blood feeding (Ferguson & Read 2004). In general it seems plausible to think that all mammal *Plasmodium* species affect the vectors' behaviour in a similar way (Paper II). There is also some evidence that the parasite attempts to avoid damaging the vector. The ookinete undergoes apoptosis, which reduces their number by 50 %. This probably decreases the damage to the vector (Al-Olayan et al. 2002). However, this remains controversial and it is not known if it is a feature characteristic to all *Plasmodia* (Paul et al. 2003).

The *Plasmodium* faces the same problems with the immune response in the vector as in the human host. In addition there is a problem with variation in temperature during the time in the mosquito. The longevity of the mosquitoes is also a challenge. In Finland, the vector species has the longevity of 9–10 months (Paper I). In tropical conditions the longevity is shorter. The long sporogonic development in the vector has been considered one of the most unexpected features (Paul et al. 2003). Principally it would be a risk factor but it has at least one advantage. The vector is quite immobile during the sporogony and (as in Finland) remains close to the human resting place. A long sporogony will then increase the possibility that the vector will bite another person than that from which it originally got the infection.

The *Plasmodium* seems to have many bottle necks during its sexual and asexual reproduction. Still, it is surprisingly successful. It is very probable that the "bottle necks" are in fact a result of inherent regulation. A seemingly exaggerated reproduction of any stage during the lifecycle only ensures that the *Plasmodium* will reach the next target. When settled in a host, it suppresses further infections to avoid too much damage to the host. For example, if all the gametocytes are successful, the mosquito would be killed before spreading the sporozoites into new humans.

Anopheles vectors of northern malaria

More than 200 species of *Plasmodium* have been described. A minor part affect mammals, 89 species occur in reptiles and 32 in birds (Paul et al. 2003). Only *Anopheles* mosquitoes are vectors of mammal malaria while Culicine mosquitoes, a Phlebotominae sand fly and *Culicoides* biting midges are vectors of avian and reptilian malaria (Martinsen et al. 2008). There are more than 400 species of *Anopheles*, but only about 20 species are major vectors of human malaria (Lal et al. 2001). The genus is divided into four subspecies of which the subgenera *Anopheles* and *Cellia* are represented in Palaearctic region (Becker et al. 2003).

Many of the important *Anopheles* vectors belong to sibling species that form species complex. The classic example is the *maculipennis* complex in Europe that was first discovered by Falleroni in 1926 (Becker et al. 2003). The most important vectors in the Northern Eurasia were *A. atroparvus*, *A. messeae*, *A. beklemishevi* and *A. sinensis* (World Malaria Report 2005, paper I and IV, Sokolova & Snow 2002, Eldrige & Edman 2004, Kampen 2005, Ramsdale & Snow 2000, Lumiaho & Itämies 1981, Ramsdale & Snow quote incorrectly Lumiaho & Itämies. They collected larvae of *A. maculipennis* sensu lato from Finland, not sensu stricto.). The first three species all belong to the *maculipennis* complex. *A. sinensis* is distributed in the Eastern part of Eurasia and it is the main vector in Korea and China (Cui et al. 2006, Lee et al. 2007).

A. atroparvus breeds both in saline and in fresh water, but shows a slight preference for brackish water. It was the primary vector in the Netherlands (Swellengrebel 1933, Jetten & Takken 1994). It occurs from the south coast of the Baltic, along the Atlantic coast from Sweden to Portugal and along the coast of the Mediterranean Sea. In Russia, it occurs in the south-western parts and in the coastal area of the Black Sea (Becker et al. 2003).

A. messeae was the main vector in Finland (Paper I, IV). It prefers large water bodies and the larvae can be found in cool, stagnant water with an abundant growth of submerged vegetation. It was also found in brackish water along the Finnish coast (Paper IV). *A. messeae* has a widespread distribution. It occurs from the Atlantic coast, through Fennoscandia, Russia as far as China. It is absent in southern Europe (Becker et al. 2003). *A. beklemishevi* is found in Siberia, Finland and northern Sweden (Korvenkontio et al. 1979, Jaenson et al. 1986, Utrio 1979). It is typical in the coniferous taiga region and the larvae seem more tolerant to cold water than *A. messeae* (Jaenson et al. 1986, Becker et al. 2003). All four species overwinter as adult females (Linton et al. 2002, Paper I, Paper III, Chong 1962). *A. messeae* undergoes a complete hibernation in cold conditions (Jaenson & Ameneshewa 1991). They often rest inside buildings and can be collected in cowsheds, stables and pigsties (Jaenson et al. 1986, Chong 1962).

