Bulletin of Entomological Research (2017) **107**, 155–164
doi:10.1017/S0007485316000766

Spread and global population structure of the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) and its larval parasitoids *Diadegma semiclausum* and *Diadegma fenestrale* (Hymenoptera: Ichneumonidae) based on mtDNA

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Abstract

The diamondback moth (DBM) (*Plutella xylostella*) is one of the main pests of brassicaceous crops worldwide and shows resistance against a wide range of synthetic insecticides incurring millions of dollars in control costs every year. The DBM is a prime example of the introduction of an exotic species as a consequence of globalization. In this study we analyzed the genetic population structure of the DBM and two of its parasitic wasps, *Diadegma semiclausum* and *Diadegma fenestrale*, based on mitochondrial DNA sequences. We analyzed DBM samples from 13 regions worldwide ($n = 278$), and samples of the two wasp species from six European and African countries ($n = 131$), in an attempt to reconstruct the geographic origin and phylogeography of the DBM and its two parasitic wasps. We found high variability in COI sequences in the diamondback moth. Haplotype analysis showed three distinct genetic clusters, one of which could represent a cryptic species. Mismatch analysis confirmed the hypothesized recent spread of diamondback moths in North America, Australia and New Zealand. The highest genetic variability was found in African DBM samples. Our data corroborate prior claims of Africa as the most probable origin of the species but cannot preclude Asia as an alternative. No genetic variability was found in the two *Diadegma* species. The lack of variability in both wasp species suggests a very recent spread of bottlenecked populations, possibly facilitated by their use as biocontrol agents. Our data thus also contain no signals of host-parasitoid co-evolution.

Keywords: Cabbage pest, parasitic wasp, COI, biocontrol, biological control, primers

(Accepted 11 July 2016; First published online 30 August 2016)

Introduction

The diamondback moth (DBM) *Plutella xylostella* (Linnaeus, 1767) (Plutellidae) is one of the main pests of brassicaceous crops and weeds worldwide (Talekar & Shelton, 1993). Recent estimates put the costs of DBM control at 1.4 billion US dollars per year, excluding crop loss (Furlong *et al.*, 2012). The DBM shows high levels of resistance against a wide range of

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pesticides, and had become resistant against almost all insecticides by 1980 (Sun *et al.*, 1986; Talekar & Shelton, 1993; Furlong *et al.*, 2012). Due to this resistance alternative control strategies, such as integrated pest management or conservation biological control, have become important. The aim of integrated pest management is to keep pesticide applications to a minimum and to actively increase the natural enemies of pests, e.g. their parasitoids (Ehler, 2006; Furlong *et al.*, 2012), while conservation biological control attempts to increase control by natural enemies entirely by habitat manipulation without pesticide application (Wäckers *et al.*, 2005; Balmer *et al.*, 2013; Balmer *et al.*, 2014; Juric *et al.*, 2015). In regions where *P. xylostella* was introduced accidentally, it has profited from a lack of natural enemies or a low parasitation rate by indigenous species, giving the pest a high probability to establish and become a serious problem (Talekar & Shelton, 1993).

The geographic origin of the DBM is still debated. It has been argued that it originated in Europe, which is largely based on the existence of a high number of Lepidopteran pest species in comparison with other regions. Over 300 Lepidopteran pest species have been recorded on the British Isles alone (Carter, 1987). Cultivated *Brassica* species are also thought to have originated in Europe, mostly in the Mediterranean area (Hardy, 1938; Vaughan, 1977). Kfir (1997), however, suggested that the origin of DBM is in South Africa, as there are many indigenous *Brassica* species (175 species) and because a substantial number of DBM parasitoids (21 species) occur there. Liu *et al.* (2000) on the other hand suggested that the moth originated in China, based on a rich native DBM parasitoid fauna and a wide range of genera of the family Brassicaceae including many endemic species (71 species).

The identification of the origin of DBM is complicated further by its repeated unintentional introduction in many regions of the world, most likely through the import of infested crops. Some of these faunal translocations into non-native areas are well documented. Around 1850 DBM was first introduced from Europe to Canada (Dosdall *et al.*, 2004). In the late 19th century DBM was brought from England to New Zealand, where parasitation by indigenous parasitoids was very weak. As a consequence DBM could proliferate and became a serious issue (Hardy, 1938; Sarfraz *et al.*, 2005). Today, DBM is considered to be the most widely distributed Lepidopteran pest and is found whereever suitable host plants are present (Shelton, 2004).

