Vector-borne Disease Risk Assessment in Times of Climate Change: The Ecology of Vectors and Pathogens

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“Es ist kein Reichtum zu vergleichen einem gesunden Leibe.“

Jesus Sirach

(2. Jh. v. Chr.)

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Glossary of Used Abbreviations

AIC	Akaike Information Criterion
Arbovirus	Arthropod-borne Virus
AUC	Area under the Receiver Operator Characteristic Curve (Model Quality Criteria)
CBD	Convention on Biological Diversity
CCLM	Regional Climate Model COSMO-CLM that is hosted by the CLM community COSMO - Consortium for Small-scale Modelling CLM - Climate Limited-area Model
CDC	Center for Disease Control and Prevention (United States Authoritie)
CHIKV	Chikungunya Virus
CRU	Climate Research Unit
DENV	Dengue Virus
DHF	Dengue Haemorrhagic Fever
DKRZ	German Climate Computing Centre (Deutsches Klimarechenzentrum)
DENV	Dengue Virus
ECDC	European Centre for Diseae Control and Prevention, Stockholm
ECHAM	Global Climate Model developed by the the Max Planck Institute for Meteorology
EIP	Extrinsic Incubation Period (Time between the acquisition of a pathogen by a vector and the vector's ability to transmit the pathogen to other susceptible hosts).
EKBM	Expert Knowledge Based Model (model that is derived by selection of variables using already understood species´climatic constraints)

GARP	Genetic Algorithm for Rule Set Production (Species distribution modelling software)
GCM	Global Climate Model (syn. General Circulation Model)
GIS	Geographical Information System
IPCC	Intergovernmental Panel on Climate Change
ISSG	Invasive Species Specialist Group
IUCN	International Union for Conservation of Nature
LST	Land Surface Temperature
MaxEnt	Software for species distribution modelling based on Maximum Entropy approach
MCDA	Multi Criteria Decision Analyses
MD	Mahalanobis Distance
MESS	Multivariate Environmental Similarity Surface
MODIS	Moderate Resolution Imaging Spectroradiometer
MRPP	Multiresponse Permutation Procedure
NDVI	Normalised Difference Vegetation Index
RCM	Regional Climate Model
RCP	Representative Concentration Pathway
RKI	Robert Koch Institute, Germany
SBM	Statistic Based Model (model that is derived by selection of variables using statistical test of variable´s importance)
SRES	Special Report on Emissions Scenarios
VBD	Vector-borne Disease

1. Compendium

1.1 Summary

Evidence suggests that European climate change in the 21st century will support a spread of disease vectors and vector-borne diseases. Ectothermic arthropods make up the largest group of vectors. They cannot self-regulate their body temperatures and are therefore considered to be very sensitive to changing climatic conditions. The duration of pathogen development inside the vector is also directly linked to the ambient temperature. This thesis addresses a multidisciplinary approach to improve risk assessment for vector species establishment and pathogen emergence based on ecological knowledge. The objective is to elucidate possible new hotspots of disease transmission.

In the first part of the thesis (articles 1 to 3), climatic factors for disease vectors are identified via literature analysis, statistical procedures in species distribution models, and vector life history trade experiments. Of particular interest here, is the mosquito Aedes albopictus is an invasive disease vector which originates in the tropics and subtropics, it is a competent vector of pathogens such as dengue and chikungunya, among others. By means of correlative species distribution models, suitable European regions are identified. The comparison of published results for various *Aedes albopictus* risk models, including the aforementioned, shows that beside the regions for which there are a consensus risk levels, there is a great deal of uncertainty in other regions about the future development.

These uncertainties in risk model projections indicate that existing knowledge of mosquito ecology needs to be expanded and deepened, in particular with regard to the temperate European situation. The second step was therefore, the integration of detailed ecological knowledge on thresholds during vector life history to improve the correlative risk models. Here, of special interest is the ability of *Aedes albopictus* to survive winter conditions in Europe, as in its native range no frost temperatures occur. Here, the low-temperature threshold for egg survival was experimentally tested for post-diapause and non-diapause European eggs of *Aedes albopictus* and non-diapausing eggs of *Aedes aegypti*. Hatching success after the cold treatment was significantly increased in European eggs which have undergone a diapause compared to non-diapausing European eggs. The experiments help to detect potential regions capable of overwintering populations. Thresholds for sur-

vival can be derived by simulating extremes, which then can be related to climate change scenarios.

In the second part of the thesis (articles 4 to 6), climatic factors for pathogens are identified. Using the example of dengue, the temperature requirement for pathogen amplification is determined via statistical analysis of extrinsic incubation period experiments found in literature. The extrinsic incubation period is the time at a defined temperature needed to render the vector infective after a contaminated blood meal. Out of all described dengue extrinsic incubation period experiments a continuous temperature-time profile is provided which allows, via highly resolved spatio-temporal climate change projections, a detailed characterization of potential regions at risk in Europe. A second approach, demonstrated with the example of chikungunya is the analysis of temperature requirement for disease transmission during an outbreak.

Once these climatic factors are identified, climate-derived risk maps are generated by combining vector and pathogen requirements. As a general tendency for Europe, it can be expected that the risk of *Aedes albopictus* establishment and vector-borne virus transmission will increase, especially for the latter decades of the 21st century. Concerning the evolving climatic suitability for *Aedes albopictus*, it can be inferred that Western Europe will provide especially favourable climatic conditions within the next decades. Furthermore, climatic suitability can be expected to increase in Central Europe and the southernmost parts of the United Kingdom. Climatic conditions will continue to be suitable in Southern France, as well as most parts of Italy and Mediterranean coastal regions in South-eastern Europe. Differences in results for scenarios become obvious, regarding the temporal scale in this century, but the spatial patterns remain the same. The climatic risk of chikungunya transmission will increase in Europe by the end of the century along the western coast of the Mediterranean Balkan States and Greece as well as in the Pannonian Basin and the Black Sea coast of Turkey. A persisting high suitability for Chikungunya transmission throughout the 21st century is projected for Northern Italy.

Finally, emerging tools and concepts are elucidated (article 7) by the means of specific examples in order to identify new multidisciplinary approaches in vector-borne disease risk assessment. However, this is difficult to achieve as scientists are part of specialised and mostly discrete scientific networks, it is necessary that results from other disciplines are understood and considered. To give a first impression of the current scientific cooperation, discipline specific citation behaviour for research on vector-borne diseases with respect to climate change is evaluated.

This thesis offers an ecologically focused evaluation of the spatially and temporally changing risk patterns of invasive vector establishment and emerging disease exposure for Europe during the 21st century. Disease surveillance and vector control measures can now be implemented effectively at locations and times to mitigate possible transmission.

1.2 Zusammenfassung

Viele Hinweise deuten darauf hin, dass sich Krankheitsvektoren (Krankheits-überträger) und die von ihnen übertragenen Krankheiten aufgrund der sich verändernden klimatischen Bedingungen im Europa des 21. Jh. neu etablieren oder ausbreiten können. Die größte Gruppe der Krankheitsvektoren bilden die ektothermen Arthropoden. Diese können ihre Körpertemperatur nicht selbst regeln und werden daher als besonders sensibel gegenüber sich ändernden klimatischen Bedingungen angesehen. Auch die Dauer der Entwicklung des Pathogens im Vektor ist direkt an die Umgebungstemperatur geknüpft. Ausgehend von diesen Überlegungen, sollen in der vorliegenden Doktorarbeit ökologische Kenntnisse in die Risikoanalyse für die Etablierung von Vektoren und die Übertragung von Pathogenen einbezogen werden. Ziel ist es, mögliche neue Hotspots vektor-übertragener Krankheiten in Europa zu identifizieren.

Im ersten Teil der Arbeit (Artikel 1 bis 3) werden die klimatischen Faktoren analysiert, die die Krankheitsvektoren beeinflussen. Dies geschieht durch Literaturanalyse, statistische Verfahren der bioklimatischen Nischenmodelle und Experimenten zu klimarelevanten biologisch-ökologischen Eigenschaften der Vektoren. Von besonderem Interesse ist hier die Asiatische Tigermücke (*Aedes albopictus*), die ursprünglich in den Tropen und Subtropen beheimatet ist und innerhalb von nur 30 Jahren auf allen Kontinenten der Welt mit Ausnahme der Antarktis zu finden ist. Diese Stechmücke ist, neben einer Vielzahl weiterer Humanpathogene, kompetenter Vektor des Dengue- und Chikungunyavirus. Mit Hilfe der bioklimatischen Nischenmodelle werden klimatisch geeignete Gebiete Europas identifiziert. Vergleicht man diese Ergebnisse mit den Ergebnissen weiterer veröffentlichter Risiko-analysen für die Etablierung der Asiatischen Tigermücke in Europa, zeigt sich, dass einige Gebiete über alle Untersuchungen hinweg eine weiter bestehende oder sich neu entwickelte klimatische Eignung aufzeigen, in anderen Gebieten hingegen werden große Unsicherheiten bezüglich der zukünftigen Entwicklung deutlich.

Diese Unsicherheiten in der Risikoanalyse legen nahe, dass bisheriges ökologisches Wissen zu den krankheitsübertragenden Stechmücken gerade im Hinblick auf die Situation in temperaten Gebieten erweitert und vertieft werden muss. In einem zweiten Schritt werden daher Grenzwerte ökologischer Eigenschaften der Art in die korrelative Risikoanalyse integriert. Von speziellem Interesse ist hier die Überlebensfähigkeit im Winter, da die Art im ursprünglichen Verbreitungsgebiet keine Frosttemperaturen ertragen muss. Die Überlebensfähigkeit bei verschiedenen Frosttemperaturen über verschiedene Zeiträume hinweg wird für post-diapause Eier von *Aedes albopictus* europäischer Herkunft und solche, die keine Diapause durchlaufen haben experimentell getestet. Außerdem wird *Aedes aegypti* (Gelbfiebermücke) tropischer Herkunft zum Vergleich in die Experimente eingebunden. Der Schlupferfolg nach der Kältebehandlung ist in den europäischen Mückeneiern, die eine Diapause durchlaufen haben, signifikant erhöht gegenüber denen, die keine durchlaufen haben. Diese Experimente unterstützen die Identifizierung von Risikogebieten, in denen die Überwinterung von *Aedes* Populationen und damit eine langfristige Etablierung möglich wäre, indem die Grenzwerte der Überlebensfähigkeit mit Hilfe zeitlich hochaufgelöster Klimadaten unter Nutzung verschiedener Klimaszenarien europaweit projiziert werden.

Im zweiten Teil der Arbeit (Artikel 4 bis 6) werden klimatische Faktoren bei der Übertragung von Pathogenen näher betrachtet. Am Beispiel von Dengue werden alle in der Literatur beschriebenen Experimente zur Extrinsischen Inkubationsperiode ausgewertet, um die Temperaturabhängigkeit der Virusamplifizierung im Vektor bestimmen zu können. Die Extrinsische Inkubationsperiode ist die Zeit nach einem Blutmahl, die das Virus im Vektor bei einer bestimmten Temperatur für die Amplifizierung benötigt, so dass der Vektor seinerseits wieder einen Wirt infizieren kann. Aus allen verfügbaren publizierten Daten wird ein kontinuierliches Temperatur-Zeit-Profil erstellt. Mithilfe raum-zeitlich sehr hoch aufgelöster Klimadaten können so potentielle Dengue Risikogebiete in Europa aufzeigt werden. In einem zweiten Ansatz werden am Beispiel von Chikungunya die Temperaturverhältnisse während eines Krankheitsausbruchs für die Risikoanalyse genutzt.

Sind die klimatischen Faktoren sowohl für Vektor als auch Pathogen bekannt, können diese für die detaillierte räumliche und zeitliche Projektion von Risikogebieten kombiniert werden. Für Europa wird erwartet, dass Ende dieses Jahrhunderts das Risiko für die Etablierung von *Aedes albopictus* und die damit verbundene Übertragung von Viruserkrankungen zunimmt. Bereits in den nächsten Dekaden werden vor allem West- und Zentraleuropa und die südlichsten Bereiche Großbritanniens zunehmend Gebiete klima-tischer Eignung für den Vektor aufweisen. Auch weiterhin als klimatisch günstig gelten Südfrankreich, große Teile Italiens und die südosteuropäischen Küstenregionen des Mittelmeeres. Zwar unterscheiden sich die Projektionen verschiedener Klimaszenarien im zeitlichen Ablauf der Risiko-einstufung, die räumlichen Muster jedoch bleiben gleich. Das Risiko der Chikungunya-Übertragung steigt gegen Ende des Jahrhunderts an den westlichen Mittelmeerküsten der Balkanstaaten und Griechenlands, außerdem im Pannonischen Becken und an der türkischen Schwarzmeerküste. Eine durchgehend hohe klimatische Eignung für die Übertragung von Chikungunya über das gesamte 21. Jh. hinweg zeigt sich in Norditalien.

Abschließend werden anhand spezieller Beispiele neue Konzepte vorgestellt (Artikel 7), die durch ihre mulitdisziplinäre Vorgehensweise die Risikoanalyse im Bereich der Vektoren und vektor-übertragenen Krankheiten unterstützen können. Es ist jedoch schwierig, das Wissen verschiedener wissenschaftlicher Disziplinen zusammenzuführen, gerade weil Wissenschaftler Teil sehr spezialisierter Netzwerke sind. Um einen ersten Eindruck des aktuellen wissenschaftlichen Austausches zu erhalten, wird das Zitations-verhalten einzelner Wissenschaftsdisziplinen im Bereich vektor-übertragener Krankheiten in Zeiten des Klimawandels ausgewertet.

Die Bedeutung der vorliegenden Arbeit liegt in der Einbindung ökologischen Wissens in die geographisch expliziten und sich zeitlich ändernden Muster bestehender Risiken für die Ansiedlung invasiver Vektoren und das damit verbundene mögliche Auftreten von Infektionskrankheiten im Europa des 21. Jh.. Maßnahmen der Krankheitsüberwachung und der Vektorkontrolle können so zeitlich und räumlich effektiv eingesetzt werden, um mögliche Krankheitsübertragungen zu vermeiden.

2. Introduction

In response to global change, range shifts of organisms are observed (Parmesan 2006). Ectotherm arthropods are considered to respond most to the on-going changes in the environment regarding distribution and abundance changes (Parmesan and Yohe 2003). These species are unable to regulate their body temperature. Thus, they are directly depending on thermal site conditions. Notably this trait – the limited ability to have a bearing on the own body temperature – also influence directly blood-feeding arthropods which transmit pathogens to humans or animals. Some of these arthropods are expanding or expected to expand their range into new areas followed by an emergence or re-emergence of vector-borne diseases in these regions (Gratz 1999, Weaver and Reisen 2010, Ibáñez-Justicia et al. 2014, IPCC 2012).

Climate change is likely to alter the climatic suitability in already existing and newly emerging regions for the individual factors involved in the chain of vector-borne disease infection (Githeko et al. 2000). Climate change will also modify arthropod's ability to transmit pathogens mediated by shifts in life cycles and life histories (Gould and Higgs 2009). However, the arthropod-pathogen-host epidemiological cycle is complex (Tabachnick 2010) and thus the evaluation of potential effects of global change requires an interdisciplinary understanding and approach (Mills et al. 2010). In addition, communities are becoming increasingly vulnerable due to the ongoing loss of drugs and pesticides. This is a result of the selection for resistant strains of pathogens and vectors, which will depend on specie's adaptive capacity (Sutherst 2004). Globalisation has many facets. Most prominent is the high degree of continental interconnectivity that has been reached worldwide. It results in the regular exchange of goods infested with eggs or larvae of arthropod vectors as well as the exchange of infected travellers (Hay et al. 2006, Tatem et al. 2012, Thomas et al. 2014).

Recently the Schmallenberg virus, which is transmitted by *Culicoides spp.*, has spread into Europe and has caused a huge veterinary health impact mostly on cattle and sheep. This is just one example that illustrates that novel diseases may even emerge in Europe. Modelling the spread of the virus after a windborne introduction or movement of infected animals, Bessell et al. (2013) found that the temperature at the time of introduction and the remaining duration of the season of vector activity affected the establishment success and subsequent spreading tendencies. Only ten years ago dirofilariosis, which is transmitted by the heartworms *Dirofilaria repens* and *Dirofilaria immitis*, was endemic to the Mediterranean region, mainly to Italy (Pampiglione et al. 1995). However, *D. repens* is now found in Brandenburg, Germany in three different mosquito species. This is the northernmost location it has ever been found (Czajka et al. 2014).

In this study, new methodological approaches are developed and implemented first to identify current and future areas at risk for the establishment of competent and hitherto alien vector species and second to evaluate the options for pathogens to replicate inside the mosquito and to be transmitted then under conditions of climate change. This is done using the examples of aedine mosquito species that exhibit a high invasive potential and their most relevant pathogens for human health, the dengue and chikungunya virus.

2.1 Ecological and Medical Entomology of the Addressed Mosquito Vectors

2.1.1 *Aedes albopictus* – Asian Tiger Mosquito

Aedes (Stegomyia) albopictus (Skuse, 1984) (*Diptera: Culicidae*) (syn. *Stegomyia albopicta* in Reinert et al. 2004, common name Asian tiger mosquito), originated in subtropical and tropical Southeast Asia. Since the 1980s, *Ae. albopictus* has spread globally and is now established in North- and South America, Africa, Oceania and Europe even in temperate regions (Benedict et al. 2007, additional own compilation see Fig. 2). This broad spectrum of biomes and habitats underpins the fact that *Ae. albopictus* is highly adaptive (Lounibos et al. 2003) and competitive in new environments if it succeeds to overcome biogeographical barriers (Juliano 1998). The rapid spread of this species was and is still facilitated by global transport of goods. The drought resistant eggs are mainly found in deposited used tires coming from *Aedes* endemic countries (Lounibos 2002, Tatem et al. 2006). Rainwater gathers there, warms up quickly due to the dark colour of the tires and eggs hatch under ideal conditions. In addition, larvae can survive long journeys with oceanic vessels, too. They are shipped in small water bodies used for the transport of plants such as Lucky Bamboo (*Dracaena sanderiana*) (Scholte et al. 2007).

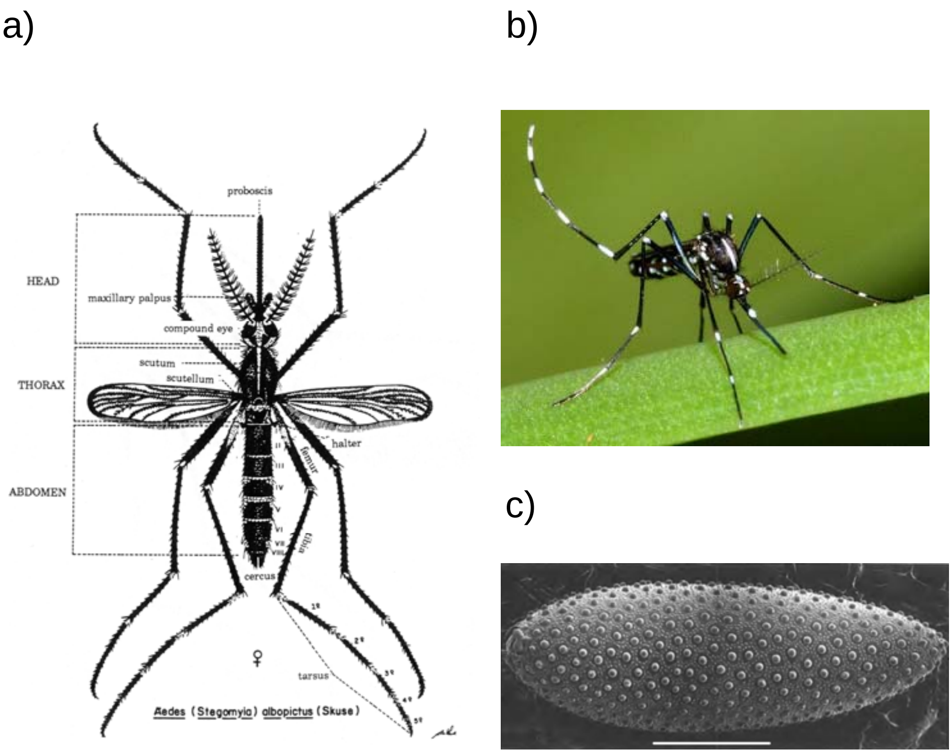

Fig. 1

a) Morphology of a female *Aedes albopictus* (Arthur Botelho de Barros, Superintendencia da Campanhas de Saúde Pública, Brasilien ex Estrada-Franco and Craig 1995).

b) Adult *Aedes albopictus* (Susan Ellis Courtesy, InsectImages.org (#1366026)).

c) Egg of *Aedes albopictus*, Electron micrograph scale = 100 µm (Dr. John Linley, Florida Medical Entomology Laboratory, University of Florida at Vero Beach ex Estrada-Franco and Craig 1995).

Ae. albopictus is a highly competent disease vector (see below for more details). The vector mosquito is regarded as a very aggressive daytime biter with peak feeding times during the early morning and late afternoon. This mosquito has a rapid bite and bites any exposed human skin surface (CDC 2012). Only the females are able to transmit the pathogens to another host, as only females take bloodmeals for the mature of the eggs. Mosquitoes' sex is easily discernable by the fine hairs at mosquitoes´ antennae, the antennal flagellum. The flagellum of male mosquitoes is much denser than that of female mosquitoes.

Ae. albopictus is clearly distinguishable from other aedine species because of the striking black and white pattern of the mosquito with a median silver-scale line at the dorsal side of the thorax (Fig. 1). The thin basal pale bands of the abdomen are laterally enlarged. Males have a more hairy and thinner abdomen than females. The last segment of the third leg is entirely white and thereby differs from *Ae. japonicus, Ae. triseratus* and *Ae. kroeicus,* but not from *Ae. atropalpus* and *Ae. aegypti* (ECDC 2012).

European Distribution

Ae. albopictus was first recorded in Europe in 1979 in Albania (Adhami and Reiter 1998): A second introduction appeared in 1990 in Genoa (Sabatini et al. 1990) followed by the subsequent spread all over Italy. Today, *Ae. albopictus* is widespread throughout most parts of the Mediterranean (Fig. 2): Spain, Mallorca, France, Corsica, Italy, Sardinia, Malta, Croatia, Slovenia, Bosnia, Greece, Turkey, Syria, Lebanon and Israel (Sabatini et al. 1990, Romi et al. 1999, Müller et al. 2012, Samanidou-Voyadjoglou et al. 2005, Aranda et al. 2006, Cristo et al. 2006, Klobučar et al. 2006, Haddad et al. 2007, Delaunay et al. 2009, ECDC 2009, Gatt et al. 2009, Moutailler et al. 2009, Kalan et al. 2011, Miquel et al. 2013, Oter et al. 2013). Besides the fact that the species reaches high population density connected with nuisance, this has raised serious health concerns due to the competence of the species for transmitting VBDs.

In addition, *Ae. albopictus* was collected for the first time in the in the Bolshoi Sochi region in the Russian Federation (Eastern Black Sea region) (Ganushkina et al. 2012). The detection of the mosquito from Bulgaria was reported at a scientific meeting in 2011 (Ognyan Mikov), but remains unpublished. Cursory records of new establishments are common. Coordinated monitoring of the spread of the species at the European scale is still missing. An initial basis for the documentation of mosquito’s establishment is provided by ECDC´s mosquito maps compiled and developed by the “Vbornet” Initiative (European Network for Arthropod Vector Surveillance for Human Public Health). However, the use of these maps is restricted by the fact that the spatial occurrence data of the vector mosquito is based on ‘regional’ administrative level of the EU (NUTS3 regions) rather than point data.

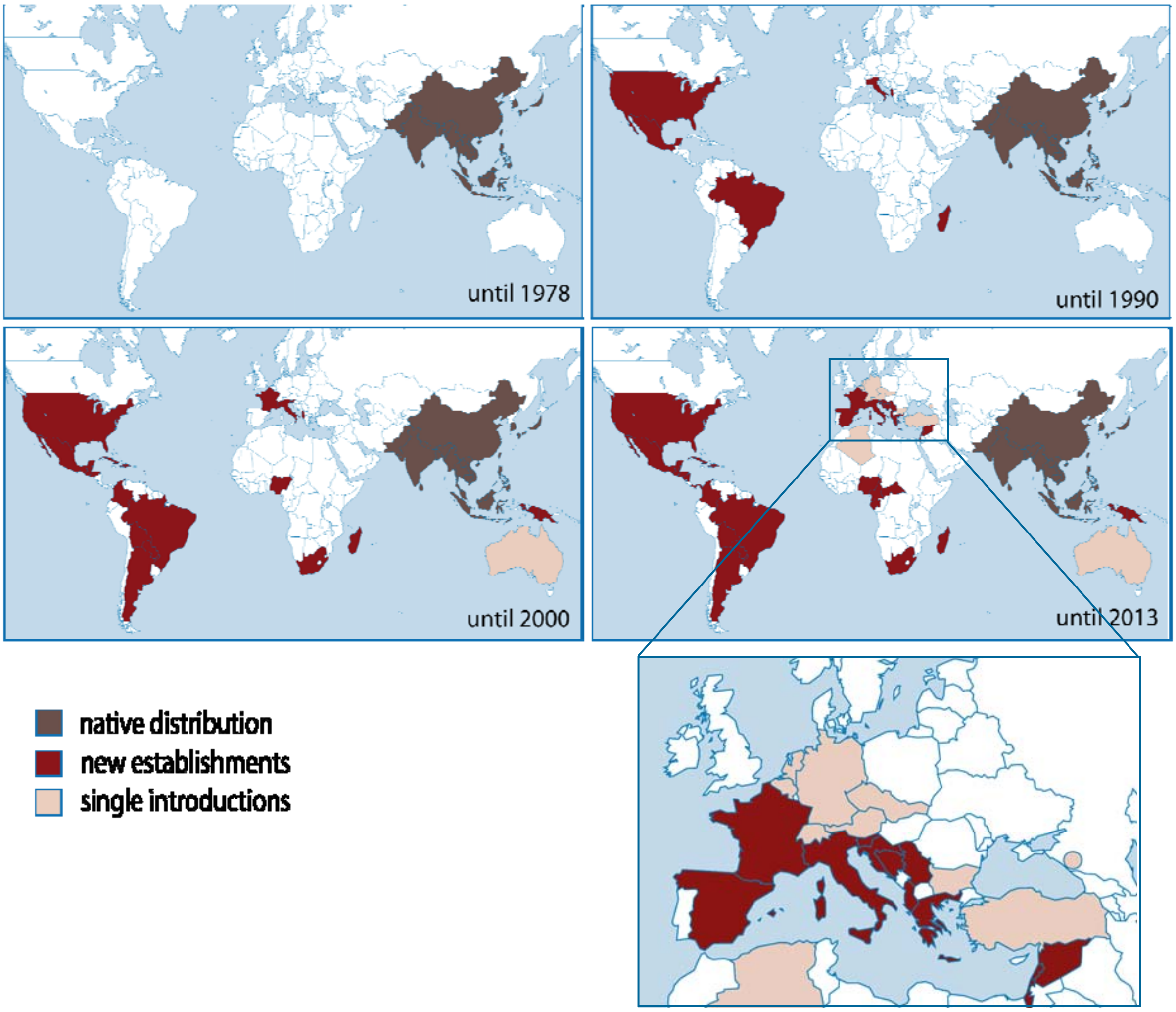

Fig. 2: Worldwide distribution of *Ae. albopictus* for the years 1978, 1990, 2000 and 2013. The native range is located in Southeast Asia. During the last 35 years new establishments of the vector mosquito are present at nearly all inhabited continents of the world. Only Australia avoided an establishment of the species as a result of intensive biocontrol measures at the ports of entry.

It is just due to the establishment of nationwide mosquito monitoring programs in Germany that adult *Ae. albopictus* have been recorded at the German-Austrian (motorway A5 near Rosenheim) and German-Switzerland border region (rest area on the A5 motorway entering Germany from Switzerland near Weil am Rhein and truck-railway transhipment station near Freiburg) (Werner et al. 2012, Becker et al. 2013, Kampen et al. 2013). Larvae have also been detected at the Czech-Austrian border region (motorway E461, near Mikulov)) in 2011 and 2012 and in Austria in the Inn Valley (Šebesta al. 2012, Seidel et. al. 2012). However, so far there is no evidence of an established population of the mosquito

North of the Alps, but it can be assumed that there is a continuous introduction from Southern Europe especially by trucks.

Vector Competence

The spread of the Asian tiger mosquito raises public health concerns as it is a competent vector for a large number of human and veterinary pathogens (Gratz 2004). A distinction is made between its (1) proven vector competence in situ, (2) *Ae. albopictus* mosquitoes found infected in situ with proven laboratory vector competence, and (3) vector competence has only been shown in laboratory experiments (Medlock et al. 2012).

Ae. albopictus is a proven vector in situ (i.e. under natural conditions) for dengue and chikungunya virus (Paupy et al. 2010) and also for the two heartworms *D. repens* and *D. immitis* (Cancrini et al. 2003, Calzolari et al. 2013). In addition, *Ae. albopictus* is found infected in situ with the alphaviruses Eastern equine encephalitis virus, Venezuelan equine encephalitis virus, La Crosse virus, Usutu virus, Japanese encephalitis virus, West Nile virus and the orthobunyaviruses Cache Valley virus, Potosi virus, Tensaw virus, Keystone virus, Jamestown Canyon virus (reviewed in Gratz 2004 and Medlock et al. 2012, Calzolari et al. 2013). Laboratory vector competence of *Ae. albopictus* has been shown for Ross-River virus and Yellow fever virus (Mitchell and Gubler 1987, Gratz 2004). First cases of autochthonous dengue were described in Southern France (La Ruche et al. 2010) and Croatia (Schmidt-Chanasit et al. 2010; Gjenero-Margan et al. 2011). Autochthonous transmission of chikungunya is proven for Southern France (Grandadam et al. 2011).

Habitat Requirements

In its native habitat, *Ae. albopictus* is a tree-hole breeder. However, in urban and rural environments it has a wide range of small artificial breeding sites (Hawley 1988, Knudsen 1995). Key breeding sites are bamboo stumps, plastic drums, and rubber tires (Stein et al. 2002). The mosquito's aquatic development from egg to larvae (first to fourth instar), pupae and adult stage is temperature and food dependent (Hien 1975) and takes from ten days at 25 °C to 25 days at 18 °C, whereby starvation can extend the larval development to an average of 42 days (reviewed in Estrado-Franco and Craig 1995).

2.1.2 *Aedes aegypti* - Yellow Fever Mosquito

The Yellow fever mosquito *Aedes aegypti* (Linaeus, 1762) (*Diptera: Culicidae*) (*Ae. aegypti*, synonym *Stegomyia aegypti* in Reinert et al. 2004) was considered in the experimental part of this study in addition to the main species *Ae. albopictus*. This closely related species which shows also invasive behaviour is also an important disease vector. The mosquito prefers to bite indoors several hours after sunrise and before sunset. The females take its bloodmeals mainly from humans and domestic animals (CDC 2012)

Ae. aegypti is clearly distinguishable from *Ae. albopictus* due to its silver scales in a shape of a lyre at the dorsal side (Fig. 3). The thin basal pale bands of the abdomen are laterally enlarged in a shape of a half-moon. As with *Ae. albopictus*, the last segment of the third leg of *Ae. aegypti* is entirely white (ECDC 2012).

European Distribution

The mosquito, originally native to Africa, is currently established in tropical and subtropical regions of America, Middle East, Asia, the Pacific Ocean islands, Indian Ocean islands, and Northern Australia (Fig. 4, Almeida et al. 2007, Fontenille et al. 2007, Gubler 1998, Hawaii Conservation Alliance 2005).

Although *Ae. aegypti* was abundant in the Mediterranean until the 1940s due to historical introductions in harbours, its current European distribution is limited to Madeira (Portugal) and the Eastern part of the Black Sea region (South Russia, Abkhazia and Georgia) (Almeida et al. 2007, Yunicheva et al. 2008, Medlock et al. 2012). It is assumed that the decline of the Mediterranean distribution in the middle of the last century was caused on the one hand by the intensive use of the pesticide DDT due to malaria vector control indoor treatments, on the other hand by the improvement of the urban water collections (Toma et al. 2011). Similar to the Asian tiger mosquito (*Ae. albopictus*), the Yellow fever mosquito (*Ae. aegypti*) is continually introduced by global shipping of goods and has been found at the

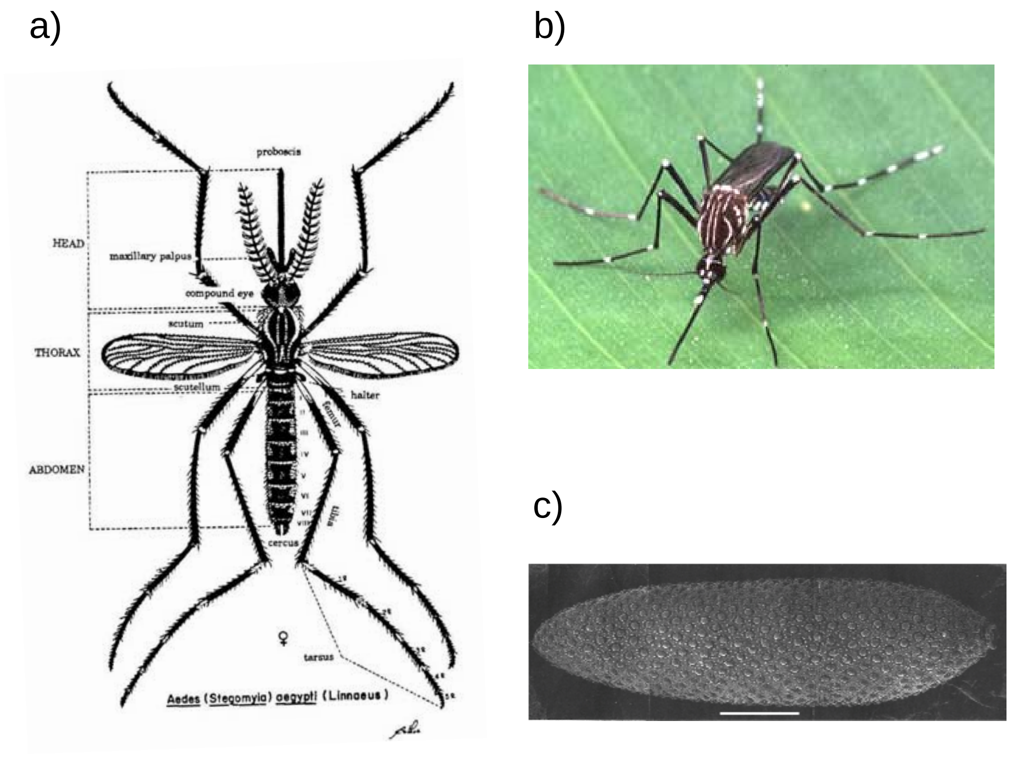

Fig. 3:

a) Morphology of a female *Aedes aegypti*, the flagellum of the female mosquitoes is less dense than that of male mosquitoes. (Arthur Botelho de Barros, Superintendencia da Campanhas de Saúde Pública, Brasilien ex Estrada-Franco and Craig 1995).

b) Adult *Aedes aegypti* (Paul Zborowski, www.co.galveston.tx.us/Mosquito_Control/ aedes_aegypti %20narr.htm).

c) Egg of *Aedes aegypti*, Electron micrograph scale = 100 µm (Dr. John Linley, Florida Medical Entomology Laboratory, University of Florida at Vero Beach ex Estrada-Franco and Craig 1995).

harbour of Rotterdam, Netherlands, but was immediately controlled to inhibit establishment (Scholte et al. 2010). Although cold winter temperatures seem to prevent large parts of Europe from a year-round establishment of the mosquito (Hanson and Craig 1995), it has been recorded in the Eastern United States as far North as New York State (Darsie and Ward 2005). Winter survival of *Ae. aegypti* at sheltered sites, which provides protection against environmental conditions, is therefore suggested (Toma et al. 2011, Medlock et al. 2012).

Fig. 4: Worldwide distribution of *Aedes aegypti*. Originally native in Africa, the mosquito is spread mainly by human trade and travel, already since back the times of slavery. A strong decline in population densities of *Ae. aegypti* has been observed at sites where *Ae. albopictus* managed to become established.

Vector Competence

As the common name implies, the Yellow fever mosquito is a highly competent vector for the Yellow Fever Virus. In West-, Central-, and East-Africa Yellow Fever epidemics result in tens of thousands deaths (Medlock et al. 2012). Also, Yellow Fever is a major public health concern in South America. A large outbreak in Europe was historically reported in Barcelona in 1821, one- sixth of the in habitants died (Chastel 1999).

Most importantly, *Ae. aegypti* is the primary dengue vector in the Americas, South East Asia, and the West Pacific (Jansen and Beebe 2010). *Ae. aegypti* was also the responsible vector for the large-scale dengue epidemic in Greece 1927 to 1928 (Rosen 1986). Starting in autumn 2012, Madeira experienced an epidemic dengue outbreak (Sousa et al. 2012), with 2187 people infected until September 2013 (Governos de Portugal – Direçao Geral da Saúde 2013). In contrast, the unexpected autochthonous dengue cases in France (La Ruche et al. 2010) and Croatia (Schmidt-Chanasit et al. 2010) were attributed to the transmission via *Ae. albopictus*. The Yellow Fever Mosquito (*Ae. aegypti)* also transmits chikungunya virus. Severe outbreak events of chikungunya transmitted by *Ae. aegypti* and

Ae. albopictus were observed in Kenya in 2004; Comores, Mayotte, Seychelles, La Réunion, and Mauritius in 2005; in Madagascar in 2006 (Gould and Higgs 2009).

In addition to Yellow fever, dengue and chikungunya, *Ae. aegypti* is a laboratory vector for Chandipura virus, Murray Valley encephalitis virus, Ross River virus and Zika virus (Kay et al. 1979, Mavale et al. 2005, Schaffner et al. 2013). Filarial infections of Wuchereria bancrofti and Diroilaria immitis have also been found in *Ae. aegypti* (Russell et al. 2005).

Habitat Requirements

Ae. aegypti is adaptive to human environments and breeds in diverse water retaining containers. Its key breeding sites close to humans are plastic and metal containers (Stein et al. 2002). Development, growth, and survival of the mosquito are temperature dependent. Rueda et al. (1990) found body size respond directly to temperature for different constant temperatures (15 to 34 °C). Body size generally decreased as temperature increased. In addition, survival to adult stage was high at 20 °C (92 %) and 27 °C (90 %) and collapsed remarkably at 15 °C (3 %). Mohammed and Chadee (2011) found that egg hatching success declined rapidly from 98 % at 24–25 °C to 1.6 % at 34–35 °C. A significant difference in the male to female ratio is found under diurnal temperature regimens ranging from 24 °C to 35 °C with significantly more females emerging at higher temperatures (Mohammed and Chadee 2011). In contrast to *Ae. albopictus*, the survival of eggs is limited in temperate regions as the *Ae. aegypti* is not able to produce diapausing eggs.

2.2 Epidemiological and Virological Description of the Addressed Viruses

2.2.1 Dengue

Dengue is a mosquito-borne Flavivirus (family Flaviviridae). Four closely related serotypes of the virus with specific pathogenity occur (DENV-1 to DENV-4). Whereas until the 1970s all four serotypes only circulated in Southeast Asia and DENV-1 and DENV-2 in West Africa and Central America, nowadays all four serotypes can be found in almost all tropical and subtropical areas of the world (Guzman et al. 2010). This has been considered particularly important, because a secondary infection with another serotype leads to a significantly higher risk for developing dengue hemorrhagic fever (DHF). Children are mostly affected by this severe course of disease (Halstead 2008). Clinical features of DHF are high fever, often with liver enlargement, and in severe cases accompanied by circulatory failure. Further detailed medical information about virologic features, immunopathogenesis, differential diagnosis, disease classification, and leading dengue vaccine candidates are currently reviewed in Simmons et al. (2012). The incubation period is 3 to 14 day, with the majority of cases taking 4 to 7 days (RKI 2011). There are currently no licensed vaccines or antiviral therapies available (Vasilakis et al. 2011) and about 2.5 % of hospitalised patients with severe dengue die (WHO 2012). Considering these facts, the potential spread of this disease supported by climate change and globalisation must be seen as a big societal challenge.

The transmission of dengue by mosquitoes was first discovered by Graham in 1902 (Peters 2010). Today, two transmission cycles of dengue are differentiated. The sylvatic cycle involves non-human primates as the reservoirs and the transmission occurs by different arboreal aedine mosquito vectors. In the human cycle, highly anthropophilic vectors, mostly *Ae. aegypti* and *Ae. albopictus*, transmit the virus only between humans (Vasilakis et al. 2011). In zones of emergence—particularly in rural areas of West Africa and Southeast Asia—these two transmission cycles are linked (Vasilakis et al. 2011). Female mosquitoes remain infective during their entire life (Watts et al. 1987). During interepidemic periods the dengue virus can maintain via vertical transmission (transovarial transmission), this means the virus is transferred from mosquitoes to their eggs and persists in mosquito populations. Rosen (1983) first showed vertical transmission of dengue virus in a laboratory experiment in 1983.

Currently, natural evidence of vertical transmission of dengue is observed in populations of *Ae. aegypti* and *Ae. albopictus* collected in Fortaleza, Brazil (Martins et al. 2012). Globally, in total 14 *Aedes* species transmit dengue: Besides the two main vector species (*Ae. aegypti* and *Ae. albopictus*), *Ae. luteocephalus*, *Ae. opok*, *Ae. africanus*, *Ae. luteus*, *Ae. furcifer* and *Ae. taylori* are vectors in West Africa; *Ae. mediovittatus* in Purerto Rico and the Caribbean; *Ae. niveus* in Southeast Asia; *Ae. polynesiensis* in French Polynesia, Samoa, Cook-Islands and Society islands; *Ae. scutellaris* in New Guinea; *Ae. cooki* in Niue and *Ae. hebrideus* in Vanuatu (Dobler and Aspöck 2010).

The geographical range of areas where dengue epidemics occur has expanded dramatically during the last decades. From the 1960s to 2007 the number of countries that experienced dengue epidemics has increased sevenfold, from 9 to 65 countries respectively (WHO 2013). The global burden of dengue has been evaluated by niche mapping approaches with nearly 400 million dengue infections per year (Bhatt et al. 2013). Hence, the total number of dengue infections is more than three times the current burden estimate of the World Health Organization (WHO 2009).

Up to now, Europeans tend to exclusively consider dengue as a travel-related disease. The most important travel destination where Europeans become infected is Southeast Asia, especially Thailand, followed by Latin America, the Indian subcontinent, the Caribbean and Africa (Heddini et al. 2009, Jelinek 2009). However, first European cases of autochthonous dengue were reported in 2010 (La Ruche et al. 2010, Schmidt-Chanasit et al. 2010, Gjenero-Margan et al. 2011) indicating the re-appearance of dengue in the Mediterranean after its epidemic outbreak in Greece in the 1920ies with more than 90 % of the metropolitan population affected in Athens and more than 1.500 people died (Louis 2012). Also New York reported the 1st locally acquired dengue infection in 2013 (ProMED-mail 2013) and in the same year the 1st laboratory confirmed case imported from Japan to Germany was described (ProMED-mail 2014).

Madeira experienced an epidemic of dengue with about 2.000 cases (Sousa et al. 2012) followed by about 80 cases introduced into 13 other European countries via travellers departing Madeira (Wilder-Smith et al. 2014). *Ae. albopictus* is the suspected vector species of the hitherto only few Mediterranean dengue cases. However, in Madeira *Ae. aegypti* is found to be the transmitting mosquito (Gould et al. 2010, Sousa et al. 2012). *Ae. albopictus* does not occur on this island. However, the latest transmission studies for European aedine populations prove that *Ae. albopictus* from Southern France exhibits unexpectededly high transmission efficiency for dengue (Vega-Ruga et al. 2013).

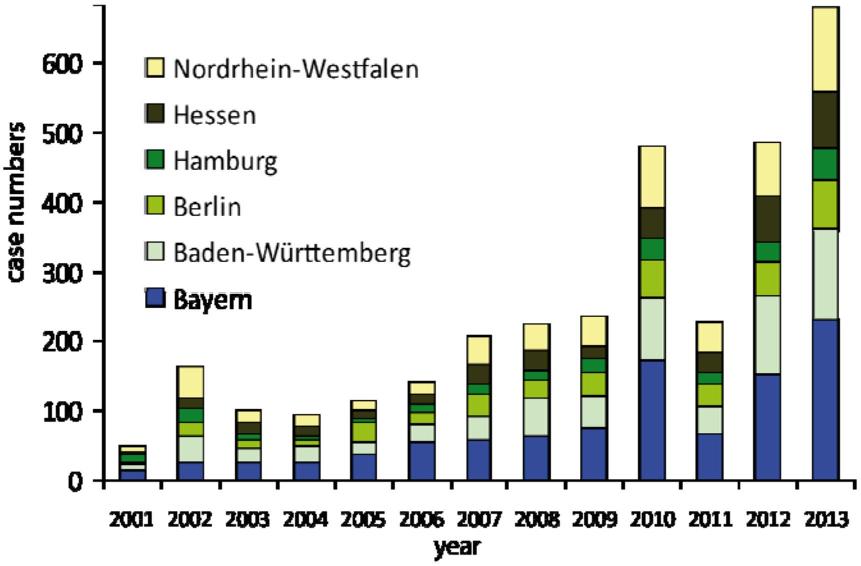

Fig. 5: Number of travel-related dengue cases in the six most affected German federal states from 2001 to 2013. Bavaria reported the highest number of cases followed by Baden-Wuerttemberg and North Rhine-Westphalia. In relation to the population, also the case numbers in the two city states Berlin and Hamburg are high. (Robert Koch-Institut: SurvStat, www3.rki.de/SurvStat, data: 14/05/2014).

Dengue is a notifiable infectious disease in Germany since 2001. Hemorrhagic and non-hemorrhagic cases shall be notified, but it is assumed that the case numbers of travel-related dengue infections (more than 3.500 cases since 2001) are underestimated due to lack of diagnosis and reduced reporting activities. Bavaria, Baden-Wuerttemberg and North Rhine-Westphalia are the most affected German states (Fig. 5, Robert Koch-Institut: SurvStat, www3.rki.de/SurvStat, 14/05/2014). Due to the large number infected tourists who came back from Madeira in winter 2012/13, about 200 travel-related dengue cases have been diagnosed form January to April of 2013. This is as much as otherwise documented in one year.

An autochthonous dengue infection in Europe is more likely to occur, when high population densities of vector mosquitoes in periods that are favourable for a short Extrinsic

Incubation Period (EIP) in summer (May to October) face a high number of infected travellers returning from vacation. The temporal variation of travel-related dengue cases in Germany is therefore interesting (Fig. 6). The cases are more or less evenly distributed over the year, but a slight increase in April and September becomes apparent. A complex interplay between the frequency of outbound travel, travel destinations and season of dengue epidemics determines the temporal characteristics of travel-related dengue cases. Overall, the tourism sector in Asia-Pacific and South America, dengue endemic areas, records last year a growth of 7 % and 12 %, respectively (ITB 2013). Dengue epidemics occur usually in Australia from November to March, in Brazil from January to May, in Vietnam, Philippines, Honduras, and Mexico from June to September, whereas in Malaysia and Singapore the case numbers remain on a relatively constant level (WPRO 2013, San Martin et al. 2010).

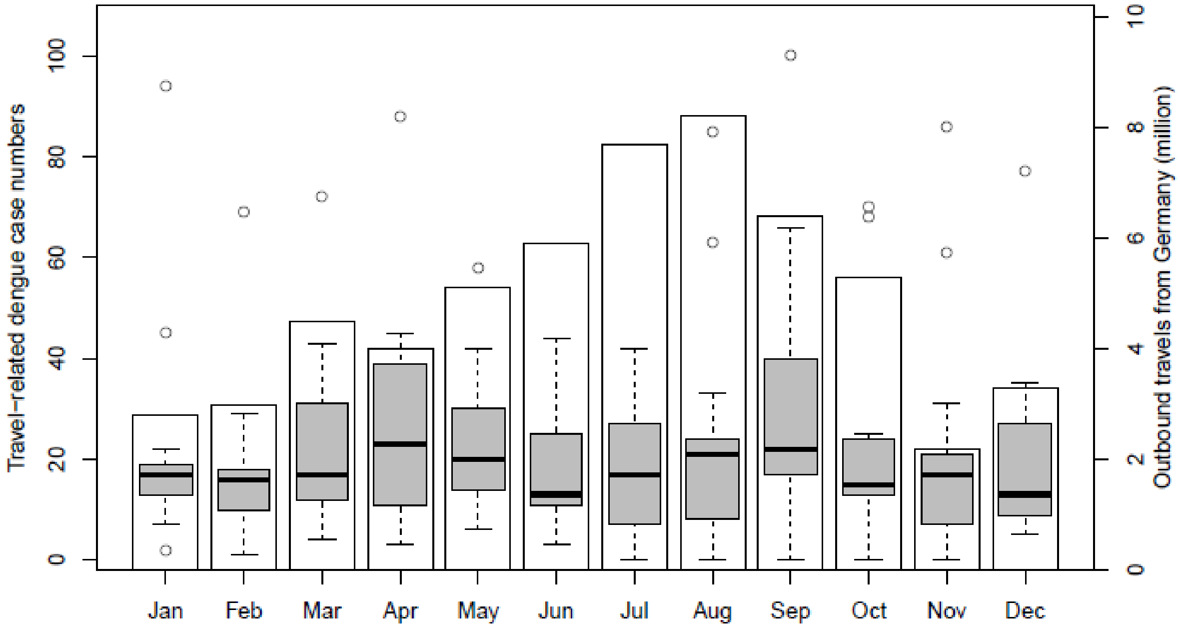

Fig. 6: Monthly number of dengue cases (grey) in Germany from 2001 to 2012 from returning travellers (Robert Koch-Institut: SurvStat, www3.rki.de/SurvStat, data: 26/04/2013) and outbond travels from Germany (white), avaraged for the period from 2008 to 2010 (Statistisches Bundesamt 2010 and 2011). Travel associated dengue cases remain at a relatively constant level during the year, despite a sharp peak of outbond travel in July and August.

2.2.2 Chikungunya

The arthritogenic, mosquito-borne chikungunya virus is an alphavirus (family Togaviridae). This group of viruses cause a wide range of diseases in humans and animals: Arthralgia is also caused by Ross River (Australia, Papua New Guinea and other South Pacific islands), Barmah Forest, Mayaro (tropical South America), o'nyong-nyong (Africa), and Sindbis viruses (Australia, South Africa, Europe, Middle East). Eastern equine encephalitis virus, Western equine encephalitis virus, and Venezuelean encephalitis virus, also alphaviruses, cause encephalitis in the Americas.

Chikungunya was first described in Tanzania in 1953 (Robinson 1955). Besides humans there are rare cases where vertebrates such as rodents or primates could serve as reservoir hosts (RKI 2011). The disease is characterised by a rapid rise in fever, headache, conjunctivitis and muscle aches. 5–10 % of infected patients have joint complaints for months, which rarely persists (RKI 2011). However, a closer look at India, the main affected country, shows that 69 % of the national burden of chikungunya measured in disability-adjusted life years were attributed to persistent arthralgia (Krishnamoorthy et al. 2009, data from 2006). During the huge epidemics in India and the Indian Ocean Islands from 2005 to 2007 more than 2 million people were infected, of which alone India reported 1,5 million cases (Burt et al. 2012). The incubation period of this pathogen is usually 7 to 9 days, sometimes between 3 and 12 days (RKI 2011). Recently, for the first time chikungunya virus-like particles where produced in insect cells, which offers a basis for an effective vaccine against chikungunya virus infections in the future (Metz et al. 2013). However, still there is neither an antiviral therapy, nor vaccines available.

Two transmission cycles were described from times when chikungunya was less common in the tropics (about 1953 and 2000) (Simon et al. 2011). The classical African cycle in rural areas shows small epidemics associated with the forest-dwelling aedine mosquitoes, mostely *Ae. furcifer* and *Ae. taylori*. In addition to humans, wild primate hosts serve as reservoirs. The second cycle, the classical Asian profile, involved anthropophilic mosquitoes (mainly *Ae. aegypti*) that caused outbreaks in urban centres. A third transmission cycle occurred during the large outbreak in La Réunion where a newly mutated chikungunya strain was transmitted by peridomestic *Ae. albopictus*, which was not known to be an efficient vector before (Simon et al. 2011). An exchange of a single amino acid has allowed *Ae. albopictus* to become the main vector mosquito, which than caused the epidemic

(Schuffenecker et al. 2006, Tsetsarkin et al. 2007). This chikungunya virus mutation increase midgut infectivity, dissemination to the salivary glands, and transmission to a vertebrate species in *Ae. albopictus* compared to *Ae. aegypti* (Tsetsarkin et al. 2007). *Culex* species cannot become infected. *Anopheles* species are found with high viral load, but these mosquitoes are unable to transmit the virus (Dobler and Aspöck 2010).

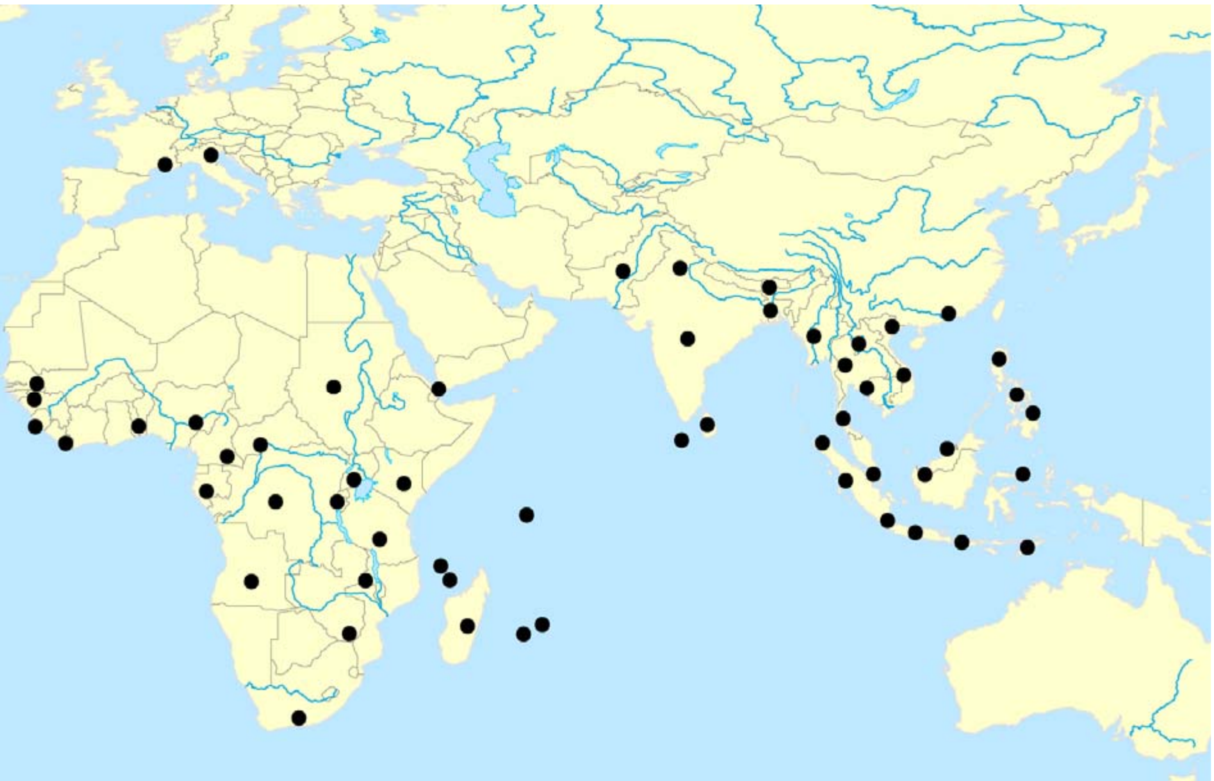

Fig. 7: Worldwide distribution of chikungunya (after Burt et al. 2012 and own data compilation out of Pubmed, Promedmail and Web of Knowledge).