During the 19th century, people often lived in log houses and cottages, with warm, dark and humid condition. The cattle were usually kept in separate cowsheds (Valonen 1963). At the end of September, they were brought inside for winterfeeding from the pastures, which could be quite far away (Vilkuna 2001). The overwintering *Anopheles* females entered the overwintering site already in the beginning of the same month (Paper I, IV). Cattle could therefore not have made the cattle sheds more attractive as an overwintering place compared with other buildings on the farm.

A. beklemishevi is considered to be a vector in the mid and south taiga in European Russia (Sokolova & Snow 2002). On the other hand *A. beklemishevi* is reported to undergo complete winter diapause of a duration that is inversely correlated with photoperiod (White 1978, Stegnii & Kabanova 1978). White also claims that *A. beklemishevi* is zoophilic and that it would not be significant as a potential malaria vector (White 1978). It is however probable that the flexibility of the species was not established in 1978. It is not known if the diapause is complete in warm surroundings indoors. Females of at least some species of the *maculipennis* complex can take blood-meals during winter (Becker et al. 2003).

STUDY AREA, MATERIAL AND METHODS

The efforts to stamp out malaria were different among the European countries. The world's first national campaign against malaria was organized in Italy in 1900, but the disease was eradicated only in 1965 (Snowden 2006). Effective anti-malarial measurements were taken in the Soviet Union where the eradication campaigns were combined with intensive research (Lysenko & Kondraschin 1999).

To examine the behaviour of the populations of *Plasmodium vivax* it was necessary to find long time series with unbiased data. For this purpose Finland was ideal. Geographically Finland is defined by the treaty of Stolbova 1617. That included Karelian Isthmus, but not the Petsamo area by the Arctic Sea. The latter became a part of Finland only after 1920. Although the border between Sweden and Russia changed in 1745 and 1810, the local ecclesiastical organisation did not change and the source material remained uniform.

The distributions of the *Anopheles* species were interpreted from literature records. The overwintering behaviour of the main species, *Anopheles messeae*, was interpreted according to literature records and samplings or observations in the field during various months of the year (Paper IV).

Chronologically the study period starts in 1749. At that time, the Swedish authorities started to systematically collect demographic statistics from the local parishes. Most of the parish registers have preserved. The validity of the data was tested both by quantitative and historical methods (Paper I, IV). The official malaria statistics have been published since 1884. The period from 1850 to 1884 was constructed from parish registers and the annual reports of the district physicians (Paper I, IV). A more detailed description of the malaria data from 1749 to 2008 is found in the original papers.

The different European theories on the cause of the decline were tested using several variables. It should be emphasized that research in Finnish history before the independence in 1917 traditionally favours local history and the use of local archives. Also the large bonds of mostly local cameral sources were moved from Stockholm to Helsinki in the 19th century. Historical statistics on economics have been published and are easily available (Vattula 1983).

The phenology of the vectors and of *P. vivax* was analyzed in the first paper (Paper I). The ecology of *P. vivax* was examined in the second paper (Paper II). The aim was to explain the mechanism behind the activation of the dormant stage. It was done by a simple graphical analyze of spatial correlation trends, which were tested for spurious correlation. The method has not previously been used in biological sciences.

Control of *P. vivax* is difficult because the parasite has a dormant stage. Although it seems to be under control epidemics can suddenly appear. To understand the set backs in the eradication process positional warfare malaria in Finland was compared with the re-emerging malaria situation in South and North Korea (Paper III).

To determine why *P. vivax* disappeared from Finland the long time series from 1749 was analyzed (Paper IV). Correlation analysis was done with several variables (climate, animal husbandry e.g.) when they could not be ruled out by logical reasoning. The malaria statistics were also compared with sociological variables. The decline of the parasite can be then interpreted as a metapopulation dynamic process. Metapopulation biology is often expressed with mathematical models (Hanski 1998, Ariey et al. 2003, Ovaskainen & Hanski 2003). The aim with this case study was to find the essential factors that affect the populations of the parasite in a long time perspective (Paper IV). It can then serve as background for modelling in deciding which variables to parameterize.