Around 60 parasitoid species are known to attack DBM (Delvare, 2004). In some regions where no natural enemies of DBM existed, or where parasitation rates by indigenous parasitoids were low, parasitoid species were introduced secondarily for biocontrol: *Diadegma semiclausum* (Hellen, 1949) and *Diadromus collaris* (Gravenhorst, 1829) were first imported from England to New Zealand as a biological control agent in the 1930s (Talekar & Shelton, 1993; Furlong *et al.*, 2012). After their establishment and spread in New Zealand both species were introduced from there to Australia and Tasmania (Hardy, 1938; Talekar & Shelton, 1993). It is further known that *D. semiclausum* was introduced from England to South Africa, but the date is unknown (Ullyett, 1947). In the early 1950s *D. semiclausum* and *D. collaris* were introduced from New Zealand to the highlands of Java (Indonesia), in 1977–1978 from Indonesia to Malaysia and in 1985 from Indonesia to Taiwan (Talekar & Shelton, 1993; Sarfraz *et al.*, 2005). In 1991, the Philippine-German Biological Plant Protection Project (PGBPPP) succeeded to introduce and establish *D. semiclausum* from Indonesia to the Philippines (Amend & Basedow, 1997). In 2001, *D. semiclausum* was translocated from Taiwan to Kenya, Tanzania and Uganda to study how a new exotic species can proliferate (Gichini *et al.*, 2008). Gichini *et al.* (2008) showed that a low number of *D. semiclausum* (125–350 females) was sufficient to establish a population. Two years after release, *D. semiclausum* was observed more than 30 km away from the release site. It was also observed that *D. semiclausum* almost entirely replaced the indigenous species D. *mollipla*, which also parasitizes DBM. We are aware of only one report of the targeted introduction of the congeneric DBM parasitoid *Diadegma fenestrale* (Holmgren, 1860) for biocontrol (Hardy, 1938, Azidah *et al.*, 2000), although there are records for its appearance in Europe and Asia (Furlong *et al.*, 2012). In field studies in Switzerland, where DBM were analyzed for parasitation by molecular methods, around a third of all parasitation events involved *D. fenestrale*, pointing to a significant role of this species as a biocontrol agent (Juric *et al.*, 2015). Since the two species are morphologically very similar (Azidah *et al.*, 2000; Wagener *et al.*, 2006) and previously published molecular markers for *D. semiclausum* (Traugott *et al.*, 2006) are actually not species-specific (Juric *et al.*, 2015), it is conceivable that *D. fenestrale* has inadvertently been introduced to some countries together with – or even instead of – *D. semiclausum*.

In the present study we analyzed mitochondrial COI sequences of DBM from field-caught individuals and available sequences from GenBank to study its worldwide phylogeography and to identify its center of origin. In a second step, we analyzed sequences from the two parasitoids, *D. semiclausum* and *D. fenestrale*, to test whether the parasitoids' population structures reflect that of their host species. In doing so, we wanted to assess patterns of co-evolution between the host and its two parasitoids. Analyzing DBM samples from all over the world, *D. semiclausum* from Europe and Kenya and *D. fenestrale* from Europe, we specifically addressed the following questions: (1) What is the geographical origin of DBM? (2) What is the population genetic structure in DBM and its parasitoids *D. semiclausum* and *D. fenestrale*? (3) Is there evidence for unintentional introductions of *D. fenestrale* alongside *D. semiclausum*? And, (4) are there signs of co-evolution between host and parasitoids?

Materials and methods

Sampling

We collected larvae and adults of DBM, *D. semiclausum* and *D. fenestrale* in Switzerland and obtained field samples from various colleagues worldwide (see Supplementary table S1) adding up to a total of 171 DBM, 70 *D. semiclausum* and 61 *D. fenestrale* fresh field samples. All field samples were of non-protected and non-endangered insect species collected on private agricultural lands with consent of the land owner and therefore did not require collection permits or ethical clearance. The field samples were complemented by all sequences of *P. xylostella* we could identify in NCBI GenBank that covered the same COI region and unambiguously originated from field-collected individuals (not laboratory-reared, to avoid overrepresentation of specific genotypes) that allowed an exact geographical localization, in total 107 sequences (fig. 1). For the two *Diadegma* species, no sequences fulfilling these criteria were found in GenBank. The samples collected in Switzerland were also used for a second study on the effects

https://doi.org/10.1017/S0007485316000766 Published online by Cambridge University Press

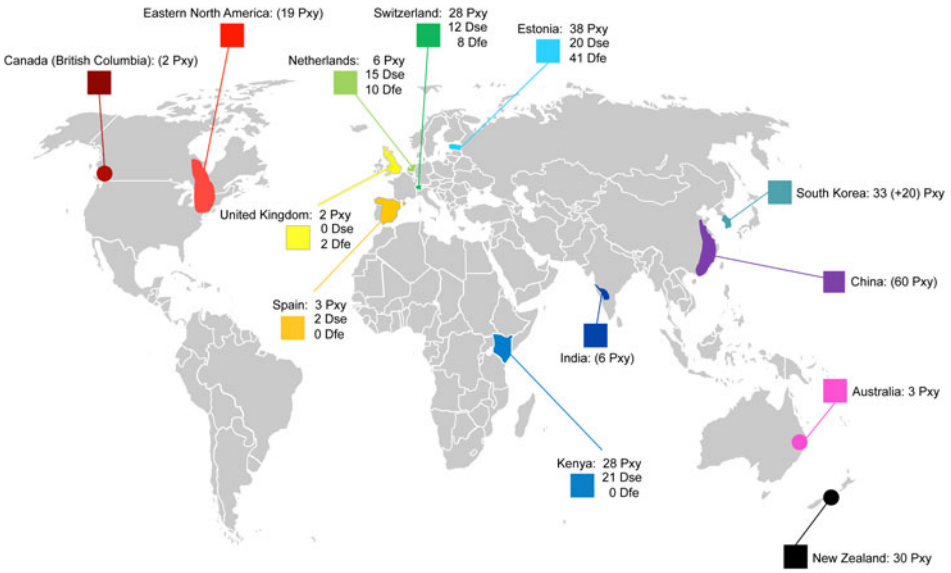

Fig. 1. Number of *P. xylostella* (Pxy, $n = 278$), *D. semiclausum* (Dse, $n = 70$) and *D. fenestrale* (Dfe, $n = 61$) individuals per region included in this study. Sequences retrieved from NCBI GenBank are in parentheses. See Table S1 for sample details.

of non-crop flowering plants on parasitization rates of *P. xylostella* by *D. semiclausum* and *D. fenestrale* (Juric *et al.*, in press). Numbers of specimens per locality of all species are listed in table S1.