Large outbreaks of the disease were rarely seen before 2000. However, since this time an increase in frequency of epidemics has become evident. After decades of apparent absence, chikungunya is now found in Africa, the Indian Ocean Islands (particular La Réunion), India, and Southeast Asia (Renault et al. 2007, Burt et al. 2012, Fig. 7). During the outbreak in La Réunion (February 2005 to June 2006) more than 266.000 people, a third of the island population, became infected (Tsetsarkin et al. 2007). This huge amount of affected people led to a temporary breakdown of social life on the island. Being part of France and highly developed, La Réunion shows that the appearance of this disease is not a matter of developing countries but will be difficult to avoid if climatic conditions are supporting an outbreak.

In an European context, it is important to notice that under laboratory conditions temperate *Ae. albopictus* from Southern France and Corsica are able to transmit the chikungunya virus (Vazeille et al. 2008, Moutailler et al. 2009). A higher proportion of *Ae. aegypti* individuals from India is able to transmit the virus in comparison to *Ae. albopictus* from South France, but this European *Ae. albopictus* strain shows a higher number of viruses in saliva (Vega-Rua et al. 2013), resulting in a higher likelihood of transmission per mosquito bite. A key finding is the unexpected high vector competence of *Ae. albopictus* under optimal conditions compared to the typical vector *Ae. aegypti* (Vega-Rua et al. 2013). The EIP of chikungunya is unusually short, it only takes two days at 28 °C from digesting an infectious bloodmeal until the female mosquito itself becomes infective (Moutailler et al. 2009).

Astonishingly, also the temperature experienced at the larval stage can influence the vector competence for arboviruses in adult mosquitoes. The cooler the temperatures in the larval habitat the larger the mosquito's body size and the more likely females are infected with chikungunya (Westbrook et al. 2010). This may have a positive effect on chikungunya virus infection rates in temperate regions.

The first epidemic of chikungunya fever in Europe occurred in Ravenna, Northern Italy, with more than 200 affected humans after virus introduction from India (Rezza et al. 2007). Another two autochthonous cases were reported from Provence-Alpes-Côte d'Azur, Southeastern France, where two children without travel history became infected originating from a travel-related case coming from an outbreak area in India (Grandadam et al. 2011). The suspected vector mosquito was *Ae. albopictus* in Italy as well as in France.

In Germany, the number of travel-related chikungunya cases is reported since 2006 and is noticeably lower than the number of dengue cases (Fig. 8). Bavaria, Baden-Wuerttemberg and North Rhine-Westphalia are the most affected German States with 44, 43 and 39 cases between 2006 and 2013, respectively (RKI survstat 14/05/2014).

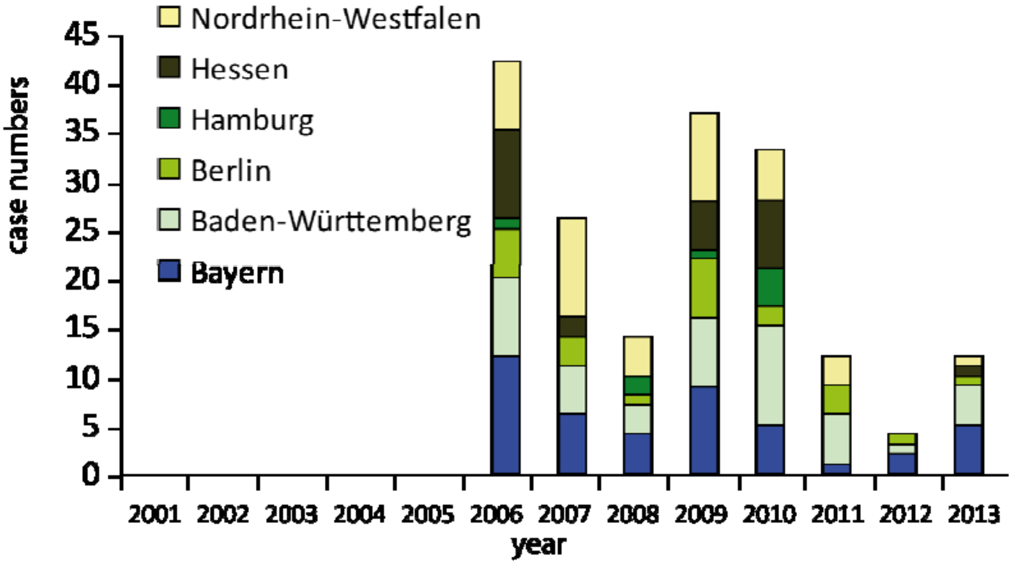

Fig. 8: Number of travel-related chikungunya cases in Germany from 2006 to 2013 (Robert Koch-Institut: SurvStat, www3.rki.de/SurvStat, data: 14/05/2014).

2.3 Climate Change Impacts on Vector Mosquitoes and their Pathogens

Climate change affects human and animal health in various ways. This has been addressed from different viewpoints and scientific disciplines such as human and veterinary medicine, ecology, and entomology (Harvell 2002, Haines et al. 2006, Ebi et al. 2007, Laaksonen et al. 2010). A range of vector-borne diseases has been linked to climate, including dengue, malaria, Hantavirus, Bluetongue and Ross River Virus (Patz et al. 2005) and have been projected to increase in geographic (IPCC 2012).

2.3.1 Climate Change in Europe

Both, regional climate projections based on global and regional climate models project strong warming **trends** for for Europe with a pronounced effect in winter in Northern Europe and in summer in Southern Europe. Less precipitation is expected in the South, while an increase in precipitation is projected in the North during winter (Meehl et al. 2007, Christensen and Christensen 2007, Kjellström et al. 2011, Déqué et al. 2012). Significant changes of seasonal temperatures in regional-scale climate signals are projected to emerge around 2020, whereas signals in precipitation changes are much more variable until the end of the century (Kjellström et al. 2013).

The frequency of **heat waves** has likely increased in Europe since the 1950s within long historical temperature series (IPCC 2013a). Also during the so-called global warming hiatus, the identified ‘pause’ in the increase of global mean temperature, observational data reveal no pause in the evolution of hot extremes over land since 1997 (Seneviratne et al. 2014). Due to poor quality and/or consistency of data heatwave trends may have been underestimated over Western Europe and the Mediterranean (Della-Marta et al. 2007a, Kuglitsch et al. 2009). There is evidence that the duration of extreme summer heat waves in Southeastern Europe during the latter part of the 20th century was extended by soil moisture-temperature feedbacks (Hirschi et al. 2011)

Models project that during the next three decades the intensity of **hot extremes** increase significantly in about half of the land fraction (Fischer et al. 2013). Furthermore the frequency of warm days and warm nights will likely increase in most regions (IPCC 2013a). By the end of this century an in-20 year maximum temperature event will likely be seen in ten years again, or become an annual or a one -in -two year event. High-percentile summer temperatures are projected to warm faster than mean temperatures (IPCC 2013a).

Also the frequency or intensity of **heavy precipitation** shows likely increases in more European regions than decreases (IPCC 2013a). But regional and seasonal variations are apparently. While decreasing trends are found in winter, increase is found in Northern Italy, Poland and some Mediterranean coastal sites (Pavan et al. 2008, Lupikasza 2011, Toreti et al. 2010). Depending on the region and season the reduction in return periods of heavy precipitation events ranges between 2% and 58% (Van den Besselaar et al. 2012). In Central Europe, the likelihood of extreme precipitation events was found to be increased between 1901 with about 1% and in 2003 about 25% (Trömel and Schönwiese 2007). Variable yearly precipitation trends are observed in the last half of the last century in the Mediterranean; while it is negative in the East, it is non-significant in the West *(*Norrant and Douguédroit 2006*)*. Decreasing relative humidity In Southern Europe is thought to lead to significant decreases in precipitation occurrence (Kendon et al. 2010).

Also projections of **drought** at a regional to global – scale remain relatively uncertain compared to other aspects of the water cycle. An improved models´ ability to predict topical sea surface temperature would support future drought projections (Dai 2010). Under the RCP8.5 scenario soil moisture drying is most prominent in the Mediterranean. Thereby precipitation deficits and / or high evapotranspiration can led to dry soil-moisture conditions (Della-Marta et al. 2007b, Vautard et al. 2007, Black and Sutton 2007, Fischer et al. 2007, Seneviratne et al. 2010). Increased drought severity is projected for Southern Europe, defined as the fraction of land under drought conditions (Prudhomme et al. 2014, global multimodel experiment).

Winter conditions in Europe are subject to rapid climate change; here low temperature quantiles rise more clearly than the global average (Orlowsky and Seneviratne 2012). The duration of cold spells is significantly reduced since the 1950s (Donat et al. 2013) and is expected to continue to decrease (IPCC 2013a). However, the global analyses of climate models suggest cold extremes may not be less severe or shorter under future climatic conditions (Kodra et al. 2011, A1B scenario for 2091–2100 period versus 1991–2000). The ecological effects of winter climate change in temperate zones are generally underrepresented

(Kreyling 2010); this also applies for questions regarding the spread of vector-borne diseases.

Various mosquito-borne diseases are considered to be sensitive to changing climatic conditions due to the fact that temperature and precipitation regulate the lifecycle of ectothermic vectors (e.g. Alto and Juliano 2001, Kilpatrick et al. 2008) as well as the development and transmission of their pathogens (e.g. Watts 1987, Dohm et al. 2002, Lambrechts et al. 2011, Carrington et al. 2013).

2.3.2 Temperature and Precipitation Effects on Vector Mosquitoes

Arthropod vectors are often considered as invasive species (e.g. *Aedes albopictus*, *Aedes aegypti, Aedes japonicus* ex ISSG (2005)). The interplay of global warming and biological invasions generally influences biodiversity and ecosystem services (Walther et al. 2009), particularly the emergence and spread of vector-borne diseases. New opportunities for the introduction of invasive arthropods can be offered, colonization facilitated, reproduction and population persistence enabled, and spread enhanced under conditions of climate change (Walther et al. 2009). Also, temperature influences the gonotrophic cycle and therewith the offspring and abundance of mosquitoes.

In Israel, Paz and Albersheim (2008) found that extreme heat in the early spring has influenced *Culex pipiens'* population to increase, resulting in increased frequency of West Nile fever cases a few weeks later. Also seasonal flight activity is directly dependent on environmental temperature. The peak in flight activity of *Culex erraticus*, a suspected bridge vector of Eastern equine encephalitis virus, varied from day to day, and was largely a function of temperature (Gray et al. 2011). Likewise, cold temperatures determine the survival rates of mosquito eggs or adults. Egg number and hatching rate of *Ae. aegypti* were drastically reduced after an El Niño event in Argentina 1998 with 1-2,5 °C lower monthly mean summer temperatures (December to March, Micieli et al. 2006). On the other hand, it was recorded for the first time in Peoria (United States) that *Ae. albopictus* populations survived the mild El Niño winter 1997-1998 (Swanson et al. 2000).

Warming can also trigger an altitudinal and northward shift of vector species. Fewer days with temperatures below -12 °C caused a northward shift of the northern distribution limit of the tick *Ixodes ricinus* in Sweden (Lindgren et al. 2000). An expansion in the altitudinal range of this tick is given by field-based evidence from Bosnia Herzegovina (from below 800 m a.s.l. in the 1950ies to 1190 m.a.s.l. by 2010, Omeragic 2011) and the Czech Republic (700 m.a.s.l. in 1957 to 1100 m.a.s.l in 2001, Daniel et al. 2003).

Intensity and frequency of precipitation are also influencing the occurrence and abundance of vector species by determining the availability of breeding sites, the longevity of eggs and adults, the flight activity, and the blood meal uptake. The monthly precipitation significantly determines the frequency of cemetery vases occupied by aedine mosquito vectors in Florida (USA) (Lounibos et al. 2010). Interestingly, the sign of the regression coefficient was opposite for the two investigated species: While *Aedes albopictus* increased, *Aedes aegypti* decreased in vase occupancy with increasing precipitation (Lounibos et al. 2010). Generally, heavy rainfall events tend to reduce *Culex* spp. catches (DeGaetano 2005). Day and Curtis (1993) report that the abundance of gravid females of *Culex nigripalpus* (St. Louis encephalitis vector) decreased after heavy rainfall events (>50 mm), whereas the number of blood-fed females increased.

Survival of eggs under different humidity conditions (43 % to 98 %) show that *Aedes aegypti* embryo survival inside the eggs is clearly diminished at the lowest humidity tested and at all humidities after increasing periods of exposure (Luz et al. 2008).

Surprisingly, drought positively promotes the abundance of *Aedes aegypti* in Brazil in an indirect way, because new breeding sites evolved in water storages (Pontes et al. 2000). This is an example of unexpected anthropogenic feedback mechanisms which are mostly ignored in ecological studies. In general, precipitation effects on vectors development and abundance are more complex, sometimes contradictory and therefore more difficult to assess than temperature driven effects.

2.3.3 Temperature and Precipitation Effects on Pathogens and Disease Incidences

As arthropods are ectothermic species, the environmental temperature directly determines the amplification of the pathogen inside the vector and the subsequent ability to transmit the pathogen. The EIP is the temperature dependent time required for the development of a disease agent in a vector, from the time of pathogen uptake to the time when the vector is infective (Fig. 10). The EIP includes virus replication, maturation, and migration within the mosquito's body to its salivary glands. Temperature is considered to be the main factor regulating the EIP and thus warmer temperatures will shorten the EIP (Watts et al. 1987, Barbazan et al. 2010). If minimum temperature thresholds for the EIP are not exceeded the virus cannot accomplish its amplification inside the vector and transmission to humans can be excluded (Ooi and Gubler 2010).

It is known that favourable weather conditions significantly influence dengue incidences in endemic regions such as South America (Luz et al. 2008) and Southeast Asia (Shang et al. 2010). When modelling dengue transmission in Rio de Janeiro (Brazil), the two most important parameters were found to be the EIP and the mortality rate of the vector (Luz et al. 2003).

Evidence suggests that global warming is extenting the latitudinal and altitudinal range as well as the intensity of dengue transmission (Jetten and Focks 1997). A special case seems to be dengue transmission on Pacific islands: Hales et al. (1999) found a positive correlation between Southern Oscillation Index and dengue cases in 10 out of 14 island nations of the South Pacific. However, in Australia decrease in the average Southern Oscillation Index (means warmer conditions) during the preceding 3-12 months was significantly associated with an increase in dengue affected areas (Hu et al. 2010).

The spatial-temporal variability of human West Nile cases in Florida is found to be associated with the spatio-temporal variability of drought and rewetting (Shaman et al. 2011). A similar finding is reported from Rhode Island for infected mosquitoes and birds: Above-average total rainfall amounts from May to June in 1996 and 1998 were significantly correlated with increased arbovirus activity (Takeda et al. 2003, Eastern equine encephalitis virus, Highlands J virus, California encephalitis serogroup, Jamestown Canyon, Cache Valley, Flanders viruses and West Niles virus). Landesman et al. (2007) found that human outbreaks of West Nile Virus are preceded by above-average rainfall in the prior year in the Eastern United States and below-average rainfall in the prior year in the Western United States.

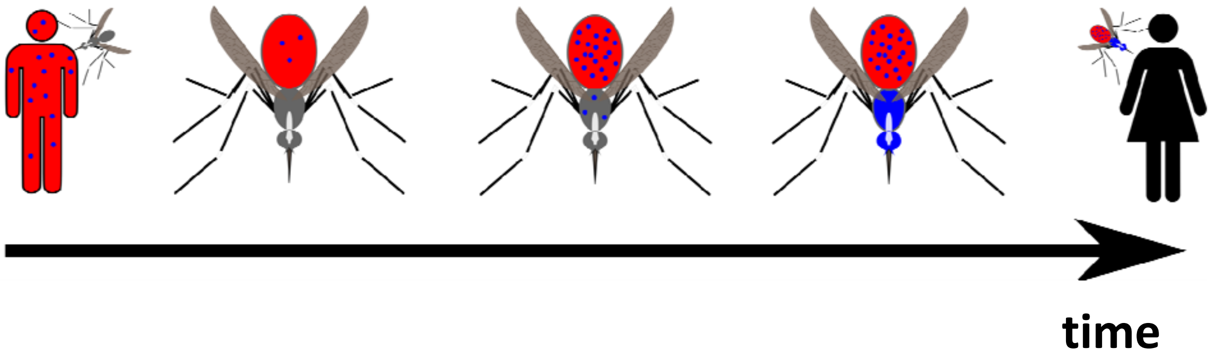

Fig. 9: Extrinsic Incubation Period (EIP) is the temperature dependent time required for the development of a pathogen (blue) in a vector, from the time of uptake to the time when the vector itself is infective (drawing by Nils Tjaden).

In Israel, positive anomalies of the temperature and extreme heat in the early spring had a positive effect on *Culex pipiens* abundance, followed by a West Nile emergence a few weeks later (Paz and Albersheim 2008). Increased Tick-borne Encephalitis incidences in Sweden are significantly related to milder winters, early arrival of spring, and extended autumn activity in the year prior to the incidence year (Lindgren and Gustafson 2001).

Correlating disease incidences with temperature reflects the temperature effects on the pathogen and indirectly also the temperature effects on vector species. Temperature effects on pathogens in detail, however, are estimated by the experimental examination of the EIP. This enables conclusions regarding future suitable areas at risk for pathogen´s transmission independently from vector population dynamics.

3. Synopsis of the Thesis

3.1 Outline of the Articles

Vector-borne disease risk assessment in times of climate change has to take into account both, vector’s ecology and pathogen’s temperature requirements. The first three Articles concentrate on the identification of the ecological niche and mapping of the possible suitable areas for **vector’s establishment**. Likewise, special climatic limitations for vector’s establishment are determined by the means of laboratory experiments to support model approaches.

Within **Article 1** the current bioclimatic niche of *Ae. albopictus* is modelled for the native range and the global range using maximum entropy approach. The aim is to project current and future climatically suitable European regions for an establishment of the mosquito. The effects of different selections of bioclimatic variables (expert knowledge vs. statistic based) are evaluated. Niche similarity between the native and the global range is analysed. The global models (regardless the chosen bioclimatic variables) fitted best with the recent regions of occurrence of the mosquito and were hence used to project species future climatic suitability in Europe. Projections are based on data provided by the regional climate model COSMO-CLM (A1B and B1 Scenario) for three time-frames 2011-2040, 2041-2070, and 2071-2100. Potential failure of projections due to non-analogue climate is excluded via Multivariate Environmental Similarity Surface analysis.

During the last seven years only four studies aim to determine the potential future climatic suitability of the invasive vector mosquito *Ae. albopictus* for the European continent. In two studies climatic suitability is projected via mechanistic models, while the results of the two other studies are based on correlative approaches (including Article 1). **Article 2** compares these different approaches with special emphasis on model set up and study design. Uncertainties in projections of the different studies are evident and areas with deviations between the future climatically suitability are identified. Benefits and drawbacks of individual approaches are discussed in order to develop guidelines for future studies.

The aim of **Article 3** is to detect low temperature thresholds for aedine mosquito egg survival, because winter temperature seems to be one of the most limiting factors regulating the establishment. So, modelling efforts are supported by expert knowledge. Here, the minimum survival temperature of eggs for two strains of *Ae. albopictus* and for one strain of

Ae. aegypti is determined in a climate chamber experiment. To the best of our knowledge there are no comparable studies on the cold tolerance in *Ae. albopictus* eggs which have undergone a diapause. Temperature was the main controlling factor, whereas the duration of the cold treatment only influenced the hatching response significantly at the thermal limits of survival for each strain or species. The data can be used to make evidence-based decisions on the temporal resolution of temperature data needed for modelling approaches. Furthermore the compilation of risk maps for temperate regions can substantially be improved by considering areas where an establishment of a vector population is unlikely due to winter conditions.

Article 4, 5 and 6 focus on the **temperature requirements of pathogen's amplification and disease transmission**. On the one hand the directly measurable and temperature dependent extrinsic incubation period (EIP) of a pathogen is used to map areas at risk of transmission. On the other hand given temperature conditions during disease outbreaks are transferred. In a **final step suitable areas for vector's establishment and disease transmission are combined** to achieve clearly refined risk maps.

The EIP indicates the time in which an infected vector becomes infectious this means be capable to transmit the pathogen. This is one of the main factors regulating transmission of a vector-borne disease. In **Article 4** two laboratory findings concerning the temperature dependent period for dengue virus amplification within the vector *Ae. aegypti* are used for risk assessment. The risk analyses are based on projected temperature data in daily resolution obtained from the regional climate model COSMO-CLM. European areas, where dengue virus amplification can be expected and the longest potential season for virus amplification are identified. The affected area sizes various widely between the two mapped EIPs. This clearly shows that before experimental findings of the EIP can be used for vector-borne disease risk assessment, the experimental design has to be developed carefully.

Therefore, in **Article 5** the results and uncertainties of the experimental studies addressing the temperature-dependence of dengue EIP are analysed and discussed. Experimental studies on this topic are rare and their results appear to some point inconsistent. However, the implementation of a realistic, temperature-dependent EIP will greatly improve the precision of epidemiological dengue modelling. Methodological challenges are identified and suggestions are formulated for the design of future studies to improve mechanistic as well as correlative modelling approaches from a spatio-ecological point of view. Since the lack of knowledge on temperature-dependence of the EIP seems to be even bigger when it comes to other arthropod-borne viral diseases such as Chikungunya, the identified challenges and suggestions may turn out to be of relevance beyond the example of dengue.

In a second disease based approach, **Article 6**, temperature conditions during Chikungunya outbreaks are spatially transferred. European areas facing current and future climatic risk of Chikungunya transmission were identified for the first time. In addition, the longest potential intra-annual season for Chikungunya transmission was estimated for areas with expected vector occurrences. This was done by combining the climatic suitability for the European distribution of the vector *Ae. albopictus* and known temperature requirements for pathogen transmission. The results for the climate change scenarios A1B and B1 were compared.

The last Article **summarizes benefits from multidisciplinary approaches** and directions forward in predicting ectotherm disease vector spread.

Article 7 reflects on the wide range of different drivers that are discussed causing the emergence of vector-borne diseases. It is quite evident, that various scientific disciplines have to be involved to solve the complex questions. Here, it is evaluated to what extent scientists of different disciplines consider results of other disciplines to extend their expertise by conducting a literature survey and evaluating the citation behaviour within different scientific communities. Emerging powerful tools for risk assessment originated in the diverse scientific disciplines are highlighted.

3.2 Main Conclusions

Risk Assessment of Disease Vector Mosquitoes

In **Article 1** it is shown that the bioclimatic envelope models built by MaxEnt of the native range of *Ae. albopictus* show high model performance for the native range, but the European occurrences are not well reflected. Models based on the global distribution of the species, however, were able to identify all regions where *Ae. albopictus* is currently established, including Europe. This was true for (1) statistically selected climatic input variables and (2) input variables selected with expert knowledge from the literature.

For both global-driven models, the results indicate that climatically suitable areas for the establishment of *Ae. albopictus* will increase in Western and Central Europe already in 2011-2040 and with a certain temporal delay in Eastern Europe. In some regions of Southern Europe climatically suitable areas are expected to decrease, which is especially project in the Expert knowledge based model. Comparing both climate change scenarios A1B and B1 used for the projection, higher values of climatic suitability are generally referred to the A1B scenario.

We observe a “West coast phenomenon”: Areas along the west coast of invaded continents seem to be climatically suitable for *Ae. albopictus.* But no occurrences have yet been documented in these areas. On the one hand, this may indicate that introduction of the species has not yet occurred or has been avoided. On the other hand, further climate factors could play a role under oceanic climatic conditions, which contribute to suppress the mosquito populations, such as wind or sea salt aerosols.

Article 2 provides a comprehensive comparison of all previous studies assessing the climatic suitability of European regions for *Ae. albopictus* establishment under current conditions and in a rapidly changing climate. The studies differ in the underlying occurrence data, model algorithm, climate data, emission scenarios, time-frames, geographical and projected coordinate systems, and spatial resolution. The models under investigation are capable to predict the current distribution of *Ae. albopictus* in Europe in good quality. General trends can be derived: especially Western Europe will provide favourable climatic conditions within the next decades. In Central Europe and in the southernmost parts of the United Kingdom climatic suitability can be expected to increase. This leads to the assumption of a possible northward spread of the species. Climatic conditions will continue to be suitable in coastal regions of Southeastern Europe. However, areas with deviations in risk classification show uncertainties in future projections of climatic suitability: France, Germany, and western parts of the United Kingdom (Wales) range from persistently unsuitable to increasingly suitable; central parts of the Iberian Peninsula, Sardinia and Sicily range from persistently suitable to increasingly unsuitable; Southwestern parts of the Iberian Peninsula, south-eastern Italy and parts of eastern parts of Greece including also the west coast of the Black Sea range from increasingly unsuitable to increasingly suitable.

These uncertainties in model risk projections indicate that existing knowledge on mosquitoes´ ecology needs to be expanded and deepened in particular with regard to the temperate European situation. In **Article 3** the low-temperature thresholds for the survival of post-diapausing tiger mosquito eggs of European populations are identified under laboratory conditions. In addition, hatching success after the cold treatment (0 °C to -15 °C) for different durations (1h, 4h, 8h, 12h and 24h) is measured. Hatching success of eggs which have undergone a diapause is significantly increased compared to non-diapausing European *Ae. albopictus* eggs. Temperature was the main controlling factor, whereas the duration of the cold treatment only influenced the hatching response significantly at the thermal limits of survival. The experiments help to detect potential regions, capable for overwintering populations. Thresholds for survival can be derived by simulating extremes, which then can be related to climate change scenarios.

Risk Assessment of Pathogens and Diseases

Vector mosquito’s presence alone does not necessarily imply disease outbreaks. The temperature requirements of the pathogen to replicate inside the vector or of disease transmission in nature must also be taken into consideration. This is demonstrated on the two examples of dengue virus and chikungunya virus.

Summarising the results of **Article 4,** remarkable differences are conspicuous in the resulting areas at risk of dengue virus amplification in Europe considering three different laboratory findings of the EIP in *Ae. aegypti*. Generally, results indicate a growing threat of virus amplification in Europe especially towards the end of this century. For the period 2011-2040, almost the whole Mediterranean region and countries in the Southeast of Europe bordering the Black Sea are likely to meet the temperature requirements. Even in some parts of

Central Europe dengue virus amplification can no longer be excluded at the end of the century. Dependent on the selected EIP exteriment and the choosen climate scenario the duration of virus amplification per year vary. The maximum period of dengue amplification lasts 146 days per year applying the A1B scenario and a minimum period of 34 days per year applying the B1 scenario.

The approach to project EIP via spatio-temporal highly resolved climate change projections allows a detailed characterization of potential areas at risk for Europe, which is currently missing. However, the limited availability of sound and realistic research on the EIP in *Ae. albopictus* is a crucial limiting factor for the bioclimatic modelling of VBDs.

Towards the aim to map areas at risk for dengue amplification by the means of a continuous temperature- time profile, all existing dengue EIP experiments were evaluated in **Article 5**. Five experimental studies were found that explicitly addressed the temperature dependence of the EIP of dengue. These papers are spanning over a long time period from 1930 to 2009. All experiments have in common that they examined the EIP of dengue virus type 2 in *A. aegypti*; type 1 and type 4 were investigated in one single experiment, only. The differences of the experiments are apparent: The origin of the used mosquitoes ranges from recently captured ones to more than 30 years lasting laboratory colonies. Pathogen uptake is supplied via intrathoracic injection or, at best, via infective bloodmeal. In addition, the amount of virus ingested varies strongly. In consequence, the way of testing the ability of an infected mosquito to transmit the virus yields different results. Various recommendations are put forward on how these experiments are more useable for climate change disease risk projections.

In a further approach, developed in **Article 6**, the temperature requirement of disease transmission during an outbreak is taken into account. The results are then combined with vector’s climatic suitability. Currently, the risk of chikungunya transmission is highest for the southernmost parts of Europe. An increase in risk of chikungunya transmission is projected for Western Europe in the first half of the 21st century, and from mid-century onwards for central parts of Europe. This general tendency is depicted in both climate change scenarios (A1B and B1). The longest possible period of transmission is described along the Mediterranean coast line with a maximum of three months for the current climatic conditions. In the second half of the century the season of transmission might even rise up to five months. Surveillance and vector control measures can now implemented effectively on locations and times to mitigate possible chikungunya transmission.

Combining Multidisciplinary Knowledge in Vector-borne Disease Risk Assessment

Finally, methods used for the identification of areas at risk of vector-borne diseases are brought into a bigger scientific context. In **Article 7** emerging tools and concepts are elucidated by the means of specific examples. Regression-based models, machine learning techniques, and mechanistic models are discussed. In addition, methods are presented, which enable spatial regression, interaction and interpolation of given information such as geographical weighted regression, least-cost path analysis, and connectivity analysis. The integration of remotely sensed data is described.

Further steps towards multidisciplinary approaches are concluded: Bayesian model-based geostatistic, parameter fitting of mechanistic models with detailed observations or experiments, combination of correlative and mechanistic models, hierarchical modelling framework, and multi-criteria decision analysis. In order to identify new multidisciplinary approaches, it is necessary that scientists consider results of other disciplines. However, this is difficult to achieve as it stands against traditional working procedures. We evaluated disciplinary citation behaviour for research on mosquito vectors and the transmitted diseases in times of climate change. The majority of references are attributed to the human medical literature, in which results of environmental sciences and geosciences receive surprisingly high attention. Veterinarians cited frequently human medical articles, but this is not apparent vice versa. An unexpected low proportion of cited references belong to the biosciences category. In each scientific discipline multidisciplinary and interdisciplinary articles are cited with at least 10%.

3.3 Authors´ Contribution to the Articles

Article 1:

Fischer D, Thomas S.M., Niemitz F., Reineking B., Beierkuhnlein C. (2011): Projection of climatic suitability for *Aedes albopictus* Skuse (Culicidae) in Europe under climate change conditions. Global and Planetary Change 78, 54-64. doi:10.1016/j.gloplacha.2011.05.008

Stephanie Thomas, Franziska Niemitz and Carl Beierkuhnlein had the initial idea of climate change projections for *Ae. albopictus* with the regional focus of Bavaria. Franziska Niemitz carried out first analyses and wrote a first draft as part of her Master thesis. She organized an initial data set of species presence records. Stephanie Thomas completed this data set with a search for additional infestations of the species from the year 2003 onwards and provided these references in the Supplemental Material. She wrote main parts of the introduction, especially the parts concerning the species' ecology. Dominik Fischer implemented the species distribution models and future projections for whole Europe and tested of non-analogue climatic conditions by Multivariate Environmental Surface analysis. He wrote the main parts of the results, discussion and conclusion and prepared the figures. Dominik Fischer and Björn Reineking wrote the methodology chapter and generated the tables. Additionally, Björn Reineking tested the data sets for niche similarity and provided expertise for further issues concerning the modelling procedure. Björn Reineking and Carl Beierkuhnlein (both supervisors of the Master thesis from Franziska Niemitz) gave both critically comments on the Article and were responsible for the final editing.

Article 2

Fischer D., Thomas S.M., Neteler M., Tjaden N.B., Beierkuhnlein C. (2013): Climatic suitability of *Aedes albopictus* in Europe referring to climate change projections: Comparison of mechanistic and correlative niche modelling approaches. Eurosurveillance. Accepted 30/07/13

Stephanie Thomas had the idea to compare all previous approaches and results regarding the climatic suitability of *Ae. albopictus* in Europe. Stephanie Thomas and Dominik

Fischer selected the specific approaches given in the six studies. Stephanie Thomas compiled study region, model approach, input data, model validation and details regarding the climate projection of the different studies (Table 1) and gave detailed information to variables and methods used (Table 2). Dominik Fischer and Nils Tjaden created the maps. Dominik Fischer and Stephanie Thomas wrote the Article. Carl Beierkuhnlein and Markus Neteler gave critically comments on the Article.

Article 3:

Thomas S.M., Obermayr U., Fischer D., Kreyling J., Beierkuhnlein C. (2012) Low temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, *Aedes albopictus* (Diptera: Culicidae). Parasites & Vectors, 5 (100). doi:10.1186/1756-3305-5-100

Stephanie Thomas designed the study, carried out the cold treatment of eggs, performed the statistical analysis and wrote the Article. Ulla Obermayr carried out the induction of diapause in mosquito eggs and wrote the subchapter "Induction of diapausing eggs". Jürgen Kreyling conceived of the study design and participated in the performance of the statistical analysis. Carl Beierkuhnlein, Jürgen Kreyling and Dominik Fischer gave critical comments on the Article.

Article 4:

Thomas S.M., Fischer D., Fleischmann S., Bittner T., Beierkuhnlein C. (2011): Risk assessment of dengue virus amplification in Europe based on spatio-temporal high resolution climate change projections. Erdkunde 65, 137-150. doi:10.3112/erdkunde.2011.02.03

Stephanie Thomas and Dominik Fischer contributed equally to this work. Stephanie Thomas had the idea of the paper and searched for profound studies concerning the EIP of the dengue virus. She wrote the introduction with biological-ecological background of the study and the discussion. Dominik Fischer developed the code for the analysis in GIS and arranged the figures 1-4. He wrote the methods, results and the conclusion. Stefanie

Fleischmann practiced the final analysis in GIS and wrote a first draft (in German) as her Bachelor-thesis. Torsten Bittner prepared scripts written in Python and R in order to standardize the procedure in GIS. He arranged figure 5. Carl Beierkuhnlein was the supervisor of Bachelor-thesis of Stefanie Fleischmann, gave critical comments and edited the Article.

Article 5:

Tjaden N.B., Thomas S.M., Fischer D., Beierkuhnlein C. (2013): Extrinsic incubation period of dengue: Knowledge, backlog and applications of temperature-dependence. PLOS Neglected Tropical Diseases 7(6): e2207. doi:10.1371/journal.pntd.0002207

Stephanie Thomas had the initial idea to review systematically laboratory studies concerning the EIP of dengue virus in order to provide reliable data for dengue risk modelling. She compiled the results of an initial literature search. Nils Tjaden performed of the statistical analysis. He wrote the first draft of the Article and prepared the figures. Stephanie Thomas helped to draft the Article and gave critically comments. Dominik Fischer and Carl Beierkuhnlein did the final editing.

Article 6:

Fischer D., Thomas S.M., Suk J.E., Sudre B., Hess A., Tjaden B., Beierkuhnlein C., Semenza J.C. (2013): Climate change effects on Chikungunya transmission in Europe: Geospatial analysis of vector´s climatic suitability and virus´ temperature requirements. International Journal of Health Geographics 12:51. doi:10.1186/1476-072X-12-51

Jonathan Suk, Jan Semenza and Bertrand Sudre initiated the project “Climate modelling for Chikungunya” (OJ/08/02/2012-PROC/2012/012). Stephanie Thomas, Dominik Fischer and Carl Beierkuhnlein developed the idea for the specific analysis. Dominik Fischer, Stephanie Thomas, Andreas Heß and Nils Tjaden practised the analysis. Carl Beierkuhnlein, Jonathan Suk, Jan Semenza and Bertrand Sudre contributed to the model design. Dominik Fischer, Stephanie Thomas and Andreas Heß prepared figures and tables. All authors contributed to the Article, commented on drafts critically. Stephanie Thomas and Dominik Fischer edited the final version of this paper.

Article 7:

Thomas S.M., Beierkuhnlein C. (2013): Predicting ectotherm disease vector spread - Benefits from multi-disciplinary approaches and directions forward. Naturwissenschaften 100(5):395-405. doi 10.1007/s00114-013-1039-0.

Carl Beierkuhnlein had the idea to compare the citation behaviour of different disciplines working in the field of vector-borne diseases. According to the broad spectrum of involved disciplines, tendencies to ignore findings in other fields must be taken serious. Stephanie Thomas added the detailed methodological dimension, analysed the literature, and compared the methodological tasks of the disciplines in an interdisciplinary context. She wrote the Article and prepared the figures. Carl Beierkuhnlein gave critically comments on the Article and did the final editing.

4. Critical Reflection and Outlook

4.1 Concerns about Correlative Niche Modelling of Invasive Species, Life History Experiments, and Climate Change Models and Scenarios

Correlative niche modelling of invasive species

Both, *Ae. albopictus* and *Ae. aegypti*, which are considered in this thesis, are listed in the Global Invasive Species Database, managed by the Invasive Species Specialist Group (ISSG) of the International Union for Conservation of Nature (IUCN) and were duly considered in the Technical Report of the European Environment Agency (EEA) regarding the impacts of invasive alien species in Europe (EEA 2012). The IUCN describes invasive species as "animals, plants or other organisms introduced by man into places out of their natural range of distribution, where they become established and disperse, generating a negative impact on the local ecosystem and species." (IUCN 2008). This IUCN definition tackles impacts of invasive species on biodiversity, ecosystem services, economic activities and human health and is therefore on a broader basis than the definition of the Convention on Biological Diversity (CBD) which says "an alien species whose introduction and/or spread threaten biological diversity […]" (CBD 2014). A critical reflection on the concept of invasion biology and the related separation of native and alien species is given by Davis and colleagues (Davis et al. 2011).

Invasive species are characterised through specific traits such as short life cycle, rapid growth, high dispersal ability, phenotypic plasticity and ability to survive a wide range of environmental conditions (IUCN 2008, CBD 2014). The regulation of invasive processes in nature is subject to current research and is reflected in the fact that a number of hypotheses are developed; such as the shifting defense hypothesis, enemy release hypothesis, enhanced weapony hypothesis, novel weapons hypothesis and the evolution of increased competitive ability hypothesis (Joshi and Tielbröger 2012), which are not all necessarily applicable to arthropod vector species. Important processes which may act as barriers to invasion of mosquito vectors are intraspecific and interspecific interactions such as competition or predation (Armistead et al. 2008, Juliano and Lounibos 2005). However, these interactions are space and time dependend and varying according to the involved species, so that even for the best-studied invasive mosquito species, *Ae. albopictus*, a predominating ecological process has not yet been found (Juliano and Lounibos 2005).

The invasion process encompasses transport, establishment, spread, and impact (Williamson 1996). During this invasion process the ecological requirements of a species may change and adaption may evolve (Pearman et al. 2008). Data from the native range is frequently used to train a niche model and project the ecological niche of an invasive species to new areas (Mau-Crimmins et al. 2006). But, this only is a convenient approach in an early phase of the invasion process. *Ae. albopictus* is known to be highly adaptive and niche shifts occur (Juliano and Lounibos 2005, Medley 2010). The ecological niche in its native range cannot be directly transferred to the invaded area. On the other hand, the native range may not necessarily cover a species' entire fundamental niche (Broennimann et al. 2008).

Consideration should also be given to limits of correlative niche modelling at temporal scales such as activity periods, individual life history thresholds of species and timing of biological processes. Moreover, climatic information used in the model can be restricted by a biased spatial coverage of weather stations in the target area, data gaps in time series, incomplete data on rare climatic events or exclusion of potential side effects such as wind speed (Jaeschke and Beierkuhnlein, personal communication). The prediction of the current distribution of an invasive species is improved using true-absence data (Lobo et al. 2010). However, the use of true-absence data should be avoided when the estimation of the potential distribution is modelled in order to develop risk maps of areas with a possible invasion (Jiménez-Valverde et al. 2011).

Further general sources of uncertainty in correlative niche approaches are detailed discussed in the Articles: the problem of non-analogue climate which can be assessed by the MESS analysis (Elith et al. 2010), the selection of parameters via Jack knife test, expert knowledge or AIC values (Bozdogan 1987), the exclusion of colinearity of selected parameters (Dormann et al. 2013); the sensitivity of model accuracy regarding spatial auto-correlation of the sampling effort (Veloz 2009).

Experiments on Life History Traits

Changing winter temperature conditions are known to have far reaching implications on insects overwintering (Bale and Hayward 2010). In this thesis the low temperature survival of aedine mosquito eggs is determined experimentally. Using long-lasting laboratory colonies of *Ae. albopictus* and *Ae. aegypti* may yield deviating results compared to natural populations. However, such colonies are more appropriate to serve for a series of different life cycle thresholds experiments, which can then be compared directly. Furthermore, in some cases – shown for the major vector of visceral leishmaniasis in the Americas *Lutzomyia longipalpis* - long term maintenance of a laboratory colony has had little effect on the level of isozyme variability and genetic distance between these populations was not significantly different from natural populations (Lanzaro et al. 1998).

Many athropods survive predictable, unfavorable environmental conditions, such as temperature extremes or drought by using the mechanism of diapause. Diapausing athropods pass through various stages starting with the induction phase and followed by the preparation phase, initiation, maintenance phase, termination and post-diapause quiescence. Photoperiod is the most important stimulus initiating diapause. Stimuli such as freezing or contact with water terminate diapause or termination may occur spontaneously. Producing diapausing eggs is the main life strategy of *Ae. albopictus* to come through unfavourable winter conditions and it is also subject to evolutionary processes during the northward spread (Lounibos et al. 2003, Leisnham et al. 2011, Lounibos et al. 2011). The successful production of diapausing eggs in the laboratory is unlikely. Hatching tests in our experiments during diapause, however, showed only about 10% spontaneous hatching.

Desiccation resistance is closely linked with the cold resistance of mosquito eggs. Mosquitoes, producing desiccation resistant eggs, are more likely to become established in new areas; possibly due to a better survivorship during long-distance transports (Juliano and Lounibos 2005). Hatching success under desiccation stress is increased in diapausing *Ae. albopictus* eggs relative to non-diapause eggs (Sota and Mogi 1992). Generally, diapause is not a physiological deadlock, but rather a physiologically dynamic state with different patterns of gene expression through the time (Denlinger 2002, Urbanski et al. 2010). The molecular physiology of egg desiccation resistance is investigated for *Ae. albopictus* and *Ae. aegypti* (Rezende et al. 2008, Urbanski et al. 2010). Urbanski et al. (2010) provide evidence that the expression and a higher quantity of fatty acyl coenzyme-A elongase transcript is an important component of increased desiccation resistance during diapause in *Ae. albopictus*. Findings of Rezende et al. (2008) confirm that chitinized serosal cuticle contributes to desiccation resistance of embryos of *Ae. aegypti*.

Climate Change Models and Scenarios

Global climate models also known as general circulation models GCMs, simulate the response of the global climate system to increasing greenhouse gas concentrations including physical processes in the atmosphere, ocean, cryosphere and land surface. They have a quite coarse three dimensional grid with a horizontal resolution of between 250 and 600 km, and about 20 to 50 vertical layers (IPCC 2013b). Main sources of uncertainties in model projections are the disregard of feedback mechanisms such as clouds or the snow albedo effect, the parameterisation to average the output over large scales, and physical processes which occur on smaller scales than the model resolution.

For climate change estimates on a regional scale which are required in impact and/or risk analysis regional climate models RCMs are advanced tools. One differentiates between RCMs based on statistical downscaling (e.g. Wilby et al. 1998) and those based on dynamical downscaling (e.g. Wang et al. 2004). For European regions WETTREG (Wetterlagen-basiertes Regionalisierungsverfahren) and STAR (Statistical Regional Model) are the two widely used statistical downscaled RCMs (Werner and Gerstengarbe 1997, Enke et al. 2005).

In this thesis the simulations of the dynamical downscaled RCM COSMO-CLM or CCLM (COSMO - Consortium for Small-scale Modelling, CLM - Climate Limited-area Model) are applied for climate projections of Europe. Dynamical downscaling is preferred as it is the more physical consistent way of modelling in comparison to statistical downscaling methods (Giorgi and Mearns 1991). Dynamical downscaled RCM are driven by a GCM which give the input for the atmospheric processes at the boundaries of a RCM.

CCLM simulations are driven by the well established global climate model ECHAM5. The CCLM model comprises whole Europe and African regions adjacent to the Mediterranean Sea and it provides data on land surface and oceanic water bodies. A major advantage lies in the very good spatial resolution with about 18km^2 (Smiatek et al. 2009). But, uncertainties depending on the choice of the climate model and the applied scenarios must be taken into account when future species response to climate change is projected (Beaumont et al. 2008, Buisson et al. 2010, Kjellström et al. 2011).

Uncertainties of model biases of the CCLM are assessed by Roesch et al. 2008. They calculate the intra-ensemble difference of the mean and interannual variability of both 2 m temperature and total precipitation for the period 1959–2000. CCLM has a marked cold bias in spring and summer over large parts of Europe (north of 40N). Those regions undergo on the other hand a warm bias in winter. Cold summer bias contributes to an underestimation of the number of consecutive summer days with maximum temperatures above 25 °C. These findings of model bias have to be taken into account when assessing the results of the future projections of climatically suitable areas for *Ae. albopictus* establishment and the duration of vector's active phase. This concerns also areas at risk of dengue transmission and the longest period of pathogen transmission to a certain extent, although daily mean instead of maximum temperatures are used. In particular, for Central Europe colder summer leads to an underestimation of the risk of vector establishment. On the other hand warmer winters overestimate this risk, due to an improved likelihood of egg overwintering. A dry summer bias is found extending from Italy to the Black Sea, whereas the precipitation is generally overestimated by CCLM during all seasons in Europe (Roesch et al. 2008). As a consequence, the availability of mosquito breeding sites and survival rates in South and Southeastern Europe are likely to be underestimated. Like other regional climate models, the performance of the CCLM in mountainous regions may be reduced. Suklitsch et al. (2008) found depending on the model setup for the European Alps an annual mean error -0.14 and -0.42 mm/d for precipitation and -0.98 and -1.44 K for temperature.

In this thesis two IPCC emission scenarios are applied, to minimise limitations of the regional climate model and to compare possible high risk and low risk developments. The A1B scenario assumes that the future world faces a very rapid economic growth and the global population will peak in mid-century. The technological change in the energy system is expected toward a balance situation across fossil and non-fossil sources. The B1 scenario assumes that rapidly a service and information economy will evolve associated with the introduction of clean and resource-efficient technologies (IPCC 2007).

4.2 Emerging Research Challenges

Next generation of IPCC emission scenarios

The next generation of IPCC emission scenarios, the so called representative concentration pathways RCPs, were developed and published by the end of 2013. In spring 2014 databases provide for the first time simulations based on these new scenarios. Nearly a decade of new economic data, emerging technologies, and observations of environmental factors are reflected in the new scenarios (Moss et al. 2010). The process of developing the climate scenarios is innovated: now socio-economic scenarios are directly linked to the new climate scenarios in parallel approach. This parallel procedure replaces the earlier sequential approach. Four representative concentration pathways were determined to create near-term climate projections (to 2035) and long-term climate projections to the year 2100, with some extensions to 2300 (Moss et al. 2010).

Overall four anthropogenic radiative forcing scenarios are differentiated; the high RCP8.5 (4.5°C by 2100), the medium-high RCP6, the medium-low RCP4.5 and the low RCP2.6 which represents a strong mitigation scenario with a projected global-mean surface temperature increases of 1.5°C by 2100. RCP4.5 is only slightly higher than the SRES B1 scenario (Meinshausen et al. 2011). For Europe a new high-resolution regional climate change ensemble has been established and the first set of simulations with a horizontal resolution of 12.5 km was completed for two of the new representative concentration pathways RCP4.5 and RCP8.5 (Jacob et al. 2014).

Expectedly, projections based on climatic extremes and their ecological consequences will be improved. Modified climatic variability and associated sporadic extreme conditions are likely to create new windows of opportunity for the establishment of disease vectors such as *Ae. albopictus* (IPCC 2012). Vector-borne disease risk assessment could be futher improved by using these new scenarios to project climatic suitability.

Improving Experimental Studies on Vector's Life History Traits and Pathogen's Extrinsic Incubation Period

The integration of diurnal temperature ranges in prospective experimental studies will strongly improve the knowledge on vector-borne disease transmission in particular for temperate regions. Currently, Carrington et al. (2013) show, that large temperature fluctuation at low temperatures (~18°C swings around 20°C) significantly shortened dengue virus EIP in *Ae. aegypti*, compared to constant temperature conditions at the same mean. Previous experiments conducted under constant temperatures may therefore underestimate the risk of dengue transmission in temperate regions (Carrington et al. 2013). Modelling dengue transmission of *Ae. aegypti* under fluctuating temperature conditions by the means of a thermodynamic model, Lambrecht et al. 2011 found, that at mean temperatures <18 °C transmission increases as diurnal temperature range increases, whereas at mean temperatures >18 °C, larger diurnal temperature range has the opposite effect. In addition, low temperature fluctuations likely effect egg survival in winter. Future studies should pay attention to winter conditions by incorporating realistic freeze-thaw cycles. Generally, studies on temperature thresholds for the EIP of the dengue virus in *Ae. albopictus* are missing. However, potential differences in the EIP between *Ae. aegypti* and *Ae. albopictus* have to be identified to improve risk maps of dengue transmission in Europe.

To exclude qualitative and quantitative food effects in laboratory experiments Müller et al. (2013) has recently standardized an optimal larval feeding procedure for *Ae. albopictus* to minimize larval mortality and to optimize pupae size. Planning new life history traits experiments, these results should be taken into account.

Important vectors beyond *Ae. albopictus* and *Ae. aegypti* should be considered. Recently, the invasive mosquito *Ae. japonicus* was found in a surprisingly huge area in Germany and also established in Belgium (Kampen et al. 2012, Ibáñez-Justicia et al. 2014). Yet, its vector competence under natural conditions is unclear, but was found infective in field with West Nile virus (Medlock et al. 2012).

Enhancing the Use of Correlative Models

Not only the potential current and future distribution of arthropod vector species can be modelled by correlative model approaches, but also the occurrences of a disease itself (Bhatt et al. 2013). Given all the links in a chain of infection, vertebrate host species can also play a role. Including the distribution of hosts in the model development can improve the final risk maps. Until now, only presences of vector species are documented. Absences are often not published or only recorded for a short period of time, so it is unclear whether they are true absences. True absences data of vector species, however, would enhance vector-borne disease risk assessment as further modelling approaches such as boosted regression trees or random forests could be applied (Peters et al. 2011, Simmons et al. 2012)

Factors others than Climate Change

Apart from overall climatic conditions, other drivers contribute to the distribution of vector-borne diseases. Microclimate and habitat availability influence the success of the vector's establishment (Romi et al. 2006, Lounibos et al. 2010). In addition, land use and land cover can modify the realised ecological niche (Vanwambeke et al. 2011). Human behaviour and socio-economic conditions have an impact on the incidences of vector-borne diseases (Sumilo et al. 2008). Finally, although it is not claimed to be a complete list, the globalisation of trade and traffic with distinct routes and ports of entry for invasive species should be considered in vector-borne disease risk assessment (Thomas et al. 2014).

4.3 Concluding Remark

Vector-born disease risk assessment is not only a scientifically highly interesting, but also a topically public health demand. Tackling these complex issues requires a broad range of expertise from the medical, biological, environmental, mathematical, social and economic sciences. Only the intensified exchange between research disciplines will help to improve methods, tools, and research approaches in general. Moreover, transparency coupled with cooperation between e.g. laboratory work, field experiments, monitoring and modelling is required.

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6. Articles

Projection of climatic suitability for *Aedes albopictus* Skuse (Culicidae) in Europe under climate change conditions

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6.1 Article 1:

Projection of climatic suitability for *Aedes albopictus* Skuse (Culicidae) in Europe under climate change conditions

With 3 Tables and 5 Figures in the main text as well as one Reference list and 7 Figures in the Supplemental Material.

Abstract

During the last decades the disease vector *Aedes albopictus* (*Ae. albopictus*) has rapidly spread around the globe. The spread of this species raises serious public health concerns. Here, we model the present distribution and the future climatic suitability of Europe for this vector in the face of climate change. In order to achieve the most realistic current prediction and future projection, we compare the performance of four different modelling approaches, differentiated by the selection of climate variables (based on expert knowledge vs. statistical criteria) and by the geographical range of presence records (native range vs. global range).

First, models of the native and global range were built with MaxEnt and were either based on (1) statistically selected climatic input variables or (2) input variables selected with expert knowledge from the literature. Native models show high model performance (AUC: 0.91-0.94) for the native range, but do not predict the European distribution well (AUC: 0.70-0.72). Models based on the global distribution of the species, however, were able to identify all regions where *Ae. albopictus* is currently established, including Europe (AUC: 0.89-0.91).

In a second step, the modelled bioclimatic envelope of the global range was projected to future climatic conditions in Europe using two emission scenarios implemented in the regional climate model COSMO-CLM for three time periods 2011-2040, 2041-2070, and 2071-2100. For both global-driven models, the results indicate that climatically suitable areas for the establishment of *Ae. albopictus* will increase in Western and Central Europe already in 2011-2040 and with a temporal delay in Eastern Europe. On the other hand, a decline in climatically suitable areas in Southern Europe is pronounced in the Expert knowledge based model.

Our projections appear unaffected by non-analogue climate, as this is not detected by Multivariate Environmental Similarity Surface analysis. The generated risk maps can aid in identifying suitable habitats for *Ae. albopictus* and hence support monitoring and control activities to avoid disease vector establishment.

