

Changes in Species Richness and Spatial Distribution of Mosquitoes (Diptera: Culicidae) Inferred From Museum Specimen Records and a Recent Inventory: A Case Study From Belgium Suggests Recent Expanded Distribution of Arbovirus and Malaria Vectors

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ABSTRACT Mosquito (Diptera: Culicidae) distribution data from a recent inventory of native and invading mosquito species in Belgium were compared with historical data from the period 1900–1960 that were retrieved from a revision of the Belgian Culicidae collection at the Royal Belgian Institute of Natural Sciences. Both data sets were used to investigate trends in mosquito species richness in several regions in Belgium. The relative change in distribution area of mosquito species was particularly important for species that use waste waters and used tires as larval habitats and species that recently shifted their larval habitat to artificial larval habitats. More importantly, several of these species are known as vectors of arboviruses and *Plasmodium* sp. and the apparent habitat shift of some of them brought these species in proximity to humans. Similar studies comparing current mosquito richness with former distribution data retrieved from voucher specimens from collections is therefore encouraged because they can generate important information concerning health risk assessment at both regional and national scale.

KEY WORDS Culicidae, vector community, change index, distribution area, artificial larval habitat

Increasing worldwide transport of people and goods create suitable conditions for the introduction of exotic mosquito species as well as the (re)emergence of mosquito-borne diseases in western Europe (Romi et al. 2004, Benedict et al. 2007, Moutailler et al. 2008, Lancelot et al. 2009). As a consequence, a better knowledge of the taxonomic and functional biodiversity of both native and invading vector species in all countries becomes urgent. Exotic and invasive species might act as introduced vectors of new virus (Crowl et al. 2008), as can native species (Aspöck 2008). Moreover, because of land use and climatic changes, native species may have expanded their distribution during the last decades and should be taken into account for risk assessment in virus transmission. For example, *Anopheles plumbeus* Stephens was recently shown to be a potential pest species in urban environments. This urbanization was caused by an expansion

in larval habitat choice from tree-holes in forests to abandoned, uncleaned manure tanks of pig stables (Dekoninck et al. 2011b). This expansion was the first documented case of a human induced distribution expansion of a native mosquito species in Belgium. Hence, it is of considerable importance to investigate whether other native mosquito species, known to be vectors of arboviruses and *Plasmodium* sp. elsewhere also have changed their larval habitat or expanded their distribution due to changes in human land use. Within the framework of the MODIRISK project, a national inventory of the current distribution of mosquito species was conducted (Coosemans et al. 2011, Versteirt et al. 2012a). To obtain a better insight into recent population expansion or decline of several native species, data on their historical distribution also are needed. This information comes from the reidentification and digitization of all specimens in the Royal Belgian Institute of Natural Sciences (RBINS) collections, belonging to 24 species of the 32 so far recorded in Belgium (Dekoninck et al. 2011a). Most of the specimens (77%) were collected between 1910 and 1960, providing a good time slice to compare the historic distribution of the majority of mosquito species for some regions in Belgium.

Here, we compare current and historic mosquito species richness, with particular attention to native vector species. Species recently introduced to Bel-

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gium (Schaffner et al. 2004; Versteirt et al. 2009, 2012b) are not discussed.

Materials and Methods

Identification and Nomenclature. All voucher specimens from RBINS collections and recently collected mosquitoes were identified at the species level by using the identification key developed by Schaffner et al. (2001). For a few species, no reliable identifications could be made, such as for *Aedes cinereus* Wiedemann and *Aedes geminus* Peus that we refer to as *Ae. cinereus* sensu lato and all specimens belonging to *Anopheles maculipennis* sensu lato (i.e., *Anopheles atroparvus* van Thiel, *Anopheles messeae* Falleroni, and *Anopheles maculipennis* sensu stricto).

Comparison of Current and Historic Mosquito Species Richness. Current mosquito species richness and distribution was estimated based on a nationwide sampling throughout Belgium in >900 randomly selected sites (Coosemans et al. 2011, Versteirt et al. 2012a). Individuals were collected with the Mosquito Magnet Liberty Plus trap (Woodstream Corporation, Lititz, PA). Results of this large-scale survey provided us with an estimation of the current observed mosquito richness, being the recent observed species richness (hereafter S_{curr}). Historic species richness was obtained by revising the RBINS mosquito-collection (hereafter S_{hist}). All current and historic records (i.e., labeled specimens of species collected at a given site on a given date) were summarized by assigning the locality to 10- by 10-km grid cells of the Universal Transverse Mercator (UTM) projection (in Belgium 385 grid cells).