DNA extraction, PCR amplification and sequencing

Individual DBM larvae or adults and *D. semiclausum* and *D. fenestrale* adults were crushed in 80 µl T1 buffer and 8 µl of proteinase K [20 mg ml^{-1}] of the XS-Tissue kit (Qiagen), using a bead beater (Qbiogene Bio101/Savant FastPrep® FP120) with two 2.6–3.3 mm SiLibeads Type ZY zircon beads (Sigmund Lindner GmbH, Warmensteinach, Germany) per Eppendorf tube at a speed of 5.5 m sec^{-1} for 15 s. The crushed samples were incubated at 56°C for 4 h and extracted following the manufacturer's protocol.

Partial COI of DBM was PCR-amplified with the universal primer pair LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al.*, 1994), producing a fragment of 658 bp in all species. To sequence *Diadegma* spp. in parasitized host larvae, we combined the same forward primer LCO1490 with a newly designed *Diadegma*-specific reverse primer DsspIJSr (5′-TTGATCAAGTAAATAAAGTTAATTGTTCA-3′), amplifying all *Diadegma* species but not the host and producing a fragment of 540 bp. For the construction of the *Diadegma*-specific reverse primer, we included a wide range of *Diadegma* species to ensure that the primer would amplify all *Diadegma* species. A range of pest and other parasitoid species co-occurring with *Diadegma* were included to exclude the possibility that primers cross-amplify other species – particularly the hosts. The included *Diadegma* species (with their GenBank accession number where appropriate) were: *Diadegma armillata* (AJ888014.1), *Diadegma blackburni* (AJ888021.1), *Diadegma chrysostictos* (AJ888023.1), *D. fenestrale* (field samples, accession numbers pending) *Diadegma incompletum* (HM020594.1), *Diadegma insulare* (AJ888015.1), *Diadegma leontiniae* (AJ888018.1), *Diadegma mollipla* (AJ888008.1), *Diadegma rapi* (AJ888019.1), and *D. semiclausum* (field samples, accession numbers pending). The additional wasp and pest species included were: *Cotesia glomerata* (DQ411831.1), *Cotesia plutellae* (AY934817.1), *Cotesia rubecula* (DQ411830.1), *Microplitis mediator* (DQ411833.1), *Microplitis varicolor* (GU141329.1), *Autographa gamma* (FN907987.1), *Mamestra brassicae* (GQ892871.1), *P. xylostella* (DQ076358.1), *Pieris brassicae* (HQ004913.1), *Pieris rapae* (AY954581.1). Polymerase chain reaction (PCR) tests were performed with parasitized pest samples and product bands from gel electrophoresis were sequenced to confirm the proper function of the primer.

Each PCR reaction (10 µl) contained 5 µl of Qiagen multiplex master mix (QMMM) (Qiagen, Hilden, Germany), 0.2 µl of each primer (LCO1490 and DsspIJSr (see above)) [10 µM], 3.8 µl of ultrapure water, and 0.8 µl of DNA template. The following PCR conditions were applied; Hot start for 5 min at 95°C followed by 35 cycles of denaturation at 94°C for 30s, annealing at 54°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min. PCR product was directly used for sequencing with Big Dye (Applied Biosystems) after purification with ExoSapIt (Amersham Biosciences). Sequencing was performed on an ABI 3130xl Sequencer (Applied Biosystems).

Analysis

Sequences were edited with ChromasPro version 1.5 (Technaysin Pty. Ltd., Queensland, Australia) and BioEdit version 7.0.9.0 (Hall, 1999). All sequences are available from GenBank under the accession numbers xxxxx-yyyyy (will be provided before publication). Alignments were performed with ClustalW in BioEdit resulting in an alignment length of 559 bp for *P. xylostella* and 398 bp for both *Diadegma* species.

Summary statistics including the number of segregating sites (S), haplotype diversity (Hd), and estimates of current (Θ_π) and historical (Θ_w) genetic diversity were computed using DnaSP version 5 (Librado and Rozas, 2009). Ratios of

