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Short Communication

The Citizen Science Project ‘Mueckenatlas’ Helps Monitor the Distribution and Spread of Invasive Mosquito Species in Germany

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Abstract

The citizen science project ‘Mueckenatlas’ (mosquito atlas) was implemented in early 2012 to improve mosquito surveillance in Germany. Citizens are asked to support the spatiotemporal mapping of culicids by submitting mosquito specimens collected in their private surroundings. The Mueckenatlas has developed into an efficient tool for data collection with close to 30,000 mosquitoes submitted by the end of 2015. While the vast majority of submissions included native mosquito species, a small percentage represented invasive species. The discovery of *Aedes albopictus* (Skuse) (Diptera: Culicidae), *Aedes japonicus japonicus* (Theobald) (Diptera: Culicidae) and *Aedes koreicus* (Edwards) (Diptera: Culicidae) specimens via the Mueckenatlas project prompted targeted monitoring activities in the field which produced additional information on the distribution of these species in Germany. Among others, Mueckenatlas submissions led to the detection of three populations of *Ae. j. japonicus* in West, North and Southeast Germany in 2012, 2013, and 2015, respectively. As demonstrated by on-site monitoring, the origins of *Ae. j. japonicus* specimens submitted to the Mueckenatlas mirror the distribution areas of the four presently known German populations as found by active field sampling (the fourth population already reported prior to the launch of the Mueckenatlas). The data suggest that a citizen science project such as the Mueckenatlas may aid in detecting changes in the mosquito fauna and can therefore be used to guide the design of more targeted field surveillance activities.

Key words: citizen science, Germany, invasive mosquitoes, Mueckenatlas, passive mosquito surveillance

Invasive mosquitoes, such as the yellow fever mosquito *Aedes aegypti* (Linnaeus) and the Asian tiger mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae), and their associated disease agents have recently (re)gained scientific, political and public attention in areas of the world where mosquitoes were not considered a severe public health threat. In some cases, diseases quickly followed the invasive species which served as vectors of non-endemic pathogens in new areas of the world. While several viruses of minor pathogenicity transmitted by native mosquitoes have been detected in Europe since the late 1950s (Hubálek 2008), only West Nile virus, isolated in 1964 from both mosquitoes and humans in southern France (Hannoun et al. 1966), caused serious disease outbreaks (e.g., Tsai et al. 1998, Pervanidou et al. 2014). However, in 2007, a series of cases and outbreaks of chikungunya and dengue started in southern Europe with invasive mosquitoes serving as the primary virus vectors (Schaffner et al. 2013, Medlock et al. 2015).

Representing the first record of an invasive mosquito vector species in Germany, eggs of *Ae. albopictus* were detected in 2007 in the southwestern part of the country (Pluskota et al. 2008). In 2008, *Aedes japonicus japonicus* (Theobald) (Diptera: Culicidae) larvae were discovered in the same region (Schaffner et al. 2009). The finding of invasive mosquitoes in Germany together with the emergence and resurgence of mosquito-borne disease cases in southern Europe triggered the initiation of a nation-wide mosquito monitoring program in Germany in 2011. This was meant to update knowledge on the occurrence and distribution of culicids in Germany, disregarded for decades due to lack of endemic transmission of life-threatening mosquito-borne pathogens, and to contribute to risk analyses for mosquito-borne diseases. Within this program, active monitoring using BG Sentinels (Biogents, Regensburg, Germany) and EVS (encephalitis virus surveillance) traps (BioQuip Products, Compton, CA) was supplemented in 2012 by the citizen science project ‘Mueckenatlas’ (mosquito atlas) (Werner et al. 2014, Kampen et al. 2015).

Citizen science has become an important data source in many scientific disciplines (Gura 2013). Science is required by the general public to become more transparent, and tax payers may not only want to know how public money is spent but also may like to be involved in pertinent processes, such as actively participating in science. Voluntary support by interested citizens may provide a huge body of valuable scientific data at low costs.

Citizen science is not completely new or unique in entomology. There are community-based international butterfly projects (e.g., 'Monarch Joint Venture', <http://monarchjointventure.org/>), a U.S. ant project (e.g., 'School of Ants', <http://www.schoolofants.org/>) and a German firefly project (<https://sachsen.lpv.de/gluehwuermchen.html>), just to mention a few. To the best of the authors' knowledge, however, the Mueckenatlas is the first and most successful citizen science project (in terms of annual participants and scientific outcomes) focusing on potential vector species. In contrast to many other citizen science projects where mere observations are reported or, in some cases, pictures are sent to the scientists via smart phones, the Mueckenatlas scheme requires the mosquitoes to be submitted physically, in order to perform reliable identification (expert quality control), be able to conduct genetic analyses and have the material available for long-term voucher collections.

