Spatial distribution of malaria transmission in relationship to *Anopheles gambiae* complex members in Sudan savanna and irrigated rice cultivation areas of Mali.

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Summary

Malaria remains a major public health problem that is exacerbated by poor implementation of control measures, and by the spread of drug-resistant parasites and insecticide resistant vectors. Preventive measures, including those targeted at vectors, are one of the four basic elements of the global malaria control strategy. The control methods to use should be selective and specific to the control area. The success of the approach of selective and targeted interventions requires a good stratification of control areas, which should be based on mapping of malaria risk and vector species distribution.

The goal of this thesis was to enhance our understanding of the relationship between the distribution of members of *Anopheles gambiae* complex and climatic and environmental conditions, to describe their spatial and temporal distribution, to quantify their unique contribution to malaria transmission, and to produce attributed malaria risk maps of Mali. We used Bayesian geostatistical modeling, implemented via Markov chain Monte Carlo simulation (MCMC), which can quantify the relationship between environmental factors and the species distribution by taking into account the spatial dependence present in the data in a flexible way that allows simultaneous estimation of all model parameters. In addition, Bayesian kriging enables model-based prediction together with the prediction error, a feature which is not possible in the classical kriging.

The analyses described in chapters 2 and 3 identified environmental factors related to the distribution of a) the two major species (*An. arabiensis* and *An. gambiae s.s.*) which compose the *An. gambiae* complex and b) the chromosomal (Bamako, Mopti, Savanna Hybrids) forms of *An. gambiae s.s.*, and produced maps of the geographical distribution of the species and chromosomal forms. Estimation of the contribution of species and chromosomal forms to malaria transmission in Mali is described in Chapter 4; the spatio-temporal

distribution of *An. gambiae* complex densities and its chromosomal (Mopti, Bamako, Savanna, Hybrids) forms in a Sudan savanna village is examined in Chapter 5; the investigation of malaria vector ecology during the dry season and its implication for vector control is described in Chapter 6, and Chapter 7 presents the spatial pattern of malaria transmission in the rice cultivation area of the Office du Niger.

The maps produced in chapters 2 & 3 showed higher frequencies of *An. arabiensis* in the drier Savanna areas and *An. gambiae s.s.* in the flooded/irrigated areas of the inner delta of Niger river, the southern Savanna, along rivers and in the Sahel. The Mopti form was found in the same ecological area as *An. arabiensis*. In addition, it occupied the flooded/irrigated areas of the inner delta of Niger River. The Savanna form prefers the Sudan Savanna areas and the Bamako form was confined around Bamako city and in part of Sikasso region (South of Mali). Analyses in Chapter 4 indicated that high malaria risk was associated with insecticide resistance gene (*kdr*) carriers (Bamako/Savanna chromosomal) and Hybrids compared to the non-carriers *An. arabiensis* and the Mopti chromosomal form, although the association was not significant. The attributed risk maps of the different species and subspecies indicated that in the middle West and South East part of the country malaria transmission risk is mainly due to *An. arabiensis*, in the irrigated/flooded areas malaria risk is attributed to the Mopti form, in the southern part to the Savanna/Bamako forms and in the southern areas of the region of Kayes to the hybrids. Thus these results suggest that insecticide control measures must be strengthened in the Sahelian (epidemic prone area) and irrigated/flooded areas where *An. arabiensis* and the Mopti chromosomal form, which have no or lower frequency of insecticide resistance gene, prevail. Any vector control by means of insecticides in the Southern part of the country, where the S molecular form (Savanna and Bamako) predominates, must be accompanied by a close insecticide resistance monitoring system.

The analyses carried out in Chapter 5 and 6 on the spatial distribution of the sibling species of *An. gambiae* complex in a savanna village showed that the distribution of mosquito densities was concentric with higher densities clustering at the periphery of the village at the beginning of the rainy season and during the dry season. This distribution was patchy during the middle and the end of the rainy season. The chromosomal forms were sympatric throughout the seasons. There was a spatial clustering in their relative frequency distribution changing over time in the village. The Mopti chromosomal form was the most abundant at the beginning and middle of the rainy season and the Bamako form at the end of the rainy season. Larval habitats monitoring showed that in the main village of Bancoumana nearly all larval habitats were human-made, rain-dependent and dried out 10-12 weeks after the end of the rainy season. At the same time, numerous natural puddles highly productive for anopheline larvae even during the dry season were located in the fishermen's hamlets. These were adjacent to the receding Niger River bed and 5 km away from the main village. Larval habitats in Bancoumana were re-colonized shortly after rainfall suggesting that mosquitoes emerging from the riverbed are an important source for the rain-fed water bodies of Bancoumana. This observation indicates that control interventions targeting the Mopti form should be implemented at the beginning and middle of the rainy season, while those targeting the Bamako form should be done at the end of the rainy season. In addition, appropriate vector control implemented in the fishermen's hamlet during the dry season and at the periphery of the main village at the beginning of the rainy season may be feasible, sustainable at low cost and may ameliorate malaria transmission in this area.

In chapter 7, the analyses of malaria transmission parameters in the rice cultivation area of the Office du Niger indicated a strong spatial correlation in mosquito densities, which is related to the rice cultivation environment. However, the spatial correlation observed in the parous rate (PR) and human blood index (HBI) was weak suggesting that these parameters are

more closely related to local conditions such as population behavior and economic status, and/or the presence of animals rather than similar environment over large areas. Since both the PR and HBI measure the vector-human contact rate, and hence the potential for malaria transmission intensity, attention must be paid to the local variations when implementing control strategies in rice cultivation areas.

This work makes a substantial contribution to the mapping of the spatial distribution of malaria vector species and subspecies which was previously limited by the lack of field data and appropriate statistical analyses. It also provides valuable information for conventional vector control as well as future implementation for genetically manipulated mosquitoes control method.

Zusammenfassung

Malaria ist noch immer eines der größten Probleme der Gesundheitswissenschaften, welches durch uneffiziente Ausführung von Kontrollmaßnahmen und durch die Verbreitung von Resistenzen in Parasiten und Vektoren gegen Medikamente und Insektizide verschlimmert wird. Vorbeugende Maßnahmen, wie Vektorregulierungen, sind eines von vier Hauptelementen der weltweiten Malaria Regulierungsstrategie. Der Wahl der Kontrollmethoden sollten Entscheidungen zur Zusammenstellung einer gezielten Vorgehensweise zur zeitlichen und räumlichen Vektorregulierung vorausgehen. Der Erfolg der gezielt ausgewählten Interventionen benötigt eine Aufteilung von Kontrollgebieten mit Hilfe von räumlich, aber auch zeitlich beachtenden Kartierungen des Malariarisikos und den Verteilungen der Vektorarten.

Das Ziel dieser Arbeit war es erstens das Verständnis von den Beziehungen zwischen den relativen Häufigkeitsverteilungen von Abstammungen des *An. gambiae* Komplexes und klimatischen und ökologischen Faktoren zu erweitern, zweitens deren räumliche und zeitliche Verteilungen zu bestimmen, und drittens ihren einmaligen Beitrag zur Malaria Verbreitung zu quantifizieren und die darauf zurückzuführenden Karten des Malariarisikos für Mali zu erstellen. Wir nutzten Bayes'sche geostatistische Modellierungen, die durch Markov Ketten und Monte Carlo Simulationen (MCMC) umgesetzt wurden, welche die Beziehung zwischen ökologischen Faktoren und der Artenverteilung unter Beachtung der flexiblen räumlichen Abhängigkeit der Daten widerspiegeln. Dies erlaubte eine simultane Abschätzung aller Modelparameter. Zusätzlich liesen Baye'sches Kriging eine modelbasierte Vorhersage samt Vorhersagefehler zu, was nicht durch klassisches Kriging ermöglicht worden wäre.

Die Analysen in den Kapiteln 2 und 3 identifizieren die ökologischen Faktoren die mit der Verteilung der zwei häufigsten Arten (*An. arabiensis* und *An. gambiae s.s.*) des *An. gambiae*

Komplexes und ausserdem der chromosomalen Formen (Bamako, Mopti, Savanna, Hybrids) von *An gambiae s.s*. in Verbindung stehen. Zudem wurden in diesen Kapiteln die Karten der geografischen Verteilung der Arten und der chromosomalen Formen erstellt. Kapitel 4 bestimmt die Mitwirkung von Spezien und chromosomalen Formen an der Malariaverbreitung in Mali. Kapitel 5 untersucht die räumlich-zeitliche Verteilung der *An. gambiae* Komplex-Dichte und ihrer chromosomalen Formen (Mopti, Bamako, Savanna, Hybrid) in einem Dorf in der sudanesischen Savanne. Kapitel 6 erforscht die Malariavektor-Ökologie während der Trockenperiode und ihre Folge auf die Vektorkontrolle. Zum Schluss, wird in Kapitel 7 das räumliche Muster der Malariaverbreitung in der Reiskultivierung im Office du Niger untersucht.

Die erstellten Karten aus Kapitel 2 und 3 zeigen höhere Häufigkeiten von *An. arabiensis* in den trockeneren Regionen der Savanne auf und für *An. gambiae s.s.* in den gefluteten/bewässerten Teilen des inneren Niger Deltas, der südlichen Savanne, entlang der Flüsse und im Sahel. Die Mopti-Form teilt sich die selben ökologischen Regionen wie An. Arabiesis. Zusätzlich belegt sie allerdings noch die gefluteten/bewässerten Teile des inneren Niger Deltas. Die Savanna-Form bevorzugt die sudanesischen Savannen und die Bamako-Form ist begrenzt auf das Gebiet um Bamako Stadt und Teile der Sikasso Region (im Süden Malis). Die Analysen aus Kapitel 4 machen deutlich, dass hohe Malaria Risiken mit den Insektizid-Resistenzgenen (kdr) tragenden chromosomalen Formen (Bamako/Savanna) assoziiert sind, im Gegensatz zu der nicht Resistenzgen tragenden Form Mopti. Allerdings war dieser Zusammenhang nicht statistisch signifikant. Die entsprechenden Risikokarten der verschiedenen Arten und Unterarten lassen den Schluss zu, dass im mittleren Westen und im Südosten des Landes das Malariaverbreitungsrisiko hauptsächlich auf *An. arabiensis* zurückzuführen ist. In gefluteten/bewässerten Gebieten ist das Malariarisiko gekoppelt mit der Mopti Form in den südlicheren Regionen bis zu den Savanna/Bamako Formen und mit der

Hybrid-Form in den südlichen Gebieten der Region Kayes. Diese Resultate legen nahe, dass Kontrollmaßnahmen besonderes im Sahel (epidemischen abgelegenes Gebiet) und gefluteten/bewässerten Gebieten mit *An. arabiensis* und der Mopti Form, welche nur selten oder gar keine Resistenzgene gegen Insektizide trägt, überwiegen. Jegliche Vektorkontrolle, die Insektizide im südlichen Teil des Landes einsetzen will, wo die S-molekulare Form (Savanna und Bamako) vorherrscht, muss von einem intensiven Insektizid-Überwachungsprogramm begleitet werden.

Die Untersuchungen der räumlichen Verteilungen der Geschwisterarten des *An. gambiae* Komplexes in einer Ortschaft der Savanne aus Kapitel 5 und 6 zeigten, dass die Verteilung der Moskitodichte konzentrisch war, mit hohen Vorkommen an den Grenzen der Ortschaft zu Beginn der Regenzeit und während der Trockenzeit. Diese Verteilung war lückenhaft während der Mitte der Regenzeit und zu deren Ende. Die chromosomalen Formen waren über alle Zeiten sympatrisch. Es fand eine räumliche Ballung in ihren relativen Häufigkeitsverteilungen innerhalb des Dorfes statt, die sich mit der Zeit veränderte. Die Mopti chromosomale Form war die am häufigsten vorkommende Form zu Beginn und in der Mitte der Regenzeit und die Bamako Form herrschte am Ende der Regnzeit vor. Untersuchungen der Lebensräume für die Larve zeigten, dass im Hauptort von Bancoumana nahezu alle Habitate vom Menschen gemacht wurden, sie vom Regen abhängig waren und nach 10 bis 12 Wochen nach Ende der Regenzeit wieder austrockneten. Es gibt aber auch noch zahlreiche natürliche Wasseransammlungen die selbst während der Trockenzeit noch besonders günstig für die Larven der Anopheles sind und sich in den Fischereigebieten befinden. Diese liegen benachbart zum Flussbett des Nigers und sind etwa 5 km vom Hauptort entfernt. Die Lebensräume der Larven in Bancoumana wurden bereits kurzzeitig nach einem Regenfall erneut besiedelt, was darauf schließen lässt dass Moskitos aus dem Flussbett einen wichtigen Träger für diese regengespeisten Wasserquellen in Bancoumana

darstellen. Die oben genannten Beobachtungen zeigen, dass Kontrollmaßnahmen, die auf die Mopti Form abzielen, zu Beginn und in der Mitte der Regenzeit gestartet werden sollten. Dahingegen sollten die Maßnahmen die auf Bamako abzielen am Ende der Regenzeit eingeführt werden. Zusätzlich könnten entsprechende Vektorkontrollen in Fischereigebieten innerhalb der Trockenzeit und an der Grenze zum Hauptort am Beginn der Regenzeit auf einem geringen Kostenniveau ausführbar sein, die die Malariaverbreitung in diesem Gebiet verbessern.

In Kapitel 7 wurden Analysen zu Parametern der Malariaausbreitung in Gebieten der Reiskultivierung im Office du Niger durchgeführt, der einer starken räumliche Korrelation zur Moskitodichte zugrunde liegt, vermutlich begründet durch die Umgebung der Reiskultivierung. Die räumliche Abhängigkeit, welche durch die Parous-Rate (PR) und den menschlichen Blutindex (HBI) gemessen wurde, war gering. Das legt den Schluss nahe, dass diese Parameter stärker mit den lokalen Bedingungen wie Bevölkerungsverhalten und ökonomischen Status, der Anwesenheit von Tieren usw. zusammen hängen als mit ähnlichen Umgebungen über weite Flächen. Da beide Messungen (PR und HBI) die Vektor-Mensch-Kontaktrate widerspiegeln, und daher auch das Potential haben die Malariaverbreitungsintensität darzustellen, muss die Aufmerksamkeit auf lokalen Veränderungen liegen wenn Kontrollmaßnahmen in Gebieten der Reiskultivierung durchgeführt werden.

Diese Arbeit steuert einen großen Teil zur Kartierung der räumlichen Verteilung von Malariaüberträgerarten und –unterarten bei, die bisher durch das Fehlen von Felddaten und geeigneten statistischen Analysen begrenzt war. Zudem stellt es außerdem wertvolle Informationen für konventionelle Vektorkontrollen bereit, sowie auch für zukünftige genetisch manipulierte Moskitokontrollmaßnahmen.

Résumé

La malaria demeure un problème important de santé publique qui est aggravé par une mauvaise mise en œuvre des mesures de contrôle, et à la résistance des parasites aux antipaludiques et des vecteurs aux insecticides. Les mesures préventives, y compris celles de la lutte contre les vecteurs, sont l'un des quatre éléments de base de la stratégie globale de lutte contre la malaria. Les méthodes de lutte à utiliser devraient être sélectives et spécifiques à la zone d'intervention. Le succès de l'approche des interventions sélectives et ciblées exige une bonne caractérisation eco- épidémiologieques des zones d'intervention qui devrait être basées sur la cartographie de la distribution du risque et des espèces vectrices de la maladie.

L'objectif de ce travail était d'approfondir notre compréhension de la relation entre les facteurs climatiques et environnementales et la distribution des membres d'*An. gambiae* complex , de décrire leur distribution spatiale et temporale, de quantifier leur contribution à la transmission du paludisme, et de produire des cartes de risque de la maladies due à chacun d'entre eux au Mali. Nous avons employé la méthode de modélisation Bayésienne utilisant la chaînes de simulation de Markov Monte Carlo (MCMC), qui est capable d'estimer la relation entres les facteurs environnementaux et la distribution des espèces de vecteurs en tenant compte de la dépendance spatiale présente dans les données d'une manière flexible permettant l'évaluation simultanée de tous les paramètres des modèles. En outre, le kriging Bayésien permet de faire la prédiction tout en estimant les erreurs commises, ce qui n'est pas possible avec la méthode de kriging classique.

Les analyses décrites dans les chapitres 2 et 3 ont identifié des facteurs environnementaux liés à la distribution : a) des deux principales espèces (*An. arabiensis* et *An.gambiae s.s.*) qui composent le complexe *An. gambiae* au Mali, et b) des formes

chromosomiques (Bamako, Mopti, Savane Hybrides) d'*An. gambiae s.s.*, et ont permises de produire les cartes de distribution géographique des espèces et des formes chromosomiques du complex. L'évaluation de la contribution des espèces et des formes chromosomiques à la transmission de la malaria au Mali est décrite dans le chapitre 4 ; la distribution spatio-temporelle des densités d'*An. gambiae* complex et de ses formes chromosomiques (Mopti, Bamako, la savane, hybrides) dans un village de la savane soudanienne du Mali est examinée dans le chapitre 5 ; l'étude de l'écologie des vecteurs et son implication pour la stratégie de lutte contre les vecteurs est décrite dans le chapitre 6, et enfin le chapitre 7 présente les résultats de l'analyse spatiale des paramètres de la transmission du paludisme dans la zone de riziculture irriguée de l'Office du Niger, Mali.

Les cartes produites dans les chapitres 2 et 3 ont montrées des fréquences élevées d'*An. arabiensis* dans les zones de savane sèches et celles d'*An. gambiae s.s.* dans les zones inondées et/ou irriguées du delta intérieur du fleuve Niger, la savane humide, le long des fleuves mais aussi dans le Sahel. La forme chromosomique Mopti partage la même zone écologique avec *An. arabiensis*. En plus elle occupe les zones inondées et/ou irriguées du delta intérieur du fleuve du Niger. La forme chromosomique Savane préfère les régions de la savane humide et la forme chromosomique Bamako était confinée autour de la ville de Bamako et une partie de la région de Sikasso (Sud du Mali). Les analyses du chapitre 4 ont montré une association entre un risque élevé du paludisme et les formes chromosomiques porteurs du gène de résistance aux insecticides (*kdr*) (Bamako/Savane) ainsi que les formes hybrides comparés aux non-porteurs que sont *An. arabiensis* et la forme chromosomique Mopti, bien que l'association n'était pas significative. Les cartes du risque du paludisme attribué aux différentes espèces et sous-espèce du complex *An. gambiae* ont montré la transmission est principalement due à *An. arabiensis* dans les parties centre-Ouest et Sud-Est du pays, dans les zone d'irrigation/ inondées, elle est due à la forme chromosomique Mopti;

dans la partie méridionale aux formes chromosomiques Savane/Bamako et dans la partie méridionale de la région de Kayes aux hybrides. Ainsi ces résultats suggèrent que des mesures de contrôle d'insecticide doivent être renforcées dans la partie Sahelienne (secteur enclin épidémique) et des zones d'irrigation et d'inondation où sévissent *An. arabiensis* et la forme chromosomique Mopti, qui ont la plus faible fréquence du gène de résistance aux insecticide jusqu'à présent. Toute méthode lutte à base d'insecticide dans la partie Sud du pays où la forme moléculaire S (Savane et Bamako) prédomine devrait être étroitement accompagnée d'un système de surveillance de résistance aux insecticide.

Les analyses effectuées dans les chapitres 5 et 6 sur la distribution spatiale des espèces du complex *An. gambiae* dans un village de la savane soudanienne du Mali ont prouvé que la distribution des densités de moustique était concentrique avec les densités les plus élevées groupées à la périphérie du village au début de la saison des pluies et pendant la saison sèche. Cette distribution était inégale en milieu et à la fin de la saison des pluies. Les formes chromosomiques étaient sympatriques tout au long des différentes saisons. Il y avait une aggregation spatiale dans la distribution de leurs fréquences relatives qui changeait au cours des saisons dans le village. La forme chromosomique Mopti était la plus abondante au début et au milieu de la saison des pluies et la forme chromosomique Bamako à la fin de la saison des pluies. Le suivi des gîtes larvaires a prouvé que dans le village mère de Bancoumana presque tous les gîtes larvaires étaient dues aux activités humaines et étaient dépendants des pluies. Ils s'asséchaient 10-12 semaines après la fin de la saison des pluies. Au même moment, de nombreux petits points d'eau fortement productifs en larves d'anophèle tout au long de la saison sèche ont été trouvés dans les hameaux des pêcheurs situés le long du fleuve Niger. Les gîtes larvaires à Bancoumana étaient recolonisés peu après la tombé des premières pluies. Ceci suggère que les moustiques émergeant du lit du fleuve pourraient être une source importante d'ensemencement des gîtes de Bancoumana. Cette observation indiquent que des

interventions de lutte visant la forme chromosomique Mopti devraient être mises en œuvre au début et au milieu de la saison des pluies, alors que celles qui visent la forme Bamako devraient être faites à la fin de la saison des pluies. En outre, une méthode de lutte antivectorielle appropriée mise en œuvre dans le hameau des pêcheurs pendant la saison sèche et à la périphérie de son village mère au début de la saison des pluies pourrait être faisable, soutenable à moindre coût et qui pourrait améliorer la transmission du paludisme dans la zone.

Dans le chapitre 7, les analyses spatiales des paramètres de la transmission du paludisme dans la zone de riziculture de l'Office du Niger ont montré une forte corrélation spatiale dans les densités de moustique, qui est probablement liée à l'environnement de la culture de riz. Cependant, la corrélation spatiale observée dans le taux parité (P.R.) et le tau d'anthropophilie (HBI) était faible suggérant que ces paramètres sont plutôt étroitement liés aux conditions locales telles que le comportement de la population et le statut économique, et/ou la présence des animaux plutôt que la similarité dans l'environnement. Puisque le PR et HBI mesurent le degré de contact vecteur-homme, et par conséquent le potentiel de transmission du paludisme, une attention particulière doit être accordée aux variations locales lors de la mise en œuvre des stratégies de lutte dans les zones de riziculture.

Ce travail apporte une contribution substantielle dans la cartographie de la distribution spatiale des espèces et sous-espèce des vecteurs de la malaria qui était précédemment limitée par le manque de données et des analyses statistiques appropriées. Il fournit également des informations précieuses pour la méthode de lutte conventionnelle des vecteurs aussi bien que pour la future méthode de lutte basée sur la manipulation génétique des moustiques.

Abbreviations

AEZ	Agro-ecological zones
AIC	Akaike's information criterion
AVHRR	Advance very high resolution radiometer
CI	Confidence Interval
CQ	Chloroquine
CTA	Combination therapy
DIC	Deviance Information Criterion
EIR	Entomological inoculation rate
ELISA	Enzyme linked immunosorbent assay
GIS	Geographic information system
GPS	Global Positioning System
HBC	Human bait collection
HBI	Human blood index
IVM	Integrated Vector Management
kdr	Knock dawn resistance
LRT	Likelihood ratio test
MARA	Mapping Malaria Risk in Africa
MBR	Man biting rate
MCMC	Markov chain Monte Carlo
MEWS	Malaria Early-Warning Systems
MRTC	Malaria Research and Training Center
NAG	Numerical algorithms group
NASA	National Aeronautic and Space Administration
NDVI	Normalized Vegetation Index

NGO	Non-governmental organization
NMCP	National Malria Control Program
NOAA	National Oceanic and Atmospheric Administration
PCR	Polymerase Chain Reaction
PR	Parous rate
PSC	Pyrethrum spray catches
SP	Sulfadoxine pyrimithamine
SWS	Soil water storage
TNF	Tumor Necrosis Factor
USGS	United States Geological Survey
VC	Vectorial Capacity
WHO	World Health Organization

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Chapter 1

1. Introduction

The term malaria means “bad air” as it was believed that it was caused by breathing the foul-smelling gases arising from marshy places. Although the signs and symptoms of malaria were known to physicians from early Egyptian times, the causative agent was first identified in 1880 by the French pathologist, Laveran. The role played by mosquitoes in the transmission of malaria was only identified in 1898 by Ronald Ross, a British bacteriologist.

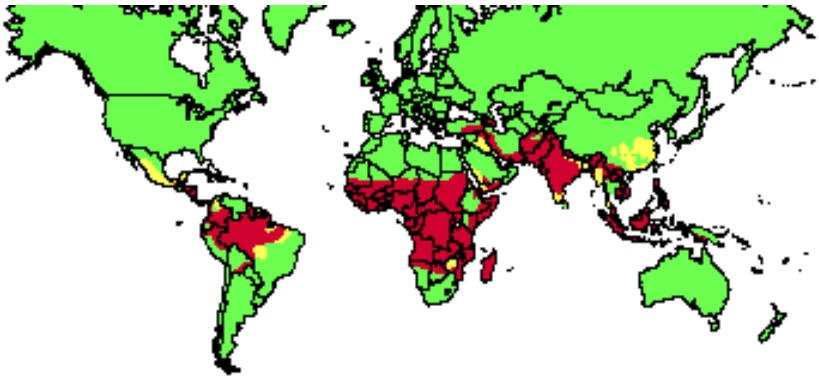
Nowadays, malaria is still one of the most severe public health problems worldwide. The disease is found across the globe in a near continuous belt through countries including India, Indonesia, and through the tropical parts of southern and central America (Figure 1.1). It is a leading cause of death and disease in many developing countries, where young children and pregnant women are the groups most affected. According to the World Health Organization (World malaria report 2005): some 3.2 billion people live in areas at risk of malaria transmission in 107 countries and territories; between 350 and 500 million clinical episodes of malaria occur every year; at least one million deaths occur every year due to malaria and about 60% of the cases of malaria worldwide and more than 80% of the malaria deaths worldwide occur in Africa, south of the Sahara.

1.2. Biology and epidemiology of malaria

Malaria is a vector born disease caused by protozoan parasites of the genus *Plasmodium*. There are four species of malaria parasites which can infect humans in natural

conditions: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The parasites are transmitted from person to person by female mosquitoes of the genus *Anopheles*.

The epidemiology of malaria results from the interaction between vector, parasite, human and physical environments and socio-economical situations. Thus, the sub-Saharan Africa region is more adversely affected than all other regions in the world because of: i) the presence of a very efficient mosquito vector (*Anopheles gambiae*) which assures high transmission; ii) the predominance of *falciparum* (*P. falciparum*), which causes the most severe form of malaria; iii) the local weather conditions which often allow transmission to occur year round; iv) the scarcity of resources and socio-economic instability which hinder efficient malaria control activities. In other areas of the world malaria is a less prominent cause of deaths, but can cause substantial disease and incapacitation, especially in rural areas of some countries in South America and South-East Asia.



Source: http://www.who.int/tdr/dw/malaria2004.htm

Figure 1.1: Global distribution of Malaria

1.2.1. Malaria parasite in human

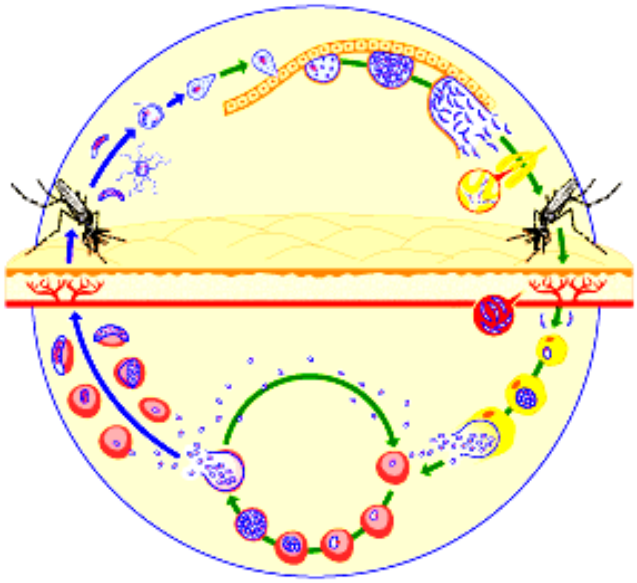
The life cycle of malaria is depicted in figure 1.2. After an infected bite from a female *Anopheles* mosquito, the sporozoïtes reach the liver in half an hour and invade the liver cells. The liver cells form a vacuole which separates the parasite from the host cytoplasm. Within this vacuole, the spororozoïtes start their intracellular asexual division leading to the schizonte. At the completion of this phase, thousands of erythrocitic merozoïtes are released in the blood from each liver cell. In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites (merozoïtes) that continue the cycle by invading other red cells.

This whole cycle of invasion-multiplication-release-invasion takes about 48 hours for *P. falciparum*. During this process, the content of the infected cells that are released with the lysis of the red blood cell stimulate the Tumor Necrosis Factor (TNF) and other cytokines, which results in the characteristic clinical manifestation of the disease. A small proportion of the merozoïtes undergo transformation into gametocytes. For *P. falciparum*, matures gametocytes appear in the peripheral blood after a period of 8-11 days of the primary attack, they rise in number in three weeks and decline thereafter, but circulate for several weeks.

1.2.2. Malaria parasite in the vector

When, during another blood meal from an infected person, gametocytes are picked up by a female *Anopheles* mosquito, they start another different cycle of growth and multiplication (sporogony) in the mosquito. The male and female gametes fuse and form into a zygote. This zygote transforms into an ookinete which penetrates the gut wall and becomes and oocyst. The oocyst divides asexually into numerous sporozoïtes which reach the mosquito's salivary glands. When the *Anopheles* mosquito takes a blood meal on another

human, the sporozoïtes are injected with the mosquito's saliva and start another human infection when they parasitize the liver cells. Thus the mosquito carries the disease from one human to another (acting as a "vector").



Source: http://www.who.int/tdr/diseases/malaria/lifecycle.htm

Figure 1.2: The life cycle of *P. falciparum*

1.2.4. The breeding cycle of the mosquito

The mosquito goes through four separate and distinct stages of its life cycle: egg, larva, pupa, and adult (Figure 1.3). Each of these stages can be easily recognize by its special appearance. Anopheline mosquitoes always lay their eggs on the surface of the water one at a time (50-200), with preference for swamps or shallow water. Mostly eggs hatch into larvae within 48 hours. The larva lives in the water and comes to the surface to breathe. Larvae shed (moult) their skins four times, growing larger after each moult. Most larvae have siphon tubes for breathing and hang from the water surface. The larvae feed on micro-organisms and organic matter in the water. The eggs development into adult requires about 7 to 14 days depending on water temperature. On the fourth moult the larva changes into a pupa. The pupal stage is a resting, non-feeding stage. This is the time the mosquito turns into an adult. It takes about 2-3 days before the adult is fully developed. When development is complete, the pupal

skin splits and the mosquito emerges as an adult. The newly emerged adult rests on the surface of the water for a short time to allow itself to dry and all its body parts to harden. The wings have to spread out and dry properly before the mosquito can fly.

Only female mosquitoes bite animals and require a blood meal to develop their eggs. Male mosquitoes do not bite, but feed on the nectar of flowers. They fly only short distances of few kilometers. Their preferred breeding location is close to human houses. Two to three days after a full blood meal is taken during the night or dawn, the female anopheline lays around hundred eggs. Thus it is clear that the ecology of mosquitoes is determined by the availability of the aquatic habitats and human environment.

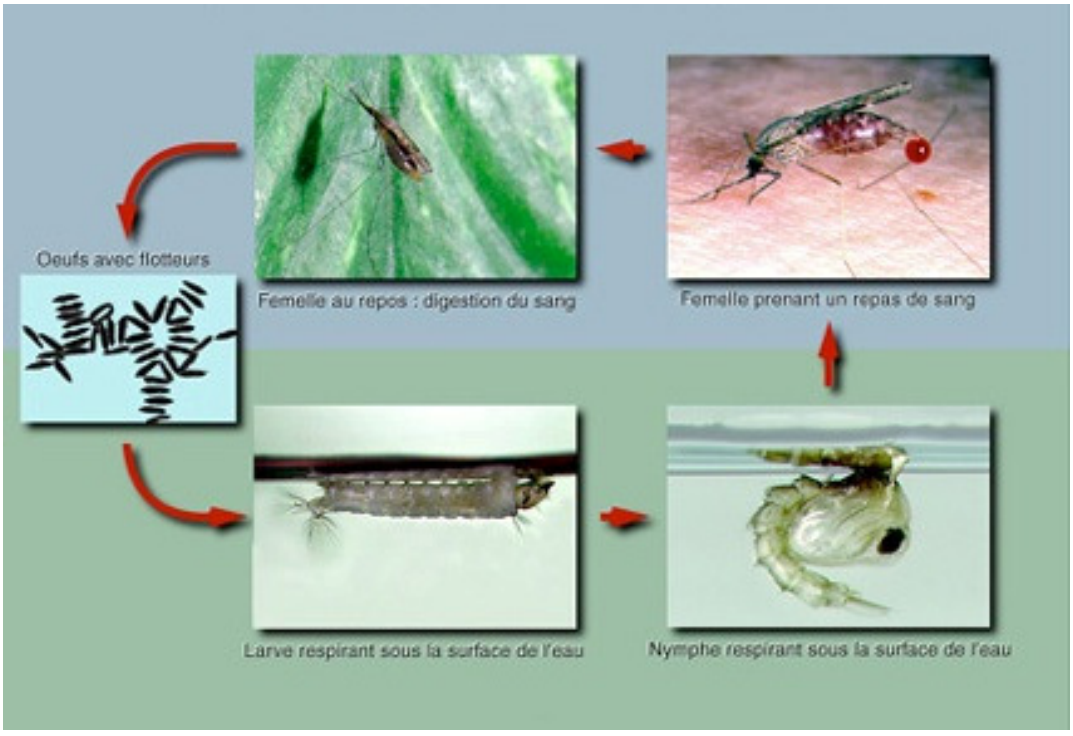


Figure 1.3: The life cycle of anopheline mosquito

1.2.5. Vector ecology

The biology and ecology of mosquitoes are intimately related to climate and environment. The survival of adult mosquitoes, hence the successful development of the malaria parasite in the mosquito, depends mainly on the ambient temperature and relative humidity. The minimum temperature required for the development and transmission of human malaria parasites by mosquito is about 15^{o}C for *P. vivax* and 18-19^{o}C for *P. falciparum*. The mean optimum relative humidity is at least 60% (Service, 1993). The altitude is a constraint for malaria transmission because of the low temperature. The near-surface humidity associated with rainfall enhances mosquito breeding habitats availability, flight activity and host-seeking behaviour. However excess rainfall can also alter the abundance and types of aquatics habitats available to mosquito for oviposition. The availability of suitable breeding habitats depends not only on rainfall but also on soil type (moisture, texture etc) (Horsfall and Porter, 1946; Peters 1965) and human activities (agriculture, construction etc). Man made ecological changes can lead to the formation of new ecological settings. Subsequently, there are changes in malaria vector species abundance and distribution and the pattern of the transmission.

1.3. Malaria vectors in Africa

Among the 30 to 40 malaria vector species in the world, *An. gambiae* complex and *An. funestus* are the primary vectors in Africa. The secondary malaria vectors are *An. nili* complex and *An. moucheti*. Here we will focus only on *An. gambiae* complex, the major malaria vector in Africa and Mali.

1.3.1. Anopheles gambiae complex

An. gambiae complex mosquitoes belongs to the order of Diptera, family Culicidae, sub-family Anophelinae and genus *Anopheles*. There is a great difficulty to identify its actual species because they are so closely related and they are virtually indistinguishable in term of shape, form and color. They can be only identified by experts who are trained to analyze the chromosome banding pattern (Figure 1.4) and the biochemical characteristics of certain enzymes in the mosquito. Why it is necessary to put so such effort for the sake of identifying malaria mosquito species? The reason is that each of these so-called sibling species has its own specific physiological requirements and these are in turn reflected in its behavior, host and ecological preferences. In turn, these characteristics have a direct bearing on its vectorial capacity.

The first suspicion of *An. gambiae s.l.* heterogeneity came from the pronounced various levels in its population vectorial efficiency in different areas coupled with some morphological variations. The heterogeneities in its responses to house spraying with insecticides for malaria control definitely confirmed its complexity (Coluzzi *et al*., 1979).

The first species recognized were the so-called salt-water forms, breeding on the West and East African Cost (Dutton, 1903; Ribband, 1944; Muirhead Thomson, 1948, 1951). *An. gambiae s.l.* was recognized by Davidson (1962) as six sibling species based on their reproductive barriers and cytotaxonomic characters. While the names *An. melas* Theobald (1903) and *An. merus* Donitz (1902) were well established for the western and eastern salt-water species respectively, a non-Linnean nomenclature was used for the others four members

Figure 1.4: The banding pattern of *An. gambiae* complex chromosomes.

of the complex, which were designed as species A, B, C and D. From synonymy with *An. gambiae*, White (1975) proposed *An. gambiae* (senso stricto) Giles (1902) for species A, *An. arabiensis* Patton (1905) for species B and *An. quadriannulatus* Theobald (1911) for species C. No formal name is yet available for species D. A seventh species was reported by White (1985) as *An. bwambae*, breeding in thermal springs and confined in Uganda.

The recognition of *An. melas* and *An. merus* was initially based on ecological evidence, coupled with slight morphological distinctions. For *An. gambiae*, *An. arabiensis*, *An. quadriannulatus* and species D, it was required complex laboratory techniques such as crossing experiments (Davidson & Jackson, 1962; Davidson, 1962, 1964; Paterson *et al*., 1963; Davidson & White, 1972), chromosomal investigation (Coluzzi, 1966; Coluzzi & Sabatini, 1967, 1968, 1969; White, 1972 and Davidson & Hunt, 1973), allozyme analysis (Miles, 1978), high performance liquid chromatography of cuticular hydrocarbons (Carlson & Service, 1980) and molecular methods (Collins *et al*. 1987; Hill & Crampton, 1994), which provided for each of the six sibling species reliable and sufficiently practical cytotaxonomic

characters. Nowadays the above characters are being used alternatively or together at operational level to distinguish the seven species of the Afro-tropical malaria vector of *An. gambiae s.l.*

An. gambiae Giles (species A) referred to as *An. gambiae* senso stricto is undergoing a complicated process of incipient speciation, particularly in West Africa, and is already characterized at both chromosomal (Coluzzi *et al*., 1985; Touré *et al*., 1998) and molecular (della Torre *et al*, 2001; Gentile *et al*., 2001) levels. Five chromosomal (Bamako, Mopti, Savanna, Forest and Bissau) and two molecular (M and S) forms have been identified.

In Mali, *An. gambiae s.l.* is the most abundant and widespread species. It is composed of *An. arabiensis* and *An. gambiae s.s. An. gambiae s.s.* comprises three chromosomal (Bamako, Mopti and Savanna) taxa and two molecular (M and S) forms. Savanna and Bamako taxa prevail in relatively humid savannas during the rainy season. Bamako is associated with riverside zones of the upper Niger River and Savanna with rain-dependent breeding sites. *An. arabiensis* and Mopti range from Sudan savannah to Sahel areas, breeding also during the dry season where permanent water is available (Coluzzi *et al*., 1985; Touré *et al*. 1998).

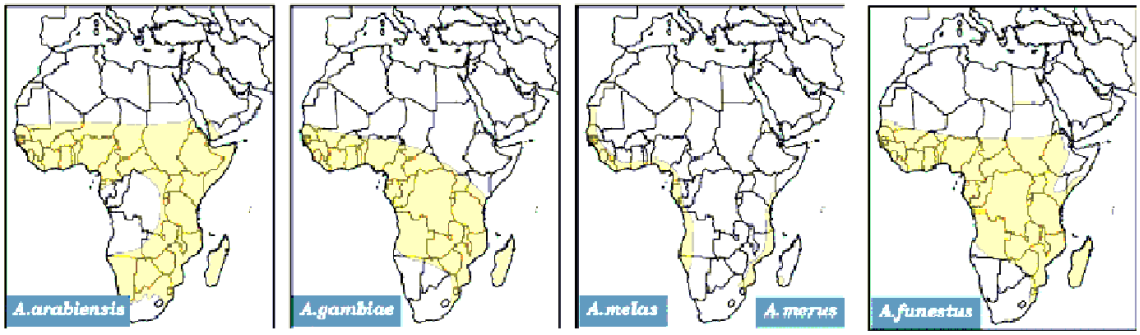
1.3.2. Anopheles funestus complex

An. funestus is the other important vector of malaria in Africa. It is a complex of nine species, with only *An. funestus funestus* recognized as a major vector. *An. funestus funestus* comprises one genetic population in several places, but two distinct chromosomally characterized populations (Kiribina and Folonzo) in Burkina Faso (Costantini *et al*., 1999)

and three populations in Senegal (Lochouarn *et al*., 1998). In Mali very few studies are done on the genetic aspects of *An. funestus*.