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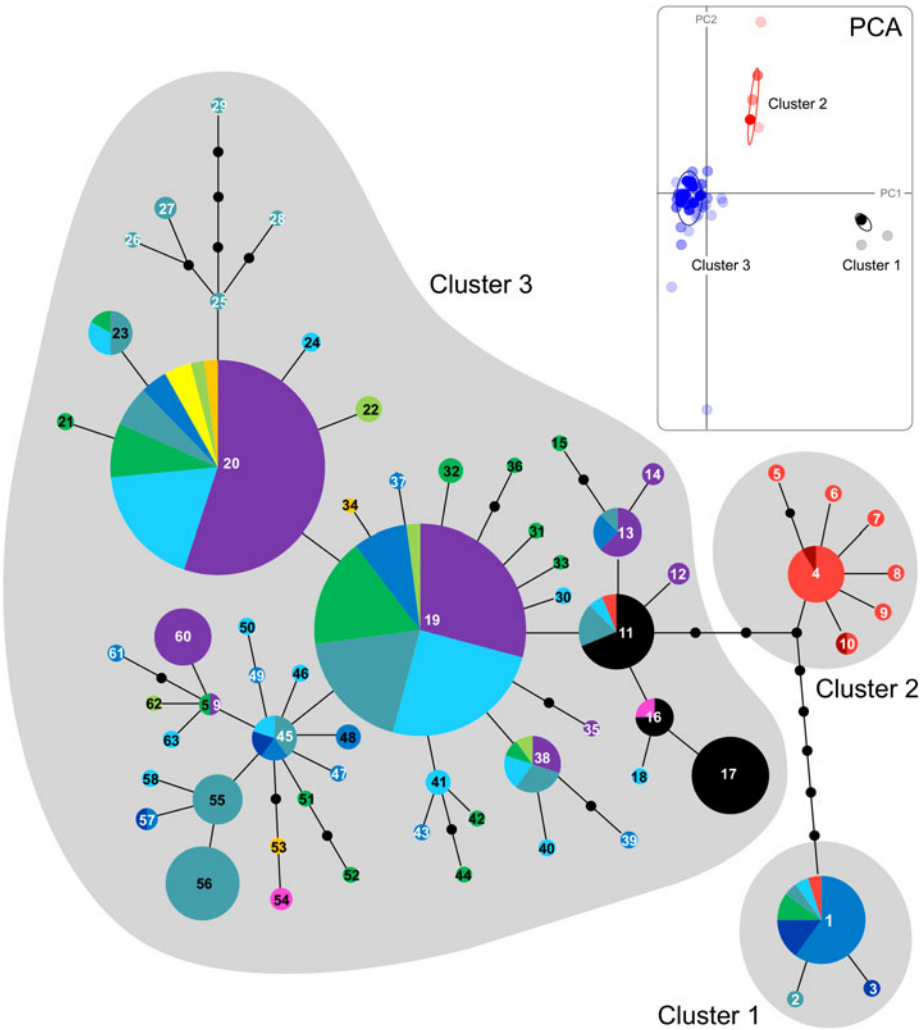

Fig. 2. Unrooted haplotype genealogy of 559 bp partial *Plutella xylostella* COI sequences. Each number corresponds to a haplotype. Sizes of the nodes are proportional to the number of samples per haplotype. Black dots symbolize hypothetical, missing haplotypes. Colors correspond to the regions in fig. 1. Grey shading highlights the three main haplotype clusters. See table 1 and table S1 for samples included. The inlay principal component analysis (PCA) displays the result of a principal components analysis including inertia ellipses per cluster as defined by the haplotype genealogy. Principal components (PC) 1 and 2 explain 13.8 and 5.3% of the variance in the data, respectively. Color saturation indicates sample density. The most distinct individual of cluster 3 (at the very bottom of the PCA plot) is haplotype 29.

$\Theta_\pi/\Theta_w > 1$ indicate recent population growth, while ratios of $\Theta_\pi/\Theta_w < 1$ indicate recent bottlenecks (Templeton, 1993; Pearse and Crandall, 2004). Phylogenetic analyses were performed with MEGA 5.05 (Tamura *et al*., 2011) using maximum likelihood. A haplotype network was constructed from a maximum likelihood tree following the strategy described in Salzburger *et al*. (2011). To corroborate the clustering identified in the haplotype network, principle components analysis was performed with R version 3.3 (R Development Core Team, 2009) packages ade4 and adegenet, using functions and setting dudi.pca(center = T, scale = T) and s.class(). We further constructed a maximum likelihood tree with *Plutella australiana* (GenBank accession 370833.1) as the closest outgroup for which a matching sequence was available to gain insights which clusters were derived from which. The tree was built in MEGA using the Tamura 3-parameter model with Gamma shape parameter $G = 0.679$ and allowing for invariant sites (T92 + G + I), identified by MEGA's model selection algorithm as the most appropriate nucleotide substitution model for our data. Mismatch analysis was performed with Arlequin version 3.5.1.2 and parameters estimated under the sudden expansion model (Excoffier & Lischer, 2010). Mismatch analysis was performed on four different datasets: (1) including all samples and haplotypes, (2) excluding haplotypes 1, 2 and 3 because these haplotypes are 9 or more mutations apart from cluster 3 (containing the majority of haplotypes) and could represent a cryptic species (fig. 2), (3) each of the three haplotype clusters identified from the haplotype network (fig. 2), i.e. cluster 1 containing haplotypes 1–3, cluster 2 containing haplotypes 4–10, and cluster 3 with haplotypes 11–63, and (4) samples from geographically close locations grouped together resulting in the following regions:

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'Asia' ($n = 111$) including China, India and South Korea; 'Europe' ($n = 74$) including Estonia, Netherlands, Spain, Switzerland and UK; 'Australasia' ($n = 31$) including Australia and New Zealand; 'Kenya' ($n = 16$) including only Kenya; and 'North America' ($n = 20$) including USA and Canada (see table 1 for haplotypes used per country). In the analysis of the samples from Australasia, haplotype 54 was excluded because it is far removed from the other haplotypes and could represent a cryptic species or an independent seeding event, biasing the results. Differentiation between clusters and geographic regions was assessed by F-statistics using DnaSP version 5 (Librado and Rozas, 2009). All analyses per region were performed excluding haplotypes from cluster 1.