We here describe the contributions of the Mueckenatlas passive surveillance scheme to mosquito mapping in Germany with particular emphasis on its role and efficacy in detecting invasive mosquito species.

Materials and Methods

Organization of the Mueckenatlas

Launched in April 2012, the Mueckenatlas is a classical citizen science project that is based on community participation. Citizens are regularly invited by press releases, articles in newspapers, radio interviews, TV appearances, public talks and flyers to contribute to mapping the German mosquito fauna by submitting mosquitoes collected in their private surroundings. Briefly, mosquitoes are to be caught within a jar or a similar closable container and to be killed by placing the container in the freezer overnight. Without being touched, the mosquitoes should then be transferred to a small nonbreakable case or vial to be sent to the research institutions involved, together with all data connected to the capture (collection date and time, precise location and environment). Upon species determination, the sender will receive a personalized feedback, usually by email, with the identification result and some biological details on the species submitted. On demand, the submitter's name or a pseudonym can be linked with a dot marking the collection site on an interactive map on the homepage of the project (www.mueckenatlas.de). The website (in German language) does not only provide instructions on how to submit mosquitoes (including a questionnaire for download, asking for specific details of the mosquito collection), but also informs about the project background and mosquitoes in general. Collection data on the endemic mosquito species obtained so far are being analyzed and will soon be presented in the form of distribution maps.

Together with data from other projects focusing on German culicids, the Mueckenatlas data are also entered into the German mosquito database 'Culbase' which facilitates the production of detailed species distribution maps, models for the future spread of the various species under preset scenarios and assessments of future risk of mosquito-borne diseases.

Mosquito Identification and Storage

The submitted mosquitoes are identified morphologically according to the determination keys by Mohrig (1969), Schaffner et al. (2001),

and Becker et al. (2010), or, in the case of cryptic species or damaged specimens, genetically by species-specific PCR assays (Proft et al. 1999, Rudolf et al. 2013, Kronefeld et al. 2014) or DNA barcoding (Folmer et al. 1994, Hébert et al. 2003). Morphological identification of invasive mosquitoes is usually also confirmed genetically by barcoding, e.g., to reliably distinguish *Ae. j. japonicus* from *Aedes koreicus* (Edwards) (Diptera: Culicidae) (cf. Werner et al. 2016). The ratio of mosquitoes undergoing genetic identification is about 20%, mainly relating to invasive species and the most common and widely-distributed native group of species, the *Culex pipiens* complex. Specimens of this complex account to about a third of all mosquitoes submitted, and roughly a third of those are genetically identified to species or biotype to give a representative overview. The success rate of genetic identification is above 95%.

After processing, a representative portion of specimens of all species and collection sites are incorporated into the Leibniz Centre for Agricultural Landscape Research (Muencheberg, federal state of Brandenburg, Germany) voucher collection of pinned mosquitoes. Extracted DNA of all specimens genetically identified is stored deep-frozen (-80°C) in the Friedrich-Loeffler-Institut (Greifswald, federal state of Mecklenburg-Western Pomerania, Germany). Both dry-pinned mosquitoes and mosquito DNA are meant to serve as reference collections for future research.

Follow-up of Invasive Species Submissions

Once invasive species have been submitted to the Mueckenatlas, the collection sites are visited as soon as possible in order to check for local reproduction of the species by screening artificial water containers for developmental life stages. The inspection starts at the sites of collection, usually on the premises of the submitters, and continues in the closest cemetery, due to easy accessibility and the high abundance of potential mosquito development sites available (Vezzani 2007). If larval or pupal stages are found, a small-scale local monitoring will be initiated using ovitraps and/or lethal gravid *Aedes* traps (GATs, Biogents) according to the ECDC guidelines for the surveillance of invasive mosquito species (ECDC 2012). To assess the spatial occurrence of the species on a wider scale, a virtual grid pattern with 10 × 10 km² cells is laid over the region and at least three cemeteries per grid cell (in different areas of the cell) are screened for larvae and pupae (cf. Kampen et al. 2016). In the case of a grid cell found colonized, all cells surrounding the positive cell are checked following the same procedure (e.g., Zielke et al. 2016).