1.4. Geographic distribution of the major malaria species in Africa

Figure 1.5 shows the distribution of the two most important malaria vectors species in Africa. The two main species of *An. gambiae* complex are *An. gambiae s.s.* and *An. arabiensis*. Largely sympatric, they are the most broadly distributed species in Africa. They are found from the southern limits of the desert to the south of the continent through-out most of the continent including Madagascar (Powell *et al*., 1999). The other species of *An. gambiae* complex are: *An. quadriannulatus* narrowly distributed in south-East Africa and Ethiopia; *An. merus* and *An. melas* confined to the East and West coast, respectively because of their ecological differentiation into salt water; *An. bwambae* known only from the Semliki forest of Uganda where it breeds in mineral springs. Confined in specific areas, the adults of *An. merus*, *An. melas* and *An. bwambae* may contact the adults of the other members of the complex.



Source: http://www.itg.be/itg/DistanceLearning/LectureNotesVandenEndenE/imagehtml/ppages/CD_1074_067c.htm

Figure 1.5: Geographic distribution of the main malaria vectors in Africa

1.5. Vector control

Vector control aims to decrease contacts between humans and vectors of human disease. The current control method recommended by the World Health Organization is an integrated vector management (IVM), which includes insecticide treated nets (ITNs), indoor residual spray (IRS), and environmental management. The two former methods have drawbacks because of the development of insecticide resistance and the difficulties in achieving high coverages (Killeen *et al* 2002, 2004) in Africa. Larval control through source reduction and routine application of larvicide, which has been a key element in eradicating malaria in many parts of the world (Kitron *et al* 1989; Killeen *et al* 2002; Utzinger *et al* 2001) is largely neglected in sub-Sahara Africa, partly because of the perceived difficulty of identifying larval habitats in rural areas.

With the completion of the *An. gambiae* genome sequence and the development of molecular tools, novel methods for malaria control are emerging that are based on the use of genetically modified mosquito species that function as vectors for parasite transmission (Catteruccia, 2006). The main principles of genetic control are based on propagation of sterility or other desirable genetic factors in successive generations (Touré *et al*., 2004). A major concern regarding the introduction and spread of refractoriness genes is the possibility that they can not be integrated into natural malaria vector populations because of gene flow barriers (Lanzaro *et al*., 2003) and/or putative genetic adaptation to the environment (Alphey *et al*., 2002; Morlais *et al*., 2005).

The morphological similarity of sibling species, their ecological and behavioral differences and their reproductive isolation highlight the values of mapping their relative frequency to support targeted control measures.

1.6. Mapping malaria vector in Africa

Because of the laborious methods to identify the sibling species, field data are sparse to support malaria vector spatial distribution models (Lindsay *et al*., 1998). However, maps of malaria vectors spatial distribution have been produced. Most of them were only displaying the relative frequency of species at sampled locations (Touré *et al*., 1998; Coetzee *et al*., 2000; Onyabe & Conn, 2001) or using climatic suitability conditions of the species (Lindsay *et al*., 1998) or climate data (Bayoh *et al*. 2001). The most elaborated distribution map produced so far is the ecological niche modeling (Levine *et al*., 2004). All the predicted distribution maps currently available are at continental or sub-regional scale.

1.6. Objectives of the thesis

The main objective of this research was to assess association between the members of *An. gambiae* complex and climatic and environmental factors and to map their distribution in relationship to malaria transmission in Sudan Savanna and irrigated rice cultivation areas of Mali. The specific objectives were:

- To assess association between climate and environmental factors and the relative frequencies of the main species (*An. gambiae s.s.* and *An. arabiensis*) of *An. gambiae* complex in Mali and to produce continuous maps of their spatial distribution.
- To assess association between climate and environmental factors and the relative frequencies of the chromosomal forms (Mopti, Bamako, Savana, Hybrids) of *An. gambiae s.s.* in Mali and to produce continuous maps of their spatial distribution
- To quantify the contribution of the different taxa of *An. gambiae* complex to malaria transmission and to produce maps of their attributed malaria risk in Mali.
- To assess the spatial and seasonal distribution of *An. gambiae* complex densities and the chromosomal forms of *An. gambiae s.s.* in Bancoumana, Mali.
- To investigate dry season malaria vector ecology in a Sudan savanna village of Mali.
- To analyze the spatial pattern of malaria transmission parameters in the rice cultivation area (Office du Niger) of Mali.
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Chapter 2

The spatial distribution of *Anopheles gambiae* sensu stricto and *An. arabiensis* (Diptera: Culicidae) in Mali.

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Abstract

Variations in the biology and ecology and the high level of genetic polymorphism of malaria vectors in Africa highlight the value of mapping their spatial distribution to enhance successful implementation of integrated vector management. The objective of this study was to collate data on the relative frequencies of *Anopheles gambiae* s.s. and *An. arabiensis* mosquitoes in Mali, to assess their association with climate and environmental covariates, and to produce maps of their spatial distribution. Bayesian geostatistical logistic regression models were fitted to identify environmental determinants of the relative frequencies of *An. gambiae s.s.* and *An. arabiensis* species and to produce continuous maps of their geographical distribution. The frequency of *An. arabiensis* was positively associated with the normalized difference vegetation index, the soil water storage index, the maximum temperature and the distance to water bodies. It was negatively associated with the minimum temperature and rainfall. The predicted map suggests that, in West Africa, *An. arabiensis* is concentrated in the drier savannah areas, while *An. gambiae s.s.* prefers the southern savannah and land along the rivers, particularly the inner delta of the Niger River. Because the insecticide knockdown resistance (kdr) gene is reported only in *An. gambiae s.s.* in Mali, the maps provide valuable information for vector control. They may also be useful for planning future implementation of malaria control by genetically manipulated mosquitoes.

Keywords: *Anopheles arabiensis*, *Anopheles gambiae s.s.,* Bayesian inference, geostatistics, kriging, malaria

2.1. Introduction

There are approximately 400 species of mosquitoes of the genus Anopheles (Culicidae) of which 30- 40 transmit human malaria. In Africa, malaria transmission is mainly associated with *Anopheles gambiae sensu lato* (*An. gambiae s.l.*) and *An. funestus. An. gambiae s.l.* constitutes a complex of seven species with different abilities to transmit the parasite (White, 1974; Coluzzi *et al*., 1979; Coluzzi, 1984, 1994). In West Africa, the *An. gambiae* complex dominates, comprising mainly *An. gambiae s.s.* and *An. arabiensis* of which the former is itself undergoing a complicated process of incipient speciation. So far, five chromosomal (Bamako, Mopti, Savannah, Forest and Bissau) and two molecular (M and S) forms of *An. gambiae s.s.* have been identified (Coluzzi *et al*., 1985; Touré *et al*., 1998).

The species of *An. gambiae s.l.* and the genetic populations of *An. gambiae s.s.* vary in relative frequency, both seasonally and geographically. These remarkable differences in the biology and ecology and the high level of genetic polymorphism of the *An. gambiae s.l.* species highlight the value of mapping their spatial distribution to enhance effective implementation of integrated vector management (IVM) (Touré *et al*., 2004).

Maps of the spatial distribution of *An. gambiae s.l.* species have been produced by displaying the relative frequency of species at sampled locations (Touré *et al*., 1998; Coetzee *et al*., 2000; Onyabe and Conn, 2001), by climatic suitability conditions of the species (Lindsay *et al*., 1998) and by ecological niche-modeling (Levine *et al*., 2004). The latter links vector data with climatic factors using artificial-intelligence algorithms. However, only sparse data are available with which to build spatial distribution maps (Lindsay *et al*., 1998) and most of the predicted distribution maps currently available have been developed at the continental or sub-regional scale.

In Mali, the Malaria Research and Training Center (MRTC), University of Bamako, have gathered a countrywide dataset on *An. gambiae s.l.* species (*An. arabiensis* and *An. gambiae s.s.*) and sub-species (Bamako, Mopti and Savanna). We have now compiled, both published (Touré *et al*., 1998) and unpublished data from this database and used Bayesian geostatistical modeling to assess the spatial distribution of the two major vector species (*An. gambiae s.s.* and *An. arabiensis*) of *An. gambiae s.l.* in Mali. To our knowledge this is the first effort to produce maps of malaria vector species distribution adjusted for climatic factors using groundtruth data, and rigorous spatial statistical modeling at the country level.

2.2. Materials and methods

2.2.1. Description of the study area

The study area covers most of the territory of Mali in West Africa, i.e. a region between the latitudes 10° and 25° north and the longitudes 12° west and 4° east. Mali has an area of 1,240,000 Km2 and a population estimated at 13,000,000 inhabitants in 2003 by the United Nations. The country is relatively flat, altitudinal variations are minimal, ranging from 200 to 350 m above sea level. The year is divided into two main seasons varying in length according to the latitude: a dry season (October–May) and a rainy season (June–September) characterized by lower temperatures and increased humidity.

Mali is drained by two major river systems (Senegal and Niger) and characterized by the following six eco-geographic strata:

1. the southern Sudan savannah with an annual rain of 1300-1500 mm from May to October and a mean annual thermal amplitude of 5-6ºC;
2. the northern Sudan savannah with about 700-1300 mm annual rainfall distributed over less than 6 months;
3. the Sahelian zones with 200-700 mm of annual rain distributed over three months and

mean annual thermal amplitude of about 12ºC;

4. the sub-Saharan zone with less than 200 mm of annual rain and 16ºC of annual average thermal amplitude;
5. the inner delta of the Niger River, a kind of "internal sea" between the northern Sudan savanna and the Sahelian zones, about 300 km long and 100 km wide, which influences the climate of the area, especially by reducing the average annual thermal amplitude; and
6. the Sahara desert where drought limits mosquito breeding.

Except in the most northerly part in the Sahara desert, the country is endemic for malaria (hyperendemic to hypoendemic when moving from South to North). The main malaria vectors are *An. gambiae s.l.* and *An. funestus*. *An. gambiae s.l.* is composed of *An. arabiensis* and three chromosomal forms of *An. gambiae s.s.* named Bamako, Mopti and Savannah (Touré *et al*., 1983).

2.2.2. Vector data

Both published (Toure *et al*., 1998) and unpublished data of the different research activities of the MRTC, University of Bamako, Mali, were collated in a unique database. The data were obtained from cross-sectional and longitudinal surveys carried out between 1981 and 2004. Most surveys were conducted during the wet season (June-November). Survey sites were mainly small human settlements located in rural areas representing various eco-climatic zones of Mali. The database includes data collected from 94 locations and contains: (i) the total number of specimens; (ii) the count of *An. gambiae s.s.* and *An. arabiensis*; and (iii) the time of the survey (month and year). The specimens were differentiated by the chromosomal identification techniques (Coluzzi, 1968; Hunt, 1973) and/or by polymerase chain reaction (PCR) (Scott *et al*., 1993). The use of similar standardised techniques for sampling and processing mosquitoes across surveys ensured data consistency.

2.2.3. Climatic and environmental data

Factors used in this study were temperature, rainfall, the normalized difference vegetation index (NDVI), distance to water bodies, soil water storage (SWS) index, land use, agro-ecological zones and suitability to malaria transmission, a binary variable defined from environmental factors (Gemperli *et al*., 2006). A list of the data sources and spatial resolution is given in Table 2.1.

Table 2.1: Climatic data sources and spatial resolution used in the study.

Factor	Spatial resolution	Source
Temperature	5 km^2	Hutchinson *et al*. (1996)
Rainfall	5 km^2	Hutchinson *et al*. (1996)
NDVI	8 km^2	NASA-AVHRR Land data sets, Agbu & James, 1994
Land use	1 km^2	USGS-NASA
Water bodies	1 km^2	African Data Sampler World Resources Institute (1995)
Soil Water Storage Index	5 km^2	Droogers *et al*. (2001)
Agro-ecological Zone	Vector Coverage	FAO (1978)

For each location, temperature and rainfall data were available as monthly long-term averages. NDVI data were also summarized by monthly long-term averages of the original decadal values during the period of 1985 to 1995. The agro-ecological zones (AEZ) were distinguished on the basis of the length of the growing period and were defined as follow: (i) the Equatorial Forest zone (>270 days); (ii) the Guinea Savannah zone (165- 270 days); (iii) the Sudan Savannah zone (90-165 days); and (iv) the Sahelian zone (<90 days). In Mali only the last three AEZs can be found.

2.3. Data analysis

A buffer zone of 2 km around each data point was created using IDRISI 3.2 (Clark Labs, Clark University, MA, USA). The mean value of all pixels (with resolutions between 1

to 8 km^2 depending on the environmental factor) in this buffer area was calculated and used as the value of the given climatic and environmental factor. To take into account the possible lag time, between the rainfall and NDVI with the mosquito abundance, four summary measures (sum for rainfall and average for NDVI) were calculated for each one of the two climatic conditions:

1. the mean climatic value during the month of collection (mean_1);
2. the mean climatic value during the previous month (mean_2);
3. the mean climatic value during the month of collection and the previous month (mean_3);
4. and the climatic value during the collection month and the two previous months (mean_4).

Vector data obtained from surveys extended over a period longer than a month were available, but cumulative for the whole period instead of monthly. In this case the midpoint month was used to relate the climatic factors.

Bivariate logistic regression models were fitted in STATA 9.0 (Stata Corporation, USA) to assess the relation between the proportion of *An. gambiae s.l.* vectors identified as *An. arabiensis* and the climatic and environmental factors. The Akaike's information criterion (AIC) was used to select the best summary measure and lag time for the rainfall and NDVI. The statistical significance of the environmental factors was assessed using the likelihood ratio test (LRT). All factors significant at the 15% significance level were entered into a Bayesian geostatistical multiple logistic regression model. The model took into account spatial heterogeneity by including the location-specific random effects φ_i at the sampling location level. In particular, we assumed that the *An. arabiensis* frequency Yi at the sampling location i follows a binomial distribution, that is $Y_i \sim Bn(p_i, N_i)$, where Ni corresponds to the total number of *An. arabiensis* and *An. gambiae s.s.* mosquitoes collected, and pi represents the *An. arabiensis* proportion at the location i. We further assumed that φ_i models a latent

spatial process, that is φ = (φi ,... φN)T ~ MVN (0,Σ), with the covariance matrix Σ a function of distance between locations, irrespective of the locations themselves (stationarity) and of the direction (isotropy). We adopted an exponential correlation function, that is $\Sigma_{ij} = \sigma 2 \exp(-\rho d_{ij})$ where σ^2 is the spatial variance, ρ the parameter that models the rate of correlation decay, and d*ij* the distance between the locations *i* and *j*. Based on the above specification, the minimum distance for which the spatial correlation becomes less than 5% is calculated by 3/ρ; (Ecker and Gelfand, 1997). The model parameters were estimated using Markov chain Monte Carlo (MCMC) simulation methods.

Bayesian kriging was used to predict the species frequency at 85,000 locations that were not sampled (Diggle and Tawn, 1998). The Bayesian model fit was carried out in WinBUGS 1.4. (Spiegelhalter *et al*., 2004), whereas the model prediction was implemented in Fortran 95 (Compaq Visual Fortran, Professional 6.6.0) using standard numerical libraries (NAG, The Numerical Algorithms Group Ltd).

2.4. Results

The results of the bivariate logistic regression analyses are shown in Table 2.2 which indicates that, among the four NDVI and rainfall measures considered in the study, the ones which fitted the *An. arabiensis* proportion best (giving smaller AIC) were the NDVI mean value during month of collection and the sum of rainfall mean value during month of collection and the two previous months, respectively.

The bivariate analyses also revealed that the agro-ecological zone, distance to water bodies, land use, transmission suitability, SWS index, minimum and maximum temperature were significantly associated with the relative frequency of *An. arabiensis*, which increases from the Guinea to the Sahelian AEZ. The crop/grass land mosaic and water body categories

of land use, the minimum temperature and the suitability to the transmission were negatively associated with the *An. arabiensis* frequency at a significant level.

All the factors above were entered into a Bayesian geostatistical model. The results of the spatial multiple regression model showed that the sum of the mean rainfall during collection month and the two previous months and the minimum temperatures were the only factors negatively associated with the relative frequency of *An. arabiensis*. None of the land use categories were significantly related to the proportional presence of this mosquito strain. Comparing the different categories of the variables between the bivariate and the multiple regression models, the following changes were observed: the Sahel category of the AEZ and the crop/grass land mosaic and water body categories of land use changed from significant in the bivariate model to not significant in the multiple regression model; the 4-10 km distance category of the distance to water bodies, the NDVI mean value during the month of collection, and the two previous months (the one included in the multiple regression model) changed from negatively significant in the bivariate model to positively significant in the multiple regression model; the mean maximum temperature which was not significant in the bivariate model became positively significant in the multiple regression model.

Assuming that spatial correlation is a function of distance between locations, irrespective of the locations themselves (stationary) and of the direction (isotropic), the minimum distance at which that correlation was less than 5% was as much as 1333.4 km (95% CI = 913.4-1520.1).

Table 2.2: Bivariate and multiple spatial logistic regression models of *An. arabiensis* relative frequency with climate and environmental variables.

		Bivariate analysis			Spatial model	
Variables	**Lag***	**Coef.**	**95% CI**	**p-value (AIC)**	**Median**	**95% CI**
Agro-ecological zones						
Guinea savannah	-	0.00			0.00	
Sudan savannah	-	2.16	2.07, 2.25		2.01	0.25, 3.49
Sahel	-	2.72	2.55, 2.89	<0.001	2.49	-1.21, 5.54
Distance to water bodies (Km)						
< 4	-	0.00			0.00	
4 - 10	-	-0.29	-0.38, -0.20		1.58	0.65, 2.58
>10 - 20	-	0.82	0.77, 0.87	<0.001	1.51	0.52, 2.50
> 20	-	1.77	1.65, 1.89		2.02	0.77, 3.36
Land use categories						
Savannah	-	0.00			0.00	
Crop/grass land/	-	-2.07	-2.38, -1.77		-0.65	-2.80, 1.53
Grass land	-	-0.02	-0.09, 0.06		0.51	-1.58, 2.91
Shrub land	-	-0.39	-1.02, 0.24		-2.50	-7.05, 1.69
Water bodies	-	-0.95	-1.72, -0.18		-0.19	-2.91, 2.46
Barren/sparsely	-	0.23	-0.14, 0.60		-1.24	-5.58, 2.32
Suitability to the transmission						
Not suitable	-	0.00			0.00	
Suitable	-	-0.15	-0.21, -0.08		0.10	-0.02, 0.22
Rainfall						
Mean_1	0	-0.0001	-0.0003,	0.57(50410.8)	-	
Mean_2	1	0.0003	0.0000, 0.0005	0.02(50405.7)	-	
Mean_3	1	0.0000	-0.0001,	0.44(50410.5)	-	
Mean_4	2	0.0001	0.0000, 0.0002	0.004(50402.9)	-0.01	-0.006, -0.004
NDVI						
Mean_1	0	-0.32	-0.49, -0.16	0.0001(50396.6	-	
Mean_2	1	-0.08	-0.25, 0.08	0.3285(50410.6	-	
Mean_3	1	-0.22	-0.39, -0.05	0.0129(50404.9	-	
Mean_4	2	-0.33	-0.52, -0.150	0.0005(50398.9	1058.0	8.67, 12.40
Temperature						
Mean minimum	-	-0.003	-0.004, -0.002	<0.001	-0.02	-0.042, -0.004
Mean maximum	-	-0.007	-0.008, 0.006	<0.001	0.21	0.18, 0.24
SWS index	-	0.22	0.13, 0.31	<0.001	1.71	1.43, 2.01
Spatial parameters						
ρ	-	-	-	-	4.00	2.63, 4.56
σ^2	-	-	-	-	0.04	0.02, 0.06

NB: Mean_1 = climatic mean value during month of collection; Mean_2 = climatic mean value during the previous month; Mean_3 = climatic mean value during month of collection and the previous month; Mean_4 = climatic mean value during collection month and the 2 previous months; * lag time (in month) between the environmental variables and the collection date (month) of vector data.

Figure 2.1 shows the observed relative frequencies of *An. arabiensis* and *An. gambiae s.s.* in the 94 locations. A lower frequency of *An. arabiensis* was observed in the southern and northern savannah while higher frequencies were observed in the Sahelian zone, with the exception of the inner delta of Niger.

Maps of the predicted proportions of *An. arabiensis* are shown in Figure 2.2 which depicts a south to north distribution pattern of *An. arabiensis* relative frequency with a moderate proportion of *An. arabiensis* in the southern savannah, a higher proportion in the northern savannah and Sahelian zones (apart from the inner delta of the Niger river where *An. arabiensis* was almost absent) and a lower one in the sub-Sahara zone.

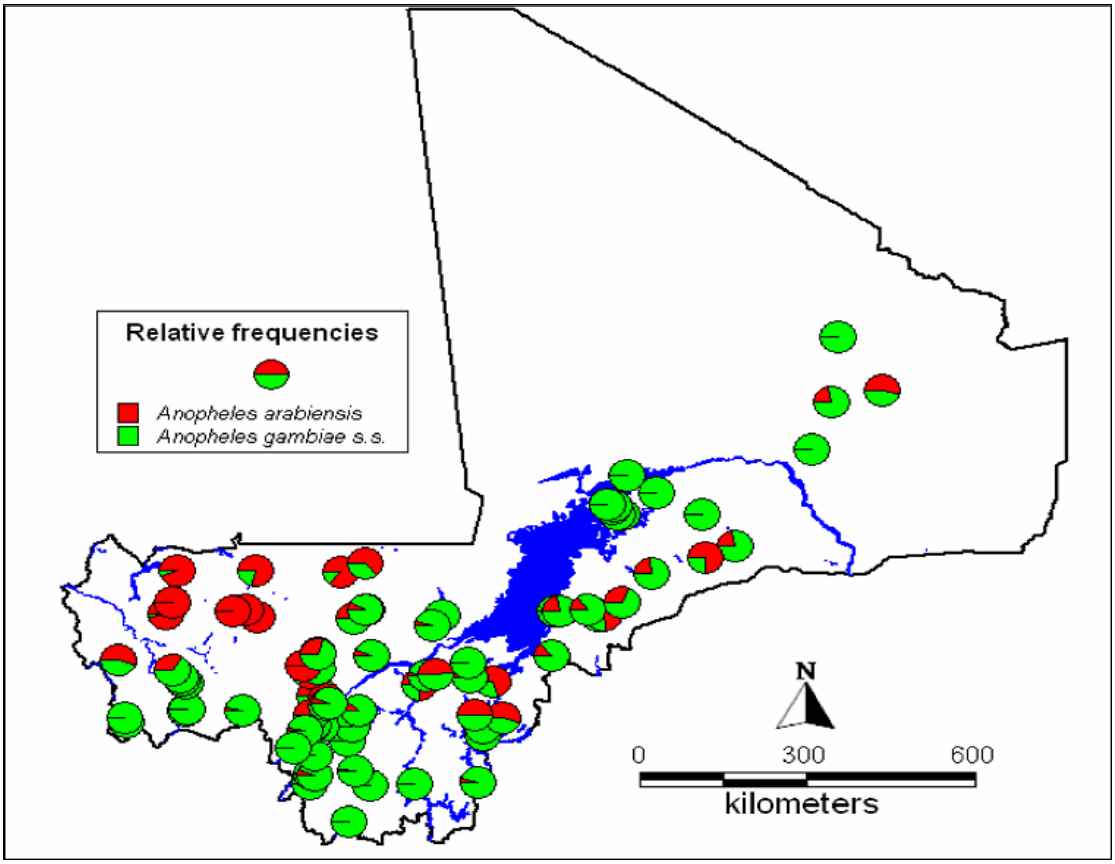

Figure 2.1: Observed relative frequencies of *An. arabiensis* and *An. gambiae s.s.* in 94 sampling locations in Mali, West Africa. The green color represents the relative frequencies of *An. gambiae s.s.* and the red the relative frequencies of *An. arabiensis*.

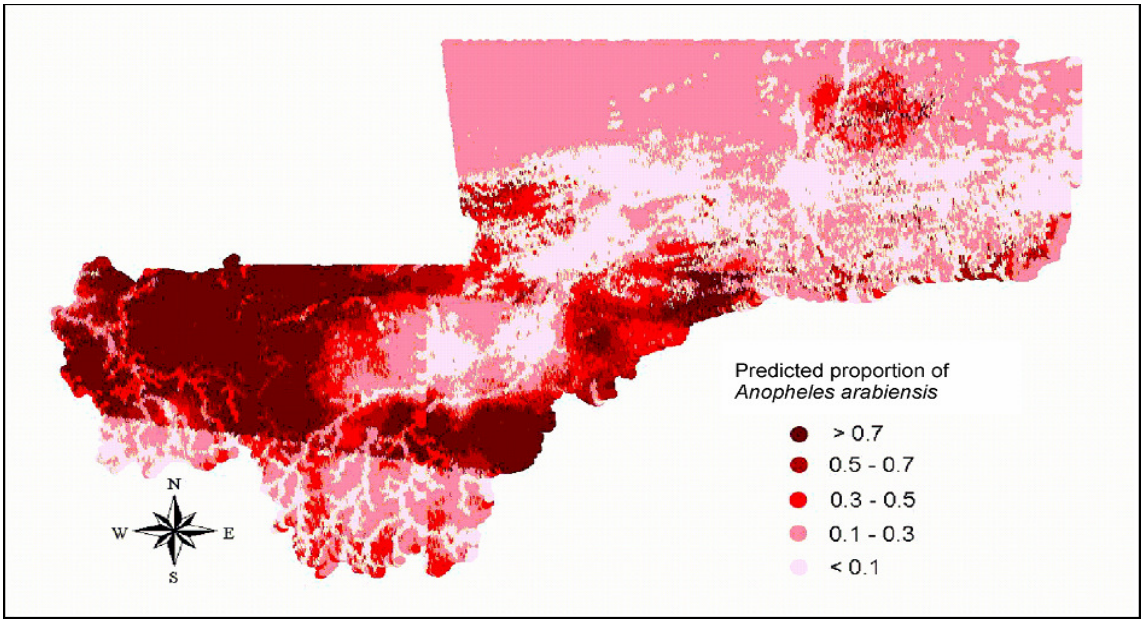

Figure 2.2: Map of predicted relative frequencies of *An. arabiensis*. The *An. arabiensis* proportion is also lower along the rivers irrespective of the eco-climatic zone. Estimates of the prediction error are shown in Figure 2.3.

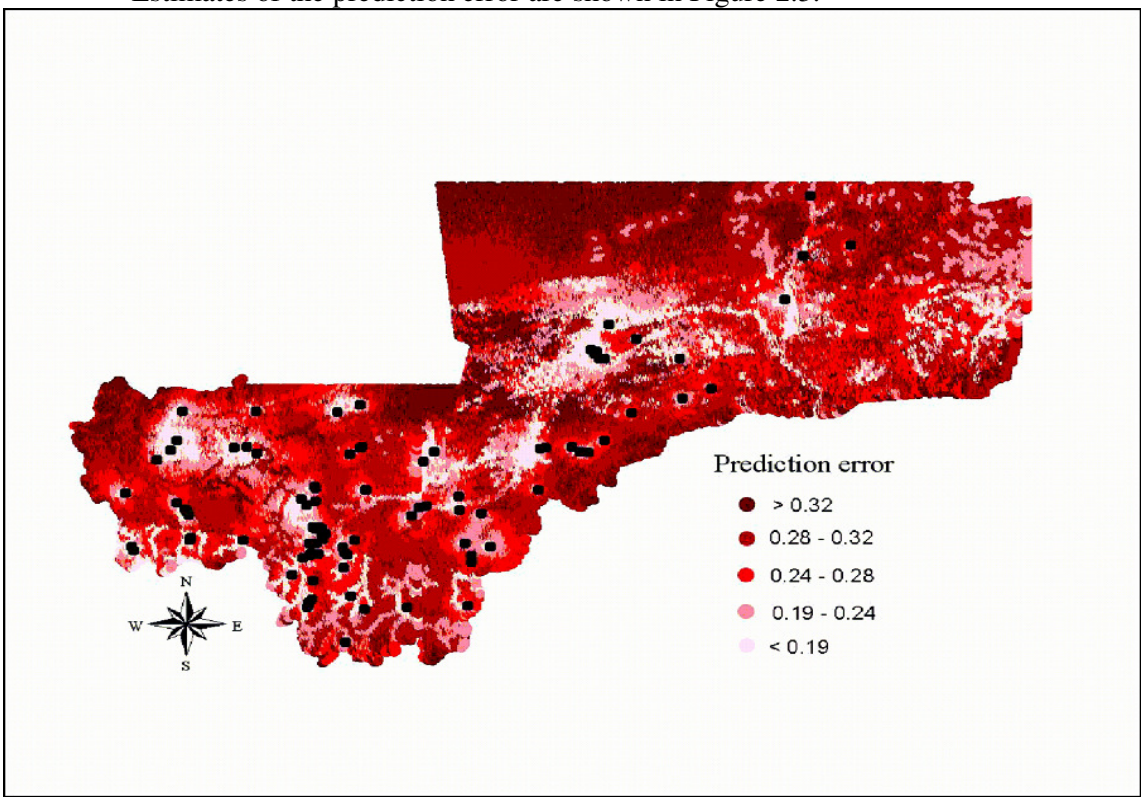

Figure 2.3: Map of prediction error of the relative frequencies of *An. arabiensis*. The prediction error is lowest along the rivers and increases with the distance from water bodies. In contrast, the prediction error is relatively high in the sub-Sahara zone where very few surveys were carried out.

2.5. Discussion

In this study, we compiled published and unpublished vector data in a unique database and using Bayesian geostatistical modeling, identified climatic and environmental factors associated with the relative frequency of the two major malaria mosquito vector species (*An. gambiae s.s.* and *An. arabiensis*) of *An. gambiae s.l.* in Mali, and assessed their spatial distribution. We used an approach considering different measures of rainfall and NDVI and performed bivariate logistic regressions to select the measures which fitted the data best using the AIC criterion. This was done to select the subset of variables to be fitted into the spatial model because Bayesian variable selection is not straightforward and requires specialized software which is not currently available. The approach adopted has been used also in other applications of spatial Bayesian modeling (Gemperli *et al*., 2006; Gosoniu *et al*., 2006). The results show that the cumulated rainfall value during the survey and during the two previous months, and the NDVI value during the survey month, fitted the data better than the other rainfall and NDVI measures assessed. This suggests that the *An. gambiae* complex species composition is more sensitive to the cumulated rainfall over previous months than to the value during the survey month. The observed lag time period between rainfall and vector abundance can enhance operational malaria earlywarning systems (MEWS) based on rainfall estimates (Grover-Kopec *et al*., 2005; Thomson *et al*., 2006).

The two sibling species of the *An. gambiae* complex (*An. arabiensis* and *An. gambiae s.s.*) exist across the whole study area. The estimates of the spatial model for the proportion of *An. arabiensis* showed a positive association between the NDVI values, the SWS index, the maximum temperature, and the distance to the water bodies. Minimum temperature and rainfall were negatively related to the relative frequencies of *An. arabiensis*. The predicted map in Figure 2.2 represents the median relative frequency of *An. arabiensis* over the transmission period (June to November). This is broadly in agreement with the ecological

distribution of *An. arabiensis* in Mali (Toure *et al*., 1998). *An. arabiensis* is concentrated in the drier savannah areas and *An. gambiae s.s.* in the inner delta of Niger, the southern savannah and along the rivers. The occurrence of *An. arabiensis* in the drier savannah reflects the known preference of this species for drier conditions. The occurrence of *An. gambiae s.s.* in the arid regions (Sahel) has been shown to be associated with the 'Mopti' chromosomal form (Toure *et al*., 1994). Many studies across Africa have described the likely adaptation of *An. arabiensis* to drier conditions than *An. gambiae s.s.* (Coetzee *et al*., 2000; Onyabe and Conn, 2001; Kirby and Lindsay, 2004; Levine *et al*., 2004). The general association of this mosquito strain with river systems is illustrated by its positive association with the SWS index and NDVI. Laboratory and field experimentation also showed that *An. arabiensis* adults are better adapted to hotter conditions than *An. gambiae s.s.* (Robert, 1998; Kirby and Lindsay, 2004). The ability for *An. arabiensis* to withstand the dry season may explain the weak and negative association of *An. arabiensis* relative frequency with rainfall.

The same pattern of south to north distribution of *An. arabiensis* relative frequencies was observed with the transmission model (Gemperli *et al*., 2006). However, in contrast to the distribution of *An. arabiensis*, the transmission model showed higher entomological inoculation rate in the south and moderate to low in the middle and northern part of country. This suggests that *An. arabiensis* may contribute less to the transmission than *An. gambiae s.s.*

Figure 2.2 depicts the spatial distribution of *An. arabiensis* and *An. gambiae s.s.* over the whole transmission period. Other studies (White, 1974; Coluzzi *et al*., 1979; Coluzzi, 1984, 1994) have shown that the temporal distribution is one of the key elements in malaria epidemiology and vector control which has valuable implication for vector stratification and adequate planning of both vector control and research activities. Our study did not take into

account temporal aspects for two reasons: firstly temporally disaggregated environmental data were not available for all survey years, especially not for the surveys conducted in the early 1980s; secondly the vector data were generally reported pooled from several surveys. Nevertheless, our effort was to produce predicted maps of the spatial distribution of *An. arabiensis* and *An. gambiae s.s.* species adjusted for climatic factors using spatial statistical modeling supported by consistently observed vector data. The advantage of our study over preceding ones is that we used statistical analysis which quantifies the relationship between environment-vector data and identifies significant determinants instead of only using geographical information system. The Bayesian approach we used takes into account the spatial dependence present in the data in a flexible way and calculates inherently the standard errors of the parameter estimates as well as the prediction error without relying on approximations or asymptotic results. The map of the prediction error indicates the confidence we can have on the model prediction for the study area.

A practical implication of our findings is their relevance in monitoring of insecticide resistance encoded by the kdr gene. In Mali resistant alleles of kdr have been reported only in the chromosomal form Savannah of *An. gambiae s.s.* (Fanello *et al*., 2003). Based on these results, insecticide resistance monitoring and management must be primarily focused on the humid savannah, along the rivers and in the inner delta of Niger where a higher frequency of *An. gambiae s.s.* is encountered. Understanding the spatial distribution of *An. gambiae s.l.* species and sub-species may also be a prelude to a successful implementation of genetic control, such as the use of transgenic technologies to make mosquitoes refractory to the parasite. IVM strategies that target particular vector populations will need information at high spatial and temporal resolutions on the distribution of the sibling species of *An. gambiae* complex.

2.6. Acknowledgements

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Chapter 3

Spatial distribution of the chromosomal forms of *Anopheles gambiae* in Mali.

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Abstract

Background Maps of the distribution of malaria vectors are useful tools for stratification of malaria risk and for selective vector control strategies. Although the distribution of members of the *Anopheles gambiae* complex is well documented in Africa, a continuous map of the spatial distribution of the chromosomal forms of *An. gambiae s.s.* is not yet available at country level to support control efforts. **Methods** Bayesian geostatistical methods were used to produce continuous maps of the spatial distribution of the chromosomal forms of *An. gambiae s.s.* (Mopti, Bamako, Savanna and their hybrids/recombinants) based on their relative frequencies in relation to climatic and environmental factors in Mali. **Results** The maps clearly show that each chromosomal form favours a particular defined ecoclimatic zone. The Mopti form prefers the dryer northern Savanna and Sahel and the flooded/irrigated areas of the inner delta of the Niger River. The Savanna form favours the Sudan savanna areas, particularly the South and South-Eastern parts of the country (Kayes and Sikasso regions). The Bamako form has a strong preference for specific environmental conditions and it is confined to the Sudan savanna areas around urban Bamako and the Western part of Sikasso region. The hybrids/recombinants favour the Western part of the country (Kayes region) bordering the Republic of Guinea Conakry. **Conclusions** The maps provide valuable information for selective vector control in Mali (insecticide resistance management) and may serve as a decision support tool for the basis for future malaria control strategies including genetically manipulated mosquitoes.

Key words:

3.1. Introduction

Malaria remains one of the main public health problems in Africa and researchers are developing new vector control methods focused on the genetic manipulation of mosquitoes. The principles of the genetic control methods are based on the propagation of sterility or other desirable genetic factors in successive generations of mosquitoes [1,2]. The most likely approach to implement genetically modified mosquitoes in malaria control is the introduction and spread of refractoriness genes in wild mosquito populations [3,4]. A major concern however regarding the spread of refractoriness genes is the possibility that they cannot be integrated into natural malaria vector populations because of gene flow barriers [5] and/or putative genetic adaptation to the environment [6]. Therefore, when developing target control methods, the structure of vector populations, the force of ecological associations and the resulting plasticity of the vectors to local environmental conditions should be considered.

The distributions of mosquito species are related to climate, and in West Africa, it appears that the different chromosomal forms of *An. gambiae s.s.* (Mopti, Bamako, Savanna, Forest and Bissau) occur sympatrically but are segregated environmentally [7-9]. In West Africa published data were compiled to demonstrate that climate variables can be used to map the distribution of *An. gambiae s.s* chromosomal forms [10]. Similar studies have been carried out in Kenya [11] and Nigeria [12]. In addition to climate, anthropogenic environmental alterations such as rice cultivation and irrigation may also affect species composition [13].

In Mali, the *An. gambiae* complex is composed of *An. arabiensis*, and *An. gambiae s.s.*. Three chromosomal (Mopti, Bamako, Savanna) and two molecular (M and S) forms of *An. gambiae s.s.* have been described and coexist [8,14-16]. The S-molecular form comprises Bamako and Savanna chromosomal forms. A map of their relative frequencies has been

produced for a number of specific locations in Mali [15]. Analysis of mosquito data from 16 sites throughout Mali showed a significant negative association between rainfall and the distribution of the Mopti chromosomal form [17]. Variation in the seasonal abundance and infection rates among chromosomal forms of *An gambiae s.s.* in Mali was also observed [18].

The ecological distribution of each chromosomal form seems to be related to a particular epidemiological pattern of the disease. The knock down resistance (*kdr*) allele in the para sodium channel gene, which confers resistance to pyrethroid insecticides, is found in the S-molecular form, but could not be detected in the Mmolecular form populations from the same localities [19]. Therefore producing a continuous map of the spatial distribution of their relative frequencies in relation to climate and environmental factors may be useful for conventional and prospective genetically manipulated vector control methods. In this study, published and unpublished vector data were compiled to assess the relationship between the relative frequencies of the different chromosomal forms of *An. gambiae s.s.* with climate and environmental factors, and to produce continuous maps of their spatial distribution.