Although mosquitoes were collected in a different way in the past (net sweeping, hand sampling), we may assume that species that were present and common during that time, were collected and that there is no evidence that the recent sampling technique, i.e., MMLP would collect several species more frequent than hand sampling did in the past. The sampling effort for both periods differentiated among most of the grid cells. There was no correlation between the number of sampling events per grid cells per period and the number of species collected (Pearson correlation test $P > 0.05$), and the total number of species collected in the first period (1900–1960) was nearly identical to the number of species collected in the recent survey.

As mentioned by Dekoninck et al. (2011a), data on historic mosquito richness are only available for a restricted number of regions. Due to that limited early data of Culicidae available in Belgium, comparative analyses will fail to take into account the whole study area. To allow a reliable comparison based on an approximately similar sampling intensity, we restricted our analyses to so-called “intensively surveyed” grid cells, where at least two species were found in both periods (S_{pres} and $S_{hist} \geq 2$). In most cases, these were cells where at least *Culex pipiens* L. and *Culiseta annulata* (Schränk) were present. This resulted in a subset of 56 grid cells, hereafter called well (intensively) surveyed grid cells and 23 mosquito species.

For each of these well surveyed grid cells, a trend metric of species richness (degree of decline or increase) was calculated (Dufrène and Desender 2007, Desender et al. 2008). The degree of change in species richness for each grid cell (TREND) was calculated by $TREND = [S_{curr} - (\alpha S_{hist})] / [(\alpha S_{hist}) + S_{curr}]$, where α is a correction factor to account for differences in sampling intensity and sampling methods between both inventory systems and periods. This correction factor was calculated by dividing the sum of S_{curr} by the sum of S_{hist} for all well surveyed grid cells. In this study, the correction factor was +1.215. By calculating this TREND we generate a value between -1 (all species richness lost) and +1 (all species richness new) for each well surveyed grid cell.

Estimating Relative Changes in Distribution Area.

To investigate whether the current observed distribution area of several mosquito species in Belgium has increased or decreased compared with the historic distribution area, one needs to account for differences in recording intensity or geographical coverage between both periods (Graham et al. 2004). To allow a proper comparison, we restricted the comparative analysis to 1) grid cells that were well surveyed in both periods and to 2) species that were recorded within these grid cells. To obtain relative changes in distribution area for those 23 mosquito species, a linear regression was performed of the logit-transformed proportion of occupied well surveyed grid cells from the recent inventory (on the y-axis) on the logit-transformed proportion of occupied well surveyed grid cells in the historic data (on the x-axis). Proportions in both surveys were calculated as $P = (x + 1) / (n + 1)$, where x is the number of occupied well surveyed grid cells for a given species and n is the total number of well surveyed grid cells surveyed, i.e., 56. The logit-transformed proportions were calculated as $\logit(P) = \ln[P / (1 - P)]$.

The index of relative change in distribution area for each species was calculated by its standardized residual from the fitted regression line (Van Landuyt et al. 2008). Species with positive values for this index are characterized by a relative extension of their distribution area. Negative residuals indicate species that decreased their distribution area relative to the other studied species.

Information on Larval Habitat and Potential Vector Capacity.

To investigate whether there is an association between the relative change of a species, and its known larval habitat, i.e., its reported use of used tires, and also its reported use of waste waters as larval habitats (Table 1; Schaffner et al. 2001), two Goodman and Kruskal's gamma coefficients were calculated and their significances were assessed by means of an exact test procedure in StatXact-5 (Cytel Software Corporation, Cambridge, MA). Goodman and Kruskal's gamma measures the strength of association of two ordered variables (i.e., variable 1, relative changes in distribution and variable 2, reported use of used tires). Gamma values range from -1 (100% negative association, or perfect inversion) to +1 (100% positive association, or perfect agreement). A value of 0 indicates

Table 1. Relative change index (a trend metric of species richness, i.e., the degree of decline or increase) in the survey area for 23 mosquito species