Keywords

Asian tiger mosquito, dengue, global change, global warming, species distribution model, invasion, vector-borne disease

Research highlights

- We model the current climatic suitability for the disease vector *Aedes albopictus*.
- We assess the potential of the mosquito to establish in Europe under climate change.
- Increasing areas for the establishment are pointed out for Western and Central Europe.
- Risk maps may support monitoring activities to avoid disease vector establishment

1. Introduction

The invasive disease vector *Aedes albopictus* (*Ae. albopictus*) has recently received much attention (e.g. Benedict et al., 2007; Enserink, 2008; Medley, 2010). The mosquito has been ranked among the first 100 of the Worlds´ worst invaders (Crans, 2008). It has spread from its original distribution area in South-east Asia (Hawley, 1988) to at least 38 countries in North and South America, Africa, Oceania and even Europe (Benedict et al., 2007), likely by global transport of goods (e.g. Lounibos, 2002; Tatem et al., 2006; Fischer et al., 2010a). The first European invasion of *Ae. albopictus* was reported in 1979 from Albania (Adhami and Reiter, 1998). Upon its second arrival in Europe in 1990 the mosquito managed to establish permanent populations in Italy (Sabatini et al., 1990) and is now found across the Mediterranean area (Spain, France, Slovenia, Croatia and Greece; see Suppl. Reference list).

The spread of *Ae. albopictus* raises serious public health concerns. Under experimental conditions *Ae. albopictus* is able to transmit 22 viruses (Gratz, 2004). In nature it is mainly known to be an important vector of dengue, chikungunya and West Nile. Also Yellow fever virus and eastern equine encephalitis virus (North America), Ross River virus (Australia), Usutu virus (Italy) and the heartworms *Diofilaria immitis* and *D. repens* (Italy) were isolated from specimens collected in the field (Mitchell et al., 1987; Cancrini et al., 2003a,b; Calzolari et al., 2010). Due to its rapid spread (Lounibos, 2002), broad ecological plasticity (Delatte et al., 2008b) and high population density, this species has the potential to serve as an epidemic vector. Furthermore, its capacity to vertically transmit dengue and La Crosse (Tesh and Gubler, 1975; Rosen et al., 1983) enhances the possibility of establishing diseases in new areas (Delatte et al., 2008a). In Europe, the medical relevance of *Ae. albopictus* was highlighted in 2007 when the occurrence of the species was related to a chikungunya-epidemic in Northern Italy in the region of Ravenna (Rezza et al., 2007). Recently, autochthonous cases of dengue fever were diagnosed in Southern France for the first time (La Ruche et al., 2010) where *Ae. albopictus* serves as vector. Furthermore, autochthonous dengue virus infections were reported from Croatia (Schmidt-Chanasit et al., 2010; Gjenero-Margan et al., 2011).

Even under conservative and optimistic scenarios, future climate change is likely to increase air temperatures. At the end of this century the number of hot days in Central Europe is projected to reach conditions that are currently experienced in Southern Europe. While heavy summer precipitation is expected to increase in north-eastern parts of Europe, it is likely to decrease in the south (Beniston et al., 2007). In addition, changes in annual cold extremes are projected, whereby the largest relative warming is expected for North-eastern Europe (Goubanova and Li, 2007). These climatic changes may support a range shift and further regional establishment of *Ae. albopictus*.

As an ectothermal arthropod, *Ae. albopictus* is unable to regulate its body temperature. Hence the species directly depends on the thermal conditions of its environment. Under laboratory conditions, changes in temperature and precipitation affect the population dynamics of *Ae. albopictus*, which suggests that climate change is likely to extend the limits of its northern distribution (Alto and Juliano, 2001). Regarding a northward shift, especially temperature constraints in the cold period and decreasing photoperiod are of outmost interest, because these factors determine diapause of eggs and thus the survival of the species. The 10 °C coldest-month isotherm coincides with the separation between continuously breeding populations and those that must undergo a period of dormancy to survive cold periods in winter (Mitchell, 1988). Larval surveillance in Northern Japan shows that the mean temperature of the coldest month below -2 °C is potentially lethal there (Kobayashi et al., 2002). Nawrocki and Hawley (1987) state that the -5 °C coldest-month isotherm describes the maximum northward expansion of *Ae. albopictus* in continental Asia and, presumably, also in North America. A risk of establishment in Europe is considered for areas with 0 °C or higher as cold-month isotherm (Mitchell, 1995; Knudsen, 1995).

But, it is not only the limitation by low temperatures that has to be considered; warm temperatures, too, play an important role for *Ae. albopictus*. Pumpuni et al. (1992) pointed out that higher temperatures greatly reduce or prevent diapause incidences in Ae. albopictus specimen that were exposed to critical photoperiods. Results from natural foci in Southern Brazil demonstrate that diapause apparently evolved from nondiapause or non-photoperiodic ancestors, whereby in southern parts of USA a diapause reduction was observed presumably due to rapid local adaptation (Lounibos et al., 2003). Sufficient precipitation or perhaps more generally a suitable local moisture regime is an additional prerequisite for the occurrence of the species. Moisture directly controls the availability of breeding sites and the relative humidity is an important factor for egg survival (Juliano et al., 2002). Annual precipitation is reported to be higher than 500 mm in the species' habitats in the Mediterranean area (Mitchell, 1995).

Previous approaches to map suitable climatic conditions for the establishment of *Ae. albopictus* in Europe mostly focused on the risk of invasion under current climatic conditions. Considering rainfall beside other factors (photoperiod, temperature and humidity), Mitchell (1995) developed a risk map for the Mediterranean Basin by comparing the climatic conditions of the region with the estimated climatic envelope of *Ae. albopictus*. Knudsen et al. (1996) investigated the distribution of *Ae. albopictus* in Italy and projected the risk for a broader distribution throughout Europe. This projection is based on climatic criteria identified by Nawrocki and Hawley (1987) including winter mean temperature, mean annual rainfall, and mean summer temperature. Eritja et al. (2005) used the same climatic limits as Mitchell (1995) and generated a detailed risk map for Spain, which considered regional climatic conditions. For the United Kingdom, Medlock et al. (2006) developed a GIS based model using mean monthly temperature, annual rainfall and photoperiod to assess the overwintering survival, spring hatching and production of overwintering eggs in autumn.

The European Centre for Disease Prevention and Control (ECDC) produced risk maps that are aiming to predict climatic suitability of *Ae. albopictus* for the years 2010 and 2030 (ECDC, 2009). On a global scale, Benedict et al. (2007) investigated the regional risk of invasion by *Ae. albopictus*. Their analysis was carried out using eleven environmental data layers of the present climate. Recently, Medley (2010) investigated environmental (including climatic) niche shifts during the global invasion of the species, by modelling niches separately for each continent. Notably, most of the relevant literature is concerned with the present distribution and risk of invasion by *Ae. albopictus* under current climatic conditions. However, the predicted increase in temperature that is based on climate change scenarios will probably extend the spatial availability of breeding sites and also enhance mosquito survival (Woodward et al., 2001).

Our analysis starts from the assumption that *Ae. albopictus* will colonise climatically suitable niches around the world. We want to identify areas that could serve as potential habitat for the species today and in the future. Here, we assess the potential of *Ae. albopictus* to establish in Europe under projected climatic trends in the 21st century. In order to account for uncertainty in the selection of presence records and environmental variables, we (1) compare projections based on the species' former native range to those based on its recent entire global range, and (2) apply variable selection by expert knowledge as well as variable selection by an automated statistical procedure.

2. Material and methods

We created distribution models with MaxEnt, using species occurrences of the native range and of the entire range across the globe. For both training areas (global and native) two sets of bioclimatic data were prepared as input variables. One set was selected using expert knowledge on species climatic constraints. The second set was selected using solely statistical criteria. The future climatic suitability of *Ae. albopictus* in Europe was projected for two climate change scenarios. In addition, niche similarity between global and native regions and climatic similarity between projections was analysed.

2.1. Spatial distribution and presence records of *Aedes albopictus*

Presence records of *Ae. albopictus* at the global scale were taken from Benedict et al. (2007). Additionally, a literature search of scientific articles and reports of mosquito surveillance was conducted for the years 2003 onwards to consider additional infestations (see Supplemental Reference list). Reported occurrences of *Ae. albopictus* without evident establishment were excluded from the dataset.

This resulted in a global dataset that consists of 6347 occurrence points with 4683 occurrence points just for Brazil, due to a detailed monitoring system in South America. Presence records for the United States are available on county level but not as precise geographical coordinates. Therefore the 1033 counties with documented presence records were converted to points by digitising the centroids for each of the counties. Considering that worldwide, apart from the USA and Brazil, less than 1000 coordinate pairs were available, a random set of ten percent of the data were extracted for Brazil and the USA. Hence, the density of presence records for Brazil and the USA was reduced to levels that correspond to the density of documented presence points in other regions with maybe less intensified mosquito monitoring systems. Without this stratified sub-sampling, the results would have been biased towards the climatic conditions of South and North America (Medley, 2010). Additionally, duplicate species records within one raster cell of the training area (described in Section 2.2) were removed. The total global number of presence records used for modelling was 1199 (including 241 records in the native range).

2.2. Selection and pre-processing of climatic data

Current bioclimatic data (19 bioclimatic variables) were taken at a spatial resolution of 5 arcmin (http://www.worldclim.com). These bioclimatic variables are derived from monthly temperature and rainfall values in order to generate more biologically meaningful variables, which are recommended to use in ecological niche modelling (Hijmans et al., 2005). Higher spatial resolution would not correspond to the spatial accuracy of occurrence data for *Ae. albopictus*. Two climatic datasets with different spatial extent were generated. The global climatic conditions of the land surfaces were used to model the global distribution. For the definition of the “native range” models, each native presence record was buffered with a circle of 1000 km radius. The native range then included all areas which were located in at least one of the circles. Hence, this range included a climatic gradient within Asia, in order to yield a clear delineation of the species' climate niche, but excluded areas that may be too far from the realised distribution, such that geographical factors rather than climate are dominant in limiting the species' distribution.

We used two sets of variables as input for global and native models. The first set is based on expert knowledge on the climatic requirements of the target species (e.g. Mitchell, 1995). Those variables are the same for the native and for the global range. Most variables represent thermal constraints (Table 1). In this model, some variables show substantial collinearity: Annual mean temperature (Bio1) is correlated with mean temperature of the warmest and coldest quarter (Bio10 and Bio11) in the native and the global range higher than r = 0.7 (Pearson correlation coefficient). Nevertheless, as a pre-selection of variables based on expertise is often useful (Elith and Leathwick, 2009), we kept these variables in our analyses, and chose a modelling approach that is known to be robust against collinearity (see Section 2.3). Hereafter, we will refer to this set as the expert knowledge based model (EKBM).

Tab. 1: Selected bioclimatic variables of native and global models, referring either to expert knowledge based or statistic based model. Listed are the training gains for the selected bioclimatic variables measured by Jackknife test. Variables without mentioned training gain were not part of the selected data set. Training gains were calculated for a single variable if used solely for the modelling procedure and additionally for the remaining dataset if this variable has been dropped from the set. Both aspects must be considered for a statistical selection of variables. For instance, altitude as variable achieved comparatively low values for the training gain is used as the single variable for modelling procedure, but training gain of the dataset decreases remarkable, if altitude is removed. Therefore it is advisable to keep this variable within the set. After selection of the variables, training gain was calculated for modelling with all selected variables. Training gain for the complete selected dataset is highest for the native datasets (expert knowledge based: 1.67, statistic based: 1.69). Training gain of the global dataset is 1.42 for the expert knowledge based selection and 1.34 for the statistic based selection.

Training gain of selected bioclimatic variables and altitude		**Expert knowledge based**				**Statistic based**			
		global		Native		global		native	
		without variable	with only variable	without variable	with only variable	without variable	with only variable	without variable	with only variable
BIO1	Annual mean temperature	1.35	0.67	1.42	0.80	0.99	0.66	-	-
BIO4	Temperature seasonality	-	-	-	-	-	-	1.43	0.84
BIO10	Mean temp. (warmest quarter)	1.27	0.70	1.42	0.72	-	-	-	-
BIO11	Mean temp. (coldest quarter)	1.33	0.64	1.42	0.90	-	-	1.50	0.91
BIO12	Annual precipitation	1.20	0.71	1.35	1.00	1.17	0.72	1.51	1.00
BIO17	Precipitation (driest quarter)	-	-	-	-	-	-	1.42	0.66
BIO18	Precipitation (warmest quarter)	-	-	-	-	1.23	0.31	-	-
BIO19	Precipitation (coldest quarter	-	-	-	-	1.21	0.48	-	-

The second set of variables was selected by using a statistical procedure for native and global range separately. First, the importance of each variable was quantified with a Jackknife test implemented in MaxEnt (Elith et al., 2011). Variable importance is calculated in a twofold manner based on the training gain for all variables in isolation and for the remaining set of variables when the isolated variable is dropped from the set (Yost et al., 2008). To reduce collinearity in the set of statistically selected variables (Dormann et al., 2008) variables were removed that had a Pearson correlation coefficient $r \geq 0.7$ with any other higher-ranking variable in the results of the Jackknife test. We applied the variable selection procedure separately for the native and global range. The statistically derived sets of variables consist mainly of variables that represent the precipitation regime for the global range and identical number of temperature and precipitation variables for native range (Table 1). Models based on this set of variables are henceforth called statistic based model (SBM).

We projected the best models (determined by AUC-value, Section 2.3) to the future European climate. Projections of climate change in the 21st century refer to the scenarios for greenhouse gas emissions implemented within global or regional climate models. A1B and B1 scenario, which were considered as marker scenarios that best illustrate the respective storyline (IPCC, 2007), were applied for our projections of the future climate suitability for *Ae. albopictus* in Europe. In short, the A1B scenario is characterised by rapid global oriented economic growth and technological change towards the balanced use of fossil and non-fossil energy resources. The B1 scenario projects a more rapid change towards a service information economy with the introduction of resource efficient technologies while assuming a similar economic growth. It matches well with the European Union target of keeping global anthropogenic warming below two Kelvin above the pre-industrial level (Jacob and Podzun, 2010). Hence warming tendencies are projected to be stronger in the A1B scenario.

On http://www.worldclim.com data of the projected climate change are provided for the global climate models CCCMA and HADCM, which originally have very coarse spatial resolution (about 250km). Climatic changes were interpolated to the high grid resolution of the current climatic conditions. As a consequence, this simple downscaling procedure does not account for climatic changes at small scales. In order to achieve more realistic projections of future climatic suitability for *Ae. albopictus* in Europe, we instead used data of climate change provided from the regional climate model COSMO-CLM (CCLM). Near-scale physical processes integrated in CCLM (spatial resolution about 18 km) are fitted at the boundaries with large-scale conditions given by the global model ECHAM5 (Rockel et al., 2008). Such a dynamical downscaling procedure enhances the quality of climate impact studies on vector-borne diseases due to integrated small-scale specifics such as topography or further landscape features (Jacob, 2008).

Climatic data were separately averaged over time periods 2011- 2040, 2041-2070 and 2071-2100 for each scenario. Bioclimatic variables for modelling future climate projections were calculated in the same way as the original variables for current conditions. The spatial resolution of CCLM was resampled to the 5 arcminutes used for the current conditions. The pre-processing of the CCLM data was done via climate data operator codes (Schulzweida et al., 2009). The spatial extension of Europe for the current and future projections is adjusted to the defined space of the CCLM data.

2.3. Species distribution models

Species distribution models were built with MaxEnt. MaxEnt is a machine-learning technique combining species occurrence data with detailed climatic and environmental datasets in order to predict species distribution (Phillips et al., 2006; Phillips and Dudik, 2008). MaxEnt is favoured among other (pseudo) presence-only species distribution models due to high predictive power across all sample sizes (Elith et al., 2006; Wisz et al., 2008). In addition, variable selection in MaxEnt is less affected by correlated variables than e.g. step-wise regression, so there is less need to remove such correlated variables or, for instance, pre-process covariates by calculating principal components (Elith et al., 2011). Model residuals were tested for spatial autocorrelation using Morans I (Dormann et al., 2007).

Several parameter settings affect MaxEnt performance: Regularisation modifiers, which reduce the likelihood of overfitting and thus increase the predictive ability of modes beyond the training region (Phillips and Dudik, 2008), were set to 1. Increasing the number of model training iterations beyond the default value of 500 enhanced model performances, and the maximum number of iterations was therefore set on 2000 for all models. Furthermore, models were run with following settings: feature types were automatically selected depending on the training sample size (auto feature), convergence thresholds were 0.00001, maximum number of background points were 10000 as more background points do generally not enhance model quality criteria, but extend running time (Phillips and Dudik, 2008).

The model performance was quantified using the area under the receiver operator characteristic curve (AUC), a threshold-independent quality criterion (e.g. Elith et al., 2006). AUC-values can be interpreted as the probability that the model assigns a higher occurrence probability to a randomly selected presence location than to a randomly selected absence location. In order to yield unbiased estimates of model performance, we employed a standard split-sample strategy. Models were trained using a random subset (70%) of occurrence data and then tested on the remaining 30% (see also Araujo et al., 2005). This procedure was replicated 100 times and finally averaged. Both native models were additionally projected onto current climatic conditions of Europe.

Those models were further analysed that yielded high model quality criteria when tested with European presence records (Table 2). Additionally, prediction quality was visualised with maps of current climatic suitability for the entire range, for the native range, and for Europe (Figs. 2-3). Climate change projections were made for three time periods at high spatial resolution for Europe in order to identify regions with future climatic suitability for the mosquito.

2.4. Niche similarity and climatic similarity

We tested for differences in environmental conditions at the occurrence points using Multiresponse Permutation Procedure (MRPP) with 999 replicates. Occurrence points were assigned to one of three groups: the native range, the invasive non-European range, or the invasive European range. The MRPP was repeated for all three sets of environmental variables that were used in the modelling, i.e. those of the EKBM, the native SBM, and the global SBM. Additionally, we tested for niche differences between models trained on the native or on the global range, using a randomization test based on a method by Warren et al. (2008). Niche overlap between two model predictions is quantified with the I statistic (Table 3).

Since our main regional interest is Europe, we evaluated niche similarity for Europe only. The test compares the niche overlap of the original data with the niche overlap of randomised data, where we randomised the assignments to the regions (native and global), for both occurrence and background points. In order to keep the number of presence records constant in both regions, we separately randomised the region assignments for occurrence points and for background points. We used a one-sided test, with the null hypothesis that niche similarity is smaller or equal in the randomised data than in the original data; 199 randomisations were performed.

Furthermore, potential non-analogue climatic conditions between all projections in space and time were calculated. If non-analogue climate is detected, this requires caution in the interpretation of the results (Fitzpatrick and Hargrove, 2009). We determined potential non-analogue climate by using Multivariate Environmental Similarity Surface (MESS) analysis (Elith et al., 2010) for all of our projections in space and time. The MESS analysis measures the similarity between those environments used to train the model and the new projected environments for any grid cell (Elith et al., 2011). Regions with dissimilar values of the used variables, representing values that are outside the range of environmental training area, can be detected (Elith et al., 2010).

Preparation of presence records, current and projected climatic data was executed in ArcGIS 9.3.1 and R 2.11.0 (R Development Core Team, 2010). Correlation analysis of bioclimatic variables and Moran's I test were done in R. This software was also used to perform MRPP using the package “vegan” (Oksanen et al., 2011), while the package “phyloclim” (Heibl, 2011) was used for calculating I statistics (Warren et al., 2008). Species distribution models and MESS as well as calculation of variables contribution and Jackknife tests were carried out in MaxEnt 3.3.3e.

3. Results

3.1. Bioclimatic envelope and current distribution

Regarding the bioclimatic envelope, the occurrence of the mosquito mainly refers to regions that exhibit more than 500 mm of average annual precipitation and annual mean temperatures above 10 °C (Fig. 1). This is found to be true for both, the native and the invasive range. Within its invasive distribution the mosquito established in areas with annual mean temperatures between 10 and 25 °C and annual precipitation that ranges from 500 mm up to 2000 mm. The native range of *Ae. albopictus* is characterised by slightly higher temperatures and rainfall compared to the invasive range. Notably, the invaded range in Europe achieved the lowest values of annual mean temperature and annual precipitation.

The EKBMs were mainly built on temperature variables. Results of the Jackknife test for the selection of meaningful variables for the SBM differ for the global and the native range: While the global-driven SBM mainly includes hydrological variables, the native-driven SBM reflects equally thermal and hydrological constraints. Altitude and annual precipitation were the only variables used in both EKBMs and SBMs, regardless of the chosen training region (Table 1).

Both native and both global models showed high model performance for their respective training region (Table 2). The global EKBM and SBM delivered a realistic representation of the global range (Fig. 2). Interestingly, large parts of the continental west coasts of South America (Chile), Mexico and the United States (California, Oregon and Washington) as well as Europe (France, Portugal) are determined as climatically suitable, although there are currently no presence records. Furthermore the eastern side of Australia has been predicted as climatically suitable in both global models where the species is not established. The SBM made better predictions of the distribution of the mosquito in India. European areas with current distributions are successfully predicted with both global models (Figs. 2,4,5).

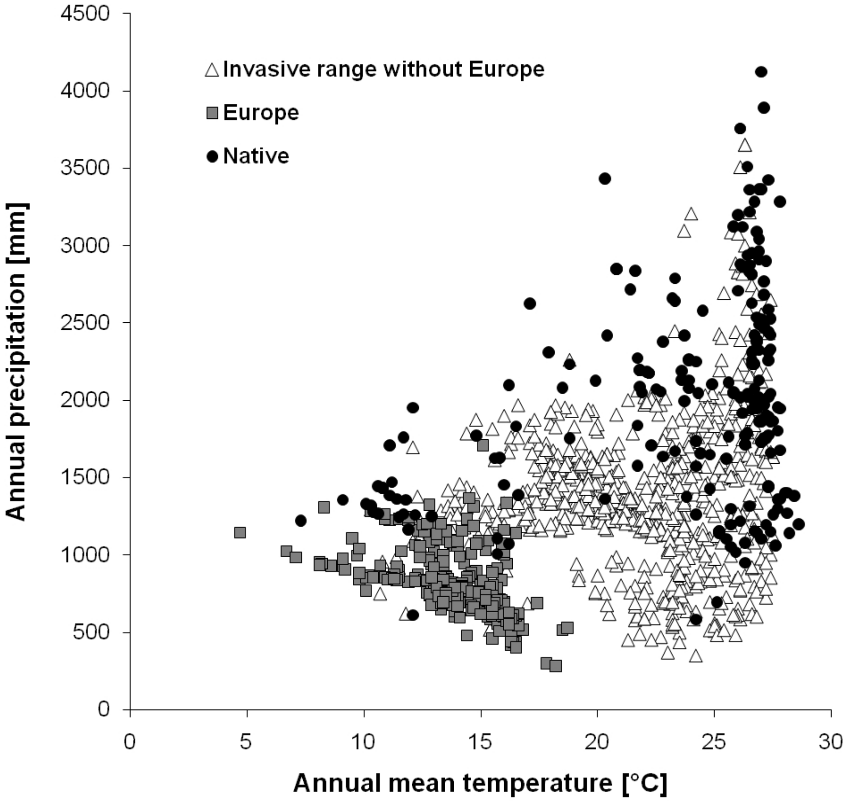

Fig. 1: Bioclimatic envelope for *Aedes albopictus* (native and invasive range), derived by geographically explicit overlay of presence records with annual bioclimatic variables. Annual mean temperature is highest in the native range (South-east Asia) with an average value of 23.7 °C (± 5.1 standard deviation) and the invasive range excluding Europe with 21.7 °C (±3.7), but lowest in Europe with 13.8 °C (± 2.2). High values of annual precipitation are characteristic for the native range (2028 mm ± 691) and for the invasive range without Europe (1392 mm ± 496). Invaded European regions obtain an average of 831 mm (± 218) of annual precipitation. Globally, the averaged annual mean temperature for regions with occurrence of the species is 20.3 °C (± 5.2) and averaged annual precipitation is 1392 mm (±632). The climatic conditions at the occurrence points differ significantly between the native range, the invaded European range, and the invaded non-European range (significance level of 0.001, Multiresponse Permutation Procedure with 999 replicates; the result holds for all three sets of environmental variables used in this study).

Tab. 2: Evaluation of model performance based on the area under the curve for the receiver-operator characteristic (AUC). AUC-values range from 0 to 1 (perfect discrimination); useful models have AUC-values above 0.7, excellent models achieve AUC-scores above 0.9. AUC values were calculated on randomly selected test and training data; the split into training and test data was replicated 100 times, reported are mean and, in brackets, standard deviation. Both global models performed best and were used for further analysis.

Evaluation of model quality via AUC-scores	Native model (trained and tested in native range)		Native model (trained in native range, tested in Europ. range		Global model (trained and Tested in global range)	
	Expert knowledge based	Statistic Based	Expert knowledge based	Statistic Based	Expert knowledge based	Statistic Based
Training data	0.93 (± 0.01)	0.94 (± 0.01)	0.94 (± 0.01)	0.94 (± 0.01)	0.91 (± 0.01)	0.90 (± 0.01)
Test data	0.91	0.91	0.72	0.70	0.90	0.89

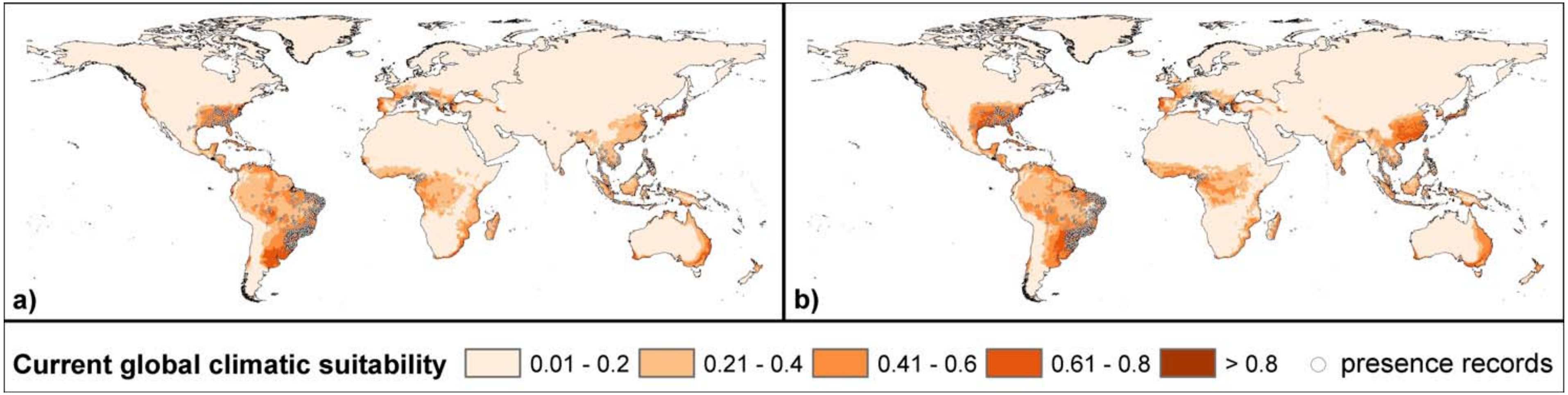

Fig. 2: Global climatic suitability, modelled based on global occurrence data under current conditions. MaxEnt suitability values range from zero to one. High values represent favourable climatic conditions for the species; values close to zero indicate unfavourable climatic conditions. Results are mapped for the global-driven: a) expert knowledge based model and b) statistic based model.

3.2. Projected climatic suitability for *Aedes albopictus* in Europe

The modelled niches for Europe differed significantly between models based on native and global occurrence records (Table 3).

Table 3: Niche similarity based on I statistic (Warren et al., 2008) for the different models, evaluated for the regional example of Europe. In all comparisons, a randomisation test indicates significant differences in the pairs of modelled niches ($p < 0.05$).

Model comparison	Niche similarity
Global expert knowledge based vs. global statistic based model	0.91
Native expert knowledge based vs. native statistic based model	0.85
Global expert knowledge based vs. native expert knowledge based	0.72
Global expert knowledge based vs. native statistic based	0.65
Global statistic based vs. native expert knowledge based	0.74
Global statistic based vs. native statistic based	0.66

Both native models that are based on records of South-east Asia do not predict the recent distribution of *Ae. albopictus* in Europe well (Table 2, Fig. 3). While the native-driven EKBM projected the north-western part of Europe (British Isles and north-west of France) as a preferable region for mosquito establishment, the SBM additionally detected Denmark and the northern part of Germany as climatically suitable.

Only the north-east of Italy and some eastern coastal Mediterranean regions are correctly predicted as climatically suitable in both native-driven models. As a consequence, European climatic suitability of *Ae. albopictus* in the 21st century was projected using the current global range of distribution as training region.

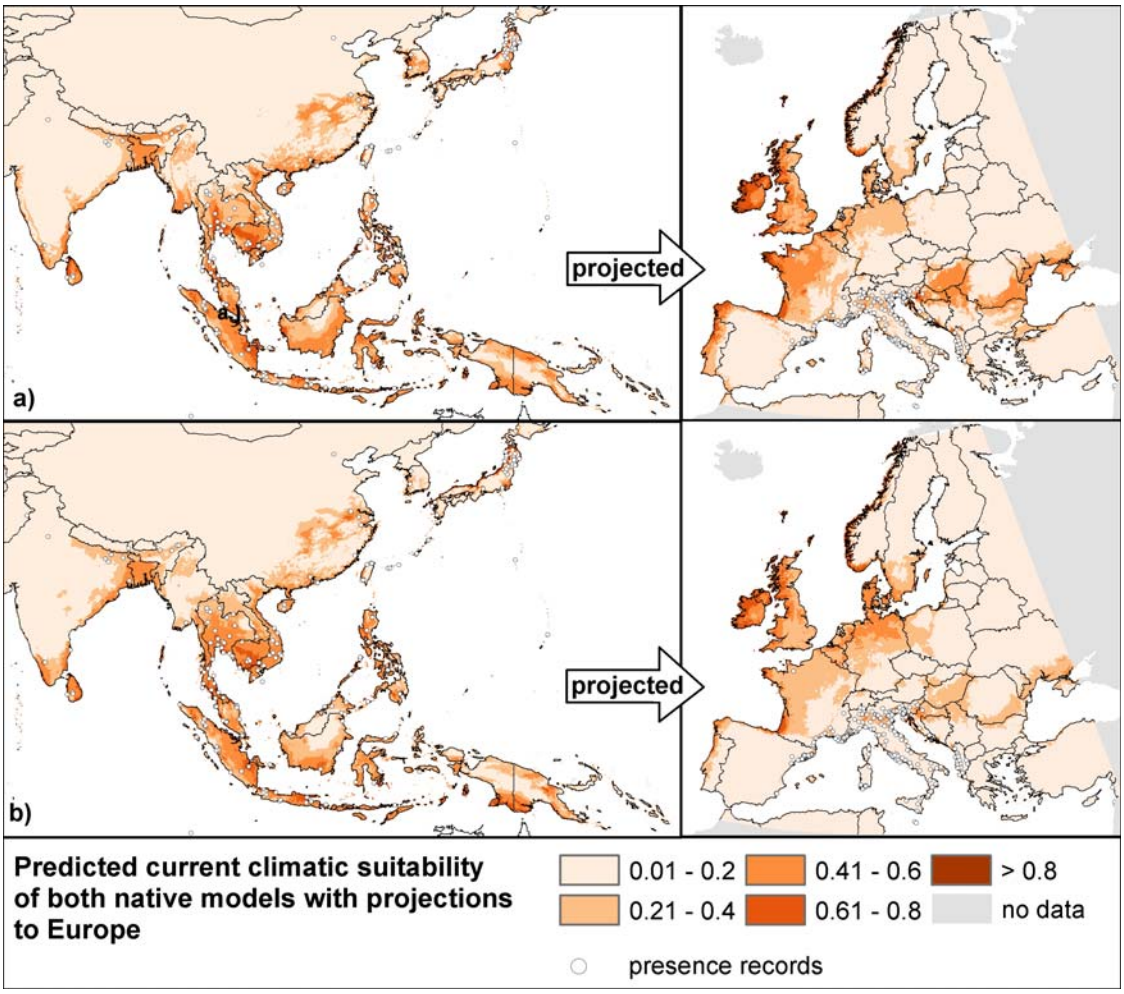

Fig. 3: Current climatic suitability modelled based on the species' occurrences in the native range and projected to the European continent. Results are mapped for the global-driven a) expert knowledge based model and b) statistic based model. Both native models failed to predict the current distribution of *Aedes albopictus* in Europe.

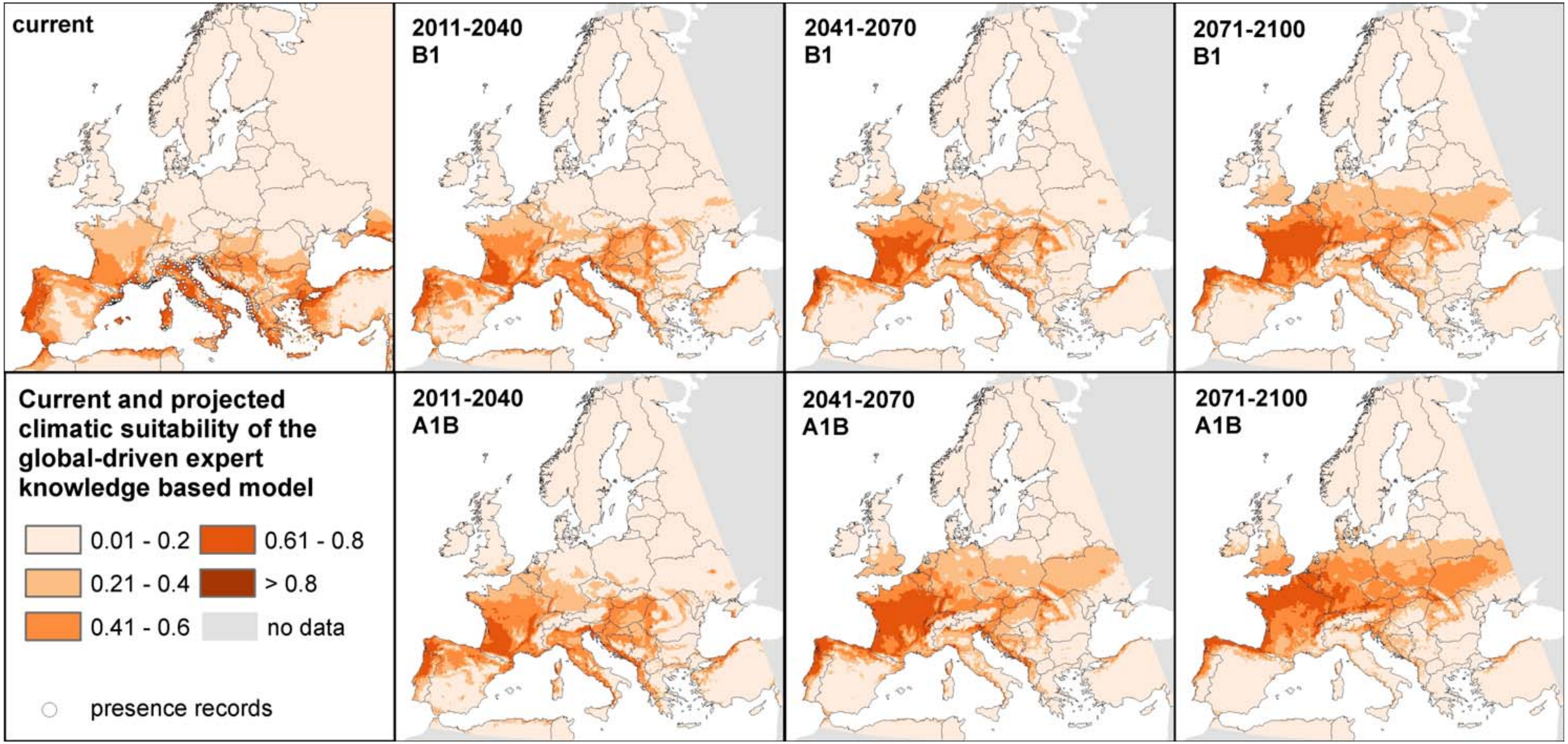

Fig. 4: Current and projected climatic suitability for *Aedes albopictus* in Europe derived from the global-driven expertknowledge based model.

Italy provides highest climatic suitability under current climatic conditions in both global models. The western Atlantic coast of Portugal, Spain and France are pointed out as climatically suitable as well, though records from these regions are still missing. Both models already project a slight decrease of climatic suitable areas in Southern Europe for 2011-2040 (Figs. 4-5). The decrease is more pronounced in south-western parts of Europe. However, Italy and south-eastern parts of Europe will still provide suitable climatic conditions for the mosquito. Passing the mid-century the Mediterranean coast of Spain seems to become unfavourable for *Ae. albopictus*.

On the other hand, climatic suitability in Western Europe is projected to increase considerably. France can be expected to become the country with the best climatic suitability, regardless of the applied model or scenario. At the end of the century, our results suggest that especially some western parts of the Mediterranean such as Spain seem to develop towards a climatically unsuitable direction for the species. Today's temperate regions of Europe will be characterised by a continental gradient of climatic suitability, with Central Europe becoming a more and more suitable habitat. The United Kingdom will be exposed to the establishment and spread of *Ae. albopictus* as well. Scandinavia is projected to remain outside of the bioclimatic niche, with the exception of Denmark, where a limited suitability is indicated at the end of the century.

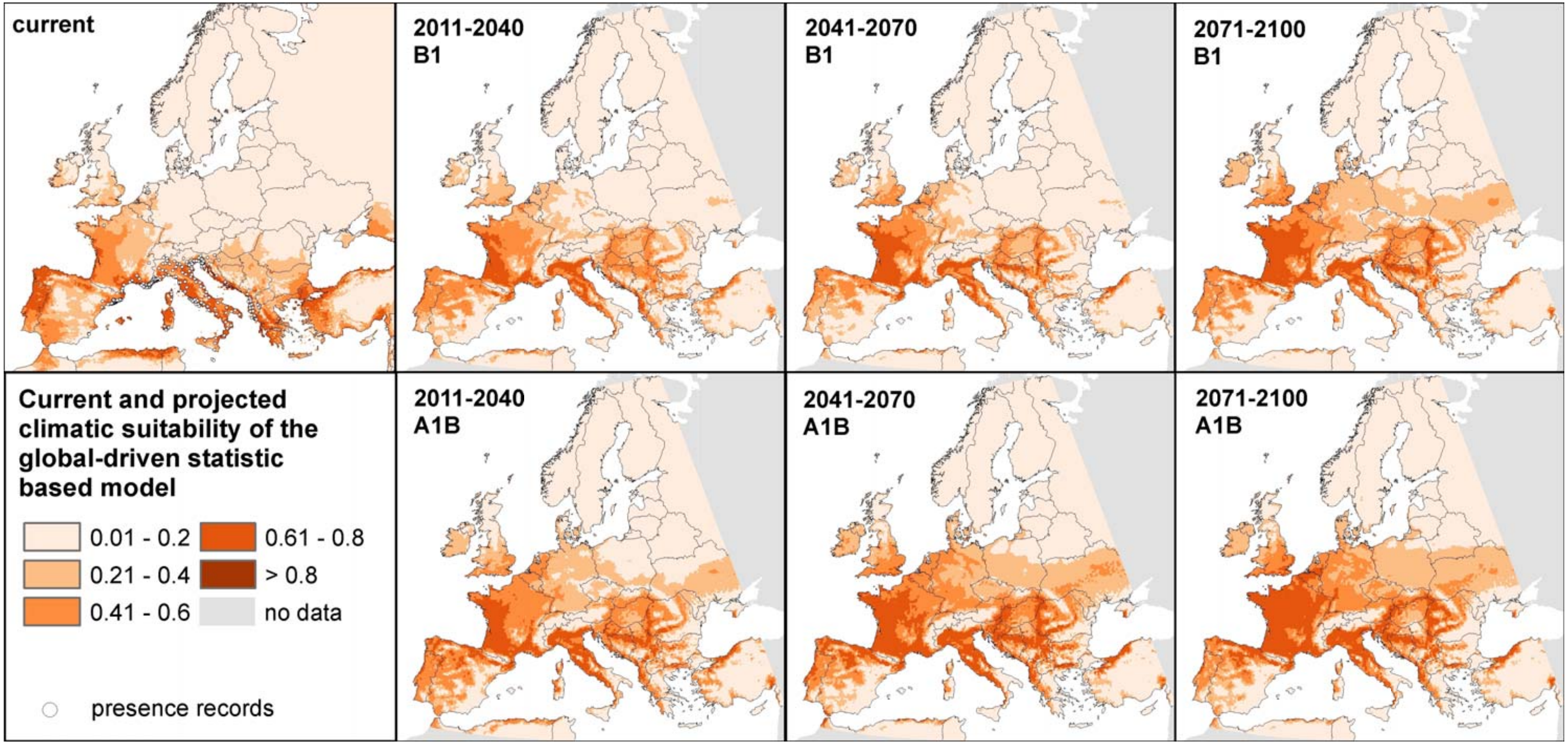

Fig. 5: Current and projected climatic suitability for *Aedes albopictus* in Europe derived from the global-driven statistic based model.

3.3. Comparison of model results

Even though the decreasing climatic suitability in the south and the increasing suitability in Central Europe are highlighted by both scenarios and models, this tendency is more pronounced in the EKBM. Notably, this model tends to attribute Central and Eastern Europe with substantially higher values of suitability throughout the 21st century than the SBM (Suppl. Fig. 1). The SBM, however, pointed out better habitat conditions for the south-west, south and south-east of Europe and additionally for the United Kingdom than the EKBM. This is true for both scenarios.

Differences between climate change scenarios are worth mentioning (see also Suppl. Fig. 2). Generally, higher values of climatic suitability for *Ae. albopictus* in both models refer to the A1B scenario. This becomes especially apparent in the SBM for Central Europe, Eastern Europe, and for the British Isles (time periods 2011-2040 and 2041-2070) and in the EKBM in the later period (2071-2100). Regionally limited areas of the Iberian Peninsula and France are detected to be more suited following the B1 scenario.

Regarding the changing climatic suitability we compare trends across different time periods. Clear temporal trends of the changing climatic suitability in projections of the EKBM exist, with a stronger emphasis in the A1B scenario (Suppl. Fig. 3). Western, Central and Eastern Europe are characterised by an increase in climatic suitability throughout the 21st century, while climatic suitability decreases in the south from one period to the following. Nevertheless, projections of the SBM do not show such a clear tendency (Suppl. Fig. 4). Although suitability also decreases generally in Southern Europe, some spatially limited regions in Central Spain and Northern Italy show increasing climatic suitability, when the time period 2011-40 is compared with current conditions. For the southern parts of Europe generally only small changes in climatic suitability are projected from the time period 2011-40 to 2041-70 in both scenarios. Instead, increasing suitability at higher latitudes is projected for the end of the century.

3.4. Climatic similarity and non-analogue climate

Climatic similarity between regions was determined by MESS analysis (Elith et al., 2010) ranging theoretically from 100 (maximum similarity) to zero (minimal similarity). Negative values represent non-analogue climatic conditions. Non-analogy may be caused either by e.g. completely divergent relationships between the chosen climatic variables in training and projected area or by completely novel relationships of the variables in the projections across space and/or time (Fitzpatrick and Hargrove, 2009). Projections of a species' climatic suitability must then be expected to be biassed. In none of our projections non-analogue climate can be identified (Suppl. Figs. 5-7). For projections of the native models to current European climate (Suppl. Fig. 5), the climatic variables selected of the EKBM show higher similarity. Regions with lowest similarity in both projections are the north-east of Italy, the coastal areas of Belgium, the Netherlands, the north-west of Germany and parts of Scandinavia as well as mountainous regions in general.

Non-analogue climate must not only be a phenomenon occurring between regions but also between time periods. But again, no European region was found to exhibit non-analogue climatic conditions in temporal projections of the two global models. The lowest values of similarity are detected in the highest mountainous regions (Alps and Pyrenees), the north-eastern parts of Italy, the coastal areas of the North Sea and the coast of Norway. Projections of the EKBM (Suppl. Fig. 6) generally result in higher values of similarity. However, these projections tend to perform dispersed spatial patterns of similarity. Using the SBM projection (Suppl. Fig. 7) France, the Iberian Peninsula and Turkey reach considerably lower values of similarity compared to the EKBM projection. Only slight deviations between scenarios and time periods were found in the results for both global climatic datasets that were used for projection.

4. Discussion

4.1. Reflection of the results and comparison to previous studies

Our aim was to identify areas that can serve as a potential habitat for *Ae. albopictus*, today and under future climate change. Two sets of bioclimatic variables were used to detect the influence of variable selections on spatio-temporal patterns of model output. In addition, we tested if records of occurrence of the former native or of the recent global range are more appropriate for predicting the current distribution in Europe.

Particularly in early stages of the invasion process, niche models are usually trained with data from the native range and applied to areas that are novel for the potentially invasive species (Mau-Crimmins et al., 2006). As invasive species are known to be adaptive (Eritja et al., 2005; Juliano and Lounibos, 2005), the habitat preferences of the species may change during the invasion process (e.g. Pearman et al., 2008; Medley, 2010). In that case, the ecology of the species in its native range cannot be directly transferred to the invaded area. On the other hand, the native range may not necessarily cover a species' entire fundamental niche (Broennimann et al., 2007).

Modelling potential spread and range dynamics based on the native habitats of a species alone has limits that can be overcome when additional data from recently occupied locations are integrated into the analyses (Broennimann and Guisan, 2008). Hence, we built native and global models in order to assess the influence on the estimated environmental niches of the invasive species. Since the global models use presence records of Europe, it was expected that they showed good results concerning the current European distribution. *Ae. albopictus* has the potential to adapt to local conditions during or after the invasion process (Medley, 2010). Hence, even using all existing species occurrence data does not guarantee per se accurate predictions of species current distribution.

We applied the regional climate model CCLM for climate projections of Europe. CCLM projects Europe to experience only a moderate warming during the first half of the century (annual mean temperature and mean winter temperatures). As mean temperature is projected to increase significantly from the midcentury onwards, certain regions reach the temperature threshold found by the global climatic envelope of the species. Precipitation, however, may be overestimated during all seasons and therefore the largest number of consecutive dry days may be higher than projected (Roesch et al., 2008), resulting in reduced habitat availability and survival rates.

An expansion of climatically suitable habitats over time could be observed in both model projections. The results raise concerns of a serious risk for the establishment of *Ae. albopictus* in Western and Central Europe. Once the species arrives there, it will be able to survive and establish permanent populations (Takumi et al., 2009). Annual mean temperature and annual precipitation are the only climatic variables that are applied in both global-driven models. Nevertheless, annual mean values should be considered as proxy-values, because the species is not active throughout the whole year in all established regions.

The risk of invasion *of Ae. albopictus* under current climatic conditions was examined in previous approaches for European countries (Knudsen et al., 1996), the Mediterranean

Basin (Mitchell, 1995), and Spain (Eritja et al., 2005). In comparison to the previously hypothesised suitable areas for establishment of *Ae. albopictus* in Spain (Eritja et al., 2005), we found a greater extent of suitable climatic conditions in the north-west (Galicia) and south-west of Spain (Western Andalucía). The European risk map of Knudsen et al. (1996) is categorised in three classes at country level: high, moderate and low risk. This does not enable for a detailed detection of suitable areas. Benedict et al. (2007) present a global risk map for the establishment of *Ae. albopictus* under current climatic conditions. When focusing on the European part of this global map it is noticeable, that the south-western areas of the United Kingdom and Ireland show a higher climatic suitability than we found in our investigation.

We observe a “coast phenomenon” beyond the one described in Benedict et al. (2007): The coastal areas of invaded continents seem to be climatically suitable for *Ae. albopictus.* This is especially true for the Americas (Pacific coastline of Washington, Oregon, California, Mexico and Chile), Europe (Atlantic coastline of Portugal, Spain and France), Africa (Atlantic coastline of Ghana, Cote d'Ivoire and for the Indian Ocean coastline of Mozambique), and for Australia (southern and western coastline). But no occurrences have yet been documented in these areas. Certainly, some of these regions have implemented pronounced mosquito monitoring and control programmes. On the one hand, these findings may indicate that introduction of the species has not yet occurred or has been avoided. On the other hand, further climate factors could play a role under oceanic climatic conditions, which contribute to suppress the mosquito populations, such as wind, sea salt aerosols, or humidity.

ECDC (2009) practised ensemble forecasting for specific years (2010 and 2030) by embedding different scenarios to detect future possible risk areas for the establishment of *Ae. albopictus* in Europe. In contrast, we used climatic averages over longer time periods, which handicap the direct comparability of the results. Nevertheless, under current conditions our global-driven models seem to project higher climatic suitability for the south-west of France and Portugal and lower suitability for Spain compared to the ECDC statistical model. Even the minimal impact scenario of the ECDC-projections (2030) determines more European regions to be climatically suitable, especially France, Belgium, Luxembourg, the Netherlands, Germany and Greece. The tendencies of the projected eastward expansion in climatic suitability in this study are in agreement with our findings.

4.2. Limitations

As with all climate impact studies, uncertainties connected to future climate projections must be taken into account (Beaumont et al., 2008). To minimise this limitation we applied two scenarios that document the respective storyline best: A1B and B1 (IPCC, 2007). Apart from overall climatic conditions, microclimate and habitat availability will strongly influence the success of *Ae. albopictus* (Romi et al., 2006; Lounibos et al., 2010). Regions that display unsuitable annual rainfall in general might nevertheless supply the mosquito with hydroponic facilities when ambient conditions are dry (Romi et al., 2006). In the same way indoor hibernation could protect the mosquito from cold extremes. Such aspects are difficult to account for on the regional scale (Kysely and Beranova, 2009). The short-term availability of suitable conditions at small scales can be responsible for local establishment events under very special conditions. In the Netherlands, *Ae. albopictus* was monitored in greenhouses of companies that imported “Lucky Bamboo” (Dracaena sanderiana) (Scholte et al., 2007). However, such anthropogenic factors cannot be covered with our analysis. Nevertheless, the number of records used in this study as well as the large geographical extent of data provides a sound basis for the detection of robust large scale patterns.

In addition to spatial aspects of uncertainty, temporal variability is relevant. Besides changes in climatic trends, extreme weather events are also expected to increase in magnitude and frequency (Semmler and Jacob, 2004; Jentsch and Beierkuhnlein, 2008), so that a temporal window of opportunity for an invasive vector could arise. Up to now, both aspects can hardly be reflected and projected accurately in climate models (Beniston et al., 2007).

Using only climatic variables as explanatory variables for scenarios may be problematic (Dormann, 2007; Wiens et al., 2009): Land use and land cover can modify the realised ecological niche in a certain region, but become more important for modelling species distribution on smaller spatial scales (Pearson and Dawson, 2003). Knowledge on interspecific competition, predation as well as quantitative life history traits may improve the understanding of the invasion processes (Juliano et al., 2004; Juliano and Lounibos, 2005; Armbruster and Conn, 2006; Armistead et al., 2008; Juliano, 2009) and could provide input data for process-based models of invader spread. As the mosquito adapts rapidly to its environment the ecological niche models will have to be updated iteratively. Mutations and even epigenetic responses have to be considered. However, this kind of knowledge is not available yet.

On a more technical level, a further source of uncertainty stems from spatial autocorrelation, which MaxEnt - at least currently - cannot take into account. Spatial autocorrelation in the residuals may for example bias parameter estimates, and lead to optimistic estimates of model performance. Based on Moran's I, we detected statistically significant spatial autocorrelation in the residuals for all models. However, estimates of AUC using 32-fold spatially structured cross-validation (e.g. Reineking et al., 2010), which is expected to be less affected by spatial autocorrelation, were only slightly lower than those by the 70/30 splitting approach. This indicates limited bias in our estimates of model performance.

4.3. Relevance

Even when considering the limits of extrapolative niche modelling, environmental envelope models remain a powerful tool to envisage potential responses in species distribution to climate change (Wiens et al., 2009).

Our projections refer to the regional model CCLM, which is integrated into the well established global simulations of ECHAM5 (Rockel et al., 2008). In comparison to their driving models, regional models project patterns of climate change at a higher spatial resolution. This is especially relevant in climate impact studies on human health, where precise geographical information is needed (Giorgi and Diffenbaugh, 2008). In particular, small-scale heterogeneity has to be considered in studies on vector-borne diseases (Jacob, 2008; Fischer et al., 2010b). We detected those regions of Europe that are especially endangered regarding a potential establishment of *Ae. albopictus* under current conditions. Subsequently, we projected for the first time geographic patterns of climatic suitability for the mosquito that can be expected to develop during the entire 21st century. These risk maps of potentially suitable areas for the establishment may serve as a valuable support for the design of monitoring and control activities. These can contribute to avoid the further spread of the disease vector and prevent the human population from unexpected disease outbreaks. Knowledge of potential future occurrences of the vector *Ae. albopictus* becomes especially relevant regarding the increasing European areas that are expected to provide suitable temperatures for dengue-virus amplification in the 21st century (Thomas et al., 2011).

Projections of species distribution in regions of non-analogue climate are a common, but still a rarely addressed problem in species distribution modelling. The consequences can be ecologically and statistically invalid studies (Fitzpatrick and Hargrove, 2009). To assess the problem of non-analogue climate, we re-analysed our dataset using the MESS analysis (Elith et al., 2010). No regions with nonanalogue climate were detected.

5. Conclusions

Our findings indicate an increasing risk of establishment by *Ae. albopictus* especially for the Atlantic Coast of the Iberian Peninsula and for the south-west of France. In addition to the detection of already potentially appropriate areas, we find additional areas of potential future establishment of *Ae. albopictus*. It is possible that the mosquito has already colonised larger areas than noticed. Large areas of Western and Central Europe that are inappropriate for the species today are projected to change during the 21st century towards a climate that can support the survival of the species. Once the species is established, it is very difficult to control.

However, unintended anthropogenic introduction (e.g. by shipping goods) can be expected as a constant source of insecurity and will very likely contribute consistently to the introduction of mosquito populations especially close to the hubs of infrastructure (harbours and large railroad terminals). Therefore, we believe in efficiency of monitoring schemes for Europe, in order to be able to limit the spread of *Ae. albopictus* and the diseases that can be transmitted by this vector.