3.2. Material and Methods

3.2.1. Description of the study area

The study area covered most of the territory of Mali in West Africa, between 10 and 25º latitude North and 12º longitude West and 4º longitude East. The Country has an area of 1,240,000 square kilometers and an estimated population (United Nations, 2003) of 13,000,000 inhabitants. It is drained by two major rivers (Senegal and Niger) and has 4 distinct eco-climatic zones: i) Southern Sudan savanna with an annual rainfall of 1300-1500 mm from May to October and mean annual thermal amplitude (difference between the mean maximum and the mean minimum temperature) of 5 to 6ºC; ii) Northern Sudan savanna with

about 700—1300 mm annual rainfall distributed over 4 to 5 months; iii) Sahelian zones with 200-700 mm of annual rainfall distributed over three months and mean annual thermal amplitude of about 12ºC; iv) Sub-Sahara zone with less than 200 mm of annual rain and 16ºC of annual average thermal amplitude.

Mali is a relatively flat country, altitudinal variations are minimal, ranging from 200 to 350 m above sea level. There are two main seasons varying in length according to latitude: a dry season (November–April) and a rainy season (May–October) characterized by lower temperatures and an increase in humidity. Except for the Sahara desert, the country is entirely endemic for malaria (hyperendemic to hypoendemic from South to North). The main malaria vectors are *An.gambiae s.l.* and *An.funestus*. *An.gambiae s.l.* is composed of *An. arabiensis* and three chromosomal forms of *An.gambiae s.s* named Bamako, Mopti and Savanna [20] and two molecular (M and S) forms [21].

3.2.2. Data sources and description

3.2.2.1. Vector data

All available published [15] and unpublished data on chromosomal forms of *An. gambiae s.s.* in Mali were collated from cross-sectional and longitudinal surveys carried out between 1981 and 2004 by the Malaria Research and Training Centre (MRTC), University of Bamako, Mali. Most surveys were conducted during the wet season (June-October). Survey sites were mainly small human settlements from 79 distinct rural sites representing various eco-climatic zones of Mali. Because of small distances separating some collection sites, data were aggregated resulting in a set of 71 locations. The database included data collected on i) the total number of *An. gambiae s.s*. specimens, ii) the count of chromosomal (Mopti,

Bamako, Savanna and their hybrids/recombinants) forms, and iii) the survey period (month and year). Mosquitoes were collected and processed across surveys following a standardized method to ensure data consistency. Identification of chromosomal forms was by cytogenetic method [22,23].

3.2.2.2. Climatic and environmental data

The climatic and environmental variables which were used in this study included temperature, rainfall, normalized difference vegetation index (NDVI), distance to water bodies, soil water storage (SWS), land use, agro-ecological zones (AEZ) and suitability for malaria transmission. The last one is a binary variable defined from environmental factors related to malaria transmission with cut-off values [24]. The data sources and spatial resolution are the same as described in previous work [25].

For each location, temperature and rainfall data were available as monthly long term averages. NDVI data were also summarized by monthly long term averages of the original decadal values during the period between 1985 and 1995. The agroecological zones (AEZ) were distinguished on the basis of the length of the crop growing period and were defined as follow: Equatorial Forest zone (> 270 days), Guinea savanna zone (165 – 270 days), Sudan savanna zone (90 – 165 days) and the Sahelian zone (< 90 days). In Mali only the last three AEZ are found.

3.3. Data analysis

Bivariate multinomial regression models were fitted in STATA 9.0 (STATA Corporation, USA) to assess the association between the relative frequencies of chromosomal forms of *An. gambiae s.s* with climatic and environmental factors. The multinomial outcome data represent the following four chromosomal forms: Mopti, Bamako, Savanna, and others

(hybrids Bamako-Savanna and Savanna-Mopti). The Mopti form was considered as the baseline category. The mosquito data obtained at a specific location were linked to the environmental and climate data by drawing a buffer of 2 km around each location and calculating the environmental value by the average of environmental values of all pixels in this buffer.

To take into account the possible lag time between the rainfall and NDVI with the mosquito abundance [26], four summary measures were calculated for each of the two climatic conditions: i) the climatic value during the month of collection (concurrent), ii) the climatic value during the previous month (lag one month), iii) the mean (or total) climatic value during the month of collection and the previous month (2 months average) and iv) the mean (or total) climatic value during the collection month and the two previous months (3 months average). The mean was used as a summary measure for NDVI and the total was considered as a summary measure for rainfall. Vector data obtained from surveys extended over a period longer than a month were available cumulatively for the whole period instead of monthly. In this case the midpoint month was used to relate the climatic factors. The Akaike's Information Criterion (AIC) was used to select the best summary measure and lag time for the rainfall and NDVI. The statistical significance of the environmental factors was assessed using the likelihood ratio test (LRT). All factors with a 15% significance level were entered in a Bayesian geostatistical multinomial regression model. The model took into account spatial heterogeneity by including location-specific random effects at the level of sampling location for each multinomial category (except the baseline). Bayesian kriging was used to assess the spatial patterns of the different chromosomal forms. A description of the geostatistical model is given in the appendix.

3.4. Results

Twenty six thousand three hundred twenty eight mosquitoes (26328) were assigned to one of the 3 chromosomal forms: Mopti, Bamako, and Savanna that represented 57.1%, 19.0% and 18.6% of the chromosomally identified mosquitoes, respectively. The remaining 5.3% were hybrids of Mopti-Savanna or Savanna-Bamako and the recombinants (Table 3.1). The three eco-climatic zones were sympatric areas for at least 2 of the chromosomal forms. Mopti form was the most abundant, prevailing in all eco-climatic areas with an increasing frequency from South to North (from 51.8% to 95.3%). The opposite situation was observed with Savanna form (1.8% to 25% from North to South). Bamako form was absent in the Sahelian zone. The highest frequency (6.3%) of hybrids/recombinants was observed in the North Sudan savanna.

Table 3.2 presents the results of the bivariate multinomial regression analyses between the chromosomal forms and the environmental and climatic factors used in the analysis. Among the four NDVI and rainfall measures considered in the study, the ones which fitted the distribution of chromosomal forms best (giving smaller AIC) were NDVI mean value and total rainfall value during the month of mosquito collection and the 2 previous months respectively. The results indicate a positive association of the suitability for transmission, the climatic values of NDVI and rainfall (Measure_4) and the SWS index with the relative frequencies of Savanna, Bamako and the hybrids/recombinants chromosomal forms, relative to the Mopti form used as baseline. The Bamako chromosomal form was positively associated with distances of 4—10 km to water bodies and crop/grass/mosaic land use categories, while the hybrids/recombinants chromosomal form was positively associated with Guinea savanna AEZ. All other parameters or category of parameters included in the analysis were negatively

associated with Savanna, Bamako and hybrids/recombinants chromosomal forms except distance of >10 – 20 km to water bodies with Savanna form were not significant.

Table 3.1: Relative frequencies of *An. gambiae s.s.*chromosomal forms by eco-climatic zone In Mali.

		Chromosomal forms of *An.gambiae s.s.*				
Eco-climatic zones	**Number of localities**	**Bamako form**	**Mopti form**	**Savanna form**	**Hybrids/ Recombinants**	**Total**
Southern Sudan Savanna	10	934 (20.4%)	2375 (51.8%)	1181 (25.8%)	91 (2.0%)	4581
Northern Sudan savanna	33	4060 (20.4%)	10907 (54.8%)	3693 (18.6%)	1248 (6.3%)	19908
Sahelian	36	0 (0.0%)	1752 (95.3%)	33 (1.8%)	54 (2.9%)	1839
Overall	79	4994 (19.0%)	15034 (57.1%)	4907 (18.6%)	1393 (5.3%)	26328

The multivariate spatial multinomial regression model showed a positive association between the SWS index and suitability for transmission and negative association between the minimum temperature and all the chromosomal forms (Table 3.3). In addition, positive association was observed between NDVI and Savanna form, between maximum temperature and Bamako form and between rainfall, maximum temperature and the hybrids/recombinants. Negative association was observed between North savanna, Sahel and Savanna form; between the minimum temperature and Bamako and between distances of 4—20 km to water bodies, AEZ and the hybrids. The SWS index and suitability for transmission were positively associated and the minimum temperature negative associated with all chromosomal forms in

both models. The AEZs significantly associated with all chromosomal forms in the bivariate analyses were no longer significant in the spatial model for Bamako form. The maximum temperature for Bamako and the hybrids/recombinants and the rainfall for the hybrids/recombinants remained significant in the spatial analysis. The distance at which correlation between 2 locations was less than 5% was 428.2 km (101.2, 1755.2), 1113.4 km (327.0, 2135.6) and 953.2 km (318.1, 2090.0) for Savanna, Bamako and the hybrids/recombinants chromosomal forms respectively, indicating a large spatial correlation in the data.

Figure 3.1 shows the observed relative frequencies of the different chromosomal forms in 71 locations across the country. The spatial distribution maps (Figs 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8 and 3.9) show clearly an ecological aggregation of the different chromosomal forms. The Mopti form (Figs 3.2-3.3) shows a South-North distribution pattern with an increasing frequency reaching up to 100% in the inner delta of Niger River and the Sahelo-Saharian part of the country. The Savanna form (Figs 3.4-3.5) is present in the Sudan savanna area at the South and South-Eastern parts of the region of Kayes and Sikasso respectively. Bamako chromosomal form (Figs 3.6-3.7) is confined to the Western part of the region of Sikasso and the hybrids/recombinants of Bamako-Savanna, Mopti-Savanna (Figs 3.8-3.9) are observed in the South-Western part of the region of Kayes.

Table 3.2: Bivariate association between chromosomal forms and climate and environmental parameters arising from multinomial regression model. Odds ratios are relative to Mopti chromosomal form.

Parameters	Savanna	Bamako	Hybrids	p-value (AIC)
	OR (95%CI)	OR (95%CI)	OR (95%CI)	
Agro-ecological zones (AEZ)				
Guinea savanna	1.00	1.00	1.00	
Sudan savanna	0.91 (0.84—0.98)	0.87 (0.80—0.94)	1.42 (1.23—1.63)	< 0.001
Sahel	0.30 (0.26—0.33)	1.03 (0.94—1.12)	0.73 (0.61—0.88)	
Distance to water bodies				
< 4 km	1.00	1.00	1.00	
4 - 10 km	0.59 (0.55—0.63)	1.10 (1.03—1.18)	0.39 (0.34—0.44)	
>10 - 20 km	0.95 (0.87—1.05	0.80 (0.72—0.89)	0.76 (0.65—0.89)	< 0.001
> 20 km	0.32 (0.25—0.42)	0.22 (0.15—0.30)	0.32 (0.21—0.49)	
Land use				
Savanna	1.00	1.00	1.00	
Crop/Grass/Mosaic land	0.34 (0.31—0.37)	1.20 (1.12—1.29)	0.60 (0.52—0.69)	< 0.001
Others	0.09 (0.07—0.13)	0.10 (0.07—0.14)	0.35 (0.25—0.49)	
Suitability to transmission				
Not suitable	1.00	1.00	1.00	
Suitable	4.44 (4.14—4.76)	1.67 (1.57—1.78)	2.90 (2.60—3.24)	< 0.001
Rainfall				
Measure_1	1.06 (1.03—1.10)	1.00 (0.96—1.03)	1.23 (1.18—1.29)	<0.001 (58461.76)
Measure_2	1.60 (1.55—1.65)	0.99 (0.96—1.03)	1.31 (1.24—1.37)	<0.001 (57481.14)
Measure_3	1.32 (1.28—1.36)	0.99 (0.96—1.03)	1.29 (1.23—1.35)	<0.001 (58134.34)
Measure_4	1.87 (1.81—1.93)	1.07 (1.04—1.11)	1.51 (1.44—1.59)	**<0.001 (56806.59)**
NDVI				
Measure_1	2.09 (2.02—2.17)	1.13 (1.09—1.16)	1.71 (1.62—1.81)	<0.001 (56443.11)
Measure_2	2.69 (2.59—2.79)	1.24 (1.20—1.28)	1.73 (1.63—1.83)	<0.001 (55413.54)
Measure_3	2.45 (2.36—2.54)	1.18 (1.15—1.22)	1.75 (1.65—1.85)	<0.001 (55830.97)
Measure_4	2.81 (2.70—2.92)	1.19 (1.15—1.23)	1.80 (1.70—1.91)	**<0.001 (55171.33)**
Temperature				
Mean minimum	0.995(0.984—0.987)	0.995(0.994—0.996)	0.992(0.990—0.994)	<0.001
Mean maximum	0.981(0.980—0.983)	0.993(0.992—0.994)	0.985(0.984—0.987)	<0.001
SWS	28.79 (23.59—35.14)	2.15 (1.67—2.77)	7.57(5.39—10.63)	<0.001

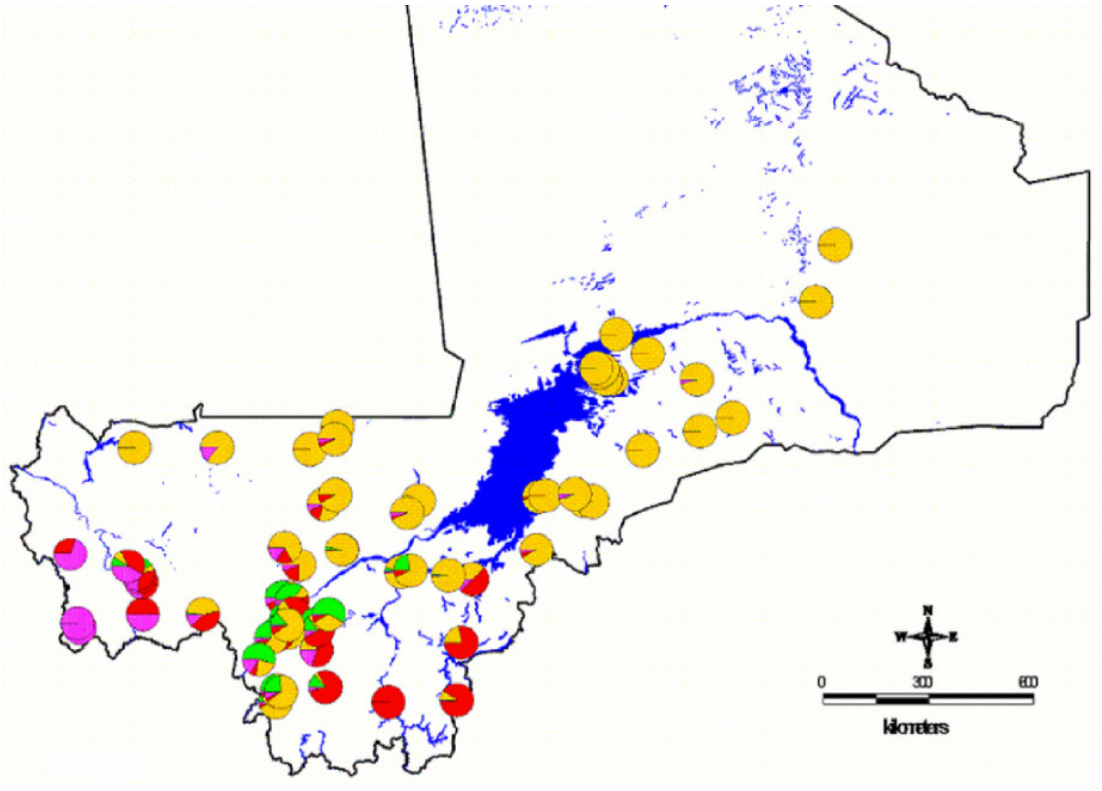

Figure 3.1: Observed relative frequencies of the chromosomal forms in 71 locations in Mali, West Africa. The orange represents Mopti, the red Savanna, the green Bamako and the purple the Hybrids/recombinants relative frequencies

Table 3.3: Odds ratios for presence of different chromosomal forms estimated from the geo-statistical Bayesian multiple multinomial regression model.

Parameters	Savanna	Bamako	Hybrids/Recombinants
	Posterior median (95%CI)	Posterior median (95%CI)	Posterior median (95%CI)
Rainfall	0.95 (0.83—1.08)	1.09 (0.99—1.20)	1.22 (1.03—1.46)
Max temperature	0.74 (0.47—1.07)	6.09 (4.29—7.99)	2.32(1.34—3.97)
Min temperature	0.41(0.22—0.99)	0.07 (0.04—0.14)	0.28 (0.14—0.58)
NDVI	1.46 (1.30—1.65)	1.04 (0.96—1.13)	1.03 (0.88—1.19)
SWS	2.02 (1.42—2.84)	5.98 (4.45—8.04)	3.25 (1.99—5.32)
Distance to water bodies			
< 4 km	1.00	1.00	1.00
4 to 10 km	0.20 (0.05—0.89)	1.52 (0.40—7.01)	0.42 (0.17—0.89)
>10 to 20 km	0.94 (0.15—7.88)	1.64 (0.14—14.05)	0.18 (0.04—0.74)
> 20 km	0.69 (0.09—4.49)	3.66(0.22—56.66)	0.31 (0.07—1.33)
Suitability to transmission			
Suitable	1.00	1.00	1.00
Not suitable	4.72(3.43—6.63)	24.76 (16.03—37.77)	3.53 (2.34—5.65)
Agro-ecological zones (AEZ)			
South savanna	1.00	1.00	1.00
North savanna	0.29 (0.07—2.00)	2.92 (0.31—29.56)	0.24 (0.06—0.82)
Sahel	0.01 (0.00—0.79)	0.00 (0.00—13.85)	0.05 (0.00—0.92)
Spatial parameters			
$3/\rho$ ** (km)	428.2 (101.2—1755.2)	1113.4 (327.0—2135.6)	953.2 (318.1—2090.0)
σ^2	9.95 (4.45—37.00)	24.95 (8.29—67.78)	8.57 (3.47—22.58)

*Odds ratios are relative to Mopti form
**Distance (km) with spatial correlation < 5%

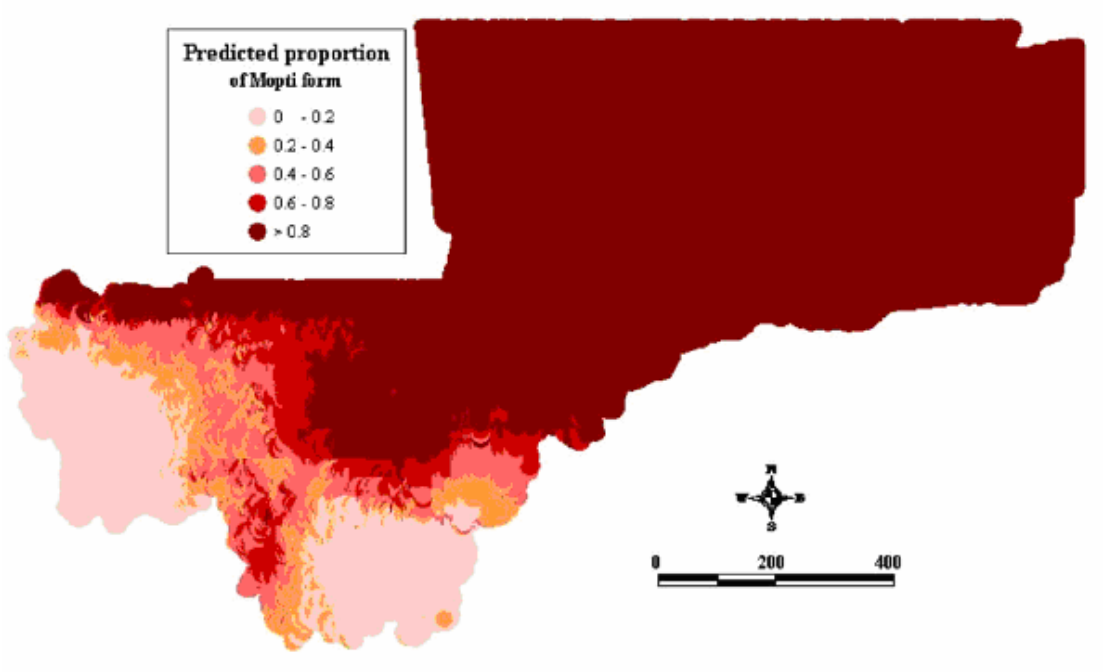

Figure 3.2: Map of the predicted proportion of the Mopti chromosomal form of *An. gambiae s.s.* in Mali, West Africa.

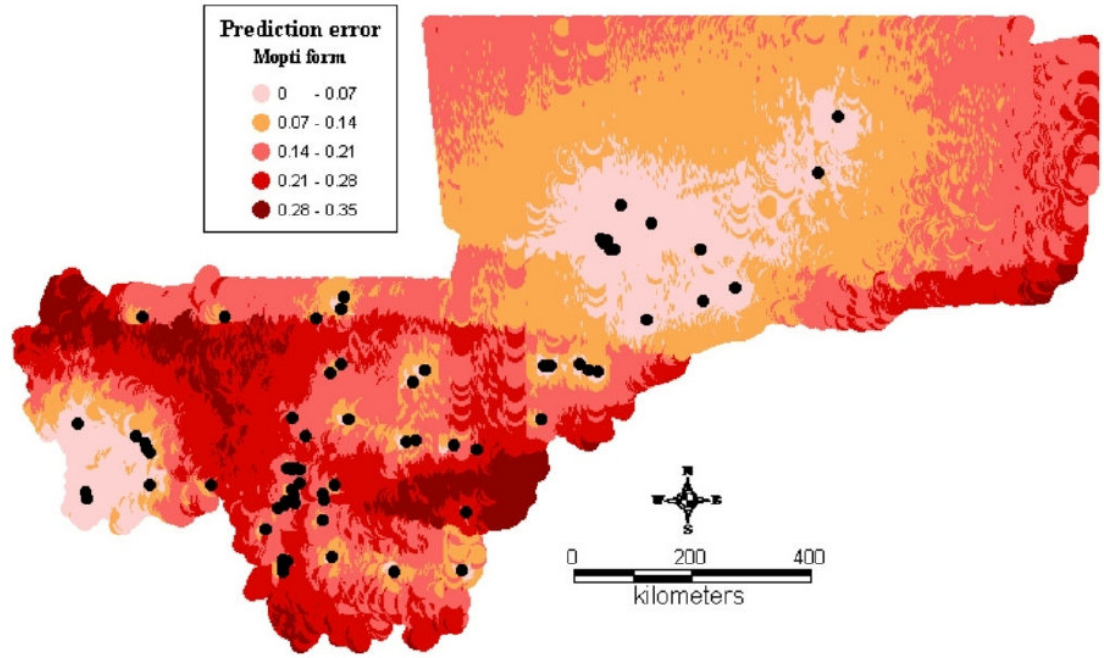

Figure 3.3: Map of the prediction errors of the Mopti chromosomal form of *An. gambiae s.s.* in Mali, West Africa. The black dots represent the data locations.

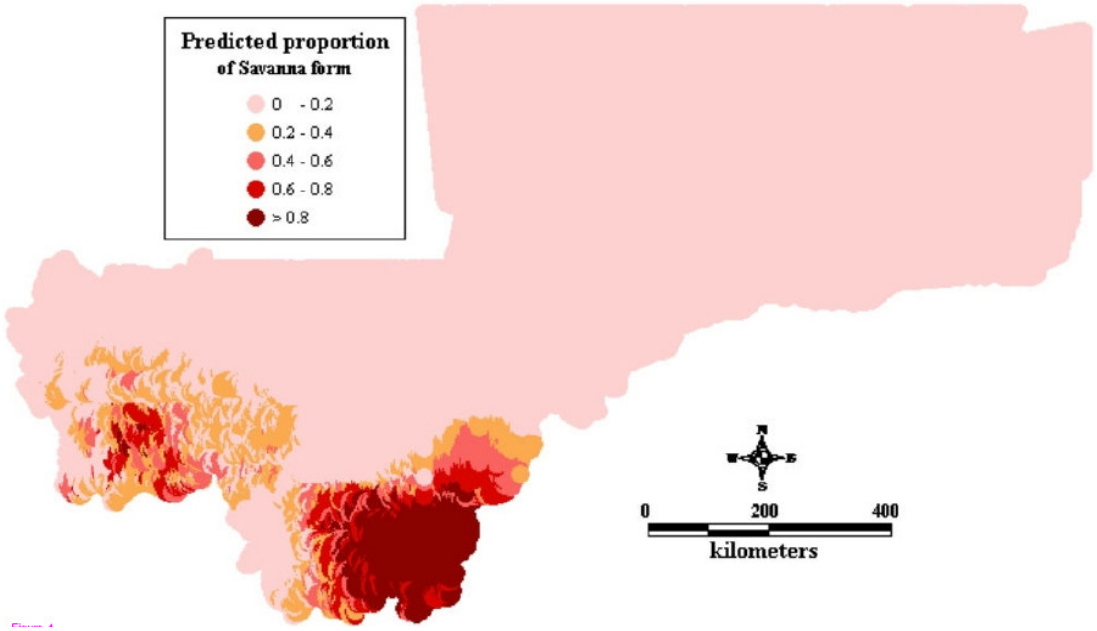

Figure 3.4: Map of the predicted proportion of the Savanna chromosomal form of *An. gambiae s.s.* in Mali, West Africa.

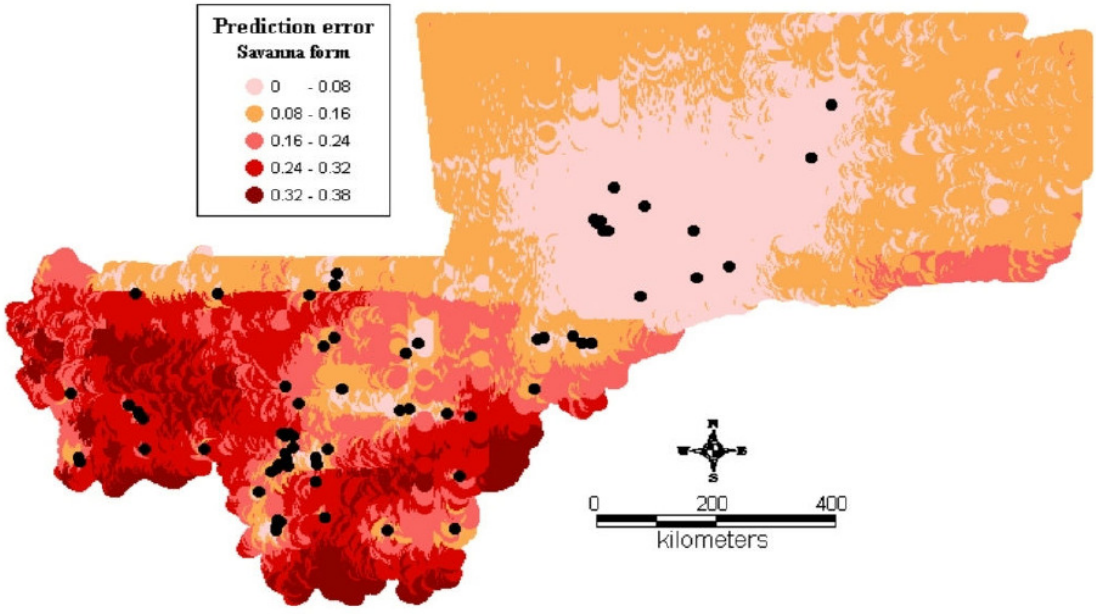

Figure 3.5: Map of the prediction errors of the Savanna chromosomal form of *An. gambiae s.s.* in Mali, West Africa. The black dots represent the data locations.

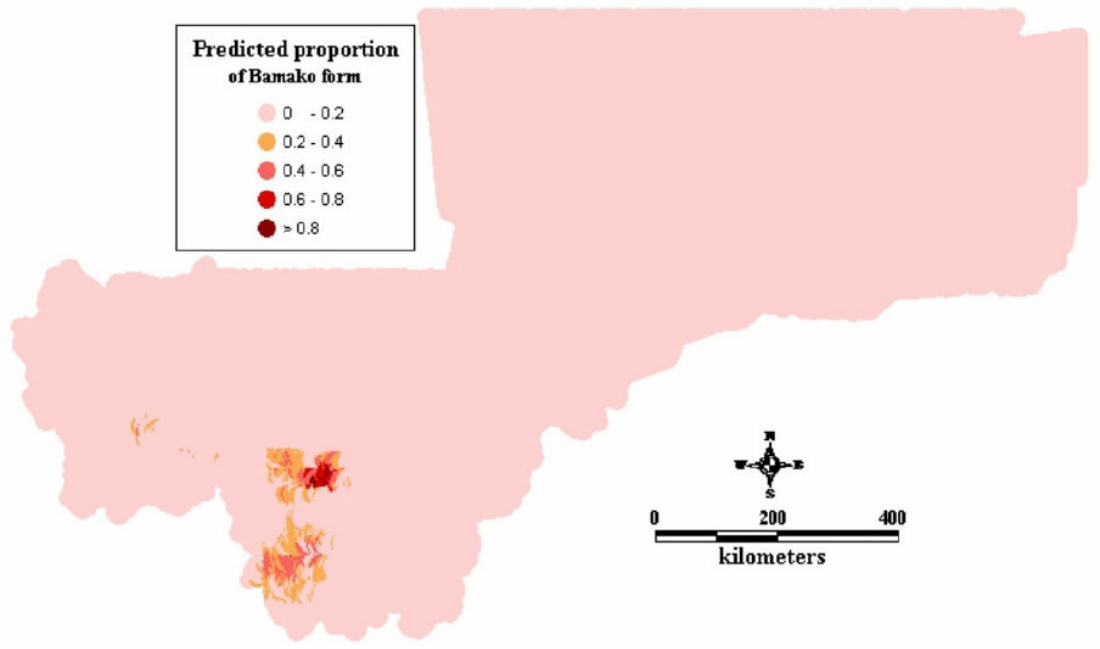

Figure 3.6: Map of the predicted proportion of the Bamako chromosomal form of *An. gambiae s.s.* in Mali, West Africa.

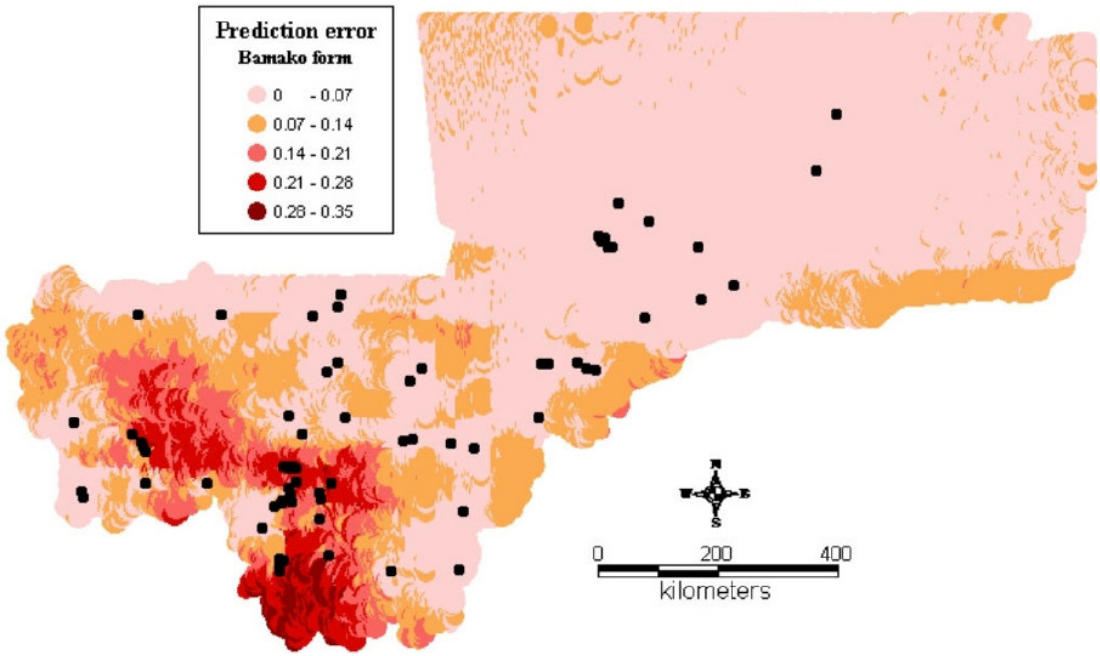

Figure 3.7: Map of the prediction errors of the Bamako chromosomal form of *An. gambiae s.s.* in Mali, West Africa. The black dots represent the data locations.

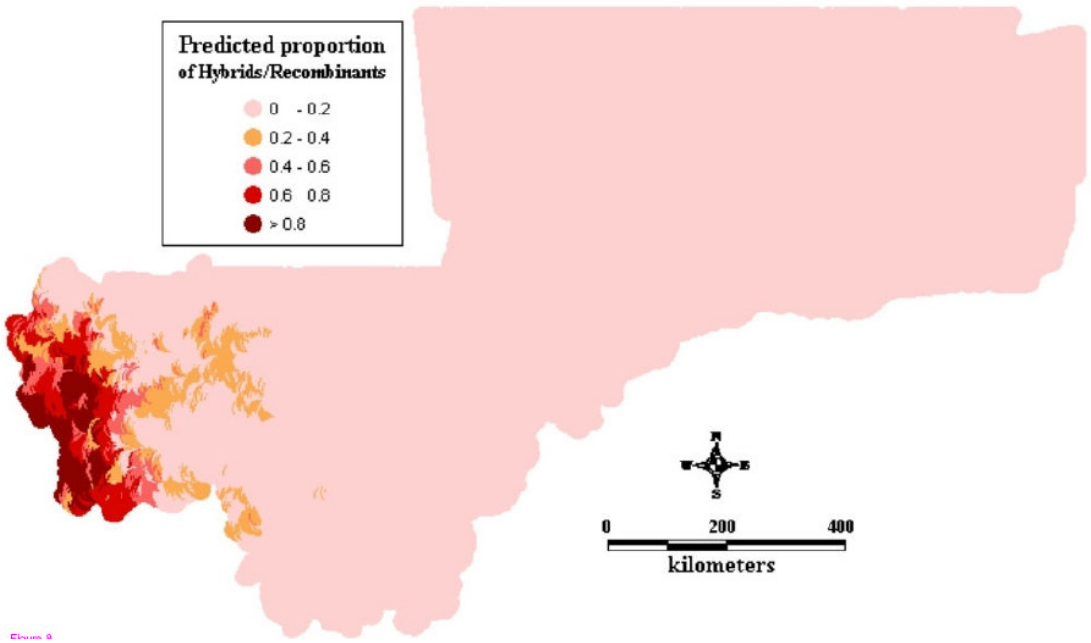

Figure 3.8: Map of the predicted proportion of the hybrids chromosomal form of *An. gambiae s.s.* in Mali, West Africa.

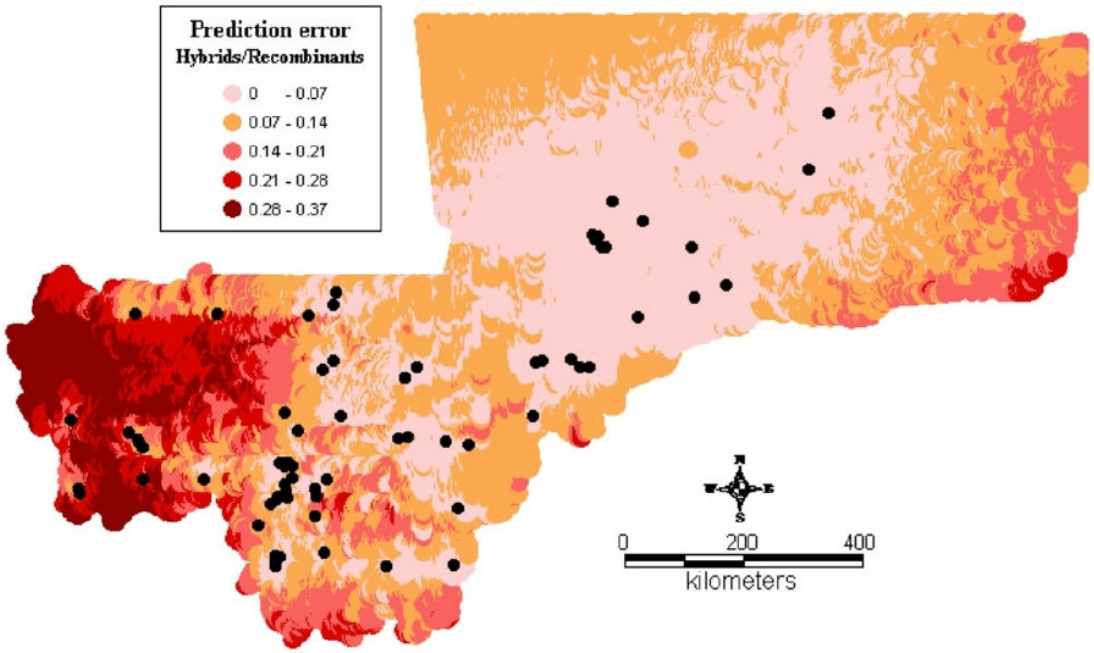

Figure 3.9: Map of the prediction errors of the hybrids chromosomal form of *An. gambiae s.s.* in Mali, West Africa. The black dots represent the data locations.

3.5. Discussion

The predicted maps of the different chromosomal forms of *An. gamabiae s.s*. represent an average relative frequency over the malaria transmission season in Mali (June to November). They may not reflect the exact situation –which is temporally dynamic– because (i) data were obtained from cross-sectional surveys carried out during a single point of time, and (ii) Long term averages of climatic and environmental factors were used because some of these factors were not available during the survey times. Despite the long duration of the data collection, standardized techniques were used for sampling and processing mosquitoes across surveys rendering the mosquito database consistent.

The analysis of the observed data showed that at least two of the chromosomal forms were sympatric in each of the three eco-climatic zones of Mali. The Mopti chromosomal form was prevalent in all eco-climatic zones indicating that this type can easily adapt to different environmental and climatic conditions. Its chromosomal arrangement bc/bc and u/u may play an important role in its adaptation to diverse environment [15]. Indeed, seasonal variations of the frequency of Mopti chromosomal arrangement show that the frequency of bc karyotype decreases in the rainy season and increases in the dry season, but the frequencies of u karyotype show the reverse variation [17]. The Bamako form which is normally present along river systems, was absent around the Niger River in the Sahelian zone showing the preference of this type to more humid climate. The Savanna form was present in all eco-climatic zones, but with higher frequency in the South Sudan savanna. The three chromosomal forms were sympatric in the Northern Sudan savanna where the highest relative frequencies of the hybrids Mopti-Savanna and Bamako-Savanna were also observed.

The spatial distribution maps clearly show that, in spite of their sympatry, the spatial distribution of the different chromosomal forms is not random. Each chromosomal form

favours a particular defined eco-climatic zone as reported by previous studies [7,10,15,27]. The Mopti form (Figs 2-3) is present country wide but prefers the dryer northern Sahel and the flooded/irrigated areas of the delta of Niger River. Because of it association with flooded plains and irrigated fields, it also breeds continuously even throughout the dry season [15]. The Savanna form (Figs 3.4-3.5) favours the Sudan savanna areas and is particularly predominant in the South and South-Eastern parts of the country (Kayes and Sikasso regions). The Bamako form (Figs 3.6-3.7) has strong preference to specific environmental conditions and it was confined in the Western part of Sikasso region and around Bamako town which also gave the name to this type [14].

The hybrids/recombinants (Figs 3.8-3.9) are observed in the Western part of the country (Kayes region), a wooded area, at the border of the Republic of Guinea Conakry. The spatial distribution of these inversions shows a strong association with ecological/climatic zones [7,27]. The border of the Republic of Guinea Conakry and Kayes is a transitional area between the forest (with high inversion diversity within mosquito populations with more standard and heterozygous carriers) and Savanna (with more homozygous carriers). Field population studies revealed a low frequency of hybrids between Mopti and Savanna and between Bamako and Savanna as well as a complete reproductive isolation between Bamako and Mopti [20]. Therefore, the hybrids/recombinants observed here are likely to be from Bamako-Savanna because these 2 forms are sympatric in this part of the country. It has also be reported that the karyotypes identified as hybrids are in fact not hybrids, but the consequence of low frequency polymorphisms in one or the other taxon [28]. The high spatial correlation observed in the data may probably be due to the effect of environmental factors which influence large areas.