Results and Discussion

From April 2012 to the end of 2015, the Mueckenatlas surveillance scheme received more than 7,300 submissions (Fig. 1). About 75% of them contained mosquitoes whereas the rest were other insects (mainly other Diptera and Hymenoptera).

Five of the six culicid genera (taxonomy according to Wilkerson et al. 2015) described for Germany were represented among the submissions: *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, and *Culiseta*. Specimens of the genus *Uranotaenia*, which is thought to occur in Germany with one species only (*Uranotaenia unguiculata* Edwards) (Diptera: Culicidae), were not submitted.

Among the more than 29,000 mosquitoes received (Fig. 1), 41 of the 51 species previously reported from Germany were recorded, only two less than recorded by trapping during the same time period (Table 1). Trapping, performed in parallel to the Mueckenatlas passive approach, was done by BG Sentinels and EVS traps operated over a 24 hr-period each week during the warm season (April to

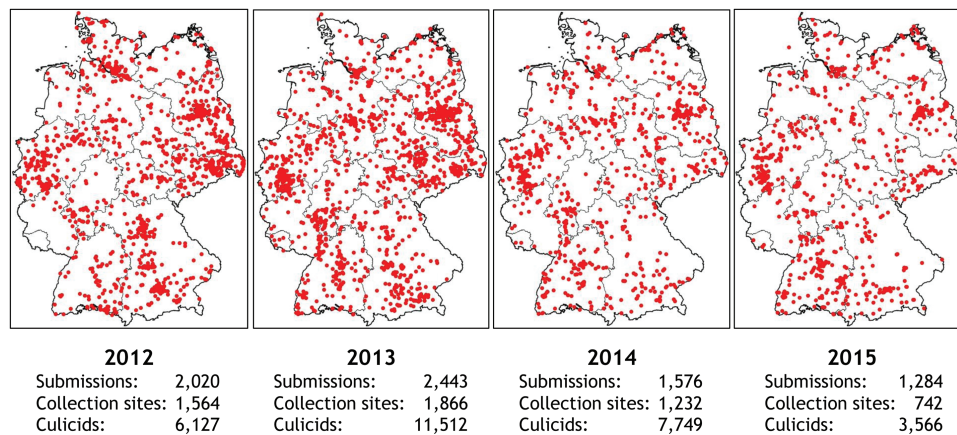


Fig. 1. Overview of origins and numbers of submissions to the Mueckenatlas, 2012–2015.

October) at 126 sites all over Germany. Trapping sites were sampled for at least 1 yr and sometimes up to 3 yr.

Five of the species not recorded by the Mueckenatlas project are rare and have been found in Germany only a few times (*Aedes cypricus* Ludlow, *Aedes nigrius* (Eckstein) (Diptera: Culicidae), *Aedes refiki* Medschid (Diptera: Culicidae), *Culex martinii* Medschid (Diptera: Culicidae)), or are neither widely distributed nor very abundant (*Ur. unguiculata*). Due to their low abundance, some of the species not submitted to the Mueckenatlas also were not collected by routine trapping since the onset of the monitoring program in 2011. By contrast, other rare species that have not been documented for decades, such as *Culiseta alaskaensis* (Ludlow), *Culiseta glaphyoptera* (Schiner) and *Culiseta ochroptera* (Peus) (Diptera: Culicidae), or that are new to the German mosquito fauna, such as *Culiseta longiareolata* (Macquart) (Diptera: Culicidae), were repeatedly recorded by the Mueckenatlas project (Kampen et al. 2013; Table 1).

In addition to *Cs. longiareolata*, three invasive *Aedes* species were detected in Germany with the help of the Mueckenatlas between 2012 and 2015: *Ae. albopictus*, *Ae. koreicus* and *Ae. j. japonicus* (Kampen et al. 2012, 2016; Werner & Kampen 2013, 2015; Werner et al. 2016; Zielke et al. 2016).

Particularly noteworthy from a public health point of view is the contribution of the Mueckenatlas to tracking the spatial occurrence of invasive *Aedes* species. *Ae. albopictus* was submitted by citizens from seven sites (two sites in 2014 and six sites in 2015, with one identical site in the 2 yr), leading to the detection of local reproduction at several sites and of the first documented overwintering in Germany at one site (Werner and Kampen 2015). In 2015, a single *Ae. koreicus* specimen was received but developmental stages of this species could not be found (Werner et al. 2016).

