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Factors determining variation in colour morph frequencies in invasive *Harmonia axyridis* populations

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Abstract The Harlequin ladybird *Harmonia axyridis* Pallas, native to eastern Asia, is an invasive, non-native species that has recently achieved an almost worldwide distribution. A conspicuous feature of this species is colour polymorphism of the elytra. In its native area, the populations consist of a recessive non-melanic morph, several dominant melanic morphs and small numbers of other (rare) morphs. The morph proportions in native populations have been intensively studied and vary with geographic area, climate and time. In contrast, colour polymorphism in invaded regions has been little studied. We examine and try to account for the morph frequencies observed across the different invaded regions. In America, monomorphic populations consist of the non-melanic morphs while European populations contain also melanic morphs. In particular geographic areas of Europe, the average percentage of the non-melanic morphs varied between 78 and 99%. It was highest in the lowlands of northern Italy and central and northern Europe and decreased in the Alps and western (Spain, UK) and eastern (southeast Russia) margins of the recently invaded area. In central Europe the frequency of the non-melanic morphs decreased over the course of the year

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but increased over the years from 2010 to 2018. The local differences might thus arise through gradual change of the morph composition of the founder invasive, non-native population. However, the variation in non-melanic morph frequency was not correlated with climatic characteristics that might affect coccinellid polymorphism. The observed rate of change in morph proportions in our data was too small to explain the diversification of what was supposedly a uniform invasive, non-native population at the point of introduction.

Introduction

The Harlequin ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is native to the east Palearctic and Oriental regions (Kovar 2007; Orlova-Bienkowskaja et al. 2015). Its recent spread into several continents where it is non-native has been well studied (Roy et al. 2016). In its native range, *H. axyridis* is an abundant and efficient predator of aphids (Kuznetsov 1975). Due to its qualities as a biological control agent, many attempts were made to introduce

H. axyridis in intensive agriculture areas outside of its native range. Several early introduction attempts of *H. axyridis* in Europe (Kuznetsov 1987; Coutanceau 2006) and North America (McClure 1987) were unsuccessful. In contrast, later unintended introductions resulted in the spread of the species in North America from the late 1980s (Chapin and Brou 1991; Tedders and Schaefer 1994; LaMana and Miller 1996) and subsequently in South America (Martins et al. 2009; Grez et al. 2010), Europe (Adriaens et al. 2003; Cuppen et al. 2004; Brown et al. 2008), Africa (Stals 2010; Nedvěd and Háva 2016), western Asia (Biranvand et al. 2019) and New Zealand (https://www.mpi.govt.nz/document-vault/12261). Thus, *H. axyridis* has now spread to all continents except Antarctica (Camacho-Cervantes et al. 2017). The invasion into Europe was evidently derived from biological control introductions mixing with an invasive population from eastern North America (Lombaert et al. 2010).

In recently colonized areas, *H. axyridis* is regarded as an efficient aphid natural enemy (Riddick 2017), but also an unwelcome competitor and predator of other members of the aphidophagous guild (Brown et al. 2015; Kenis et al. 2017; Masetti et al. 2018; Zaviezo et al. 2019). The abundance and distribution of *H. axyridis* has increased dramatically whilst the numbers of several native species have decreased,

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with *H. axyridis* implicated in the declines. This has helped increase interest in this species, with more than 1300 references since 1990 published on Web of Science concerning the life cycle, predation behaviour, distribution, and other elements of the ecology and genetics of *H. axyridis* (Roy et al. 2016).

Among the most intensively studied aspects of *H. axyridis´* biology is its conspicuous colour polymorphism, and the genetics, distribution, ecological and evolutionary factors influencing it. Interest in this issue has a long history. Patterns of variation of *H. axyridis*, its genetic determination and factors that determine morph distribution have been studied since the 1920s (Dobzhansky 1924; Komai 1956; Timofeeff-Ressovsky and Svirezhev 1967; Komai and Chino 1969) and continue today (Seo et al. 2007; Wang et al. 2009, 2011).