The only spatially-continuous map of *An. gambiae s.s.* chromosomal form distribution produced so far was for West Africa [10]. Our introduced approach, however, yielded a more finely resolved *An. gambiae s.s.* chromosomal form spatially-continuous distribution for Mali. Based on current knowledge on vector resistance to pyrethroids in Mali [19], these maps provide valuable information for selective and targeted malaria vector control in Mali. Indeed, the Mopti chromosomal form –which have not yet developed resistance to insecticide— prevails in the Sahelian and irrigated/flooded areas, while the S molecular form (Savanna and Bamako) –which carries the kdr gene— is more abundant in the southern part of the country, particularly in Sikasso and Kayes regions. Although any vector control by means of insecticides must be accompanied by a resistance monitoring system, particular attention must be paid to the southern part of the country.

The maps may also be useful for planning future implementation of malaria control by genetically manipulated mosquitoes. However, more bio-ecological and gene flow studies among the different chromosomal forms are needed before undertaking any field implementation of control by genetically manipulated mosquitoes. In addition, temporal distribution maps of the chromosomal forms would be useful to complete the stratification for targeted vector control. Indeed, in areas where the chromosomal forms occur sympatrically; their relative frequencies change seasonally, most likely in response to annual fluctuations in climate [29]. However, collecting temporal genotyped data is not an easy task because of the skilled and labor intensive techniques required for field identification of the chromosomal forms.

3.6. Conclusions

Our study represents more finely resolved spatially-continuous distribution maps of *An. gambiae s.s.* chromosomal form in Mali. The maps provide valuable information for

selective vector control in Mali (insecticide resistance management) and may serve as a decision support tool for the basis for future malaria control strategies including genetically manipulated mosquitoes.

3.7. Acknowledgements

The authors are thankful to all of those who have participated to the vector data collection and processing. They also thank the villagers for their cooperation. The data analysis was supported by the Swiss National Science Foundation project Nr.3252B0-102136/1.

3.8. Appendix

3.8.1. Geostatistical multinomial regression model

Let Y_{ik} be the observed frequency of mosquito chromosomal form *k* at location *i* where *k=1,2,3,4* denote the Mopti, Bamako, Savanna , and hybrid forms, respectively. It was assumed that Y_{ik} arise from a multinomial distribution, that is $(Y_{i1}, Y_{i2}, Y_{i3}, Y_{i4}) \sim Mult(n_i, \pi_{i1}, \pi_{i2}, \pi_{i3}, \pi_{i4})$ with parameters π_{ik} and n_i is the total number of *An. gambiae s.s* collected at location *i*. Spatial correlation was introduced on the location-specific random effects ϕ_{ik} which are modeled together with the covariate effects on the logit parameters, that is $\log\left(\frac{\pi_{ik}}{\pi_{i4}}\right) = \underline{X}_i^T \underline{\beta}_k + \phi_{ik}$ where $\underline{\beta}_k$ are covariate parameters related to the *kth* multinomial category, *k=1,2,3*.

It was also assumed ϕ_{ik} to model a latent isotropic Gaussian spatial process, that is $\phi_k = (\phi_{1k}, ...\phi_{Nk}) \sim MVN(0, \Sigma_k)$, with covariance matrix Σ_k and that spatial correlation

between any pair of locations is a function of distance between locations, that is $(\Sigma_k)_{ij} = \sigma_k^2 \exp(-\rho_k d_{ij})$ where σ_k^2 is the spatial variance related to the multinomial category k, ρ_k is the parameter that models the rate of correlation decay and d_{ij} the distance between the locations *i* and *j*. Based on the above specification, the minimum distance for which the spatial correlation becomes less than 5% is calculated by [1]. The model parameters were estimated using Markov Chain Monte Carlo (MCMC) simulation methods. Bayesian kriging was used to predict the species frequency at 85,000 unsampled locations [2]. The Bayesian model fit was carried out in WinBUGS 1.4. (Imperial College and MRC, UK), whereas the model prediction was implemented in Fortran 95 (Compaq Visual Fortran, Professional 6.6.0) using standard numerical libraries (NAG, The Numerical Algorithms Group Ltd).

3.8.2. Model fit

The parameters of the above models were estimated using Markov Chain Monte Carlo (MCMC) simulation methods. In accordance with the Bayesian model specification, prior distributions were adopted for the model parameters. Vague normal prior distributions were chosen for $\underline{\beta}$ parameters with large variances (i.e., 10,000), gamma prior for r, inverse gamma priors for σ_k and uniform priors for $\rho_k, k = 1,2,3$. A single chain sampler was run with a burn-in of 5,000 iterations. Convergence was assessed by inspection of ergodic averages of selected model parameters. Bayesian kriging was used to predict the species frequency at 85,000 unobserved locations [2]. The Bayesian model fit was carried out in WinBUGS 1.4. (Imperial College and MRC, UK), whereas the model prediction was implemented in Fortran 95 (Compaq Visual Fortran, Professional 6.6.0) using standard numerical libraries (NAG, The Numerical Algorithms Group Ltd).

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Chapter 4

Contribution of members of *An. gambiae* complex (Diptera: Culicidae) to malaria transmission in Mali.

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Abstract

Reliable maps of malaria risk and knowledge of the contribution of vector species and subspecies to transmission are important tools for selecting areas of priority for malaria control and appropriate intervention. In this study we obtained a recent malaria risk map of Mali as well as attributed malaria risk ma ps for the different chromosomal variants of *An. gambiae* complex using Bayesian geostatistical modeling. The different chromosomal forms of *An. gambiae s.s* contribute equally to malaria transmission during the dry survey period (1981-1990). During the survey period 1991-2004, *An. arabiensis* contribution was significantly lower compared to the Mopti form. The *kdr* allele carriers (Bamako/Savanna) were associated with higher malaria parasite risk. The revised malaria risk map was in agreement with the eco-geographical description of malaria in Mali. Malaria transmission is mainly due to *An. arabiensis* in the middle West and South East part of the country, to the Mopti form in the irrigated/flooded areas, to the Savanna/Bamako forms in the southern part, and to the hybrids in the southern areas of the region of Kayes (West of the country).

Keywords: *An. gambiae*, chromosomal forms, Bayesian geostatistics, multinomial, binomial, Markov chain Monte Carlo (MCMC), krigging.

4.1. Introduction

Malaria remains one of the major tropical health challenges in the world. The number of deaths dues to the disease is estimated to 1.1-1.3 million (World Health Reports 1999-2004). Almost 90% of these deaths occur in sub-Sahara Africa, especially among children and pregnant women. The majority of malaria infections in Africa are caused by *Plasmodium falciparum,* predominantly transmitted by members of *An. gambiae* complex. *An. gambiae* complex consists of, at least, six sibling species exhibiting varying degrees of ecological, behavioral and vectorial capacities (Costantini *et al*, 1999). The chromosomes of all sibling species in *An. gambiae* complex show polymorphic inversions (Coluzzi *et al* 1979). In Mali, the *An. gambiae* complex consists of *An. arabiensis* and *An. gambiae s.s.*, which has at least three inversion karyotype named Bamako, Mopti and Savanna (Touré *et al*. 1989). In previous work, we produced spatial distribution maps of the relative frequencies of *An. arabiensis* and *An. gambiae s.s* (Sogoba *et al*. 2007) as well as for the different karyotypes of *An. gambiae s.s.* (Mopti, Bamako, Savanna and hybrids/recombinants). These maps showed distinct geographical preferences of the species and the chromosomal karyotypes of *An. gambiae* complex.

The geographical distribution of vector species plays an important role in malaria epidemiology. Differences in vectorial capacity and behavior are contributing factors in the role of individual species in the epidemiology of malaria transmission (Petrarca and Beier, 1992; Fontenille *et al*., 1997). However, little is known about how the different species and chromosomal karyotypes of *An. gambiae* complex are related to malaria transmission intensity. One of the direct ways to assess this relationship is to estimate the contribution of each species and chromosomal karyotype to the entomological inoculation rate (EIR). Such exercise at larger areas will be expensive and time-consuming. Although studies have shown that the relationship between malaria prevalence and EIR is not linear (Mbogo *et al*. 2003),

the parasite prevalence in a population is related to the intensity of the transmission and it can be used to estimate EIR and *vise versa* (Gemperli *et al*. 2006). Data on parasite prevalence are available in the Mapping Malaria Risk in Africa (MARA) project database, the most comprehensive database of malaria survey data in Africa. The MARA database allows not only assessing the relation between malaria risk and vector subspecies distribution but also producing malaria risk maps attributed to each subspecies. A number of predicted malaria prevalence maps in Mali have been produced using different statistical approaches in order to improve their accuracy (Kleinschmidt *et al*. 2000; Gemperli *et al* 2006; Gosoniu *et al*., 2006). These maps are based on data collected until 1998 however some of our vector surveys took place after this year. The MARA database has not been updated over the last 10 years and it may not reflect the current situation of disease risk in Mali. The main objective of this study was to assess the relationship between malaria risk and the vector species distribution. To address this aim, we (i) linked the MARA and vector databases by predicting the subspecies distribution at the MARA locations ii) quantified the contribution of each subspecies to malaria risk and iii) produced malaria risk maps in Mali attributed to each vector subspecies.

4.2. Material and methods

4.2.1. Data description

4.2.1.1. Prevalence data

Data on malaria endemicity were obtained from the Mapping Malaria Risk in Africa (MARA/ARMA, 1998), which is a geo-referenced database of all available published and unpublished malariometric survey data in 44 countries including Mali. These surveys record the presence of *P. falciparum* in blood smears. The latest recorded data for Mali was in 1998. We updated the database for Mali with survey data collected up to 2004 using the same data extraction proforma of MARA/ARMA. For the purpose of our study, we extracted the

prevalence data for children of 1-15 years old and for the total population in epidemic prone areas (all location at altitude more than 15 degree North). Data were obtained from 121 locations well distributed widely in all the eco-climatic zones of the country.

4.2.1.2. Vector data

The vector data were obtained from published (Touré *et al*., 1998) and unpublished surveys carried out during various research activities of the Malaria Research and Training Center (MRTC) of the Faculty of Medicine, University of Bamako, Mali. They were collected from cross-sectional and longitudinal surveys from 1981 to 2004, generally conducted during the wet season (June – November). The database is described in detail in Sogoba *et al*., (2007).

4.2.1.3. Environmental data

The environmental and climatic factors which were used to predict both malaria endemicity and vector relative frequencies were obtained using remotely sensed. Data on Normalized Vegetation Index (NDVI) was extracted from the NOAA/NASA Pathfinder AVHRR Land Project. Temperature and rainfall data were obtained from the topographic and climate database for Africa (Hutchinson *et al*., 1996). Data on land use type were obtained from United State Geological Survey and the NASA's Distributed Active Archive Center (Anderson *et al*., 1979). Water bodies data and data on soil water storage were extracted from the African Data Sampler and from Droogers *et al*. (2001), respectively. For more details on the sources and spatial resolution of these data refer to Sogoba *et al*. (2007).

4.3. Statistical analysis

The main objective of this study was to assess the relationship between malaria risk and the distribution of the vector species and subspecies. To address this aim we i) linked the MARA and vector subspecies databases by predicting the vector subspecies at the MARA

locations ii) quantified the contribution of each subspecies to malaria risk and iii) produced continuous malaria risk maps in Mali attributed to each vector subspecies by combining the malaria risk map with maps of the distribution of each subspecies in the country.

The MARA and vector subspecies databases contain data at different locations. To align the databases, we developed a predictive model of the mosquito subspecies based on the relation between the frequency of subspecies and environmental factors by fitting multinomial geostatistical models on the subspecies data. This model was employed to predict the vector species and subspecies frequency at the MARA survey locations using Bayesian kriging. Bayesian kriging was also employed to produce continuous maps of the distribution of vector species and subspecies for the whole country. The multinomial categories were representing the following five species or chromosomal forms: Mopti, *An. arabiensis*, Bamako, Savanna, and Hybrids/recombinants. The Mopti form was considered as the baseline category. Summary measures of the environmental factors were used to link them with the vector data. In particular, rainfall and NDVI were summarized by long term averages as follow: i) the climatic value during the month of collection (measure_1), ii) the climatic value during the previous month (measure_2), iii) the mean (or total) climatic value during the month of collection and the previous month (measure_3) and iv) the mean (or total) climatic value during the collection month and the two previous months (measure_4). The climate value was the arithmetic mean for NDVI and the total for rainfall.

To quantify the contribution of each subspecies to malaria risk a logistic geostatistical regression model was fitted relating the MARA survey data to the frequency distributions of species and subspecies. The malaria survey data were considered as the outcome measure and the logits of each subspecies frequency with reference to the frequency of the Mopti form were treated as the explanatory variables.

In order to produce malaria risk maps attributed to each vector subspecies a spatial logistic regression model was fitted relating the malaria survey data to environmental predictors. These models were used to predict the malaria risk at the unobserved locations and produce a continuous malaria risk map. The environmental data, namely NDVI, SWS index, rainfall, minimum and maximum temperature were summarized at each survey location by long term averages during the following months: 1) January – December 2) May – November 3) May – October, 4) June – November, and 5) June – October. Time intervals 2) to 5) were linked to the malaria transmission seasons in the different eco-climatic zone in Mali. For the land use variable, a buffer of 2 km around each data point was created and the relative frequencies of the pixels of the different land use categories inside this buffer were calculated. We grouped together urban and built-up dry and barren or sparsely vegetated land (category 1), crop/grass, crop/wood mosaic shrub and grassland (category 2) and Savanna, water bodies and irrigated cropland and pasture (category 3). The predicted malaria prevalence map was combined with maps of vector subspecies distribution to obtain malaria risk maps attributed to each vector subspecies in the country as described in the appendix.

Bivariate non-spatial regression models (binomial and multinomial) were fitted in STATA v9.0 (STATA Corporation, USA) to select predictors and their summary measure which best fitted the data (malaria risk and species/subspecies frequency distributions, respectively) as indicated by the Akaike's information criterion (AIC). The statistical significance was assessed using the likelihood ratio test (LRT). All factors significant at the 15% significance level were entered into a Bayesian multivariate geostatistical model (binomial or multinomial depending on the outcome).

Model parameters were estimated using Markov chain Monte Carlo (MCMC) simulation methods. The Bayesian model fit was carried out in WinBUGS 1.4. (Spiegelhalter

et al., 2004), whereas the model prediction was implemented in Fortran 95 (Compaq Visual Fortran, Professional 6.6.0) using standard numerical libraries (NAG, The Numerical Algorithms Group Ltd).

A description of the geostatistical multivariate binomial and multinomial models is given in the Appendix.

4.4. Results

Vector data were available from 1981 onwards. During 1981-1990, a total of 15762 mosquitoes were identified in species and chromosomal karyotypes over 71 locations throughout the country. Table 4.1 presents the relative frequencies of the different taxa of *An. gambiae* complex per year in Mali. Their overall relative frequencies were 44.6%, 20.4%, 29.8% and 5.2% respectively for Mopti, *An. arabiensis*, Bamako/Savanna and the hybrids/recombinants, respectively. From 1991-2004 and in the same order the relative frequencies of members of *An. gambiae* complex were 25.3%, 28.1%, 43.2% and 3.5% for a total of 18530 mosquitoes identified. Significant difference in the overall relative frequencies of members of *An. gambiae* complex was observed.

The bivariate non-spatial multinomial regression models showed that long term averages of NDVI and rainfall during the month of mosquito collection (measure_1) fitted best (giving smaller AIC) the species and subspecies data (Table 4.2). These factors were used in the Bayesian spatial multivariate multinomial regression models. Temperature, distance to water bodies and suitability to transmission were also significantly related to the mosquito data ($P < 0.0001$).

Table 4.1: Relative frequencies of the different taxa of *An. gambiae* complex per year in Mali

Years	Total	*An. arabiensis* (%)	Bamako (%)	Mopti (%)	Savanna (%)	Hybrids (%)
			Period 1			
1981	1079	17.4	16.3	61.2	3.0	2.1
1982	2709	11.3	35.1	41.1	10.2	2.2
1983	4824	15.5	7.3	42.1	27.0	8.1
1984	2964	29.1	8.7	45.4	9.8	6.9
1985	1863	24.3	16.0	39.3	16.3	4.2
1986	409	49.1	15.6	24.7	8.8	1.7
1987	141	0.7	25.5	66.0	4.3	3.5
1988	617	10.2	0.2	88.0	0.8	0.8
1989	1156	33.8	15.7	35.6	11.0	3.9
1990	-	-	-	-	-	-
Overall	15762	20.4	14.7	44.6	15.1	5.2
			Period 2			
1991	-	-	-	-	-	-
1992	1306	11.1	15.6	22.1	48.7	2.5
1993	1828	31.2	9.2	33.9	22.8	2.9
1994	3131	23.6	22.9	37.6	10.3	5.7
1995	3178	22.5	8.4	57.0	8.7	3.4
1996	2320	31.5	10.0	48.0	6.9	3.7
1997	1287	19.3	13.6	54.0	9.5	3.6
1998	1878	15.9	20.7	50.1	12.3	1.1
1999	1967	38.5	13.3	38.5	7.5	2.2
2000	198	61.1	0.0	38.9	0.0	0.0
2001	-	-	-	-	-	-
2002	995	29.5	11.3	41.0	15.6	3.3
2003	181	36.5	4.4	32.6	23.8	2.8
2004	261	0.4	54.0	23.4	6.1	16.1
Overall	18530	25.3	14.4	43.2	13.6	3.5

The results of the spatial multivariate multinomial model are presented in Table 4.3. Rainfall was negatively associated with the frequency of *An. arabiensis* and positively related to the frequency of Savanna chromosomal form in comparison to Mopti subspecies. Except the minimum temperature and the distance of >10-20 km to water all other environmental factors were positively related to *An. arabiensis*. Maximum temperature, NDVI and suitability to transmission were positively associated with the Bamako chromosomal form. More recent entomological surveys indicated lower frequencies of Bamako and Savanna

forms and higher frequencies of *An. arabiensis* in comparison to the Mopti form. The Savanna chromosomal form was also positively associated with maximum temperature, NDVI and suitability to transmission and negatively associated with SWS index, and distance of 4-10 km from water. NDVI and suitability to transmission were positively related to the hybrid form. SWS index and distances > 10 km to water bodies were negatively related to hybrid in comparison to Mopti. Strong spatial correlation was observed in the frequency distribution of all the species and subspecies of *An. gambiae* complex.

The results of the relative contribution of the different chromosomal entities of *An. gambiae* complex to the transmission are presented in Table 4.4. During both survey periods (1981-1990 and 1991-2004), the Bamako/Savanna chromosomal forms showed higher contribution to the transmission (32.3, 95% CI = 9.1—89.1 and 38.6, 95% CI = 19.5—88.8) followed by the hybrid form (28.0, 95% CI = 6.8—82.7 and 22.1, 95%CI = 15.1—78.9), the Mopti form (23.4, 95%CI = 12.2—66.9 and 20.8, 95%CI = 22.7—61.8) and finally by *An. Arabiensis* (16.3, 95%CI = 5.1—68.4 and 18.5, 95%CI = 13.0—68.0) even though the difference was not significant. This indicate that about 83.7% and 81.5% of the transmission was due to *An. gambiae s.s.* against 16.3% and 18.5% by *An. arabiensis* during the respective survey periods. The range of spatial correlation in the potential for the different sibling species to transmit the disease was very strong during the drought period of 1981-1990 (Median = 314.58 km, 95% CI = 0.1—899.0) and weak during the relatively wet period of 1991-2004 (Median = 0.08, 95%CI = 0.05—0.37).

Table 4.2: Bivariate association between chromosomal forms and climate and environmental parameters arising from multinomial regression model. Coefficients are relative to Mopti chromosomal form.

Environmental factors	*An. arabiensis* Coef. (95% CI)	Bamako Coef. (95% CI)	Savanna Coef. (95% CI)	Hybrids Coef. (95% CI)	AIC	LRT
Rainfall						
Measure_1	-0.002 (-0.002, -0.002)	-0.002 (-0.002, -0.001)	0.002 (0.002, 0.001)	0.001 (0.001, 0.001)	**92192.53**	χ^2 = 3381.9; P<0.001
Measure_2	-0.002 (-0.002, -0.002)	-0.002 (-0.002, -0.002)	0.002 (0.002, 0.003)	0.002 (0.001, 0.002)	92909.99	χ^2 = 2664.5; P<0.001
Measure_3	-0.006 (-0.006, -0.005)	-0.004 (-0.004, -0.004)	0.005 (0.004, 0.005)	0.003 (0.002, 0.003)	92471.78	χ^2 = 3102.7; P<0.001
Measure_4	-0.003 (-0.003, -0.003)	-0.002 (-0.003, -0.002)	0.003 (0.003, 0.003)	0.003 (0.002, 0.003)	93934.03	χ^2 = 1640.4; P<0.001
Temperature						
Mean minimum	0.020 (0.019, 0.022)	0.017 (0.016, 0.018)	-0.019 (-0.021, -0.017)	-0.012 (-0.015, -0.009)	92732.36	χ^2 = 2842.1; P<0.001
Mean maximum	0.004 (0.003, 0.006)	0.068 (0.064, 0.071)	0.011 (0.009, 0.014)	0.022 (0.018, 0.027)	93484.18	χ^2 = 2090.3; P<0.001
NDVI						
Measure_1	-7.01 (-7.27, -6.74)	-3.40 (-3.70, -3.10)	2.57 (2.22, 2.92)	0.63 (0.08, 1.18)	**91430.96**	χ^2 = 4143.5; P<0.001
Measure_2	-5.77 (-6.00, -5.53)	-3.75 (-3.03, -2.48)	3.52 (3.15, 3.89)	1.79 (1.22, 2.36)	91580.5	χ^2 = 3994.0; P<0.001
Measure_3	-6.03 (-6.27, -5.80)	-2.88 (-3.15, -2.61)	2.11 (1.80, 2.42)	0.41 (-0.07, 0.91)	91784.31	χ^2 = 3790.2; P<0.001
Measure_4	-0.021 (-0.022, -0.020)	-0.010 (-0.011, -0.009)	0.013 (0.012, 0.015)	0.011 0.008, 0.013)	92144.52	χ^2 = 3429.9; P<0.001
Distance to water bodies						
< 4 km	0.00	0.00	0.00	0.00		
4 - 10 km	1.92 (1.85, 2.00)	-1.10 (-1.22, -0.95)	0.80 (0.71, 0.89)	-0.37 (-0.57, -0.18)		
>10 - 20 km	0.82 (0.75, 0.89)	-6.79 (-8.18, -5.41)	-0.28 (-0.36, -0.19)	-0.56 (-0.71, -0.42)	88207.29	χ^2 = 7383.2; P<0.001
> 20 km	1.55 (1.41, 1.69)	-4.55 (-5.94, -3.17)	-0.60 (-0.87, -0.33)	-0.68 (-1.11, -0.24)		
SWS index	-1.64 (-1.76, -1.53)	-1.36 (-1.49, -1.23)	1.17 (1.04, 1.30)	0.85 (0.63, 1.06)	93514.35	χ^2 = 7383.2; P<0.001
Suitability to transmission						
Not suitable	0.00	0.00	0.00	0.00		
Suitable	-1.03 (-1.10, -0.95)	0.49 (0.36, 0.62)	2.32 (2.04, 2.60)	1.42 (1.13, 1.81)	93541.13	χ^2 = 2033.3; P<0.001

Table 4.3: Posterior estimates for presence of *An. arabiensis* and the different chromosomal forms of *An. gambiae s.s.* estimated from the geo-statistical Bayesian multiple multinomial regression model. The Mopti form is the baseline.

Environmental factors	*An. arabiensis*	Bamako	Savanna	HYBRIDS/RECOMBINANTS
	Posterior median (95%CI)	Posterior median (95%CI)	Posterior median (95%CI)	Posterior median (95%CI)
Rainfall	-0.004 (-0.005, -0.003)	0.001 (-0.001, 0.003)	0.002 (0.000, 0.003)	0.000 (-0.002, 0.003)
Max temperature	0.010 (0.006, 0.014)	0.024 (0.017, 0.031)	0.007 (0.001, 0.013)	0.008 (-0.003, 0.018)
Min temperature	0.004 (-0.002, 0.010)	-0.001 (-0.010, 0.007)	-0.004 (-0.013, 0.005)	-0.002 (-0.019, 0.011)
NDVI	9.61 (7.81, 11.83)	6.95 (4.31, 9.40)	4.44 (1.46, 7.29)	4.21 (0.06, 8.42)
Distance to water bodies				
< 4 km	0.00	0.00	0.00	0.00
4 to 10 km	1.29 (0.26, 2.44)	0.05 (-1.19, 1.22)	-1.77 (-2.98, -0.54)	-0.75 (-1.76, 0.18)
>10 to 20 km	1.23 (-0.33, 2.70)	-0.41 (-2.32, 1.44)	-0.05 (-1.55, 1.46)	-1.68 (-3.13, -0.30)
> 20 km	1.57 (0.03, 3.00)	-0.61 (-3.20, 1.75)	-0.55 (-2.32, 1.18)	-1.45 (-3.00, -0.07)
SWS	0.61 (0.28, 0.94)	-0.55 (-1.06, 0.00)	-0.62 (-1.14, -0.17)	0.14 (-0.57, 0.85)
Suitability to transmission				
Suitable	0.00	0.00	0.00	
Not suitable	0.19 (0.02, 0.36)	2.17 (1.73, 2.67)	0.92 (0.55, 1.30)	0.73 (0.26, 1.21)
Periods				
1981-1990	0.00	0.00	0.00	0.00
1991-2004	0.67 (0.55, 0.79)	-0.65 (-0.78, -0.52)	-0.55 (-0.70, -0.40)	-0.19 (0.42, 0.02)
Spatial parameters				
$3/\rho$ (km)	219.7 (53.5, 866.3)	988.1 (246.3, 2132.2)	996.1 (312.7, 2132.2)	976.8 (307.0, 2097.4)
σ^2	4.87 (2.72, 13.77)	18.01 (5.69, 53.46)	19.05 (6.75, 48.71)	9.06 (3.58, 25.28)

Table 4.4: The relative contribution of the different chromosomal entities of *An. gambiae* complex to malaria transmission in Mali.

Periods	Chromosomal entities	95% CI
Period 1 (1981 – 1990)	Percentage of transmission	
Mopti	23.4	(12.2, 66.9)
An. arabiensis	16.3	(5.1, 68.4)
Bamako/Savanna	32.3	(9.1, 89.1)
hybrids	28.0	(6.8, 82.7)
Spatial parameters		
range = $3/\rho$ (Km)	314.58	(0.1, 899.0)
σ^2	8.70	(3.7, 22.2)
Period 2 (1991 – 2004)		
Mopti	20.8	(22.7, 61.8)
An. arabiensis	18.5	(13.0, 68.0)
Bamako/Savanna	38.6	(19.5, 88.8)
hybrids	22.1	(15.1, 78.9)
Spatial parameters		
range = $3/\rho$ (Km)	0.08	(0.05, 0.37)
σ^2	0.94	(0.60, 1.57)

The bivariate logistic regression (non-spatial) analyses indicate that suitability to transmission over the year (January-December), mean NDVI value during May-October, SWS index, rainfall and maximum temperature values during June-November and the minimum temperature value during June-October best fit the prevalence data (Table 4.5). The above summaries of environmental factors gave the smallest AIC value.

The results of the Bayesian geospatial multivariate logistic regression model are presented in table 4.6. The SWS index and maximum temperature were negatively associated with malaria prevalence. Malaria risk was lower during 1981-1990 and higher prior to 1980 than the baseline period (1991-2004). A positive association was observed between rainfall and minimum temperature with malaria prevalence. All other environmental factors included in the model did not show a significant association with malaria prevalence.

Table 4.5: Bivariate association of malaria prevalence with the climatic and environmental factors estimated by (non-spatial) logistic regression analysis.

Variables	OR	95% CI*	AIC
NDVI			
Jan-Dec	19.7	16.8, 23.2	143306.85
May-Nov	9.4	8.4, 10.5	143201.12
May-Oct	9.6	8.6, 10.7	**143016.50**†
Jun-Nov	7.8	7.0, 8.6	143218.33
Jun-Oct	7.7	7.0, 8.5	143017.93
SWS index			
Jan-Dec	12.2	9.9, 15.0	144121.70
May-Nov	4.3	3.8, 4.8	144132.01
May-Oct	3.4	3.1, 3.8	144190.80
Jun-Nov	3.6	3.2, 3.9	**144120.01**†
Jun-Oct	2.8	2.6, 3.1	144179.14
Rainfall			
Jan-Dec	1.011	1.011, 1.012	142834.81
May-Nov	1.007	1.006, 1.007	142843.43
May-Oct	1.006	1.006, 1.006	142842.96
Jun-Nov	1.006	1.006, 1.007	**142829.56**†
Jun-Oct	1.005	1.005, 1.006	142829.86
Minimum temperature			
Jan-Dec	1.005	1.004, 1.007	144614.15
May-Nov	0.962	0.960, 0.964	143031.22
May-Oct	0.960	0.958, 0.961	142481.24
Jun-Nov	0.963	0.961, 0.965	142977.06
Jun-Oct	0.961	0.959, 0.962	**142326.49**†
Maximum temperature			
Jan-Dec	0.952	0.950, 0.953	141940.53
May-Nov	0.972	0.971, 0.973	141386.14
May-Oct	0.976	0.975, 0.977	141427.29
Jun-Nov	0.970	0.969, 0.971	**141164.76**†
Jun-Oct	0.975	0.974, 0.976	141216.45
Suitability to transmission			
Jan-Dec	1.18	1.17, 1.19	142734.73
May-Nov	1.26	1.25, 1.27	142381.15
May-Oct	1.49	1.47, 1.52	**141162.97**†
Jun-Nov	1.26	1.25, 1.27	142445.83
Jun-Oct	1.49	1.47, 1.52	141249.82
Land use‡			
Category 1	0.968	0.967, 0.969	140072.36
Category 2	0.998	0.998, 999	144585.25
Category 3	1.007	1.007, 1.008	143100.81
Distances to water bodies			
< 4 km	1.00		
4- 20 km	1.64	1.59, 1.69	142917.85
> 20 km	2.19	2.08, 2.30	
Study period			
1991-2004	1.00		
1981-1990	0.47	0.45, 0.49	142667.98
< 1980	1.25	1.20, 1.31	

‡ 1=Urban/Barren/dry land, 2=crop/grassland mosaic, 3=water/irrigated crop/savanna, † Variables which best fit the data. *The P-values calculated from the Likelihood Ratio Test were all <0.001

Table 4.6: Posterior estimates of the multivariate spatial logistic regression model of malaria risk given as odds ratios.

Variables	Posterior Median (OR)	95%CI
NDVI		
May-Oct	1.48	0.83, 2.82
SWS index		
June-November	0.53	0.41, 0.71
Rainfall		
June-November	2.62	1.16, 5.75
Minimum temperature		
June-October	2.73	1.66, 4.50
Maximum temperature		
June-November	0.44	0.20, 0.99
Land use‡		
Water/irrigated crop land/savanna (cat3)	1.00	
Urban/barren/sparsely vegetated/dry land (cat1)	0.83	0.69, 1.00
Crop/grassland/mosaic (cat2)	1.14	0.89, 1.44
Length of transmission		
> 4 months	1.00	
2-4 months	2.29	0.99, 5.30
0 month	1.10	0.34, 3.43
Distance to the nearest water bodies		
< 4 km	1.00	
4- 20 km	1.32	0.90, 2.09
> 20 km	1.17	0.70, 2.05
Time periods		
1991-2004	1.00	
1981-1990	0.33	0.20, 0.54
< 1980	1.40	0.88, 2.24
Spatial parameters		
range = $3/\rho$ (Km)	0.08	0.05, 0.34
σ^2	0.91	0.68, 1.23

Figure 4.1 and 4.2 depict the spatial distribution of malaria risk and the prediction error respectively, during the survey period 1991-2004. The map showed high malaria risk in the Southern part, a moderate risk in the middle and lower risk in the Northern part of the country. This distribution pattern is in agreement with the eco-geographical description of the epidemiology of malaria in Mali.

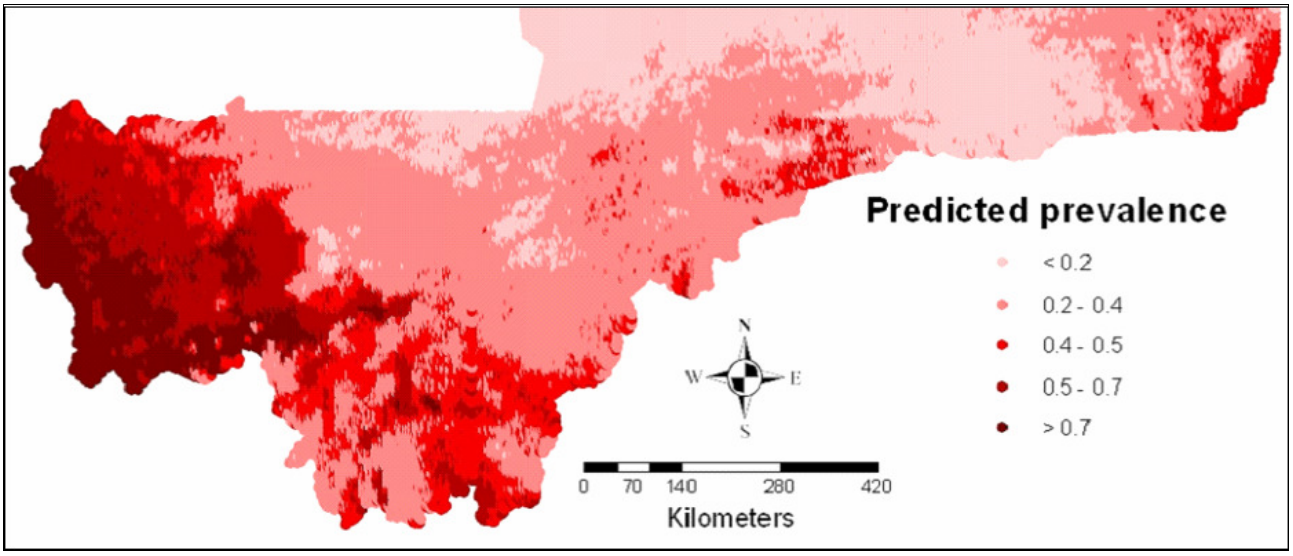

Figure 4.1: Map of predicted malaria prevalence during survey period 1991-2004.

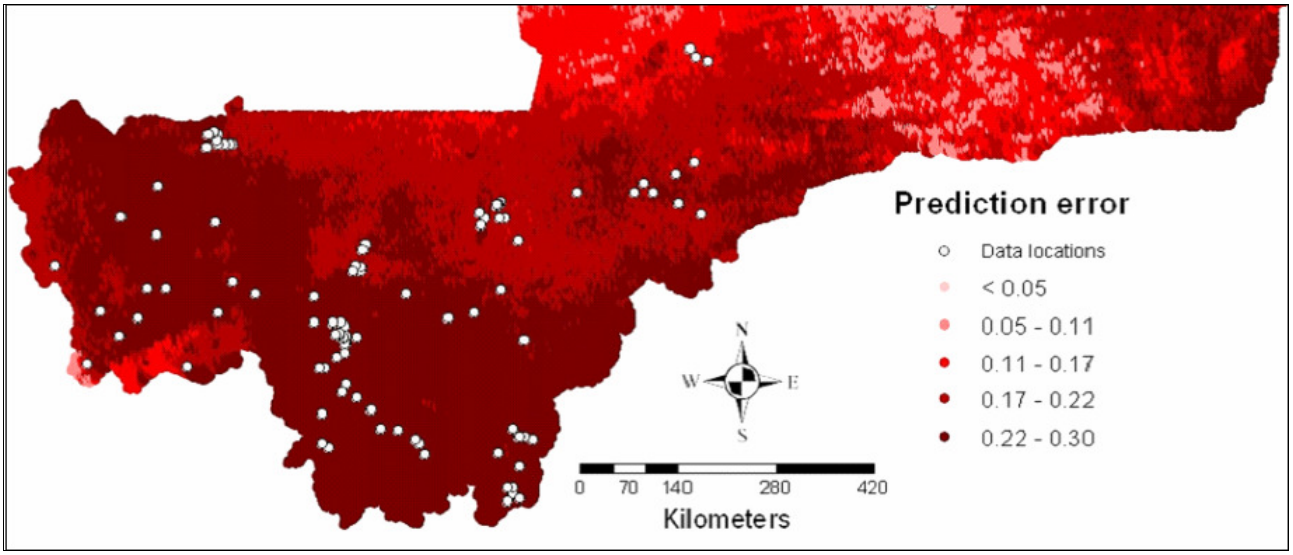

Figure 4.2: Map of prediction error of malaria prevalence during survey period 1991-2004.

Figure 4.3 to 4.6 present the attributed malaria risk to each species and subspecies. The Malaria risk is mainly due to *An. arabiensis* (Figure 4.3) in the middle West and South East part of the country, to the Mopti form (Figure 4.4) in the irrigated/flooded, to the Savanna/Bamako forms (Figure 4.5) in the southern part, and to the hybrids (Figure 4.6) the southern areas of the region of Kayes (West of the country).

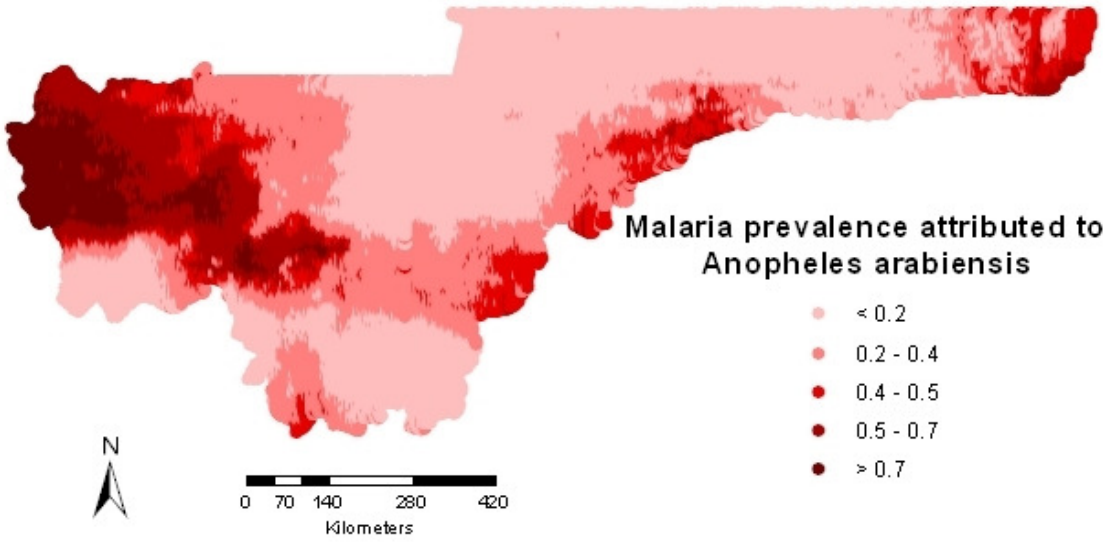

Figure 4.3: Maps of the attributed malaria risk to *Anopheles. arabiensis* in Mali.