Results

Variability of COI in DBM

We found a total of 63 distinct haplotypes among the 278 DBM samples, which differed by 1 to 17 mutations, resulting in a maximum genetic divergence of 3.0% between haplotypes. Haplotypes varied both within and between regions (fig. 2). Three main clusters were observed. Cluster 3 contained the majority of haplotypes. Four haplotypes were found in the samples from New Zealand and Australia, three of which (16, 17 and 54) were unique. The highest ratio of haplotypes per number of samples was found in Switzerland (28 samples with 16 distinct haplotypes), followed by Kenya (28 samples, 12 haplotypes), North America (21 samples, 9 haplotypes) and Estonia (38 samples, 16 haplotypes). The lowest ratios were found in Korea (53 samples, 17 haplotypes), China (60 samples, 9 haplotypes) and New Zealand (30 samples, 3 haplotypes). Cluster 2 differed from cluster 3 by four mutations and contained only North American samples (haplotypes 4–10, $n = 19$). Only two North American samples had haplotypes (haplotypes 1 and 11) not in cluster 2 (fig. 2). Cluster 1 contained haplotypes 1, 2 and 3, mostly from Kenya ($n = 12$) and India ($n = 4$) and differed from cluster 3 by nine mutations (fig. 2). The distinctness of the three identified clusters was corroborated by principal components analysis (fig. 2 inlay) and the maximum likelihood phylogenetic tree (fig. S1).

Genetic diversity as indicated by Hd was generally high in all investigated clades except in cluster 1, which exhibited very low diversity. The ratios of $\Theta\pi/\Theta w$ indicated recent bottlenecks in all investigated clades except in Australasia, where the ratio was 1 (table 2).

Variability of COI in D. semiclausum *and* D. fenestrale

We analyzed a total of 70 *D. semiclausum* and 61 *D. fenestrale* from 5 and 4 regions, respectively (fig. 1). There was no within-species variability in COI in both species. The two species differed from each other by 15 mutations in 398 bp of aligned sequence, giving a percent genetic distance (number of mutations per number of nucleotides analyzed) of 3.77%.

Mismatch analysis of DBM populations

We performed mismatch analyses to test for demographic expansions in DBM. Analysis of the entire dataset (278 samples, see table S1) resulted in a bimodal distribution, with a mean number of differences (mnd) of 4.011 ($\tau = 1.344$) (table 3). Removing haplotypes 1, 2 and 3 (cluster 1) resulted in only one clear peak (256 samples, mnd = 2.978 ; $\tau = 2.258$) (fig. 3). For cluster 1, we obtained an mnd of 0.182 ($\tau = 3.0$), for cluster 2 an mnd of 0.830 ($\tau = 0.912$), and for cluster 3 an mnd of 2.470 ($\tau = 2.742$). Mismatch distributions differed substantially among the regions (fig. 3), with the lowest mnd in Australasia (0.989, $\tau = 1.896$) and in North America (1.189, $\tau = 0.973$), suggesting a rather recent expansions, followed by Europe with an mnd of 2.004 ($\tau = 1.977$) and Asia with 2.363 ($\tau = 2.906$). The highest mnd was found in the Kenyan samples (2.650, $\tau = 2.812$).

Genetic differentiation between identified clusters and geographical regions in DBM

Strong differentiation was apparent between the three clusters with F_{ST} values from 0.73 (between clusters 2 and 3) to 0.93 (between clusters 1 and 2). No differentiation was apparent between Kenya and either Asia or Europe and only weak differentiation between Europe and Asia (table 4). The North American and Australasian clades were were strongly differentiated from all other clades (table 4).

Discussion

The aim of this study was to elucidate the phylogeography of DBM and two of its larval parasitoids. Our analyses revealed a high genetic variability in DBM but no variability within *D. semiclausum and D. fenestrale.*

The high variability in DBM suggests a relatively ancient origin of this species. Earlier studies reported a lack of genetic population structure both worldwide based on analyses of inter-simple sequence repeats (Roux *et al.*, 2007) and allozymes (Pichon *et al.*, 2006) and on regional scales based on COI sequence and microsatellite variation in China (Li *et al.*, 2006; Wei *et al.*, 2013) and on microsatellite variation in Australia (Endersby *et al.*, 2006). In contrast, there are several noticeable patterns in the DBM haplotype genealogy reported here. Haplotypes found in Europe and Asia mostly belonged to the largest and most diverse haplotype cluster 3. The Australian samples clustered with haplotypes from New Zealand as reported previously (Endersby *et al.*, 2006). At least two independent lineages appear to have seeded Australia and New Zealand: the lineage consisting of haplotypes 11, 16, and 17 and a lineage represented by haplotype 54, an exclusively Australian haplotype, which is five mutations away from the other group of Australian and New Zealand samples. North American samples primarily form their own cluster (cluster 2), suggesting a single colonization event for this haplogroup. This cluster may represent an endemic clade as these are not found anywhere else and are relatively distinct from all other haplotypes: four or more mutations from cluster 3 and seven mutations from cluster 1. Only two North American samples shared a haplotype (haplotypes 1 and 11) with the other two clusters, and may represent additional (independent) seeding events into North America. Finally, cluster 1 is distinct and might represent a cryptic species. Therefore, samples of this cluster were excluded from most mismatch analyses to avoid falsely inflated variability values. Kenya harbored a particularly large proportion of samples from cluster 1, but haplotype 1 was also found in all other investigated regions except Australasia. The phylogenetic tree recovered the same clustering as the other methods but did not provide further insights into the evolution of the species as it did not well resolve the sequence of branching of the three clusters. Future research should attempt to resolve

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Table 1. Number of samples per haplotype and country used for analysis. Numbers in parentheses indicate total sample size. Haplotypes 1, 2 and 3 were excluded from most analyses (see Materials and methods for details). ENA, Eastern North America.