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Supplemental - Reference list

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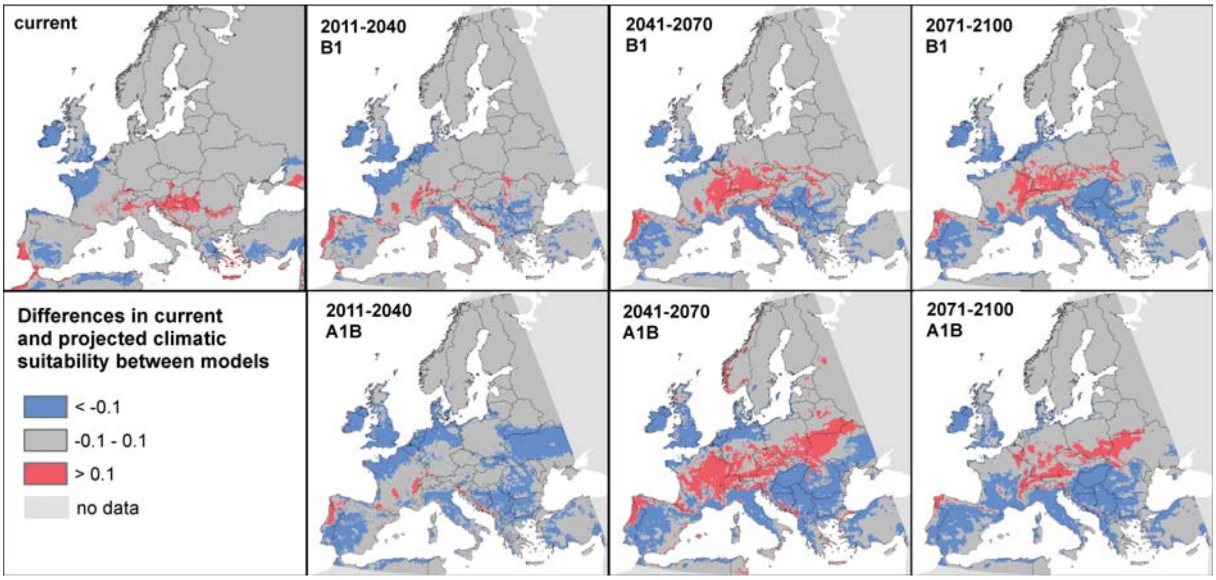
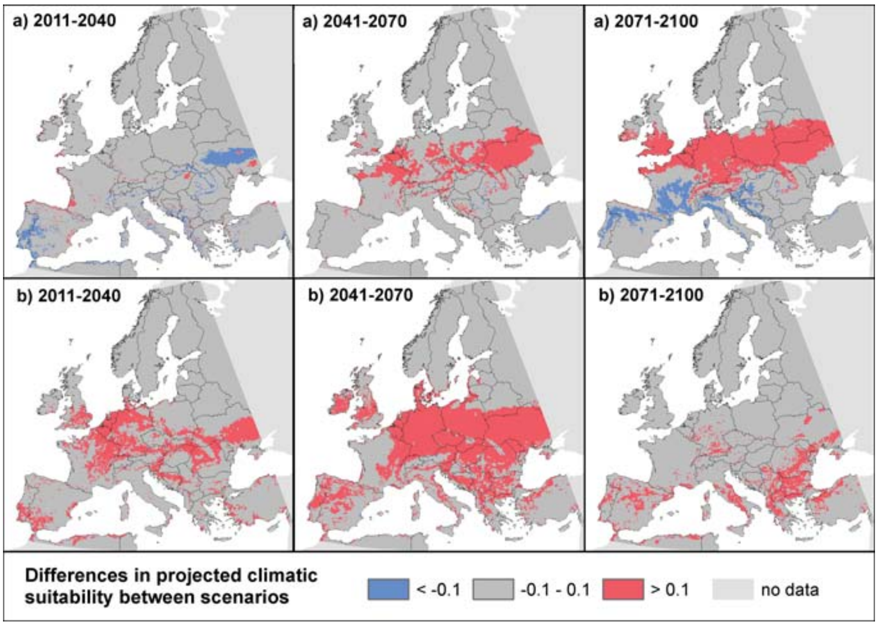
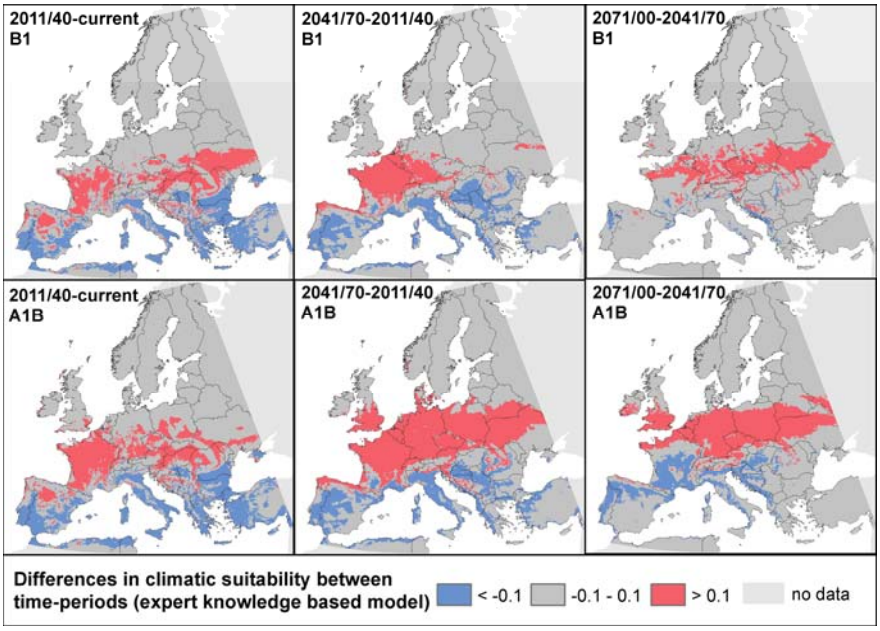
Supplemental Material Figures



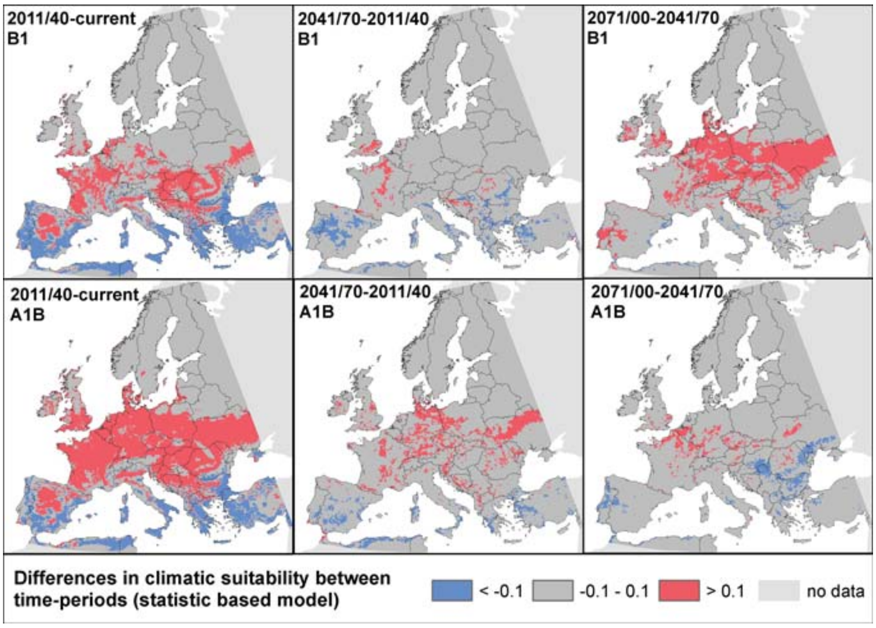
Suppl. Fig. 1: Differences in results between global-driven models. The computed values of climatic suitability from the statistic based model were subtracted from the results of the expert knowledge based model for each scenario and time period separately.



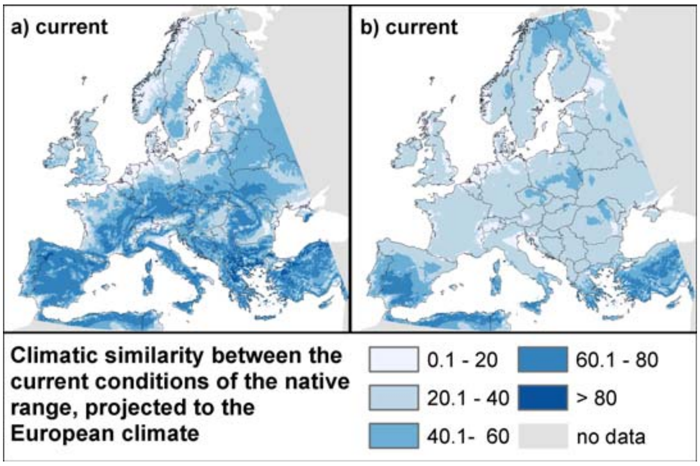
Suppl. Fig. 2: Differences in results between scenarios of global-driven models. The computed values of the climatic suitability for the B1 scenario were subtracted from the results of the A1B scenario for each model and time period separately. Differences are mapped for the a) expert knowledge based model and b) statistic based model.



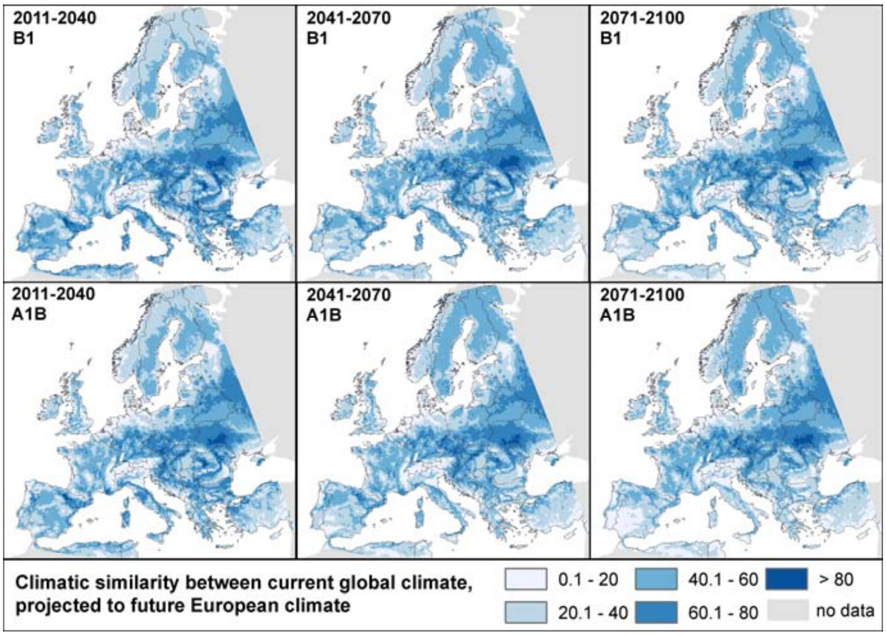
Suppl. Fig. 3: Changing climatic suitability in Europe, comparing the different time periods, calculated for the global-driven expert knowledge based model. The computed values for each time period were subtracted from the following one for each scenario separately.



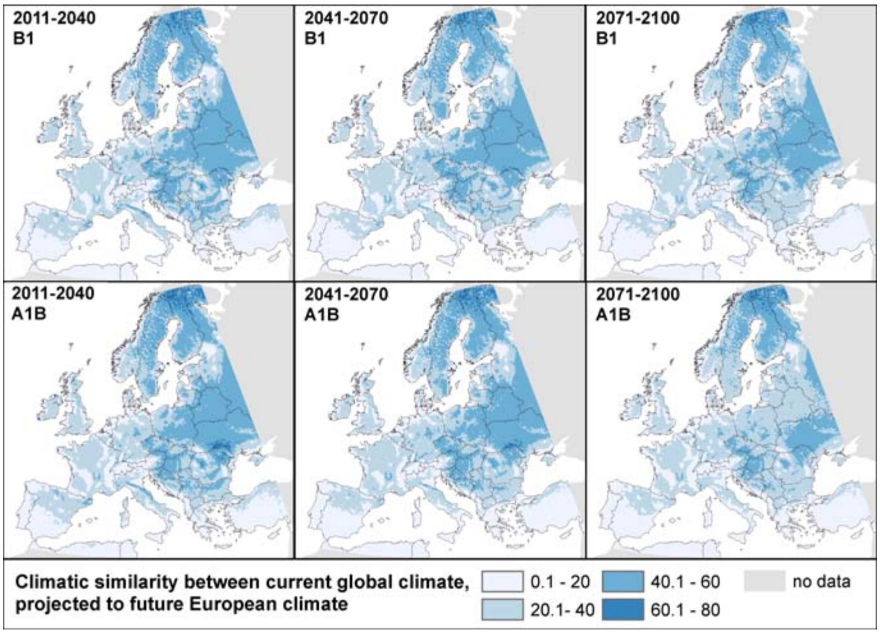
Suppl. Fig. 4: Changing climatic suitability in Europe, comparing the different time periods, calculated for the global-driven statistic based model. The computed values for each time period were subtracted from the following one for each scenario separately.



Suppl. Fig. 5: Climatic similarity detected via Multivariate Environmental Similarity Surface analysis by projecting the a) expert knowledge based and the b) statistic based native models to the current European conditions. High values represent high climatic similarity. Non-analogue climate was not detected.



Suppl. Fig. 6: Climatic similarity detected via Multivariate Environmental Similarity Surface analysis by projecting the global expert knowledge based models to future European climatic conditions. High values represent high climatic similarity. Non-analogue climate was not detected.



Suppl. Fig. 7: Climatic similarity detected via Multivariate Environmental Similarity Surface analysis by projecting the global statistic based models to future European climatic conditions. High values represent high climatic similarity. Non-analogue climate was not detected.

Climatic suitability of *Aedes albopictus* in Europe referring to climate change projections:

Comparison of mechanistic and correlative niche modelling approaches

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6.2 Article 2:

Climatic suitability of *Aedes albopictus* in Europe referring to climate change projections: Comparison of mechanistic and correlative niche modelling approaches

With 2 Tables and 2 Figures

Abstract

The Asian tiger mosquito, *Aedes albopictus*, is capable of transmitting a broad range of viruses to humans. Since its introduction at the end of the 20th century, it has become well established in large parts of southern Europe. As future expansion as a result of climate change can be expected, determining the current and projected future climatic suitability of this invasive mosquito in Europe is of interest. Several studies have tried to detect the potential habitats for this species, but differing data sources and modelling approaches must be considered when interpreting the findings. Here, various modelling methodologies are compared with special emphasis on model set-up and study design. Basic approaches and model algorithms for the projection of spatio-temporal trends within the 21st century differ substantially. Applied methods range from mechanistic models (e.g. Overlay of climatic constraints based on Geographic Information Systems or rather process-based approaches) to correlative niche models. We conclude that spatial characteristics such as introduction gateways and dispersal pathways need to be considered. Laboratory experiments addressing the climatic constraints of the mosquito are required for improved modelling results. However, the main source of uncertainty remains the insufficient knowledge about the species´ ability to adapt to novel environments.

Background

In recent years, European awareness concerning the introduction and establishment of invasive mosquitoes has increased, most notably due to the incursion of the Asian tiger mosquito, *Aedes albopictus* – the most invasive disease vector globally [1,2]. This mosquito has spread from its original distribution area in south-east Asia [3] to all continents via shipping of goods [4]. After its initial introduction to Europe at the end of the 20th century, *A.*

albopictus became well established in southern Europe [2]. Recent observations hint towards a spread of this vector to the continental interior of Europe [5]. This mosquito is capable of transmitting several viruses that are pathogenic to humans [1,6]. Most strikingly, *A. albopictus* was the vector that caused the first autochthonous transmission of Chikungunya [7,8] and dengue [9-11] in the Mediterranean area. More recently, a dengue outbreak occurred in the autonomous region of Madeira, Portugal. In this case, A. aegypti, the yellow fever mosquito, acted as the vector [12].

Several studies have aimed to determine the climatic suitability of *A. albopictus* for the outgoing 20th and the beginning 21st century [13-15] as well as the expected future tendencies in Europe [16-19]. Most recently, Caminade et al. [19] implemented three established modelling approaches [14,16,20], making use of new observations, climate data and a report on model quality. However, a comparative methodological evaluation of the different approaches was still missing. Here, we provide a comprehensive comparison of studies assessing the climatic suitability of European regions for *A. albopictus* as a result of a rapidly changing climate during the 21st century. General information as well as limitations in study design and data quality is highlighted. Uncertainties related to climate change and insect vectors are identified. In so doing, we aim to provide guidance for future research.

Review of distribution models for *Aedes albopictus*

We identified studies (up to 2012) aiming to determining the potential future distribution of *A. albopictus* in Europe as a consequence of climate change. However, we bring them into a bigger context, as we account for the (methodological) development in the creation of risk maps of *A. albopictus* in order to understand the philosophy behind the work more intuitively. Therefore, some studies from different regions are described initially, in case that they introduced a specific method or task on which subsequent studies for Europe are built on.

Generally, two methodological approaches seem to be appropriate for the projection of climatic suitability of European habitats for *A. albopictus*: mechanistic models and correlative niche models. Mechanistic models do not require geographical occurrence data for species. They are either based on the construction of overlay functions for climatic constraints in Geographic Information System (GIS) environments or process-based models with mechanistic principles. The aim of such models is to simulate and project the response of an individual organism or a population by explicitly incorporating biological processes calibrated with observations on individuals in natural populations and controlled field or laboratory studies [21]. Thus, mechanistic models rely on the implicit assumption that the model structure and process formulations are correct [22].

A second, rather statistical approach is the use of correlative environmental niche models. Here, species presence and, in some approaches, also absence locations are related to environmental or climatic variables with the aim of determining species-specific niche (synonymously used: envelope) that is defined by the parameter values - including the multivariate combinations - from the known occurrences. This niche can be interpolated or extrapolated to infer species´ geographical distribution. Advanced modelling techniques offer novel opportunities for the determination of species changing spatial distribution patterns as a response to environmental and climatic changes [23]. The main issue with correlative models is their dependence on the amount, quality and relevance of the data used [22]. Commonly, niche-modelling algorithms require presence as well as absence records. However, some models make use of pseudo-absence data or even presence-only data, as in many cases, absence data are not available. The lack of absence data may also suggest that areas where the species is missing might be suitable, but the insect may simply not be present yet. Consequently, presence-only models are appropriate to handle most of the data for mobile and invasive insects in the course of climate impact research.

Distribution models devoid of climate change projections

Several studies identified past or current climatic suitability for *A. albopictus*, based either on mechanistic [13,14,20] or correlative [15,16,24,25] distribution modelling approaches. Here, we highlight studies with relevance for Europe.

Mechanistic approaches

Kobayashi et al. identified a close connection with the annual and January mean temperature for the distribution of *A. albopictus* in northern Japan [20]. In addition, a period with daily temperature continuing above at least 11 °C during summer months (more than

186 days per year) was observed and interpreted as a requirement for larval development.

The first GIS-based risk maps were developed by Mitchell [13] for the Mediterranean Basin. Expert knowledge on temperature, rainfall and humidity as well as the photoperiod was applied in order to frame climatic constraints. For the United Kingdom, Medlock et al. [14] used temperature and daylight thresholds to simulate life cycle dynamics via overlay functions in GIS. Furthermore, they created different scenarios by altering the diurnal length of the photoperiod. This was done to assess the ability of eggs to survive in winter and predict the hatching in spring and the subsequent production of diapausing eggs in autumn. Consequently, the potential responses to these alterations in mosquito life cycle can be determined. It should be noted that the scenarios of Medlock et al. [14] do not refer to scenarios announced in the Special Report on Emissions Scenarios (SRES) [26] from the Intergovernmental Panel on Climate Change (IPCC). In the technical report of the European Centre for Disease Prevention and Control (ECDC) Development of *Aedes albopictus* risk maps [16], the approach from Medlock [14] is adapted, but was expanded to cover Europe entirely . In this ECDC report [16], two further modelling approaches were used: one further mechanistic and one correlative approach, which are described below.

Correlative approaches

Presence/absence models

Many niche modelling algorithms require both documented presence, as well as absence localities in order to build statistical relationships. In its report, ECDC deployed random forest models (based on regression trees) in order to estimate the current climatic suitability for *A. albopictus* in Europe [16]. In short, random forest is an ensemble classifier that consists of combined decision trees and gives the class that is the mode of the classes by individual trees as an output. Centroids (Geometric centers) of the European municipalities were used as presence or absence localities [27]. It should be noted that these municipalities differ in their spatial extent. The average area calculated from the political boundaries of the municipalities in Southern Europe (e.g. Italy or Spain) may be up to three times bigger than in Central Europe (e.g. Germany), which limits the ability to account for landscape heterogeneity. The centroids indicating species presences or absences are correlated with 57 (standardised) climate data layers, from which four variables are chosen as predictors via a backward stepwise procedure. All selected predictors are related to temperature.

Another approach recently published in a later ECDC technical report, The climatic suitability for dengue transmission in continental Europe, is based on multivariate discriminant analyses [15]. Again, this approach concentrates on modelling the current climatic suitability for *A. albopictus*. Here, global occurrence of this species was used as a model input. Accounting for the global dimension offers the opportunity to include the entire environmental space occupied by the species. However, this neglects the role of adaptation in regional populations. As discriminant analyses require absence records, (global) pseudo-absences were generated by evaluating localities that were geographically and environmentally dissimilar to presences. The models aim to discriminate between these two categories using the predictor variables available. The final risk maps were produced by averaging over 100 bootstrap samples [15].

Presence-only models

Many insect databases rely on documented presence localities, especially if a species is globally distributed. As the generation of pseudo-absences is ambitious [see 15], novel ways to cope with presence-only data have been developed. In presence-only models, relationships are based on comparison of a species presence with the environmental background. Within this environmental background, the species were not recorded, which could also mean that data collection was not attempted in the respective region. Thus, at those sites, no information on the suitability of the environment or climate exists.

Employing the correlative environmental niche model Genetic Algorithm for Rule-set Prediction (GARP), Benedict et al. determined the global risk of invasion by *A. Albopictus* [24]. A model built with GARP is iteratively chosen from non-random correlations between environmental and occurrence data. The non-random correlations describe environmental thresholds, depending on the chosen type of mathematical rule. Apparently, *A. albopictus* occupies different environmental niches on the invaded continents, which is revealed by Medley by applying correlative niche models for isolated geographical occurrence localities from the native and invaded range [25]. For all comparisons, the niche for introduced distributions was not equivalent to the native niche. For this purpose, Medley [25] applied the Maximum Entropy approach (implemented in MaxEnt software) [28]. MaxEnt has replaced

GARP as a preferred modelling algorithm for presence-only data during the past years, due to improved model performance [23]. The idea behind MaxEnt is to find the probability distribution of maximum entropy (most spread out) that is subject to constraints imposed by information available on the species presence and the environmental conditions across the study area [28,29].

Distribution models that consider climate change projections

Until 2012, four studies exist have aimed to determine potential future climatic suitability of *A. albopictus* in Europe (summarised in Table 1 and 2) [16-19]. In two studies [16,19], climatic suitability was projected via mechanistic models, while the results of the two other studies [17,18] were based on correlative approaches. One study [18] was applied to a limited study region, while the other three [16,17,19] cover the entire European continent. In order to detect methodological qualities and constraints, these studies are compared in detail.

Tab. 1: Studies addressing current and projected climatic suitability of *Aedes albopictus* in Europe.

Study	Region	Model	Input data: climate/environmental	Validation or data/model predictive power	Climate projection or climate model	Scenario	Time step
Medlock et al. 2006 [14]	UK	GIS overlay (MA)	• Climate data: annual mean rainfall and monthly mean temperature from 1971 to 2000 provided by the UK Meteorological Office (1 km) • Weekly weather data: derived from monthly temperature data using a continuous piecewise quadratic function	–	Own alteration	Own scenarios	–
ECDC 2009 [16]	Europe	Random forest (CA)	• World climatic zones • Temperature data archive at the University of Daytona, US: daily mean temperatures (1995–2007) • MODIS: day- and night-time LST (1 km) • CRU: monthly mean temperatures and rainfall variables averaged from 1961 to 1990 (5 km) • NDVI and EVI (1 km)	• n=1,525 (presences and absences, due to centroids of municipalities) • training sample (n=300), divided over both the presence (n=165) and absence (n=135) • AUC	No projection	–	–
	Europe	GIS overlay (CA) sensu Medlock et al. [14]	• Same climate data source as for the random forest (CA)	–	No projection	–	–
	Europe	MCDA (CA)	• Same climate data source as for the random forest (CA)	–	According to IPCC (no further details)	Minimal and maximum impact scenarios	2010, 2030
Fischer et al. 2011 [17]	Europe	MaxEnt (CA)	• Worldclim: 19 bioclimatic variables derived from monthly temperature, rainfall values and altitude (10 km)	• Presence point data worldwide (n=1,199) • Randomly selected test (30%) and training (70%) data; the split into	Regional climate model COSMO-CLM rescaled to 10 km	A1B^1 B1^1	2011–2040, 2041–2070, 2071–2100

				training and test data was replicated 100 times • AUC			
Roiz et al. 2011 [18]	Trentino (north-east Italy)	GLM (CA)	• Daily LST (MODIS Terra and Aqua satellites), reprojected to 200 m • Human population data from official population census (2001) and from Landscan Global Population Database	• Absence and presence point data at 145 sample stations • AIC	No specific climate model: increase in mean January temperature (1.5 °C) and mean annual temperature (1 °C) with respect to reference period 1961–1990	A2[1]	2040–2050
Caminade et al. 2012 [19]	Europe	GIS overlay (MA) sensu Kobayashi et al. [20] and Medlock et al. [14], MCDA sensu ECDC [16]	• Gridded climate dataset based on station measurements at daily and monthly temporal resolution (25 km^2)	• Absence and presence data at the regional administrative level of the European Union • AUC	10 selected regional climate models (ensembles), 0.25 ° step: C4IRCA3, CNRM-RM4.5, DMI-HIRAM5, ETHZ-CLM, ICTP-RegCM3, KNMI-RACMO2), METO-HC-HadRM3.0, MPI-M-REMO, OURANOSMRCC4.2.1, SMHIRCA	A1B	2030–2050
ECDC 2012 [15]	Europe	Non-linear discriminant analysis (CA)	• Fourier transformation of MODIS temperature (Terra satellite) and elevation data • Worldclim data • Human population density from Global Rural-Urban Mapping Project	• Thousands of occurrence records via existing databases and own literature search (for *A. albopictus* and *A. aegypti*) • Generation of pseudo-absences via environmental (MD) and geographical distance measure	No projection	–	–

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[1] Emissions Scenarios are based on IPCC Special Report on Emissions Scenarios (SRES), where different storylines describe the relationships between the driving forces of climate change: The A1B scenario describes a future world of very rapid economic growth, global population that peaks in mid-century and declines thereafter, and the rapid introduction of new and more efficient technologies The A2 scenario assumes a continuously increasing global population. The economic development is primarily regionally oriented and per capita, economic growth and technological changes are more fragmented and slower than in other storylines. The B1 scenario is based on the assumption that economic structures will change rapidly towards a service and information economy, and resource-efficient technologies will be introduced [26].

AIC: Akaike´s Information Criterion; AUC: (AIC), Area under the Receiver Operator Characteristic Curve; CA: Correlative Approach; CRU: Climate Research Unit; ECDC: European Centre for Disease Prevention and Control; EVI: Enhanced Vegetation Index; GIS: Geographic Information System; IPCC: Intergovernmental Panel on Climate Change; LST: Land Surface Temperature; MA: Mechanistic Approach; MCDA: Multi Criteria Decision Analyses; MD: Mahalanobis Distance; MODIS: Moderate Resolution Imaging Spectroradiometer; NDVI: Normalised Difference Vegetation Index UK: United Kingdom; US: United States.

Information concerning input data is given including: climate variables, model validation and source and steps, e.g. of climate data for the respective emission scenario as well as addressed future time steps.

Tab. 2: Variables and model set-up in studies addressing current and projected climatic suitability of *Aedes albopictus* in Europe.

Study	Variables	Method
Medlock et al. 2006 [14]	**Overwintering criteria** - Mean January temperature >0 °C - Annual mean rainfall > 500 mm **Spatio-temporal activity** **Scenario 1** Low risk - Spring mean temperature 10–10.5 °C - Spring photoperiod 11–11.25 h (daylight) - Temperature for cessation of egg/larval activity <9.5 °C - Critical photoperiod for autumn diapause 13–13.25 h Medium risk - Spring mean temperature 10.5–11 °C - Spring photoperiod 11.25–11.5 h - Temperature for cessation of egg/larval activity 9.5–10 °C - Critical photoperiod for autumn diapause 13.5–14 h High risk - Spring mean temperature >11 °C - Spring photoperiod >11.5 h - Temperature for cessation of egg/larval activity >10 °C - Critical photoperiod for autumn diapause >14 h **Scenario 2** - Critical photoperiod for autumn diapause 11 h, 11.5 h and 12 h for high, medium and low risk, respectively. The other three parameters stay the same. Photoperiod is based on astronomical equations of sunrise and sunset.	**GIS-based overlay** Assessing the potential for survival and spatio-temporal activity dynamics (number of weeks between the first hatching of overwintered eggs in spring and the production of diapausing eggs)
ECDC 2009 [16] GIS overlay	**Adapted by Medlock et al. [14] but no overwintering criteria** - Critical photoperiod for autumn diapause 13.5 h - Spring photoperiod 11–11.5 h - Spring mean temperature 10–11 °C	**GIS-based overlay sensu Medlock et al. [14]**
ECDC 2009 [16] Random forest	**Four predictor variables chosen from 57 data layers** - Maximum night-time LST - Mean annual daytime LST - Minimum daytime LST - Second amplitude of daytime LST	**Random forest** - 200 aggregated classification trees for classification - Stepwise backward reduction of the number of variables until accuracy dropped below 90%.

ECDC 2009 [16] MCDA	**Annual mean rainfall** - No suitability <450 mm - Maximum suitability >800 mm **Summer temperature (June–August)** - No suitability <15 °C or >30 °C - Maximum suitability 20–25 °C **Mean January temperature** - No suitability <–1 °C - Maximum suitability >3 °C	**MCDA** - Sigmoidal transformation of mean annual rainfall and temperature in January - Symmetrical sigmoidal transformation of summer temperatures - Linear combination for suitability data layers, whereby each factor was assigned with equal weight
Fischer et al. 2011 [17] Expert knowledge-based model	**Selection from 20 bioclimatic variables** - Annual mean temperature - Mean temperature of the warmest quarter - Mean temperature of the coldest quarter - Annual precipitation - Altitude	**MaxEnt** - Selection of variables based on expert knowledge
Fischer et al. 2011 [17] Statistic based model	**Selection from 20 bioclimatic variables** - Annual mean temperature - Annual precipitation - Precipitation of the warmest quarter - Precipitation of the coldest quarter - Altitude	**MaxEnt** - Jackknife test to measure variables´ importance - Calculations of models´ training gains for variables´ in isolation and for remaining dataset if this variable is dropped
Roiz et al. 2011 [18]	**Survival of overwintering eggs** January mean temperature >0 °C Annual mean temperature >11 °C Highly suitable: January mean temperature ($\text{JanT}_{\text{mean}}$) >0 °C and Annual mean temperature ($\text{AnnT}_{\text{mean}}$) >11 °C Moderately suitable: $\text{JanT}_{\text{mean}}$ >0 °C and $\text{AnnT}_{\text{mean}}$ <11 °C or $\text{JanT}_{\text{mean}}$ <0 °C and $\text{AnnT}_{\text{mean}}$ >11 °C Unsuitable: $\text{JanT}_{\text{mean}}$ <0 °C and $\text{AnnT}_{\text{mean}}$ <11 °C **Human population data** - Human population density - Distance to human settlements	**GLM with binomial distribution (multiple logistic regression)** - Relating species’ presences/absences to variables

Caminade et al. 2012 [19] Model 1	**Annual mean temperature** - Totally suitable >12°C - High risk 11–12 °C - Moderate risk 10–11 °C - Low risk 9–10 °C **Overwintering criterion** Highly unsuitable - Mean January temperature <0 °C - Annual mean rainfall <500 mm Medium unsuitable - Mean January temperature 0–1 °C - Annual mean rainfall 500–600 mm Low unsuitable - Mean January temperature 1–2 °C - Annual mean rainfall 600–700 mm Suitable - Mean January temperature >2 °C - Annual mean rainfall >700 mm	**GIS-based overlay sensu Kobayashi et al. [20]**
Caminade et al. 2012 [19] Model 2	See **ECDC [16]** (MCDA)	**MCDA sensu ECDC [16]**
Caminade et al. 2012 [19] Model 3	**Overwintering criterion (see model 1)** **Weeks of activity** - Mean weekly temperatures - Mean weekly photoperiods **Hatching onset (medium scenario)** - Spring temperature >10.5 °C - Photoperiod >11.25 h **Autumn diapause** - Temperature >9.5 °C - Photoperiod >13.5 h	**GIS-based seasonal activity model sensu Medlock et al. [14]** - Overwintering criterion to mask the areas where the mosquito would not be able to survive - Photoperiod calculation as the period between sunrise and sunset - Computation of the start of spring hatching and autumn egg diapause is based on medium scenario
ECDC 2012 [15]	**Clear documentation of pre-processing MODIS data; no further information about the chosen variables**	**Non-linear discriminant analysis** - Preliminary k-means cluster analysis to analyse outliers in training set for exclusion in modelling process - 100 random bootstrap samples with equal number of presences and absences - Stepwise inclusion of 10 environmental variables - 100 results were averaged to produce the final risk maps

GIS: Geographic Information System; GLM: Generalised Linear Model; LST: Land Surface Temperature; MCDA: Multi Criteria Decision Analyses.

Mechanistic approaches

Within the technical report of the ECDC, a mechanistic Multi Criteria Decision Analysis (MCDA) was performed [16]. In contrast to the correlative approaches of this report, the results of the MCDA were projected to future conditions. A MCDA is a structured tool within a decision support framework. This enables evaluation of multiple decision constraints based on previously defined estimation criteria. The exploration of such decision alternatives for complex problem settings was recently developed within GIS frameworks in order to achieve accurate spatial risk assessment of vectors and vector-borne diseases [30]. In order to detect climatic suitability for *A. albopictus*, sigmoidal or symmetric sigmoidal membership functions were generated for the standardised variables and combined linearly with equal weight [16]. This was done based on expert advice. Generally, MCDA applications for spatial pattern analysis offer an opportunity to identify gaps and limits in knowledge; however, they are limited in determining causality [30]. Projections were applied for the MCDA approach and applied to the expected situation in 2010 and 2030, using SRES-scenarios with minimal or maximal impact [25]. Detailed information concerning the climate model and scenario characteristics was not given.

The mechanistic approaches used by Kobayashi et al. [20], Medlock [14] and the MCDA by ECDC [16] were adapted by Caminade et al. [19]. In contrast to previous approaches, Caminade et al. evaluated model performance via the area under the receiver operator characteristic curve (AUC) [19]. AUC is based on signal detection theory and illustrates the performance of a binary classifier system when the discrimination threshold varies. Hence, it is typically used to determine performance of correlative niche models. Although it is a mechanistic approach, presence and absence localities based on centroids created from administrative level [are generated [27]. These data were used as an evaluation of their results of the mechanistic classification in order to measure model performance. A novel feature was that Caminade et al. considered the role of climate change in Europe in past years (1960–1989, 1990–2009, 2005–2009) in the spread of the mosquito [19]. Furthermore, ensemble data of climate change projections were used, which were given by 10 regional climate models. Regional climate models are driven, at their boundaries, by global climate models. Employing ensemble data enables variations of future projections to be assessed and, consequently, reduces uncertainty [31]. Usually, projections based on ensemble data include a multitude of potential variations by averaging over all possible developments. In the study of Caminade et al., projections were solely based on the A1B emission scenario [19]. The A1 storyline describes a future world with very rapid economic growth and a rapid introduction of new and more efficient technologies. Thereby, the global population peaks mid-century and declines thereafter. In the A1B scenario, a balanced use across all energy resources is expected [26].

Correlative approaches

Previous findings hint towards niche shifts of *A. albopictus* during the global invasion process [25]. In order to account for this, Fischer et al. applied two models built on presence-only data beyond the European distribution with MaxEnt [17]. Firstly, global occurrence was used for training. Secondly, the native (Asian) distribution served as a training region. Both models were tested for the current European climatic conditions. The database contains more than 6,000 occurrence records of which 1,200 were selected as model input. The initial database was reduced by using geographically weighted correction to minimise spatial bias and autocorrelation in data. Geographically explicit point localities were taken from the literature and completed with presences reported on county level from the United States for the generation of the global database. The problematic issue with political or administrative borders in datasets was mentioned before. While the native range models, containing the Asian distribution and environments, fail to predict the current distribution in Europe, the global-trained model predicts the current European distribution with highly satisfactory quality. This suggests the use of the entire ‘climatic niche’ for projections. Two sets of bioclimatic variables provided by WorldClim (global climate data) [32] were used as model input. The first set was based on expert knowledge on species’ ecology. The second set was chosen via statistical tests to determine the highest explanatory power of the model. All models were validated with AUC values. As both the expert knowledge- and statistical-based models of the global range yield high AUC values, they were both projected to future climate conditions in Europe. The training region seemed to be more important than the chosen set of climatic variables. Projections were based on data given by the regional climate model COSMO-CLM, applying the two scenarios A1B and B1. The A1B scenario has been described above. The B1 storyline describes the same development of the global populations in a globalised world, as in the A1B scenario, but with a rapid change in economic structures towards a service- and information-oriented economy with environmental sustainability [26]. The B1 scenario is a rather moderate scenario and corresponds to the aim of the European Union of keeping anthropogenic warming below 2 Kelvin in comparison to the pre-industrial level [33]. Non-analogue climate is a problematic issue in species distribution modelling, as the observed distribution of a species provides no information about species response under novel climates [e.g. 22,34,35]. Hence, projections (in space and/or time) to regions with non-analogue climate are biased and require caution in interpretation. In the study of Fischer et al. [17], however, non-analogue climate in projections were excluded via Multivariate Environmental Similarity Surface Analysis as state-of-the-art evaluation [see 36].

Roiz et al. focused on the potential spread of *A. albopictus* to higher altitudes in the Alps of northern Italy using binomial Generalied Linear Model (GLM) as a logistic regression [18]. They related presences and absences of *A. albopictus* in ovitraps to land surface temperature (LST) data from satellite and human population data. Multiple years of daily LST data from the Moderate Resolution Imaging Spectroradiometer (MODIS) were reprocessed at increased spatial resolution of 200 m pixels. The geographically explicit presence/absence data offers the opportunity to correlate them with the background data at this high spatial resolution. A temperature-gradient-based model was used to fill no-data areas from more than 11,000 daily MODIS LST scenes from 2000 to 2009. On the basis of this, threshold conditions for the survival of eggs in the winter, alongside the survival of the adults, were determined. The best models were selected via Akaike´s Information Criterion (AIC). AIC is grounded on the concept of information entropy and evaluates the information loss, when a given model should describe reality. It can be interpreted as a trade-off between model accuracy and complexity. In concurrence with previous results [20], Roiz et al. identified annual mean temperature (11 °C) and January mean temperature (0 °C) as best predictors for identifying areas suitable for *A. albopictus* establishment [18]. Applying the A2 scenario, they considered an increase of the annual mean temperature of 1 and 1.5 Kelvin in winter in order to simulate the expected climatic conditions in 2050. Using data obtained directly from regional climate models would be inappropriate as these data are given in a resolution of 10–20 km. The A2 storyline describes a heterogeneous regionally oriented world and economy with a continuously increasing global population. Warming tendencies are more pronounced than in the previously described A1B and B1 scenarios [26].

Evaluation of climate change effects on the habitat suitability

Evidently, several distribution modelling efforts have been used to project the future climatic suitability of *A. albopictus* in Europe, which differ in model algorithm, climate data and scenarios. Here, we generated a simple GIS overlay (Figure 1A) to compare the risk map from the technical report of ECDC [16] with the results from Fischer et al. [17] and Caminade et al. [19]. However, an accurate comparison concerning the results of future projections cannot be presented, for several reasons. Firstly, there were clear differences regarding the chosen time-steps, emission scenarios and spatial resolution (Tables 1 and 2). Secondly, both, geographical and projected coordinate systems were used in the different studies. Hence, the comparison must be considered as a schematic and qualitative generalisation rather than a quantitative detailed compilation. Furthermore, we labelled localities with documented establishments of *A. albopictus* with the colour of the local climatic suitability (Figure 1B), to indicate how accurate the models reflect these occurrences. In general, the models under investigation were capable of predicting well the current localities of *A. albopictus* in Europe (Figure 1B). Only a few presences were observed in regions with rather unsuitable conditions.

General trends arising from comparison of the studies

Regardless of the above-mentioned differences and obstacles for comparisons, some general tendencies concerning the evolving climatic suitability for *A. albopictus* in Europe within the first half of the 21st century can be derived. Projections indicate that climatic suitability will especially increase in many regions where the species is not yet established. Regions that are currently characterised by a rather low or moderate suitability have the potential for invasion by mid-century, due to increasing climatic suitability (Figure 1A). As a general tendency of all studies at the continental scale [16,17,19] it can be inferred that especially western Europe (Belgium, France, Luxembourg and the Netherlands) will provide favourable climatic conditions within the next decades. Furthermore, climatic suitability can be expected to increase in central Europe (e.g. parts of Germany) and the southernmost parts of the United Kingdom. Climatic conditions will continue to be suitable in southern France, as well as most parts of Italy and Mediterranean coastal regions in south-eastern Europe. Astonishingly, decreasing suitability for *A. albopictus* is projected for the western

Mediterranean coast of Spain. This is very likely a consequence of an increased expectancy of drier conditions during the summer months.

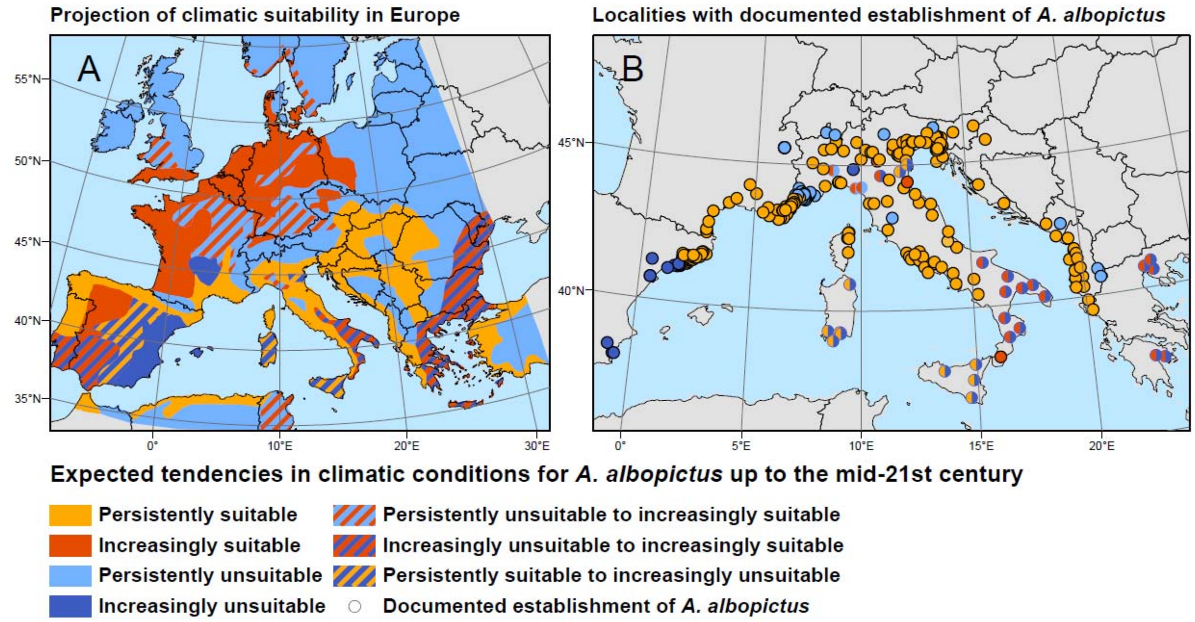

Fig. 1: Projections of climatic suitability of *Aedes albopictu*s in Europe (A) and in European localities with documented establishment of *A. albopictus* (B)

A. Evaluation of projections of climatic suitability of *A. Albopictus* within first half of the 21st century in Europe in comparison with the situation at the outgoing 20th century. Results of the mechanistic models based on Multi Criteria Decision Analyses of ECDC [16] and Caminade et al. [19] were compared with the statistical-based correlative niche model of Fischer et al. [17]. This is simply a schematic and qualitative generalisation, due to differences in time periods, scenarios and spatial resolution.

B. The records are coloured according to the evaluation of projections for climatic suitability of *A. albopictus* within the first half of the 21st century in Europe in comparison with the current condition presented in panel A.

However, some uncertainties in projections of the different studies are worth mentioning (see Figure 1A): differences between projections are evident in France, Germany, and western parts of the United Kingdom (Wales), where projections range from persistently unsuitable to increasingly suitable. In central parts of the Iberian Peninsula, Sardinia and Sicily, it is uncertain whether climatic conditions will continue to be suitable or will become less suitable in the future. Deviations between projections are most pronounced in the south-western parts of the Iberian Peninsula, south-eastern Italy and parts of eastern parts of Greece including also the west coast of the Black Sea. In these regions, uncertainties model outputs vary strongly in projections: climatic suitability is expected to persist or increase in the projections of ECDC [16] and Caminade et al. [19], while Fischer et al. [17] identified decreasing climatic suitability. Generally, projections are more sensitive to uncertainties for precipitation than for temperature, which is particularly evident in southern Europe. Compared with the studies of ECDC [16] and Caminade et al. [19], the influence of precipitation in climatic suitability is more pronounced within the statistical-based model of Fischer et al. [17] (see also Table 2).

Further trends to be expected

The general trend of increasing climatic suitability in regions that are currently rather unfavourable for *A. albopictus* establishment leads to the assumption of a northward spread in western but also central Europe up to the middle of the century. This is the time frame of results published by ECDC [16] and Caminade et al. [19]. From then on, trends can only be obtained by accounting solely for the study of Fischer et al. [17]. According to their projections, climatic suitability will further increase in central Europe and climate will become suitable for mosquito establishment in eastern Europe during the second half of the century [17].

Besides the continental dimension, potential range expansions on a local scale become crucial for the spread of *A. albopictus* in Europe as well. For instance, increasing temperatures may facilitate an upward spread in alpine regions, which has been demonstrated in northern Italy (Trentino) [18].

Future research avenues

In a warmer world, invasion processes of species may exhibit novel dynamics [37,38]. Thus, new challenges arise concerning the surveillance of invasive mosquitoes in Europe with high ability to colonise new territories as it is the case with *A. albopictus* [39]. Future research addressing invasive species that are of societal importance (e.g. regarding health issues) requires a comprehensive strategy for embedding climatic risk analyses in a broader scientific context. The main issues, such as transport mechanisms, alterations of habitats due to climatic extremes and biotic interactions, are highlighted below, as they are the most challenging tasks in modelling.

Continental dispersal pathways

None of the studies on potential future European occurrence of *A. albopictus* explicitly addresses processes such as the introduction and dispersal of the species. The introduction of this mosquito in Europe can be attributed to the global shipping of goods, especially by the world trade of used tyres or the import of tropical plants such as 'Lucky Bamboo' (Dracaena braunii) [1,2]. Undoubtedly, shipping is extremely effective in overcoming long-distance oceanic barriers [2,40,41]. Thus, the intercontinental range expansions of *A. albopictus* proved to be predictable using this combination of frequencies and traffic volumes of shipping lines in combination with climatic data at the target region around harbours [35]. The establishment of *A. albopictus* evidently took place around Mediterranean harbours, e.g. around the seaports of Genoa, La Spezia and Gioia Tauro in Italy as well as Barcelona, Spain – regions that are considered to be climatically suitable for the species today (Figure 2).

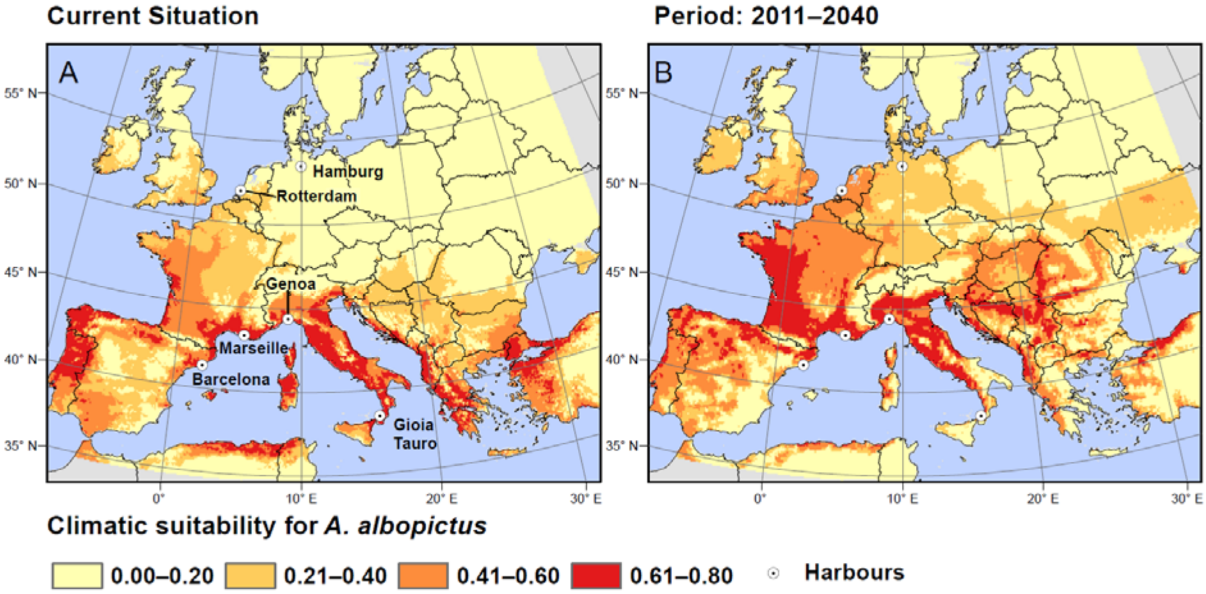

Fig. 2: Current (A) and projected (2011–2040) (B) climatic suitability of *Aedes albopictus* in Europe, with locations of important harbours

Data concerning the current and projected climatic suitability (A1B scenario) for *A. albopictus* refer to results of the statistical-based niche model of Fischer et al. [17]. Values for establishment theoretically range from 0 (completely unfavourable) to 1 (extremely favourable). Additionally, the changes in climatic suitability for 2011 to 2040 become obvious. Suitability will increase for the biggest European harbours of Rotterdam and Hamburg, which marks these as potential gateways for unintended mosquito introduction. In order to account for areas involved in cargo transport on a regional scale, we created buffer zones with different radii around the harbours of Rotterdam, the Netherlands, and Hamburg, Germany. This was done in order to detect examples of climatic suitability of the regions surrounding harbours with expected container transport. Climatic suitability was averaged for each buffer zone. Currently, climatic suitability is rather low for regions around Hamburg (radius (r) 50 km = 0.12 ± 0.01; r 100 km = 0.12 ± 0.02; r 200 km = 0.11 ± 0.03), while moderate suitability can be found for areas around Rotterdam (r 50 km = 0.21 ± 0.02; r 100 km = 0.23 ± 0.05; r 200 km = 0.23 ± 0.07). For 2011 to 2040, suitability of both regions of interest will increase remarkably. Regions around Hamburg will provide moderate suitability (r 50 km = 0.27 ± 0.03; r 100 km = 0.28 ± 0.05; r 200 km = 0.29 ± 0.06), while climatic suitability will even favour establishment of *A. albopictus* in zones around Rotterdam (r 50 km = 0.50 ± 0.03; r 100 km = 0.51 ± 0.03; r 200km = 0.48 ± 0.05).

For this reason, intensified monitoring systems are installed in harbour regions at higher latitudes, which should be pursued further. After introduction, *A. albopictus* populations were found in glasshouses in the Netherlands used by Lucky Bamboo importers [42]. Such unintended import of the mosquito to the Netherlands seems to be a repeated phenomenon [43], although no evidence consists concerning the establishment of *A. albopictus* in Dutch landscapes. This is probably related to their low climatic suitability. This is also still true for other regions around the most important European harbours of Rotterdam, the Netherlands, and Hamburg, Germany,) that are characterised by the highest number of import containers, coming from endemic regions. Obviously, the harbours are not the final destination of the containers, as they are transported to the continental interior. We calculated the averaged climatic suitability within buffer zones of different radii (50–200 km) around the harbours of Rotterdam based on the results of Fischer et al. [17]. Increasing climatic suitability within these buffer zones around the introduction gateways may become crucial for future *A. albopictus* spread (Figure 2).

Once introduced and established, the question arises how to determine the risk of *A. albopictus* spreading to further potentially climatically suitable habitats. Using the example of sandflies, it has been demonstrated that the dispersal of disease vectors on the continental scale can be evaluated by creating artificial cost surfaces that include several landscape features that are attributed with cost factors [44]. Consequently, the pathway with least costs for a species' dispersal can be considered as the most likely path of the species to move across landscapes. However, in contrast to sandflies, the dispersal of *A. albopictus* is mainly driven by unintended human transport through trade and traffic as opposed to natural dispersal. Hence, accounting anthropogenic factors in dispersal analyses is ambitious and acquires attribution of (rail-) roads and resting places in analyses. Consideration of these dispersal mechanisms, combined with current risk mapping and climate change assessments, suggests that further expansion across much of Europe is probable [2]. The necessity of dispersal analyses on the continental scale is highlighted by the recent incursion of *A. albopictus* in south-westernmost parts of Germany [45]. Thus, it has been concluded that *A. albopictus* crossed the Alps via transportation on motorways [46]. Another striking example is the recent importation of the mosquito to southernmost parts of the Czech Republic due to transit traffic [47]. Further spreading pathways need to be identified, as invasive mosquitoes may also be adaptable to new environments in a target region [2,36,48,49]. Without human transportation, the spreading potential of *A. albopictus* is limited to the local scale. In Italy, a flight range up to 300 m around their breeding containers has been observed [50]. This short-distance natural dispersal can be only assessed with high-resolution (250 m pixel resolution), gap-filled daily LST satellite data to predict areas that are potentially affected by infestation of *A. albopictus* [51,52].

Climatic constraints and novel scenarios

Integration of expert knowledge in modelling approaches demands detailed information on mosquitoes´ ecology. In temperate regions, diapausing is a strategy to maintain species' typical life cycle traits, as diapausing eggs show remarkable desiccation resistance aside from increased cold tolerance [53]. In Italy, either favourable microclimates or cold acclimation may play a decisive role in the context of overwintering [54]. Likewise overwintering was identified as a constraint also in Switzerland [52]. Under laboratory conditions, the low-temperature thresholds for the survival of eggs of European populations of *A. albopictus* have been identified [55]. Such experiments help to detect potential regions, capable of overwintering populations. To date, information is mostly obtained by field observations; however, the thresholds for survival can be derived by simulating extremes that then can be transferred to climate change scenarios.