Species	Change index	Known vector for			Artificial larval habitat	
		Human malaria	Avian malaria	West Nile	Used tires	Waste waters
<i>Aedes (Ochlerotatus) annulipes</i> (Meigen)	−1.61					
<i>Aedes (Ochlerotatus) sticticus</i> (Meigen)	−1.34					
<i>Culiseta (Culiseta) morsitans</i> (Theobald)	−1.20					
<i>Culex (Culex) territans</i> Walker	−0.94				x	
<i>Culiseta (Culiseta) fumipennis</i> (Stephens)	−0.66					
<i>Culiseta (Culiseta) subochrea</i> (Edwards)	−0.66					x
<i>Aedes (Ochlerotatus) punctor</i> (Kirby)	−0.63			(x)		
<i>Aedes (Ochlerotatus) communis</i> (De Geer)	−0.55					
<i>Aedes (Ochlerotatus) cantans</i> (Meigen)	−0.52			x		
<i>Aedes (Rusticoides) rusticus</i> (Rossi)	−0.27					
<i>Culex (Culex) hortensis</i> Ficalbi	−0.24				x	x
<i>Aedes (Ochlerotatus) detritus</i> Haliday	−0.24					
<i>Coquillettidia (Coquillettidia) richiardii</i> (Ficalbi)	0.04			x		
<i>Culiseta (Culiseta) annulata</i> (Schrank)	0.04		x		x	x
<i>Aedes (Finlaya) geniculatus</i> (Olivier)	0.08			(x)	x	
<i>Anopheles (Anopheles) maculipennis</i> s.l.	0.28	x ^a		x	x	
<i>Aedes (Aedes) cinereus/geminus</i>	0.50					
<i>Aedes (Ochlerotatus) caspius</i> (Pallas)	0.51			x		
<i>Aedes (Aedimorphus) vexans</i> Meigen	0.55			x		
<i>Anopheles (Anopheles) claviger</i> (Meigen)	0.70	x			x	
<i>Culex (Culex) pipiens</i> L.	1.46		x	x	x	x
<i>Anopheles (Anopheles) plumbeus</i> Stephens	1.72	(x)		(x)	x	x
<i>Culex (Culex) torrentium</i> Martini	2.40				x	x

Vector capacity for human and avian malaria as well West Nile virus vectors with reports from experimental studies are in perentheses (Schaffner et al. 2001). Information on artificial larval habitats was retrieved from Schaffner et al. (2001).
^a Not all species belonging to this complex are vector for human malaria.

the absence of association (Sheskin 2007). In the same way, we checked for an association of the relative change in distribution of a species and its vector capacities for human malaria, avian malaria, and West Nile virus (Table 1; Schaffner et al. 2001).

Results

Comparison of Current and Historic Mosquito Richness. Historic data could be obtained from 100 grid cells (Fig. 1, left), albeit with a strong heterogeneity in the number of specimens and species per grid

cell. In particular, in grid cells near the capital Brussels and the city of Ghent, more than seven species were collected between 1900 and 1960 (Dekoninck et al. 2011a). In the recent inventory (Fig. 1, right), nearly all cells contain more than one species. Large S_{curr} were mainly observed in the north east of the country. Despite the lower availability of specimens in the historic collections, we observed a general decline in mosquito species richness for most of the well surveyed grid cells (Fig. 2). This seemed most pronounced for the surroundings of Brussels and Ghent. Yet, also in the most eastern part of the country a