Region	Country	Haplotype																														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Asia (119)	China (60)												2	5	2					14	27													
	India (6)	3		1																														
	South Korea (53)	1	1									3		1						9	3			3		2	1	2	1	1				
Europe (77)	Estonia (38)	1										1							1	12	9			2	1						1			
	Netherlands (6)																			1	1		2											
	Spain (3)																				1													
	Switzerland (28)	2														1				8	4	1		1								1	2	1
	UK (2)																				2													
Australasia (33)	Australia (3)																1																	
	New Zealand (30)											11					3	16																
North America (21)	ENA (19)	1			11	1	1	1	1	1	1	1																						
	Canada (2)				1						1																							
Kenya (28)	Kenya (28)	12												2						4	2													
All (278)	All (278)	20	1	1	12	1	1	1	1	1	2	16	2	8	2	1	4	16	1	48	49	1	2	6	1	2	1	2	1	1	1	1	2	1
Region	Country	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
Asia (119)	China (60)					1			3																					1	5			
	India (6)															1												1						
	South Korea (53)								3							2			3							4	17							
Europe (77)	Estonia (38)								2		1	2				1	1				1								1					1
	Netherlands (6)								1																								1	
	Spain (3)				1																			1										
	Switzerland (28)	1	2	1			1		1				1		1							1	1							1				
	UK (2)																																	
Australasia (33)	Australia (3)																								2									
	New Zealand (30)																																	
North America (21)	ENA (19)																																	
	Canada (2)																																	
Kenya (28)	Kenya (28)							1		1				1		1		1		1								1				1		
All (278)	All (278)	1	2	1	1	1	1	1	10	1	1	2	1	1	1	4	1	1	3	1	1	1	1	1			17	1	1	2	5	1	1	1

Table 2. Polymorphism data calculated with DnaSP per defined *Plutella xylostella* clade, including sample size (n), number of segregating sites (S), haplotype diversity (Hd) (± 1 standard deviation), and current (Θ_π) and historical (Θ_w) genetic diversity estimates. Clusters 1–3 as defined in fig. 2. Values per geographical region calculated excluding haplotypes 1–3.

Clade	*N*	*S*	Hd (±SD)	Θ_π	Θ_W	Θ_π/Θ_W
Full data set	278	50	0.922 ± 0.009	0.0072	0.0144	0.50
Full data set excl. haplotypes 1–3	256	47	0.914 ± 0.010	0.0053	0.0137	0.39
Cluster 1	22	2	0.177 ± 0.106	0.0003	0.0010	0.30
Cluster 2	19	7	0.608 ± 0.127	0.0015	0.0036	0.42
Cluster 3	237	42	0.901 ± 0.011	0.0044	0.0124	0.35
Kenya	16	12	0.933 ± 0.048	0.0047	0.0065	0.72
Asia	113	20	0.869 ± 0.019	0.0043	0.0068	0.63
Europe	74	26	0.867 ± 0.029	0.0036	0.0095	0.38
North America	20	11	0.647 ± 0.120	0.0021	0.0056	0.38
Australasia	33	7	0.655 ± 0.053	0.0029	0.0031	0.94

Table 3. Details of mismatch analysis per defined *Plutella xylostella* clade, including the expansion parameter Tau (τ), the mismatch observed mean number of differences (mnd) and Harpending's raggedness index (hri) (Harpending, 1994). Values in brackets refer to 95% consistency intervals. P_{HARP} is the probability to get a higher value of hri by chance. P_{SSD} (sum of squared deviations) is the probability to observe a less good fit between the model and the observed distribution by chance. See fig. 3 for graphs. Clusters 1–3 as defined in fig. 2. Values per geographical region calculated excluding haplotypes 1–3.

Clade	τ	mnd	hri	P_{HARP}	P_{SSD}
Full data set	1.344 (0.264–7.602)	4.011	0.780	0.01	0.55
Full data set excluding haplotypes 1–3	2.258 (1.035–6.918)	2.978	0.017	0.77	0.76
Cluster 1	3.000 (0.471–3.500)	0.182	0.450	0.70	0.34
Cluster 2	0.912 (0.355–1.857)	0.830	0.881	0.57	0.56
Cluster 3	2.742 (1.168–3.707)	2.470	0.028	0.56	0.33
Kenya	2.812 (1.434–4.336)	2.650	0.035	0.70	0.90
Asia	2.906 (1.049–5.523)	2.363	0.033	0.57	0.31
Europe	1.977 (1.180–2.648)	2.004	0.044	0.43	0.31
North America	0.973 (0.000–1.777)	1.189	0.073	0.76	0.64
Australasia	1.896 (0.000–3.127)	0.989	0.189	0.22	0.19

the taxonomic position of cluster 1. Interestingly, Kenya also exhibited a weaker signal of recent bottleneck than all other geographical regions except Australasia and harbored the greatest variability among cluster 3 haplotypes (excluding haplotypes 1, 2 and 3), followed by Asia and Europe. New Zealand, Australia and North America exhibited the lowest variability, which is in line with reports from the literature that DBM was imported unintentionally from Europe to North America about 150 years ago and to New Zealand 120 years ago (Hardy, 1938; Dosdall *et al.*, 2004; Sarfraz *et al.*, 2005).