The impact of the Mueckenatlas is best reflected by following up on the most wide-spread invasive *Aedes* species in Germany, *Ae. j. japonicus*. This species was first detected in 2008 on the border with Switzerland (Schaffner et al. 2009) but was soon shown to have colonized a considerable area in the southwestern part of Germany (Huber et al. 2012). After the submission to the Mueckenatlas project of seven *Ae. j. japonicus* specimens in July 2012 from western Germany, one specimen in October 2012 from northern Germany and three specimens in July 2015 from southeastern Germany, regional sampling of aquatic stages led to the discovery of three additional populations of this species (Kampen et al. 2012, Werner and Kampen 2013, Zielke et al. 2016).

In terms of collection sites, Mueckenatlas submissions increased from 2012 to 2015 for all four German *Ae. j. japonicus* populations (with a small decrease in 2013 for the West German one) while numbers of specimens also increased for the southwestern population but not for the western one (Table 2). Of note, the number of submissions may vary temporally and regionally according to mosquito abundance, but is also related to media coverage of the Mueckenatlas.

The geographical extent of the colonized areas was determined by cemetery screening according to the grid pattern scheme. Since their detection, the West and North German populations of *Ae. j. japonicus* were monitored annually in the field for ongoing colonization and spatial spread. Hence, there are precise data available for the West, North and Southeast German populations (Kampen et al. 2016, Zielke et al. 2016), whereas field collections by our group were only made sporadically, but not systematically, in southwestern Germany. As updates on the distribution of the southwestern population have not been published, approximate estimates on its spatial distribution in 2015 are based on older published data (Becker et al. 2011, Schneider 2011, Huber et al. 2012, Krebs et al. 2014), own field collections and recent personal communications (Becker and co-workers, Institute for Dipterology, Speyer, Germany).

In Fig. 2, Mueckenatlas submissions from 2012 to 2015 are contrasted with areas positive for *Ae. j. japonicus* based on field-collected data, i.e., larval sampling. Although Mueckenatlas submissions concentrate in the centers of densely colonized areas, probably due to higher probabilities of capturing this species, the passive and active monitoring approaches show a high degree of matching. They also display a continuous spread, as documented for example in 2015 by the first Mueckenatlas submissions from the German federal states of Hesse and Bavaria. These eastward expansions of the western and the southwestern populations could also be subsequently verified by larval sampling.

Conclusions

A citizen science project, such as the Mueckenatlas, appears to be an efficient alternative to routine active mosquito surveillance. In the presented approach, it covered a similar species spectrum as trapping, and was able to detect changes in the mosquito fauna in due time and to display species distributions correctly. Regarding invasive species, it can constitute a valuable early warning system to trigger and help design active monitoring schemes.

Table 1. Mosquito species trapped during the German monitoring program and species submitted to the Mueckenatlas project from 2012–2015 in relation to all species ever documented for Germany by 2015 according to Dahl et al. (1999) and Werner et al. (2012, 2016)