MAIN RESULTS

1. The analysis of the phenology of *P. vivax* showed that the annual variations had a close connection with the phenology of the vector. In a stable population of *P. vivax,* in spring a malaria epidemic could be explained by a previous warm summer with a favourable development of the larvae of the *Anopheles* vector (Paper I). In a cold climate malaria was an indoor disease transmitted by the mosquitoes that overwintered in the human cottages (Paper I).
2. The triggering mechanism of the dormant stage of *P. vivax* has been unknown. It was now shown that the bites of even uninfected *Anopheles* mosquitoes trigger a relapse (Paper II). This optimizes the chances for the *Plasmodium* to reach a mosquito vector for sexual reproduction. The longevity of the hypnozoites could be shown to be at least nine years (Paper II).
3. A malaria epidemic occurred among Finnish troops during World War II. By then indigenous malaria had almost disappeared. Contrary to earlier theories (Hernberg 1947), it was not caused by mosquitoes flying over the frontier. The epidemic

could be explained by the mosquito induced relapses (Paper III). Because the epidemic started in July before infective mosquitoes existed the earliest malaria cases must have been triggered by the new uninfected mosquito generation. Later when the drafted soldiers lived in crowded quarters overwintering vectors triggered further relapses in those soldiers who still carried hypnozoites. From them, the disease became transmitted to new vectors that infected healthy soldiers. The re-emergence of malaria in Korea was explained by the same mechanism (Paper III).

4. The decline of *P. vivax* already started in the 18th century. It was shown that only sociological factors affected the long time trend. The parasite manipulated the vector, which for the declining trend became a constant (Paper IV). The main factors were the size of household, housing conditions and changes in local settlement structure (Paper IV).

Plasmodium vivax and climate

The July isotherm of 16 °C is often mentioned as the restricting barrier for *P. vivax* (Kettle 1995, de Zulueta 1987, Garnham 1980, Garnham 1988). This was established by the German physician August Hirsch even before the *Plasmodium* was known. He claimed that a summer temperature of 15 – 16 °C determined the border of indigenous malaria (Hirsch 1881). Already the distribution of the parasite in Finland shows that this assumption is not correct (Paper I, IV). The distribution of *A. messeae* and *A. beklemishevi* on the European continent shows that there is vector capacity present from the Baltic to the Arctic Sea. The only European country with no *Anopheles* species is Iceland (Erling 1991). It is also the only European country where there never has been indigenous malaria (Bruce-Chwatt & de Zulueta 1980, MacArthur 1951).

Humans cannot survive in a cold climate without sufficient shelter with a kind of tropical microclimate. Those shelters also served as overwintering places for sporadic *Anopheles* females. The *Plasmodium*, which spent most of its life in the human host, met the mosquito indoors. Inside the houses, the temperature was sufficient for the sporogony in the vector (Paper I). The parasite depends on a temperature above +16 °C only for the sporogony. When the sporozoites are developed they are not so sensitive. In Uzbekistan sporozoites of *P. falciparum* survives in overwintering *Anopheles sacharovi* at winter ambient temperature hardly above freezing (Shishlyaeva-Matova 1952).

It is easy to identify a phenological pattern of *Plasmodium vivax* in the Finnish material (Paper I, II). It started with relapses in August and had a peak in May (Paper II). The peak in spring could also be observed in northern Russia and Siberia (Lysenko & Kondraschin 1999). Several reports of malaria cases during winter from different parts of Europe were documented by the Malaria Commission in 1927 (Lane 1931). In Holland N.H. Swellengrebel collected anophelines in the same house during 1934–1937. He observed both infected and uninfected *Anopheles* females from August to February (Swellengrebel & de Buck 1938).