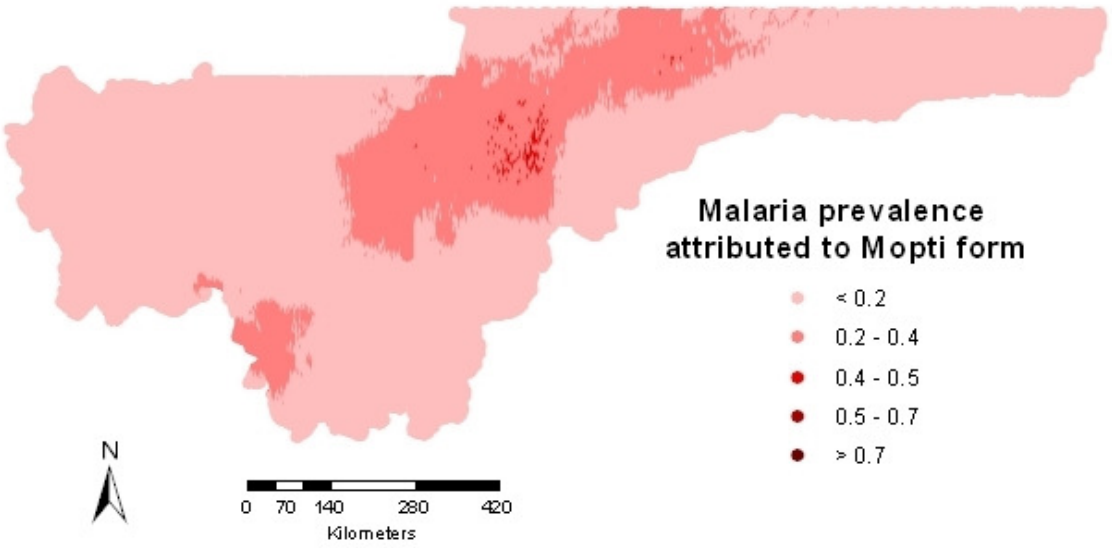

Figure 4.4: Maps of the attributed malaria risk to Mopti chromosomal form of *Anopheles gambiae s.s.* in Mali.

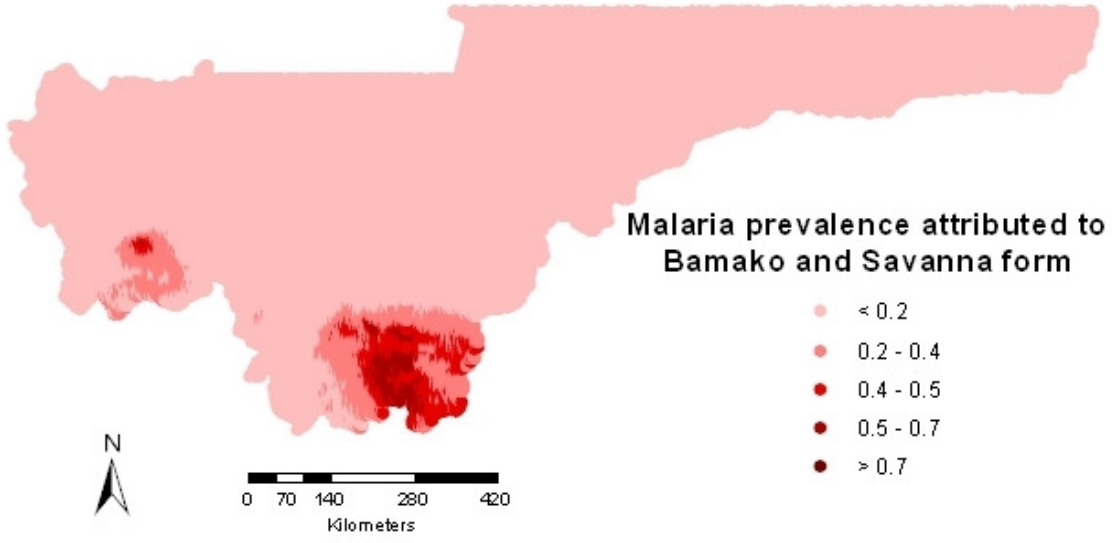

Figure 4.5: Maps of the attributed malaria risk to Bamako/Savanna chromosomal form of *Anopheles gambiae s.s.* in Mali.

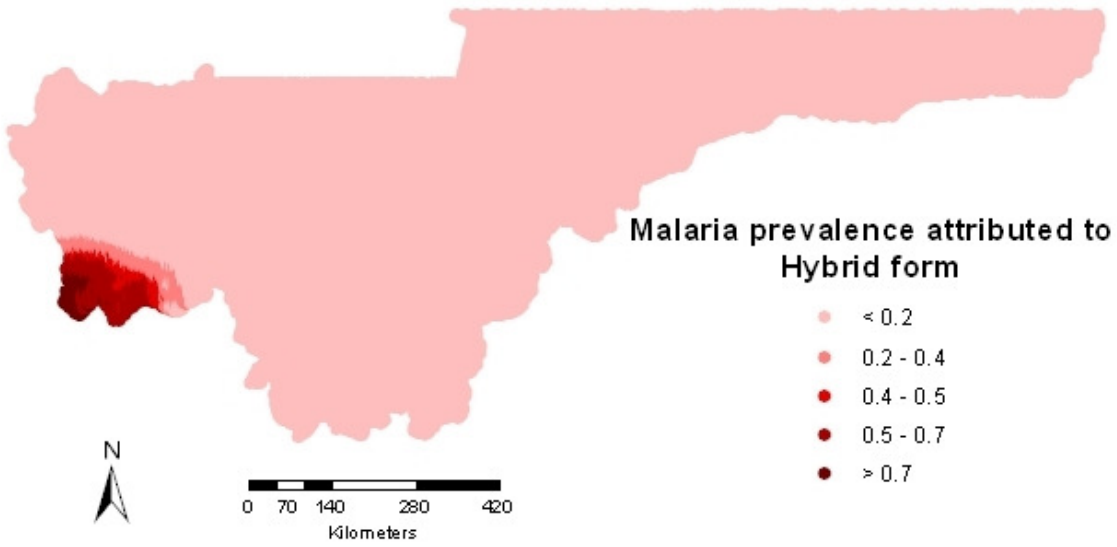

Figure 4.6: Maps of the attributed malaria risk to the hybrids/recombinant chromosomal form of *Anopheles gambiae s.s.* in Mali.

4.5. Discussion

We assessed the relationship between malaria risk and the vector species distribution, quantified the contribution of the different subspecies to malaria transmission and produced an attributed malaria risk map for each species and subspecies. Suitability to transmission and NDVI, which are influenced by rainfall in arid regions (Iwasaki, 2006) were significantly related to the frequency of all members of *An. gambiae* complex in Mali. Association of *An. arabiensis* with dry conditions and of Savanna chromosomal form with wet conditions was confirmed by our analyses (Touré *et al.*, 1998). The higher frequency of *An. arabiensis* observed during the relatively wet survey period (1991-2004) was surprising because of the usual association of this species to dry conditions (Kirby and Lindsay, 2004; Levine *et al.*, 2004). This situation could be due to the availability of breeding places preferred by *An. arabiensis*. Also this species was positively associated with most of the environmental factors included in our analyses suggesting its ability to prevail in various eco-climatic conditions

found in Mali. In addition, our data showed a strong spatial correlation between the frequencies of all member of *An. gambiae* complex supporting the adaptation of the members to diverse environmental and climatic conditions.

All the sibling species of *An. gambiae* complex were equally contributing to malaria transmission during both survey periods (1981-1990 and 1991-2004) (Table 4.4). Compared to the chromosomal forms (Mopti, Bamako/Savanna and hybrid/recombinant) of *An. gambiae s.s.*, *An. arabiensis* contribution was much lower during both survey periods probably because of its higher exophilic and zoophilic tendency (Mahande *et al* 2007). During the drought of the 1981-1990 a slightly increase in the contribution of *An. arabiensis* to transmission compared to the relatively wet period (1991-2004) was observed. During this period most of the livestock in the Sahel was decimated. Thus, *An. arabiensis*, which inherentely feeds on both animals and human (Tirados *et al*. 2006), may have been directed to human host only. This can explain its contribution to transmission as much as the other chromosomal form of *An. gambiae s.s*.

Our analyses showed a significant negative association between the malaria risk with the maximum temperature and the SWS index. The negative association with temperature can be explained by the fact that low temperature delays development of *P. falciparum* parasite in the mosquito (Macdonald, 1957; Detinova, 1962). The negative association with SWS was surprising, but it could be partly due to irrigation since it has been reported low malaria risk in the irrigated/flooded inner delta of the Niger River, Mali (Dolo *et al*., 2004, Sissoko *et al*., 2004). Rainfall was positively related to malaria prevalence. In fact, in the Sahel, the range temperature required (18-32^{o}C) for the completion of the parsite development within the mosquito (Macdonald, 1957) is observed yearound. The potential and intensity of malaria transmission is largely influenced by the rainfall, which creates the breeding habitats and

enhance adult mosquito survival (Craig *et al*., 1999). Therefore, the amount and temporal distribution of the rainfall is the main driving factor of malaria transmission in the Sahelian Africa.

The analysis of the updated MARA data (Table 4.5) showed a significant decrease in malaria prevalence during 1981-1990. Similar observations were reported from neighboring Sahelian countries of Niger and Senegal where up to 80% of reduction in malaria prevalence was observed (Faye *et al*., 1995; Mouchet *et al*., 1996). These authors explained their findings by the drought which affected the Sahel at that period limiting the availability of mosquito larval habitats. Subsequent to a slight increase in rainfall during 1991-2004 compared to the drought period (1981-1990), an increase in malaria risk was also observed. The same observation was made by Konate *et al*., (2001) in Senegal; Labbo *et al*., (2004) in Niger; Thomson *et al*. (2006), and Kent *et al*., (2007). Other factors such as environmental changes due to human activities, the resistance of parasite to drugs and of the vectors to insecticides as well as the poor implementation of control interventions could have contributed to this situation. Indeed, to response to the crucial needs of food in the Sahelian countries subsequent to the drought, governmental and non-governmental organizations (NGOs) invested in the building of small dams and irrigation systems for vegetable and rice cultivation. These agricultural activities generally create suitable conditions for vector breeding and extend malaria transmission season length. In addition, there was the spread of parasite resistance to drugs and mosquito to insecticides across the continent of Africa during the last decade. An overall of 30% of resistance to CQ was reported by the National malaria control program of Mali. A malaria epidemic investigation in Kidal, (Northern Mali) reported a resistance of 27-40% of *P. falciparum* to chloroquine (CQ) (Djimde *et al*., 2004). About 90.5% resistance to CQ and 7% to Sulfadoxine Pyrimithamine (SP) were reported in southern Mali (de Radigues

et al. 2006). Fanello *et al*. (2003) reported up to 83% of relative frequency of the Knock dawn *(kdr*) allele in the Savanna chromosomal form of *An. gambiae* complex in southern Mali.

We produced a malaria risk map only for the survey period of 1991-2004 because this may reflect more accurately the actual situation of the disease. This map showed high malaria risk in the Southern part, a moderate risk in the middle and lower risk in the Northern part of the country. This distribution pattern is in agreement with the eco-geographical description of the epidemiology of malaria in Mali (Doumbo *et al*. 1989).

The attributed malaria risk maps of the different species and subspecies indicated that malaria transmission is driven by *An. arabiensis* in many part of the country namely in the middle West and South East part. This can be explained by the ability of this sibling species to survive under different climatic conditions even throughout the dry season (Touré *et al*., 1998). In the irrigated/flooded areas malaria risk is supported by the Mopti form. In the southern part of Mali, the transmission is mainly due to the Savanna/Bamako form. Malaria risk is mainly driven by the Hybrid forms in the southern areas of the region of Kayes.

This study indicated that malaria risk varies over time in Mali with lower risk associated to the drier period. All the members of *An. gambiae* complex are contributing to malaria transmission in Mali. *An. arabiensis* contributes to transmission across most of the territory but at very low intensity compared to the populations of *An. gambiae s.s*.

4.6. Acknowledgements

We acknowledge all the MRTC/FMPOS Entomology team for their efforts and contribution to the data collection and processing. We are very thankful to the community of

all our study sites for their full collaboration. The analysis of the data was supported by the Swiss National Foundation project Nr. 3252B0-102136/1.

4.7. Appendix

We describe 1) the geospatial logistic regression model fitted to obtain a map of the malaria risk in Mali 2) the geospatial multinomial model employed to predict the frequency distribution of the subspecies at the locations we had observed malaria survey data as well as to produce a map of the distribution of subspecies in Mali 3) the geospatial logistic regression model fitted to assess the relation between malaria risk and the distribution of the subspecies and 4) the approach used to obtain the malaria risk maps attributed to each subspecies.

4.7.1. Logistic regression model for malaria prevalence

Let N_i be the number of persons examined, at location s_i, $i = 1, \ldots, n$, and Y_i be the number of those found positives with malaria parasite in a blood sample and $X_i = (X_{i1}, X_{i2}, ..., X_{ip})^T$ be the vector of p associated environmental predictors observed at location s_i. We assume that Y_i arise from a binomial distribution, that is $Y_i \sim Bn(p_i, N_i)$, with parameter p_i measuring malaria risk at location s_i and model the relation between the malaria risk and environmental covariates X_i via the logistic regression $\log it(p_i) = X_i^T \beta$, where $\beta = (\beta_1, \beta_2, ... \beta_p)^T$ are the regression coefficients. This model assumes independence between the surveys. To take into account the spatial correlation present in the data we introduce location specific random effects (error term) ϕ_i at each location s_i that $\log it(p_i) = X_i^T \beta + \phi_i$, which model a latent spatial process, that is $\phi = (\phi_i, ... \phi_N)^T \sim MVN(0, \Sigma)$. The covariance matrix Σ is a function of distance between locations, irrespective of the locations themselves (stationarity) and of the direction (isotropy).

We adopted an exponential correlation function, that is $\Sigma_{ij} = \sigma^2 \exp(-\rho d_{ij})$ where σ^2 is the spatial variance, ρ is the parameter that models the rate of correlation decay, and d_{ij} is the distance between the locations s_i and s_j. Based on the above specifications, the minimum distance for which the spatial correlation becomes less than 5% is calculated by $3/\rho$ (Ecker and Gelfand, 1990).

4.7.2. Geostatistical multinomial regression model

Let Y_{ik} be the observed frequency of mosquito chromosomal form *k* at location *i* where *k = 1, 2,3,4,5* denote the Mopti, *An. arabiensis*, Bamako, Savanna , and hybrid forms, respectively. We assume that Y_{ik} arise from a multinomial distribution, that is $(Y_{i1}, Y_{i2}, Y_{i3}, Y_{i4}, Y_{i5}) \sim Mult(n_i, \pi_{i1}, \pi_{i2}, \pi_{i3}, \pi_{i4}, \pi_{i5})$ with parameters π_{ik} and n_i is the total number of *An. gambiae* complex collected at location *i*. We introduce spatial correlation on location-specific random effects ϕ_{ik} which are modeled together with the covariate effects on the logit parameters, that is $\log\left(\frac{\pi_{ik}}{\pi_{i5}}\right) = \underline{X}_i^T \underline{\beta}_k + \phi_{ik}$ where $\underline{\beta}_k$ are covariate parameters related to the k^{th} multinomial category, *k=1,2,3,4*. We further assumed a latent isotropic Gaussian spatial process $\phi_k = (\phi_{1k}, ...\phi_{Nk}) \sim MVN(0, \Sigma_k)$ at each multinomial category k with covariance matrix Σ_k defined as above that is $(\Sigma_k)_{ij} = \sigma_k^2 \exp(-\rho_k d_{ij})$ where σ_k^2 is the spatial variance related to the multinomial category k, ρ_k is the parameter that models the rate of correlation decay and d_{ij} the distance between the locations *i* and *j*.

4.7.3. Assessing the relation between malaria risk and mosquito subspecies

We assessed the relation between malaria risk and mosquito subspecies by fitting the following logistic spatial regression model: $\log it(p_i) = b_0 + \sum_{j=1}^{4} b_j \log \frac{\pi_{ij}}{\pi_{i5}} + \phi_i$, where p_i is the malaria risk at location i, $\pi_{ij}, j = 1,...5$ are the frequencies of the *An. arabiensis*, Bamako/Savanna, hybrid, Mopti subspecies, respectively at location *i,* and ϕ_i is a spatial random effect modeled as described in Section 4.7.1, b_j are coefficients corresponding to the logits of the subspecies' frequencies.

4.7.4. Model fit

The parameters of the above models were estimated using Markov Chain Monte Carlo (MCMC) simulation methods. In accordance with the Bayesian model specification, we adopted prior distributions for the model parameters. We choose vague normal prior distributions for the regression parameters $\underline{\beta}$ having large variances (i.e., 10,000), inverse gamma priors for σ_k^2 and uniform priors for $\rho_k, k = 1, 2, 3, 4$. We ran a single chain sampler with a burn-in of 5,000 iterations. Convergence was assessed by inspection of ergodic averages of selected model parameters. The Bayesian model fit was carried out in WinBUGS 1.4. (Imperial College and MRC, UK), whereas the model prediction was implemented in Fortran 95 (Compaq Visual Fortran, Professional 6.6.0) using standard numerical libraries (NAG, The Numerical Algorithms Group Ltd).

4.7.5. Producing malaria risk maps attributed to mosquito subspecies

Maps of malaria risk and of the distribution of mosquito subspecies in Mali have been produced using Bayesian kriging (Diggle and Tawn, 1998) and the models described in 4.7.1 and 4.7.2. These maps are based on predictions made over 85,000 unsampled locations and they were converted to malaria risk maps attributed to each subspecies. In particular the malaria risk q_{ik} attributed to subspecies k at location i was calculated by $q_{ik} = p_i w_k \pi_{ik}$ where p_i is the malaria risk at location i, π_{ik} is the frequency of subspecies k at i and $w_k = \frac{\exp(a_k)}{\sum_{j=1}^{5} \exp(a_j)}$ is a weight corresponding to the transmission potentials of subspecies k. a_j are regression coefficients arised from bivariate logistic regressions of each subspecies frequency on the malaria risk.

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Chapter 5

Spatial and seasonal distribution of sibling species and chromosomal forms of *An. gambiae* complex within a Malian village.

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Working paper

Abstract

Differences in the ecology of sibling species of malaria vectors may be reflected in different spatial and temporal distributions within areas where their ranges overlap. We have now assessed the spatial and seasonal distribution of *An. gambiae* complex adult densities and the relative frequencies of the chromosomal forms of the sibling species of *An. gambiae s.s.* in relation to the local environmental factors in a Sudan savanna village in Mali. Bayesian geospatial negative binomial and multinomial models were fitted to mosquito densities and subspecies composition data, respectively. The mosquito densities were over-dispersed at the onset of the rains and during the dry season with a concentric clustering of higher densities at the periphery of the village. A patchy clustering distribution of mosquito density was observed during the middle and end of the rainy season. The chromosomal forms were sympatric over all seasons, with a spatial and temporally dynamic clustering in their relative frequency distribution. The Mopti chromosomal form was the most abundant at the beginning and middle, and the Bamako form at the end of the rainy season. The minimum distance of the spatial correlation between chromosomal forms was 1.13 km and the maximum one was up to 10 km (except for the hybrids in October). Vector densities were control targeting the periphery of the village at the onset of the rains and during the dry season can ameliorate malaria situation. More focused micro-ecological studies are required to better understand the ecological differences between the chromosomal forms and their distinct contributions to disease transmission.

Key words: *An. gambiae*, Chromosomal forms, Bayesian geostatistics, multinomial, negative binomial, Markov chain Monte Carlo (MCMC), kriging.

5.1. Introduction

Malaria transmission is a dynamic and complex process which is not yet understood enough to eradicate the disease. The degree of vector-human contact determines the malaria transmission risk. Vector abundance and transmission patterns are largely influenced by environmental and climatic factors (Thomson *et al*., 1996). The risk of transmission can vary from one geographical area to another and in the same geographical area from one village to another (MBogo *et al*., 2003). Moreover, there are local differences in malaria transmission over time and space in the same village (Staedke *et al*. 2003). Distance to breeding sites (Cano *et al*. 2005, Oesterholt *et al*., 2006) and type of houses (Bagayoko, 2001, van der Hoek *et al*. 2003) are local environmental factors frequently associated with high mosquito density.

Another important factor in malaria transmission is the distribution of vector species in space and time. The species and subspecies of *An. gambiae* complex have different breeding site preferences (Toure *et al*. 1998a, della Torre *et al*., 2002; Eidillo *et al*., 2002). The Mopti form shows the closest association with the domestic environment and larval habitats created by human activities; the Savanna form is more frequent in rain-dependent temporary breeding sites whereas the Bamako form is associated with riverine areas of the upper River Niger. Fanello *et al*. (2003) also explain differences in insecticide resistance among sympatric species and subspecies of *An. gambiae* complex in cotton cropping areas of Mali by the segregation of their breeding habitats. Thus, the availability of suitable breeding habitats for one or another species or subspecies will determine its abundance and contribution to malaria risk. Other factors such as relative humidity can affect the spatial and temporal distribution of the different chromosomal forms. For example the Mopti form can better survive in the dry season while the Savanna form tends to disappear during this period (Touré *et al*., 1998). Reliable information on the spatial distribution of *An. gambiae* complex species and/or

subspecies throughout the transmission season in relation with local environmental factors might thus be useful for targeted control.

In previous work, we analyzed the spatial distribution of *An. gambiae* complex species (Sogoba *et al*., 2007) and subspecies and assessed the relation between their frequency distributions and malaria prevalence across the whole country of Mali. These analyses showed a clear geographical preference for each species and subspecies. We have now analyzed the spatial and seasonal distribution of *An. gambiae* complex adult densities and the relative frequencies distribution of the chromosomal forms of *An. gambiae s.s.* in relation to environmental factors at local level within a single Sudan savanna village.

5.2. Materials and methods

5.2.1. Study site

The village of Bancoumana is located 60 km southwest of Bamako (12.20N, 8.20W) in the south savanna zone of Mali,5 km from the left bank of the Niger River. In 1996 a socio-demographic study conducted by the Malaria Research and Training Center of the Faculty of Medicine, Pharmacy and Dentistry of the University of Bamako (MRTC/FMPOS) indicated that the village had about 8000 inhabitants living in 1771 houses (1237 with iron roofs and 534 with straw roofs) grouped in 340 compounds.

A map of the village (Figure 1) with the location of the 340 compounds, the major larval breeding sites, the main roads, and the major communal buildings (clinics, school) with a resolution of 1-3 m has been produced using Differential Global Positioning System (DGPS) (Bagayoko, 2000). The village is subdivided into four clusters or blocks by the main roads. The primary economical activity is agriculture. There are two main seasons: the rainy

season from June to November and the dry season from December to May. Much of the land between the village and the river is flooded during the rainy season, and is used to grow rice, with millet and sorghum grown nearby. During the dry season the flooded area is used for gardening. There is also a canaal about 0.5 to 1 meter deep through the village, the legacy of a failed irrigation project.

The major malaria vector is *An. gambiae s.l.* (Touré *et al*. 1998b; Bagayoko, 2000). The malaria prevalence in children less than 5 varies from about 30 to 50% during the dry season up to 75% during the rainy season (Doumbia, 2002; Dolo *et al*., 2003).

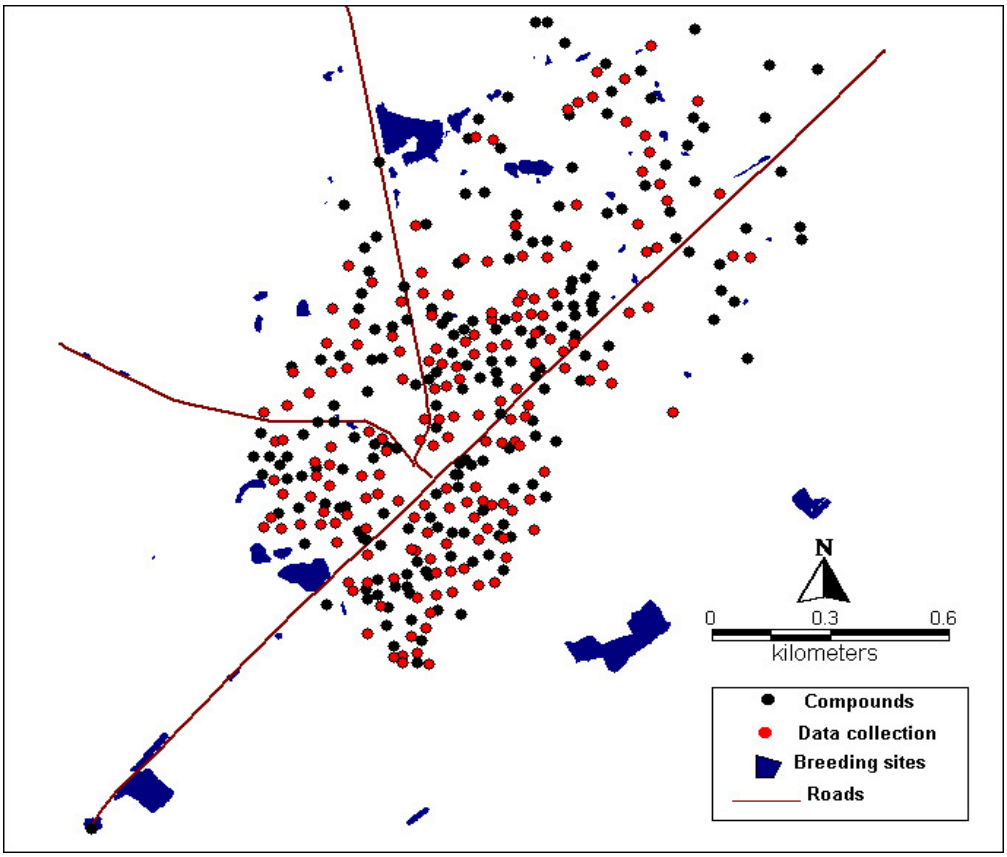

Figure 5.1: Map of the village of Bancoumana showing the location of the 340 compounds and the major potential larval breeding sites

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5.2.2. Mosquito sampling and processing

Mosquitoes were collected in monthly cross-sectional surveys using pyrethrum spray catches (PSC). Collections were performed during the day in human sleeping houses from 1996 to 1999 in June, August, October and March, representing the onset, the middle, and end of rainy season and the dry season, respectively. The collections were performed during the last fortnight of each month in 180 randomly selected houses. The sampling of these houses was constrained to respect the proportion of house-roof type (thatch roof vs. metal roof) in the study site. Sampling was also constrained to prevent multiple houses from being selected within single compound (aggregation of houses). The total number of mosquitoes, the house identification number, the type of the house and the number of people whom slept the previous night in that house were recorded. Mosquitoes were kept in the Carnoy's fixative.

Mosquito densities were measured using the count of mosquitoes sampled per house. Abdomens of the half-gravid mosquitoes were used for chromosomal identification (ovaries). Chromosome preparations were made by extracting ovaries from each abdomen sample following established protocols (Coluzzi *et al*., 1968; Hunt, 1973). Species and chromosomal form identification were carried out by examining the banding patterns of polytene chromosomes by phase-contrast microscopy using the polytene chromosome map for the *An. gambiae* complex developed by Coluzzi, *et al* (unpublished).

5.2.3. Environmental variables

The local environmental variables collected and included in the analysis were the housing type (straw and iron roofs), the distance of each collection point to the nearest potential larval habitat, canal and edge of the village. House type was recorded at the time of mosquito data collection. The minimum distance of each collection point to larval breeding sites, main canal, and edge of the village were extracted using Arc GIS.

5.3. Data analysis

The main objectives of this study were to assess the spatial and seasonal distribution of i) *An. gambiae* complex adult densities and ii) the frequency distribution of the chromosomal forms of *An. gambiae s.s.*

Mosquito density data were analyzed using spatial negative binomial regression models. These models were fitted to relate mosquito count data per house with the local environmental factors. Negative binomial regression was employed because most of the houses had no or small number of mosquito counts and only few had large numbers. These models were also used to predict mosquito density at unobserved locations and produce a density map for each season. The seasons were represented by the following months: June (beginning), August (middle), October (end) of the rainy season and March (dry season).

An. gambiae s.s. chromosomal forms data were analyzed using multinomial geostatistical models. These models related the frequency distributions of the species and subspecies data (outcome measures) to environmental predictors. They were also used to predict the vector species and subspecies data at unobserved locations. The multinomial categories represented the following four species or chromosomal forms: Mopti, Bamako, Savanna, and Hybrids/recombinants. The Mopti form was considered as the baseline category.

Both analyses considered the following environmental factors: the house type (straw or iron roof), distance to the nearest larval habitat, distance to the canal and the distance to the edge of the village.

Bivariate non-spatial regression models (binomial and multinomial) were fitted in STATA v9.0 (STATA Corporation, USA). The statistical significance was assessed using the likelihood ratio test (LRT). All factors were entered into a Bayesian multivariate geostatistical model (negative binomial or multinomial depending on the outcome). The parameters of the geostatistical models were estimated using Markov chain Monte Carlo (MCMC) simulation methods. The Bayesian model fit was carried out in WinBUGS 1.4. (Spiegelhalter *et al*., 2004), whereas the model prediction was implemented in Fortran 95 (Compaq Visual Fortran, Professional 6.6.0) using standard numerical libraries (NAG, The Numerical Algorithms Group Ltd).

A description of the geostatistical multivariate negative binomial and multinomial models is given in the Appendix

5.4. Results

Table 5.1 presents the geometric mean (GM) density of *An. gambiae s.l.* by year and season. The overall highest mean density was observed in August and the lowest in March. The mean density decreased progressively over the successive years of the study for all seasons except in June 1998 and in October 1997 where a slight increase was observed compared to the first year of study. In March there was a rapid increase in the densities between 1997 and 1998.

Table 5.1: Geometric mean (GM) density per house of *An. gambiae s.l.* by year and season (months represent the seasons).

	June		August		October		March	
Years	**GM***	**95%CI**	**GM**	**95%CI**	**GM**	**95%CI**	**GM**	**95%CI**
1996	4.4	(3.6, 5.3)	8.9	(6.9, 11.6)	2.8	(2.2, 3.4)	-	-
1997	4.2	(3.3, 5.3)	7.8	(5.9, 10.3)	3.9	(3.1, 4.8)	0.01	(-0.01, 0.02)
1998	5.5	(4.4, 6.8)	3.0	(2.4, 3.8)	3.3	(2.6, 4.2)	0.18	(0.10, 0.23)
1999	1.0	(0.8, 1.2)	5.2	(3.9, 8.0)	2.3	(1.8, 2.9)	0.02	(0.00, 0.04)
Overall	3.4	(3.0, 3.8)	5.9	(5.1, 7.7)	3.0	(2.7, 3.4)	0.06	(0.04, 0.08)

NB: N=156 *GM = geometric mean

The results of the bivariate associations between the environmental factors and the mosquito count are presented in Table 5.2. All the environmental factors were significantly associated with mosquito density in June and in October except the distance to the breeding sites and the distance to the edge of the village, respectively. In August, the year of study and the house type were the only environmental factors significantly associated with mosquito density. The house type was significantly associated to mosquito density in all months except March.

The Bayesian geostatistical negative binomial models (Table 5.3) showed that mosquito densities were higher in 1998 than 1996 and that houses with straw roof had higher densities than houses with iron roof. The association between mosquito densities and distance to potential breeding sites was weakly positive (except in March). Lower mosquito densities were observed throughout 1999. Any other factor was not significantly related to mosquito density.

There was an over-dispersion of mosquito densities in general and particularly at the beginning of the rainy season (Negative Binomial r = 1.3 [95%CI: 1.1—1.5]). A range parameter $3/\rho$ of 2.1 km (1.4, 11.8) was observed in mosquito densities during the dry season (March). During the other seasons, it was 1.7 km.

Figures 5.2 - 5.5 are maps of mosquito densities and their prediction errors during different seasons. At the beginning of the rainy season (June), there is a concentric distribution pattern with higher densities at the periphery of the village (Figure 5.2). This pattern disappears in August (Figure 5.3) and October (Figure 5.4) where patchy clusters of highest densities are observed across the village. During the dry season (March), the concentric distribution pattern reappears with higher densities at the South-Eastern part of the village facing the Niger River (Figure 5.5).

Table 5.2: Bivariate association between *An. gambiae s.l.* density and environmental parameters arising from non-spatial negative binomial regression model.

Environmental factors	Early rainy seasonJune		Mid rainy season		Late rainy season		Dry season	
	Coef.	LRT* P-Value	Coef.	LRT P-Value	Coef.	LRT P-Value	Coef.	LRT P-Value
Years								
1996	0.00		0.00		0.00	$\chi^2 = 13.97$	-	
1997	0.03	$\chi^2 = 157.26$	-0.11	$\chi^2 = 42.30$	0.25	P = 0.0029	0.00	$\chi^2 = 42.27$
1998	0.19	P < 0.001	-0.69	P < 0.001	0.13		3.02	P < 0.001
1999	-1.17		-0.40		-0.15		0.69	
House type								
Iron roof	0.00	$\chi^2 = 6.60$	0.00	$\chi^2 = 34.81$	0.00	$\chi^2 = 33.98$	0.00	$\chi^2 = 1.24$
Straw roof	0.28	P = 0.0102	0.62	P < 0.001	0.59	P < 0.001	0.39	P = 0.2647
Distance to canal								
0 – 500m	0.00		0.00		0.00		0.00	
501 – 750m	-0.49	$\chi^2 = 11.01$	-0.27	$\chi^2 = 2.80$	-0.50	$\chi^2 = 11.56$	-0.95	$\chi^2 = 8.78$
751–1000m	-0.41	P = 0.0117	-0.14	P = 0.4235	-0.19	P = 0.0091	-1.21	P = 0.0323
> 1000m	-0.17		-0.16		-0.15		-0.15	
Distance to breeding sites	0.0001	$\chi^2 = 1.34$	0.000	$\chi^2 = 0.11$	0.0002	$\chi^2 = 4.02$	0.0004	$\chi^2 = 0.95$
		P = 0.2465		P = 0.7389		P = 0.0449		P = 0.3306
Distance to village's edge	0.003	$\chi^2 = 31.93$	0.00	$\chi^2 = 0.28$	0.000	$\chi^2 = 0.01$	0.008	$\chi^2 = 14.72$
		P < 0.001		P = 0.5978		P = 0.9060		P < 0.001

Table 5.3: Association between *An. gambiae s.l.* densities and environmental parameters arising from the geo-statistical Bayesian multiple negative binomial regression model.

Environmental factors	June		August		October		March	
	Coef.	95%BCI*	Coef.	95%BCI	Coef.	95%BCI	Coef.	95%BCI
Years								
1996	0.00		0.00		0.00		-	
1997	0.04	(-0.19, 0.26)	-0.12	(-0.46, 0.19)	0.39	(0.15, 0.64)	0.00	
1998	0.32	(0.11, 0.56)	-1.22	(-1.54, -0.88)	0.21	(-0.06, 0.44)	2.96	(1.93, 4.31)
1999	-1.59	(-1.85, -1.33)	-0.27	(-0.60, 0.07)	-0.11	(-0.35, 0.13)	0.43	(-1.11, 1.93)
House type								
Iron roof	0.00		0.00		0.00		0.00	
Straw roof	0.40	(0.18, 0.64)	1.15	(0.80, 1.47)	0.81	(0.58, 1.06)	0.22	(-0.63, 1.17)
Dist.to breeding sites	4.2e-04	(6.4e-06, 4.8e-04)	1.0e-03	(4.5e-04, 1.6e-03)	6.3e-04	(1.2e-04, 1.2e-03)	-3.8e-04	(5.1e-05, 8.3e-04)
Distance to the canal								
0–500 m	0.00		0.00		0.00		0.00	
500–750 m	-0.29	(-0.93, 0.34)	0.51	(-0.58, 1.43)	-0.43	(-1.04, 0.19)	-0.25	(-1.50, 0.95)
750–1000m	0.20	(-0.83, 1.06)	0.93	(-0.47, 2.12)	-0.01	(-0.90, 0.90)	-0.07	(-1.57, 1.35)
> 1000 m	0.10	(-1.01, 1.16)	1.32	(-0.28, 2.86)	-0.03	(-1.09, 1.07)	-1.69	(-3.44, -0.06)
Dist. to village's edge	0.00	(-0.01, 0.00)	0.00	(-0.01, 0.01)	0.001	(-0.003, 0.004)	-0.01	(-0.02, 0.00)
Spatial parameters								
$3/\rho$ **(km)	1.74	(1.35, 6.92)	1.67	(1.35, 3.67)	1.69	(1.34, 4.87)	2.06	(1.36, 11.83)
r	1.27	(1.07, 1.51)	0.59	(0.51, 0.67)	0.80	(0.68, 0.95)	0.97	(0.83, 1.66)
σ^2	1.66	(0.79, 6.90)	3.67	(1.73, 11.03)	1.99	(0.82, 6.32)	1.02	(0.28, 4.18)

* Bayesian credible interval

**Distance (km) with spatial correlation < 5%

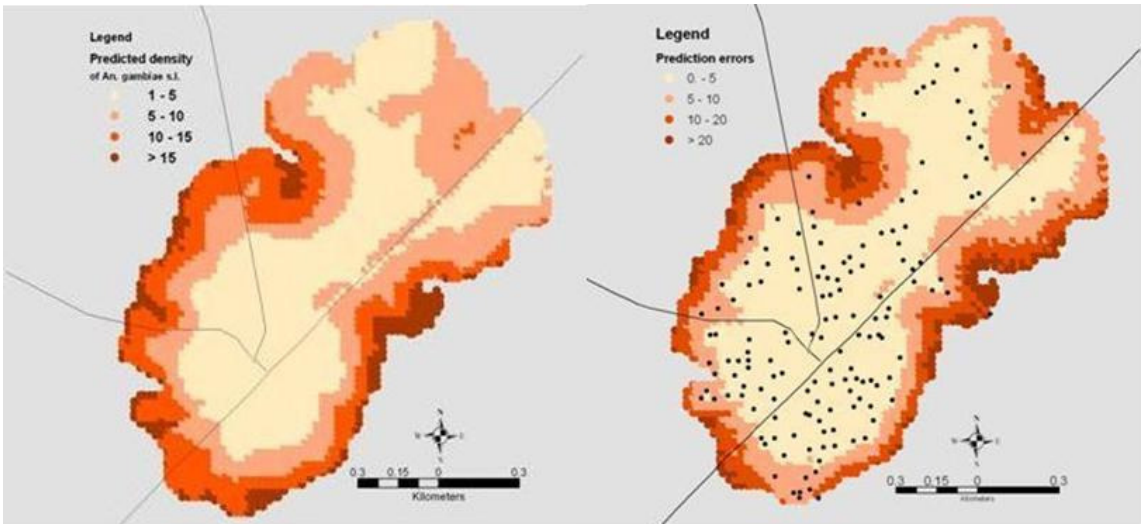

Figure 5.2: The predicted density (left) and it prediction error (right) maps of *An. gambiae s.l.* in June in Bancoumana, Mali. The gray indicates the unsampled area.

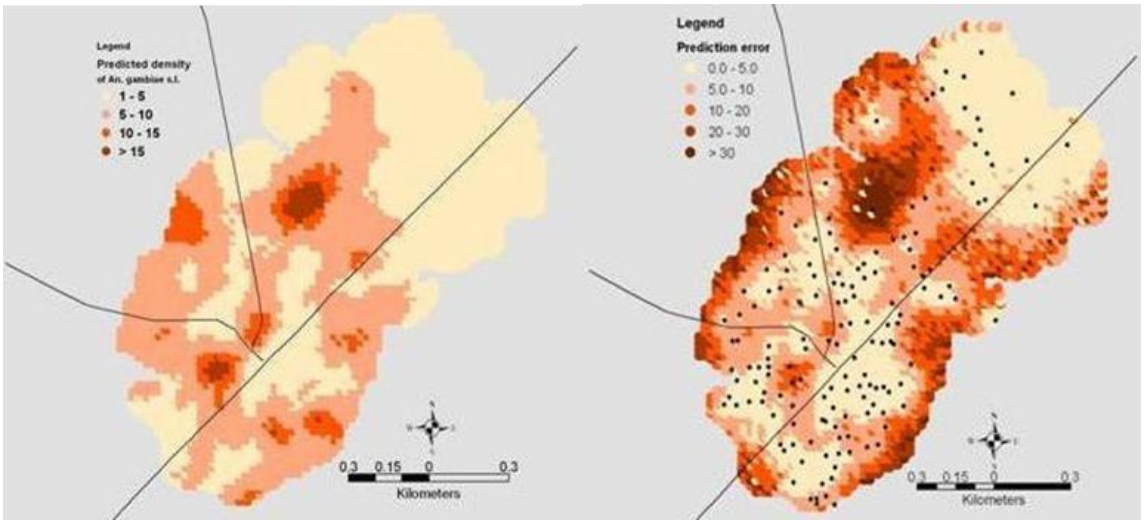

Figure 5.3: The predicted density (left) and it prediction error (right) maps of *An. gambiae s.l.* in August in Bancoumana, Mali. The gray indicates the unsampled area.