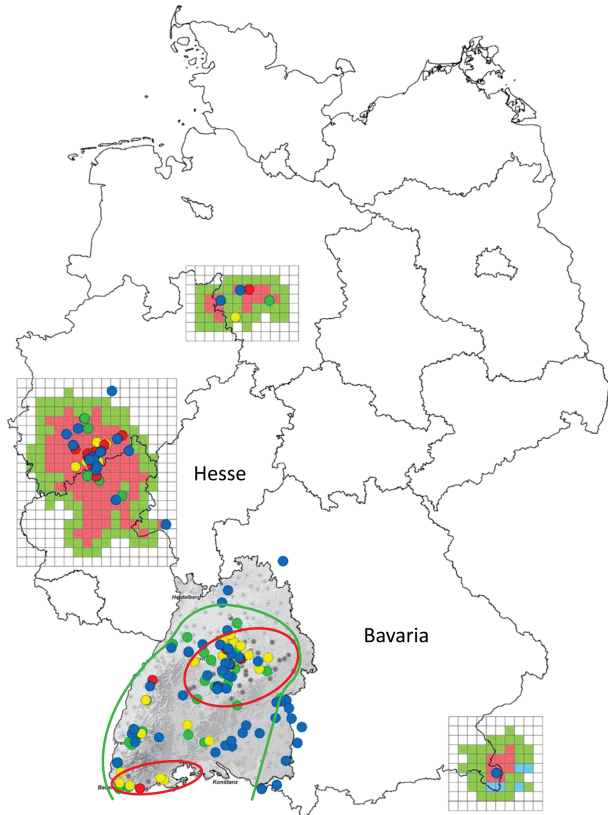
Species	Adults	
	Trap	Mueckenatlas
<i>Aedes albopictus</i>	+	+
<i>Aedes annulipes</i>	+	+
<i>Aedes cantans</i>	+	+
<i>Aedes caspius</i>	+	+
<i>Aedes cataphylla</i>	+	+
<i>Aedes cinereus</i>	+	+
<i>Aedes communis</i>	+	+
<i>Aedes cyprius</i>	–	–
<i>Aedes detritus</i>	+	+
<i>Aedes diantaeus</i>	+	–
<i>Aedes dorsalis</i>	+	+
<i>Aedes excrucians</i>	+	+
<i>Aedes flavescens</i>	+	+
<i>Aedes geminus</i>	+ ^d	?
<i>Aedes gemiculatus</i>	+	+
<i>Aedes intrudens</i>	+	+
<i>Aedes japonicus</i>	+	+
<i>Aedes koreicus</i>	–	+
<i>Aedes leucomelas</i>	+	+
<i>Aedes nigrinus</i>	–	–
<i>Aedes pullatus</i>	+	+
<i>Aedes punctor</i>	+	+
<i>Aedes refiki</i>	–	–
<i>Aedes riparius</i>	+	+
<i>Aedes rossicus</i>	+	+
<i>Aedes rusticus</i>	+	+
<i>Aedes sticticus</i>	+	+
<i>Aedes vexans</i>	+	+
<i>Anopheles algeriensis</i>	+	–
<i>Anopheles atroparvus</i>	+	+
<i>Anopheles claviger</i>	+	+
<i>Anopheles daciae</i>	+	+
<i>Anopheles maculipennis</i>	+	+
<i>Anopheles messeae</i>	+	+
<i>Anopheles plumbeus</i>	+	+
<i>Coquillettidia richiardii</i>	+	+
<i>Culex hortensis</i>	+	+
<i>Culex martinii</i>	–	–
<i>Culex modestus</i>	+	+
<i>Culex pipiens</i>		
biotype <i>pipiens</i>	+	+
biotype <i>molestus</i>	+	+
<i>Culex territans</i>	+	+
<i>Culex torrentium</i>	+	+
<i>Culiseta alaskaensis</i>	+	+
<i>Culiseta annulata</i>	+	+
<i>Culiseta fumipennis</i>	?	?
<i>Culiseta glaphyroptera</i>	+	+
<i>Culiseta longiareolata</i>	+	+
<i>Culiseta morsitans</i>	+	+
<i>Culiseta ochroptera</i>	+	+
<i>Culiseta subochrea</i>	?	?
<i>Uranotaenia unguiculata</i>	–	–
Total no. of species	43	41

+ : species found, – : species not found, ? : identification of collected species uncertain due to high morphological and genetic (COI barcode) similarity with closely related species.

^dTrapping of a male specimen allowed for reliable morphological species identification.

Table 2. Annual number of *Ae. j. japonicus* collection sites registered by the Mueckenatlas, 2012–2015 (number of submitted specimens in brackets)

Population	No. collection sites (no. specimens submitted)			
	2012	2013	2014	2015
Southwest Germany	5 (5)	28 (41)	37 (65)	46 (85)
West Germany	12 (14)	9 (22)	12 (56)	18 (33)
North Germany	1 (1)	1 (2)	1 (1)	2 (2)
Southeast Germany	–	–	–	1 (3)

**Fig. 2.** Map of Germany as of late 2015, comparing *Ae. j. japonicus* submissions to the Mueckenatlas (red dots: 2012, yellow dots: 2013, green dots: 2014, blue dots: 2015) and distribution areas of the four German *Ae. j. japonicus* populations as determined by field monitoring (grids: 10 × 10 km² cells in which cemeteries were screened for *Ae. j. japonicus* aquatic stages (cf. Kampen et al. 2016, Zielke et al. 2016); green squares: positive for *Ae. j. japonicus*, red squares: negative for *Ae. j. japonicus*; blue squares: not accessible due to mountainous regions; areas encircled in red: approximate distribution areas according to Huber et al. (2012); area encircled in green: estimated distribution area by late 2015 according to publications cited in the text, own unpublished data and personal communications).

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