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The invasive Asian tiger mosquito *Aedes albopictus* (Diptera: Culicidae) in Germany: Local reproduction and overwintering

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ABSTRACT

Within the framework of a German mosquito monitoring programme, the 'Mueckenatlas' (mosquito atlas) has been established as an instrument of citizen participation in mosquito mapping. In 2015, a strikingly large number of *Aedes albopictus*, which had not been considered established in Germany, was submitted. Three of six collection sites showed local reproduction, with demonstration of developmental stages over three months at two sites. The third populated site was checked only once in October. Developmental stages of *Ae. albopictus* were found again at these three sites in spring 2016, including one site in southeastern Germany where reproduction had already been documented in 2014. Although population genetic analyses performed on specimens collected at the latter locality in 2014 and 2015 did not provide proof for hibernation, the finding of developmental stages at this and two other very same sites as in the year before and at very early times in the season strongly suggest accomplished overwintering of *Ae. albopictus* in Germany.

Obviously, the second extremely mild winter in Germany in a row and ongoing adaptation of *Ae. albopictus* to the temperate European climate allow the species to push northwards from endemic regions in the south. Due to the vector competence of *Ae. albopictus* for numerous pathogens, including dengue, chikungunya and Zika viruses, action should be taken immediately after the detection of local reproduction to eliminate the populations.

1. Introduction

The Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus* is an efficient vector of numerous viruses of human and veterinary importance, including dengue, chikungunya and various encephalitis viruses (Paupy et al., 2009). In the laboratory, it is also vector-competent for Zika virus (e.g., Wong et al., 2013), but its role in the present Zika epidemic in South America is not clear and by all means secondary to *Ae. aegypti* (ECDC, 2015). In addition to viruses, the tiger mosquito is a potential vector of dirofilarial worms (Cancrini et al., 1995).

Aedes albopictus is considered one of the most invasive species of the world (ISSG, 2009). Facilitated by human activities, it has started its global spread from original distribution areas in the Asian-Pacific region already decades ago (Paupy et al., 2009). Major modes of movement are the worldwide trade with used tyres and ornamental plants such as 'lucky bamboo' (*Dracaena* spec.) (Reiter, 1998; Hofhuis et al., 2009). Thereby, intercontinentally displaced eggs and larvae built up new populations. In Europe, *Ae. albopictus* was first detected in 1979 in Albania (Adhami and Reiter, 1998), but continental spread is supposed to have occurred only after further introductions to Italy in the early 1990s (Knudsen et al., 1996). Meanwhile, the species has been found in 26 European countries, in 19 of which it succeeded in establishing (Medlock et al., 2015). Since 2007, *Ae. albopictus* has repeatedly been trapped on motorway service stations and on a transfer railway station for cargo trucks in southern Germany (Pluskota et al., 2008; Werner et al., 2012; Becker et al., 2013; Kampen et al., 2013), indicating an introduction by cars from Mediterranean countries, where the species is now widely distributed (Knudsen et al., 1996).

Mosquito surveillance by trapping has been supplemented in Germany since 2012 by a passive approach, the citizen science project 'Mueckenatlas' (mosquito atlas) where private persons may submit collected mosquito specimens for mapping purposes (Kampen et al., 2015). Due to roughly 1500 collection sites per year, randomly distributed all over the country, and approximately 30,000 mosquito specimens collected until the end of 2015, the

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Table 1
Mueckenatlas' submissions of *Ae. albopictus*, 2014 and 2015.

Locality	German federal state	Specimens submitted to Mueckenatlas		Field follow-up
		2014	2015	
Wuerzburg	Bavaria	–	1 (late Aug)	n.d.
Freudenstadt	Baden-Wuerttemberg	–	2 (early Sept)	n.d.
Essen	North Rhine-Westphalia	–	1 (late Aug)	no findings in Oct 2015
Jena-Lobeda	Thuringia	–	2 (mid-July)	Table 2
Heidelberg-West	Baden-Wuerttemberg	–	3 (mid-Sept)	Table 2
Freiburg-East	Baden-Wuerttemberg	2 (mid-Aug, mid-Oct)	2 (mid- and late July)	Table 2

(n.d. = not done).

'Mueckenatlas' contributed significantly to mosquito surveillance in Germany, particularly to the detection of invasive species. Based on submissions of single individuals, three populations of the Asian bush mosquito *Aedes japonicus* were detected in West, North and Southeast Germany in 2012, 2013 and 2015, respectively (Kampen et al., 2012; Werner and Kampen, 2013; Zielke et al., 2016), and a reproducing population of the Asian tiger mosquito was identified in southwestern Germany in 2014 (Werner and Kampen, 2015).