The natural behaviour of *A. messeae* is not identical with the behaviour of that small fraction of the population that acted as vectors. The typical overwintering sites for the species are cool, draught-free places without domestic animals. During winter, the hibernating females used energy stored in the fat body (Jaenson & Ameneschewa 1991). In warm conditions their behaviour change (Paper I, IV). This has already been observed by

Lewis Hackett in the beginning of the 1930's (Hackett 1933). He studied the morphological characters of the *A. maculipennis* complex and compared the hibernation habits of *A. atroparvus* and *A. messeae*. He stated that: "Placed in a warm environment, both races will suck blood in winter, showing that the need for food depends on the physical conditions (microclimate) of the place chosen for overwintering and not vice versa". He further stated that: "Both *messeae* and *atroparvus* are ordinarily cattle feeders but both, on occasion, will enter human habitations to bite" (Hackett 1933).

The Finnish data set showed that the long time trend of indigenous malaria is independent of climatic trends and that vector capacity is constant (Paper IV). This cannot, however, be observed in the short series of malaria statistics, which show a high correlation with the temperature (Paper I, II, IV). A typical feature for the mosquito fauna in Finland is large annual variations. After a summer with a favourable development of larvae, more adult females would try to hibernate. A typical spring epidemic in a malarious area could therefore be explained with the previous warm summer.

The advantage of a relapse triggered by mosquitoes

The main result of this thesis was the discovery of the triggering mechanism behind the relapse in *Plasmodium vivax,* which was studied by statistical evidence (Paper II). The key to the immense success of *P. vivax* is the ability to detect and react on the presence of anophelines. It is able to produce gametocytes at a time when the mosquito vectors are present. This has been especially important in the northern areas where the human population is scarce and there might be several years between the optimum conditions. The parasite compensated with a long dormant lifespan in its human host. It has to be emphasized that *P. vivax* probably has evolved in Southeast Asia when primitive humans still lived in small groups between 217,000 and 304,000 years ago (Cornejo & Escalante 2006). Such a nomadic group would often be on the move. Under those conditions longevity and control of the relapse was necessary for the survival of the parasite. Where ever the human group settled for a while, local *Anopheles* populations would trigger relapses to insure a new round for the *Plasmodium*.

It was shown from the Finnish data that triggered relapses could be detected at least nine years after a primary infection in the less malarious eastern parts, but in the more malarious southwestern part of the country the incubation time was shorter (Paper II). In a highly malarious area, the relapses could be statistically demonstrated only for two years after the primary infection (Paper II). The relapse from hypnozoites would in more malarious areas often be simultaneous with a primary infection caused by the bite of another infected vector.

The difficulties in eradicating *P. vivax* can be explained by the parasite's ability to control the relapses. Often widespread epidemics of *vivax* malaria have started in sociologically critical situations although the parasite seemed almost gone. This happened in many European countries during World War II (Bruce-Chwatt & de Zulueta 1980). The analysis of positional warfare malaria in Finland and the re-emerging malaria epidemic in Korea demonstrated how the epidemics can start when human carriers are accommodated in crowded surroundings together with non-carriers. It creates an excellent opportunity for the parasite to activate and transmit to the vector and other human hosts (Paper III). During crises, it is often impossible to carry out strict mosquito control. Therefore the risk of re-emergence mainly depends on the social circumstances of the healthy human carriers, not on changes in mosquito populations.

The parasite-controlled relapse has several advantages. The formation of hypnozoites decreases the degree of in-host competition between the sporozoites. They will then undergo asexual reproduction separately from each other. Because the hypnozoite can be triggered by the presence of biting *Anopheles* mosquitoes, the *Plasmodium* is able to maximize the chances for sexual reproduction when mosquitoes are abundant. The hypnozoites from one batch of sporozoites are not genetically identical and the gametocytes from different hypnozoites will be genetically slightly different. This also maximizes the chances for the parasite to adapt to the possible changes of vector species. It would also partly explain the fast growing emergence of drug resistance. Standard primaquine dosing has in many cases been too low (Schwartz et al. 2000, Baird & Hoffman 2004). Sometimes not all of the hypnozoites have been affected. In such a case, a human carrier would after several unsufficiently treated infections get a relapse from hypnozoites that won't be affected by further use of the drug.

The considerable genetic diversity in *P. vivax* has been emphasized in several studies (de Souza-Neiras et al. 2007, Maestre et al. 2004, Cui et al. 2003). The observed polymorphism of the parasite is also one of the major problems in malaria vaccine development (de Souza-Neiras et al. 2007). The studies, however, do not explain how the parasite manages recombination. Theoretically, recombination can occur when the human host is infected by two different plasmodia strains during a time window of one week, before the emerging of merozoites in the blood which suppresses further infections from sporozoites (Paul et al. 2003, Kettle 1995). This mechanism would not alone explain the genetic diversity and population structure of *P. vivax*.