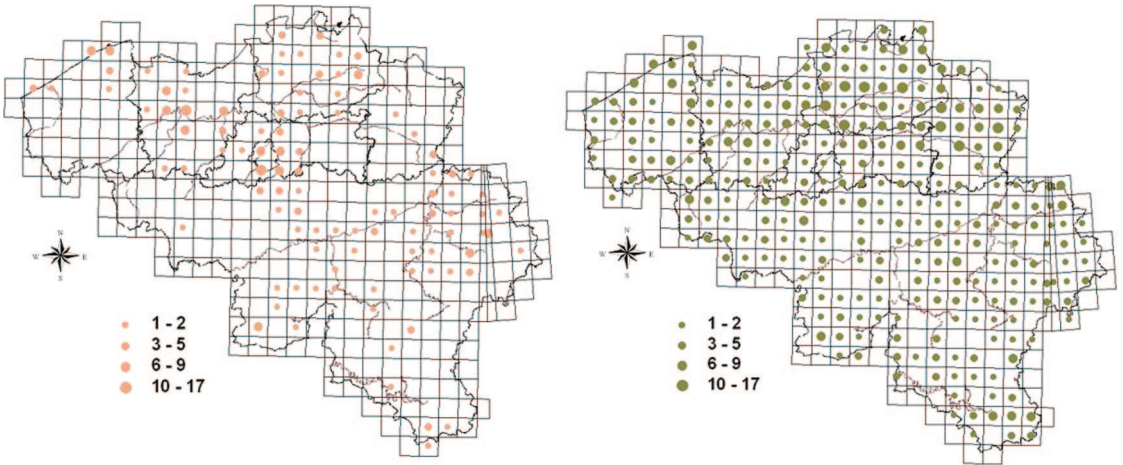


Fig. 1. Historic (<1960; left) and recent observed (2007–2008) number of mosquito species (S) per 10 km × 10 km UTM square in Belgium. (Online figure in color.)

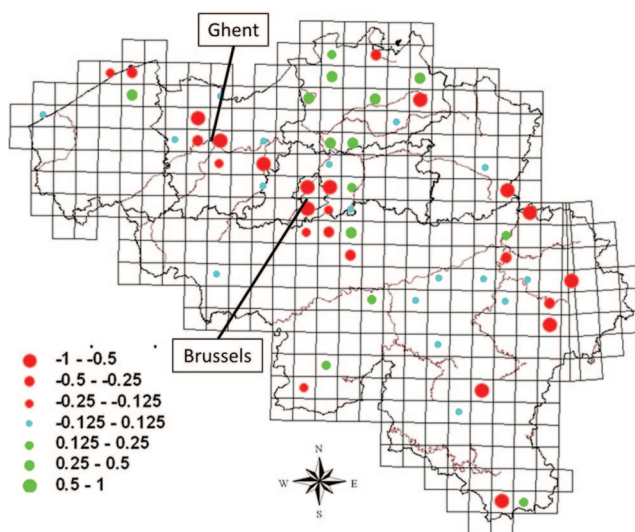


Fig. 2. Changes in mosquito species richness between both time periods (1900–1960 and 2007–2008) for a subset of grid cells ($n = 56$) that showed sufficient coverage in both time periods in Belgium. Red dots are grid cells with a decrease and green dots are grid cells with an increase of mosquito species richness; blue dots are grid cells with a stable species richness. (Online figure in color.)

strong decline in mosquito species richness is observed. An apparent increase in species richness was mainly restricted to the northern part of the country.

Relative Changes in Distribution Area of Belgian Mosquito Species. Eight species increased their distribution relative to the other species (Table 1). Among them are *Anopheles* species such as *An. claviger* (Meigen), *An. plumbeus*, and the *An. maculipennis* s.l. Also, *Ae. vexans* Meigen and *Ae. cinereus* s.l. have a positive relative index of change. The highest increases in relative distribution were observed for *Culex torrentium* Martini, *Cx. pipiens*, and *An. plumbeus*.

Negative indexes of relative distributional change were found for most of the *Aedes* and *Culiseta* species, with *Aedes* (*Ochlerotatus*) *annulipes* (Meigen), *Aedes* (*Ochlerotatus*) *sticticus* (Meigen), and *Culiseta moritani* (Theobald) retrieving the largest negative indices. Three species, *Cs. annulata*, *Aedes* (*Finlaya*) *geniculatus* (Olivier), and *Coquillettidia richiardii* (Ficalbi), showed no apparent change in distribution area as their indices approached 0.

Association of Larval Habitat, Potential Vector Capacity of Species, and Relative Index of Distributional Change. There is a positive association between the positive relative distribution change of a species (increase), and its reported use of used tires as larval habitats ($\gamma = 0.603$, $P = 0.016$). Moreover, there is a trend that larvae of these species with a positive index are found in waste waters ($\gamma = 0.49$, $P = 0.09$).

Also, species that are known to be potential vectors for West Nile virus and human malaria are associated with a higher relative index of distributional change ($\gamma = 0.51$, $P = 0.046$ and $\gamma = 0.73$, $P = 0.047$, respectively), but this is not the case for avian malaria ($\gamma = 0.52$, $P = 0.28$).

Discussion

Our analyses show 1) a change in mosquito species richness for some restricted regions in Belgium, 2) evidence for increasing relative change in distribution area of mosquitoes that use artificial larval habitats, and 3) an increase in the relative distribution area of several vector species native to Belgium. Methodological aspects and the actual results of the use of museum specimens and contemporary data collections to assess changes in species richness and spatial distribution of mosquitoes are discussed below.