We here report recent findings of *Ae. albopictus*, not only in southern Germany, but also in more central parts of Germany, remote from previous collection sites, including several cases of local reproduction and evidence for overwintering.

2. Materials and methods

2.1. Mosquito collection

Insects received in the scope of the citizen science project 'Mueckenatlas' are usually accompanied by a filled questionnaire asking for details of the collection such as date, locality and contact data of the submitter. After registration, georeferencing and identification of the mosquitoes, all data connected to a collection are entered into the German national mosquito database 'Culbase' which allows for generating distribution maps and dispersal models. Since the launch of the 'Mueckenatlas' in 2012, mosquitoes belonging to invasive *Aedes* species have been submitted increasingly often. In 2015, *Ae. albopictus* females were submitted to the 'Mueckenatlas' from six German sites (Fig. 1, Table 1), including one (Freiburg-East) which had been under observation already since 2014 (Werner and Kampen, 2015). Four of the six sites were subsequently inspected while two sites, brought to attention only late in the year, could not be visited anymore in 2015. On-site inspections concentrated on searching immature developmental stages in cemeteries in close proximity to the collection sites, due to the numerous breeding opportunities offered (Vezzani, 2007). Cemeteries with larval findings were continued to be monitored in more or less biweekly intervals for ongoing colonisation. Ovitraps with wooden spatula as oviposition supports were distributed in two cemeteries according to ECDC guidelines for monitoring invasive mosquito species (ECDC, 2012).

Collected larvae were kept in jars for finishing aquatic development. Emerged adults were identified morphologically using the keys by Schaffner et al. (2001a) and Becker et al. (2010). Identification of at least one specimen per locality was genetically confirmed by CO1 (cytochrome oxidase subunit I gene)-barcoding according to published protocols (Werner et al., 2016).

Local temperatures were retrospectively analysed at the sites of presumed overwintering. For this purpose, daily minimum and daily mean temperature data from October to March, interpolated to a grid cell of 1×1 km^2, were acquired from the German Weather Service (DWD).

2.2. Genetic analyses

As the emergence of reproducing *Ae. albopictus* in 2014 and 2015 at the very same site in Freiburg-East, remote from common introduction pathways, suggested overwintering, population genetic analyses were carried out, assuming that the 2014 and 2015 specimens from this locality were genetically identical or at least revealed higher genetic similarity than to individuals collected elsewhere in or outside Germany. Included in those analyses were specimens from the other German collection sites described plus from a German population, 'Freiburg-West', detected in 2015 by colleagues in an allotment garden complex (Pluskota et al., 2016), as well as from laboratory strains from Italy (Rimini, Turin) and Mauritius. Specifically, mitochondrial CO1, *nad*4 (NADH dehydrogenase subunit 4) and *nad*5 (NADH dehydrogenase subunit 5) gene sequences were comparatively examined according to protocols by Birungi and Munstermann (2002), Zielke et al. (2014) and Egizi and Fonseca (2015). In addition, ten microsatellite loci were analysed as described by Beebe et al. (2013).

For DNA extraction, legs of adult mosquitoes were processed by means of the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Obtained gene sequences were aligned and compared using Geneious version 8.1.3 (Kearse et al., 2012). Microsatellite results were visualized applying the same Geneious software and interpreted with STRUCTURE, STRUCTURE HARVESTER and GenAlEx as described in Zielke et al. (2014).

3. Results

3.1. Collection of Aedes albopictus

In 2015, *Ae. albopictus* females were submitted to the 'Mueckenatlas' from six sites (Fig. 1, Table 1). The submissions from 'Wuerzburg' and 'Freudenstadt' were not followed up in 2015.

In Essen (German federal state of North Rhine-Westphalia), the 'Mueckenatlas' collection locale was inspected in early October 2015 (Table 1). Aquatic stages of *Ae. albopictus* could neither be detected in water containers on the submitter's premises or their close surroundings nor in three cemeteries within a radius of 1.2 km.

In Jena (federal state of Thuringia), a cemetery adjacent to the collection site of the submitted specimens was scrutinized in mid-July within a few days after the submission of the *Ae. albopictus* specimens. As no larvae could be found, 30 ovitraps were distributed over that cemetery with distances of about 5 m each. During every single of the following visits from late July until late October, eggs, larvae and/or pupae of *Ae. albopictus* were found in flower vases and ovitraps (Table 2). At the end of August, two adult females were captured while soaring up from a flower vase and one female while entering the collector's car in front of the cemetery. In 20 further cemeteries checked in the municipality of Jena in mid-August 2015, no *Ae. albopictus* were found, but *Ae. japonicus* adults developed from one of the larval samples.