A mosquito-induced relapse optimizes the possibility for the meiotic recombination of the *Plasmodium* in the vector (Paper III). A human that carries hypnozoites and moves to another region can be infected by a vector carrying a different strain of *P. vivax*. Except for the sporozoites that cause the primary infection some will develop into hypnozoites. A human host can therefore carry hypnozoites of several strains. If the human carrier is bitten by an infected mosquito, the bite might activate a hypnozoite at the same time with the primary infection. The presence of uninfected vectors can in turn activate hypnozoites synchronously from different strains. In both cases different strains produce gametocytes nearly simultaneously and enable meiotic recombination during the sexual reproduction in the vector.

Triggered relapses in *P. vivax* could be used in the battle against malaria. By artificially producing the active components of the anopheline saliva, the whole infection process could be controlled. A deliberate triggering of the hypnozoites and simultaneous use of blood stage medication would be a more effective treatment of malaria. The *Plasmodium* cannot develop resistance against the trigger because it is dependent on that mechanism to survive. Artificially triggered relapses with medication could also be used in the process of eradicating *P. vivax* locally in regions with vector seasonality. By treating the whole population, first with the activation of possible hypnozoites and then, in case of malaria, by medication, the transmission rate of *P. vivax* would drop radically during the next mosquito season.

Traditionally, malaria has been described as a result of two mosquito bites: the ingestion of gametocytes from humans and the inoculation of sporozoites into humans. The results from Finland indicate that for *P. vivax* three mosquito bites are needed: the activation of the dormant stage in humans, the ingestion of gametocytes from humans and the inoculation of sporozoites into humans. A simplified schema of the new life-cycle of *P. vivax* is presented in Fig. 3. It is suggested that a corresponding life-cycle is valid for all malaria species in conditions with distinct seasonality in climate.

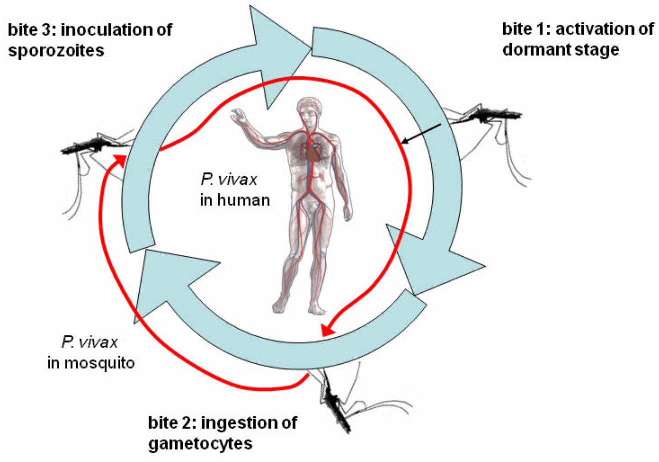


Figure 3. Proposed three-step malaria life-cycle

The decline and extinction of *Plasmodium vivax* in a metapopulation dynamic concept

The classic metapopulation concept has been an important tool for conservation strategy and for examining populations in highly fragmented landscapes (Hanski 2004, 2001). The distribution of many species can be described as a system of local (meta) populations. A metapopulation has been defined as a group of subpopulations that live in habitat fragments or patches balanced by colonization and extinction (Levins 1969). Each of them faces extinction and recolonization by dispersing individuals. The key processes; colonization and extinction, have to be in balance to maintain a stable population. Most of the applied work with real metapopulations has been done on insects, mammals, birds and a reptile with limited distribution (Hanski 1999, 2001). The direct impact of the theory on infectious diseases has been limited. Nevertheless epidemiologists have had the same approach to understand diseases. Both conservation biology and epidemiology are concerned with the persistence of patchily-distributed species (Grenfell & Harwood 1997).