An Asian origin of DBM has previously been suggested based on the large numbers of natural enemies and food plants occurring there (Liu *et al.*, 2000), but also a European origin has been proposed (Hardy, 1938; Carter, 1987). The mismatch analysis showed higher values of mnd for Asia than Europe, and the highest mnd in Kenya. This would argue against a European origin of the species, but suggests an African or Asian origin instead (on the basis of its high haplotype diversity). Additional samples from across Africa would be valuable to verify this hypothesis. Some authors have argued before that the origin of DBM is in South Africa (Kfir, 1997). Based on our results, an origin of DBM in North America, New Zealand and Australia is very unlikely. On the other hand, an African origin is consistent with the great genetic variability (despite the restricted geographical extent of sampling), the weak signal of bottlenecking among Kenyan samples, and the observation that African haplotypes are spread across the entire haplotype network and occupy central positions therein. However, our data do not permit to exclude Asia as an alternative origin since Asian samples also exhibited elevated variability and the two regions show no genetic differentiation as measured by F-statistics.

The complete lack of variability in the COI sequences of *D. semiclausum and D. fenestrale* suggests that the species were introduced to the various regions only recently starting from very small numbers of individuals. The genetic difference between the two species is 3.77% and hence similar to that reported by Wagener *et al.* (2006) who found a difference of 3.9%. Previous studies indicate England as the most likely origin of introduction of *D. semiclausum* to other regions (Hardy, 1938; Talekar & Shelton, 1993; Furlong *et al.*, 2012). The fact that the two *Diadegma* species are closely related might serve as evidence that the two wasp species have a direct common ancestor and a single region of origin.

We are aware of only one report that *D. fenestrale* was introduced as a biocontrol agent (Hardy, 1938; Azidah *et al.*, 2000). The morphological differences between the two wasp species are subtle and for a long time they were even considered as the same species (Hardy, 1938; Azidah *et al.*, 2000). It is thus possible that *D. fenestrale* was mistaken for *D. semiclausum* and distributed together with it. Although morphological keys for different *Diadegma* species are available (Azidah *et al.*, 2000), the samples that we received for the present study were in several cases incorrectly identified. Sequencing revealed 24 misidentifications in 131 *Diadegma* samples coming from five different sources. In most cases, *D. fenestrale* were misidentified

https://doi.org/10.1017/S0007485316000766 Published online by Cambridge University Press