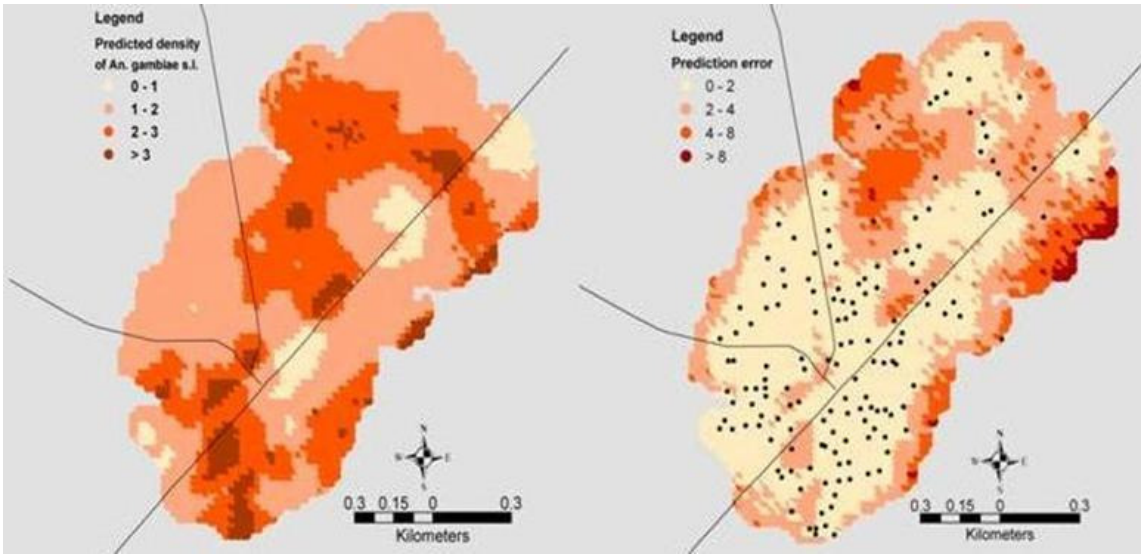

Figure 5.4: The predicted density (left) and it prediction error (right) maps of *An. gambiae s.l.* in October in Bancoumana, Mali. The gray indicates the unsampled area.

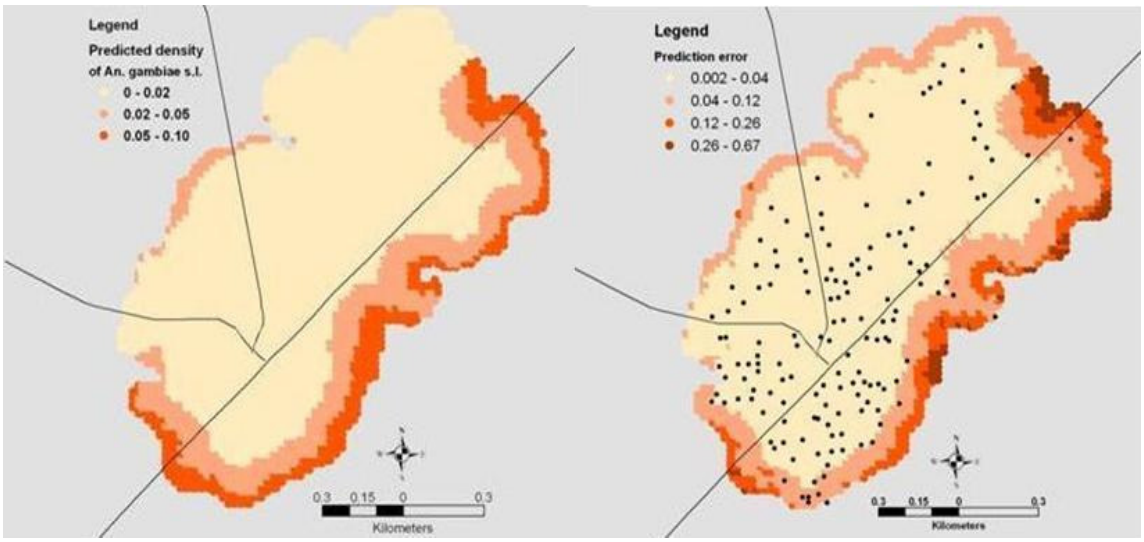

Figure 5.5: The predicted density (left) and it prediction error (right) maps of *An. gambiae s.l.* in March in Bancoumana, Mali. The gray indicates the unsampled area.

Table 5.4 shows the relative frequency of the different chromosomal forms of *An. gambiae s.s.* The Mopti chromosomal form was by far the most abundant over the study period and during all seasons except in October where the Bamako chromosomal form was the most prevalent. The highest relative frequency of the hybrid chromosomal form was in October.

Bivariate (non-spatial) multinomial regression models were used to analyze factors associated with the relative frequencies of different chromosomal forms by season (Table 5.5). Significantly associated factors were the house type in August and October, and the distance to the edge of the village in June and August.

In the geostatistical multinomial models (Table 5.6) housing type was the only good predictor of the karyotype composition. Straw roof houses are positively associated with the Savanna form in June and August and the Bamako form in August and October. The distances at which spatial correlation between were less than 5% (range) are shown by karyotype and season in Table 5.6, These range from 1.13 km to a maximum of 11.3 km (except for the hybrid in August where the estimate of the range is very imprecise).

Table 5.4: Relative frequencies of the chromosomal forms (Mopti, Bamako, Savanna, Hybrids) of *An. gambiae s.s.* by year and seasons (months represent the seasons).

	Early rainy season				Mid rainy season				Late rainy season			
Years	**Count**	**Bamako (%)**	**Savanna (%)**	**Hybrids* (%)**	**Count**	**Bamako (%)**	**Savanna (%)**	**Hybrids (%)**	**Count**	**Bamako (%)**	**Savanna (%)**	**Hybrids (%)**
1996	233	13.7	5.2	4.7	642	9.0	5.1	3.4	202	44.6	7.9	6.4
1997	542	13.7	8.9	3.7	115	12.2	8.7	1.7	116	44.8	6.0	2.6
1998	262	17.6	16.4	0.0	158	32.3	24.1	0.0	120	50.0	9.2	1.7
1999	49	40.6	6.1	2.0	74	29.7	10.8	4.3	85	82.4	5.9	4.7
Overall	1086	15.8	9.8	2.9	989	14.7	9.0	2.7	523	52.0	7.5	4.2

* Hybrids between Mopti-Savanna, Bamako-Savanna and the recombinants

Table 5.5: Bivariate association between chromosomal forms and environmental parameters arising from multinomial regression model. The coefficients are relative to the Mopti chromosomal form.

Environmental	June				August				October			
factors	Bamako	Savanna	Hybrids	LRT*	Bamako	Savanna	Hybrids	LRT	Bamako	Savanna	Hybrids	LRT
House type												
Iron	0.00	0.00	0.00	$\chi^2 = 6.8$	0.00	0.00	0.00	$\chi^2 = 15.4$	0.00	0.00	0.00	$\chi^2 = 9.5$
Straw	0.02	0.50	-0.53	P=0.0767	0.67	0.26	1.01	P=0.0015	0.59	0.04	0.13	P=0.0232
Distance to				$\chi^2 = 3.2$				$\chi^2 = 1.9$				$\chi^2 = 5.5$
breeding sites	0.0002	-0.0002	-0.0007	P=0.3606	0.0002	0.0004	0.0003	P=0.6018	0.0001	0.0007	0.0009	P=0.1379
Distance to village's edge												
0-200 m	0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00	0.00	
201-300 m	-0.23	-0.14	-0.51	$\chi^2 = 9.3$	0.37	0.12	-0.17	$\chi^2 = 20.5$	0.16	0.35	0.12	$\chi^2 = 5.0$
301-400 m	-0.08	-0.32	0.15	P=0.0050	0.67	0.14	-0.40	P=0.0152	-0.39	-0.40	-0.03	P=0.8332
> 400 m	-0.52	-0.24	0.82		1.22	1.11	1.03		-0.15	-0.40	0.19	
Distance to the canal												
0–500 m	0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00	0.00	
500–750 m	-0.29	-0.13	-0.02		0.48	0.03	0.39		0.62	0.41	0.61	$\chi^2 = 15.6$
750–1000m	-0.39	-0.27	-0.13	$\chi^2 = 9.3$	0.79	-0.19	0.40	$\chi^2 = 16.1$	0.07	0.18	0.52	P=0.3009
>1000 m	0.82	1.15	0.51	P=0.4102	0.87	0.53	0.86	P=0.0641	0.32	-0.82	0.08	

* Likelihood Ratio Test

Table 5.6: Posterior estimates of the parameters of the multiple geostatistical multiple multinomial regression model.

Environmental factors	June			August			October		
	Bamako Median (95%BCI*)	Savanna Median (95%BCI)	Hybrids Median (95%BCI)	Bamako Median (95%BCI)	Savanna Median (95%BCI)	Hybrids Median (95%BCI)	Bamako Median (95%BCI)	Savanna Median (95%BCI)	Hybrids Median (95%BCI)
House type									
Iron roof	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Straw roof	0.16 (-0.33, 0.66)	0.34 (0.24, 0.97)	-0.21 (-1.37, 1.03)	0.54 (0.04, 1.09)	0.59 (0.01, 1.16)	0.64 (-0.29, 1.57)	0.89 (0.34, 1.39)	0.12 (-0.81, 1.01)	0.57 (-0.59, 1.89)
Distance to breeding sites	-1.4e-04 (-9.1e-04, 5.1e-04)	-3.1e-04 (-1.4e-03, 7.2e-04)	-2.2e-04 (-2.5e-03, 3.0e-03)	-2.1e-03, (-1.3e-03, 1.3e-03)	5.5e-04 (-7.7e-04, 2.3e-03)	2.5e-04 (-1.5e-03, 2.4e-03)	1.0e-04 (-7.4e-04, 8.1e-04)	2.1e-04 (-1.4e-03, 1.7e-03)	-7.6e-04 (-2.8e-03, 1.7e-03)
Distance to village's edge									
0-200 m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200-300 m	-0.12 (-0.69, 0.33)	0.03 (-0.63, 0.67)	-0.37 (-2.01, 1.67)	0.26 (-0.38, 1.07)	0.38 (-0.61, 1.30)	1.15 (-0.40, 3.50)	0.08 (-0.40, 0.61)	-0.44 (-1.36, 0.52)	-0.08 (-1.40, 1.27)
300-400 m	0.19 (-0.59, 1.03)	0.01 (-1.17, 1.13)	-0.58 (-3.26, 2.20)	0.00 (-0.93, 1.06)	-0.20 (-1.63, 1.05)	1.11 (-0.78, 4.01)	0.52 (-0.15, 1.32)	-0.37 (-1.99, 0.89)	0.66 (-1.84, 3.05)
>400 m	-0.22 (-1.46, 1.08)	0.58 (-0.63, 2.01)	0.98 (-1.74, 4.14)	-0.24 (-1.18, 1.11)	-0.92 (-2.65, 0.52)	1.47 (-0.86, 4.44)	0.10 (-0.67, 0.92)	0.11 (-1.19, 1.51)	1.46 (-0.84, 4.27)
Distance to the canal									
0-500 m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
500-750	-0.45 (-1.37, 0.38)	-0.30 (-1.44, 0.73)	1.85 (-0.77, 6.33)	0.70 (-0.32, 1.16)	0.96 (-0.26, 2.09)	0.26 (-1.57, 2.34)	0.78 (0.01, 1.59)	0.12 (-1.61, 1.82)	-1.04 (-4.06, 1.21)
750-1000	-0.60 (-2.03, 0.62)	-0.17 (-1.40, 1.35)	2.01 (-1.00, 7.06)	0.61 (-0.62, 1.68)	0.51 (-1.14, 2.03)	-0.10 (-2.52, 2.16)	0.42 (-0.53, 1.30)	0.12 (-1.53, 2.39)	-2.24 (-6.26, 0.36)
>1000	-0.31 (-1.81, 0.78)	0.25 (-1.07, 1.94)	2.20 (-1.24, 8.74)	0.58 (-0.84, 1.92)	0.02 (-2.02, 1.46)	0.55 (-1.39, 2.81)	0.83 (-0.09, 1.76)	0.39 (-1.38, 2.73)	-051 (-4.79, 1.82)
Spatial parameters									
Range = 3/ ρ (km)	1.64 (1.13, 5.79)	1.56 (1.13, 4.28)	1.77 (1.15, 11.31)	1.73 (1.13, 7.71)	1.64 (1.13, 6.17)	1.67 (1.13, 6.15)	1.66 (1.14, 6.04)	1.81 (1.16, 9.20)	1.70 (1.14, 459)
σ^2	0.64 (0.06, 4.80)	0.92 (0.06,4.50)	8.82 (0.22, 134.0)	0.30 (0.02, 7.77)	0.80 (0.03, 8.14)	0.58 (0.02, 7.18)	0.13 (0.01, 1.22)	0.46 (0.02, 11.0)	2.49 (0.02, 23.1)

* Bayesian credible interval;

The different subspecies are sympatric over all seasons with clear spatio-temporal patterns (Figures 5.6-5.8). Overall, the Mopti chromosomal form was the most abundant, particularly during the beginning (Figure 5.6) and middle (Figure 5.7) of the rainy season (June and August). The Bamako form was clustered in the North-Eastern part of the village at the beginning of the rainy season, occupied the South-Western part during the middle of the rainy season and was found almost everywhere in the village at the end of that season (except the South-Eastern part). The Savanna chromosomal form was concentrated in the Northern part of the village at the beginning of the rainy season. During the middle of the rainy season, it was found from South-West to North-East part of the village having the highest frequency in the center. At the end of the rainy season (Figure 5.8), the Savanna form was present mainly at the periphery of the village. The hybrid chromosomal forms showed low frequencies in the center of the village at the beginning of the rainy season. The highest frequencies were observed in the middle and at the end of the rainy season and it were present everywhere in the village, particularly in the South-Eastern part.

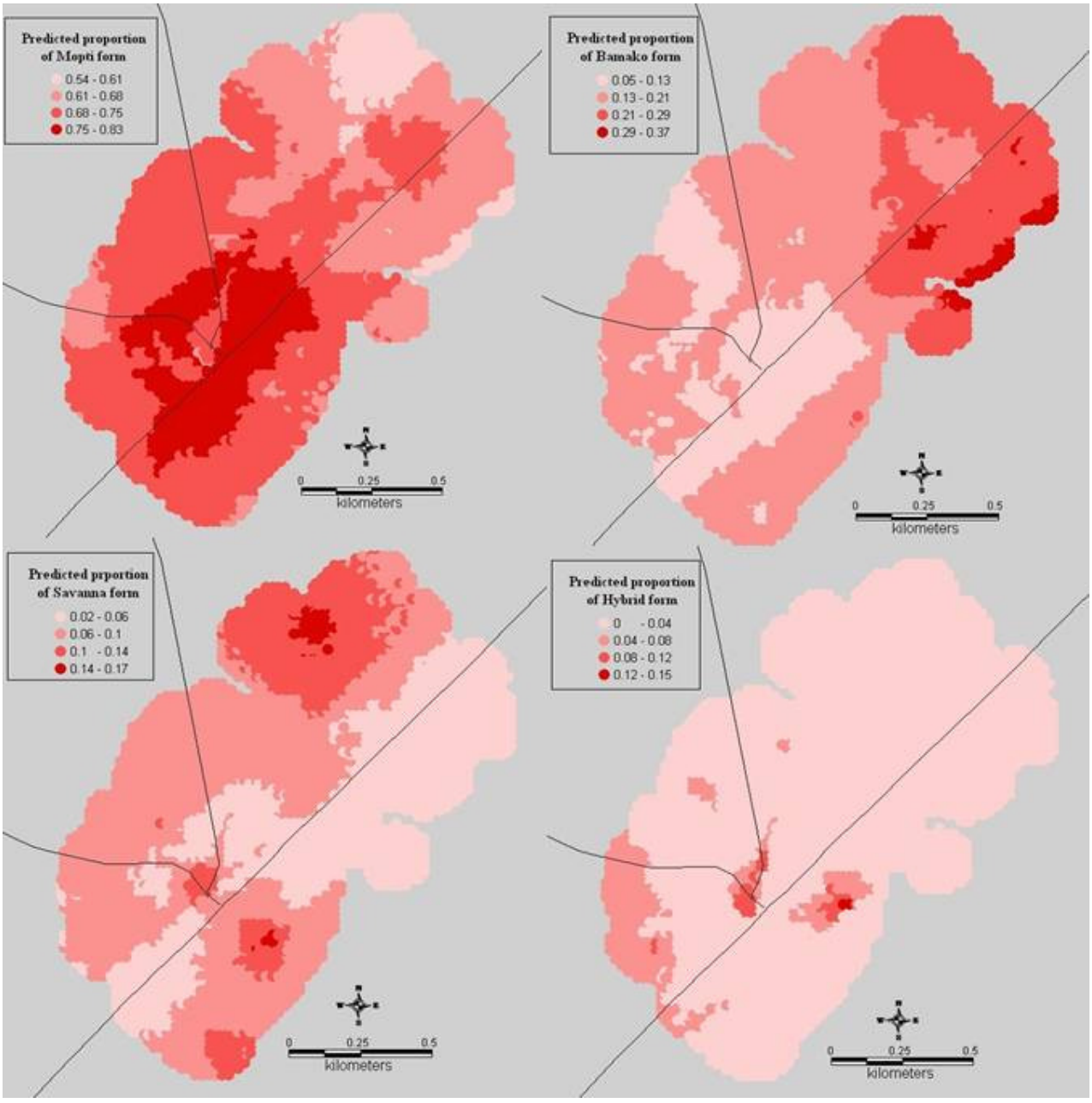

Figure 5.6: Spatial distribution of the proportion of the chromosomal of *An. gambiae s.s.* in June in Bancoumana, Mali.

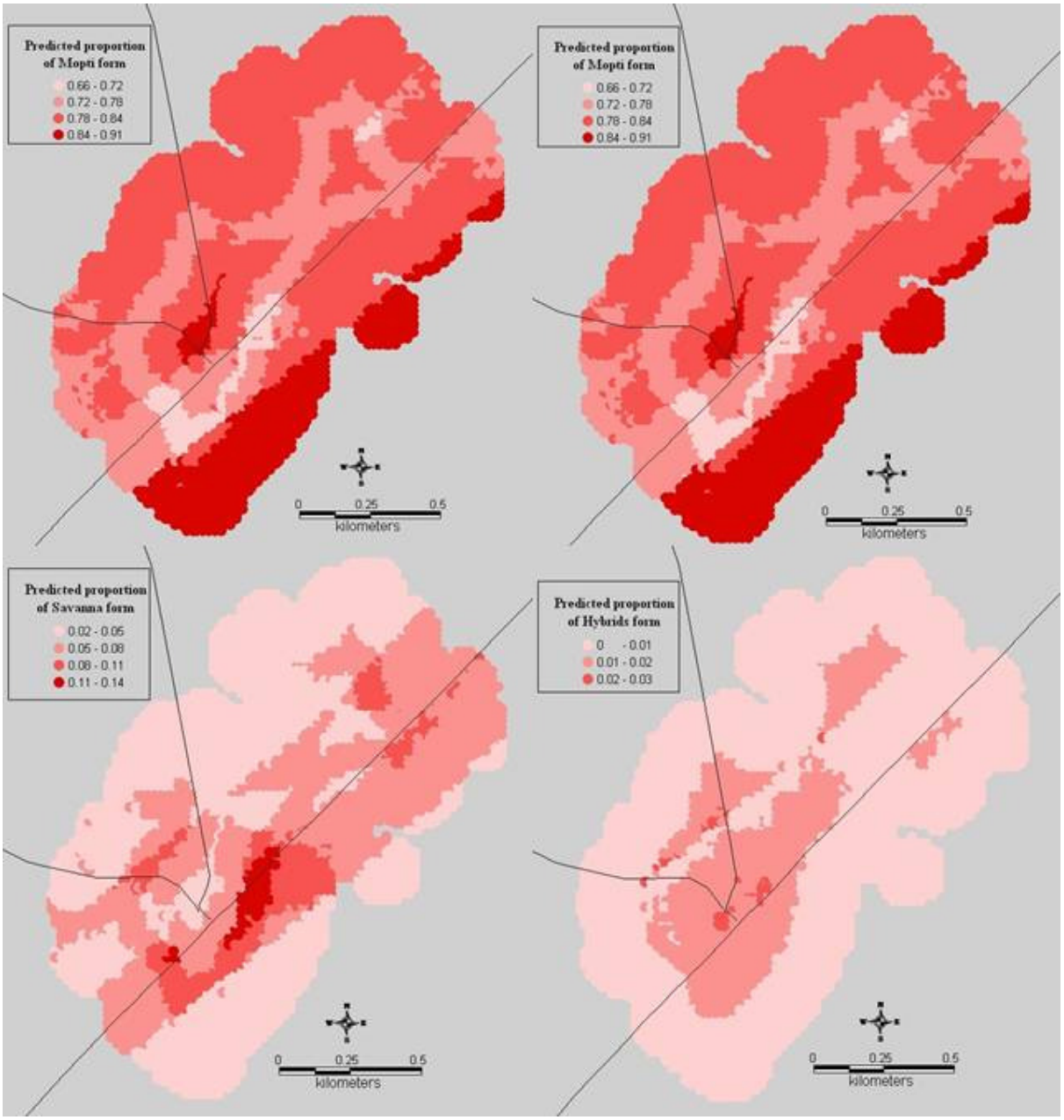

Figure 5.7: Spatial distribution of the proportion of the chromosomal of *An. gambiae s.s.* in August in Bancoumana, Mali

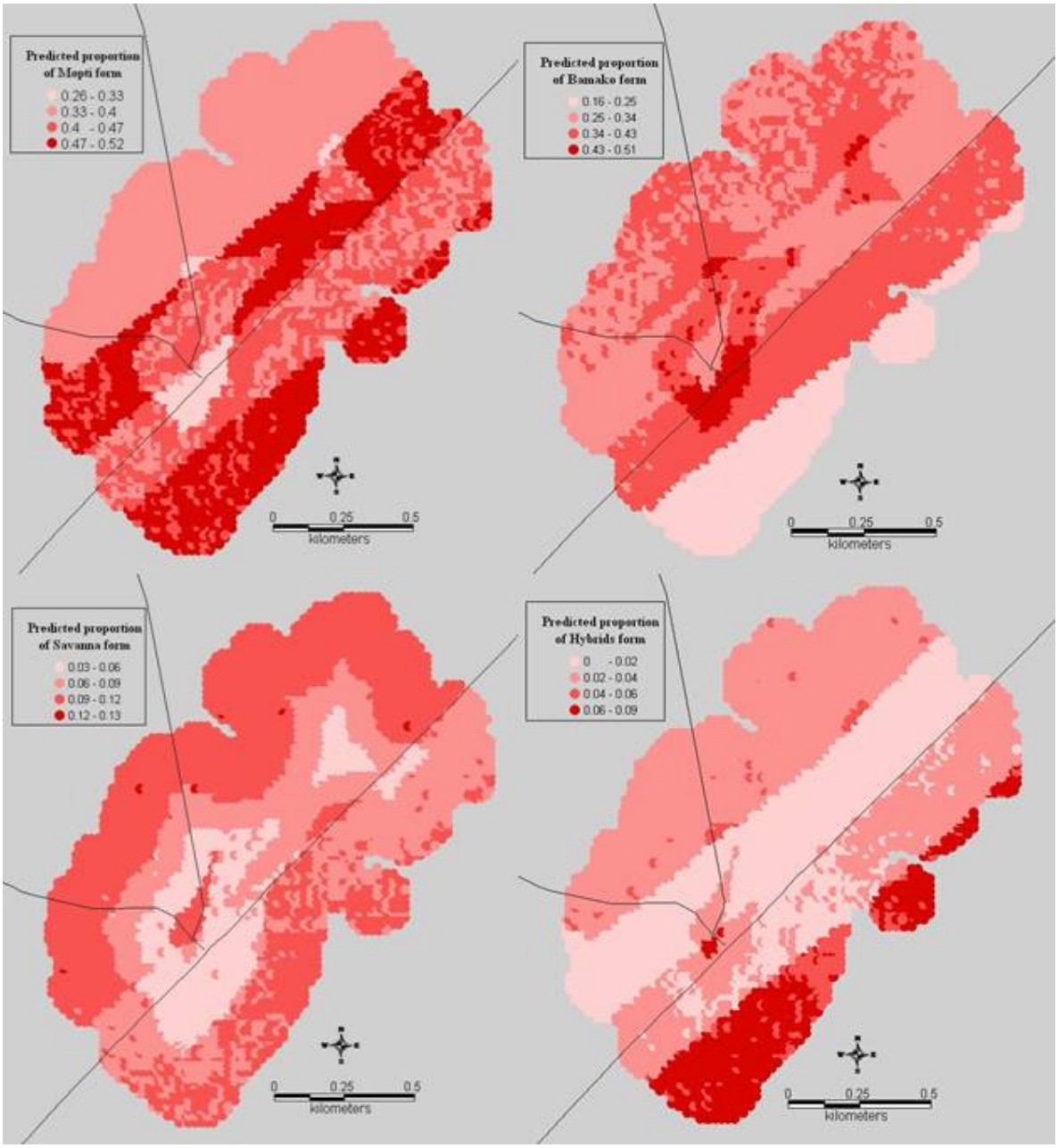

Figure 5.8: Spatial distribution of the proportion of the chromosomal form of *An. gambiae s.s.* in October in Bancoumana, Mali

5.5. Discussion

In this study, we investigated the spatial and seasonal distribution of *An. gambiae* complex densities and the chromosomal variants of *An. gambiae s.s.* in a savanna village of Mali in relation with the local environmental conditions. Our data showed spatial, seasonal and year to year variations in the distribution of mosquito densities. The annual and seasonal variations could be explained by annual and seasonal variations in the rainfall. There was a positive association between the number of mosquitoes found in a house and its distance from the nearest breeding habitat. This observation is contrary to previous results (Minakawa *et al*., 2002; Zhou *et al*., 2007). However, it is supported by a number of other studies (Trape *et al*., 1992; Oesterholt *et al*., 2006)

There was an over-dispersion in the distribution of mosquito densities at the beginning and during the dry season, with concentric clustering of higher densities at the periphery of the village as has been seen elsewhere (Smith *et al*. 1995; Ribeiro *et al*., (1996). These findings can be explained by results from Sogoba *et al*. (2007) who reported mainly man-made breeding sites around Bancoumana that were replenished at the start of the rainy season. There were very few breeding habitats during the dry season at the side of the village away from the Niger River. On the other hand, there were many and active dry season breeding sites in the bed of the Niger. The patchy distribution pattern observed in August (middle of rainy season) and October (end of rainy season) can be explained by numerous foot and tire prints everywhere in the village at that time (Sogoba *et al*. 2007).

The maps confirm the typical seasonal variations in mosquito densities in savanna areas (Taylor *et al*., 1993; Shililu *et al*., 2004). The positive association of mosquito densities with the straw roof housing type (poorly constructed) has been reported by many other studies

(Bagayoko, 2000; van der Hoek *et al.* 2003) and can be explained by the suitable microclimatic and resting conditions they may offer to mosquitoes.

The geostatistical multivariate multinomial models confirmed the relationship between housing type and the relative frequencies of the different karyotypes. The maps of the proportions of the different chromosomal forms also show spatial and seasonal clustering, with the Mopti form being the most abundant at the beginning and the middle of the rainy season and the Bamako form taking over at the end of the rainy season. There are many possible explanations for these patterns, including stochastic effects of choice of oviposition sites, or unobserved parameters such as indoor relative humidity and temperatures, microecology of the breeding sites, or differential effects of personal protection measures. The positive association of straw roof housing type with Savanna in June and Bamako forms in August and October, respectively, is probably related to the high humidity and moderate temperatures generally observed in these houses (Gamage-Mendis *et al.* 1991; Bagayoko *et al*., 2001) which are the prefered conditions for the above chromosomal forms (Touré *et al*., 1984).

The range parameters for the models for the karyotypic composition are relatively high compared to mosquitoes flying range, indicating that they are not explained by patterns of active dispersion. This is explained by the fact that karyotype frequencies are similar in neighboring areas because of environmental similarities. Passive migration directed by the wind could also contribute to the high values of the range parameters.

Our results suggest that interventions targeting the Mopti form should concentrate at the beginning and in the middle of the rainy season, while those targeting the Bamako form should be at the end of the rainy season. In addition, appropriate vector control targeting the

periphery of the village at the beginning of the rainy season and during the dry season can ameliorate the malaria situation in seasonal malaria transmission areas. However, more studies focused on micro-environmental factors at house level are required to better understand the micro-ecological difference between the chromosomal forms and their unique contribution to the disease transmission.

5.6. Acknowledgements

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5.7. Appendix

5.7.1. Geostatistical negative binomial regression model

Let Y_i be the mosquito count in house *i*. We assumed that Y_i arises from a negative binomial distribution, $Y_i \sim Nb(\mu_i, r)$ with mean μ_i, dispersion parameter *r* and probability density function

$$f(Y_i = y_i \mid r, \mu_i) = \frac{(y_i + r - 1)!}{y_i!(r-1)!}\left(\frac{r}{r+\mu_i}\right)^r \left(\frac{\mu_i}{r+\mu_i}\right)^{y_i}, r > 0 \qquad (1).$$

The negative binomial model assumes that the variance of the counts, $\mathrm{var}(Y_i)$ is equal to

$$\mathrm{var}(Y_i) = \mu_i + k * \mu_i^2 \quad (2)$$

with the aggregation parameter $k = 1/r$. The Poisson distribution arises as $r \to \infty$ (or equivalently $k \to 0$) and thus $\text{var}(Y_i) = \mu_i$.

We introduce covariates $\underline{X}_i$ and house-specific spatial random effects ϕ_i on the $\log(\mu_i)$, that is $\log(\mu_i) = \underline{X}_i^T \underline{\beta} + \phi_i$, where $\underline{\beta}$ is the vector of regression coefficients. We assume that the random effects model a continuous spatial process that is $\underline{\phi} = (\phi_1, \phi_1, ..., \phi_N)^T \sim MVN(\underline{0}, \Sigma)$, has a multivariate normal distribution with variance-covariance matrix $\Sigma_{il} = \sigma^2 \exp(-\rho d_{il})$, where d_{il} is the shortest straight-line distance between house *i* and *l*, σ^2 is the geographic variability (the sill), and ρ is a smoothing parameter that controls the rate of correlation decay with increasing distance.

5.7.2. Geostatistical multinomial regression model

Let Y_{ik} be the observed frequency of mosquito chromosomal form *k* at location *i* where *k=1,2,3,4* denote the Mopti, Bamako, Savanna , and hybrid forms, respectively. We assume that Y_{ik} arise from a multinomial distribution, that is $(Y_{i1}, Y_{i2}, Y_{i3}, Y_{i4}) \sim Mult(n_i, \pi_{i1}, \pi_{i2}, \pi_{i3}, \pi_{i4})$ with parameters π_{ik} and n_i is the total number of *An. gambiae s.s* collected at location *i*. We introduce spatial correlation on location-specific random effects ϕ_{ik} which are modeled together with the covariate effects on the logit parameters, that is $\log\left(\frac{\pi_{ik}}{\pi_{i4}}\right) = \underline{X}_i^T \underline{\beta}_k + \phi_{ik}$ where $\underline{\beta}_k$ are covariate parameters related to the k^{th} multinomial category, *k=1,2,3*.

We further assumed that ϕ_{ik} model a latent isotropic Gaussian spatial process, that is $\phi_k = (\phi_{1k}, ...\phi_{Nk}) \sim MVN(0, \Sigma_k)$, with covariance matrix Σ_k and that spatial correlation between any pair of locations is a function of distance between locations, that is $(\Sigma_k)_{ij} = \sigma_k^2 \exp(-\rho_k d_{ij})$ where σ_k^2 is the spatial variance related to the multinomial category k,

ρ_k is the parameter that models the rate of correlation decay and d_{ij} the distance between the locations *i* and *j*. Based on the above specification, the minimum distance for which the spatial correlation becomes less than 5% is calculated by $3/\rho_k$ (Ecker and Gelfand, 1997).

5.7.3. Model fit

Model parameters were estimated using Markov Chain Monte Carlo (MCMC) simulation methods. We chose vague normal prior distributions for $\underline{\beta}$ parameters with large variances (i.e., 10,000), gamma priors for *r*, inverse gamma priors for σ_k and uniform priors for $\rho_k, k = 1,2,3$. We ran a single chain sampler with a burn-in of 5,000 iterations. Convergence was assessed by inspection of ergodic averages of selected model parameters. Bayesian kriging was used to predict the species frequency at 85,000 unsampled locations (Diggle and Tawn, 1998). The Bayesian model fit was carried out in WinBUGS 1.4. (Imperial College and MRC, UK), whereas the model prediction was implemented in Fortran 95 (Compaq Visual Fortran, Professional 6.6.0) using standard numerical libraries (NAG, The Numerical Algorithms Group Ltd).

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Chapter 6

Monitoring of larval habitats and mosquito densities in the Sudan Savanna of Mali: Implication for malaria vector control

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Abstract

In Mali, anopheline mosquito populations increase sharply during the rainy season, but are barely detectable in the dry season. This study attempted to identify the dry season mosquito breeding population in and near the village of Bancoumana, Mali, and in a fishing hamlet 5 km from this village and adjacent to the Niger River. In Bancoumana, most larval habitats were human made, and dried out in January–February. In contrast, in the fishing hamlet, productive larval habitats were numerous and found mainly during the dry season (January–ay) as the natural result of drying riverbeds. Adult mosquitoes were abundant during the dry season in the fishermen hamlet and rare in Bancoumana. To the extent that the fishermen hamlet mosquito population seeds Bancoumana with the advent of the rainy season, vector control in this small hamlet may be a cost-effective way to ameliorate malaria transmission in the 40-times larger village.

Key words: Anopheline, larvae, larval habitat, dry season, Bancoumana, fishing hamlet.

6.1. Introduction

Vector control is one of the major elements of the World Health Organization (WHO) global malaria control strategy in 2005 that primarily focused on indoor residual spraying and the use of insecticide-treated nets. However, these control measures have drawbacks, including insecticide resistance and difficulties in achieving high coverage (Killeen *et al*., 2002; 2004). Larval control through source reduction and routine application of larvicides was a key intervention in eradicating malaria in many parts of the world (Kitron and Spielman, 1989; Utzinger *et al*., 2001; Killeen *et al*., 2002), but this control has been largely neglected in recent decades in sub-Saharan Africa, partly because of the perceived difficulty of identifying larval habitats in rural areas. Larval control can be effective where larval habitats occur seasonally or are relatively limited and well defined (Fillinger and Lindsay, 2006).

In areas of Sudan savanna with seasonal malaria transmission, larval habitats of the *Anopheles gambiae* complex are considerably reduced during the dry season (Taylor *et al*., 1993; Charlwood *et al*., 2000). Adult vector densities are thus also very low in the dry season, but increase sharply at the onset of the rainy season (Lindsay *et al*., 1991; Mbogo *et al*., 1995). Permanent breeding sites during the dry season may serve to seed the additional larval habitats formed during the rainy seasons (Toure *et al*., 1998; Charlwood *et al*., 2000). Therefore, dry season larval control might prevent this sharp increase, and thus play an important role in integrated vector control strategies (Fillinger *et al*., 2004). However, it has also been suggested that adult mosquitoes survive the dry season by estivating in yet undetermined locations (Omer *et al*., 1970; Taylor *et al*., 1993).

We report the mapping, characterization, and monitoring of larval habitats for the presence of anopheline larvae and the monitoring of the distribution of adult anopheline mosquitoes in a rural savanna area of Mali. We consider whether analyses of the factors

influencing the fluctuations of adult and larval abundance, and in particular the dry season ecology, provide a basis for a selective larval control strategy.

6.2. Materials and methods

6.2.1. Description of the study site

The study was carried out in the village of Bancoumana, which is located at 60 km southwest of Bamako (12° 20 N, 8° 2 W) and in a fishing hamlet 5 km from this village adjacent to the Niger River (Bozokin) (Figure 6.1).

The total population of Bancoumana is approximately 8,000 inhabitants, predominantly of the Malinké ethnicity living in 340 compounds. The main economic activity is agriculture. The fishing hamlet has approximately 300 inhabitants of Bozo ethnicity living in 10 compounds. The land between the village and the river is used for growing rain-fed rice during the rainy season (usually June to October) and for growing other crops (onions, tomatoes) during the dry season (November to May).

There is intense malaria transmission during the rainy season and for the next two months (Dolo *et al*., 2003). The major vectors are *An. gambiae* (approximately 95.5%) and *An. arabiensis* (approximately 4.5%) (Touré *et al*., 1998). The mean monthly entomologic inoculation rate was 2.8 infectious bites per person with marked seasonal variations (Bagayoko M, 2000). The prevalence of *Plasmodium falciparum* infection in children less than five years of age varies from approximately 30–50% during the dry season to 75% during the rainy season (Doumbia S, 2002).

6.2.2. Identification and characterization of potential anopheline breeding sites

From June 2004 to December 2005, we performed a monthly active search to identify and geo-locate all larval habitats in both Bancoumana and the fishing hamlet. The search was extended to a perimeter 2 km around the two study sites and also included the Niger River riverbed. The search was carried out by three entomologists assisted by two local guides who had good knowledge of the area. Villagers were questioned about their awareness of open water bodies around the villages, particularly during the dry season.