In metapopulations of parasites the definition of a patch has to be discussed. Usually, each host organism can be considered as a habitat patch, which contains a local population of the parasite (Lawton et al. 1994, Hanski & Gilpin 1997). This definition was also used by Ariel et al. in their model of *Plasmodium falciparum* for the interpretation of the spreading of resistance against chloroquine (Ariey et al. 2003). However, the definition of a patch in metapopulations of parasites using vectors is more complicated. In many cases, the metapopulation analogy applies when groups of hosts are considered as patches (Grenfell & Harwood 1997). To survive the *Plasmodium vivax* needs two hosts; the human and the vector. It is therefore meaningless to define only the “human container” for the parasite as the patch. The life of the parasite in the vector is of equal importance. Only the vector can transmit the parasite from an infected human host to an other human host. The habitat needs therefore to cover the interaction between the host and the vector. That aspect has been analyzed in the extensive Soviet research of medical geography. The Russian concept of “nosofocus” would for a vector borne disease as vivax malaria in a metapopulation dynamic system correspond to the patch. It has been defined as the region or place of interaction between groups of people and the population of the causative agent (Beklemishev & Zhelokhovtsev 1945, Beklemishev 1959, Lysenko & Yashkul 1967).

The sporozoites from the original vivax infection have, as hypnozoites, a lifespan of several years (Paper II). For the sporogony of *Plasmodium vivax* to be completed in the vector, 16 days in +20 °C are needed (Kettle 1995). A patch/nosofocus in this context would therefore be a human bedroom or a human nocturnal resting place with *Anopheles* mosquitoes present. A bedroom with only one person is an unavailable isolated patch even though the sleeping person carries the parasite and gets relapses and new infections. That patch will evidently meet with extinction with the death of the carrier. The number of vectors needed is small, in theory one is enough. For the parasite in the malarious Finland, the landscape was highly fragmented. Because of the cold climate, the vector could not switch patch during the transmission season (Paper I). Only the human host was mobile.

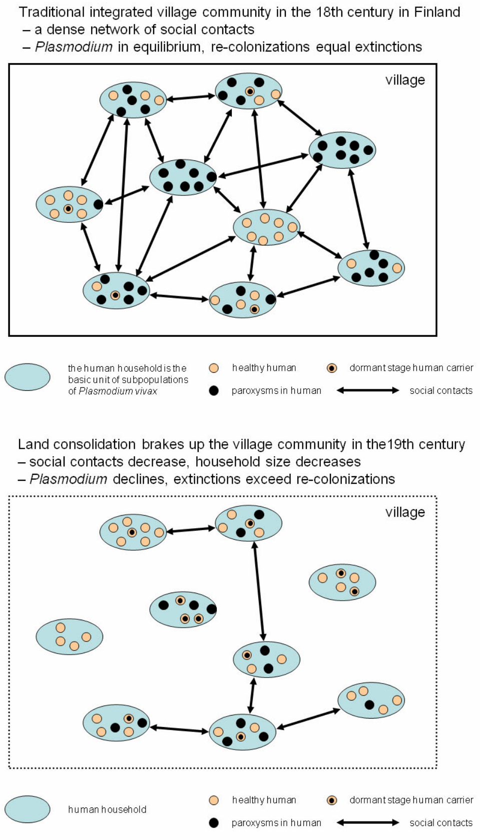
The decline and extinction process of *vivax* malaria in Finland lasted about 200 years. There were no radical changes in the trend of malaria frequency in spite of large annual variations. The disappearance of malaria was the result of slow sociological changes in human society. When the household size had dropped from 8–10 to 4 members, indigenous malaria finally disappeared (Paper IV). The inability of the parasite to re-colonize the country during the epidemics in World War II indicated that the housing and sociological conditions in peace time prevented malaria from being an indigenous disease (Paper III). The analysis of the decline showed that only factors that affected the human host were significant (Paper IV).

Overwintering *Anopheles* mosquitoes are still present in Finnish summerhouses though they no longer transmit malaria (Paper I, IV). It is therefore safe to presume that *Anopheles* mosquitoes could be found in practically every cottage in the traditional Finnish rural society before World War II, except for the first summer months. For the long time trend of malaria in Finland mosquitoes can be considered a constant (Paper IV). In the 18th century, a temporary decline of the vector populations would not have had any influence on the long time malaria trends. It would have been seen as a temporary drop in the number of malaria cases, but the disease would immediately recover because of the hypnozoites.