Currently, the development of the next generation of IPCC climate change scenarios is under way. Until now, a sequential approach has been used for scenario development [56]. These scenarios depict a linear chain of causes and consequences of anthropogenic climate change, handed from one research community to the next in a lengthy process, leading to inconsistencies. The new parallel process begins with the identification of radiative forcing characteristics that support modelling a wide range of possible future climates. In parallel, new socio-economic scenarios will be developed to explore important socio-economic uncertainties affecting both adaptation and mitigation. This is directly linked to, and integrated within, the new climate scenarios [5,57]. The extensive exchange between scientific disciplines acquired a more sophisticated design matching. Then, projections based on climatic extremes and their ecological consequences will be improved. To date, projections concerning future climatic suitability of *A. albopictus* in Europe are based on long-term changes and do not consider the decisive role of rather short-term extremes. Modified climatic variability and associated sporadic extreme conditions are likely to create windows of opportunity for the establishment and reproduction of disease vectors such as *A. albopictus*, even if this is not reflected in trends of long-term average values [58].

Projections for the climatic suitability of *A. albopictus* can be combined, for instance, with the temperature-dependent extrinsic incubation period of an arbovirus, the time between pathogen infection of the insect vector and the vector's ability to infect the next vertebrate host. An accurate risk assessment of a climate-driven shift or spread of a vector-borne disease can then be obtained by combining risk maps of vector and transferred pathogen amplification in the light of a rapidly changing European climate for dengue [15,59,60,] or Chikungunya [61,62].

Further challenges for risk assessment

Aside from the above-mentioned novel opportunities, some challenges pertaining to future developments and their analyses need to be mentioned. A combination of phylogenetic analyses with distribution models was used to reconstruct the spatial occurrence of *A. albopictus* during the Pleistocene [63]. Such combined approaches seem to be a promising effort to support future projections. However, mutations and rapid adaptations of short-lived species to changing environment must be expected. Furthermore, outside of its native range *A. albopictus* acts as a strong competitor to local mosquitoes [49]. This not only affects the vectors' occurrence, but also the activity phase and population dynamics [64].

As *A. albopictus* prefers anthropogenic habitats, modified human behaviour is also a source of uncertainty. For instance, humans provide breeding sites for this container-breeder that enable survival in dry regions due to water storage [40]. Thus, changes in human behaviour or more general in human societies demand a comprehensive philosophy that must be implemented in risk assessments of climate change effects on emerging diseases. Estimating climatic suitability should be considered as a first step in risk assessment. Once future climatic suitability is detected for specific regions, societal and demographic aspects must be considered and regional specifics of healthcare systems can then be designed in a more specific and efficient way [65-67]. Such hierarchical and logical strategies may contribute to lowering the risks of vector spread and disease transmission.

Recently, ECDC has launched the E3 Geoportal as a (spatial) data dissemination platform to facilitate data sharing and usability [68]. In order to guarantee accuracy for environmental risk mapping of *A. albopictus*, a proof of concept was given beneficially [69]. Furthermore, ECDC initiated research activities on assessing the related risk of Chikungunya [62] and dengue transmission in Europe [70].

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Low-temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, *Aedes albopictus* (Diptera: Culicidae)

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6.3 Article 3

Low-temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, *Aedes albopictus* (Diptera: Culicidae)

With 2 Figures in the main text as well as one Figure in the Supplemental Material.

Abstract

Background

The interplay between global warming and invasive arthropods in temperate zones is of utmost interest in terms of the potential expansions of vector-borne diseases. Up to now, investigations on the recent establishment of mosquito vectors have focused on temperatures during their phases of activity. However, cold temperatures may also act as a strong ecological constraint. Projected changes in winter climate indicate an increase of mean minimum temperatures of the coldest quarter, less frequent days with frost and a shorter frost-season in Europe at the end of the century. Nevertheless, single cold extremes are also expected to persist under warming scenarios, which have a strong impact on reproduction success.

Methods

Here, the temperature constraints of European *Aedes albopictus* eggs, which had passed through a diapause, compared to non-diapausing eggs were examined systematically under controlled laboratory conditions. Additionally, one tropical strain of *Ae. albopictus* and of *Ae. aegypti* was used in the comparison.

Results

The lower temperature threshold tolerated by the European eggs of *Ae. albopictus* which have undergone a diapause, was -10°C for long term exposures (12 and 24h) and -12°C for 1h exposure. Non-diapausing eggs of European *Ae. albopictus* were found to hatch after a -7°C cold treatment (8, 12 and 24h exposure). Both tropical aedine species only tolerated the long term treatment at -2°C. Neither *Ae. albopictus* nor *Ae. aegypti* eggs hatched after being exposed to -15°C. Survival was mainly influenced by temperature (F = 329.2, df =

1, $p < 0.001$), whereas the duration of the cold treatment only significantly influenced the hatching response at the thermal limits of survival (F = 5.6, df = 1, $p = 0.031$) but not at 0°C (F = 0.1, df = 1, $p = 0.730$). Hatching success after the cold treatment was significantly increased in European eggs, which have undergone a diapause compared to non-diapausing eggs (F = 14.7, df = 3, $p < 0.001$). These results illustrate rapid adaptation.

Conclusions

Here, low temperature thresholds for aedine mosquito egg survival were detected. The compilation of risk maps for temperate regions can substantially be improved by considering areas where an establishment of a vector population is unlikely due to winter conditions.

Background

The interplay between invasive arthropods and global warming is of utmost interest in terms of potential expansions or shifts of vector-borne infectious diseases [1,2]. In Europe, six non-European aedine mosquito species have been found, quite recently Aedes koreicus [3,4]. Almost all were accidentally imported by the used tire trade [4]. *Aedes albopictus* (Diptera: Culicidae) is a prominent example of an invasive arthropod vector. It is a competent vector for 22 arboviruses (among others Chikungunya and dengue) and for Dirofilaria worms [5]. Almost 30 years after the first recorded introduction of this vector to Europe, the first autochthonous cases of Chikungunya [6] and dengue [7] have been detected in southern Europe. Originating from subtropical and tropical regions, the aedine vector has overcome a wide range of continental and oceanic barriers during the last decades [8]. Today, among other regions [9] the European continent is affected by the invasion of this species. Laboratory experiments [10-12] and field observations [13,14] on the climatic constraints and on the climate-driven population dynamics of this mosquito have focused on the role of temperature during the phase of larval and adult activity, but rarely during the total life cycle [15]. Mortality of eggs has been examined under different levels of relative humidity and warm temperatures [16]. However, low temperatures during inactive periods may also act as an ecological constraint for the range expansion of mosquitoes [17]. In the case of shifts of populations to higher latitudes, the minimum temperatures and the mean temperature of the coldest month or quarter have been discussed as a proxy for the ecological constraints of *Ae. albopictus* [18]. But sensitivity against temperature thresholds and the ability to cope with thermal constraints is not a permanent trait during the life cycle of this species. The capability to develop cold-tolerant diapausing eggs under specific environmental conditions is one key factor for *Ae. albopictus* to establish in higher latitudes [19]. Photoperiod and temperature are known to modulate the production of diapausing and non-diapausing eggs in *Ae. albopictus* [13,20]. During diapause, desiccation resistance in eggs increases due to higher concentrations of hydrocarbons at the egg surface [21].

Projected changes in winter climate indicate an increase of mean minimum temperatures of the coldest quarter by 2.2-4.2°C in south-western Europe, and by up to 4.0-6.4°C in northern Europe (IPCC scenario A2, 1961–1990 to 2071–2100) [22]. However, these values do not display changes in absolute minimum temperatures. Furthermore, less frequent days with frost (a decrease of 60-80% and 30-45%, in south-western and northern Europe, respectively) and a shorter frost-season (a decrease of 35-50% and 20-30%, in south-western and northern Europe, respectively) are expected at the end of the century [22]. Nevertheless, single cold extremes seem to persist by the end of this century also under warming scenarios because of increasing climatic variability [23,24]. These cold spells are usually short term events with a strong impact on the population dynamics of mosquitoes and thus on mosquito-borne disease transmission. Effects are expected especially when late frost events happen to occur in spring [25]. Hence, the influence of short frost events on egg survival when mosquito diapause is already broken is highly relevant for populations to establish and in terms of population dynamics.

Up to now, investigations on the recent establishment of the mosquito vector focused on temperatures during the phases of activity [10-14,16]. However, cold temperatures may also act as a strong ecological constraint in terms of possible range expansions [18]. Winter warming may increase the survival rate of mosquito eggs, but as evidence suggests that climatic variability and extreme temperature events will become more important, reproduction success and population dynamics can also be negatively affected [26]. As a matter of fact, the minimum temperature constraints of European *Ae. albopictus* eggs have not yet been systematically examined under controlled laboratory conditions. Because regional invasive populations of the species are known to adapt rapidly to the climatic conditions such knowledge on low temperature constraints is urgently needed. Here, we hypothesized that: (1) the eggs of European *Ae. albopictus* which have undergone a diapause tolerate minimum temperatures down to -10°C, (2) the duration of exposure to frost influences the survival of eggs and (3) hatching success after a cold treatment increases in eggs which have passed through a diapause compared to non-diapausing eggs.

Methods

Species, strains and standard protocol

Overall, we tested eggs from two strains of *Ae. albopictus* (temperate European and tropical Asian origins) and one strain of *Ae. aegypti* (tropical Asian origin). The European *Ae. albopictus* strain originated from eggs collected in the field in Rimini (Italy) and reared in the laboratory (Rimini F43). The tropical aedine species were obtained from a long-lasting laboratory colony. Laboratory colonies were used so that further threshold experiments with the same strains could be combined as a basis for epidemiological and environmental modeling.

The temperate strain of *Ae. albopictus* was artificially introduced to diapause. For all strains, we determined the survival of eggs after being exposed to low temperatures. In a climate chamber trial we applied a gradient of minimum temperatures (0 to -15°C). Each temperature treatment was replicated for different durations (1 to 24h).

Female mosquitoes were reared according to the standard protocol at a temperature of 27°C, a relative humidity of 60 – 80% and a 12:12 h (L:D) photoperiod. The light period was set from 8:00 to 20:00 and held constant at 150 Lux. After hatching from the eggs, larvae were kept in a water basin (30 x 30 x 10cm) that was filled with a 1:1 mixture of tap- and deionised water. Larvae were fed with fishfood flakes (Tetra Min®). Before adult emergence, the pupae were transferred to a cage (40 x 30 x 20cm). Adult mosquitoes had constant access to sugar solution (10% dextrose). At the age of seven to ten days after emergence from the pupae, females received their first blood-meal with human blood derived from expired blood preservations. The blood was heated up to 38 ± 1°C and transferred into sheep intestines to be exposed to the mosquito cage population. After three to four days eggs were laid on moistened filter paper, subsequently removed from the cage, left to dry for 2 days and then stored in plastic bags at 27°C with a minimum relative humidity of 85% before being exposed to the frost manipulation.

Induction of diapausing eggs

In *Ae. albopictus*, the production of diapausing eggs is known to be induced by low temperatures and shortened photoperiods [27,28]. According to Hanson and Craig [29], the induction of diapause under laboratory conditions can be achieved by transferring mosquito pupae from rearing conditions at 27°C and a 16:8 h (L:D) photoperiod to 21°C and a 8:16 h (L:D) photoperiod. We applied this procedure with some minor modifications: After hatching from the eggs, European *Ae. albopictus* larvae were kept at a temperature of 27°C, a relative humidity of 60 – 80% and a 12:12 h (L:D) photoperiod until they reached the second instar. The basins were then transferred to a room at 21-22°C, 30-40% rH and a 8:16 h (L:D) photoperiod [29]. The light period (150 Lux) was set from 8:00 to 16:00. In contrast to [29], mosquito larvae were kept at 21-22°C and at a 8:16 h (L:D) photoperiod as soon as they reached the second instar larval stage. By doing so the chances of diapause induction are increased while overall development is slowed down. Before emergence the pupae were transferred to a cage (40 x 30 x 20 cm) and provided with sugar solution (10% dextrose). Adults were held under the same conditions and were fed blood after 7-12 days. Eggs were laid on moistened filter paper after three to four days and left to dry for 2 days. They were then stored for 14 weeks in plastic bags at 21-22°C with a minimum relative humidity of 60% until spontaneous hatching occurred again with about 90%.

Treatment

We manually placed 20 eggs (with each egg inspected under a stereo microscope to exclude capped eggs or those with signs of desiccation) from each Aedes population onto a moist filter paper pad, which was then deposited in a glass vial. This vial was then sealed by a rubber plug to reduce the loss of moisture and, hence to minimize any possible desiccation effects. The experimental design aimed to maximize temperature levels and cold duration in order to determine lethal thresholds as precisely as possible. The eggs were exposed to seven different temperatures: 0°C, -2°C, -5°C, -7°C, -10°C, -12°C and -15°C by using a climate chamber (Licht-Thermostate Typ 1301, RUMED, Rubarth Apparate, Laatzen, Germany). The duration was 1, 4, 8, 12 and 24h (16:8 (L:D) photoperiod) for each temperature treatment, respectively.

Hatching and counting

After the cold treatment, the eggs were acclimated at room temperature (18-20°C). The glass vials containing the filter paper pad with 20 eggs were filled with 20 ml of nutrient broth solution (cooled boiled water with the addition of powdered bacto-nutrient broth, 0.1% in weight, according to Novak and Shroyer [30]). Furthermore, the temperature of the nutrient broth solution was set to 25°C to stimulate the hatching response. The submerged eggs of the tropical strains were kept at 25°C with a 16:8 h (L:D) photoperiod and the eggs of the temperate strains were kept at 20°C. Hatching at each level of temperature was determined after 12, 24, 48, 72 and 96h. Larvae were removed at these times, respectively. The minimum survival temperature was quantified as the lowest temperature at which successful hatching was observed. The hatching rate was also quantified as a percent for each combination of temperature and duration.

Statistics

The interaction between temperature level and the duration of exposure on survival was explored by a linear least squares regression of the minimum survival temperature for each category of duration. Inter-specific differences in minimum survival temperature were compared by analyzing the slopes of the linear regression between the duration and the minimum survival temperature. This was evaluated by a Monte-Carlo permutation procedure to compare the true difference in the slope of two strains with the differences in 1,000 permutations of randomly-assembled groups. The importance of temperature and duration on hatching success was further investigated by linear models while using the species/strain identity as a co-variate. Differences between species/strains were evaluated by the same linear models combined with analyses of variance (ANOVA). Prior to statistical analysis, data were log-transformed if conditions of normality were not met or to improve the homogeneity of variances. Both characteristics were tested by examining the residuals versus fitted plots and the normal qq-plots of the linear models. Statistical analysis was carried out using R 2.12.0 [31] supported by the package simba [32].

Results

Minimum survival temperature

The minimum survival temperature tolerated by the European eggs of *Ae. albopictus* after a diapause (Figure 1) was -10°C for long term exposures (12 and 24h), while they survived short term exposure (1h) at -12°C. Non-diapausing eggs of European *Ae. albopictus* were found to hatch even after a 4h treatment at -12°C. However, with mean and long term exposures (8, 12 and 24h) hatching only occurred at less extreme temperatures (-7°C). The tropical aedine species only differed in its hatch response for the 1h cold treatment: While tropical *Ae. albopictus* survived at -10°C, *Ae. aegypti* hatched at -7°C.

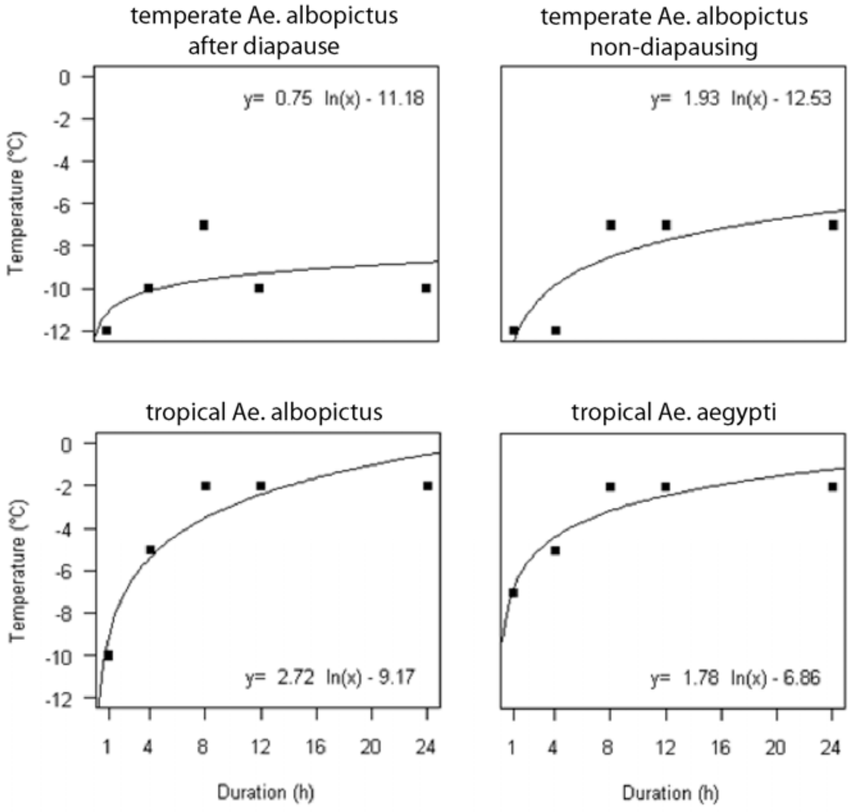

Figure 1: Minimum survival temperature of Aedes eggs after cold treatment. Minimum survival temperature in comparison to duration of treatment for European eggs which have undergone a diapause and non-diapausing eggs of *Ae. albopictus* and tropical eggs of *Ae. albopictus* and *Ae. aegypti*. Eggs of European *Ae. albopictus* which passed through a diapause survive lower minimum temperatures than non-diapausing eggs when exposed for more than 12h, whereas no differences occurred for short exposure (1h). Eggs of tropical *Ae. albopictus* survive lower minimum temperatures than *Ae. aegypti* eggs when exposed for 1h, whereas no differences occurred for longer exposure (>8h).

In contrast to the short term exposure to cold temperatures, both species only tolerated the long term treatment at -2°C. The minimum survival temperature for different durations of exposure did not differ significantly between strains/species according to the Monte-Carlo permutation procedure of the slopes of the linear regression.

Duration of exposure

Survival was mainly influenced by temperature (F = 329.2, df = 1, p < 0.001). In comparison to temperature, the duration of exposure only had a minor impact on the hatching rate (F = 16.2, df = 1, p < 0.001). The duration of the cold treatment only significantly influenced the hatching response at the thermal limits of survival for each strain/species (F = 5.6, df =1, p = 0.031) but not at 0°C (F = 0.1, df =1, p = 0.730).

Hatching success after a cold treatment

Hatching success after the cold treatment was significantly increased in European eggs that have undergone a diapause compared to non-diapausing eggs (F = 14.7, df = 3, p < 0.001; Figure 2). Differences within the same geographical range but between species (tropical *Ae. albopictus* and Ae. aegypti) were less pronounced than differences between geographically different strains of the same species (tropical and European *Ae. albopictus*). Hatching success of European *Ae. albopictus* after the total cold treatment was 45% for eggs after diapause and 30% in non-diapausing eggs. Only eggs of the European strain hatched after exposure to -12°C for 1h: Eggs after a diapause showed a high hatching success (75%), whereas hatching success was decreased in non-diapausing eggs (10%). Tropical *Ae. albopictus* and *Ae. aegypti* total hatching success was 19 and 25%, respectively. Surprisingly, hatching of one tropical *Ae. albopictus* egg occurred after exposure to -10°C for 1h. The hatching success of *Ae. aegypti* was almost unaffected when exposed to -7°C for 1h, whereas an extension of the -7°C cold period for more than 1h or a further reduction of temperature down to -10°C caused a complete breakdown of hatching. Neither *Ae. albopictus* nor *Ae. aegypti* eggs hatched after being exposed to -15°C.

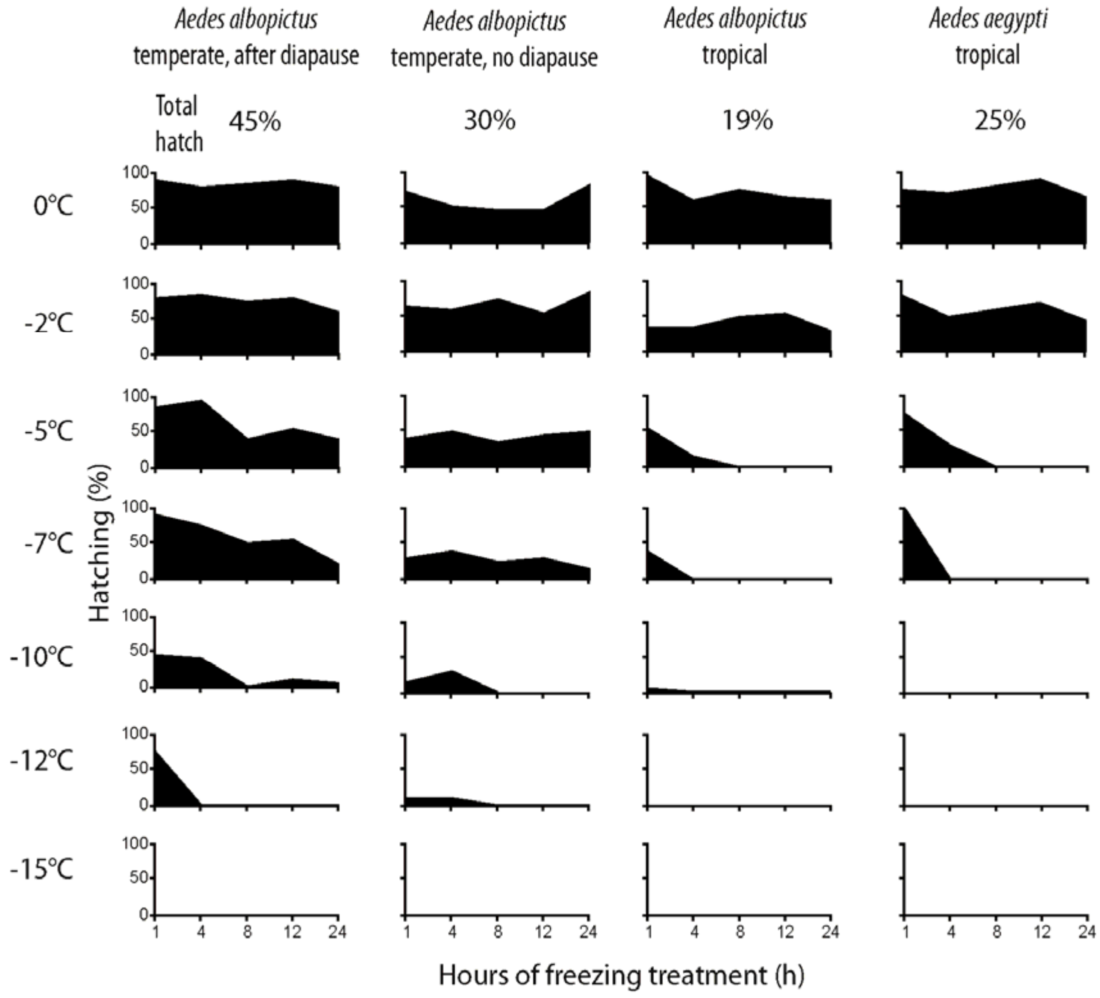

Figure 2: Hatching success of Aedes eggs after cold treatment. Hatching success of Aedes after exposure to cold temperatures (0°C to -15°C) for different durations (1 to 24h).

Discussion

We determined the minimum survival temperature of eggs for two strains of *Ae. albopictus* and for one strain of *Ae. aegypti* and showed that the hatching success after the cold treatment was ignificantly increased in European eggs which have undergone a diapause compared to non-diapausing European eggs after exposure to cold temperatures (0°C to -15°C), for different durations. Overall, we focused on relatively short exposure times (1h, 4h, 8h, 12h and 24h), because these are the time spans on which minimum temperatures are available from climatology and meteorology. Consequently, our data can be used to make evidence-based decisions on the temporal resolution of temperature data needed for modeling approaches. Temperature was the main controlling factor, whereas the duration of the cold treatment only influenced the hatching response significantly at the thermal limits of survival for each strain/species.

To the best of our knowledge there are no comparable studies on the cold tolerance in *Ae. albopictus* eggs which have undergone a diapause. Previous studies on non-diapausing, non cold-acclimated eggs of a North American strain of *Ae. albopictus* have shown that no hatching occurs after a 24h cold period of -10°C [18]. Our results confirmed this threshold at which no eggs survive for European non-diapausing *Ae. albopictus* eggs. However, the survival of the European strain in this study is reduced more strongly with decreasing temperature than the survival of the North American strain: for the European strain we found 50% egg mortality at -5°C, whereas for the North American strain 50% egg mortality at -8°C was observed [33]. The minimum survival temperature of *Ae. aegypti* eggs in the field (Houston, Texas, USA) was found to be 7°C for a 24h cold period [33]. But in nature short term temperature fluctuations occur. Additionally, at one sampling site small scale differences across short distances can be substantial. Ecological complexity contributes to statistical noise. This is why controlled experiments are needed to identify physiologically relevant thresholds.

In the laboratory -3°C for 24h was identified as a threshold for *Ae. aegypti* eggs, respectively [33]. The present results show only a slight increase in the minimum survival temperature for *Ae. aegypti* (-2°C for 24h). Using a very long-lasting laboratory colony may yield deviating results compared to natural populations. However, such colonies are more appropriate to serve for experimental proof of life cycle thresholds.

It was suggested that the duration of exposure to temperatures below a certain value is an important factor influencing the hatching rate of mosquito eggs [18]. Cold acclimation tends to increase cold hardiness in *Ae. albopictus* eggs [29,33]. In this study, previous cold acclimation was avoided to distinguish between the direct impact of cold temperatures and the duration of exposure (see also [34]). The role of the duration of low temperatures is confirmed at the thermal limits of egg survival by the present study.

Up to now, the risk of *Ae. albopictus* to establish in Europe was assumed to be relevant only for regions with cold-month mean temperature of 0°C or higher [35,36]. The -5°C coldest-month isotherm was suggested to characterize the maximum northward expansion for continental Asia and also for North America [37]. The results presented in this study emphasize the ecological importance of absolute minimum temperatures. Up to now, thermal minima are neither adequately considered in environmental niche models nor in epidemiological models. Yet, vector niche modeling is mainly based on long-term average conditions such as annual mean temperature and annual precipitation (e.g. [38]). Winter conditions in terms of the mean minimum temperature of the coldest month and days with ground frost per month are considered in the niche model of *Ae. albopictus* [39]. Although the incorporation of the absolute minimum temperatures would considerably improve vector risk maps, this would be difficult to implement in large scale projections due to the limited availability of meteorological data in an hourly resolution. Recent epidemiological models consider temperature and season dependent population dynamics of vectors [40]. However, those models start each annual cycle with the same initial number of mosquito individuals. Knowing the minimum survival temperature and survival success after frost events has therefore the potential to improve epidemiological models in temperate zones substantially.

The present study has certain specifics that need to be taken into account: First, the long colonization history of the European *Ae. albopictus* strain raises the question to what extent this strain actually now represents the characteristics of its founding population. Second, the successful production of diapausing eggs in the laboratory in all females under the recorded circumstances is unlikely. Hatching tests during diapause still showed about 10% spontaneous hatching.

Nonetheless, winter conditions, or more specifically absolute minimum temperatures, play a decisive role for the distribution limits of a species [41]. Spatial quantification of absolute minimum temperatures, however, is non-trivial. Microclimate may vary up to 10°C at small spatial scales [42]. The specific thermal conditions of microsites were found to matter also for the occurrence of aedine species [43]. For these species, indoor breeding sites [44] are adding anthropogenic habitats with specific conditions that do not refer to landscape traits. Thus, it would be naive to concentrate on natural and semi-natural site conditions alone.

Future studies should pay attention to winter conditions by incorporating realistic freeze-thaw cycles to identify how far these temperature fluctuations are affecting egg survival. Furthermore, the knowledge on winter survival of viruses such as dengue and Chikungunya within the eggs of vector insects is of utmost importance and should be intensively addressed in future research.

Conclusions

Until now, low temperatures have not been considered adequately in the modeling of vector species. Here, low temperature thresholds for aedine mosquito egg survival were detected. The compilation of risk maps for temperate regions can substantially be improved by considering areas where an establishment of a vector population is unlikely due to winter conditions (see Additional file 1).

Authors' contributions

ST participated in the design of the study, carried out the cold treatment of eggs, performed the statistical analysis and drafted the Article. UO carried out the induction of diapause in mosquito eggs. DF helped to draft the Article. JK conceived of the study and participated in the performance of the statistical analysis. CB coordinated the study and helped to draft the Article. All authors read and approved the final Article.

Acknowledgements

This study would not have been possible without Romeo Bellini who provided the eggs of *Ae. albopictus* from Northern Italy. In particular, we are grateful to Manuel Steinbauer for valuable comments on the statistical analysis. Laboratory work was strongly supported by Sebastian Hohmeyer and Sven Boese. The authors also thank the 'Bavarian State Ministry of the Environment and Public Health' for their financial support in the project "Vector-borne infectious diseases in climate change investigations (VICCI study)" and the 'Bavarian Food and Health Safety Authority' for coordination of the project. This publication was funded by the German Research Foundation (DFG) and the University of Bayreuth in the funding programme Open Access Publishing.

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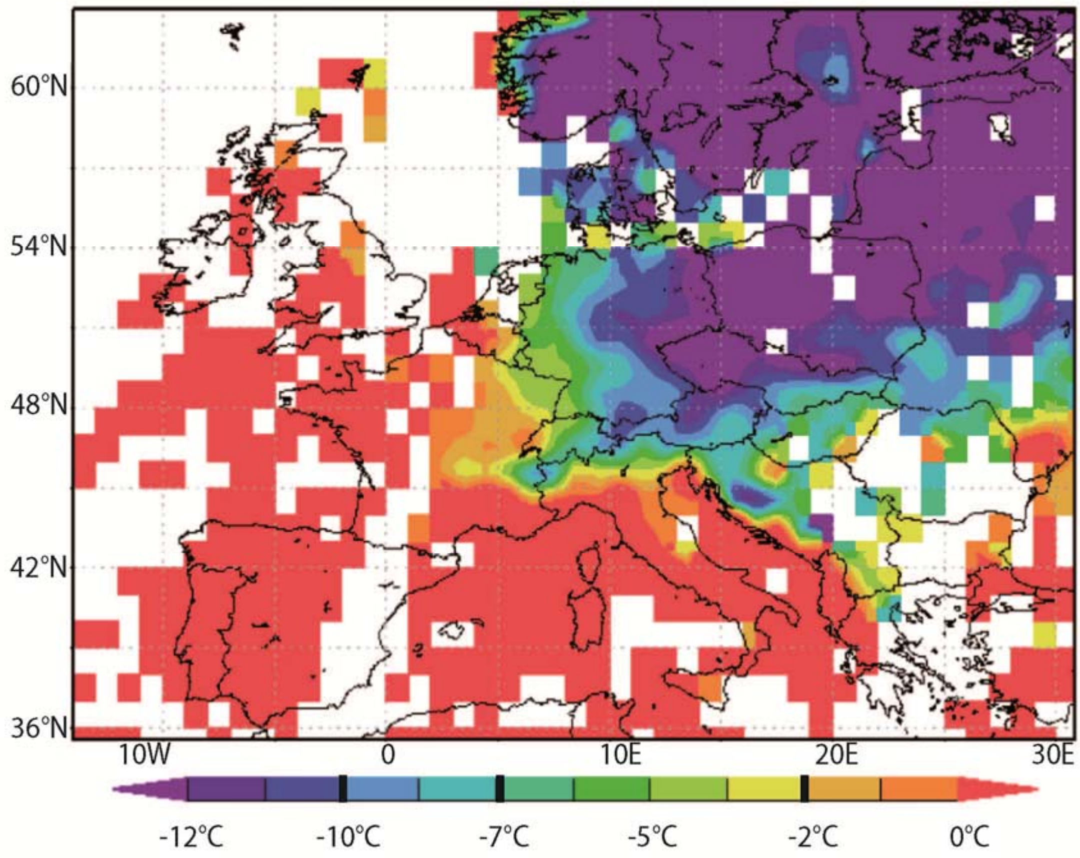
Additional file



Additional file 1: Surface air temperature at night in Europe on one of the coldest nights in winter 2011, which was one of the coldest winters of the last decade (2011-02-23; http://daac.gsfc.nasa.gov/giovanni, [45]). This map shows where the distribution limits of aedine species in Europe are to be expected due to their minimum survival temperature of eggs. In the experiment, European diapausing *Aedes albopictus* survived -10°C, European non-diapausing *Ae. albopictus* -7°C, tropical *Ae. albopictus* and *Aedes aegypti* -2°C for 12h. Obviously, there is only a narrow margin between regions with a possible winter survival of non-diapausing eggs and those where winter survival of diapausing eggs seems to be possible.

Risk assessment of dengue virus amplification in Europe based on spatio-temporal high resolution climate change projections

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6.4 Article 4:

Risk assessment of dengue virus amplification in Europe based on spatio-temporal high resolution climate change projections

With 1 Table and 5 Figures.

Summary

During the last decades dengue incidences are emerging significantly around the globe. Currently, about one fifth of the human population lives in dengue risk zones, which are mainly located in (sub-) tropical regions of Southeast Asia and the Western Pacific. Dengue infections in European population mainly referred to returning travellers from tropical endemic regions. Nevertheless, vector establishment in Europe already took place and therefore the risk increases. Currently, autochthonous cases of dengue fever have been reported in Europe.

Studies estimating the risk of dengue epidemics regarding changing climatic conditions in Europe are missing. Therefore, we close this gap by using the temperature constraints for virus amplification within the vector *Aedes aegypti* from two laboratory experiments. We transfer these findings to the changing European climate based on data provided from a regional climate model (COSMO-CLM; A1B and B1 scenario). Daily mean temperature were averaged for the time-steps 2011-2040, 2041-2070 and 2071-2100 in order to reduce natural variability but rather point out climatic trends for risk assessments. For both scenarios the strongest increase of temperature is projected after mid-century.

Results indicate a growing threat of virus amplification in Europe especially towards the end of this century. Larger parts of the Mediterranean will be at risk. The southwest of the Iberian Peninsular appears to be especially threatened. Even in some parts of Central Europe, such as Southwest Germany, dengue virus amplification can no longer be excluded at the end of the century. However, it is unlikely that *Aedes aegypti* will serve as an efficient vector in Europe. In fact, it is *Aedes albopictus* that is an invasive species in Europe and potential differences in extrinsic incubation period between *Ae. aegypti* and *Ae. albopictus* have to be identified. Policy and public health authorities have to consider these emerging

biorisks in order to establish surveillance systems and develop counteraction strategies. Hence, we strongly emphasize the need for a growing European awareness in the face of biological hazards that are responding to climatic changes.

Zusammenfassung

Dengue-Fieber ist eine durch Stechmücken übertragene Infektionskrankheit, deren Gefährdungspotenzial innerhalb der letzten Jahrzehnte dramatisch zunahm. Mittlerweile lebt ein Fünftel der Weltbevölkerung in Dengue-Risikogebieten, welche sich insbesondere in den (sub-) tropischen Gebieten Südostasiens und dem Westpazifik befinden. Regelmäßig wird das Dengue-Virus von infizierten Reisenden aus Endemiegebieten nach Europa importiert. In jüngster Vergangenheit treten auch vereinzelte autochthone Fälle in Europa auf. Ein kompetenter Überträger hat sich in Südeuropa bereits Ende des letzten Jahrhunderts etabliert (*Aedes albopictus*); ein Weiterer ist sporadisch wieder neu aufgetreten (*Aedes aegypti*).

Zu Risikoabschätzungen möglicher Dengue-Epidemien in Europa fehlen allerdings bislang Studien. Für eine thermisch abgeleitete Gefährdungsabschätzung nutzen wir Temperaturanforderungen des Virus zur Entwicklung im Vektor (*Ae. aegypti*) aus zwei verschiedenen Laborexperimenten. Diese Anforderungen der sogenannten extrinsischen Inkubationsperiode des Virus werden auf die projizierte Erwärmung Europas im 21. Jahrhundert übertragen. Hierzu bereiten wir das projizierte Klimaänderungssignal der Szenarien A1B und B1 des Regionalen Klimamodells COSMO-CLM in täglicher Auflösung auf. Um signifikante klimatische Trends herauszufiltern und Unsicherheiten in den Projektionen der täglichen Durchschnittstemperaturen zu minimieren, werden diese für die Zeitabschnitte 2011-2040, 2041-2070 und 2071-2100 gemittelt.

In beiden Szenarien wird eine stärkere Erwärmung ab Mitte des Jahrhunderts und speziell gegen Ende des Jahrhunderts projiziert. Insofern steigt die thermische Eignung im Verlaufe des 21. Jahrhunderts abhängig von der verwendeten extrinischen Inkubationsperiode an. Ende des Jahrhunderts ist eine Amplifikation des Virus in den wärmsten Regionen Mitteleuropas wie dem Oberrheingraben im Südwesten von Deutschland nicht mehr auszuschließen. In weiteren Studien bleibt zu klären, ob sich die extrinsische Inkubationsperiode in *Ae. albopictus* im Vergleich zu *Ae. aegypti* unterscheidet. Frühzeitig erkannte potenzielle Gefährdungsgebiete verhelfen politischen Entscheidungsträgern und dem

Gesundheitssektor dazu, rechtzeitig Adaptions- bzw. Gegenmaßnahmen initiieren zu können. Unsere Ergebnisse verdeutlichen, dass Europa gewappnet sein muss, um nicht von Epidemien scheinbar exotischer Tropenkrankheiten überrascht zu werden.

Keywords

Dengue fever, emerging infectious disease, GIS, global change, global warming, mosquito-borne disease, surveillance, vector-borne disease

1. Introduction

Globally, the importance of vector-borne diseases has increased significantly during the last decades. Today, this group represents about one third of all outbreaks of emerging infectious diseases (Jones et al. 2008). Changing spatial patterns of occurrence are observed. The reasons for such changes are manifold, ranging from globalization of travel and trade to environmental and climatic changes or modified human behaviour (e.g. Maier 2003; Sutherst 2004; Fischer et al. 2009; Fischer et al. 2010a; Pfeffer and Dobler 2010; Randolph and Rogers 2010).

The dengue virus is mainly transmitted by the mosquitoes *Aedes aegypti* and *Ae. albopictus*. The latter ranks among the first 100 of the “World’s Worst” invaders (Crans 2008) and has been mostly introduced by trade of goods, especially used tires (Mitchell 1995). A distinction is drawn between different cycles of dengue: a primitive enzootic transmission cycle which involves lower primates, an epidemic transmission cycle in rural villages and the urban endemic/epidemic cycle in large urban centres, which is most relevant for public health (Gubler 1998). Four closely related serotypes of the arbovirus occur (DENV-1 to DENV-4), with specific geographical distribution and pathogenity (Halstead 2008). Furthermore, different dengue genotypes (American and Asian DENV-2) show different ability of the virus to grow in mosquitoes (Halstead 2007). Dengue fever is characterised either by mild fever or high fever combined with severe headache, pain behind the eyes, muscle and joint pains and rash. Patients that suffer a secondary infection with another dengue virus serotype have a significantly higher risk for developing dengue haemorrhagic fever (DHF). Especially young children are concerned (Halstead1988; Guzman et al. 2002). Clinical features of DHF are high fever, often with liver enlargement and in severe cases accompanied by circulatory failure. The number of countries that experienced DHF epidemics has quadrupled between 1970 and 1995. Without intensive care, affected human population can exceed mortality rates of 20% (WHO 2009; Cummings 2010).

In Northern America, outbreaks have arisen along the Texas-Mexican border for about three decades (Reiter et al. 2003). Recently, locally acquired dengue infections were reported for Florida (CDC 2010). Up to now, Europeans tend to consider dengue as a travel-related disease only. Southeast Asia, especially Thailand, is the most important region of travel-related dengue infections, followed by Latin America, the Indian subcontinent, the Caribbean and Africa (Heddini et al. 2009; Jelinek 2009). The last dengue epidemic in Europe occurred in Greece during the years 1927 and 1928. At that time, *Ae. aegypti* was transmitting the virus (DENV-1) (Rosen 2006). During the following decades, dengue was no longer established in Europe. However, exotic arbovirus are thought to become a future public health concern in Europe (Pfeffer and Dobler 2009). In September 2010, the French Ministry of Health reported the first cases of dengue fever from autochthonous origin in Europe (La Ruche et al. 2010). Furthermore, a dengue virus infection was reported for a German traveller returning from Croatia (Schmidt-Chanasit et al. 2010) and there upon autochthonous cases were found in Croatia too (Gjenero-Margan et al. 2011).

The potential rate of transmission depends on the daily survival rate and duration of the gonotrophic cycle of the mosquito (including searching for a host, blood feeding, blood meal digestion, eggs maturation, and oviposition). Virus amplification is determined by the extrinsic incubation period (EIP). EIP is defined as the time interval between the acquisition of an infectious agent (pathogen) by a vector and the vector's ability to transmit the agent to a susceptible vertebrate host. The EIP includes virus replication, maturation and migration within the mosquito body to its salivary glands. Females remain infective during their entire life. Temperature is considered to be the main factor regulating the EIP and thus warmer temperatures shorten the EIP (Watts et al. 1987; Barbazan et al. 2010). If minimum temperature thresholds for the EIP are not exceeded, the virus can not accomplish its amplifycation inside the vector and transmission, for instance to humans, can be excluded (Ooi and Gubler 2010).

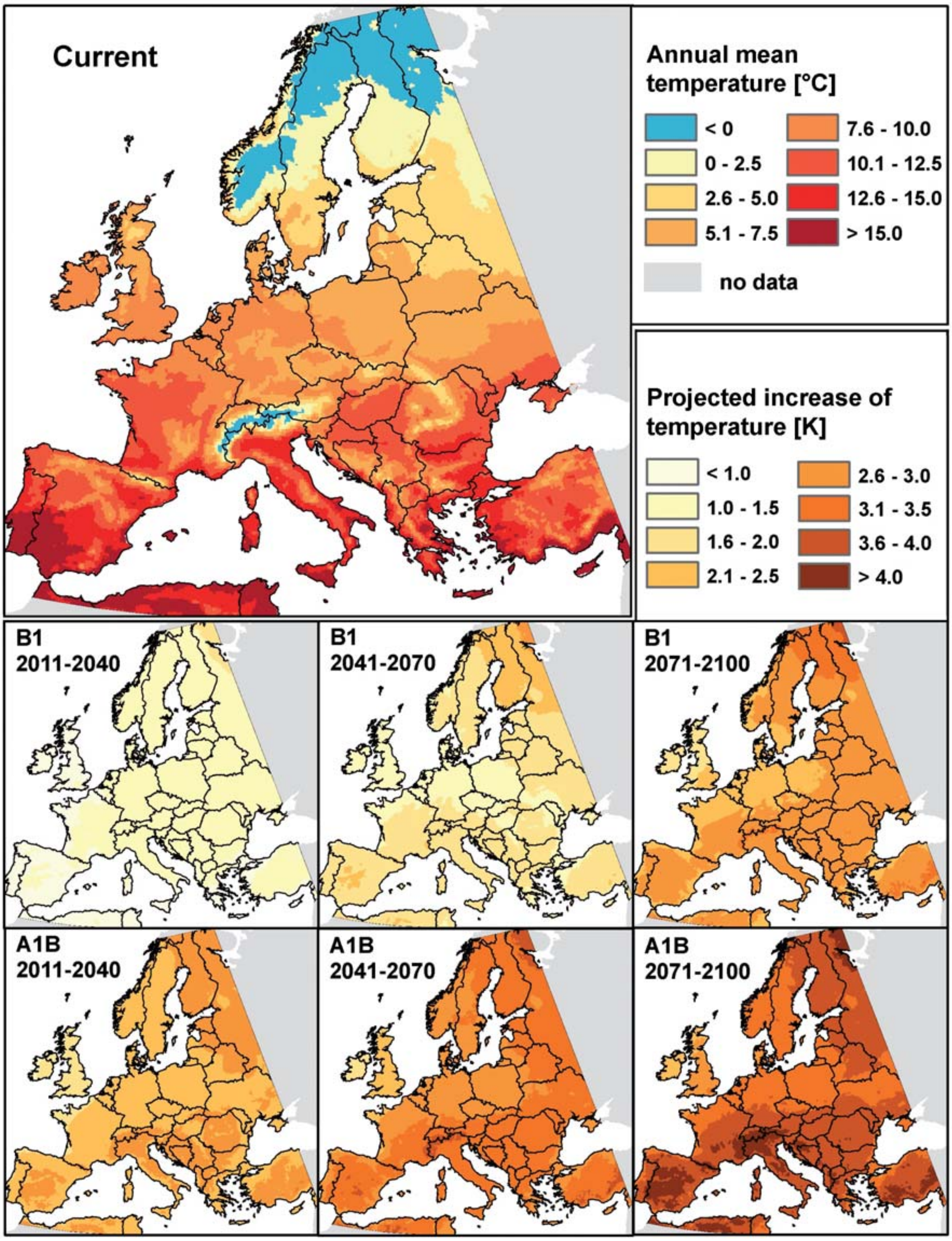

Fig. 1: Current annual mean temperature in Europe and projected warming in Kelvin during the 21st century based on two IPCC emission scenarios. Projections are based on the regional climate model COSMO-CLM. Generally, projected warming is less severe for the B1 scenario than for A1B. Highest increase in annual mean temperature is projected for Central parts of the Iberian Peninsular, the Alps and the northernmost parts of Scandinavia. Instead, the British Isles seems to be less affected by the projected increase of annual mean temperature.

It is known that favourable meteorological conditions significantly influence dengue incidences in endemic regions such as South America (Luz et al. 2008) and Southeast Asia (Shang et al. 2010). Evidence suggests that global warming increases the latitudinal and altitudinal range as well as intensity of dengue transmission (Jetten and Focks 1997). At the end of the 21st century, about 5-6 billion people can be expected to live in risk areas of potential dengue transmission including present-day's temperate regions (Hales et al. 2002).

Identifying the climatic constraints of the organisms that are involved in a chain of infection on spatio-temporal scales is the first step in determining risk areas (Fischer et al. 2010b).

Although the WHO (2009) declared dengue as one of the main public health concerns, it is surprising that no study exists that geographically analyzes the risk of dengue for Europe. Especially the availability of highly resolved regional climate models, both in terms of spatial and temporal resolution, gives us the option to detect possible developments in the run-up to climatic changes.

There is no doubt that Europe will be confronted with increasing temperatures in the 21st century (Fig. 1). The question arises whether climate change will assist a potential re-establishment of dengue in Europe. Here, we survey the risk of virus amplification by using the EIP. Our aim is to explore:

i.) Which areas will provide suitable temperature conditions?

ii.) At what time will these regions be at risk?

iii.) Which longest seasonal duration of risk has to be expected?

2. Material and methods

First we took documented temperature requirements for EIP from literature. Then, we prepared climatic data of a regional climate model in a daily resolution for the 21st century and transferred the determined temperature requirements to three time-steps and two scenarios. We detect areas at risk in the 21st century and identify the longest temperature-dependent intra-annual season of potential dengue virus amplification in Europe.

2.1 Temperature constraints

In this study we applied the temperature relationship for the EIP of the dengue virus. *Ae. albopictus*, a known vector of dengue virus, is already established at the European continent (mainly in Italy and the eastern shore of the Adriatic Sea). Studies on the EIP of this species are actually missing. Therefore, experiment-derived knowledge of EIP and temperature relationships was taken for *Ae. aegypti* (Blanc and Caminopetros 1930; Watts et al. 1987). This mosquito was already endemic in Europe up to the Second World War and extinct thereafter. Currently, *Ae. aegypti* is established in Madeira (Portugal) (Almeida et al. 2007). The species was also introduced into the Netherlands (Scholte et al. 2010). This gives rise to concern regarding a re-establishment of this dengue vector in continental Europe. Knowledge on temperature thresholds for virus amplification in *Ae. aegypti* generates from two experimental studies:

a) Blanc and Caminopetros (1930) detect an EIP of eight days with temperatures of at least 22 °C for dengue virus amplification. They aimed to identify the required EIP with special respect for Europe. For this laboratory study *Ae. aegypti* mosquitoes were taken from the Greek outbreak of dengue in 1927/28.

b) In contrast to this, Watts et al. (1987) found temperature requirements for dengue virus amplification in a Bangkok strain of *Ae. aegypti* of at least 30 °C mean temperature at twelve consecutive days for mosquitoes with low virus dose or seven consecutive days with daily mean temperature between 32 and 35 °C for those with a high virus dose.

As these studies yielded remarkably differing results, we compared projections based on both studies, respectively. Additionally, we evaluated both temperature requirements found by Watts et al. (1987) in order to determine, whether the frequency of highest daily mean temperatures over short time-periods (seven consecutive days between 32-35 °C) increases more rapidly than those of moderate high temperatures over a longer time-period (twelve consecutive days of at least 30 °C) in regional climate model projections.

2.2 Application of regional climate change projections

2.2.1 The regional climate model COSMO-CLM

Spatially explicit data on projected climate change are supplied by climate models on regional to global spatial scales. In contrast to their driving global models, regional climate models are capable to consider topography and further landscape features. They offer a much higher spatial resolution which enhances especially the quality of climate impacts studies (Rummukainen 2010). Consequently, such regional projections can be applied to impact studies on human health (Giorgi and Diffenbaugh 2008) and to assessments of climate-sensitive vector-borne diseases (Jacob 2008).

Our projections refer to the regional climate model COSMO-CLM (CCLM), which is driven by ECHAM5 and dynamically downscaled for Europe (Rockel et al. 2008). The quality of the driving data has a larger impact on simulation results than spatial resolution or physical parameterization (Meissner et al. 2009). CCLM addresses the scenarios A1B and B1, which both expect continuous human population growth until mid-century in a global oriented homogeneous world.

The A1B scenario is characterized by an equal use of fossil and non-fossil energy resources and the introduction of efficient technologies. The moderate and hence rather optimistic B1 scenario supposes a development towards service orientated societies with regional focus on ecological changes by introduction of renewable energies. Hence, projected temperature increase is less severe in B1 than in A1B (Fig. 1). Both were considered as marker scenarios that best illustrate the respective storyline (IPCC 2007). The B1 scenario matches well with the European Union target of keeping global anthropogenic warming below two Kelvin above industrial level (Jacob and Podzun 2010).

2.2.2 Pre-processing of the data

In our calculations, the original model output for projected daily mean temperature data was applied for both scenarios and for the complete 21st century in the binary net.cdf format (network common data form). We used the data stream D3 (run 2), which is the only one that organises on a regular grid and does not require conversion from the usually used rotated grid. This data stream was previously also used for model evaluation (Smiatek et al.

2009). The spatial resolution is 0.2°, which is about 18 km.

In order to reduce statistical noise and natural variability and to detect significant climatic trends in both scenarios we averaged the daily values separately for the time-steps 2011-2040, 2041-2070 and 2071-2100. By calculating the averages over the time-intervals we receive more robust and veritable hints for the expected temperature increase of every day in the year. Averaging of daily temperature data as well as interpolation of the available binary format net.cdf to a horizontal grid as text files was done via Climate Data Operators code (Schulzweida et al. 2009). This resulted in text files incorporating temperature data for each julian day for the respective time-step and scenario. Each text file was then attributed with an identical header indicating the spatial resolution and geographical extent. Hence, the text files could be imported by conversion to raster files for further processes in ArcGIS 9.3.1. In a second step the raster of the first of January for each scenario and time-step and scenario was converted to a point shapefile, locating points at the centre of each raster cell. These point shapefiles were used to extract the raster files representing other days of the year (January 2 - December 31) for the respective time-step and scenario.

The conversions of the text files to raster grids and the extraction of the raster values for each day via the point shapefiles were standardized and carried out with scripts written in Python 2.5.5 and R 2.11.0 (R Development Core Team 2010).

2.3 Modelling the spatio-temporal risk of dengue virus amplification

We received point shapefiles for each time-step and scenario including the daily temperatures of the whole year for Europe. This allowed a selection via attributes. We generated three selection codes to determine at which locations temperature requirements are fulfilled for:

- eight consecutive days with temperatures of at least 22 °C (Blanc and Caminopetros 1930)
- twelve days of at least 30° C (Watts et al. 1987)
- seven days with temperatures between 32-35 °C (Watts et al. 1987)

for the respective time-step and scenario.

The selection principles was a moving temporal window beginning on the first of

January (and consecutive days), while the second selection then started on the second of January and the last ended on the 31st of December. Those points were selected where the mentioned temperature requirements are at least fulfilled one time. In a second step, the longest potential intra-annual period, where the temperature requirements for virus amplification are fulfilled, was identified for the three time-steps and two scenarios separately. The beginning and the end was recorded for those points with the longest temporal fulfilment of temperature requirements without interruptions. Resulting selections were exported and converted to raster grids with the usual raster grid size of 0.2° (10 arcminutes) for cartographical visualization. We quantified areas at risk for three countries that represent a climatic gradient in Europe (Spain, France and Germany). Risk areas were calculated in comparison to the total country area.

Selection codes to model the spatial risk of virus amplification and to determine the longest intra-annual period as well as calculating percentages of areas at risk, for specific countries were performed in ArcGIS 9.3.1.

3. Results

3.1 Areas at risk according to dengue virus amplification

Apparently, the risk of virus amplification is likely to generally increase in the course of the 21st century, regardless of the chosen EIP and climate change scenarios. The highest percentage of areas located in risk zones is identified for the end of the century due to the projected increase in daily mean temperature from mid-century onwards in both scenarios (Tab. 1). For the A1B scenario the total areas at risk does exceed the risk areas for the B1 scenario. Remarkable differences in the results for virus amplification are conspicuous between EIP determined by Blanc and Caminopetros (1930) and Watts et al. (1987).

Following the constraint of Blanc and Caminopetros (1930) with temperature requirements of eight consecutive days with at least 22 °C for virus amplification, big parts of Europe would provide suitable temperature conditions during the 21st century (Fig. 2). This is true for both scenarios, even if the A1B scenario entails larger areas. The spatial hotspots are the same for both scenarios. For the period 2011-2040, almost the whole Mediterranean region and countries in the Southeast bordering the Black Sea seem to meet the temperature requirements.