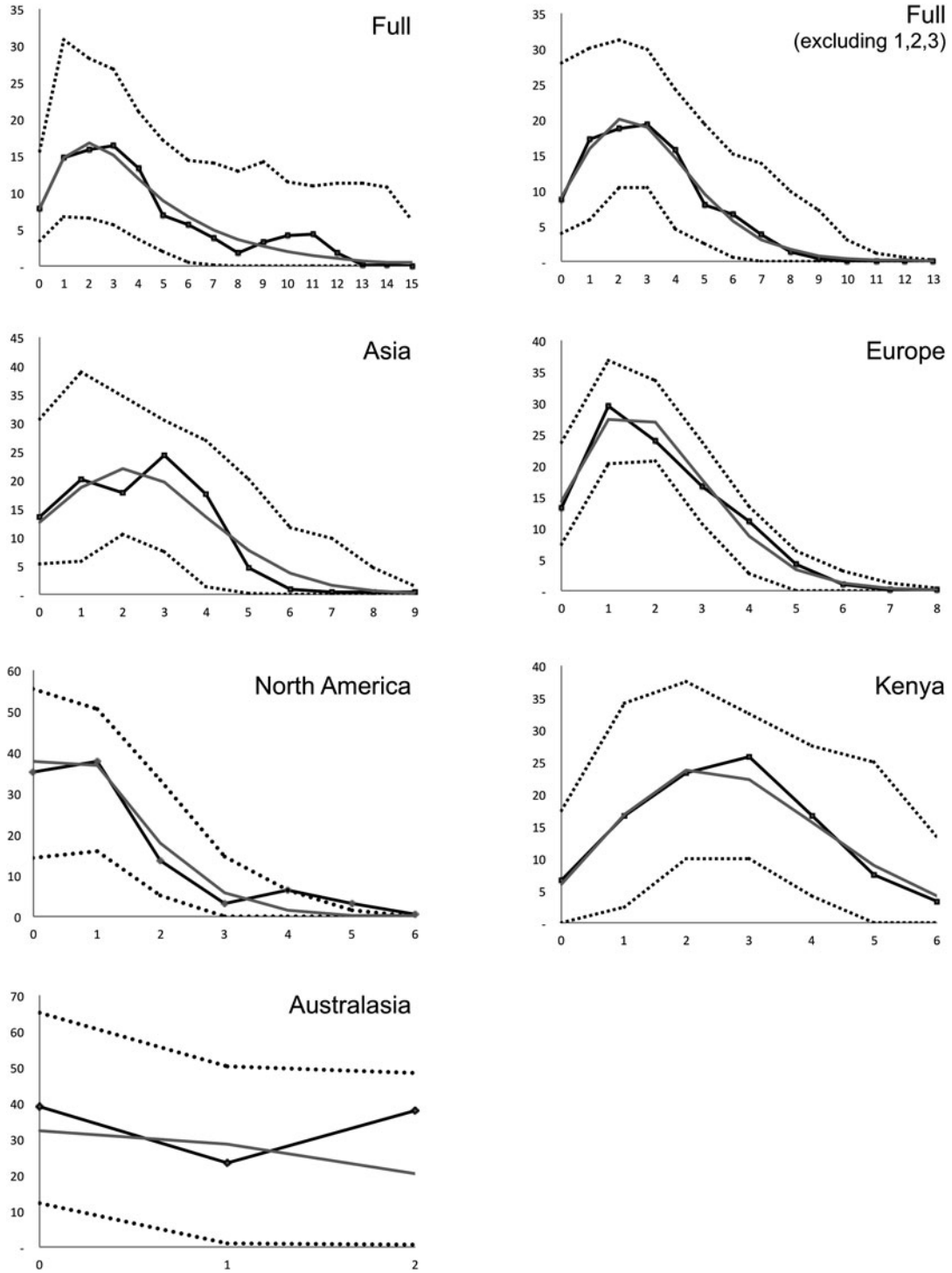

Fig. 3. Frequency distribution of mutational differences between individuals of *P. xylostella* obtained by mismatch analyses of the full data set ('full'), the full data set excluding haplotypes 1–3 ('full (excluding 1–3)'), and for each region (excluding haplotypes 1–3, see table 1 for countries included). The black curve delineates the observed values, the grey curve is the model fitted to the data, and the dotted lines represent the 5 and 95 percentile values based on 100 simulations. The percentage of mutational frequency is depicted on the *y*-axis, the number of mutational differences on the *x*-axis.

https://doi.org/10.1017/S0007485316000766 Published online by Cambridge University Press

Table 4. Genetic differentiation of *Plutella xylostella* populations from different geographical regions measured by pairwise F_{ST}. See table 1 for countries included per region.

	Asia	Europe	North America	Australasia
Kenya	0.002	0.005	0.696	0.314
Asia		0.038	0.706	0.356
Europe			0.738	0.418
North America				0.743

as *D. semiclausum*. We thus suggest caution in interpreting data found in the literature and in handling these species. At the same time, our results once more highlight the importance of species-specific molecular markers in biocontrol to prevent such mistakes (Juric *et al.*, in press). Field studies on parasitation rates of *P. xylostella* by *D. semiclausum* and *D. fenestrale* showed higher parasitation rates by *D. semiclausum* in Switzerland but also substantial frequencies of *D. fenestrale* (Juric *et al.*, in press). Stronger parasitation of cabbage pests by *D. semiclausum* may have been the reason why *D. semiclausum* was primarily considered as a biocontrol agent and why *D. fenestrale* may have been missed in several cases.

Our data do not allow us to draw conclusions on the co-evolution of *Diadegma* species and DBM because samples from too restricted a geographic range were available and because each wasp species showed no genetic intraspecific variability. Unfortunately, we could get no *Diadegma* samples from outside Europe, despite it being known that these species are distributed at least across Japan and India (Furlong *et al.*, 2012). However, the complete lack of variability in the *Diadegma* species, even across the restricted region investigated and the very high variability in DBM are not consistent with prolonged co-evolution between the taxa. To uncover undocumented variability in *D. semiclausum* and *D. fenestrale*, more extensive sampling is needed. Microsatellite or SNP analysis would further increase our power to resolve how populations dispersed for these two wasp species.

Conclusions

In conclusion, the geographical origin of DBM is still not resolved due to the scarcity of African samples. The high haplotype diversity in Kenyan samples corroborates Africa as most probable origin of the species but an Asian origin remains a possible alternative. Our data further suggest that DBM has been introduced to North America and New Zealand/Australia relatively recently, most likely from a genetically highly variable population from Europe. In the case of *D. semiclausum* and *D. fenestrale* the lack of genetic variability at the locus investigated does not permit any speculation on their geographic origins.

Supplementary material

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0007485316000766.

Acknowledgements

We thank Leon Westard and Jetske de Boer (Wageningen, Netherlands), Eve Veromann and Gabriella Kovacs (Tartu, Estonia), David George (North Yorkshire, England), Anne-Marie Cortesero (Rennes, France), Oscar Alomar (Barcelona, Spain), Annete Reineke (Geisenheim, Germany) and Lisa Knolhoff (Jena, Germany), Brigitte Nyambo (Nairobi, Kenya), Megan Herlihy (Amherst, USA), Dean O'Connell (Christchurch, New Zealand), Nancy Schellhorn (Brisbane, Australia) and Tim Haye (Delémont, Switzerland), Kim Ju Il and Minho Lee (Songwon, South Korea) for providing insect samples, all farmers and Rathgeb's Bioprodukte (Unterstammheim, Switzerland) for access to their cabbage fields in Switzerland, Kirsten Oude-Lensferink, Juliane Preukschas, Daniel Eglin, and Anna Moesch for help with collection of the Swiss field samples, Gerda Förderer, Moritz Fürst, Marco Colombo and Christoph Gerber for help in the laboratory and during analyses, two anonymous reviewers for helpful comments, and the Singenberg Foundation for their generous financial support. The authors declare that they have no competing interests.

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