Evidence for Recent Changes in Mosquito Richness in Belgium. The comparison of the mosquito richness in two time periods indicates that for most regions in the study area the mosquito richness decreased, although a few regions showed an increase in species richness. In particular after World War II, the area of undisturbed forest sites in northern Belgium decreased dramatically and resulted in the loss of many insect species characteristic for these habitats (De Bruyn et al. 2003). In the surroundings of Brussels and Ghent, the strongest decline in mosquito species richness was found, probably due to increasing urbanization in the neighborhood of these cities leading to the destruction of suitable habitats (Tack et al. 1993; Honnay et al. 1999, 2002; Gaubomme et al. 2008). Whether the loss of biodiversity in some regions might imply a risk for creating empty niches that might be easily colonized by exotic, recently settled species (as *Ae. japonicus* (Theobald) cf. Versteirt et al. 2009 and *Ae. koreicus* (Edwards) cf. Versteirt et al. 2012b) need to be explored further.

Evidence for Increasing Relative Change in Distribution Area of Mosquitoes That Use Artificial Larval Habitats. Besides changes in species richness, the distribution area of many species also has changed. A

relative decline in distribution area is most pronounced for *Ochlerotatus* species and some *Culiseta* species (except *Cs. annulata*), suggesting a general decline of mainly forest and nonurban species in Belgium. Nevertheless, the relative distribution area of some other species has expanded, mainly species that use waste waters and used tires i.e., artificial larval habitats. In Belgium, the total area of urban and rural habitat has increased since 1950 (Dumortier et al. 2007), creating suitable conditions for species that can adapt to anthropogenic habitats and landscapes. Mosquito species that are able to use artificial larval habitats, such as used tires, flower pots, and waste waters, might profit from this land cover change. Artificial habitats differ in many respects from the conditions encountered in their original, natural environment. For example, competition and predation may be reduced in such environment, leading to favorable conditions for a rapid population expansion of previously less common species (Hobbs et al. 2009). This is of particular relevance if these species are a potential risk for human health (Simon et al. 2008). This was already reported in Belgium for *An. plumbeus* that showed a habitat shift from tree-holes in forests to manure pits of abandoned pig stables, contributing to recent severe pest outbreaks in certain regions in Belgium (Dekoninck et al. 2011b). Consequently, the presence of a numerous population of *An. plumbeus* at proximity of human activities increases also the risk for disease transmission and could be responsible for autochthonous malaria cases in the future (Takken et al. 2007). Moreover, local huge population densities in this novel artificial habitat might facilitate colonization success toward other novel human-created habitats (e.g., flower pots, water butts, sewerage), resulting in an even more expanded distribution of this species.

Another native *Anopheles* species, *An. claviger*, seems to have enlarged its distribution area in Belgium and recently its larvae were found in second hand tires (Versteirt et al. 2009). Two other species that frequently use artificial larval habitats, *Cx. pipiens* and *Cx. torrentium*, also have a high relative index of change in distribution area. These two species are morphologically nearly identical, and especially ancient specimens are very difficult to distinguish (Onyeka 1982). Therefore, correct species identification and conclusions on distribution and vector status are very difficult and often neglected (Lundström 1990, Hesson et al. 2010). The presences of prealar scales in female *Cx. torrentium* and their absence in *Cx. pipiens* are used to distinguish between the two species (Schaffner et al. 2001). However, scales fall off easily and with time all *Cx. torrentium* might appear as *Cx. pipiens* (Hesson et al. 2010). Probably, some voucher specimens of *Cx. torrentium* were identified as *Cx. pipiens*, resulting in a very high relative index of change in distribution area of *Cx. torrentium*. Maybe when used in the near future on samples from good stored voucher specimens, the reliable mitochondrial cytochrome *c* oxidase subunit I gene restriction enzyme assay (Hesson et al. 2010) can elucidate the true distribution and

relative proportions of the two species in Belgium before 1960.