Tab. 1: Area (in per cent) at risk of dengue virus amplification for a climatic gradient across Spain, France and Germany. Novel threats are projected to be most important for Spain and France. Germany will be at risk only if the extrinsic incubation period that was determined by Blanc and Caminopetros (1930) (eight consecutive days with minimum temperatures of 22 °C) is relevant, but not if applying the findings of Watts et al. (1987) (twelve consecutive days of at least 30 °C or seven consecutive days between 32–35 °C.

	Time-step	Area at risk per country in per cent					
		Spain		France		Germany	
		B1	A1B	B1	A1B	B1	A1B
8 days ≥ 22 °C	2011-2040	74	76	22	23	-	-
	2041-2070	83	85	46	54	< 1	< 1
	2071-2100	86	94	70	83	1	12
12 days ≥ 30 °C	2011-2040	5	5	-	-	-	-
	2041-2070	18	16	-	-	-	-
	2071-2100	21	35	-	< 1	-	-
7 days ≙ 32-35 °C	2011-2040	1	1	-	-	-	-
	2041-2070	5	4	-	-	-	-

In addition, the Rhone valley in France will already be suitable. During the mid of the century there is a considerable increase of risk areas in Western Europe, especially in France. There, the area at risk is nearly doubled in the period 2041-2070 in comparison to the time-step 2011-2040 (Tab. 1). Temperature requirements will be met during this period also in parts of Central Europe, for instance in the Southwest of Germany. At the end of the century, larger parts of Belgium and the North of France will provide suitable temperature conditions for the A1B but not for the B1 scenario as well.

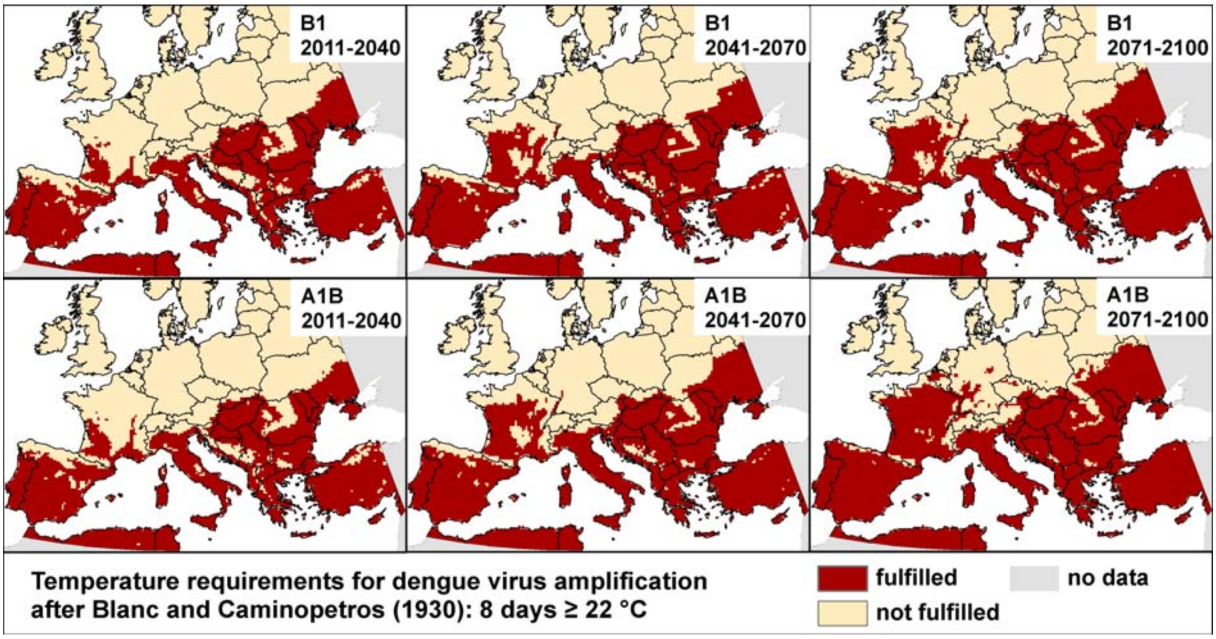

Fig. 2: Projection of the extrinsic incubation period for dengue virus amplification within the vector *Aedes aegypti*, determined by Blanc and Caminopetros (1930) with eight consecutive days of at least 22 °C.

When assuming an EIP of 12 days above 30 °C (Watts et al. 1987), the Southwest of the Iberian Peninsular (Valleys of Tajo, Guadalquivir and Guadiana) and Sicily are exposed to high risks during the time-step 2011-2040 (Fig. 3). During the following decades, the risk areas increase further in the Southwest of Europe and additionally spatially limited areas will be threatened in Greece (region of Thessaly) and coastal zones of Turkey. Furthermore, confined areas in Southeast Europe are expected to exceed thresholds. Between 2071 and 2100 considerable parts of Italy will also be appropriate.

The temperature constraints with daily mean temperatures between 32-35 °C (Watts et al. 1987) are rather extreme and only few regions will achieve daily mean temperatures between 32-35 °C over seven consecutive in the 21st century (Fig. 4). Following these assumptions, for Seville and regions along the Tajo River in the Southwest of Spain, dengue virus amplification can be assumed already during the first half of the 21st century. The risk area would extend slightly within the time-step 2041-2070 and reach up to 20% (A1B) of the total area of Spain. At the end of the century spatially limited risk is projected for the Italian regions (Apulia, Lombardy, Piedmont and Venetia). In south-eastern Europe, the valley of the Danube in Romania and the Aliakmon in Greece as well as the coastal region of Turkey will provide suitable temperatures.

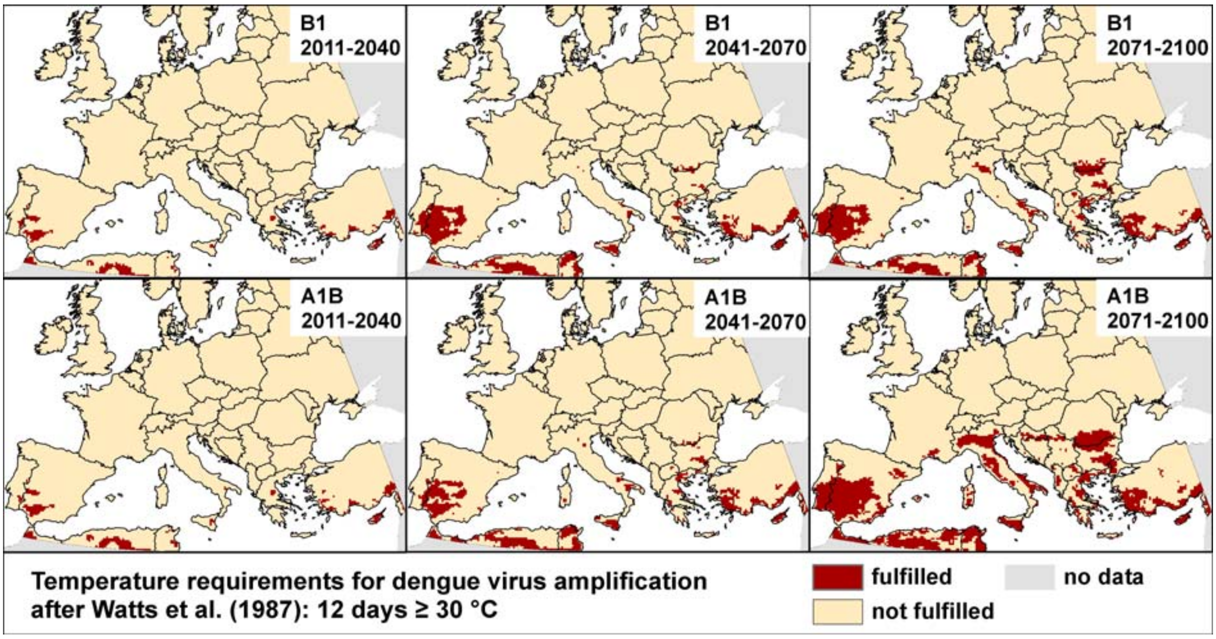

Fig. 3: Projection of the extrinsic incubation period for dengue virus amplification within the vector *Aedes aegypti*, determined by Watts et al. (1987) with twelve consecutive days of at least 30 °C.

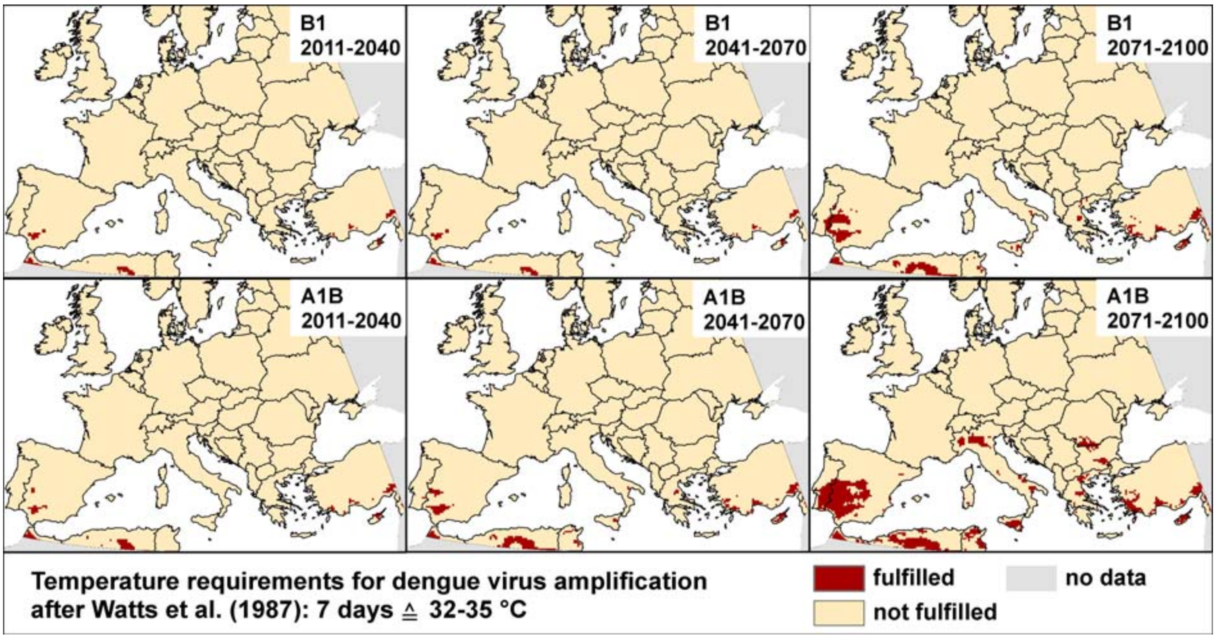

Fig.4: Projection of the extrinsic incubation period for dengue virus amplification within the vector *Aedes aegypti*, determined by Watts et al. (1987) with seven consecutive days between 32-35 °C.

3.2 Longest potential period of dengue virus amplification

The longest suitable period is detected in the southwest of the Iberian Peninsula - the region around Seville. We expect the annual duration of periods that are providing suitable temperatures for virus amplification to increase during the 21st century in general and especially towards the end of the century (Fig. 5).

This is true for all temperature requirements, time-steps and scenarios. As expected, the duration of the longest period mainly depends on the chosen EIP. Moreover, the longest intra-annual period of virus amplification varies more between time-steps than between scenarios. On the regional example of southwest Europe, the longest duration with suitable temperature conditions are noted:

Virus amplification based on the findings of Blanc and Caminopetros (1930) can last 146 days (A1B) or 136 (B1) during the coming decades (2011-2040). The increase of the length of suitable intra-annual periods from the early 21st century to mid-century is surprisingly higher for the B1 scenario. As consequence, at mid-century, the maximum temporal range for virus amplification will last up to 160 days in both scenarios. However, differences in the projections of the two scenarios are again from mid-century onwards to the end of the century, when a further increase of up to 179 days is projected in the A1B scenario, while the B1 scenario is characterised by a slight decrease to 157 days.

Concerning the EIP found by Watts et al. (1987) of at least 30 °C mean temperature over twelve consecutive days, the longest potential period is limited to 70 days (A1B) or 58 days (B1) within the years 2011 to 2040. The period for dengue virus amplification is longer in B1 scenario (88 days in comparison to 80 days in A1B scenario) for the years 2041 to 2070. A temporal extension can be expected (A1B: 93 days, B1: 90 days) for the last time-step.

When applying the finding of Watts et al. (1987) with daily mean temperatures of 32 to 35 °C over seven consecutive days, we identify the shortest window for virus amplification. Regarding the A1B scenario, the period will last longer in all time-steps than in B1 scenario. Starting at 2011-2040 the maximum period will last 41 (A1B) or 34 (B1) days respectively. At mid-century a period of 59 (A1B) or 48 days (B1) can be expected, while the virus amplification will be extended up to 85 days in A1B and 72 days in B1 scenario.

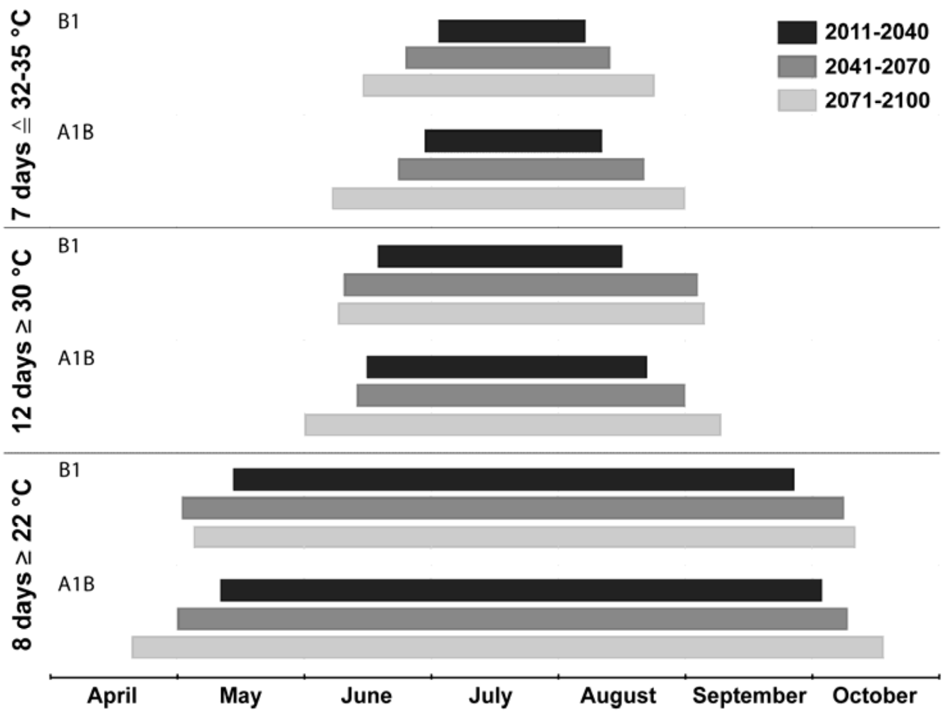

Fig. 5: Longest possible intra-annual period of dengue virus amplification in Europe.

4. Discussion

4.1 General tendencies in projected temperature thresholds

In this study, we used temporally high resolved data (daily resolution) from a regional climate model. We detect where and when dengue virus amplification can be expected to take place with respect to climate change in Europe. We indicate increasing areas at risk for all temperature requirements of dengue virus in both scenarios. Especially towards the end of the century the negative trend that we find is expected to speed up.

The results are based on experimentally identified temperature constraints. Differences between these laboratory studies are considerable. First of all, the temperature ranges for dengue virus transmission via *Ae. aegypti* is influenced by the titer of the mosquito-infecting virus dose. In the classic study, Blanc and Caminopetros (1930) experimentally infected mosquitoes by feeding them on infected humans at subsequent days of illness with low virus dose. Using low virus dose in monkey blood, Watts et al. (1987) determined extended EIP in comparison to high virus dose. Applying the comparatively low temperature threshold determined by Blanc and Caminopetros (1930) resulted consequently in an earlier threat and more European areas at risk, than in the projections based on the much higher temperature requirements that were detected by Watts et al. (1987). Comparing the results for the two alternative temperature regimes of Watts et al. (1987), most European regions would not achieve these extremely high daily mean temperatures (corresponds to EIP found for high virus dose) over short periods. More regions will experience lower but nevertheless rather high temperatures over longer periods (corresponds to EIP found for low virus dose).

4.2 Other factors for dengue transmission and comparison of aedine dengue vectors

Various factors and processes are contributing to the performance of mosquito-borne diseases besides climatic constraints. Thus, our results should not be misinterpreted as factual risk maps but rather as temperature-derived risk maps for dengue virus amplification, assuming the presence of a competent vector.

For the potential introduction of dengue virus in Europe, increasing risks are related to increasing intercontinental travel and trade (Kuno 1995; Reiter 2008). The number of virus-carrying human hosts in Europe increases due to close connections with endemic (sub-) tropical regions (Randolph and Rogers 2010; Reiter 2010). Socioeconomic factors play an important role in dengue transmission, as shown in Texas, where human behaviour (use of air-conditioning, evaporative coolers) lowers dengue prevalence (Reiter et al. 2003).

Regarding the risk of transmission, the mean age and the life expectancy of the mosquito population have to be taken into account, as older females show higher probability to transmit the virus (Holmes and Birley 1987; Carbajo et al. 2001).

Ae.aegypti, which is one main vector of the dengue virus, was recently introduced and established in Madeira (Almeida et al. 2007). Mosquito control actions inhibited an establishment in the Netherlands (Scholte et al. 2010), whereas *Ae. albopictus*, also a potential vector, is already established in Southern Europe. This invasive species is observed to rapidly spread into warm regions of the continent (Knudsen et al. 1996; Benedict et al. 2007). Survival during wintertime will be crucial regarding the further expansion of *Ae. albopictus* in Europe. Depending on the origin of the species, cold tolerance and the production of diapausing eggs differ (Hawley 1988). Moreover, diapause apparently evolved from nondiapause or nonphotoperiodic ancestors (in Brazil), whereby a diapause reduction could be observed presumably due to rapid local selection (in USA) (Lounibo set al. 2003). Furthermore, a distinct competitive advantage is found for *Ae. albopictus* compared with *Ae. aegypti* especially in the larval stadium (Brakset al. 2004).

Unfortunately, studies on temperature thresholds for the EIP of the dengue virus in *Ae. albopictus* are missing. As a consequence, our study is based on the temperature constraints for the EIP in *Ae. aegypti* only. These two mosquito species differ in habitat preference, desiccation resistance of eggs (Sota et al. 1992) and, most notably, in feeding patterns. Female *Ae. aegypti* take more than one blood meal during each gonotrophic cycle and prefer feeding on humans. Feeding rates of *Ae. aegypti* vary geographically depending on climatic conditions (Scott et al. 2000). Also the oral receptivity of *Ae. aegypti* to dengue is significantly higher than that of *Ae. albopictus.* Generally, colonisation of these vectors in laboratory increases their susceptibility for dengue virus (Vazeille et al. 2003). Moreover, differences in feeding patterns and susceptibility of both aedine mosquitoes could lead to different dengue incubation times.

Both vectors are capable of transmitting the dengue virus transovarial (vertical) to the offspring, which determines the starting point for further infections (Rosen et al. 1983; Rosen 1987; Shroyer 1990). *Ae. albopictus* and *Ae. aegypti* are also capable of transmitting various other virus such as chikungunya, Rift-Valley, Ross-River, West Nile and yellow fever (Gratz et al. 2004). Recently, invasive populations of *Ae. albopictus* were involved in a chikungunya outbreak in the region of Ravenna, Northern Italy (Rezza et al. 2007). After more than six decades autochthonous dengue cases have been reported in Europe again (Southern France La Ruche et al. 2010, Croatia Gjenero-Margan et al. 2011).

4.3 Previous models regarding the role of changing temperature in dengue transmission

Focks et al. (1995) provided a dengue simulation model with EIP as the most influencing parameter in the transmission dynamics in areas with suitable vector habitat conditions. Even slight fluctuations in temperature significantly affect the EIP and hence seasonal risk of dengue transmission. In contrast, further parameters such as the length of gonotrophic cycle or the probability of multiple feeding stay more or less unchanged (Patz et al. 1998).

Based on this previous study, Patz et al. (1998) applied global climate change effects to project the basic reproduction rate (R_O) originally representing the vectorial capacity multiplied by the length of time that a person remains pathogenic. Hence, R_0 is interpreted as the average number of secondary human infections produced from one infected person among a susceptible human population. In their study Patz et al. (1998) excluded the multiplication by duration of a pathogenic person assuming this factor as relatively constant in the case of dengue. They indicate an increasing risk of potential seasonal dengue transmission for temperate regions at mid 21^{st}-century. This is in accordance with the projections based on global climate change of Haleset al. (2002) who additionally integrated further climatic factors and projections of human population.

As temperature effects on EIP have previously been pointed out as crucial factor, our approach to project EIP via spatio-temporal highly resolved climate change projections allows a more detailed characterization of potential areas at risk for Europe, which is currently missing. In addition, our methodological proposal offers the opportunity to calibrate recently proposed dengue models (e.g. Barbazan et al. 2010; Degallier et al. 2010; Erickson et al. 2010) to the expected regional climate change in Europe. Those regional climate change projections are also applied in order to project the risk of malaria transmission in Germany using a R0-model, although not in a daily resolution (Holy et al. 2011).

4.4 Data quality of the regional climate model

In order to cope with uncertainties regarding future climate change (IPCC 2007), we focused on two scenarios (A1B and B1) integrated into the regional climate model CCLM. This is driven by the global model ECHAM5 (Rockel et al. 2008). An accurate downscaling of the spatial resolution of ECHAM5 improves model performance (Roeckner et al. 2006). Hence, the uncertainty that is related to the boundary conditions of a regional climate model is reduced (Déqué et al. 2007; Meissner et al. 2009).

Comparing the observed present-day climate with the current conditions simulated by CCLM, a cold summer bias becomes obvious for Western and Central Europe (Brockhaus et al. 2008; Jaeger et al. 2008). This leads to an underestimation in the longest continuous period of summer days with maximum temperatures above 25 °C (Roesch et al. 2008). A potential underestimation in the projected longest period of dengue virus amplification for

Europe may occur in our study, although we used daily mean instead of maximum temperatures. Nevertheless, these biases are documented - and even more pronounced - for other state-of-the-art models of European regions (Christensen et al. 2007; Jacob et al. 2007; Jaeger et al. 2008).

The earlier version (CLM) was nominated as a community model for the German climate research by the steering committee for the German Climate Computing Centre (DKRZ) in 2005 (Rockel et al. 2008). Additionally, CCLM offers the advantage of including the entire area of Europe. Therefore, in this study CCLM is used.

4.5 Impact of weather extremes

As it has been stressed for ecological impact studies in the face of climate change (Jentsch et al. 2007; Jentsch and Beierkuhnlein 2008), also for the evaluation of risks related to mosquito-borne diseases in Europe, studies are needed on the relevance of short-term weather extremes and increasing climatic variability. During the 21st century, the continental interior of Europe is very likely to experience a rapid increase in the intensity of extreme temperatures (Beniston et al. 2007). However, projections for temperature and precipitation extremes differ significantly between models (Kjellströmet al. 2007). Only if this uncertainty is reduced in the climate models, both climatic trends and weather extremes can be considered. This would improve the risk assessments for mosquito-borne diseases.

5. Conclusions

Here, we identified potential future risk areas for dengue virus amplification. Climate change can be connected with spatial as well as temporal extension (longer potential intra-annual period for dengue transmission) of this novel threat for European regions. Our proposed methodological task to integrate climate change data in daily resolution seems promising to benefit impact studies on mosquito-borne diseases. Such projections necessarily require profound knowledge on climatic constraints of vectors or/and pathogens. Therefore experimental studies should take this issue into account in future research in order to reduce uncertainties in projections.

Climate change is expected to cause repercussions in the distribution of pathogens and vectors resulting in novel threats for human societies and challenges for healthcare. The recent example of an outbreak of chikungunya virus in Italy was a first wake-up call in Europe. Obviously, infectious diseases that were thought to be restricted to tropical regions can expand northwards.

Introduction of virus and vector already took place at certain European gateways, such as harbours and airports. Obviously, the expected spread of mosquito-borne diseases refers not solely to climatic changes. Other aspects of globalization have to be taken into account as well and strict biocontrol may help to delay or even avoid further accidental carry-overs. Policy and public health authorities urgently require profound knowledge on the potential responses of mosquito-borne diseases to climatic changes for decision making. The design of specific monitoring and surveillance systems can only be efficient if it can be concentrated to risk areas.

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Extrinsic incubation period of dengue:

Knowledge, backlog and applications of temperature-dependence

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6.5 Article 5

Extrinsic incubation period of dengue: Knowledge, backlog and applications of temperature-dependence

With 2 Figures in the main text as well as one Table in the Supplemental Material.

Background

Dengue is generally believed to be one of the most hazardous vector-borne diseases, with over 40% of the world's population at risk of an infection [1]. While in the past the disease has mainly been observed in the tropical regions, recent studies suggest that, under the pressure of future climate change, new areas as far north as Europe may become endangered. In fact, in 2010 the first European cases of autochthonous dengue since the epidemic outbreak in Greece in the late 1920s [2] were reported from Croatia [3] and France [4]. Recently, Madeira experienced a severe epidemic of dengue fever, with about 2,000 cases within two months [5].

When it comes to determining the risk of dengue occurring in a given region, the extrinsic incubation period (EIP) plays an important role. The EIP is commonly defined as "the interval between the acquisition of an infectious agent by a vector and the vector's ability to transmit the agent to other susceptible vertebrate hosts" [6]. In the case of dengue, after the virus is ingested by a mosquito through a blood meal, some time is required for the virus to replicate, escape the midgut, and spread through the mosquito's body until it ultimately reaches the salivary glands (SG), from where it can be passed on to another host during the next blood meal.

For dengue, the duration of the pathogen's EIP is known to be temperature-dependent, but very few mechanistic risk models (usually based on the basic reproductive number R0, i.e., the number of secondary cases produced by one primary case in a completely susceptible population [7]) have taken that into account until now. In fact, most of the models implemented for dengue use fixed values for the duration of the EIP or rather rough estimates of temperature dependence [8].

This may be due to the fact that experimental studies on this topic are rare, and their results may appear to some extent inconsistent or even contradictory. However, the implementation of a realistic, temperature-dependent EIP will greatly improve mechanistic dengue modeling: since EIP appears as an exponent in the equations used for the determination of R0 and vector capacity [7,9,10], even small changes in EIP can have a large impact on the results of mechanistic dengue models that build on the concept of R0. The practical relevance of this issue has been demonstrated for dengue [9] as well as other vector-borne diseases such as malaria [11] and bluetongue [12].

In addition, correlative models based on environmental factors and vector distributions (also referred to as “climate envelope models” or “environmental niche models”) have to be revised and enhanced. Currently, these models usually focus on the spatial distribution of vector species. But if temperatures do not support amplification and establishment of the virus even though the vector is present, risk assessment based solely on vector distributions leads to an overestimation of areas at risk. Combining such models with information on temperature requirements for the virus derived from the EIP can reduce uncertainty [13].

Here, we give a short overview of the few experimental studies that are explicitly addressing the temperature dependence of the EIP of dengue. We analyze the implications of these studies and discuss current uncertainties in modeling dengue risk in face of climate change. We identify methodological challenges and formulate suggestions for the design of future studies from a spatio-ecological point of view.

What Has Been Done So Far?

In order to assess current knowledge about the temperature dependence of the EIP of dengue, we conducted an extensive literature search, using the Thomson Reuters Web of Knowledge research portal (which includes the databases Web of Science, BIOSIS, Current Contents Connect, MEDLINE, and Journal Citation Reports) as well as Google Scholar and Google Books. Search terms were built from all possible combinations of the keywords “dengue,” “DENV,” “extrinsic,” “EIP,” “incubation period,” and “temperature.” Journal articles and books that were found to provide secondary information on the topic were scanned for references to experimental studies, and a forward and reverse literature search was performed for experimental studies.

We found five experimental studies that explicitly addressed the temperature dependence of the EIP of dengue. The first one was carried out by Blanc and Caminopetros in Greece during the winter of 1928–1929 [14]. This was followed by two publications by McLean et al. in the mid-1970s [15,16] and another article by Watts et al. in 1987 [17]. Rohani et al. revived the topic in 2009 [18]. In addition to these works, we include two further studies in the dataset that examine the duration of the EIP at a single, fixed temperature: Salazar et al. [19] studied the spread of dengue virus within the body of *Aedes aegypti* at 28 °C, and Anderson and Rico-Hesse [20] examined the effect of viral genotype on the vector capacity of *A. aegypti* at 30 °C.

All experiments have in common that they examined the EIP of dengue virus type 2 in *A. aegypti*, with the exception of Blanc and Caminopetros, who did not provide information about the serotype examined (retrospective studies suggest dengue virus types 1 and 2 occurred during the Greece epidemic [21]), and Rohani et al., who additionally examined dengue virus type 4. However, the experimental approaches vary considerably in many respects within and between the studies. An overview of the durations of the EIP as observed by the different studies is given in Figure 1A; a detailed list can be found in Table S1.

Differences start with the study material used: the provenance of the mosquitoes used ranges from recently captured wild animals [14] to colonies that had been held in the laboratory for more than 30 years [18]. Since populations that have been held in the laboratory for a longer time may develop adaptions to the artificial environment, field-relevant mosquitoes are preferred for determining EIP, in order to yield results that reflect natural processes as closely as possible [19]. This is also true for viruses that have been maintained in the laboratory for longer periods [19]. Additionally, it is highly important to cover the whole range of genetic variations that occur in nature, since it has been demonstrated that different genotypes or strains of the dengue virus can show significant differences regarding their EIP [10,15,20].

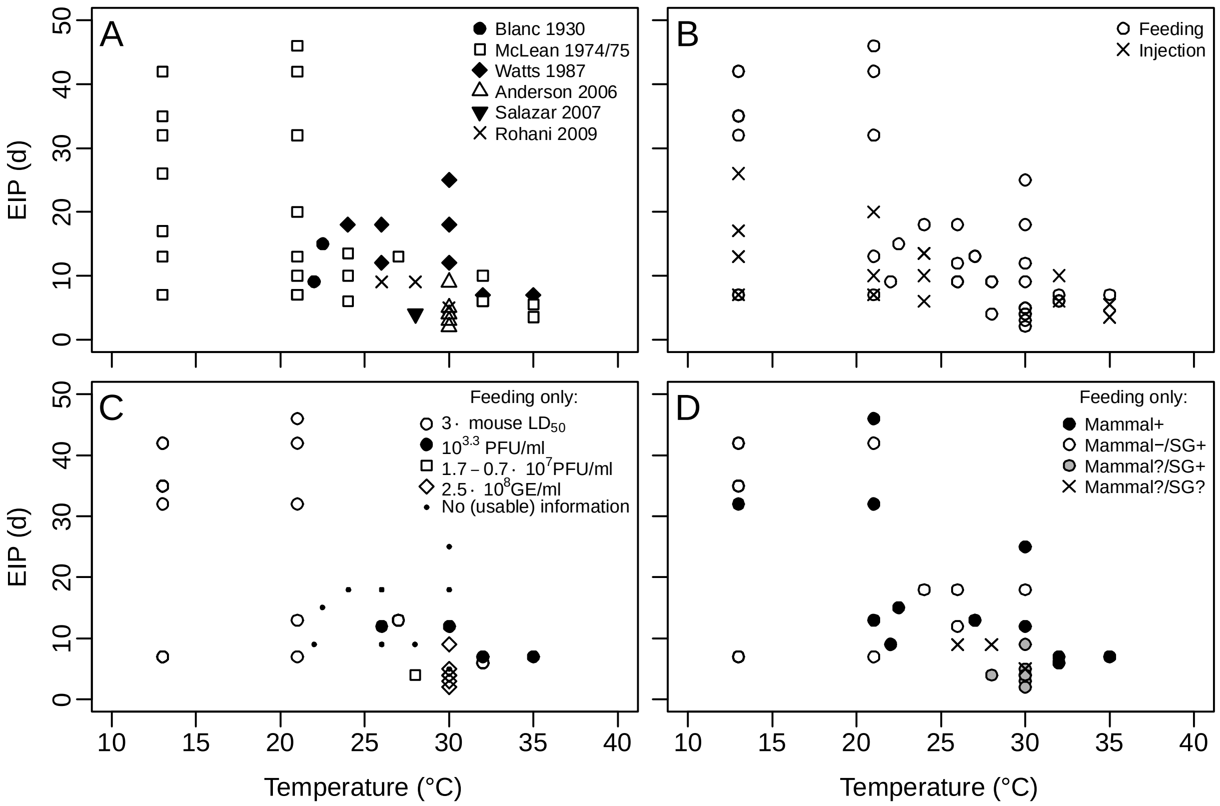

Figure 1. Overview of the available data for the temperature dependence of the EIP of dengue.

Each point represents the duration until the first observed transmission or infection of SG at a given temperature in a single experiment. (A) Complete dataset, divided by study. (B) Complete dataset, divided by method used to infect the mosquitoes: results obtained by letting mosquitoes feed on infected mammals or artificial blood meals versus results obtained via intrathoracic injection of virus solution. (C) Data from mosquitoes infected via feeding, divided by the amount of virus ingested by mosquitoes. GE, genome equivalents; LD50, mean lethal dose; PFU, plaque forming units. (D) Data from mosquitoes infected via feeding, divided by method of demonstration of transmission. Black circles: Transmission was demonstrated by allowing infected mosquitoes to feed on mammals. White circles: Tests on mammals yielded negative results, but SG contained virus. Grey circles: Tests on mammals were not done, but SG contained virus. Xs: Neither transmission to mammals nor SG were tested.

Moreover, differences in experimental techniques for infecting the mosquitoes became obvious: while intrathoracic injection of virus solution provides the opportunity to exactly determine the amount of virus a mosquito receives, it bypasses the midgut infection and escape barriers. This drastically shortens the EIP [15,22], leading to overestimation in the process of risk assessment. In the case of dengue, this problem affects about 60% of the data points by McLean et al. [15,16] (Figure 1B). Hence, we strongly suggest the use of more natural and realistic feeding techniques that use viremic vertebrates or artificial blood meals.

Since the duration of the EIP also depends on the amount of virus ingested during the blood meal, ideally the complete range of virus titers observed in vertebrate hosts in nature should be considered. The methods and units used for determining and presenting the amount of virus differ across the experiments, making it difficult to conduct an adequate comparison (see Figure 1C for an overview and Table S1 for the details). While a consistent methodology would surely help to make the results of such experiments more comparable and more accessible for scientists from other fields, in our eyes the most important issue is to make sure that future experiments resemble nature as closely as possible.

Furthermore, the method used to test the ability of an infected mosquito to transmit the virus should be chosen carefully. Allowing the mosquito to take a second blood meal from uninfected mammals such as mice [15,16], monkeys [17], or even humans [14], and then monitoring the mammals for dengue symptoms or virus content may seem desirable, since it gives rather clear evidence of transmission. However, because of ethical as well as logistical restraints, in most cases this cannot be considered as an option anymore today. Consequently, other methods have been developed that focus on the detection of virus content in the SG of the mosquito. While it is generally assumed that transmission can occur as soon as the SG are infected, the literature provides some cases where the SG tested positive for virus content but additional transmission tests with mammals gave negative results [15–17]. A possible explanation for this may be the existence of a “salivary gland escape barrier,” which has been shown to exist for other viruses [23] but which is considered controversial for dengue [24]. However, new techniques exist that circumvent this potential problem by causing mosquitoes to spill their saliva, which can then be assayed for virus content [22]. Equally, methods that use complete heads or even full bodies to extract virus RNA are not suitable for the assessment of the EIP. The latter method was used by Rohani et al. [18], unfortunately making their data unsuitable for real-life modeling approaches even though the data seem to be consistent with the rest of the dataset. An overview of the implications of this issue for the dataset is presented in Figure 1D; additional details are given in Table S1.

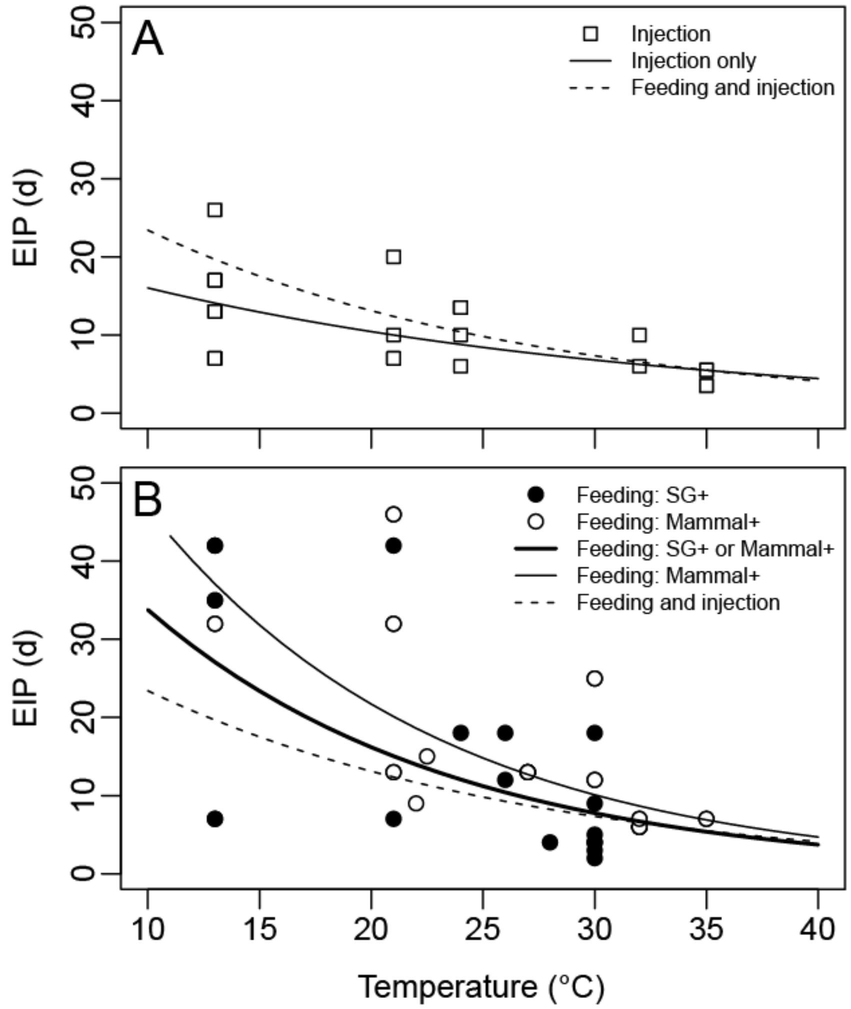

Figure 2. Estimated temperature dependence of the EIP of dengue based on the dataset used.

Each point represents the duration until the first observed transmission or infection of SG at a given temperature in a single experiment. Estimation was done via a simple linear model in R 2.14.1 [31], using log-transformed values of the duration of the EIP. (A) Results obtained from experiments with mosquitoes infected via intrathoracic injection; the solid line depicts the linear model for those data (adjusted $R^2 = 0.40$, $p < 0.001$). (B) Results obtained from experiments with mosquitoes infected via feeding. Filled circles: SG tested positive for virus content, but transmission to mammals was either negative or not tested. Unfilled circles: Transmission to mammals was observed. Thick solid line: Linear model for cases where either transmission to mammals was observed or SG tested positive for virus content (adjusted $R^2 = 0.34$, $p < 0.001$). Thin solid line: Linear model for cases where transmission to mammals was observed (adjusted $R^2 = 0.46$, $p < 0.01$) For better comparability, in both panels the dashed line shows the linear model for all data (injection as well as feeding) combined (adjusted $R^2 = 0.32$, $p < 0.00001$).

Careful preprocessing is crucial in order to gain meaningful results from the data that are currently available. First, experimental results that were obtained using intrathoracic injection to infect mosquitoes should be discarded, since their inclusion would lead to underestimation of the EIP and thus overestimation of areas at risk (Figure 2A). Then, data points for which verification of transmission does not exist by either examination of vertebrates bitten during a second blood meal or by examination of the SG should be discarded, too. Whether one wants to include data points for which transmission was verified only via examination of the SG may depend on the context the data are being used in: Figure 2B shows that the inclusion of these points in general leads to a shorter mean EIP, particularly at the lower end of the temperature range. Hence, risk maps based on a dataset that includes those points may overestimate the threat in regions with lower temperatures—which from an ethical point of view would be preferable to the underestimation that would probably result from the exclusion of those data. Additionally, data obtained from experiments at low temperatures (<20 °C) are especially scarce, so that further reduction must be carefully weighed for statistical reasons.

Design of Future Experiments with Respect to Interdisciplinary Research

Apart from the specific problems that arose in analyzing the experiments that have been done so far, there are some other things that might be worth considering when it comes to planning future works. Because the EIP varies between single mosquitoes, usually a batch of mosquitoes is examined for each time point during the experiment. The EIP can then be estimated as the period of time between the infectious blood meal and the point in time when (1) for the first time at least one mosquito of the batch is able to transmit the virus, (2) a given fraction (typically 50%) of the mosquitoes are transmitting, or (3) all mosquitoes are transmitting. A more advanced approach has been applied by Paaijmans et al. [25] that considers the fact that even after long incubation periods not all mosquitoes of a batch are able to transmit the virus. Here, we decided to use the time until the first observed occurrence of transmission or infection of the SG for the data shown in Figures 1 and 2 for two reasons. First, this is the most conservative approach, as it utilizes the shortest possible EIP and hence is unlikely to underestimate risk. Second, in some cases batches consisted of only five or fewer mosquitoes [15–17], which is too few to derive statistically meaningful fractions. In order to facilitate the application of advanced statistical methods, this issue should be taken into account during the design of future experiments: in our opinion, batches of 20 to 30 mosquitoes, as used by Salazar et al. [19] and Paaijmans et al. [25], are desirable.

Another important issue to note is that past laboratory studies usually held temperatures constant over the whole experiment. This neglects the fact that in nature diurnal temperature is far from constant. Recent studies imply that diurnal fluctuations in temperature may play a more decisive role for pathogen amplification than previously thought [26,27]. Including thermal fluctuations in future experiments and comparing the results with those from identical experiments with constant temperatures may prove rewarding.

Furthermore, not only the current main vector of dengue, *A. aegypti*, deserves attention: *A. albopictus* has undergone a vast global spread over the last decades [28] and is being considered as serving as a potential future main vector of dengue in Europe [29]. Until recently, knowledge about the EIP of dengue for *A. albopictus* was scarce and was mentioned only in a side note in the study by McLean et al. stating that “comparable results were obtained with *A. albopictus* mosquitoes” [16]. In 2012, Richards et al. compared the vector competence of *A. albopictus* and *A. aegypti* for dengue at different temperatures [30]. Even though the duration of the EIP was not explicitly examined (a fixed incubation period of 14 days was used), this study can be regarded as a step in the right direction, since experiments focusing on *A. albopictus* are urgently needed.

In conclusion, further studies on the EIP of dengue based on experiments with modern methodology and adequately high resolution in time and temperature may facilitate risk assessment by improving mechanistic as well as correlative modeling approaches. Since the lack of knowledge on the temperature dependence of the EIP seems to be even bigger when it comes to other arthropod-borne viral diseases such as Chikungunya, the identified challenges and suggestions may turn out to be of relevance beyond the example of dengue.

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Supporting Information

Table S1

Summary of the data obtained from the literature. This table provides information about the different experimental studies, including study material used and methodological details. The duration until the first observed transmission or infection of SG at a given temperature is given for each study.

Table S1: compilation of five experimental studies that explicitly address the temperature-dependence of dengue EIP.

Author	**Year**	**Mosquitoes taken from**	**Dengue type**	**Dengue genotype**	**Dengue strain**	**Amount of virus ingested by or injected into mosquitoes**	**Path of infection**	**Feeding: Living hosts vs. artificial blood meal**	**Feeding: Type of host/blood used**	**Salivary glands contain virus?**	**Trans-mission to mammals occur?**	**Mammals used for testing transmission**	**Temp. (°C)**	**EIP in days (first transmission or infection of Salivary glands)**
Blanc	1930	captured in Athens	NA	NA	NA	NA	feeding	feeding[*]	human	not tested	yes	human	22	9
Blanc	1930	raised in the lab	NA	NA	NA	NA	feeding	feeding[*]	human	not tested	yes	human	22,5	15
McLean	1974	BC Research Council lab*	DEN-2	NA	NC-6	3 * LD50 (mouse)	feeding	artificial bm	sheep	yes	yes	suckling mice	32	6
McLean	1974	BC Research Council lab	DEN-2	NA	40173	3 * LD50 (mouse)	feeding	artificial bm	sheep	yes	yes	suckling mice	32	6
McLean	1974	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	feeding	artificial bm	sheep	yes	yes	suckling mice	27	13
McLean	1974	BC Research Council lab	DEN-2	NA	40173	3 * LD50 (mouse)	feeding	artificial bm	sheep	yes	yes	suckling mice	27	13
McLean	1974	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	feeding	artificial bm	sheep	yes	no	suckling mice	13	35
McLean	1974	BC Research Council lab	DEN-2	NA	40173	3 * LD50 (mouse)	feeding	artificial bm	sheep	yes	no	suckling mice	13	35
McLean	1974	BC Research Council lab	DEN-2	NA	193-72	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	24	13 to 14
McLean	1974	BC Research Council lab	DEN-2	NA	193-72	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	13	13
McLean	1974	BC Research Council lab	DEN-2	NA	193-72	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	35	5 to 6
McLean	1974	BC Research Council lab	DEN-2	NA	40173	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	13	25 to 27
McLean	1974	BC Research Council lab	DEN-2	NA	40173	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	24	13 to 14
McLean	1974	BC Research Council lab	DEN-2	NA	40173	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	32	10

Author	Year	Mosquitoes taken from	Dengue type	Dengue genotype	Dengue strain	Amount of virus ingested by or injected into mosquitoes	Path of infection	Feeding: Living hosts vs. artificial blood meal	Feeding: Type of host/blood used	Salivary glands contain virus?	Trans-mission to mammals occur?	Mammals used for testing transmission	Temp. (°C)	EIP in days (first transmission or infection of Salivary glands)
McLean	1974	BC Research Council lab	DEN-2	NA	40173	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	35	3 to 4
McLean	1974	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	13	13
McLean	1974	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	24	6
McLean	1974	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	24	13 to 14
McLean	1974	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	32	6
McLean	1974	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	13	17
McLean	1974	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	24	10
McLean	1974	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	35	5 to 6
McLean	1974	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	35	3 to 4
McLean	1975	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	feeding	feeding*	suckling mice	yes	yes	suckling mice	13	32
McLean	1975	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	feeding	feeding*	suckling mice	yes	no	suckling mice	13	42
McLean	1975	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	feeding	feeding*	suckling mice	yes	yes	suckling mice	21	46
McLean	1975	BC Research Council lab	DEN-2	NA	NC-6	3 * LD50 (mouse)	feeding	feeding*	suckling mice	yes	yes	suckling mice	21	32
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	feeding	feeding*	suckling mice	yes	no	suckling mice	13	42
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	feeding	feeding*	suckling mice	yes	no	suckling mice	13	7
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	feeding	feeding*	suckling mice	yes	no	suckling mice	13	7

Author	Year	Mosquitoes taken from	Dengue type	Dengue genotype	Dengue strain	Amount of virus ingested by or injected into mosquitoes	Path of infection	Feeding: Living hosts vs. artificial blood meal	Feeding: Type of host/blood used	Salivary glands contain virus?	Trans-mission to mammals occur?	Mammals used for testing transmission	Temp. (°C)	EIP in days (first transmission or infection of Salivary glands)
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	feeding	feeding[*]	suckling mice	yes	yes	suckling mice	21	13
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	feeding	feeding[*]	suckling mice	yes	no	suckling mice	21	42
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	feeding	feeding[*]	suckling mice	yes	no	suckling mice	21	7
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	13	17
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	13	7
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	13	7
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	21	10
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	21	7
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	yes	suckling mice	21	20
McLean	1975	BC Research Council lab	DEN-2	NA	PR-159	3 * LD50 (mouse)	injection°	NA	NA	yes	no	suckling mice	21	7
Rohani	2009	lab colony (> 30 years old)	DEN-2	NA	NA	100µl virus #	feeding	artificial bm	human	not tested	not tested	NA	26	9
Rohani	2009	lab colony (> 30 years old)	DEN-2	NA	NA	100µl virus #	feeding	artificial bm	human	not tested	not tested	NA	28	9
Rohani	2009	lab colony (> 30 years old)	DEN-2	NA	NA	100µl virus #	feeding	artificial bm	human	not tested	not tested	NA	30	5
Rohani	2009	lab colony (> 30 years old)	DEN-4	NA	NA	100µl virus #	feeding	artificial bm	human	not tested	not tested	NA	26	9
Rohani	2009	lab colony (> 30 years old)	DEN-4	NA	NA	100µl virus #	feeding	artificial bm	human	not tested	not tested	NA	28	9
Rohani	2009	lab colony (> 30 years old)	DEN-4	NA	NA	100µl virus #	feeding	artificial bm	human	not tested	not tested	NA	30	5

Author	Year	Mosquitoes taken from	Dengue type	Dengue genotype	Dengue strain	Amount of virus ingested by or injected into mosquitoes	Path of infection	Feeding: Living hosts vs. artificial blood meal	Feeding: Type of host/blood used	Salivary glands contain virus?	Trans-mission to mammals occur?	Mammals used for testing transmission	Temp. (°C)	EIP in days (first transmission or infection of Salivary glands)
Salazar	2007	Yucatan/Mexico (F3-F6)	DEN-2	NA	NA	1,7+- 0,7 *10^7 PFU/ml	feeding	artificial bm	sheep	yes	not tested	NA	28	4
Watts	1987	Bangkok (F2 progeny)	DEN-2	NA	NA	10^3.3 PFU/ml blood	feeding	feeding˟	rhesus monkey	yes	no	rhesus monkey	26	12
Watts	1987	Bangkok (F2 progeny)	DEN-2	NA	NA	10^3.3 PFU/ml blood	feeding	feeding˟	rhesus monkey	yes	yes	rhesus monkey	30	12
Watts	1987	Bangkok (F2 progeny)	DEN-2	NA	NA	10^3.3 PFU/ml blood	feeding	feeding˟	rhesus monkey	yes	yes	rhesus monkey	32	7
Watts	1987	Bangkok (F2 progeny)	DEN-2	NA	NA	10^3.3 PFU/ml blood	feeding	feeding˟	rhesus monkey	yes	yes	rhesus monkey	35	7
Watts	1987	Bangkok (F2 progeny)	DEN-2	NA	NA	0.5ml with 10^5 PFU/ml ~	feeding	feeding˟	rhesus monkey	yes	yes	rhesus monkey	30	25
Watts	1987	Bangkok (F2 progeny)	DEN-2	NA	NA	0.5ml with 10^5 PFU/ml ~	feeding	feeding˟	rhesus monkey	yes	no	rhesus monkey	24	18
Watts	1987	Bangkok (F2 progeny)	DEN-2	NA	NA	0.5ml with 10^5 PFU/ml ~	feeding	feeding˟	rhesus monkey	yes	no	rhesus monkey	26	18
Watts	1987	Bangkok (F2 progeny)	DEN-2	NA	NA	0.5ml with 10^5 PFU/ml ~	feeding	feeding˟	rhesus monkey	yes	no	rhesus monkey	30	18
Anderson	2006	McAllen, Texas (F4)	DEN-2	Southeast Asian	CO489	2.5*10^8 GE per ml blood ≯	feeding	artificial bm	rabbit	yes	not tested	NA	30	3
Anderson	2006	McAllen, Texas (F4)	DEN-2	Southeast Asian	K0049	2.5*10^8 GE per ml blood ≯	feeding	artificial bm	rabbit	yes	not tested	NA	30	4
Anderson	2006	McAllen, Texas (F4)	DEN-2	Southeast Asian	Mara3	2.5*10^8 GE per ml blood ≯	feeding	artificial bm	rabbit	yes	not tested	NA	30	2
Anderson	2006	McAllen, Texas (F4)	DEN-2	American	IQT2913	2.5*10^8 GE per ml blood ≯	feeding	artificial bm	rabbit	yes	not tested	NA	30	5
Anderson	2006	McAllen, Texas (F4)	DEN-2	American	131	2.5*10^8 GE per ml blood ≯	feeding	artificial bm	rabbit	yes	not tested	NA	30	9
Anderson	2006	McAllen, Texas (F4)	DEN-2	American	Ven2	2.5*10^8 GE per ml blood ≯	feeding	artificial bm	rabbit	yes	not tested	NA	30	4

* lab colony at the British Columbia Research Council lab

° intrathoracic injection

100µl virus containing solution in 1ml blood

~ rhesus monkeys artificially infected by injection of 0.5ml with 10^5 PFU/ml

˟ feeding on infected animal

≯ 2.5*10^8 genome equivalents per ml blood

Climate change effects on Chikungunya transmission in Europe: Geospatial analysis of vector´s climatic suitability and virus´ temperature requirements

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6.6 Article 6

Climate change effects on Chikungunya transmission in Europe: Geospatial analysis of vector´s climatic suitability and virus´ temperature requirements

With 5 figures and 3 additional files

Abstract

Background

Before the first European outbreak of Chikungunya in Northern Italy in 2007, Chikungunya was, from the European perspective, considered to be a travel-related tropical mosquito-borne disease. In 2010, cases of autochthonous transmission were reported from South-eastern France. These events occurred after the introduction and subsequent establishment and expansion of the Chikungunya-competent and highly invasive disease vector *Aedes albopictus* (Asian tiger mosquito) in Europe. There is a need to further examine the relationship between potential transmission of Chikungunya and climatic factors in Europe.

Methods

Climatic suitability for Chikungunya outbreaks was determined by using bioclimatic factors that influence both vector and pathogen. Climatic suitability for the European distribution of the vector *Aedes albopictus* was based upon previous correlative environmental niche models. Climatic risk classes are derived by combining climatic suitability for the vector with known temperature requirements for pathogen transmission, obtained from outbreak regions. In addition, the longest potential intra-annual season for Chikungunya transmission was estimated for regions with expected vector occurrences.