The connection between household size and malaria was already made several decades ago. In 1929, James explained that the spread of malaria depended on the factors that brought the source, the carrier and the recipient into the necessary close association with each another. It was therefore obvious, that in an English cottage where the whole family slept together in one room this association was closer than in a house containing several bedrooms. This was the main reason why the number of malaria cases always was higher among the poor than among those who could afford better houses (James 1930).

In the habitat patches of a stable system, there were frequent extinctions and recolonizations of *Plasmodium*. This was the case in 18th century Finland (Fig. 4). It is not possible to form a consistent mathematical model of the declining pattern of *P. vivax* with the Finnish data. Several factors had an impact on parameters like colonization and extinction rate. Colonization rate is defined as a sum of contributions from all fragments surrounding the focal fragment weighted by their area, distances from the focal fragment and their probability of beeing occuped (Hanski 2001). In the Finnish metapopulations of *P. vivax* colonization and re-colonization were always done by humans. Distance in this case is predominantly a social concept, and only secondarily a spatial factor. During the decline of malaria in Finland, the sociological changes affected the possibility for the parasite to find a new patch. In spite of the human host's increasing mobility, the possibilities for colonization decreased.

In the 18th century Finland, the *Plasmodium* had many possibilities to colonize empty patches. Travelling was often done during the transmission time in winter. It was slow and a day's journey by horse was usually 25 km (Talaskivi 1939). The inns usually had only one heated room for travellers, which meant a lot of people sleeping together. It was also common to seek temporary accommodation in other people's household (Nenonen 1999). In those cases, the travellers slept together with the household members on the floor. The communication became faster with railroads, canals and better roads in the 19th century. The quality of the inns also improved. Travellers needed less accommodation for the same journey and it became less common to seek temporary lodging among the peasants (Mauranen 1999). The number of nights spent together with people from other households decreased. This meant that the isolation of the patches grew and became more inaccessible for the parasite. The problems of how to differentiate between unoccupied and inaccessible patches has been discussed by Doncaster et al. (1996) They argued that a suitable habitat should be considered unoccupied only if it was accessible. Isolation should, as a potentially limiting factor, be included in the analyses.



Traditional integrated village community in the 18th century in Finland
– a dense network of social contacts
– *Plasmodium* in equilibrium, re-colonizations equal extinctions

Land consolidation brakes up the village community in the19th century
– social contacts decrease, household size decreases
– *Plasmodium* declines, extinctions exceed re-colonizations

Figure 4–5. The isolation of *P. vivax* populations in Finland.

The colonization rate was also affected by migration. Marriages, the employment of maids and hired hands would have affected the extinction in one patch and the colonization in another. The disintegration of the villages also changed the success of colonization of the *Plasmodium*. In the traditional villages with cottages close to each other, there must have been a lot of sleep over among the children. When the houses were built further apart, that became more difficult (Fig. 5).

To calculate the extinction rate would be impossible in the Finnish data. The most important factor must have been the death of a carrier. The general death rate was high (30.4 ‰ in 1761–1770, Vattula 1983). How often the *Plasmodium* was eliminated without being able to reach sexual reproduction is unknown. Only 4.7 ‰ of the death cases 1750–1850 was caused by malaria.

Equally important as the changes in the possibility for the colonization of the patches were the changes in the patches themselves. The total number of potential human hosts increased from 421,500 in 1750 to 4,029,803 in 1950 (Statistical Yearbook of Finland 2003). During the same time, the household size decreased (Fig. 4–5, Paper IV). The extented family household became gradually replaced by the nuclear family household. The standard of the houses became gradually higher. They were built bigger with several bedrooms. The social differentiation grew and it became less common for the maids and hired hands to sleep together with the family (Paper IV). Since there were fewer people sharing a bedroom, the possibility for the *Plasmodium* to find another human host decreased. From the parasite's point of view the quality of the patch decreased although there still were available vectors. It became increasingly more difficult to find a new human host.