Evidence for Increasing Relative Change in Distribution Area of Mosquitoes That Are Known as Vector for Arboviruses and Malaria. Some *Anopheles* species that have an increased relative distribution area, are known as potential vectors for *Plasmodium* sp. and arboviruses (Table 1; Schaffner et al. 2001), and it is therefore recommended to monitor the distribution of these *Anopheles* species. Within the *An. maculipennis* s.l. complex (also enlarged distribution area), only *An. messeae* and *An. maculipennis* s.s. were recently found (Versteirt et al. 2012a). A third species of this complex, *An. atroparvus*, the former primary vector of human malaria in Belgium, seems to have a decreased distribution area because there are no recent records of this species in Belgium (Versteirt et al. 2012 a). Similar observations were recorded from other European countries (Schaffner 2003, Roiz et al. 2007).

Two other species, *Ae. vexans* and *Aedes cinereus* s.l., have a positive relative index of change in distribution area. Both species are known to be vectors of different arboviruses (both as vector of Tahyna, the first as vector for West Nile and Lednice infection, the latter as Sindbis vector; see Lundström 1990, Schaffner et al. 2001). In contrast to the other species, the relative increase of both *Aedes* species is probably not linked to habitat shifts toward novel artificial habitats (Becker et al. 2010) but rather is due to other factors such as changes in water regime or quality and the creation of new natural landscape due to nature restoration projects in Belgium (Lock and Goethals 2012). Because they are vectors for arboviruses, their abundances should be monitored, especially in nature reserves that act as resting places for migratory birds (Reusken et al. 2011).

Differences in Sampling Strategy and Differences in Mapping Intensity When Comparing Museum Specimens and Contemporary Data Collections. The difficulty of making valid comparisons between historical and current data are one of the main barriers for documenting biodiversity change in general (Graham et al. 2004). Therefore, caution must be used when directly comparing species richness maps that originate from different time periods, implying a different sampling intensity or a different sampling method. The main problems are the variation in the details recorded (detailed information on locality and data on the labels) and methodological characteristics of historical occurrence data. The MMLP collection method used during the recent survey, collects mosquitoes differently than methods such as sweeping and manual collections (Silver 2008), methods that were most often used in the past (Dekoninck et al. 2011a). However, besides small differences (*An. atroparvus* [see above] and some introduced exotic species), the historical species list does not differ from that of the recent species list. Indeed, we may assume that species that were present and common during 1900–1960, were collected and that there is no evidence that the recent sampling technique, i.e., MMLP would collect

several species more frequently than hand sampling did in the past.

Another problem is the difference in spatial extent of the two inventories. Only relative changes in species richness and distribution are possible with accurate and cautious methods as used in this study. Indeed, the limited historic data of Culicidae in Belgium does not allow us to extrapolate over the whole study area as this might give an over- or underestimation of estimated historic species richness for several regions and therefore we only used well surveyed grid cells. Such paucity of detailed data on the past distribution of vectors can be a major limiting factor for global modeling of vector-borne diseases (Rogers and Randolph 2003, Tatem et al. 2006.). Notwithstanding, also limited data sets can give general information on changes in vector communities as demonstrated in the current study. The sampling effort for both periods differentiated among most of the grid cells. However, there was no correlation between the number of sampling events per grid cells and the number of species collected. Therefore, we can assume this differences do not bias the species richness estimated. Moreover, the difference in effort between the two periods was adjusted by the correction factor α .

Future Research. Documenting shifts in species richness is an integral part of understanding how species and communities responded to past environmental change and how they might respond to future environmental modifications (Heller and Zavaleta 2009). Factors such as environmental modifications, i.e., the loss of the appropriate habitat (Van Dyck et al. 2009) or the introduction of exotic species (Clark and Samways 1997) and even climate change (Travis 2003) and their interactions are all likely explanations for changes in mosquito richness. Also, the creation of novel artificial habitats is becoming more and more important and might clarify such changes. This might be of particular importance if these habitats become dominated by species that are also known as potential vector for *Plasmodium* sp. and arboviruses. The most important epidemiological parameters for risk assessments include vector diversity, spatial and temporal variation in vector abundance, and pathogen prevalence in the vector population (Braks et al. 2011). Here, we discussed and highlighted a change in the relative distribution area of several vector species, and we suggest a further monitoring of the distribution of these species in Belgium in the near future. We are convinced that the methods presented here, although they have some shortcomings, can be advantageous also for non-Belgian researchers interested in changes in mosquito distribution and how to investigate them. Similar studies comparing current mosquito diversity with former distribution data retrieved from voucher specimens from collections is therefore encouraged because they can generate important information concerning health risk assessment at both regional and national scale.

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