In order to analyse spatio-temporal trends for risk exposure and season of transmission in Europe, climate change impacts are projected for three time-frames (2011-2040, 2041-2070 and 2071-2100) and two climate scenarios (A1B and b1) from the Intergovernmental Panel on Climate Change (IPCC). These climatic projections are based on regional climate model COSMO-CLM which builds on the global model ECHAM5.

Results

European areas facing current and future climatic risk of Chikungunya transmission are identified. An increase in risk is projected for Western Europe (e.g. France and Benelux-States) in the first half of the 21st century and from mid-century onwards for central parts of Europe (e.g. Germany). Interestingly, the southernmost parts of Europe will not provide suitable conditions in its entirety in these projections. Nevertheless, many Mediterranean regions will persist to be climatically suitable for transmission. Overall, the highest risk of transmission by the end of the 21st century was projected for France, Northern Italy and the Pannonian Basin (East-Central Europe). This general tendency is depicted in both, the A1B and B1 climate change scenarios.

Conclusion

In order to guide preparedness for further outbreaks, it is crucial to anticipate risk as to identify areas where specific public health measures, such as surveillance and vector control, can be implemented. However, public health practitioners need to be aware that climate is only one factor driving the transmission of vector-borne disease.

Keywords

Asian tiger mosquito, Chikungunya, climate change, dengue, globalization, global warming, infectious disease, invasion, public health, vector-borne disease

Background

Chikungunya virus (CHIKV) is an alphavirus (family Togaviridae) and was first isolated during an outbreak in Tanzania in 1953 [1]. The virus causes a disease form that typically consists of an acute illness with fever, rash and long-lasting incapacitating arthralgia [2,3]. In recent years, CHIKV has re-emerged in Africa, the Indian Ocean islands (especially on Reunion Island) and the Indian subcontinent as well as South-eastern Asia [3]. The main disease vectors are the two aedine mosquito species, *Aedes aegypti* and *Aedes albopictus* [2,4,5]. In the past, large epidemics were related to the presence of the primary vector *A. aegypti*, the Yellow fever mosquito, which is also the main vector of the dengue virus [2,6,7]. *A. aegypti* was established in southern parts of continental Europe until the mid-1900s but subsequently disappeared for reasons that are not completely understood [7].

During the last few years, *A. aegypti* has established a permanent population in Madeira, Portugal [8], where a recent dengue outbreak occurred [9]. A. aegypti has also re-established in the Caucasian region, bordering the Black Sea [10]. It was also introduced further north, such as around the harbour of Rotterdam, Netherlands, in 2010, but mosquito control activities resulted in its eradication in that area [11]. Establishment of *A. aegypti* might be more difficult in colder climates, however, as this appears to be a limiting factor in continental Europe [12].

Similarly, temperate regions have proven, thus far, to be of limited suitability for autochthonous CHIKV transmission. The disease was predominantly perceived as travel-related risk in continental Europe until the outbreaks of 2005 and 2006, in which Reunion Island and several neighbouring islands in the Indian Ocean were affected, raising concerns about novel trends of the CHIKV transmission cycle. During this time, genomic micro-evolution of CHIKV enabled transmission by a secondary mosquito vector, *A. albopictus* [4], with the consequence that Chikungunya epidemics can now also occur in regions where the primary vector, *A. aegypti*, is missing [4,5].

The possibility of transmission of CHIKV by *A. albopictus* is significant for continental Europe due to the anthropogenically-faciliated expansion of this mosquito [6,13]. The first introduction of *A. albopictus* in Europe took place in Albania in 1979 [14] and later into the port town of Genoa, Italy, in 1990 due to the importation of used tires [15]. Upon its second arrival, *A. albopictus* became well established in Southern Europe [16,17]. This increases the risk that autochthonous CHIKV transmission may arise, as European populations of *A. albopictus* exhibit a remarkable high vector competence for CHIKV [18, 19]. Indeed, the first epidemic of Chikungunya fever in Europe occurred in Ravenna, Northern Italy, with more than 200 affected humans after virus introduction from India [20]. Very recently, two children without travel history became infected in Provence-Alpes-Côte d'Azur, South-eastern France, all originating from a travel-related case coming from an outbreak area in India [21]. In both, the Italian and French outbreaks, *A. albopictus* is believed to have acted as the vector. In another example, *A. albopictus* transmitted dengue virus in Southern France [22] and Croatia in 2010 [23,24]. In light of such developments, along with intensive exchange of travellers between epidemic areas and Europe, the European Centre for Disease Prevention and Control (ECDC) launched a project to assess the risk of introduction and transmission of CHIKV in Europe [25].

Several studies have previously highlighted the increasing climatic suitability for *A. albopictus* in Europe as a consequence of climate change [12,26-28]. Until now, however, the risk for CHIKV transmission has been deduced from the current climatic situation [29]. Climatic requirements for pathogen circulation in outbreak regions and vector suitability must then be addressed as crucial factors [30]. Surprisingly few studies evaluate the spatio-temporal future trends in the risk of CHIKV transmission under European climate change scenarios through the 21st century. Here, we close this research gap by pursuing the following questions:

I) Which continental European regions are at risk (currently and under climate change scenarios), based upon temperature conditions from endemic Chikungunya areas?

II) Which continental European regions are at risk (currently and under climate change scenarios), when accounting for temperature requirements for CHIKV replication and the climatic suitability (including temperature and precipitation) of the vector *A. albopictus*?

III) How long would the potential season of CHIKV transmission last in European areas with assumed *A. albopictus* establishment?

Methods

Methodological challenge and strategy in brief

The focus of this study was to determine spatial and temporal climate-derived risk exposure for European regions facing potential transmission of CHIKV. Temperature requirements were derived from the literature based on areas where CHIKV circulated during past outbreaks (1995-2007). These temperature requirements were then used to model the current and expected future climatic suitability in continental Europe for CHIKV transmission.

The climatic suitability for CHIKV transmission was then combined with the climatic suitability of habitats for the vector *A. albopictus* that is based on temperature and precipitation requirements. This was done in order to determine climatic risk classes of CHIKV transmission for European regions by considering both pathogen and vector requirements. In addition, the longest potential intra-annual season for CHIKV transmission was estimated for regions, where vector occurrence has been observed or can be expected in the future. Differences between future projections are evaluated. All analyses were carried out in ArcGIS 10.0TM.

Mapping temperature requirements for the Chikungunya virus

Temperature requirements for CHIKV were obtained from a previous study [29]. Tilston et al. [29] examined progression of several Chikungunya epidemics in relation to local monthly mean temperatures (Tmean) and derived minimum Tmean needed for an outbreak. Interestingly, outbreaks started at different Tmean in different geographical localities: 20 °C in Italy and Reunion Island, 22 °C in India, 24 °C in Africa, and 26 °C in (Southeast) Asia, respectively.

One conclusion from this is that a Tmean of 20 °C appears to be the minimum threshold for Chikungunya outbreaks. However, in Italy and Reunion Island, Tmean at the beginning of the outbreak was at least 22 °C. Hence, we assume a higher CHIKV transmission risk in regions with mean temperatures greater than 20 °C for a period of at least one month. This assumption is supported by the fact that an amplification of CHIKV within the vector *A. albopictus* may occur if at least seven days provide temperatures of 26 °C [31]. Therefore, higher temperatures will likely increase the risk of transmission as they leads to shorter Extrinsic Incubation Periods (EIP), defined as the time interval between acquisition of an infectious agent by a vector and the vector's ability to transmit the agent to other susceptible vertebrate hosts.

In order to produce the analysis, the first step involved working with rastered data for Tmean for the current situation in Europe, obtained from worldclim.org [32]. For each raster cell, Tmean of the warmest month was selected and classified according to the requirements noted above. Projected data for future development of Tmean was obtained from the regional climate model COSMO-CLM [33] and classified same way. Pre-processing of the netCDF (network Common Data Form) files of COSMO-CLM demands climate data operator codes [34], before transformation into raster format capable for a Geographical Information System (GIS) was possible. Spatial resolution of the latter was lower, so climatic data coming from worldclim.org [32] was up-scaled to the 18 km resolution of the COSMO-CLM data via cubic convolution. The COSMO-CLM regional climate model is derived from the driving global model ECHAM5 by dynamical downscaling procedures and covers continental Europe in its entirety [33]. The advantage of working with regional climate models is that they simulate climate change effects more precisely than global models do (resolution > 100 km), which is especially useful for climate change impact studies of ecological processes and vector-borne diseases [35].

Two of the emission scenarios implemented in COSMO-CLM (A1B and B1) were used for climate projections assessment. The A1 scenario family is based on the assumption of an integrated world with single scenarios being characterised by rapid economic growth and a quick spread of new and efficient technologies. Human energy use in the A1B scenario itself is based on a balanced emphasis on all energy sources [36]. The B1 scenario also assumes a globalised world with rapid economic growth, but with changes towards an economy primarily based on service and information. The emphasis is global solutions to economic, social and environmental stability [36]. The B1 scenario corresponds with the ambitious target of the European Union of keeping anthropogenic warming below 2 Kelvin up to the end of the 21st century in comparison to preindustrial level [37]. To derive climatic trends, Tmean data were averaged over intervals of thirty years (2011-2040, 2041-2070, 2071-2100).

Climatic suitability for the vector *Aedes albopictus*

Data concerning climatic suitability for the vector *A. albopictus* for current and future conditions in Europe was obtained from a previous study [27]. For the purpose of this study, the Maximum Entropy approach, implemented in the MaxEnt software (latest version 3.3.3k) [38], was applied as correlative species distribution model. MaxEnt does not work with real absences, but with an “environmental background”. This approach accounts for both types of “absence” information: either the species does not occur at a given location, or no one has been tried to find it there.

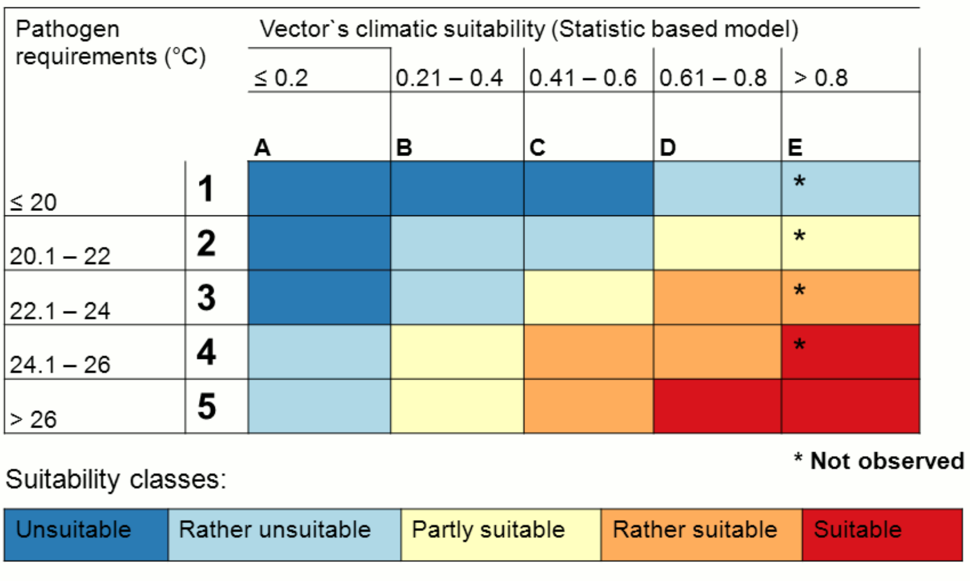
We used the results from the global Statistic-based model (SBM). In short, from a global database of 6347 documented occurrence records of *A. albopictus* a stratified subsampling was conducted resulting in 1119 records that were used as model input in order to avoid inflated results [see 27 for details]. Multiple records within one grid cell were additionally removed. The importance of each variable was quantified in a twofold manner with a Jackknife test implemented in MaxEnt. First, models training gain was measured for all variables in isolation and for the remaining set of variables when the isolated variable is dropped from the set. The gain indicates how closely the model is concentrated around the presence samples and can be compared with deviance as a measure of goodness of fit [38]. To reduce collinearity in the data set, variables that had a Pearson correlation coefficient r > 0.7 with any other higher-ranking variable in the results of the Jackknife test were removed. Variables were tested for collinearity before and after upscaling of the climatic data from worldclim.org [32] to the resolution of the COSMO-CLM data. The final input variables of the model are annual mean temperature, annual precipitation, and precipitation of the warmest as well as of the coldest quarter and altitude. Models were trained using a random subset (70%) of occurrence data, tested on the remaining 30% and procedure, replicated 100 times and finally averaged [see 27 for details]. The model performance was quantified using the area under the receiver operator characteristic curve (AUC).

The study outputs are climate suitability maps with values ranging from 0 (completely unsuitable) to 1 (extremely favourable conditions). For this study, climate suitability maps were reclassified into five probability classes in equal breaks from zero to one (0.2, 0.4, 0.6, 0.8). Projections of climatic suitability refer to data from the climate model COSMO-CLM [33], time-frames (2011-2040, 2041-2070, 2071-2100) and scenarios (A1B and B1), which were used for projections of CHIKV temperature requirements.

Risk classification for potential Chikungunya transmission

In order to address the second research question (which regions are at risk and will be at risk under climate change scenarios), pathogen temperature requirements and vector climatic suitability were combined via an overlay procedure. This type of risk classification to detect transmission potential of a vector-borne disease embedded in a GIS environment has been described previously [39].

We postulate the simple relationship that higher temperatures for the virus and higher climatic suitability for the vector result in higher risk for CHIKV transmission in European regions (Figure 1).



Pathogen requirements (°C)		Vector`s climatic suitability (Statistic based model)				
		≤ 0.2	0.21 – 0.4	0.41 – 0.6	0.61 – 0.8	> 0.8
		A	B	C	D	E
≤ 20	1	🟦	🟦	🟦	🔹	🔹 *
20.1 – 22	2	🟦	🔹	🔹	🟨	🟨 *
22.1 – 24	3	🟦	🔹	🟨	🟧	🟧 *
24.1 – 26	4	🔹	🟨	🟧	🟧	🟥 *
> 26	5	🔹	🟨	🟧	🟥	🟥

* Not observed

Suitability classes:

🟦 Unsuitable	🔹 Rather unsuitable	🟨 Partly suitable	🟧 Rather suitable	🟥 Suitable

Fig. 1: Climatic-derived risk classes for Chikungunya-transmission. Temperature requirements for the occurrence of Chikungunya virus were obtained from the analysis of Tilston et al. [29]. Chikungunya virus occurrences are observed for values of the mean monthly temperature in different regions. Virus information is combined with the spatial climatic suitability of the vector *Aedes albopictus* from Fischer et al. [27].

Based on this, we created five climatic-derived risk classes upon values for Tmean, representing pathogens constraints at different geographical regions and five suitability classes for the vector *A. albopictus*. Projections were done for each climate change time-frame and scenario. As precipitation was a variable in the analysis of the vector climate suitability, this ensured that misleading projections of high-risk areas in hot but dry areas are excluded. The results from this overlay were mapped to illustrate risk of CHIKV transmission in Europe, using the raster calculator implemented in ArcGIS 10.0TM. Additionally, we calculated the percentage of affected area of each risk class for specific European countries.

Determining the length of season for Chikungunya transmission

The potential length of the intra-annual season of CHIKV transmission was determined by tallying the number of months in which thermal virus´ requirements are fulfilled for each cell of the environmental raster. In order to gain the most conservative estimate, the threshold was set to a minimum Tmean of 20 °C (minimum temperature where transmission has been observed according to Tilston et al. [29]). The procedure was carried out for current climatic conditions, each time-frame (2011-2040, 2041-2070, 2071-2100) and scenario (A1B and B1). Once this information was obtained, the number of months with respective minimum Tmean (≥ 20 °C) were displayed as raster maps.

However, presenting solely number of months suited for pathogen threshold without consideration of potential vector occurrences would overestimate the risk for CHIKV. Consequently, the potential season of transmission was identified for regions with assumed presence of the vector *A. albopictus*. We reclassified the suitability maps of the SBM for the vector *A. albopictus* [27] into binary maps by determining a certain suitability threshold to categorise in regions with expected absence or presence. In environmental niche modelling, a number of procedures for choosing such thresholds exist [40]. Thus, in order to account for the effect of such a threshold choice for species range shifts under climate change [41], we used two established procedures for threshold estimation.

First, a rather classical choice of threshold is separating indices at 0.5, where suitability values range theoretically from zero to one [42,43]. This fixed choice of threshold is not adapted to specific data and modelling results. Second, equalisation of sensitivity and specificity (SeSpeql) by minimising the absolute difference between sensitivity and specificity is another established method [44-47]. Sensitivity and specificity are statistical measures of performance of a binary classification test. Sensitivity measures the proportion of actual positives, which are correctly identified as such. Specificity measures the proportion of negatives which are correctly identified. The probability threshold was chosen at the level where sensitivity (number of true positives divided by the sum of true positives and false negatives) equals specificity (number of true negatives divided by the sum of true negatives and false positives). We calculated the percentage of affected areas for the season of CHIKV transmission for respective European countries (based on SeSpegl-method to determine the threshold of assumed vector occurrence).

Results

Temperature requirements for Chikungunya virus

European regions at risk were identified based upon temperature conditions from endemic Chikungunya areas (Figure 2). According to previously detected temperature requirements [29] of Tmean, the warmest month for each time-frame and for both climate change scenarios were mapped for the current situation and projected to the future (2011-2040, 2041-2070, 2071-2100). Currently, western, central, eastern and northern parts of Europe do not have mean temperatures higher than 20 °C during the warmest month. Such conditions were solely fulfilled in southern parts of Europe. Generally, large parts of Southern Europe will exceed the lowest observed requirements for Tmean of 20 °C and achieve values of 26 °C. The size of these regions will expand during the 21st century. Interestingly, there are no remarkable differences between the two scenarios concerning temperature conditions for half a century. In the three last decades of the century (2071-2100), in comparison to the B1 scenario, the A1B scenario predicts temperatures of the warmest month to be up to two Kelvin higher in Western, Central and Eastern parts of Europe. This may have severe consequences for Central and Eastern Europe (e.g. Czech Republic, Germany and Poland) as there the lowest requirements were not projected to be fulfilled in the B1 projection while they were for large parts of the countries in the A1B scenario.

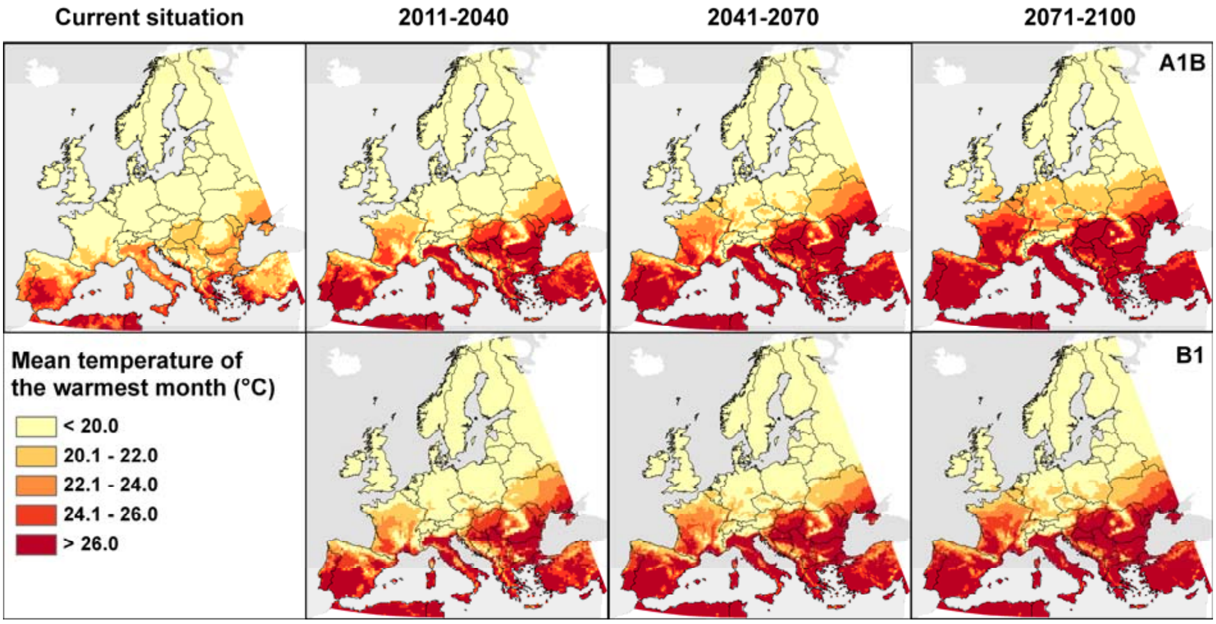


Fig. 2: Fulfilling of temperature requirements for the Chikungunya virus in Europe. Projections for different time-frames are based on two emission scenarios (A1B and B1) from the Intergovernmental Panel on Climate Change, implemented in the regional climate model COSMO-CLM.

Risk classification by overlaying of vector and virus maps

Assessing which European regions are at risk was done by accounting for temperature requirements for CHIKV replication and the climatic suitability of the vector *A. albopictus*. The models demonstrated high model performance, as indicated by an AUC value of 0.89 (±0.01) for the SBM of the vector. Currently, the risk of CHIKV transmission is highest for the southernmost parts of Europe. As a general tendency, the climatic risk of CHIKV will increase in Europe and the increase in risk exposure is more pronounced in the A1B scenario in comparison to the B1 scenario (Figure 3 and Additional file 1). A persisting high suitability for CHIKV transmission throughout the 21st century is projected for the Po valley in Emilia Romagna, Northern Italy. The climatic risk for CHIKV transmission is moreover projected to increase in the Western coastal Mediterranean areas of the Balkan States and Greece as well as in the Pannonian Basin. The Black Sea coast of Turkey must be aware of increasing climatic suitability for CHIKV transmission.

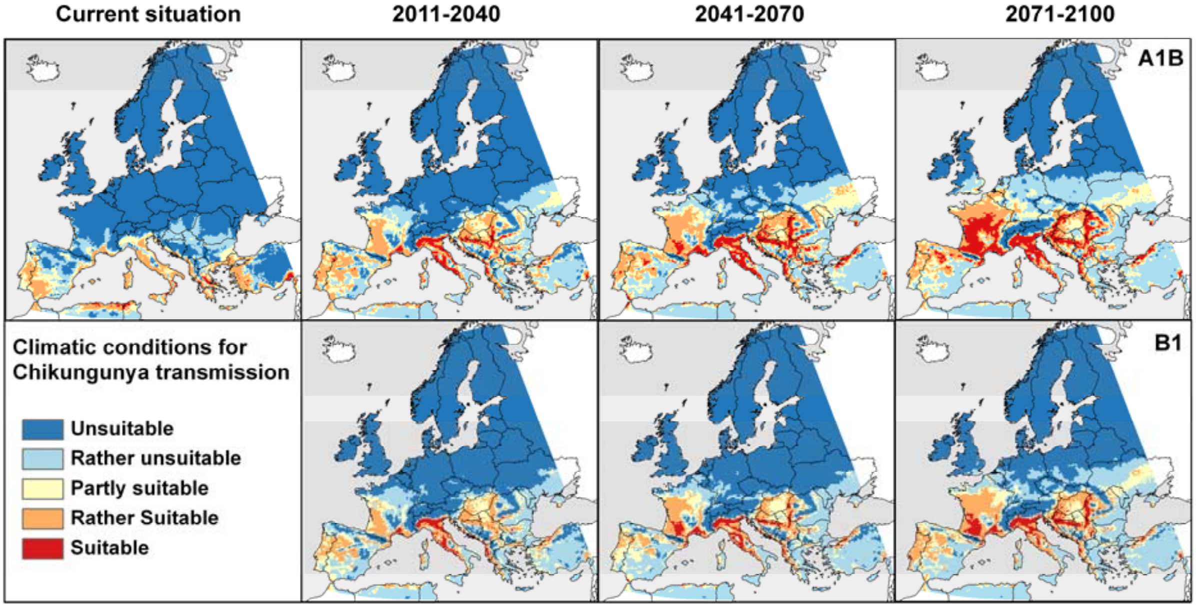


Fig. 3: Risk map for Chikungunya transmission in Europe generated by combining temperature requirements of the Chikungunya virus with the climatic suitability of the vector *Aedes albopictus*. Projections for different time-frames are based on two emission scenarios (A1B and B1) from the Intergovernmental Panel on Climate Change, implemented in the regional climate model COSMO-CLM.

A spatially limited risk is projected for mid-century conditions in Central Europe. South-eastern parts of the British Isles will be at limited risk at the end of the 21st century, according to both currently available scenarios. The northernmost parts, Scandinavia and the Baltic states, will not likely be subject to climate-induced risk.

Potential season of transmission

A final research question for this paper relates to the potential season of CHIKV transmission in Europe. First, we present only the number of months with suitable temperatures for CHIKV replications, without consideration of the vector. Obviously, the number of months with suitable temperatures increases for many European regions (Figure 4 and Additional file 1). Currently, a Tmean of 20 °C or higher in at least one month is restricted to countries in Southern Europe. Yet, by the end of the current time-frame (2011-2040), up to three months can be expected in Western Europe, regardless the chosen climate change scenario. With temporal delay, temperature requirements will be fulfilled for at least one month in Central (2041-2070) and many parts of Eastern Europe (2071-2100). By the end of the century, five months with minimum temperatures of at least 20 °C are projected for many parts of Southern Europe (in both scenarios). Differences in scenarios do, however, arise for the end of the 21st century (2071-2100) in Central and Eastern Europe. In the A1B scenario, most of the regions are identified with at least one month of fulfilled requirements, while in B1 scenario only spatially limited regions are highlighted there. In addition, the risk in the south-easternmost part of the United Kingdom is more extended in A1B projection. The tendencies in Western and Southern Europe are the same throughout the 21st century.

The number of months with suitable temperatures for CHIKV replication is only one part of the story to determine the potential season of transmission. Therefore, we also present the number of months with suitable temperatures for CHIVK replications for those regions with expected presence of *A. albopictus*. This determination of potential season of transmission is based on the described number of months with the lowest observed temperature requirements of 20 °C and on the assumed distribution of the vector *A. albopictus.*

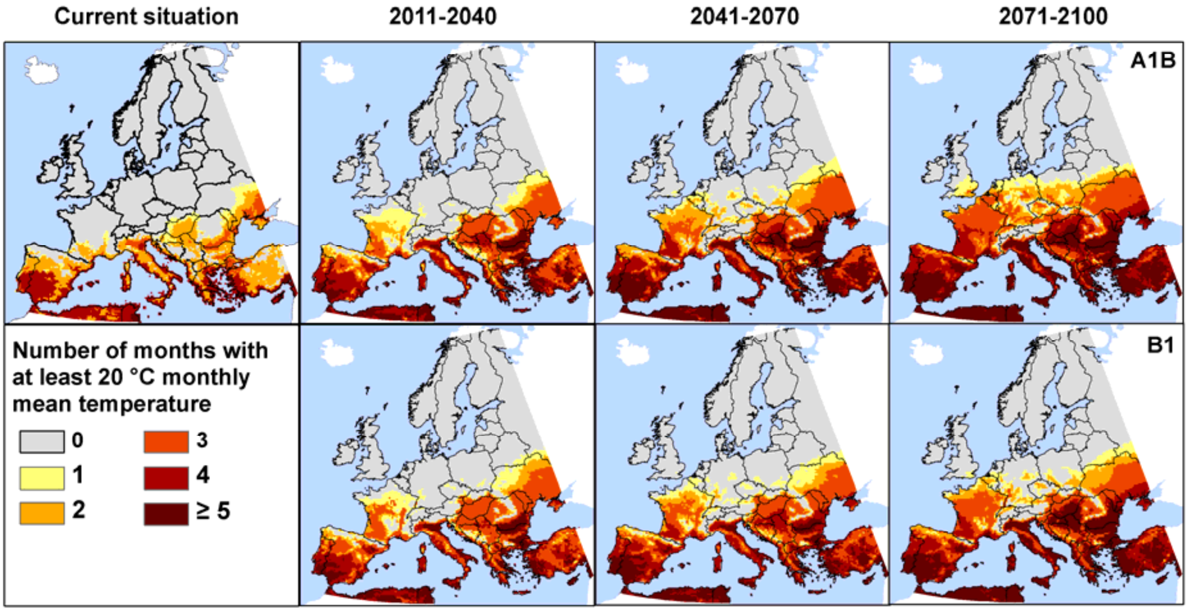


Fig. 4: Number of months with mean monthly temperature of at least 20 °C as minimum requirement for the transmission of Chikungunya virus. Projections for different time-frames are based on two emission scenarios (A1B and B1) from the Intergovernmental Panel on Climate Change, implemented in the regional climate model COSMO-CLM.

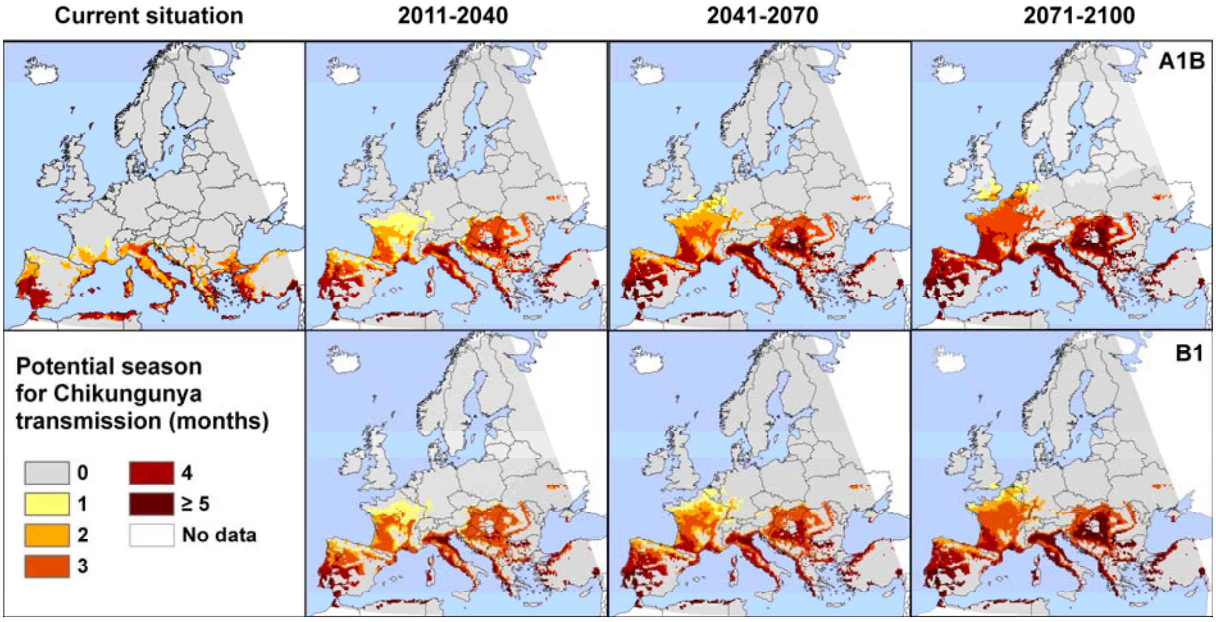


Fig. 5: Potential season of Chikungunya transmission based on Figure 4, but filtered by areas, where the presence of the vector *Aedes albopictus* can be expected. The threshold for occurrences from continuous values of suitability was obtained by minimising the absolute difference between the sensitivity and specificity, resulting in a specific value (0.371) as threshold for vector occurrences. Projections for different time-frames are based on two emission scenarios (A1B and B1) from the Intergovernmental Panel on Climate Change, implemented in the regional climate model COSMO-CLM.

The threshold for vector presences via SeSpeql-method was calculated to be 0.371. We also used the classical fixed value of 0.5. Presence of the species can be expected if these thresholds are met or exceeded in the respective region. Due to lower threshold value for the occurrence of *A. albopictus* via SeSpeql-method, more areas are identified where *A. albopictus* may be present, in comparison the conservative fixed threshold of 0.5. This resulted in more regions under consideration for the potential season of transmission by applying the SeSpeql-method (Figure 5) than by applying the conservative threshold (Additional file 2).

The following detailed interpretation is for the results of the SeSpeql-method (Figure 5). Currently, the longest possible period of transmission is identified along the Mediterranean coast line with a maximum of three months. Regardless of the chosen climate change scenario, areas of widened transmission windows will be the Pannonian Basin, the Po Valley, where the season of transmission might even rise up to five months from mid-century onwards. Moreover, three months of transmission will be possible in wide ranges of Central and Western Europe. In large parts of France the potential season of transmission could be four months. Interestingly, some regions where the potential transmission exists are geographically close to regions where the vector *A. albopictus* is not expected to occur. This is especially apparent in south-eastern parts of the Iberian Peninsula.

Discussion

Relevance, novelty and uncertainty in modelling approaches

The European-wide projections for *A. albopictus* account for changing patterns of activity phase or/and climatic suitability [12,26-28]. Frequently, spatial risk analyses for CHIKV transmission are based on calculating (and mapping) the basic reproduction number R0 [30,47-49]. However, in the case of CHIKV in Europe this type of modelling can be very misleading as one key factor for such models is the vector density, which is not yet known [50]. Therefore, in this study we pursued an alternative approach, in which we indicate regions where the climate is favourable for CHIKV transmission. Combined risk maps for the climatic suitability of the vector (*A. albopictus*) and the temperature thresholds for CHIKV transmission are derived via classification functions in order to identify spatial patterns at different future time-frames. This insight guided the climatic risk maps presented here of potential

CHIKV transmission zones for all of Europe that address both the current and expected future climatic conditions. We provide an additional map indicating all of the mentioned regions and localities of the mentioned European regions or localities in the text for an easy interpretation (Additional file 3). Differences in projected time-frames and scenarios are evaluated. As European climates become more permissive in the future, further spread of *A. albopictus* to higher latitudes on the continental European scale [12,26-28] and to higher altitudes on the local scale is anticipated [51,52].

The objective of this study has been to further assess the potential role of climate in CHIKV transmission in continental Europe, both now and in the future. For this purpose, we applied climate change data from a regional climate model, to enable the best accuracy available [35]. One of the main benefits of this study is the consideration of different temperature requirements for Chikungunya outbreaks during current conditions and at different time horizons. Additionally, the approach followed in this paper allowed for the seasonal (intra-annual) trend in potential transmission to be identified. The model results of this current and future climatic risk analyses by combining habitat suitability for the vector *A. albopictus* in Europe [27] and the temperature requirements for the CHIKV [29] are all based on the same climatic data basis, worldclim.org [32] and COSMO-CLM [33], respectively.

We identify the effect of spatial autocorrelation in MaxEnt models for the vector *A. albopictus* based on Moran's I calculation as a source of uncertainty [see 27]. However, up-scaling of the bioclimatic data coming from worldclim.org [32] to the resolution of the data from COSMO-CLM [33] had no significant effect on that. In accordance with the findings of other authors [53], we figured out that geographical corrections of clustered data my improved reliability of predictions due to lower values of Moran's I, but did not resolve the problem entirely. Furthermore, the choice of a threshold for determining species presence is one of the most challenging issues in species distribution modelling [40]. Reliability of risk analysis for CHIKV transmission that is based on the presence of a competent mosquito vector is highly sensitive to this issue. In order to consider the range of approaches, two established settings were applied. First, we compared the results to those that were derived from a conservative and fixed choice of threshold of 0.5 [42,43]; and second, in order to consider the findings that the general threshold contributed to uncertainty in predictions under climate change [40], data-adapted thresholds choice based on SeSpeql was used that is considered as one of the most accurate threshold choices [54,55]. The results from this study demonstrate that differences concerning vector occurrences are remarkable when applying different threshold criteria. Consequently, interpretation of results is intricate for those regions where the intra-annual season for transmission is determined on the basis of assumed occurrences of the vector *A. albopictus*.

Climate data: limitations and assumptions for risk analyses

The role of climate change in the recent outbreak of CHIKV in Northern Italy, the first recorded outbreak in a temperate region, is, for example, uncertain [56]. The impact of globalisation, however, is clearer, as travel and trade lead to the introduction of *A. albopictus* in Europe and the subsequent introduction of CHIKV into a formerly non-endemic area [3]. Although partially a consequence of the greater reliability and availability of future climatic projections as opposed to, say, future projections of public health capacity or human susceptibility, it must be stressed that the future-orientated models in this paper are based upon climatic factors. Climate-impact studies are inevitably vulnerable to some degree of climate reductionism, in which climatic drivers of change are prioritised vis-à-vis other important disease drivers [57]. Projections cannot include the role that either biological, socio-economic or other factors might play in either exacerbating or mitigating the future spread of CHIKV. In this study, socioeconomic factors principally influence results through the way in which they factor into the different emissions assumptions underpinning the IPCC A1B and B1 scenarios.

Currently, the next generation of climate scenarios are in development, which will be helpful for future climate impact studies [58]. The new parallel process for the development of such scenarios is characterised by an extensive exchange between scientific disciplines. One major advantage is that socio-economic uncertainties affecting both adaptation and mitigation appear to be better accounted for, perhaps particularly in the rainfall induced climate extremes [58, 59]. This becomes even more crucial, as the impact of precipitation on vector distribution is elusive. In general, precipitation signals depend on local phenomena [59], leading to temporary increases of breeding sites for mosquitoes after e.g. heavy rains. Any deviations in the relationship between heavy rains and breeding sites can reasonably be assumed to be caused by human activities [60].

In coastal Kenya, the epidemic Chikungunya fever emergence after unusually warm, dry conditions, whereas previous epidemics in Africa and Asia followed heavy rain [61]. The applied niche model for vector´s potential distribution does also account for a certain amount of rainfall as important explanatory variable in a global dimension. However, reality on the regional or local scale is more complex. In Kenya, infrequent replenishment of water stores during drought may have led to an increase of domestic *A. aegypti* populations, thus heightening the risk for CHIKV circulation. In the Mediterranean an increase of frequency of droughts has already been observed [62]. Here, private water storages may create additional breeding sites for the container breeding mosquitoes in regions where occurrences are not projected yet. However, within this study we do not address local and short-term phenomena but focus on general tendencies in a longer temporal dimension on a continental scale of Europe.

Vector´s and pathogen´s related specifics in disease transmission

One assumption of this study is that evidence from the current climatic situation can help to detect risk zones of vector-borne diseases. However, the vector as well as the virus could evolve to their changing environment in space and/or time, with unpredictable results. In the case of the vector, climatic data were used as explanatory variables of a species distribution model for the vector *A. albopictus* [27]. It is worth mentioning that *A. albopictus* prefers anthropogenic habitats and has further environmental or biological preferences which are not accounted for in our niche model. Nevertheless, it is shown that climatic-derived distribution models can predict the current distribution of this mosquito in Europe at a high spatial resolution (< 20 km) in a valid quality [26-28].

The risk analysis is exclusively based on one possible vector species, namely *A. albopictus*. In addition to the vector competence of *A. albopictus* for CHIKV, it must be taken into account that further aedine species are also capable of transmitting this alphavirus [2,3]. Biotic interactions e.g. between competitive mosquitoes in larval or adult stage may play a decisive role in species establishment. The primary vector is thought to be *A. aegypti*. The risk of re-establishment of *A. aegypti* in the continental interior of Europe is on one hand related to permanent populations of the mosquito in Madeira [9] and the Caucasian region of the Black Sea [10], and on the other hand to continual introductions by intercontinental transportation. In particular past experiences of the Netherlands showed introduced populations [63] originated from Miami, Florida, USA which are currently eliminated due to intensified mosquito control activities [11]. This highlights the necessity to account for a Europe-wide control of intercontinental transportation systems [64]. In order to detect areas for mosquito control activities, the minimum survival temperature of mosquito eggs over the winter should be taken into account. *A. aegypti* only tolerates long term cold treatments not lower than -2 °C; a -7 °C cold period for more than one hour causes a complete breakdown of hatching [65]. Therefore the establishment and spread of *A. aegypti* in temperate Europe seems to be mitigated by European winter temperatures. In any case*, A. albopictus* is probably the mosquito that replaces resident and further invasive mosquito most effectively [e.g. 66], justifying the focus on this vector in this study.

The frost tolerance of *A. albopictus* may be crucial for risk analyses. In Italy, cold acclimation as overwintering strategy has been observed for *A. albopictus* [67]. Under laboratory conditions, the low-temperature thresholds for the survival of diapausing and non-diapausing eggs of *A. albopictus* have been identified [65]. It can be assumed that besides changes in long-term trends the frequency and intensity of climatic extremes will increase [59], which will have serious effects for the alteration of vector habitats, which has not been accounted so far in projections of distribution.

The temperature required for CHIKV transmission was adapted from the compilation of endemic regions given in Tilston et al. [29]. This contains the risk that temperature requirements used here may be superimposed by other factors, which were not accounted for. A more accurate way to determine a temperature threshold for transmission would be to identify the extrinsic incubation period (EIP) via laboratory experiments [68]. For the dengue virus, this temperature-dependent EIP has been mapped and projected for the European continent [69]. In the case of CHIKV, concrete laboratory controlled studies aiming to determine the temperature-dependent EIP of different CHIKV strains in different vectors are currently missing.

Outlook and concluding remarks

In general, there is a growing consensus that infectious diseases transmitted via vectors are especially affected by climate change, when regarding the northern limits of distribution [70], which is also shown within this study. Additional work should be conducted to improve the models and/or with laboratory data about the temperature requirement in light of virus evolution and changing vector distribution [71]; it should combine both information on pathogen requirements and bioclimatic conditions of the vector(s) *A. albopictus* and *A. aegypti*. It would be furthermore of particular interest to distinguish between areas of possible establishment of aedine vectors in Europe and areas with sufficiently long weeks of activity (ranging from spring hatching to autumn diapause).

As a consequence of global transport and travelling, several exotic viruses and/or disease vectors were introduced in Europe and became established thereafter [13]. This caused necessity for control strategies [72]. In current years, the number of travel-related CHIKV infections increased in many European countries [73]. Combined assessment of potential virus introduction by using e.g. the VBD-Air tool [74] with climatic zones may form an evidence base for concepts of efficient mitigation strategies.

Once climatic risk zones and potential introduction gateways have been identified, a comprehensive CHIKV risk assessment needs to be expanded to account for societal and demographic drivers in order to adapt public health systems [75,76]. Then an overall view of all relevant impacts can be used to evaluate the way in which surveillance ought to be implemented or modified [77]. If diseases emerge, then adaptation strategies are required to be available in order to protect public health from the impending threat [78].

List of abbreviations

A. aegypti: *Aedes aegypti* (Yellow fever mosquito)

A. albopictus: *Aedes albopictus* (Asian tiger mosquito)

AUC: Area under the receiver operator characteristic curve

CHIKV: Chikungunya virus

ECDC: European Centre for Disease Prevention and Control

EIP: Extrinsic Incubation Period

GIS: Geographical Information System

netCDF Network Common Data Form

SBM: Statistic-based model

SeSpeql: Equalisation of sensitivity and specificity (as threshold-setting method)

Tmean: Mean monthly temperature

Authors' contribution

JCS, JES and BS initiated the project "Climate modelling for Chikungunya" (OJ/08/02/2012-PROC/2012/012). DF, SMT and CB developed the idea for the specific analysis. DF, AH, SMT and NBT practised the analysis. CB, JCS, JES and BS contributed to the model design. DF, SMT and AH prepared figures and additional files. All authors contributed to the Article, commented on drafts critically and edited the final version of this paper. All authors read and approved the final version of the Article.

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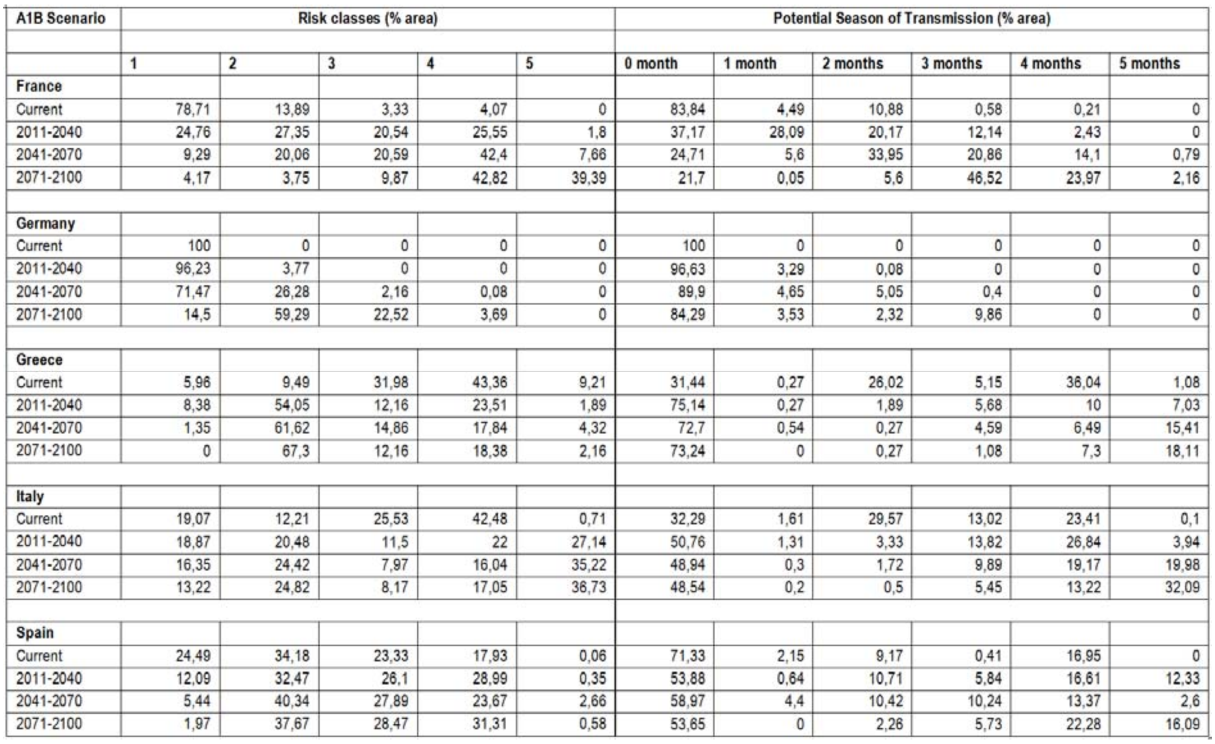
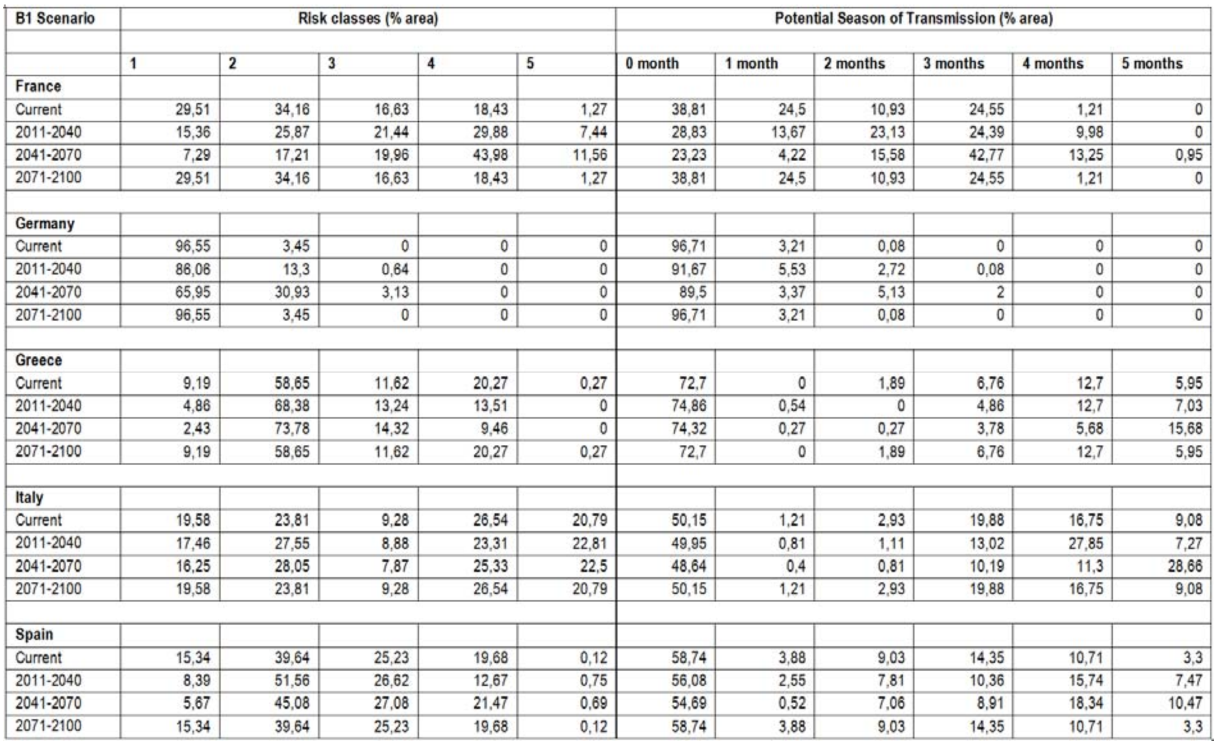
Additional files

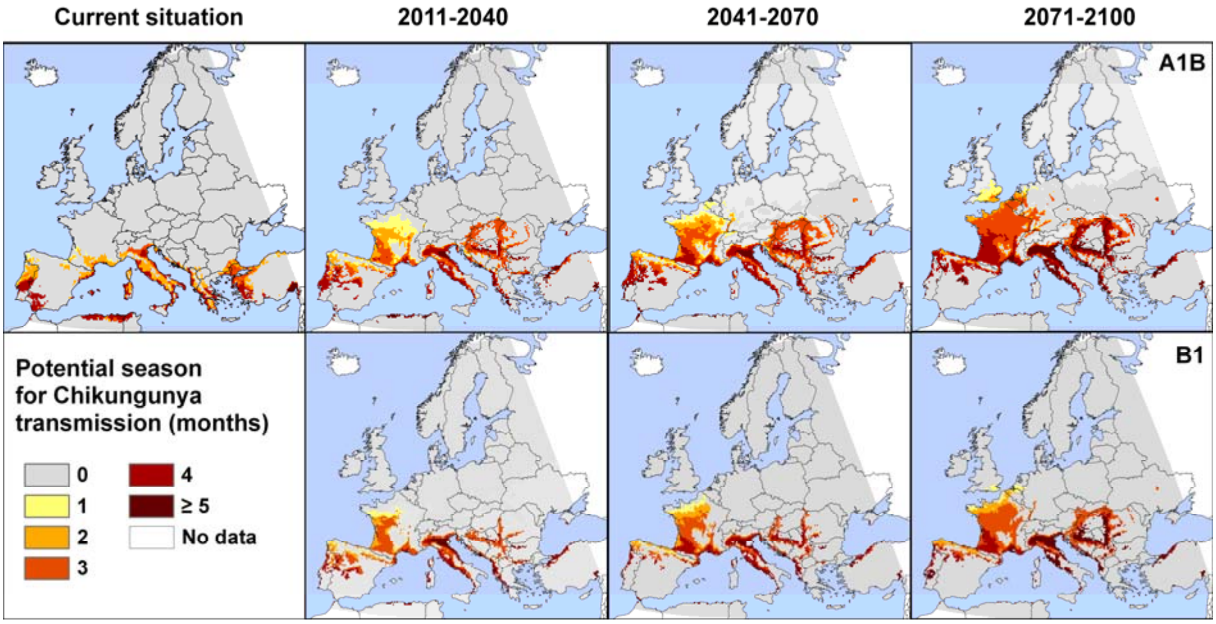
Table 1: Percentage of affected area of France, Germany, Greece, Italy and Spain in the respective risk classes and the potential season of transmission periods for the A1B and B1 scenario during this century according to Figures 3 and 5. The calculation of transmission is based on the method of equalisation of sensitivity and specificity as threshold-setting method to determine vector´s occurrence.



A1B Scenario	Risk classes (% area)					Potential Season of Transmission (% area)					
	1	2	3	4	5	0 month	1 month	2 months	3 months	4 months	5 months
France											
Current	78,71	13,89	3,33	4,07	0	83,84	4,49	10,88	0,58	0,21	0
2011-2040	24,76	27,35	20,54	25,55	1,8	37,17	28,09	20,17	12,14	2,43	0
2041-2070	9,29	20,06	20,59	42,4	7,66	24,71	5,6	33,95	20,86	14,1	0,79
2071-2100	4,17	3,75	9,87	42,82	39,39	21,7	0,05	5,6	46,52	23,97	2,16
Germany											
Current	100	0	0	0	0	100	0	0	0	0	0
2011-2040	96,23	3,77	0	0	0	96,63	3,29	0,08	0	0	0
2041-2070	71,47	26,28	2,16	0,08	0	89,9	4,65	5,05	0,4	0	0
2071-2100	14,5	59,29	22,52	3,69	0	84,29	3,53	2,32	9,86	0	0
Greece											
Current	5,96	9,49	31,98	43,36	9,21	31,44	0,27	26,02	5,15	36,04	1,08
2011-2040	8,38	54,05	12,16	23,51	1,89	75,14	0,27	1,89	5,68	10	7,03
2041-2070	1,35	61,62	14,86	17,84	4,32	72,7	0,54	0,27	4,59	6,49	15,41
2071-2100	0	67,3	12,16	18,38	2,16	73,24	0	0,27	1,08	7,3	18,11
Italy											
Current	19,07	12,21	25,53	42,48	0,71	32,29	1,61	29,57	13,02	23,41	0,1
2011-2040	18,87	20,48	11,5	22	27,14	50,76	1,31	3,33	13,82	26,84	3,94
2041-2070	16,35	24,42	7,97	16,04	35,22	48,94	0,3	1,72	9,89	19,17	19,98
2071-2100	13,22	24,82	8,17	17,05	36,73	48,54	0,2	0,5	5,45	13,22	32,09
Spain											
Current	24,49	34,18	23,33	17,93	0,06	71,33	2,15	9,17	0,41	16,95	0
2011-2040	12,09	32,47	26,1	28,99	0,35	53,88	0,64	10,71	5,84	16,61	12,33
2041-2070	5,44	40,34	27,89	23,67	2,66	58,97	4,4	10,42	10,24	13,37	2,6
2071-2100	1,97	37,67	28,47	31,31	0,58	53,65	0	2,26	5,73	22,28	16,09



B1 Scenario	Risk classes (% area)					Potential Season of Transmission (% area)					
	1	2	3	4	5	0 month	1 month	2 months	3 months	4 months	5 months
France											
Current	29,51	34,16	16,63	18,43	1,27	38,81	24,5	10,93	24,55	1,21	0
2011-2040	15,36	25,87	21,44	29,88	7,44	28,83	13,67	23,13	24,39	9,98	0
2041-2070	7,29	17,21	19,96	43,98	11,56	23,23	4,22	15,58	42,77	13,25	0,95
2071-2100	29,51	34,16	16,63	18,43	1,27	38,81	24,5	10,93	24,55	1,21	0
Germany											
Current	96,55	3,45	0	0	0	96,71	3,21	0,08	0	0	0
2011-2040	86,06	13,3	0,64	0	0	91,67	5,53	2,72	0,08	0	0
2041-2070	65,95	30,93	3,13	0	0	89,5	3,37	5,13	2	0	0
2071-2100	96,55	3,45	0	0	0	96,71	3,21	0,08	0	0	0
Greece											
Current	9,19	58,65	11,62	20,27	0,27	72,7	0	1,89	6,76	12,7	5,95
2011-2040	4,86	68,38	13,24	13,51	0	74,86	0,54	0	4,86	12,7	7,03
2041-2070	2,43	73,78	14,32	9,46	0	74,32	0,27	0,27	3,78	5,68	15,68
2071-2100	9,19	58,65	11,62	20,27	0,27	72,7	0	1,89	6,76	12,7	5,95
Italy											
Current	19,58	23,81	9,28	26,54	20,79	50,15	1,21	2,93	19,88	16,75	9,08
2011-2040	17,46	27,55	8,88	23,31	22,81	49,95	0,81	1,11	13,02	27,85	7,27
2041-2070	16,25	28,05	7,87	25,33	22,5	48,64	0,4	0,81	10,19	11,3	28,66
2071-2100	19,58	23,81	9,28	26,54	20,79	50,15	1,21	2,93	19,88	16,75	9,08
Spain											
Current	15,34	39,64	25,23	19,68	0,12	58,74	3,88	9,03	14,35	10,71	3,3
2011-2040	8,39	51,56	26,62	12,67	0,75	56,08	2,55	7,81	10,36	15,74	7,47
2041-2070	5,67	45,08	27,08	21,47	0,69	54,69	0,52	7,06	8,91	18,34	10,47
2071-2100	15,34	39,64	25,23	19,68	0,12	58,74	3,88	9,03	14,35	10,71	3,3



Additional file 2: Potential season of transmission for Chikungunya Virus. From the map of the number of months with at least 20 °C as mean temperature, only those areas were considered, where the presence of the vector *Aedes albopictus* can be expected, according to the fixed threshold (0.5) for vector occurrences from the continuous scale for climatic suitability. Projections for different time-frames are based on the two IPCC-scenarios (A1B and B1), implemented in the regional climate model COSMO-CLM.