The urbanization during the first decades of the 20th century (Vattula 1983) created a situation in which there were human carriers with hypnozoites in the liver without getting a relapse. The political crises changed the development. During the continuation war, temporary "good" patches were created. The soldiers, of whom some still were hypnozoite carriers, slept in crowded quarters together with overwintering vectors. Consequently, an epidemic of malaria broke out (Paper III). When the situation returned to normal after the war, it again became difficult for the parasite to survive. The low number of people sleeping in the same bedroom made the patches too small and isolated for indigenous malaria.

The use of metapopulation theory as a tool for conservation biologist has been questioned. The main critic argued that many species have metapopulations only towards the range limits and that the usability of the models are therefore limited (Baguette 2004). The statements by Baguette have been thoroughly questioned (Hanski 2004). The spatially realistic version of the classical metapopulation theory is useful for examining the dynamics in highly fragmented landscapes (Hanski 2001, 2004). The critique still seems to get some superficial support when the dynamics of the whole distribution area of *Plasmodium vivax* is discussed. Lysenko & Semashko have analyzed the dynamics of the distribution area. They stated that the reasons for regression depended on two main reasons: factors connected with man's economic activity, and activities carried out to combat malaria. The former was considered spontaneous and took place in countries with temperate climates on the peripheries of the distribution area (Lysenko & Semashko 1968). They also emphasized themselves that spontaneous regression did not take place in Russia, which was an underdeveloped country (Lysenko & Semashko 1968). However, the difference between conscious and unconscious measures against malaria is irrelevant when the parasite is studied in a metapopulation dynamic system.

Although the principles of metapopulation theory can be used to explain the decline of *vivax* malaria in Finland, it cannot readily be quantified in a model. Several important variables cannot be sufficiently estimated. The social variables could, together with the effect of medication and mosquito control indoors, be quantified locally in a modern society. Metapopulation models should therefore be developed and used as a tool for planning the eradication of malaria. They could also be used for projecting future seasonal epidemics in critical situations like refugee camps. The Finnish case study shows that by systematically increased isolation of the *Plasmodium* in the patch with human carriers the possibilities for eradication increases.

The adaptation of the concept of metapopulations of *Plasmodium* allows new approaches for the fight against malaria. Land development and social planning should be analyzed for their eventual impact on the patches of the parasite. The number of bedrooms with mosquitoes could be reduced by building multi-storeyed houses. To increase the fragmentation of the landscape for the parasite it would be important to prevent or reduce the number of vectors that seek blood meals in more than one house. That would be the case when villages are split and houses are built further apart. Other local solutions like fences or walls with climbing vines that offer resting places could also make the mosquitoes to return to the same house. Conservation biologists have with the development of metapopulation dynamic models created a powerful tool for the protection of endangered species. That same tool should be adapted for the opposite: the eradication of human parasites.

CONCLUSIONS

The relapse mechanism in *vivax* malaria has long been an enigma to the scientific community. The demonstration that the hypnozoite responds to the bite of an *Anopheles* mosquito gives a new tool for the understanding of malaria epidemics and will constitute a new base for medication. If hypnozoites can be artificially activated under controlled conditions, the medication will be more effective and further relapses prevented.

The extinction of *P. vivax* was caused by sociological changes. The vector was a constant during the long time process. Although the principles of metapopulation theory can be used to explain the decline, it could not be quantified in a model. Several important variables could not be sufficiently estimated. The social variables could, together with the effect of medication and mosquito control indoors, be quantified locally in a modern society. Metapopulation models should therefore be developed and used as a tool for planning the eradication of malaria. They could also be used for projecting future seasonal epidemics in critical situations like refugee camps. The Finnish case study shows that by lowering the quality of the patch by reducing the number of human hosts within it and at the same time increasing the isolation between the patches, the possibilities for eradication increases.

The adaption of the concept of metapopulations of *Plasmodium* allows new approaches for the fight against malaria. Land development and social planning should be analyzed for their eventual impact on the patches of the parasite. The number of bedrooms with mosquitoes could be reduced by building multi-storeyed houses. To increase the fragmentation of the landscape for the parasite it would be important to prevent or reduce the number of vectors that seek bloodmeals in more than one house. That would be the case when villages are splitted and houses are built further apart. Other local solutions like fences or walls with clinging vines that offer resting places could also make the mosquitoes return to the same house. The development of metapopulation models could offer a tool that, in an efficient way, evaluates the different measurements in the struggle against malaria.

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