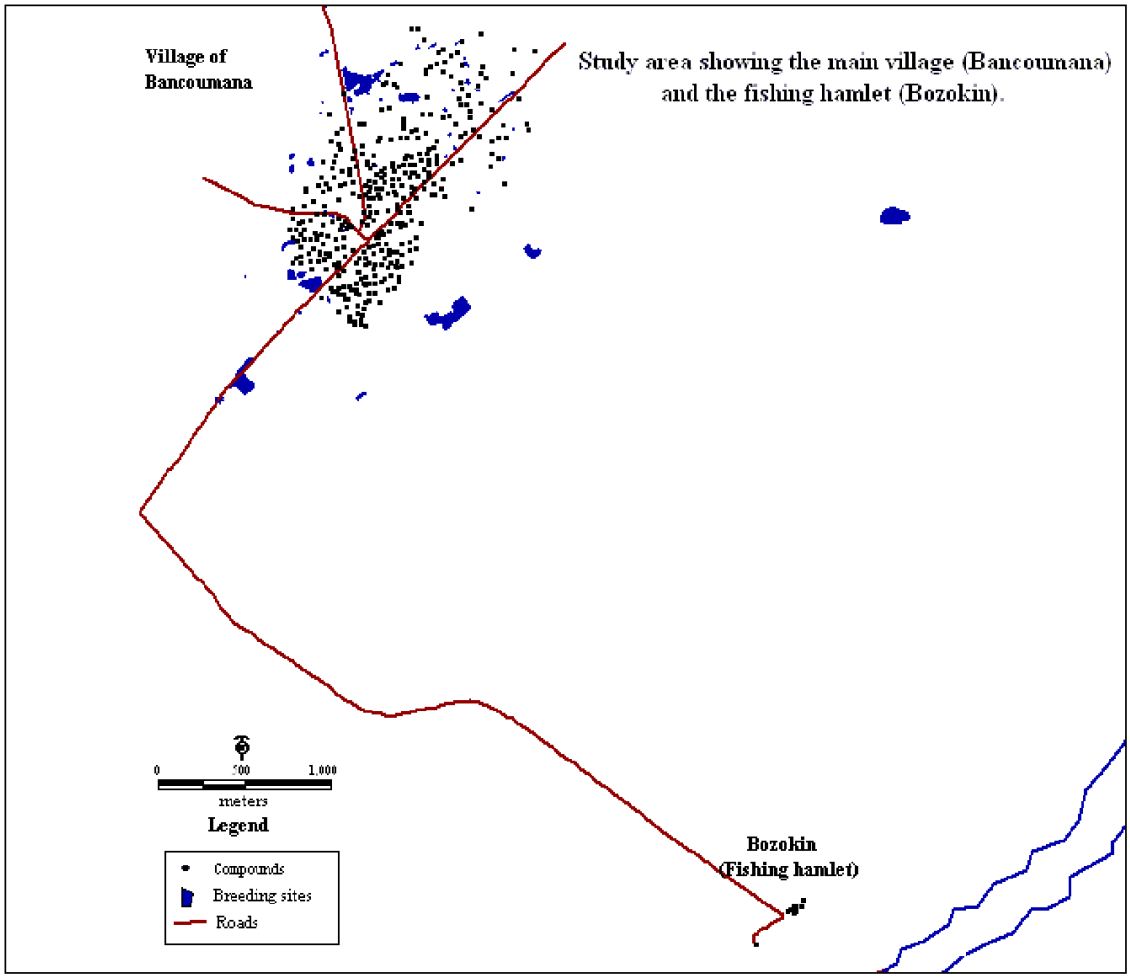

Figure 6.1: Map showing the village of Bancoumana, Mali, and the fishing hamlet (Bozokin) adjacent to the Niger River with the location of the compounds in both villages and the larval habitats (Bancoumana)

A unique identification number was assigned to each water body according to its location (block), type (ponds, brick pit, puddles, and tire prints), and the order in which it was identified. Geographic coordinates for all identified water bodies were recorded using a global positioning system (GPS) (GeoXM; Trimble Navigation Ltd., Sunnyvale, CA) with a spatial margin of error of 2–5 meters. All surface waters were mapped and sampled with a WHO standard mosquito dipper to determine the presence or absence of immature mosquitoes. During each monthly survey, investigators recorded information on characteristics of the water bodies (type of larval habitat, the presence of vegetation and other co-occurring arthropods, exposure to sunlight, water turbidity and transparency, and the color of the bottom), and productivity (presence or absence of anopheline larvae). *An. gambiae s.l.* mosquito larvae were morphologically identified and separated from other species by experienced entomologists. A polymerase chain reaction method was used to identify molecular forms (M and S) (Favia *et al*., 2001) on a random sample of anopheline larvae selected from each monthly collection.

6.2.3. Monitoring adult mosquito density.

In both Bancoumana and the fishing hamlet, standard indoor pyrethrum spray catches (PSC) (Service 1993) were used to collect adult mosquitoes during the dry season (December 2004 and May 2005). Collections were performed during the last two weeks (16th–27th) of each month.

In Bancoumana, we updated the existing geo-referenced base map established with GeoExplorer 3^R GPS receivers (Trimble Navigation Ltd.) with an accuracy of 1–3 m (Bagayoko M, 2000. Thèse de Doctorat de Spécialité de l'ISFRA). This map includes landmarks and all housing compounds and larval habitats. A unique identification number

was assigned to each compound. Adult mosquito collections were conducted in 180 houses sampled to represent the two types of housing (thatch roof versus metal roof) and located in 180 different compounds randomly selected from the list of the 340 compounds of the village. The identification number assigned to each selected compound was marked on the door of the house using a permanent marker. In the fishing hamlet, mosquito collection was performed in all 10 housing compounds that composed the hamlet.

Adult *An. gambiae s.l.* and *An. funestus* from both sites were identified morphologically. The total number of *An. gambiae s.l.* and *An. funestus*, the identification number, the type of the house, and the number of people sleeping in them were recorded onto appropriate data sheets. In the laboratory, a sample (at least 120 specimen) of *An. gambiae s.l.* was further identified to species (*An. gambiae s.s.* and *An. arabiensis*) and sub-species (molecular forms M and S) (Favia *et al.*, 2001).

6.3. Data analysis

Statistical analysis was carried out with STATA version 9.0 (Stata Corporation, College Station, TX). Logistic regression models were used to determine the key factors influencing anopheline larvae presence in larval habitats. The key factors included in the models were type of water bodies, their size and depth, turbidity and transparency of the water, bottom color, presence and abundance of vegetation, and the co-occurrence of other arthropods. The chi-square test was used to compare the proportion of the different type of water bodies positive for anopheline larvae.

6.4. Ethics

This study did not involve human subjects. The inherent ethical considerations with the execution of this research were related to pyrethrum spray catches. No house was sprayed

without the approval of its owner. The insecticide used was a pyrethroid marketed under the name of Premium Killer (NIRA BVBA, Antwerp, Belgium). This product has a weak persistence, has no human toxicity under normal conditions of use, and is intended for use as an indoor spray. The treated house is reusable a few minutes after spraying. The study was reviewed and approved by the ethical committee of the Faculty of Medicine and Pharmacy of the University of Bamako, Bamako, Mali.

6.5. Results

6.5.1. Characteristics of water bodies

6.5.1.1. Bancoumana

From June 2004 to December 2005, 63 major water bodies were identified in and around the village of Bancoumana. Overall, these belonged to four major types (Figure 6.2) comprising brick pits (74.6%), tire prints (14.3%), puddles (9.5%), and ponds (1.6%). There were temporal variations in the number of water bodies in the village of Bancoumana (Figure 6.3). During the rainy season (June–September), tire prints formed a slightly increased proportion (16.4%). There were innumerable small water bodies created by human footprints and cattle hoof prints; however, these usually did not persist for more than two weeks and were not counted. At the end of the rainy season (October–November), brick pits accounted for up to 83.3% (n = 8) of the water bodies. Ten weeks after the rainy season ended (January and February corresponding to the dry cold season), we did not find any additional water bodies. Figure 6.4 shows the frequency distribution of the different type of water bodies positive and negative for anopheline larvae during the dry season (December 2004–May 2005). In March 2005, subsequent to 60 mm of rain, 26 of the 63 water bodies (41.3%) were replenished. These comprised brick pits (65.4%), ponds (19.2%), tire or foot prints (11.5%), and rain puddles (3.8%).

There was no significant difference (χ^2 =7.5, P = 0.058) between brick pits (59.0%), tire prints (62.9%), puddles (80.0%) and ponds (42.9%) for anopheline larvae. The highest proportion of anopheline-positive water bodies was observed in August 2005 (92.7%, n = 55) and the lowest was observed in November 2005 (37.5%, n = 8). In December 2004, the only potential breeding site was a single brick pit but this was negative for anopheline larvae. Among the replenished water bodies after the rainfall of March 2005, 66.7% of the brick pits (n = 21) and the tire prints (n = 3) were positive for anopheline larvae. In April, three additional ponds were found between Bancoumana and the fishing hamlet, but all were negative for anopheline larvae. Accordingly, in the immediate surroundings of Bancoumana, only four potential larval habitats were found, although we could not find anopheline larvae in them. In May, at the onset of the rainy season, 45 water bodies were found, mainly composed of brick pits (73.3%), but no anopheline larvae were found in any of them.

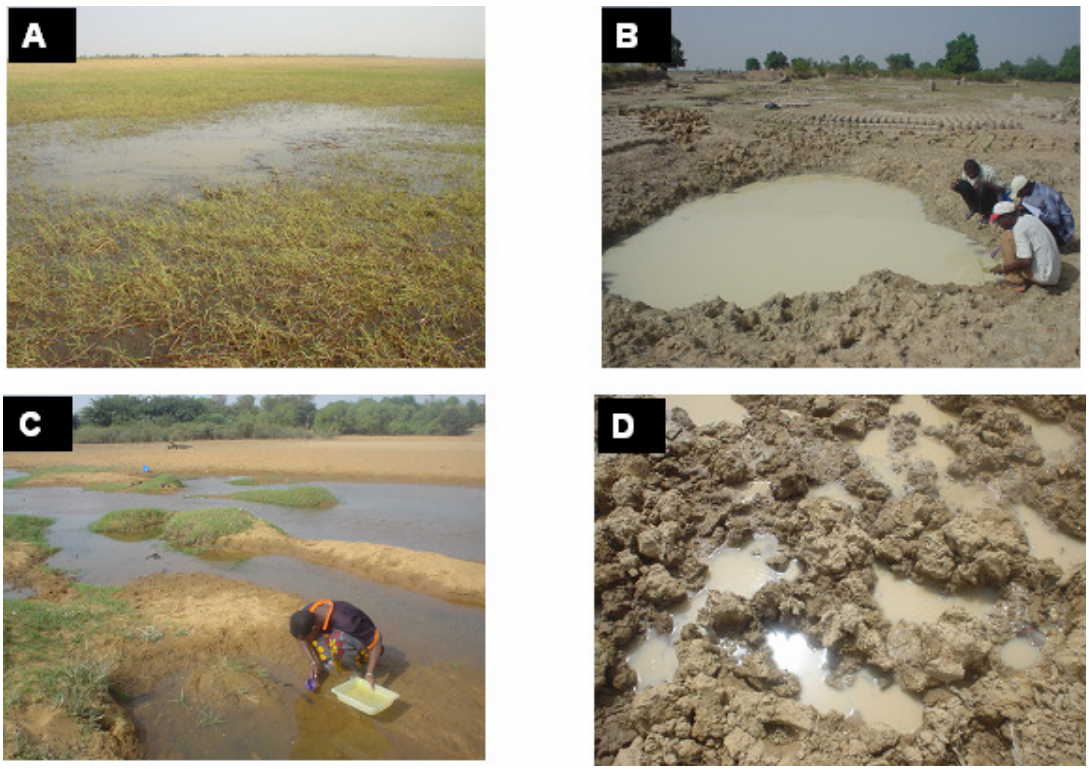

Figure 6.2: Typical potential larval habitats in Bancoumana and Bozokin: ponds (A), brick Pits (B), river bed puddles footprints (C).

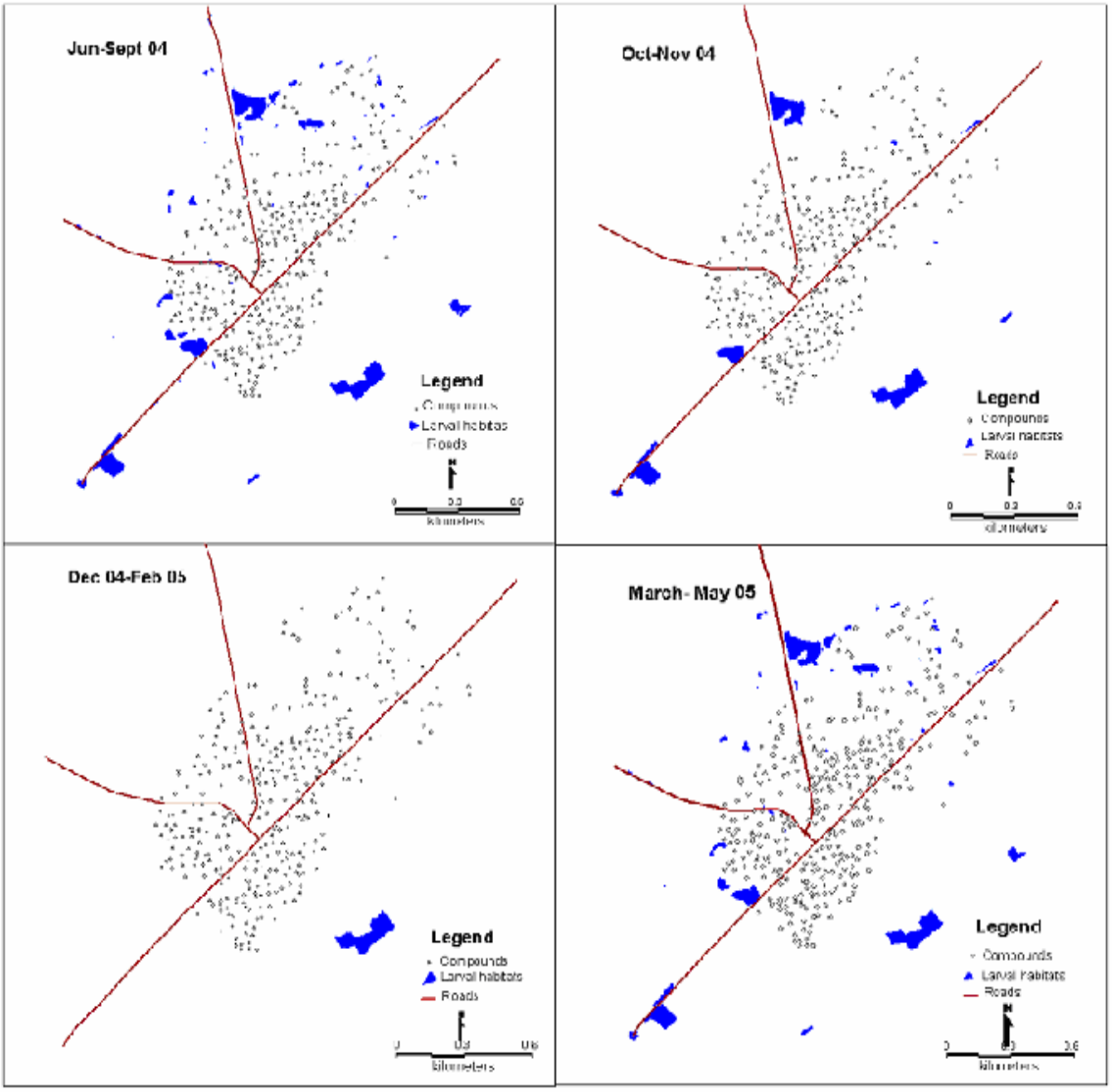

Figure 6.3: Temporal variation of watered major larval habitats in the village of Bancoumana : June-September (rainy season), October-November (end of rainy season), December-February (cold dry season), March-May (hot dry season

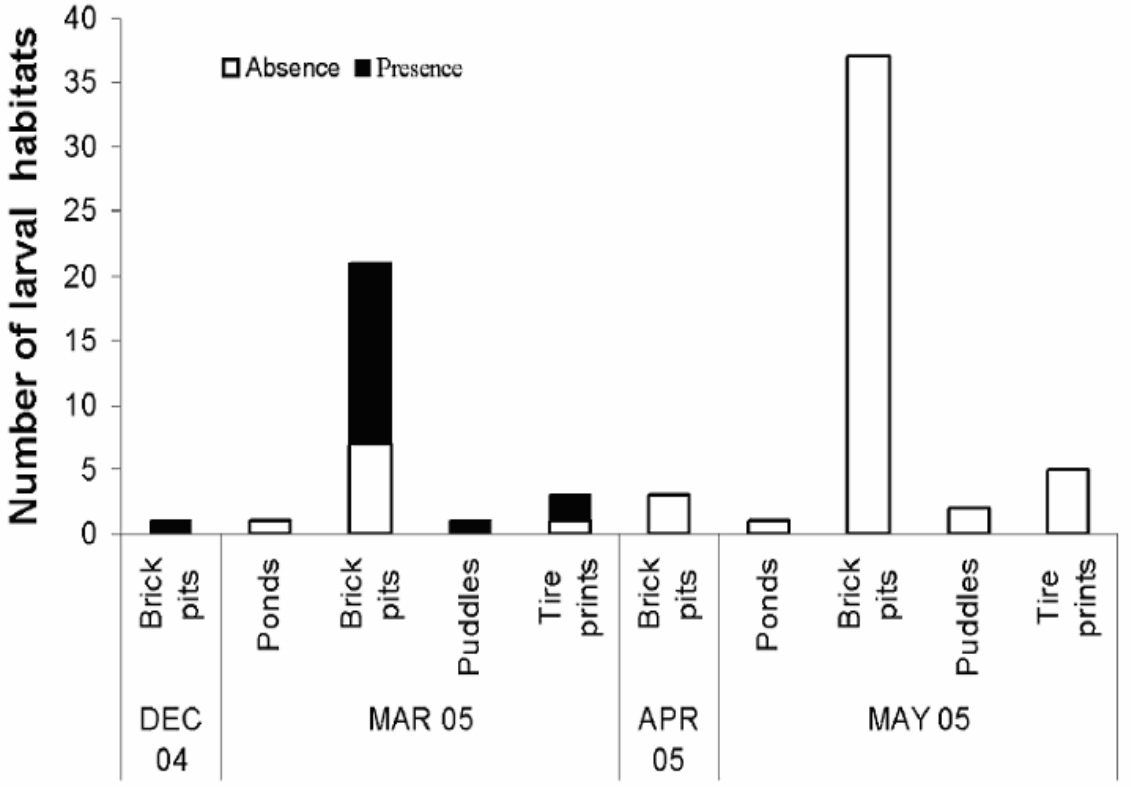

Figure 6.4: Frequency of the different type of larval habitats positive and negative for anopheline larvae during the dry season in Bancoumana village.

6.5.1.2. Fishing hamlet

During the period when all larval habitats were almost dried out in the village of Bancoumana (dry season), numerous water puddles (Figure 6.2) created in the riverbed by the drying river were found highly positive for anopheline larvae. Unlike the larval habitats observed in Bancoumana, all water bodies found in the fishing hamlet were natural and most often full of larvae. No vegetation was found in these larval habitats but other cooccurring arthropods were often present, and the water was always clear.

6.5.2. Key environmental factors associated with anopheline larvae in water bodies.

6.5.2.1. Bancoumana.

Table 6.1 shows the results of the bivariate regression between the presence/absence of anopheline larvae and the environmental variables in the village of Bancoumana. Water turbidity and transparency, other co-occurring arthropods, and vegetation presence and abundance were significantly associated with the presence/absence of anopheline larvae in the water bodies. Water bodies with vegetation (odds ratio OR = 5.1, 95% confidence interval [CI] = 3.4–7.5), other co-occurring arthropods (OR = 3.0, 95% CI = 1.9–4.6), and a brownish bottom (OR = 2.4, 95% CI = 1.5–3.6) were much more likely to contain anopheline larvae than when vegetation and other co-occurring arthropods were absent, and when the bottom was a different color. Compared with opaque but non-turbid water, both turbidity and transparency (OR = 0.5, 95% CI = 0.4–0.8) decreased the chance of finding anopheline larvae. The multivariate logistic regression analysis indicated that only larval habitats with other co-occurring arthropods (OR = 3.0, 95% CI = 1.8–4.9) and vegetation (OR = 8.7, 95% CI = 4.7–16.3) were much more likely to contain anopheline larvae than all other larval habitats; vegetation abundance was negatively associated with larvae.

6.5.2.2. Fishing hamlet

The water bodies were exclusively natural puddles with clear water, not vegetated, and highly positive for anopheline larvae. No further analysis to assess associations between anopheline larvae presence and the environmental variables was performed.

6.5.3. Monitoring adult mosquito density during the dry season

6.5.3.1. Bancoumana.

During the dry season (December 2004 to May 2005) in Bancoumana, mosquitoes were nearly undetectable in human dwellings (Figure 6.5). Overall, only 175 mosquitoes were collected in 1,078 spray collections (mean mosquito density = 0.16, 95% CI = 0.11–0.21). The few mosquitoes collected in Bancoumana were clustered at the side of the village facing the fishing hamlet (Figure 6.6).

6.5.3.2. Fishing hamlet

During the dry season (December 2004 to May 2005) in the fishing hamlet, mosquito density was relatively high throughout the study period (Figure 6.5), and peaked in December when the mosquito density in Bancoumana was very low. The mean mosquito density was 8.16 per house (95% CI = 7.4–9.0). Overall, 506 mosquitoes were collected in only 62 spray collections compared with 175 in 1,078 collections in Bancoumana.

6.5.4. Estimates of larval An. gambiae molecular form frequencies in the two villages

A comparison between the molecular forms frequencies of anopheline larvae collected in the riverbed and in the rain-fed larval habitats of the main village was done after the heavy rain in March 2005. The mosquito population was identical in the fishing hamlet and Bancoumana village with a predominance of the M form, 79.0% (n = 286) and 79.4% (n = 34), respectively.

Table 6.1: Bivariate analysis between the presence of anopheline larvae and environmental factors

Parameters	Total	Positives	Odds Ratio	95% CI
Type of water bodies				
Ponds	14	8	1.0	
Brick pits	368	214	1.1	(0.4–3.2)
Puddles	40	32	3.0	(0.8–11.1)
Tire prints	70	44	1.3	(0.4–4.1)
Size categories				
< 1m	21	14	1.0	
1–5 m	220	125	0.7	(0.3–1.7)
>5–10 m	120	72	0.8	(0.3–2.0)
> 10m	126	87	1.1	(0.4–3.0)
Turbidity of water				
Clear	165	117	1.0	
Turbid	322	181	0.5	(0.4–0.8)
Water transparency				
Opaque	360	181	1.0	
Transparent	161	117	0.5	(0.4–0.8)
Water bodies bottom's color				
Not visible	327	181	1.0	
Brownish	141	105	2.4	(1.5–3.6)
Other	19	12	1.4	(0.5–3.6)
Vegetation presence				
Absent	196	76	1.0	
Present	291	222	5.1	(3.4–7.5)
Vegetation abundance				
None	196	76	1.0	
Less abundant	155	109	3.7	(2.4–5.9)
Abundant	136	113	7.8	(4.6–13.2)
Co-occurrence arthropods				
Absent	339	183	1.0	
Present	148	115	3.0	(1.9–4.6)
Depth of water bodies				
< 25 cm	360	209	1.0	
25–50 cm	91	59	0.9	(0.5–1.6)
51–75 cm	19	14	1.1	(0.5–2.2)
76–100 cm	15	7	1.0	(0.5–1.8)
100–150 cm	5	4	1.2	(0.0–11.0)
> 150 cm	4	3	2.0	(0.7–2.1)

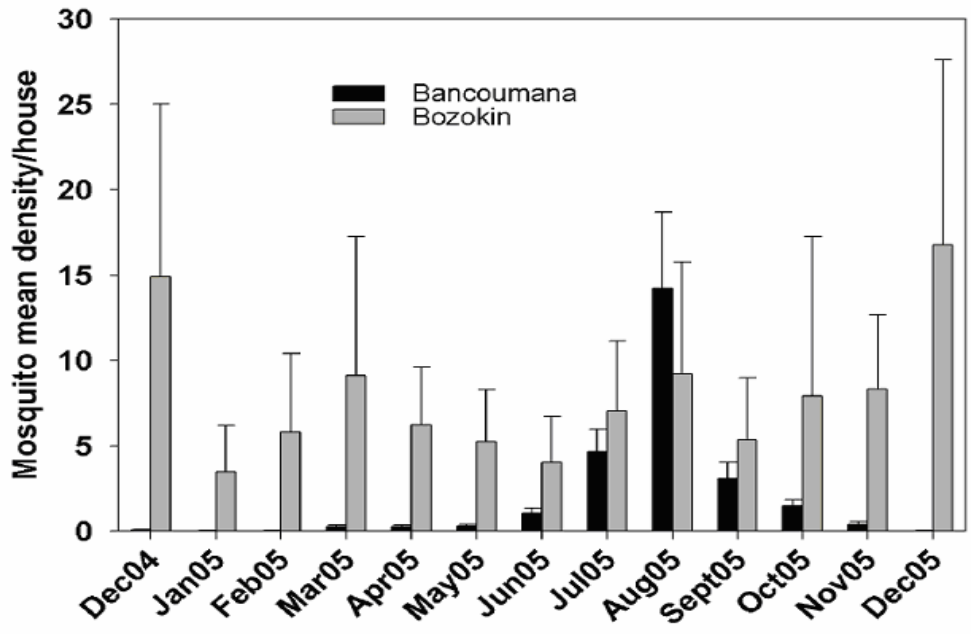

Figure 6.5: Variation in *An. gambiae s.l.* mean density per house in the village of Bancoumana (dark barplots) and the fishermen's hamlet (white barplots) during the dry season. The error bars represent the 95%CI.

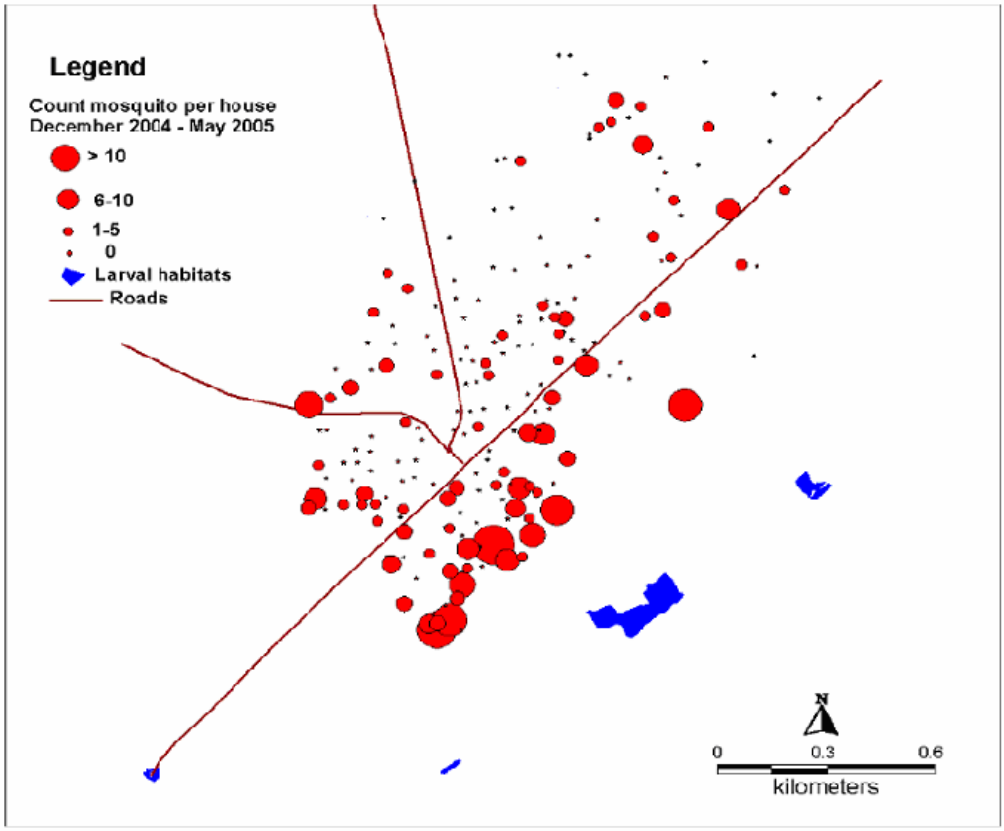

Figure 6.6: Spatial distribution of *An. gambiae s.l.* total count per house and potential larval habitats during the dry season in Bancoumana (December 2004 – May 2005).

6.6. Discussion

We mapped and characterized larval habitats in two ecologic settings: Bancoumana, where no permanent water is present, and a fishing hamlet lying adjacent to the Niger River. Our study focused on *An. gambiae s.l.*, which is the main vector for malaria transmission and accounts for 98% of the mosquitoes versus only 2% for *An. funestus* (Diuk-Wasser *et al*., 2005). *An. funestus* is mostly observed towards the end of the rainy season (October–November). In Bancoumana, nearly all larval habitats were human-made and rain-dependent, attesting to the human-dependent ecology of Afrotropical *Anopheles* (Coluzzi, 1999). By 10–12 weeks after the end of the rainy season, most water bodies have dried and few mosquito larvae can be found. As a result, the number of adult mosquitoes collected in the houses became very small. The study confirmed previous reports of undetectable transmission in Bancoumana during the dry season (Toure *et al*., 1998).

Although the dry season in the study area typically lasts from November through April–May, a short rainfall lasting a few days often occurs in March or April. Because this period corresponds to the maturation of mangoes, this phenomenon is called “mango-rain”. After such rainfall in March 2005, larval habitats, mainly composed of brick pits, were replenished with water and became positive for anopheline larvae. This shows the rain dependency of overwhelmingly human-made larval habitats and indicates that in Bancoumana mosquitoes probably laid their eggs quickly at the onset of the rainy season. The near absence of watered larval habitat in January and February, when no rain was observed, supports the hypothesis. However, this refilling of most larval habitats does not translate into high anopheline larvae productivity in the subsequent months of April and May 2005, presumably because of higher temperature (> 40°C), and low relative humidity (minimum = 26%, maximum = 62%) occurring in these months. Studies in Kenya showed a reduction from 55–57% in the survivorship of *An. gambiae s.l.* larvae in open larval habitats associated with an

increase of 3–3.4°C in their average daily water temperature compared with full forest-canopy coverage (forest habitats) and partial canopy coverage (forest edge habitats) larval habitats.

The greater dependency of *An. gambiae s.l.* on humid conditions has also been described (Charlwood *et al*., 2000). However, at the same period (dry season) in the fishing hamlet adjacent to the receding Niger River riverbed; there were numerous small natural puddles that were highly productive for anopheline larvae. As a result, the mosquito density was higher in the hamlet during the dry season than in Bancoumana. The quick re-colonization of the larval habitats shortly after a rainfall in Bancoumana suggests that mosquitoes that emerged from the riverbed are an important seed of the rain-fed water bodies of Bancoumana. The distance of 3–5 km that separates the river and the village is well within the flight range of *An. gambiae* (Kaufmann and Briegel, 2004). We did not find any potential obstacles to the flight of mosquitoes between Bancoumana and the fishing hamlet. Moreover, the different molecular forms of *An. gambiae* larvae after the first rain after the dry season had near identical frequencies in the two sites. It thus appears that the vectors in the two villages are from a common population. If the small fishing village was targeted for larval and adult mosquito control during the dry season (February and March), it could have a substantial impact on malaria transmission in surrounding areas such as the main village of Bancoumana.

The high anopheline larvae productivity of the larval habitats created by the receding riverbed parallels the ecology of *An. culicifacies* in Sri Lanka (Konradsen *et al*., 1998) more than the usual situation of *An. gambiae* in Africa. However, in various areas with seasonal malaria transmission in Africa, it has proved possible to identify local reservoirs of transmission during the dry season (Omer and Cloudsley-Thompson, 1970; Charlwood *et al*., 2000). Identifying sources of mosquito larvae during the dry season may provide a basis for

selective larval control, which may impact on subsequent malaria transmission in the rainy season.

In this area of seasonal malaria transmission, most productive larval habitats are human-made and rain-dependent, drying out within 10–12 weeks after the rainy season ends. Not very far away, numerous highly productive anopheline larvae may be found in favorable ecologic conditions (e.g., along the receding riverbed), which may sustain malaria transmission at a low level during the dry season and may serve as inoculums in surrounding areas. This scenario is similar to those in other areas of seasonal malaria transmission and provides an opportunity for a mosquito control strategy targeting dry season larval control and environmental management.

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Chapter 7

Spatial analysis of malaria transmission parameters in the rice cultivation area of Office du Niger, Mali.

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Abstract

The effects of rice growth environment on malaria transmission, taking into account spatial correlation, were assessed in the Office du Niger, Mali. Between April 1999 to January 2001, 8 quarterly entomological surveys were conducted in 18 villages in 3 agricultural zones. Vector densities in sleeping houses were related to rice crop, rice development stages, vegetation abundance, water state and seasons. They were high throughout the rice growing seasons, increased as the rice crop developed and decreased as vegetation became abundant. They also showed large spatial correlations (up to 30.6 km). The vectorial capacity exhibited both seasonal and village to village variation. Parity and the human blood index were weakly related to adult densities and showed low spatial correlations (up to 3.4 km), suggesting that small area variation in malaria transmission results mainly from variations in vector-human contact. Control strategies in rice cultivation areas should pay attention to this local variation.

Keywords: Malaria transmission, *An. gambiae s.l.*, *An. funestus*, Office du Niger, Mali.

7.1. Introduction

Many studies have been carried out in Africa to assess the impact of rice cultivation on malaria. However, no consistent association has been found between irrigated rice fields and malaria transmission measured by classical entomological methods (Ijumba *et al*., 2001). It has been reported that transmission intensity in irrigated settlements is higher, similar or lower than in neighboring villages outside the irrigation scheme depending on the malaria situation before the implementation of the irrigated projects (Ijumba *et al*., 2001; Carnevale *et al*., 1999).

Little attention has been paid to the spatial variation in malaria transmission in the rice agro-ecosystems because they are generally monocultures and are considered to be homogenous. However, rice-growing environments change during rice development and vary significantly within and between countries (Khush 1984; Bambaradeniya *et al*., 2001). This variability affects the risk of malaria transmission in large irrigated rice cultivation areas.

The Office du Niger, in the district of Niono, Mali, represents one such area where 2 main environments result from an ongoing renovation process: renovated and non-renovated (Figure 7.1). Our previous study used remote sensing data to map anopheline breeding sites and described the relation between mosquito densities, survival rates, zoophilic rates, and vectorial capacity in order to explain the low prevalence of malaria (Diuk-Wasser *et al*., 2004; 2005). In the current study we reanalyze the same data to assess the effects of rice growth environmental features on malaria transmission in order to get an insight into the spatial variation of malaria risk within a large-scale irrigated rice cultivation area. This work was complemented by repeated cross-sectional anopheline larval collections in selected rice plots, which will be published elsewhere.

7.2. Materials and methods

7.2.1. Study area.

The study was carried out in the Office du Niger area (Figure 7.1) located in the inner delta of the river Niger, 350 km northeast of Bamako in the prefecture of Niono, in the region of Segou. This area comprises a colonial-era irrigation system that has undergone upgrading and repair since the 1980s. At the time of the study the Niono and Ndebougou zones were renovated, unlike the Molodo zone, and a surface of 68,000 hectares (ha) was used for rice cultivation (Coluzzi and Petrarca, 1973).

The district of Niono has about 360,000 inhabitants with 180,000 living in the irrigated area. About 44% of the population is under 15 years old and only 20% is literate. The production system is based on animals (cow, donkey etc) which are used for ploughing, for producing organic fertilizer and as pulling. Some farmers are also involved in the production of meat and milk.

Depending on the quality of water supply and regimen control, there are 3 categories of rice plots: controlled, shallow water regimen plots which are cropped either once or twice a year and unbounded plots with maximum sustained water depths. The first 2 categories have adequate delivery and disposal of excess water, whilst the last one has a poor draining system. In the renovated zones, all plots are shallow controlled water plots while in the un-renovated zone of Molodo all 3 plot types are encountered.

7.2.1.1. Study sites

18 villages were selected in the 3 agricultural zones of Niono, Ndebougou and Molodo (Figure 7.1). The selection criteria were: 1) a minimum distance of 2-km between 2 selected villages; 2) accessibility in all seasons, and 3) village cooperation. Each selected village was geo-referenced using handheld GPS receivers (Trimble ® Geo-Explorer II). A population census indicated that the median number of inhabitants per village was 963 (Minimum 600, Maximum = 2080).

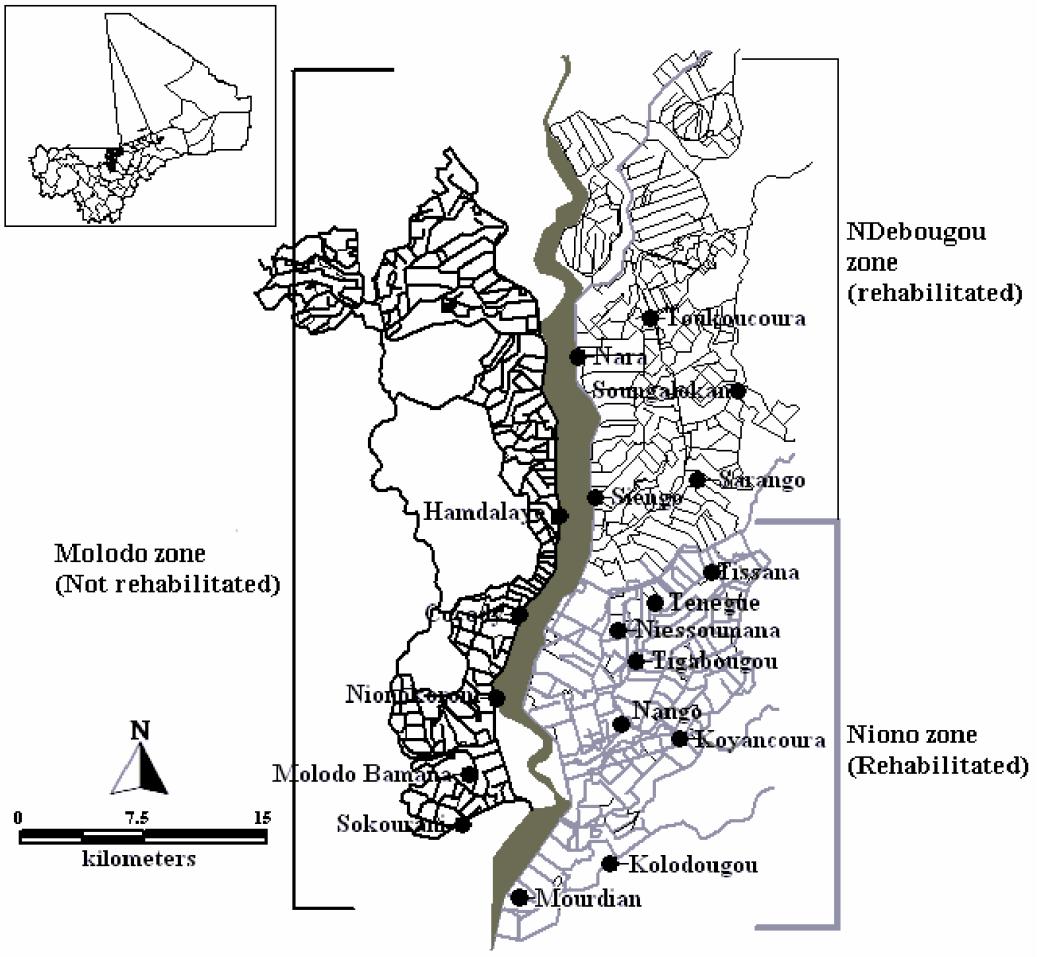

Figure 7.1: Study area showing the irrigation scheme, the agricultural zones, and the study villages.

7.2.1.2. Rice growth cycle

The typical rice cultivation cycle occurs from June to December and includes 1) a sowing-transplanting phase (June-August), 2) a growing phase (August-November) and 3) an after-harvest phase (November- December). A second and shortercultivation cycle (or off-season crop) takes place from January to May. The duration of the rice cycle varies between 120 to 150 days, depending on the rice variety.

Following the practice of the Office du Niger administration we categorized the growing stages of the rice as follows: (i) fallow/ploughing (no rice), (ii) early vegetative (tilling), (iii) vegetative (elongation), (iv) reproductive/flowering (gaining), (v) maturation (mature grain). In addition we recorded whether fields were fertilized, pre-irrigated, or were undergoing irrigation.

We also recorded crop type (rice/vegetable/fallow), vegetation abundance, rice state (sparse/dense), water turbidity, soil type and rice plot types (Table 7.1).