Additional file 3: Map of mentioned European regions and localities in the main text.

Predicting ectotherm disease vector spread

- Benefits from multidisciplinary approaches and directions forward

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6.7 Article 7

Predicting ectotherm disease vector spread - Benefits from multi-disciplinary approaches and directions forwards

With 3 figures and three tables in the supplemental material.

Abstract

The occurrence of ectotherm disease vectors outside of their previous distribution area and the emergence of vector-borne diseases can be increasingly observed at a global scale and are accompanied by a growing number of studies which investigate the vast range of determining factors and their causal links. Consequently, a broad span of scientific disciplines is involved in tackling these complex phenomena.

First, we evaluate the citation behaviour of relevant scientific literature in order to clarify the question “do scientists consider results of other disciplines to extend their expertise?”. We then highlight emerging tools and concepts useful for risk assessment. Correlative models (regression-based, machine learning and profile techniques), mechanistic models (basic reproduction number R0) and methods of spatial regression, interaction and interpolation are described. We discuss further steps towards multidisciplinary approaches regarding new tools and emerging concepts to combine existing approaches such as Bayesian geostatistical modelling, mechanistic models which avoid the need for parameter fitting, joined correlative and mechanistic models, multi-criteria decision analysis and geographic profiling. We take the quality of both occurrence data for vector, host and disease cases, and data of the predictor variables into consideration as both determine the accuracy of risk area identification.

Finally, we underline the importance of multidisciplinary research approaches. Even if the establishment of communication networks between scientific disciplines and the share of specific methods is time consuming, it promises new insights for the surveillance and control of vector-borne diseases worldwide.

Keywords

arthropod vectors, climate change, global change, species distribution model, zoonoses

1. Challenges and directives for multidisciplinary research

The occurrence of competent disease vectors outside of their historic distribution area and the emergence of vector-borne diseases are increasingly observed on a global scale (Weaver and Reisen 2010). Changes in the environment (Fig 1) such as long-term climate change, intensification and increasing frequency of extreme weather events, biodiversity loss or changes in land use management, such as farming practices influence the establishment and outbreak of vector-borne diseases (Weaver and Reisen 2010, IPCC 2012). Particularly, the intensified exchange of people and goods across continental barriers supports the accidental establishment and spread of vectors and of pathogens. Aside from intercontinental travelling and migration, it is the trade of agricultural products, livestock, wildlife and of goods (such as tires or lucky bamboo) that serve as temporal habitats facilitating insects that made formerly regional vectors and diseases in becoming global players (Rosenthal 2009). Additionally, social factors such as the rate of unemployment and the state of healthcare in terms of vaccinations, sanitation and also quantity and quality of vector control actions influence the emergence, distribution and incidence of vector-borne diseases (Sumilo et al. 2008). These fundamental changes have occurred within a time span of just a few years. In most cases, the exposed regions and societal systems were found to be under prepared in the face of these novel threats. Strategies to avoid outbreaks of hitherto unexperienced diseases are not elaborated.

Tackling these complex phenomena requires a broad range of expertise, which is by definition not covered by one single person, institution, or solely by one scientific discipline. Scientists of human medicine, veterinary sciences, and biology have built networks that deal with questions of causal connections within the chain of infection. On the other hand, global environmental change issues and their ecological impacts are primarily investigated by ecologists and scientists from geosciences and environmental sciences. Here we critically discuss whether the exchange of knowledge and expertise between these scientific groups that address research questions in the field of vectors and vector-borne diseases and climate and global change issues are effective.

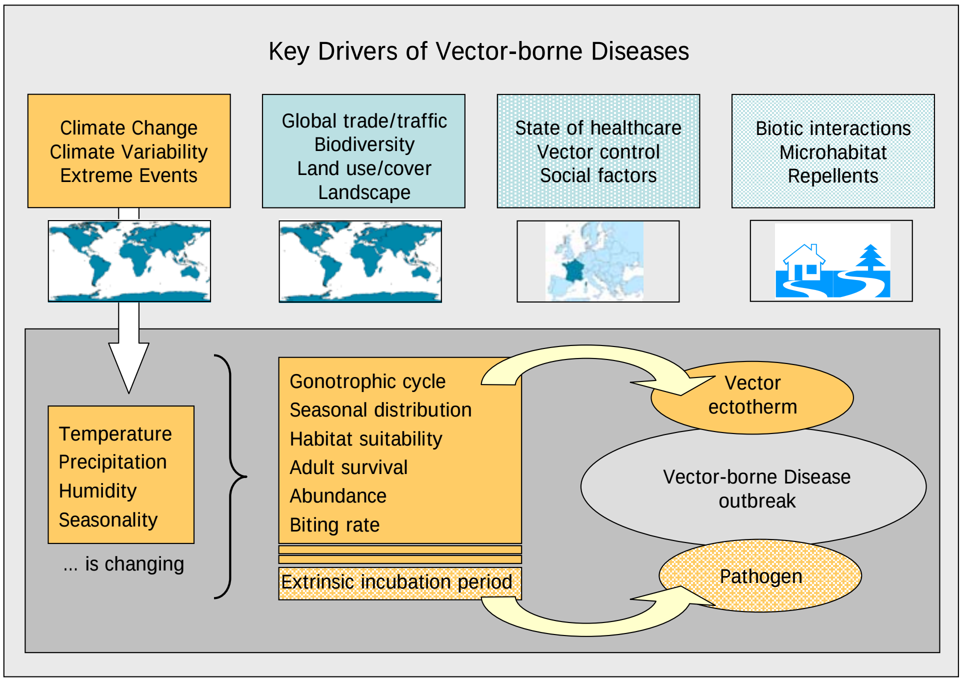

Fig 1 Impact of different drivers on ectotherms within the chain of infection. Thereby, the impact of climate change is directly modulating the life history and transmission probability of ectotherm vectors as these species are unable to regulate their body temperature

Due to the broad spectrum and complexity of research questions and approaches involved in the topic of global change and vector-borne diseases, a large number of disciplines deal with this topic. They exhibit intrinsic paradigms and established individual forums for the publication of research output. Therefore, we evaluated the extent to which scientists of specific disciplines consider the output of other research fields and whether multi-disciplinarity takes place or is still underdeveloped. If knowledge is not referred to or ignored in a domain of high societal importance, strategies must be implemented to overcome potential inefficiency of cross-disciplinary communication.

We highlight emerging tools and concepts useful for risk assessment and discuss further steps towards multidisciplinary approaches regarding new tools and emerging concepts to combine existing approaches. We take the quality of both occurrence data for vector, host and disease cases, and data of the predictor variables into consideration as both determine the accuracy of risk area identification.

2. Citation behaviour - Do we consider results of other disciplines?

In order to evaluate the efficiency of information exchange, we conducted a literature survey in the ISI Web of Knowledge, containing global research databases, namely Web of Science, Medline (PubMed, NCBI) and BIOSIS (Biological Abstracts, Biological Abstracts RRM – Reports, Reviews, Meetings and Zoological Record) over all mosquito-borne viruses and their vectors in Europe in the context of changing climate and global change (additional information is given in Online Resource 1, Tab 1 and Tab 2).

The citation behaviour within a scientific community was considered for reviews and research papers separately. For this purpose ten papers of the highest ranked journals of each scientific discipline were analysed using the backward citation map function. All records directly cited by those papers were grouped according to their journal affiliation and evaluated by journal subject categories given in the Journal Citation Reports. As a result about 5000 cited references were evaluated (Fig 2).

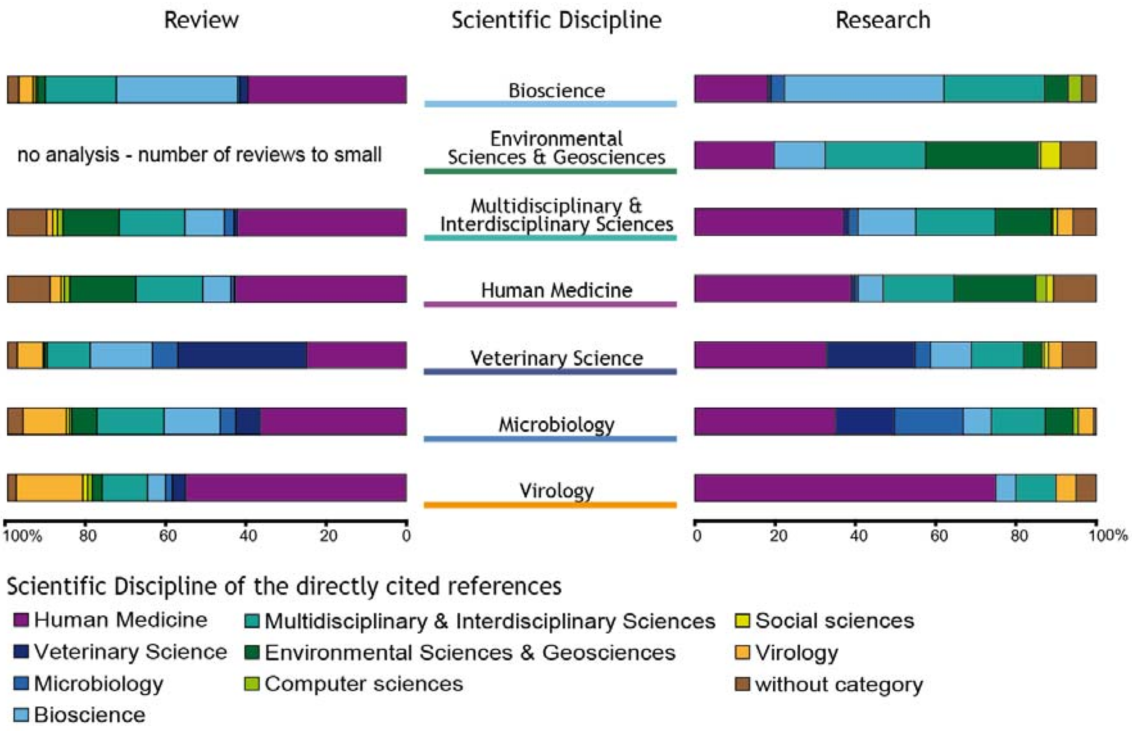

Fig 2 Citation behaviour of authors, who published high ranked research papers or reviews dealing with human mosquito-borne viruses and their vectors in Europe regarding climate and global change issues

The majority of references are attributed to the human medical literature, an unexpected low proportion of cited references belong to the biosciences category. Multidisciplinary and interdisciplinary articles are cited in each scientific discipline with a proportion of at least 10%. Results of environmental sciences and geosciences receive surprisingly high attention in human medicine publications. Interestingly veterinarians cited frequently human medical articles, but this cross-disciplinary citation behaviour is not apparent vice versa. Social sciences received mainly consideration in articles of environmental sciences and geosciences. Computer sciences were only slightly represented in all fields.

Already in 2008 Moore stated that no scientist has the required level of expertise in all of the important disciplines regarding vector-borne diseases but rather should see himself as a specialist at some level able to communicate across disciplines and work efficiently across scales, organisms and different application scenarios. In the last decades, the increasingly pronounced differentiation of disciplines in the last decades has hampered interaction and collaboration across disciplinary barriers. Coming from different scientific communities, having its own language and acronyms and its specific refereed journals to publish in, it is a non-trivial task to effectively communicate with each other, to develop straightforward research strategies, and to gain novel insights that are reaching beyond individual fields. Scientific careers are made within and not between disciplines. However, fascinating new fields of research are rather emerging in the interfaces.

3. Emerging tools and concepts

Current efforts aim to establish multidisciplinary research in the field of vector-borne diseases: ‘One World - One Health’ encourages wildlife, domestic animal and human health professionals to work multidisciplinary together in order to combat for instance emerging infectious diseases or antibiotic resistance. The One Health concept supports worldwide collaborations between physicians, veterinarians and other scientific-health and environmentally related disciplines. The International Association for Ecology & Health (IAEH) advances research that recognizes ecology and the health of all species as inter-related and explores new ways of transdiciplinary approaches on health, natural and social sciences and the humanities.

Also Wilcox and Colwell (2005) stated in their review that the diversity and unexpected occurrence of environmental factors as well as the interactions of human and natural systems cause typically nonlinear inherently unpredictable responses. These authors call for a holistic perspective on emerging infectious diseases integrating social, physical, chemical and biological dimensions. In their theoretical framework disease emergence is described as involving processes operating on the population, natural community and landscape level; and relevant key concepts for emerging infectious diseases such as resilience, surprise, barriers and bridges are highlighted. Currently Beltrán-Alcrudo (2013) takes up this issue of "biocomplexity of emerging pathogens" and supports the idea that ecosystems, wildlife, and human factors have to be taken into account as specific drivers of disease emergence and outbreaks; and that this multidisciplinary approach will minimize the impact of epidemics.

Different scientific communities currently develop a range of tools and concepts which address the challenge of spreading vectors and emerging vector-borne diseases and hence enrich options to detect areas at risk. Vector control management and health care strategies such as creating medical awareness or identifying the need for vaccination can then be guided and improved. A common and frequent procedure of risk detection is to construct distribution maps of vectors, hosts, or diseases. Subsequently these may be combined with geoinformation and remote sensing data on abiotic conditions or vegetation indices (Ostfeld et al. 2005). Subsequently, spatial variables which are found to be highly correlated with the distribution data are used to project the future potential distribution of a vector, host, or disease (Ostfeld et al. 2005). This fundamental concept was extended in various ways including different philosophies of modelling (Fig 3).

3.1 Correlative models: Regression-based models and machine learning techniques

Spatial epidemiology concentrates on the spatial distribution of infectious diseases (and of all organisms involved in the chain of transmission) and their changes through time. The basis is spatially explicit information on presence or abundance of vectors and hosts and disease case presence or incidence (Eisen and Eisen 2011), reflecting the environmental niches of biota. Environmental factors are extracted and a predictive model is applied to extrapolate a risk surface.

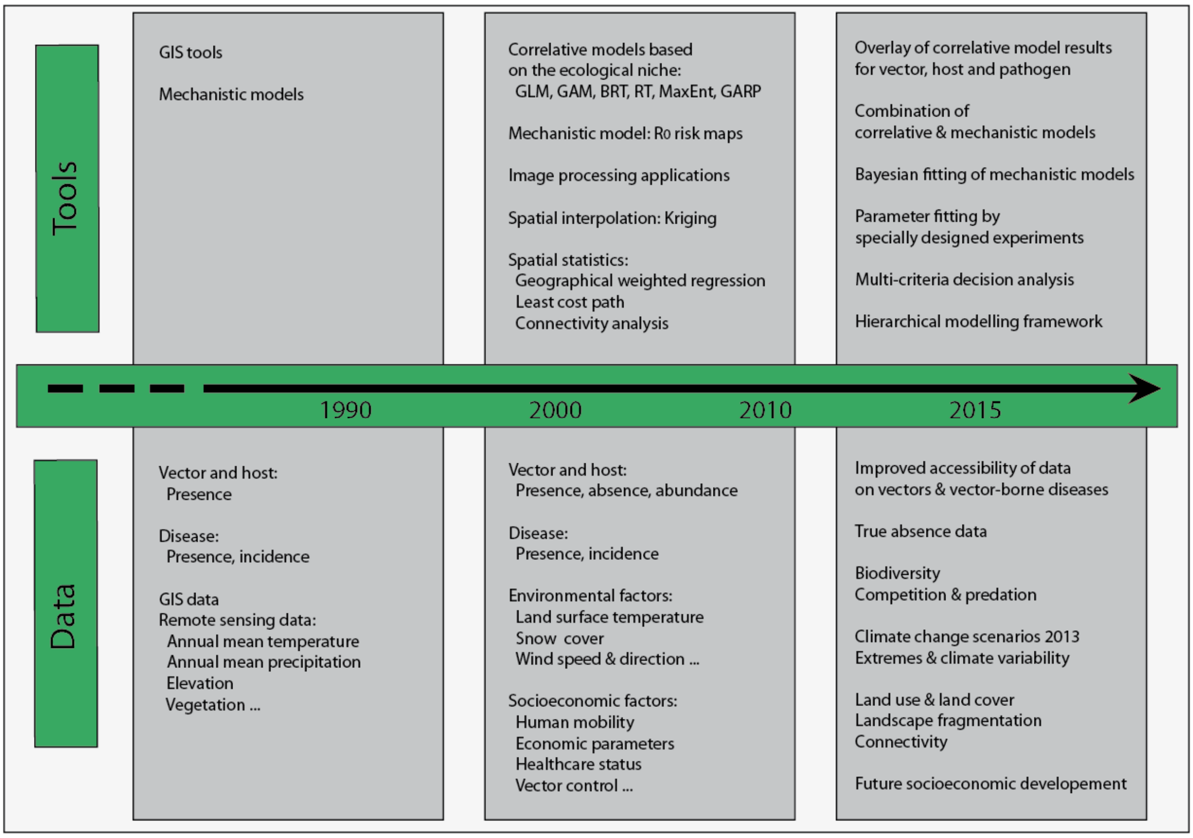

Fig 3 Emerging tools and concepts for evaluating areas at risk of vector-borne diseases

Regression-based models

Commonly regression-based models such as generalized linear models (GLM) and generalized additive models (GAM) are used. In recent years machine learning techniques such as boosted regression trees (BRT) (e.g. Simmons et al. 2012) or random forests RF (Peters et al. 2011) are applied to epidemiological research questions. These models require a binary response variable. In consequence, they refer to both presence and absence data. In 2001 Breiman proposed RFs, a combination of tree predictors, whereby each node is split using the best among a subset of predictors randomly chosen at that node. The method is deemed to be more robust with respect to noise and against overfitting (Breiman 2001). BRTs also aim to improve the performance of a single model by fitting many simple tree models, but combining them by boosting to optimize the predictive performance (Elith et al. 2008).

Machine learning techniques

Aside from this presence/absence models so called presence only models (such as maximum entropy model, MaxEnt, and genetic algorithm for rule set production, GARP) were developed, using true presence data and information about a background matrix where species do not occur by generating pseudo-absences. Mostly, pseudo-absences are generated from a random sample of the entire study area (background) or by subjective expert opinion. The relationship between the geographic extent from which pseudo-absences are taken and model performance and shape and importance of predictor variables is evaluated by VanderWal et al. (2009). A model built with GARP is iteratively chosen from non-random correlations between environmental and occurrence data. The non-random correlations describe environmental thresholds, depending on the chosen type of mathematical rule. Using this model algorithm Peterson (2003) analysed the geographic and ecological distributions of three Lutzomyia sand fly vectors and Benedict (2007) determined the global risk of invasion for *Aedes albopictus*. MaxEnt, originated in the information theory, estimates the projected distribution by finding the distribution of maximum entropy, i.e. the distribution closest to uniform, given that the expected value of each feature (environmental variables or functions thereof) is in its empirical average, whereby the weights on features are sequentially updated. This approach has supported epidemiological research in many different ways: combining spatially explicit projections for the climatic and habitat suitability of specific regions for vectors (e.g. Medley 2010) and hosts (e.g. Andreo et al. 2011) can deliver risk maps for the current potential occurrence or emergence of diseases (Peterson 2008). Furthermore new insights regarding possible new vector species can be obtained (González et al. 2011) and disease outbreaks can be modelled directly (e.g. Machado-Machado 2012).

In addition, future projections on modified risks in face of changing climate conditions are based comparatively on various time steps and emission scenarios (Fischer et al. 2011b). The emergence of climatic conditions which do not presently exist both in space and time (non-analogue climates) is thought to limit the reliability of projections (Fitzpatrick and Hargrove 2009). To identify where climates are non-analogue, i.e. novel, means to know where the model is most uninformed (Elith et al. 2010). In order to asses and map those areas, multivariate environmental similarity surfaces analysis (MESS) was implemented in MaxEnt (Elith et al. 2010).

Spatial autocorrelation

Spatial autocorrelation in species distribution data reflects the fact that locations close to each other display more similar values than those further apart. Mobility of organisms, species-specific dispersal mechanisms and behavioural factors, but also observer bias, differences in sampling schemes and sampling effort can cause spatial aggregation of documented species distribution (Dormann et al. 2007). This spatial aggregation violates the key assumptions of statistical analyses, that residuals are independent and identically distributed. In presence-only niche models spatial autocorrelation of sampling effort between subsets of a larger dataset, training and test data, inflates measures of prediction accuracy (Veloz 2009). Established procedures in Ecology and Geography to check for spatial autocorrelation are Moran's I plots, Geary's c correlograms and semi-variograms. Additional statistical approaches are autocovariate regression, spatial eigenvector mapping, generalised least squares, autoregressive models and generalised estimating equations, which are explained and discussed in Dormann et al. (2007).

Comparison of correlative models

The performance and robustness of some important species distribution models was evaluated in detail regarding presence only versus presence/absence data (e.g. Brotons et al. 2004) and among presence-only models (e.g. Tsoar 2007). It was found that species with narrow niches, i.e. restricted ecological requirements, were modelled more accurately than species with wider niches. In terms of vector-borne diseases, Zeimes (2012) compared logistic regression, BRT, MaxEnt and cross-validated logistic regression stressing advantages and disadvantages of individual approaches.

3.2 Mechanistic model: The basic reproduction number R0

The fundamental limitation of correlative geostatistical approaches is related to the disregard of biological processes and functions. The calculation of the basic reproduction number R0 is a common concept in epidemiology to include those processes. The measure takes into account the duration of infectivity, the infectiousness of the organism, number of susceptible vectors and hosts. It serves as a threshold parameter that predicts whether an infection is likely to spread in a susceptible population arising from the introduction of a single infected individual (Heffernan et al. 2005). A disease is able to invade, i.e. increases in frequency, a host population if R0>1. For vector-borne diseases R0 is determined by the number and mortality of hosts and vectors, biting rate, the probability that host or vector becomes infectious and the transmission probability per vector bite.

Currently spatially varying values of R0 are applied for the construction of risk maps. These R0 maps support health policy makers and public health authorities to assess the effects on disease patterns and the risk of outbreak of vector-borne diseases under changing climatic and environmental conditions. Using the example of canine leishmaniasis, Hartemink et al. (2011) analysed vector abundance by high- and low-resolution remotely sensed data, respectively. These abundances were integrated in a mechanistic temperature dependent R0 model. The R0 value was calculated for each raster cell of the investigated area resulting in a GIS based raster R0 map. Such a mechanistic approach allows the interpretation of parameters used in the R0 equation in a biological and epidemiological way and the identification of temperature dependent effects in general (Hartemink et al. 2011). But there are also drawbacks such as uncertainties in parameter estimation and often, the explanatory power is restricted by the lack of data (Hartemink et al. 2011).

Regarding climate warming, the temperature dependent parameters of ectotherm vectors such as mosquitoes, midges, or sandflies are crucial for R0 modelling. These are biting rate, mortality rate, birth rate, transmission rate and rate of becoming infectious (Gubbins et al. 2008, Rubel et al. 2008, Hartemink et al. 2009, Hartemink et al. 2011, Barbazan et al. 2010). By now, only a few approaches connect climate change scenarios with temperature dependent parameters when calculating R0 (Rubel et al. 2008, Barbazan et al. 2010). Determining malaria transmission intensity, Paaijmans et al. (2010) call attention to the fact that mosquito related traits are sensitive to daily variation in temperature. Small changes could have large biological significance due to quadratic or exponential effects of these traits in the R0 equation (Paaijmans et al. 2010). Spatial variations in people/vector contacts at the landscape scale are implemented in R0 models by coupling with multi-agent simulations. The agent-based approach allows simulating and understanding complex system responses through the modelling of discrete events (Linard et al. 2009).

3.3 Spatial regression, interaction and interpolation

Emphasizing this spatial dimension of epidemiology, landscape structure is assigned a strong potential to influence disease dynamics in terms of dispersal barriers, habitat availability, and suitability for a vector or a host. Hence, landscape ecology and landscape metrics are considered in modelling approaches by including landscape composition (land use, land cover) and configuration (spatial relationships such as the degree of habitat fragmentation or habitat connectivity) (Ostfeld et al. 2005). Consequently, landscape structure, land use, and land cover are implemented in epidemiological research first of all supported by remotely sensed data (such as Normalized difference vegetation index NDVI, Enhanced Vegetation Index EVI, digital elevation models, land cover data bases), image processing applications and GIS applications (Geographic information system) such as geographical weighted regression (GWR), least cost path analysis, or connectivity analysis.

Geographical weighted regression

The GWR is used to explore the geographical heterogeneity of spatial relationships by calibrating a multiple regression model, which allows different relationships to exist at different points in space (Brunsdon et al. 1996). This technique is loosely based on kernel regression, a non-parametric technique to find a non-linear relation between pairs of random variables. Lin and Wen (2011) applied a GWR model to geographically differentiate the relationships of dengue incidence with densities of mosquito larvae/pupae and human densities. However, they point to the limitations of the method: the results strongly depend on the chosen kernel type and the number of neighbours used for each local estimation, i.e. the degree of smoothing in the model (Lin and Wen 2011). Moreover, non-linear terms cannot be added and inference cannot be done in the GWR.

As most dispersal processes are controlled by spatial filters, which are species-specific, modelling devices are needed which consider supporting and restraining structures.

Least-cost path analysis

Least cost path analysis is a method used for measuring the functionally effective distance between habitats. It is based on the resistance surface derived from spatially explicit variables such as topography or physical barriers. The method is capable to yield a spatially differentiated image for the likelihood of specific spreading vector-borne diseases in complex landscapes. This promising tool is able to yield more realistic risk projections concerning a vector or a host species' future distribution (Fischer et al. 2011a). To account for species-specific dispersal abilities, Jaeschke et al. (2012) applied observed maximum dispersal distances for modelling and considered dispersal lags by allowing dispersal only after the completion of the development cycle of an insect.

Connectivity

Connectivity measures are reviewed in detail by Kindlmann and Burel (2008) indicating that only a combination of the two dimensions of connectivity, namely landscape structure and species-specific movement behaviour guide towards meaningful values of connectivity. Non-Euclidean distance but rather ecologically determined distance measures have been used to quantify connectivity in heterogeneous landscapes by the means of Schistosoma japonicum and its intermediate snail host Oncomelania haupensi. In this case, novel relationships between nodes became apparent, which were not evident when using mere Euclidean distance metrics (Remais et al. 2010).

Interpolation

When aiming at the reduction of statistical noise in space or at the transformation of point-based data into smooth risk surfaces at fine spatial scales, spatial interpolation methods such as kriging are applied in spatial epidemiology (Eisen and Eisen 2011). Kriging can be done by linear or nonlinear approaches. It provides predictions and kriging errors or kriging variances for each location between data points. Haining et al. (2010) recently give an overview of kriging applications and software for geostatistical analysis.

3.4 Remote sensing data

Frequently, remote sensing data are taken for models and spatial statistics. NDVI, EVI, land cover, land use, elevation, slope, aspect, lithofacies, lakes, rivers and other water bodies, temperature, rainfall and humidity are integrated in data layers for ecological analyses. Bergquist (2011) reviewed the current spatial, temporal and spectral resolution feasibilities of satellite data that are applied in epidemiological research. The present state of the art of how geostatistics are used in remote sensing studies is reviewed by Van der Meer (2012). He discusses the issue of stationarity, the use of variograms and kriging-based estimation techniques and cokriging techniques. A topical example is given by Neteler (2010) for using MODIS Land Surface Temperature (LST) data to project tick and mosquito distribution in Northern Italy. There, incomplete time series of LST data are reconstructed by subsequently interpolating the input data points with volumetric splines. Moreover, by combing high resolution elevation maps and temperature gradient based models, the resolution of the LST maps is improved from 1,000 m to 200 m pixel size.

4. Further steps towards multidisciplinary approaches

Risk evaluation of vector-borne diseases is expected to be improved by multidisciplinary collaborations. Emerging concepts to combine established approaches and new tools are highlighted below. Subsequently, the quality of occurrence and environmental data is described.

4.1 New tools and emerging concepts to combine existing approaches

Both, correlative models and mechanistic models have been shown to currently be prominent tools for risk assessment. However, on the one hand the main issue with correlative models is their dependence on amount, quality and relevance of the data. On the other hand mechanistic models rely on empirical parameterizations of the processes considered. Projecting models in time or space introduces the problem that the causality of observed correlations is not necessarily assured, because a parameter could be a proxy for an unrecognized and not implemented parameter (e.g. temperature versus solar radiation) (Dormann et al. 2012). Dormann et al. (2012) highlights three avenues for research on species distributions: (1) Bayesian fitting of mechanistic models (allowing the uncertainty of model parameters to enter the fitting process), (2) mechanistic models which avoid the need for parameter fitting by determining them by detailed observations or experiments (such as the state-of-the-art mechanistic model “Niche Mapper”), (3) joined correlative and mechanistic models: first generate hypotheses on potentially underlying processes by exploring species distribution by correlative models, which can then be tested by mechanistic models. In this way the unknown parameters in mechanistic models could guide experimental research. Dormann et al. (2012) stated that “In such a comprehensive approach, researchers with different interests, expertise and focus can synergistically progress the field in a way neither correlative nor process-based approaches can do by themselves.”

Bayesian model-based geostatistic

Bayesian model-based geostatistic has been used recently for managing uncertainty due to limited data in spatial epidemiology by determine probability distribution maps (Eisen and Eisen 2011). The probability of correctness for each map of a large set of candidate maps reflects the probability distribution map: In other words with the help of Bayesian geostatistics a large sample of maps that can explain a dataset is found, in which maps that explain the data best are more likely to be represented (Patil et al. 2011). The method is frequently based on Markov chain Monte Carlo (MCMC) algorithms. Parameters and inferences can be estimated. Bayesian geostatistic methods in health cartography are reviewed in (Patil et al. 2011) using the example of malaria.

Mechanistic model - parameter fitting with detailed observations or experiments

A mechanistic model, which uses detailed observations and experiments for parameter fitting has recently been applied to predict energy expenditure and food requirements of seabird species (Fort et al. 2009). This is also a promising approach for detecting host species occurrence. Another example would be the laboratory determination of the extrinsic incubation period (EIP) of a pathogen inside its vector under a defined temperature regime. This data could then be used as a temperature dependent parameter in a mechanistic risk model (Tjaden et al. 2013). So far, Hartemink et al. (2011) used simple, linear models to describe parameters known to vary with temperature, such as the biting rate, vector mortality and duration of the EIP in the R0 map of canine leishmaniasis.

Combination of correlative and mechanistic models

Joining correlative and mechanistic models will yield a new quality of projections and risk maps. In a first correlative modelling step, those parameters with important individual effects and those with the most information not represented by other parameters are identified (e.g. by Jackknife tests of parameter importance in MaxEnt). Doing so, new hypotheses can evolve. Especially parameters which determine species distribution in a way that was not expected can be scrutinized in more detail. Subsequently mechanistic models can test these novel hypotheses. Furthermore, the application of two independent modelling approaches provides a more robust basis for forecasts of climate change impacts on habitat and population parameters if convergent lines of independent evidence are detected. Kearney et al. (2010) illustrate this principle for the projection of current and future species distributions by applying mechanistic (Niche Mapper) and correlative (MaxEnt, Bioclim) models. Moreover, mechanistic models can be substantially improved when considering the output of correlative models. For example, vector abundance can be modelled by a species distribution model first and then these findings can be used for mapping the R0.

Hierarchical modelling framework

Bearing in mind all drivers of vector-borne diseases (see Fig°1) the hierarchical modelling framework presented by Pearson and Dawson (2003) enable to involve those drivers which interact at different scales from local to global dimension. Modelling climate change effects at a global or continental scale narrows the range of climatically suitable areas. Subsequent modelling of regional and local factors including topography, land cover type and in addition biotic interactions, evolutionary change and species dispersal will then improve the reliability of model results (Pearson and Dawson 2003).

Implementation of ecological processes

The integration of ecological processes is enabled by mechanistic models. Evidently, such processes are strongly involved in the transmission of vector-borne infectious diseases. Considering ecological concepts and theories makes modelling approaches and risk assessments complex but also realistic. For instance, species diversity of ecological communities of pathogens, vectors and reservoir hosts plays a decisive role for the prevalence of a disease. “Disease risk is more likely to decrease under high reservoir host diversity and this is especially likely when pathogen transmission is frequency-dependent” (Keesing et al. 2006). The reduction of disease risk due to increased species diversity is seen as a ‘dilution effect’ (Keesing et al. 2006), because it is correlated with the probability of contact between potentially supporting organisms. Consistent with the dilution effect hypothesis due to intra-host diversity Johnson and Hoverman (2012) extended this hypothesis in a way that an increase in parasite richness leads to reduced overall infection success, including infections by the most virulent parasite they have examined. Their experiments emphasized also the importance of parasite community composition and assembly when evaluating pathology.

Moreover, ecological processes which are apparent at small scales such as interspecific competition and predation have to be taken into account, especially when vectors are thought to be invasive. Juliano and Lounibos (2005) evaluate the application of basic ecological theory to invasions by mosquito vectors and review the knowledge on ecological interactions such as interspecific competition, apparent competition and predation operating during invasions. Using the example of *Aedes albopictus*, they develop a model for effects of resource competition and predation. These may act as barriers to invasion. With this approach it is possible to identify community and ecosystem characteristics which support invasion processes. In this example, a trade-off between competitive ability and vulnerability to predation affects the success of *Aedes albopictus* in an invaded ecosystem.

Multi-criteria decision analysis

Knowing the spatial distribution of risk is instrumental to prioritizing surveillance and intervention strategies. Multi-criteria decision analysis (MCDA) combines both data-driven and qualitative indicators for evaluating alternative strategies taking into account stakeholder participation. Although widely used in a number of disciplines (e.g. Mendoza and Martins 2006) over the last few decades, the use of spatially explicit MCDA remains relatively limited in public health related fields such as vector-borne disease prevention and control. Hongoh et al. (2011) reviews applications of this tool and illustrates recent advances in the development of the framework, and how it can be applied to public health decision support for vector-borne diseases. General steps in an MCDA process, selection of alternatives and site selection are discussed and examples of software and algorithms are presented (Hongoh et al. 2011).

Geographic profiling

A new statistical tool borrowed from criminology is the geographic profiling. This method allows the backward description of the trajectories in which pathogens spread from a central source (Le Comber and Stevenson 2012). Thereby, current spatial patterns are used to identify the sources. Distance decay, a measure that refers to the decrease of similarity between two observations as the distance between them increases and buffer zones are combined in this method to narrow down the possible area of origin rather than making a point estimate.

4.2 Quality of data and data resources

The quality of presence and abundance data for vector, reservoir host and disease cases as well as the quality of data for the predictor variables determines the accuracy of risk area identification. Today, records on disease cases and vectors are becoming globally available. But, up to now there is no standard protocol or data format. A surveillance system for a range of arborviral diseases was established in the United States as a result of the West Nile Virus emergence at the end of the 20th century, compiling data for infections from humans, domestic animals, vertebrates and vectors (ArboNET, Eisen and Eisen 2011). Global georeferenced records of mosquitoes, ticks and sandflies, and species distribution models derived from those records are public domain (http://www.vectormap.org/). Those data collection is often only made possible by the huge commitment and initiative of individual scientist; however a secured long-term funding on a global scale would be important in this context. In Europe currently guidelines for the monitoring and control of invasive mosquitoes and associated vector-borne diseases are developed (ECDC 2012, WHO 2011). The target is to acquire confirmed quality controlled data of vector presence (VBORNET 2012). Networks such as the European Network for Arthropod Vector Surveillance for Human Public Health (VBORNET) produces distribution maps of the major arthropod disease vectors and are thus striking starting points. However, political borders are still playing a considerable but sometimes distorting role in the monitoring of vectors and also for the surveillance of vector-borne infectious diseases. Nations establish individual monitoring approaches and data banks. In addition, presence and abundance data, and especially incidences are often given at county level only, the latter to protect patient privacy or to restrict data access for other researchers. However, georeferencing of serological data collected on vectors and reservoir hosts is essential. For human cases, knowing the place of infection (in addition to the place of residence of patients) is a prerequisite for tracking the infection process (Lambin et al. 2010).

A best practice example that combines detailed and up to date data on vectors and pathogen is the Malaria Atlas Project (MAP). It highlights among other issues the spatial variability of different malaria vectors and spatial transmission limits of Plasmodium species in a global map (http://www.map.ox.ac.uk/browse-resources/). Cutting edge monitoring regarding the biodiversity of the mosquito fauna is found in the MODIRISK project, a cross-sectional field study with adult trapping and modelling of vector species in all of Belgium (Van Bortel et al. 2007).

Joint efforts towards the improved accessibility of occurrence data on vectors and their vector-borne diseases are urgently needed. The recent appearance of citable data papers may contribute to this. Every study is a step towards a more open-minded culture of data sharing with the ultimate goal of improving disease risk evaluations. And, often overlooked, such data bases should also consider true absence data (Braks et al. 2011) to expand the options of modelling. Until now, tools are restricted by the fact of having access only to “presence” information in space. This means that merely the occurrence of a vector or pathogen is recorded and reported but not a missing evidence for their appearance at a certain site.

The quality of data for the environmental predictor variables is critical to identify realistically areas that are suitable for disease outbreaks or vector establishment. Here, the positive development of rapidly increasing availability of open ecological and climate data is an encouraging fact. A generic list of data platforms providing open climate, ecological and social data for model input is given in Online Resource 2 (Tab 3).

More and more researchers take advantage of building and testing models by using shared open-source codes (e.g. R Language and Environment for Statistical Computing, http://www.R-project.org or Geographical Resources Analysis Support System (GRASS) GIS, http://grass.osgeo.org). Code robustness and reliability of results can then be controlled by the scientific community (Rocchini and Neteler 2012). This philosophy of sharing and transparency is encouraging especially when such complex questions have to be addressed as in the case of vector-borne diseases.

5. Cooperate

"It is only by forming multidisciplinary groups to focus on specific vector-host-pathogen systems that we will be able to answer the most interesting (and pressing) problems in our field" states Moore (2008). Thus tackling these pressing problems requires a broad range of expertise from the medical, biological, environmental, mathematical, social and economic sciences. In addition to the necessary developments required in specialised research, an intensified exchange between research disciplines will help to improve methods, tools, and research approaches in general. This is needed to find solutions for the pressing problems regarding vector-borne diseases in times of a rapidly changing world. Moreover, transparency coupled with cooperation between e.g. laboratory work, field experiments, monitoring and modelling is required.

Scientific networks must integrate researchers of different disciplines. Only then, research gaps and scientific problems can be identified and addressed. And in consequence efficient ways of target-oriented troubleshooting will be implemented.

Desired improvements of data quality, data quantity and modelling algorithms should not conceal the fact that powerful methods are available. Combining projected distributions of vectors and pathogens generate for instance detailed risk maps for the potential future occurrence of diseases. However, research frontiers exist mainly according to the iterative exchange of findings and ideas across disciplinary borders. Even if the establishment of communication networks between scientific disciplines and the share of specific methods is time consuming, it promises new insights for the surveillance and control of vector-borne diseases worldwide.

Increasingly, scientific journals are supporting multidisciplinary approaches. Even specific platforms evolve that focus on the multidisciplinary dialogue. Obviously, the increasing complexity of societal problems and research questions asks for forums that stimulate and encourage the communication and collaboration of various disciplines not only in the field of vector-borne diseases. However, there, it is absolutely crucial and urgently needed.

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Online Resource 1 and Online Resource 2

Tab 1: Search strings of the literature survey: combining vector and pathogen of mosquito-borne diseases in Europe (see Hubalek 2008) with changing climate and global change issues.

Vector species		**Diseases**	**Umbrella terms**
Aedes albopictus	*Culiseta impatiens*	Batai	arthropod-borne
Aedes cinereus	*Culiseta inornata*	Chikungunya	insect-borne
Aedes vexans	*Culiseta morsitans*	Dengue	mosquito-borne
Anopheles claviger	*Ochlerotatus cantans*	Inkoo	vector-borne
Anopheles hycranus	*Ochlerotatus caspius*	Sindbis	
Anopheles maculipennis	*Ochlerotatus cataphylla*	Snowshoe Hare	
Coquillettidia richiardii	*Ochlerotatus communis*	Tahyna	
Culex modestus	*Ochlerotatus excrucians*	West Nile	
Culex pipiens	*Ochlerotatus flavescens*	Yellow fever	
Culex torrentium	*Ochlerotatus hexodontus*		
Culiseta annulata	*Ochlerotatus punctor*		

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Tab 2: Classifications of journal categories in scientific disciplines.

Scientific Disciplines	**Journal Categories**
Bio Sciences	Biology; Cell Biology; Ecology; Entomology; Evolutionary Biology; Genetics and Heredity; Parasitology; Zoology
Computer Sciences	Information Systems, Interdisciplinary Applications, Software Engineering, Theory & Methods, Mathematical & Computational Biology
Environmental Sciences and Geosciences	Environmental Engineering; Environmental Sciences; Physical Geography; Multidisciplinary Geosciences; Meteorology and Atmospheric Sciences
Human Medicine	Immunology; Infectious Diseases; Medicine; General and Internal Medicine; Research and Experimental; Neurosciences; Pathology; Paediatrics; Public, Environmental and Occupational Health
Microbiology	Microbiology
Multidisciplinary and Interdisciplinary Sciences	Multidisciplinary Sciences and Interdisciplinary Sciences: Journals in more than one cluster category
Social Sciences	Education, Psychology
Veterinary Sciences	Veterinary Sciences
Virology	Virology

Most journals are classified in one journal category, e.g. Journal of Vector Ecology belongs to the category “Entomology” and will be clustered in “Bio Sciences”. Basically journals could belong to more than one journal category: Emerging Infectious Diseases is listed in the journal categories “Immunology” and “Infectious Diseases” for instance. Both categories are clustered in “Human Medicine”. In some cases journals belong to various journal categories of different scientific disciplines. Examples are Environmental Health Perspectives (“Environmental Sciences”, “Public, environmental and occupational Health”) or Microbes and Infection (“Immunology”, “Microbiology”, “Virology”). These were classified as “Interdisciplinary Sciences”. “Multidisciplinary Sciences” is a journal category of the Journal Citation Report (ISI Web of Knowledge) which includes resources of a very broad or general character in the sciences. Typical examples of the “Multidisciplinary Sciences” are Nature and Science, covering a spectrum of major scientific disciplines.

Tab 3: Generic list of data platforms providing open climate, ecological and social data for model input.

Product	Description	Spatial resolution	Source	Link
Land cover & Land use				
Corine Landcover	European wide inventory of land cover in 44 classes	300m	Envisat MERIS	http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster
Globcover	global land cover	300m	Envisat MERIS	http://ionia1.esrin.esa.int/
Culture-MERIS	global land management activities, for which a quick and updated overview of the land’s state is needed	300m	Envisat MERIS	sftp://cm2011@culturemeris.esrin.esa.int
UMD Land cover classification	global land cover classification fourteen land cover classes (14981-1994)	1km	AVHRR	http://glcf.umiacs.umd.edu/data/landcover/
Land cover/Land cover change	17 categories of land cover following the IGBP global vegetation database: natural vegetation developed lands, mosaic lands and nonvegetated lands	500m	MODIS	http://modis.gsfc.nasa.gov/data/dataprod/index.php
High-resolution images	Panchromatic, colour merge, multispectral	10m	SPOT	https://earth.esa.int/web/guest/data-access/browse-data-products/

Vegetation				
Normalized difference vegetation index NDVI	changes in terrestrial vegetation	250m 8km	MODIS AVHRR NOAA	http://glcf.umiacs.umd.edu/data/ http://glcf.umiacs.umd.edu/data/gimms/index.shtml http://glcf.umiacs.umd.edu/data/gimms/
Enhanced vegetation index EVI	classify and quantify the various concentrations of vegetation	250m	MODIS	http://modis.gsfc.nasa.gov/data/dataprod/index.php
Vegetative cover conversion VCC	Annual data on changes in global land cover by burning or by deforestation (5 years frequencies, Tropics)	250m	MODIS	http://glcf.umiacs.umd.edu/data/vcc/
Vegetation continuous fields VCF	Vegetative cover types: woody vegetation, herbaceous vegetation, and bare ground	500m	MODIS	http://glcf.umiacs.umd.edu/data/vcf/
Tree cover continuous fields	Vegetation is represented as continuous fields of land cover, resulting in every pixel having a percentage value for tree cover	1km	AVHRR NOAA	http://glcf.umiacs.umd.edu/data/treecover/
Global production efficiency model dataset GloPEM	Terrestrial net primary production and gross primary production at the global scale.	8km	AVHRR NOAA	http://glcf.umiacs.umd.edu/data/glopem/
Forest change	Humid tropical deforestation in Amazon and Central Africa	30-60-m	Landsat	http://glcf.umiacs.umd.edu/data/pathfinder/
Global vegetation index MGVI	Fraction of absorbed photosynthetically active radiation; measure of the amount and vigour of vegetation at the surface	1km	MERIS	http://gpod.eo.esa.int/

Climate & Weather				
Worldclim	Set of global climate layers past (downscaled), current (1950- 2000) and future (IPCC 4) conditions	approx 1km	Observations Simulations	http://www.worldclim.org/
Climatic research unit CRU	Temperature, precipitation, pressure & circulation indices, UK climate indices, Mediterranean climate, Alpine climate data, high-resolution gridded datasets, paleoclimate, drought indices	20-500km	Observations Simulations	http://www.cru.uea.ac.uk
Land surface temperature	Land Surface Temperature	1km	MODIS Aqua, Terra	http://modis.gsfc.nasa.gov/data/dataprod/index.php
Temperature & precipitation	precipitation and minimum, maximum, and mean surface temperature for the period 1950–2006, daily climate observations	25-50km	Observations ENSEMBLES	http://www.ecad.eu/download/ensembles/ensembles.php
Extreme indices	Cloudiness, cold, compound, drought, heat, humidity, pressure, rain, snow, sunshine, temperature, wind	various	Observations ENSEMBLES	http://www.ecad.eu/download/millennium/millennium.php
ERA-Interim	45 micrometeorological Parameters/Variables, hourly, daily or monthly	50-100km	ECMWF Reanalysis	http://rda.ucar.edu/datasets/ds627.0/#metadata/detailed.html?_do=y
Merra Land	historical analyses of the hydrological cycle, 58 micrometeorological Parameters/Variables, hourly or daily or monthly means	50-150km	NASA Reanalysis	http://gmao.gsfc.nasa.gov/research/merra/merra-land.php
NCEP /CFSR	72 micrometeorological Parameters/Variables, hourly time series	50-300km	NOAA Reanalysis	http://nomads.ncdc.noaa.gov/data.php?name=access#cfsr

Hydrology				
Total precipitable water	Water vapour	1km	MODIS	http://modis.gsfc.nasa.gov/data/dataprod/index.php
Evapotranspiration	Transpiration by vegetation and evaporation from canopy and soil surfaces	1km	MODIS	http://modis.gsfc.nasa.gov/data/dataprod/index.php
Water mask	Global map of surface water	250m	MODIS	http://glcf.umiacs.umd.edu/data/watermask/
Flood maps	Daily maps of flooded areas (examples)	250m	MODIS	http://glcf.umiacs.umd.edu/data/flood/
Snow cover	Snow Cover	500m-1km	MODIS	http://modis-snow-ice.gsfc.nasa.gov/ (North America)
Water vapour	Water vapour	1km	MERIS	http://earth.eo.esa.int/pcs/envisat/meris/maps/watervapour/
Soil moisture	Soil moisture in land surfaces	35-50 km	SMOS	https://earth.esa.int/web/guest/missions/esa-operational-eo-missions/smos
Soil				
Harmonized World Soil Database (HWSD)	Soils and soil parameters (such as organic Carbon, pH, water storage capacity, soil depth, cation exchange capacity, total exchangeable nutrients, salinity, textural class)	600m	Observation	http://www.fao.org/nr/land/soils/harmonized-world-soil-database/en/
European Soil Database	Soil geographical database of Eurasia, Database of hydraulic properties	1km	Observation	http://eusoils.jrc.ec.europa.eu/ESDB_Archive/ESDBv2/fr_intro.htm
Acidification of soils in Europe	acidification, PH, chemical, physical and biological soil properties	1km	Observation	http://eusoils.jrc.ec.europa.eu/library/data/ph/
Elevation & relief				
Landform classification	relief classes based on the relief roughness (slope, exposition)	1km	Digital elevation models	http://eusoils.jrc.ec.europa.eu/projects/landform/
Global Land Survey Digital Elevation Model GLSDEM	elevation	90m	Landsat	http://glcf.umiacs.umd.edu/data/glsdem/index.shtml
Global Digital Elevation Model	elevation	30m	ASTER	https://lpdaac.usgs.gov/products/aster_products_table/astgtm

Socioeconomic data				
Global human influence index	Anthropogenic impacts on the environment	1km	NASA Earth data	http://sedac.ciesin.columbia.edu/data/collection/wildareas-v2/sets/browse
Global Human Footprint	Anthropogenic impacts on the environment	1km	NASA Earth data	http://sedac.ciesin.columbia.edu/data/collection/wildareas-v2/sets/browse
Urban Extents Grid	The urban extent grids distinguish urban and rural areas based on a combination of population counts, settlement points, and the presence of night-time lights	1km	NASA Earth data	http://sedac.ciesin.columbia.edu/data/set/grump-v1-urban-extents
Small Area Estimates of Poverty and Inequality	high spatial resolution subnational estimates of poverty and inequality	subnational	NASA Earth data	http://sedac.ciesin.columbia.edu/data/set/povmap-small-area-estimates-poverty-inequality
Urban Landsat	composite images of 66 urban areas	30m	ENVI Landsat	http://sedac.ciesin.columbia.edu/data/set/ulandsat-cities-from-space
U.S. Population Grids	demographic data, including characteristics of age, race, ethnicity, and housing, for US metropolitan statistical areas	250m	NASA Earth data	http://sedac.ciesin.columbia.edu/data/set/pend-summary-file1-2000-neworleans-msa

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8. List of Publications

9.1 Articles of this Thesis

Fischer D., **Thomas S.M.**, Neteler M., Tjaden N.B., Beierkuhnlein C. (2014): Climatic suitability of *Aedes albopictus* in Europe referring to climate change projections: Comparison of mechanistic and correlative niche modelling approaches. Eurosurveillance 19(6): pii=20696.

Fischer D., **Thomas S.M.**, Suk J.E., Sudre B., Hess A., Tjaden B., Beierkuhnlein C., Semenza J.C. (2013): Climate change effects on Chikungunya transmission in Europe: Geospatial analysis of vector´s climatic suitability and virus´ temperature requirements. International Journal of Health Geographics 12(51). doi:10.1186/1476-072X-12-51.

Thomas S.M., Beierkuhnlein C. (2013): Predicting ectotherm disease vector spread - Benefits from multidisciplinary approaches and directions forward. Naturwissenschaften 100(5):395-405. doi: 10.1007/s00114-013-1039-0.

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8.2 Further Publications of the Author on Vector-borne Diseases

8.2.1 Peer-reviewed Journals

Thomas SM, Tjaden N, van den Bos S, Beierkuhnlein C (2014) Implementing Cargo Movement into Climate Based Risk Assessment of Vector-Borne Diseases. International Journal of Environmental Research and Public Health 11(3):3360-3374. doi:10.3390/ijerph110303360.

Becker, N., Krüger, A., Kuhn, C., Plenge-Bönig, A., **Thomas, S.M.**, Schmidt-Chanasit, J., Tannich E. (2014) Stechmücken als Überträger exotischer Krankheitserreger in Deutschland. Schwerpunktheft "Gesundheitsschädlinge" des Bundesgesundheitsblattes 57: 531-540. doi: 10.1007/s00103-013-1918-8.

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Fischer, D., Moeller, P., **Thomas, S.M.**, Naucke, T.J., Beierkuhnlein, C. (2011): Combining climatic projections and dispersal ability: a method for estimating the responses of sandfly vector species to climate change, PLoS Neglected Tropical Diseases, 5(11), e1407. doi:10.1371/journal.pntd.0001407.

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Hiermit erkläre ich, dass keine Tatsachen vorliegen, die mich nach den gesetzlichen Bestimmungen über die Führung akademischer Grade zur Führung eines Doktorgrades unwürdig erscheinen lassen.

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Stephanie Thomas Bayreuth, im Juni 2014