7.2.2. Mosquito collections and processing

Between April 1999 to January 2001, 8 crosssectional surveys were carried-out in 18 villages to determine mosquito adult abundance, manbiting (MBR or *ma*) rates, parity rate (PR or *P*), human blood index (HBI or *a*), and hence, the vectorial capacity (VC or *C*). The surveys were scheduled according to rice cropping activities and carried-out in March 1999 and 2000 (dry hot season), August 1999 and 2000 (rainy season), October 1999 and 2000 (end of rainy season) and January 2000 and 2001 (dry and cold season). Mosquitoes were collected using pyrethrum spray catches (PSC) and human bait catches (HBC).

PSC was carried-out during daytime in houses using an aerosol of 0.3% pyrethrum sold under the label of Timor. During each survey, 30 compounds (conglomerate of houses) were randomly selected from the list of compounds in each village. The collection was performed in 1 house per compound by 2 teams of 3 collectors each, during 2 consecutive days in each village. The total number of mosquito collected and the number of sleepers in the house were recorded.

In each village, HBCs were performed at night by 2 collectors using a mouth aspirator (Detinova, 1962). and sitting inside and outside of each 1 of 2 sentinel houses, at least 200 m distant from each other, from 6:00 pm. to 6:00 AM (Coluzzi and Petrarca, 1973).

Mosquitoes were morphologically identified and malaria vectors (*An. gambiae s.l.* and *An. funestus*) were selected from other *Anopheles*. Mosquitoes from HBC ovaries were dissected and their tracheoles examined to determine their physiological parity[8]. Blood meals of blood fed and semi- gravid mosquitoes, from PSC, were used to determine the human blood index by enzyme linked immunosorbent assay (ELISA) (Beier *et al*., 1988). The Polymerase Chain Reaction (PCR) method was used to determine the species of *An. gambiae s.l.* (*An. gambiae s.s.* vs *An. arabiensis*). The potential malaria transmission was estimated by the vectorial capacity, $C = ma^2P_n / (\_\log P)$ of *An. gambiae s.l.,* which is the abundant vector (Garett-Jones, 1964). *C* represents the expected number of inoculations to human from an infected person per time unit; *ma* is the human-biting density; *a* is the product of the human-biting habit (estimated to be 2 days in Mali) and the human blood index (proportion of mosquitoes fed on human); *P* is the average daily survival of the female mosquito and *n* is the mean extrinsic period of development of the parasite in the mosquito (estimated to be 12 days in the study area). We applied the parity status

method to estimate mosquito longevity (Davidson, 1954). This approach does not incorporate effects of unstable age structure of mosquito population or irregular feeding pattern (Service, 1976). However the large time intervals of 3 months between our surveys did not allow us to apply alternative methods (Charlwood and Alecrim, 1997; Mehugh, 1990; Briet, 2002).

7.3. Statistical analysis

The data were entered and cleaned in SPSS 11.0 (SPSS Inc. Chicago, USA) and analysed in STATA 8.0 (Stata Corporation, USA) and WinBUGS 1.4 (Imperial College and MRC, UK). Mosquito densities and man-biting rates (*ma*) were summarised by geometric means. Poisson regression analyses were performed to assess the bivariate relations between mosquito density and a set of rice-growth related predictors. A Bayesian spatial Poisson model was fitted in WinBUGS on the vector density data with explanatory those variables which appeared significant at a 15% significance level in the bivariate regressions. This model was used to quantify spatial correlation in mosquito density and to adjust the significance of the predictors under the presence of these correlations. In particular, we assumed that the mosquito density Y_{it} in village i and survey t follow a Poisson distribution, that $Y_{it} \sim P_o(\mu_{it})$. Spatial correlation was modelled by village-specific random effects ϕ_i, $i = 1,.....N(N = 72)$ that assumed to arise from a multivariate normal distribution $\phi = (\phi_1,...\phi_N)^T \sim MVN(0,\sum)$, with covariance matrix $\sum$. We further assumed that spatial correlation is a function of distance between locations, irrespective of the locations themselves (stationarity) and of the direction (isotropy). We adopt an exponential correlation function, that is $\sum_{ij} = \sigma^2 \exp(-\rho d_{ij})$, where σ^2 is the spatial variance, ρ models the rate of correlation decay and d_{ij} the distance between the centroïds of villages i and j. For the exponential correlation structure specified above, the minimum distance that correlation becomes

less than 5% is given by $3/\rho$ (Ecker and Gelfand, 1997). Temporal correlation was introduced by assuming an autoregressive process $AR(1)$ of order 1 on fortnight-specific random effects $\nu_t = 1,...48$. The predictors as well as the spatial and temporal effects were modeled on the log scale of the mean parameter μ_{it} of the Poisson distribution which corresponds to the average mosquito density in village i and fortnight t. that is $\log(\mu_{it}) = \underline{X}_{it}^{T}\underline{\beta} + \phi_i = \nu_t$ where $\underline{X}_{it}$ is the predictors of vector and $\underline{\beta}$ are the coefficients of the predictors. A non spatial-temporal Bayesian model was also fitted in WinBUGS. The Deviance Information Criterion (DIC) was used to assess the goodness-of-fit of the models (Spiegelhalter *et al*., 2002). The smaller the DIC is, the better the fit.

In a separate analysis we linked the larval density data with the vector adult data using a Bayesian spatial Poisson model in order to assess the relation between larvae and vector related transmission indicators. In particular, we extracted from the larvae data set those collections made a fortnight prior to the adult data collection allowing a 2 week lag for the larvae to become adults. The Pearson's chi-square test was applied to assess seasonality in the parous rate (PR) and human blood index (HBI). A Bayesian spatial logistic regression was employed to look at the relation between HBI and mosquito density. The Kruskal Wallis (KW) test was used to compare the median vectorial capacity by season and by agricultural zone. Bayesian spatial Poisson models were fitted in WinBUGS to assess the relation between the adult density and environmental factors as well as adult density and larval density. Previous studies (Service, 1976) have already shown that *An. gambiae complex* and *An. funestus* were responsible for malaria transmission in the area, therefore we focused on these species only.

7.4. Results

7.4.1. Vectors population composition and structure

A total number of 366,657 specimens of malaria vectors (*An. gambiae s.l.* and *An. funestus*) were collected. *An. gambiae s.l.* was the predominant species with a relative frequency of 90.2%. Higher frequencies of *An. funestus* were observed at the end of the rainy and during the dry cold season, specifically in villages located in the non-renovated zone of Molodo.

Results from the PCR identification-based method show that *An. gambiae s.l.* was composed of 93.1% of *An. gambiae s.s.* and 6.9% of *An. arabiensis* (n = 891). The highest relative percentage of *An. arabiensis* (31.2%, n = 93) was observed at the end of the rainy season.

7.4.2. Malaria transmission parameters

Figure 7.2 presents the variation of the geometric mean density per house, the parity ratio (PR) and the human blood index (HBI) of both *An. gambiae s.l.* and *An. funestus*. Over the study period, the mean density per house was 69.5 (95%CI: 52.7—86.3) for *An. gambiae s.l.* and 5.6 (95%CI: 4.4—6.8) for *An. funestus*. The mean PR and HBI were 60.3% (95%CI: 59.4—61.3, n = 10705) and 34.7% (95%CI: 33.9—35.4, n = 15980) for *An. gambiae s.l.* and 74.4% (95%CI: 72.9—75.9, n = 3323), 32.2% (95%CI: 30.9—33.4, n = 5854) for *An. funestus,* respectively. On average, the daily survival rate was 77.7% for *An. gambiae s.l.* and 86.3% for *An. funestus*. The highest mosquito density period corresponded to the lower HBI and PR for both *An. gambiae s.l.* and *An. funestus*. Particularly in August 2000 where the highest density (252.5, 95%CI: 205.4—299.6) for *An. gambiae s.l.* was observed, the HBI (17.0% 95%CI: 15.6—18.5) and the PR (57.8, 95%CI: 56.0—59.5) were also very low.

The median vectorial capacity (interquartile range) was 0.33 (0.01—1.03), 0.11(0.01—0.79), 0.00 (0.0—0.10) and 0.01(0.0—0.10), during the dry cold, the dry hot season, the rainy and at the end of the rainy season respectively. The vectorial capacity differs significantly between the seasons (KW = 21.33, df = 3, $P < 0.001$). In particular, the highest vectorial capacity was observed in the dry cold season which showed the lowest mosquito density. The median vectorial capacity was not significantly higher (KW = 4.97, P = 0.083) in the nonrehabilitated agricultural zone of Molodo (0.1, 0.0—0.61) than in the rehabilitated zones of Niono (0.02, 0.0—0.44) and NDebougou (0.0, 0.0—0.54).

7.4.3. Spatial analysis of malaria transmission parameters

Bivariate and multiple nonspatial and spatial Poisson models were fitted to assess the association between mosquito density and rice growth related environmental features (Table 7.1)**.** The goodness of fit criterion indicates that the spatial multiple model fits the data better (DIC = 4360.0) than the non-spatial one (DIC = 5092.8). The good predictors of vector density were rice crop, rice development stages, vegetation abundance, water state, and seasons. Field types, which was a good predictor of mosquito density in the non-spatial model was no longer significant in the spatial multiple Poisson model. Tilling stage of rice, which was not significantly correlated with mosquito density in the multiple independent model became negatively related in the spatial model. The association of abundant vegetation category to mosquito density changed from positive in the multiple independent model to negative in the spatial one. This clearly illustrates how the standard statistical method, which assumes independence of observations, can over or underestimate the standard error, hence the significance, of the covariates when they are used to analyze spatially correlated data (Cressie, 1993). In fact, the data reveal a spatial correlation up to

distance of 30.0 km (95% CI = 22.2, 133.2), which was not accounted for in the non-spatial model.

A separate multiple spatial Poisson model (detailed results not shown) was fitted to assess association between larval density in rice fields and adult density in human settlements. The model estimated a density ratio, DR = 1.005 (95% CI = 1.0013, 1.0016) for every increase of adult density by 1 mosquito. When adjusted for the environmental covariates, the larval density was no longer significant. Spatial correlation was strong and diminished to less than 5% at 35.5 km (95%CI =21.1, 427.4).

Spatial logistic models showed that seasonality was significantly associated with PR and HBI for both species, *An. gambiae sl* and *An. funestus* (Table 7.2). Also both species were less likely to be fed on human during the rainy, end of rainy and dry hot seasons compared to the dry cold season. *An. gambiae s.l.* was less likely to be parous during the dry hot and rainy seasons compared to the dry cold season. The odds of parity in *An. funestus* was significantly higher during the dry hot season (OR = 8.27, 95%CI = 4.95—13.29) and significantly lower during the rainy and end of rainy season relative to the dry cold one. Mosquito density was significantly associated with the PR and HBI only for the *An. funestus* species but not for the *An. gambiae s.l.* The minimum distances at which there was no spatial correlation in the PR and the HBI were 3.36 km (1.41—21.29), 3.17 km (1.41—19.96) for *An. gambiae s.l.* and 2.56 km (1.39—15.13), 2.17 km (1.39—7.31) respectively for *An. funestus*.

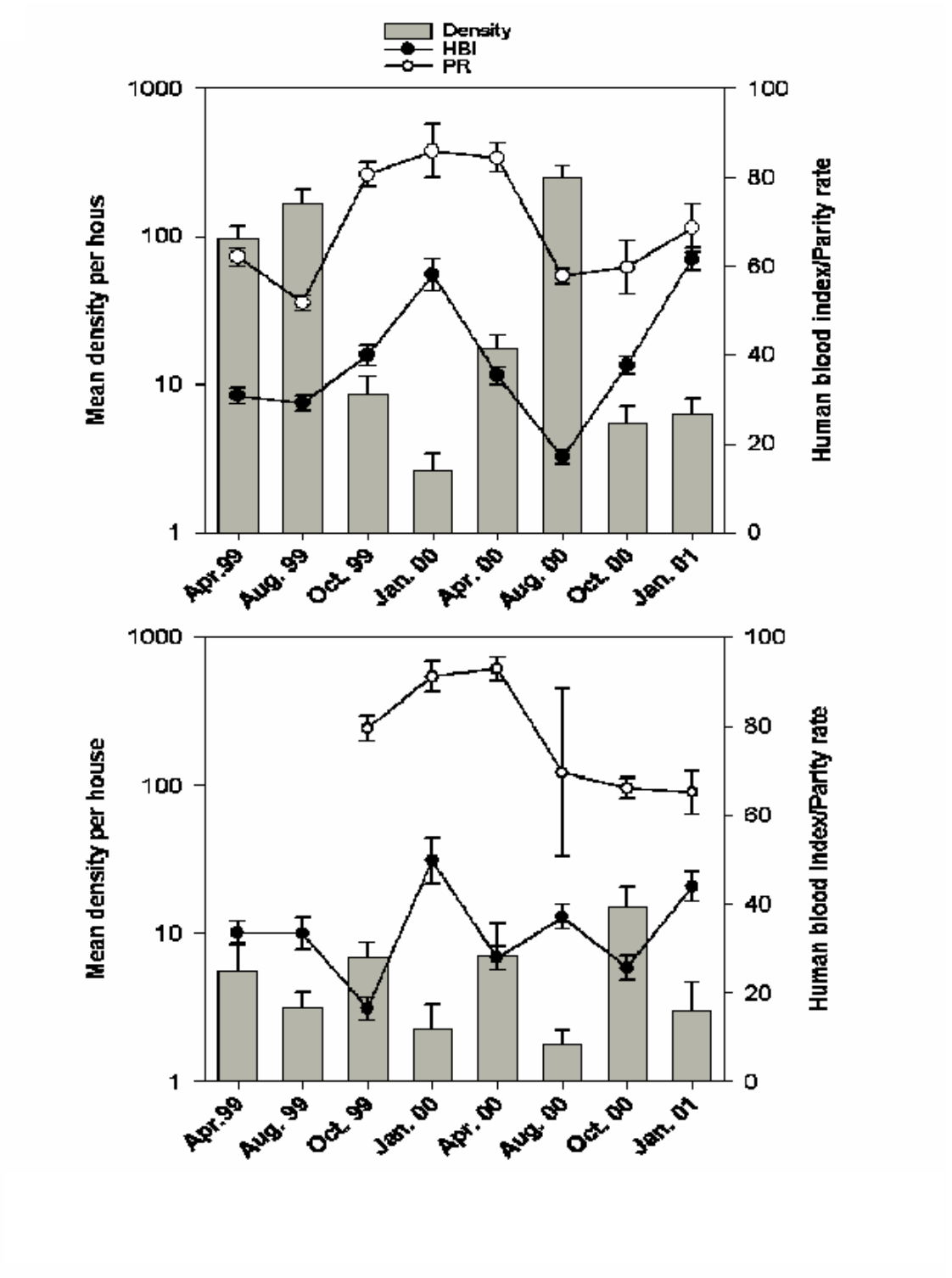

Figure 7.2: Variation in *An. gambiae s.l.* (top) and *An. funestus* (bottom) density (bars), parity rate (white dots) and human blood index (black dots) over the study period. The bars represent the 95%CI.

Table 7.1: Estimates of the effects of rice growth on adult mosquito densities

Variables		Bivariate independent		Multiple independent		Multiple spatial	
		Estimates	95% CI	Estimates	95%CI	Estimates	95%CI
Rice crop							
	No rice	1.0		1.0		1.0	
	Rice	1.2	1.2—1.3	1.2	1.1—1.3	1.4	1.3—1.5
Rice stages							
	No rice	1.0		1.0		1.0	
	Tilling ⊗	2.1	2.1—2.2	1.04	0.95—1.13	0.8	0.7—0.8
	Elongation	1.3	1.2—1.3	1.7	1.5—1.9	1.6	1.4—1.9
	Gaining	0.8	0.8—0.9	3.9	3.4—4.5	2.5	2.1—3.1
	Maturation	0.3	0.3—0.4	1.3	1.1—1.6	0.9	0.7—1.1
Field types							
	Single crop	1.0		1.0		1.0	
	Double crop	0.9	0.9—1.0	1.1	1.0—1.1	1.4	0.8—2.4
	Ind. Managed	1	0.9—1.1	1.3	1.2—1.4	1.2	0.5—3.1
Seasons							
	Dry cold	1.0		1.0		1.0	
	Dry hot	12.8	11.3—14.4	12.2	10.6—14.1	14.9	12.8—17.3
	Rainy	29.8	26.6—33.4	37.6	32.7—43.2	39.4	34.0—45.7
	End rainy	2.8	2.5—3.2	2.3	1.8—2.8	2.5	1.9—3.2
Vegetation abundance							
	No vegetation	1.0		1.0		1.0	
	Less abundant	0.7	0.6—0.7	0.3	0.3—0.3	0.3	0.3—0.3
	Abundant	3.2	3.1—3.4	1.2	1.1—1.3	0.8	0.7—0.8
	Very abundant	2.1	1.9—2.4	1	0.9—1.1	0.9	0.8—1.1
Rice state							
	No rice ±	1.0		-	-	-	-
	Sparse	16.9	13.9—20.5	-	-	-	-
	Partly Covered	14.2	11.6—17.3	-	-	-	-
	Covered	5.6	4.6—6.9	-	-	-	-
Agricultural activities							
	No rice	1.0		1.0		1.0	
	Pre-irrigation	2.1	1.9—2.4	0.7	0.6—0.8	0.7	0.6—0.8
	Transplanting	2.1	1.9—2.4	0.6	0.5—0.7	0.7	0.6—0.8
	Grass removal	-	-	1.0	0.0—3.40E+08	1.0	0.0—3.2E+8
	Fertilizing	1.5	1.3—1.7	0.2	0.1—0.2	0.1	0.1—0.2
	Irrigation	1.1	1.0—1.2	0.2	0.2—0.3	0.2	0.2—0.2
	No activity	0.7	0.6—0.8	0.6	0.6—0.7	0.5	0.4—0.5
	Protect birds	0.1	0.09—0.2	0.2	0.1—0.2	0.2	0.1—0.2
	Canal cleaning	0.3	0.2—0.3	0.3	0.2—0.3	0.4	0.3—0.6
	Water drainage	0.3	0.2—0.4	0.7	0..5—1.0	1.1	0.8—1.6
	Harvesting	0.1	0.08—0.1	0.7	0.6—0.9	0.8	0.6—1.0
	Market gardening	0.5	0.4—0.5	1	0.0—3.50E+08	1.0	0.0—3.2E+8
Water state							
	No water	1.0		1.0		1.0	
	Dusty	0.5	0.5—0.6	0.1	0.1—0.1	0.1	0.1—0.1
	Turbid	1.8	1.7—1.9	0.6	0.5—0.7	0.8	0.7—0.9
	Clear	1.1	1.0—1.1	1.1	1.0—1.1	1	0.9—1.1
Soil types							
	Clay	1.0		-	-	-	-
	Mixed	0.9	0.9—0.9	-	-	-	-
Spatial parameters							
	Correlation decay (ρ)	-	-	-	-	10.9	2.4—15.8
	Spatial Variance (σ^2)	-	-	-	-	0.7	0.2—2.1
Goodness of fit							
	DIC		-		5092.81		4359.99

** Covariant effects are density ratios. Estimates are posterior means. CI = confidence interval; BCI = Bayesian credible interval; DIC = deviance information criteria. ⊗ Two decimal places guiven to show non-significance ± Excluded because of collinearity*

7.5. Discussion

The aim of this study was to assess malaria transmission parameters in a large scale irrigated rice cultivation area taking into account the spatial correlation present in the data. The main species, which we were found, were *An. gambiae s.l.* and *An. funestus*. These are also the most common species in West African rice cultivation areas (Dolo *et al*., 2004; Koudou *et al*., 2005). Among these 2 species, *An. gambiae s.l* was predominant accounting on average for 90% (n = 366,657) and was particularly abundant during the rainy season of 1999 and 2000 and the dry hot season of 1999 (second agricultural cycle). The lowest density of *An. gambiae s.l.* during the second agricultural cycle period of 2000 was related to restrictions imposed in rice cropping by the agricultural department to clean the draining system. During this period, the remaining stagnant water in the canals was used by *An. funestus* as breeding habitats (Klinkenberg *et al*., 2003). At the end of the rainy and during the dry cold season, the frequencies of both species reached similar levels. This seasonal variation in the frequency ratio of the 2 species is commonly observed and it is related to their ecology (Mouchet and Brengues, 1990). The sun-loving *An. gambiae s.l.* colonizes rice fields at the transplanting period and is replaced by the shade-loving *An. funestus* when rice plants cover the fields.

The negative association between the adult density with the PR and HBI in the Office du Niger has been already reported and has been also observed in neighboring Burkina Faso (Doannio *et al*., 2002; Dolo *et al*., 2004; Diuck-Wasser *et al*., 2005). The most likely explanation is that when the mosquito density increases individuals take more protective measures (i.e. bed net use) which may divert mosquitoes to animals such as cattle. This argument is supported by the exceptionally low HBI of the very anthropophilic species of *An. funestus* in spite of its very high parity rate. Whereas a negative association between adult density and HBI has been

observed in the Office du Niger, Mali (Klinkenberg *et al*., 2003; Dolo *et al*., 2004) and Burkina Faso (Daonni *et al*., 2002), a recent study conducted in Côte d'Ivoire suggested a positive association reporting HBI up to 95% during high density periods (Koudou *et al*., 2005).

The vectorial capacity was relatively low with a seasonal and village to village variation. The median vectorial capacity was higher in the non-renovated zone of Molodo than the renovated zones of Niono and NDebougou but the statistical significance was borderline. The inadequate water disposal system of the non-renovated zone may have raised the relative humidity that aids mosquito survival[1] and therefore the vectorial capacity. The higher prevalence of *An. funestus* in this zone may have also contributed to this finding. The deficiency in the draining system of this agricultural zone has created deep, vegetated and persistent water bodies which are used by *An. funestus* as breeding habitats. However it is important to note that in this study our estimate of vector survival did not take into account the recruitment rate in mosquito population which can have an impact on the parity ratio and hence on season specific vectorial capacity estimates. Unfortunately, the large sampling interval of our data did not allow us to use alternative approaches. However the possible bias in the estimates of the vectorial capacity should not reflect in the comparison between locations since the same method was applied.

Table 7.2: Multiple spatial logistic regression of parity ratio (PR) and human blood index (HBI) on adult mosquito density adjusted for seasonal effects

Parameters	*An. gambiae s.l.*		*An. funestus*	
	Parous rate OR (95%CIs)*	HBI OR (95%CIs)	Parous rate OR (95%CIs)	HBI OR (95%CIs)
Seasons				
Dry cold	1.0	1.0	1.0	1.00
Dry hot	0.63 (0.49—0.80)	0.41 (0.37—0.46)	8.27 (4.95—13.29)	0.47 (0.40—0.55)
Rainy	0.32 (0.24—0.41)	0.37 (0.31—0.44)	0.68 (0.24—1.63)	0.69 (0.59—0.80)
End of rainy	1.12(0.80—1.46)	0.48 (0.43—0.54)	0.75 (0.58—0.96)	0.27 (0.23—0.33)
Density	1.00 (1.00—1.00)	1.00 (1.00—1.00)	0.97 (0.96—0.98)	1.01 (1.01—1.02)
Correlation decay (ρ)	99.03 (15.66—235.50)	104.90 (16.70—235.80)	130.00 (22.03—238.20)	153.50 (45.59—240.00)
Spatial variance (σ^2)	0.17 (0.08—0.36)	0.12 (0.06—0.24)	0.23 (0.09—0.52)	0.07 (0.03—0.16)

Our data showed that shallow controlled plots used for the 2 agricultural cycles (twice a year) produced fewer larvae than all other plots types. The better draining system has certainly shortened the time they serve as breeding sites for anopheline. Indeed research on water management in rice plots reported the numerous and lasting breeding habitats even after harvesting in inefficiently drained plots (Klinkenberg *et al*., 2003; Temel, 2004). However more studies are required to rigorously support this observation because restriction was made in cropping during the second year of our study period.

Adult densities showed marked seasonality however they were large enough to sustain transmission throughout the year. This is almost certainly due to the current cultivation methods, characterized by overlaps between several agricultural cycles (Klinkenber *et al*., 2003; Koudou *et al*., 2005). In spite of the high densities during the rainy season, the potential for transmission was lower than in the dry season. This could be explained by the decreases in the HBI (a measure of vector–human contact) and in the PR (a measure of vector longevity) at that time period (Figure 7.2). In the dry season, lower vector densities may lead to relaxation of individual protection. Vector-human contact may also be higher during the dry hot season because people spend longer periods outside.

Spatial correlation in mosquito density data was significant in distances up to 30.6 km indicating that the number of mosquitoes per house is related to the number of mosquitoes up to 30.6 km apart. This strong spatial correlation is likely to be related to the rice cultivation environment which is associated with mosquito abundance because of the suitable conditions it creates. In addition our analysis does not include climate related parameters such as rainfall and

temperature which are spatially structured and might also explain the residual spatial correlation. Spatial correlation in PR was relatively low (up to 3.36 km and 3.17 km for *An. gambiae s.l.* and *An. funestus*, respectively). Similarly spatial correlation in HBI is up to 2.56 km and 2.17 km for *An. gambiae s.l.* and *An. funestus*, respectively. This weak spatial correlation suggests that PR and HBI are more related to local conditions such as population behavior and economical status, presence of animals rather than similar environment over large areas. A spatial analysis performed to assess the effect of mosquito density on the PR and the HBI did not show any significant association other that between PR of *An. funestus* and mosquito density (OR = 0.97, 95%CI = 0.96—0.98). The importance of local environment may also explain the difference we observed in the vectorial capacity from village to village and between the agricultural zones. A separate model linking larvae and adult density suggested that larvae density was significantly related to the mosquito density per house. This association disappeared when we adjusted the density for rice growth environmental factors.

This study is the first to quantify the amount of spatial correlation in rice cultivation areas and to assess the effect of rice growing on malaria transmission taking into account this correlation. Our results show that in the Office du Niger, rice cultivation has created environmental conditions favorable to the occurrence of the 2 major malaria vectors which, with current agricultural practices is leading to a year round transmission with a marked seasonality.

Local variation was observed in mosquito parity ratio and human blood index, which both measure the vector-human contact rate and hence the potential for malaria transmission intensity. Attention must be paid to this local variation when implementing control strategies. Similar

studies elsewhere in Africa are needed if we are to understand whether these are general features of malaria transmission in large scale irrigated ecosystems.

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Chapter 8

General discussion and conclusions

The wide spectrum of malaria transmission patterns resulting from heterogeneity of vector populations and the limitations of current control tools underscore a need to address the challenges of vector control with tailored interventions responsive to local conditions and transmission patterns. The success of such an approach requires accurate information on vector biology and ecology, malaria transmission and epidemiology, in relationship to local environmental conditions, and a good stratification of control areas with respect to time and space (Toure *et al*., 2004). The goal of this thesis was to enhance our understanding of the relationship between the relative frequencies distribution of members of *An. gambiae* complex and climatic and environmental conditions, to produce their spatial and temporal distribution, to quantify their unique contribution to malaria transmission, and to produce their attributed malaria risk maps in Mali. More specifically we: (i) identified environmental factors related with the distribution of a) the two major species (*An. arabiensis* and *An. gambiae s.s.*) which compose the *An. gambiae* complex and b) the chromosomal (Bamako, Mopti, Savanna Hybrids) forms of *An. gambiae s.s.*; (ii) produced maps of the geographical distribution of the species and chromosomal forms; (iii) assessed the contribution of species and chromosomal forms to malaria transmission in Mali; (iv) examined the spatio-temporal distribution of *An. gambiae* complex densities and its chromosomal (Mopti, Bamako, Savanna, Hybrids) forms in a Sudan savanna village; (v) investigated the malaria vector ecology during the dry season and its implication for vector control, and (vi)

assessed the spatial pattern of malaria transmission in the rice cultivation area of the Office du Niger.

Previous studies have produced maps of the distribution of the *An. gambiae* complex by displaying the relative frequency of its species at sampled locations (Coetzee *et al*., 2000; Onyabe & Conn, 2001; Toure *et al*. 1998). Maps of the distribution of species (Lindsay *et al*., 1998) and subspecies (Bayoh *et al*., 2001) at continental and regional scales have been produced based on climatic suitability conditions, standard statistical models assuming independence of the observations and ecological niche-modeling (Levine *et al*., 2004). However, malaria vectors species frequencies are spatially correlated because neighboring areas are sensitive to similar climatic and environmental factors influencing species distribution in a similar way. Analyzing spatially correlated data assuming independence lead to overestimation of the statistical significance of the covariates (Cressie, 1993). In our study, we used Bayesian geostatistical modeling and vector field data from village to country level. The Bayesian geostatistical modeling, implemented via Markov chain Monte Carlo simulation (MCMC) quantifies the relationship between environmental factors and the species distribution by taking into account the spatial dependence present in the data in a flexible way that allows simultaneous estimation of all model parameters. In addition, Bayesian kriging enables model-based prediction together with the prediction error, a feature which is not possible in the classical kriging.

A detailed discussion on the findings was given in each chapter previously. Here we report a summary of the main contributions of the work and their implication in malaria control. In chapter 2, we found that the relative frequencies of the two major species (*An. arabiensis* and *An. gambiae s.s*) of *An. gambiae* complex were associated with the cumulative rainfall during the

survey and during the two previous months instead of the rainfall of the survey month. *An. arabiensis* was associated with dry and *An. gambiae s.s.* with wet conditions. Thus, the spatial distribution map of their relative frequencies showed higher frequencies of *An. arabiensis* in the drier Savanna areas and *An. gambiae s.s.* in the flooded/irrigated areas of the inner delta of Niger river, the southern Savanna, along rivers and in the Sahel. The occurrence of *An. gambiae s.s* in the arid regions (Sahel) has been shown to be associated with the 'Mopti' chromosomal form (Touré *et al*. 1994), which also prefers dry conditions.

Using the same geostatistical approach in chapter 3, we analyzed data of the relative frequencies of the chromosomal forms (Mopti, Bamako, Savanna and theirs Hybrids) of *An. gambiae s.s.*, which showed that at least two of the chromosomal forms were sympatric, though each of them had a preference for one of the three eco-climatic zones of the country. The Mopti form was sharing the same ecological area with *An. arabiensis*. In addition, it occupied the flooded/irrigated areas of the inner delta of Niger River. The Savanna form prefers the Sudan Savanna areas and the Bamako form was confined around Bamako city and in part of Sikasso region. The ecological distribution of the different chromosomal forms seems to be related to difference in their preference for larval breeding habitats. The Savanna chromosomal form breeds more frequently in temporary rain-dependent breeding places, which are more likely to be present in savanna areas; the Mopti form was observed more frequently in semi-permanent to permanent breeding places, which are in general man-made; and the Bamako form breeds more often on the edges of temporary streams (Touré *et al*., 1998; Edillo *et al*., 2002). Fanello *et al*. (2003) explained the higher frequency of the pyrethroid *kdr* gene observed in the savanna compared to its sympatric and synchronous Mopti and Bamako forms by the differences in their preference for different breeding habitats. The chromosomal arrangement *bc/bc* (associated to dry conditions)

and *u/u* (associated to wet conditions) of the Mopti chromosomal form may play an important role in its adaptation to diverse environments (Touré *et al*. 1998).

The practical implication of the findings of chapter 2 & 3 is that they provide valuable information for monitoring insecticide resistance encoded by the *kdr* gene and selective and targeted malaria vector control in Mali. Indeed, based on the current knowledge, the *kdr* gene has been frequently reported in *An. gambiae s.s.* and particularly in its chromosomal form Savanna and not yet in the other taxa in Mali (Fanello *et al*., 2003). Therefore, insecticide control measures must be emphasized in the Sahelian (epidemic prone area) and irrigated/flooded areas where *An. arabiensis* and the Mopti chromosomal form prevail. Any vector control by means of insecticides in the Southern part of the country, where the S molecular form (Savanna and Bamako) predominates, must be accompanied by a close insecticide resistance monitoring system. Even though more bio-ecological and gene flow studies among the different species and chromosomal forms are needed before undertaking any field implementation of genetically manipulated mosquito control, the maps may be useful for planning future implementation of this control method.

Malaria control resource allocation must be proportional to the risk of malaria transmission if decision makers are aware of which areas are at higher risk than others and which species are responsible of the transmission. Maps of malaria risk of Mali have been produced (Kleinschmidt *et al*. 2001; Gemperli *et al*. 2006; Gosoniu *et al*. 2006). These maps are based on data collected until 1998. Chapter 4 includes a revised malaria risk map based on more recent data. This map should reflect more accurately the current malaria risk in Mali. The malaria risk map was combined with maps of the geographical distribution of subspecies to produce attributed

malaria risk maps for each subspecies. Our analyses showed that all the chromosomal forms of *An. gambiae s.s.* were equally contributing to malaria transmission during the survey period of 1981-1990. However, during the relatively wet period (1991-2004), High malaria risk was associated with insecticide resistance gene (*kdr*) carriers (Bamako/Savanna chromosomal) compared to the non-carrier Mopti chromosomal form, though the association was not significant. The revised predicted malaria prevalence map based on surveys during 1991-2000 showed a South to North distribution of malaria risk. Higher malaria risk was observed in the Southern part, a moderate risk in the middle and lower risk in the Northern part of the country. This distribution pattern is in agreement with the eco-geographical description of the epidemiology of malaria in Mali (Doumbo *et al.* 1989). The attributed risk maps of the different species and subspecies indicated that in the middle West and South East part of the country malaria transmission risk is mainly due to *An. arabiensis*, in the irrigated/flooded areas malaria risk is attributed to the Mopti form in the southern part to the Savanna/Bamako forms and in the southern areas of the region of Kayes to the hybrids.

The analysis of the updated MARA data showed a significant decrease in malaria prevalence during 1981-1990 which could be due to low rains in that period. Similar observations were reported from neighbor Sahelian countries of Niger and Senegal where up to 80% of reduction in malaria prevalence was observed (Faye *et al.*, 1995; Mouchet *et al.*, 1996). An increase in malaria risk was observed during 1991-2004 in comparison to the drought period (1981-1990) which can partly be explained by the high amount of rainfall during this period (Konate *et al.*, 2001; Labbo *et al.*, 2004; Thomson *et al.* 2006; Kent *et al.*, 2007). Other factors such as environmental changes due to human activities, the resistance of parasite to drugs and of

the vectors to insecticides as well as the poor implementation of control interventions could have contributed to this situation.

Integrated Vector Management (IVM) strategies targeting a particular area and vector populations in time require information at high spatial and temporal resolutions on the distribution of adult vector densities as well as its sibling species and subspecies. In chapter 5, we assessed the spatial and seasonal distribution of *An. gambiae* complex adult densities and the relative frequencies of the chromosomal forms of *An. gambiae s.s.* in relation with the local environmental factors in the village of Bancoumana, Mali using data over four years. Our data showed spatial, seasonal and year to year variations in the distribution of mosquito densities. Spatial and seasonal variations in the relative frequencies of the chromosomal forms of *An. gambiae s.s.* were also observed. The annual and seasonal variations could be explained by annual and seasonal variations in the rainfall. Surprisingly, we found a positive, but weak association between the number of mosquitoes found in a house and its distance from the nearest breeding habitat. This observation is contrary to previous results (Minakawa *et al*., 2002; Zhou *et al*., 2007). Nevertheless, the spatial distribution maps of mosquito densities showed a concentric clustering pattern with higher densities at the periphery of the village at the beginning of the rainy season and during the dry season. This distribution was patchy during the middle and the end of the rainy season. Temporal dynamics of larval habitats may explain such distribution pattern. The chromosomal forms were sympatric over all seasons. There was a spatial clustering in their relative frequency distribution changing over time in the village. The Mopti chromosomal form was the most abundant at the beginning and middle of the rainy season and the Bamako form at the end of the rainy season. The range parameters for the frequencies of the chromosomal forms were relatively high compared to mosquito flying range. This is explained by the fact that the

frequencies of the chromosomal forms are similar in neighboring areas due to related environmental and climatic factors which favor the breeding and survival of mosquitoes. Passive migration directed by the wind could also contribute to the high values of the range parameters. Our results suggest that control interventions targeting the Mopti form should be implemented at the beginning and middle of the rainy season, while those targeting of the Bamako form should be done at the end of the rainy season. In addition, appropriate vector control targeting the periphery of the village at the beginning of the rainy season and during the dry season can ameliorate the malaria situation in seasonal malaria transmission areas.

In Chapter 5, we monitor larval habitats and mosquito densities in and around the village of Bancoumana, Mali, in order to provide a basis for the development of vector control strategies during the dry season. Our results showed that in the main village of Bancoumana nearly all larval habitats were human-made, rain-dependent and dried out 10-12 weeks after the end of the rainy season. As a result, the number of adult mosquitoes collected in the houses became very small. In the fishermen's hamlets adjacent to the receding Niger River riverbed, there were numerous small natural puddles that were highly productive for anopheline larvae even during the dry period. As a result, the mosquito density was higher in those hamlets than in the main village. Larval habitats in Bancoumana were re-colonized shortly after a rainfall suggesting that mosquitoes that emerged from the riverbed are an important seed of the rain-fed water bodies of Bancoumana. Although the distance of 3–5 km that separates the river and the village seems to be out of the flight range of *An. gambiae* complex in Mali (Dolo *et al*), studies from elsewhere reported that this distance is within the flight range of *An. gambiae* complex (Kaufmann *et al.* 2004). These findings suggest that vector control in the fishermen's hamlet during the dry season may be feasible, sustainable, at low cost and may ameliorate malaria transmission in the main

village. Similar scenarios may exist in other areas with seasonal malaria transmission as in the main village and can provides an opportunity for a mosquito control strategy targeting dry season larval control and environmental management in sub-Sahara Africa.

A challenge for African countries is accommodating irrigated agriculture required to respond to food needs, and diseases associated with irrigation. Rice cultivation is traditionally related to vector-borne diseases, especially malaria. The changes in the malaria transmission pattern due to the development of irrigation are subject of debate (Ijumba *et al*. 2002). However, little attention is paid to the spatial variations in malaria transmission in rice cultivation areas. In chapter 7, we assessed malaria transmission parameters in a large scale irrigated rice cultivation area taking into account the spatial correlation present in the data. The data showed a strong spatial correlation in mosquito densities certainly related to the rice cultivation environment. However, our analysis does not include climate related parameters such as rainfall and temperature which are spatially structured and might also explain the residual spatial correlation. The most interesting findings were the weak spatial correlation observed in the parous rate (PR) and human blood index (HBI) suggesting that these parameters are more related to local conditions such as population behavior and economical status, presence of animals etc rather than similar environment over large areas. Since both PR and HBI measure the vector-human contact rate and hence the potential for malaria transmission intensity, attention must be paid to this local variation when implementing control strategies in rice cultivation areas.

Conclusion

The Bayesian geospatial analyses used in these studies enable the analysis of complex data like the morphologically indistinguishable species and subspecies of *An. gambiae* complex

in relation to environmental and climatic factors in Mali. This enhances our understanding of the relationship between climatic and environmental factors and the relative frequency distribution of *An. gambiae* complex species and subspecies. We were also able to assess the relative contribution of the different chromosomal variants to malaria transmission as well as to map their attributed malaria risk.

This work makes a substantial contribution in the mapping of the spatial distribution of malaria vector species and subspecies which was limited by the lack of field data and appropriate statistical analyses. Our findings provide relevant information for both operational control and academic research activities.

In the control context, the species and chromosomal forms distribution maps are useful for insecticide based vector control because they identified areas were insecticide resistant and susceptible species or subspecies are present. In addition, they provide information for targeted control of a specific species or subspecies. The results of this work provide the basis for malaria control strategies during the dry season which may cost less and showed that large areas of rice cultivation must not be considered as a whole when implementing control interventions

In the academic research context, though more focused research still needed in order to better understand the micro-ecology and gene flow among the different chromosomal variants, the produced maps provide the basis for future implementation of genetically manipulated mosquitoes in malaria control.

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