In its native area the species has over 200 described colour morphs, grouped into 15 classes (Tan and Li 1934; Hosino 1940; Tan 1946). The individuals are classified according to the colour pattern of their elytra. Four major morph groups, light-coloured non-melanic *succinea* and dark-coloured melanic *axyridis*, *spectabilis* and *conspicua* (Fig. 1) are among the most frequent in the species' native area, where there is extensive variation in morph proportions among local populations (Gautier et al. 2018). *Succinea* morphs have elytra with yellow to red ground colour and on each elytron up to nine black spots organised in four transversal rows. Pale coloration of the dorsal side leads these morphs to be referred to as the "non-melanic morphs". The ground colour of the three other morphs mentioned is black. These morphs are distinguished by the number of red spots, i.e. one (*conspicua* morph), two (*spectabilis* morph) or six (*axyridis* morph) on each elytron (see Gautier et al. 2018). The mostly black dorsal side leads these morphs to be classified and further referred to as "melanic morphs". Elytral colour pattern in *H. axyridis* is determined by a multiple-allelic series, with melanic morphs dominating non-melanic morphs in the order of dominance

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conspicua > *spectabilis* > *axyridis* > *succinea* (Tan and Li 1934; Tan 1946). In the native (and to an extent, introduced) ranges, there are a number of other morphs found in low proportions, the genetics of which have not been well studied (e.g. Hosino 1940; Komai 1956; for a review see Sloggett and Honek 2012). Morph identity is determined by genetic factors. Specifically the morph is determined by mosaic dominance, which itself is shaped by both the dominance relationships between colour morph alleles and the expression of a transcription factor (pannier); this determines the formation of melanic elements on the elytra (Gautier et al. 2018). A large inversion in the cis-regulatory regions of this transcription factor exists between colour morphsand is thought to underly the maintenance of so much variation within populations (Gautier et al. 2018). An additional factor of phenotypic variation within a morph is temperature during pre-imaginal development, which modifies the degree of melanisation. In the non-melanic morphs low temperature increases the size and number of black spots, while its effect on the size of red spots in melanic morphs is below the limit of resolution (Michie et al. 2010).

The morph frequencies in the native area of *H. axyridis* differ among three geographic regions: the insular region (Japan) is characterized by a mixture of non-melanic and melanic morphs (Komai et al. 1950; Komai 1956; Noriyuki and Osawa 2015), the east continental region (China, Korea and the Russian Far East) is characterized by a high frequency of the non-melanic morphs, while the central Siberian region is dominated by the *axyridis* morph (Dobzhansky 1924; Komai et al. 1950; Komai and Chino 1969; Kholin 1988, 1990; Vorontsov and Blehman 2001; Zakharov and Blekhman 2001; Korsun 2004; Blehman 2009). This coarse pattern of morph distribution slightly varies among localities and in time. Likely causes of this variation are differences in local climate (Purse et al. 2015) and a complex of biotic factors that manifest in variation of morph frequencies among host plants (Komai and Chino 1969). Temporal variation in morph frequency was observed several times (Komai et al. 1950; Komai and Chino 1969). Seasonal trends include an increase in the proportion of non-melanic morphs in the growing season, and vice versa during the winter (Osawa and Nishida 1992; Wang et al. 2009). While in the short term (a few years) the difference was not significant (Kholin 1990), after

many years there were significant changes in morph frequency (Komai et al. 1950; Komai and Chino 1969; Bogdanov and Gagalchij 1986).

Polymorphism in native populations is balanced by seasonal variation in mating preferences (Osawa and Nishida 1992; Ueno et al. 1998): in populations in Japan (Kyoto), females breeding in the spring preferred mating with non-melanic males and this preference led to a c. 4% increase in the proportion of the non-melanic morphs in the summer generation (Osawa and Nishida 1992). In summer, females showed no preference for males of a particular morph and consequently the frequency of melanic morph progeny in the autumn generation was relatively higher (Osawa and Nishida 1992). Seasonal changes in mating preferences also influenced morph frequencies in east continental Asia (Beijing, China) where the percentage of non-melanic morphs increased over the growing season to c. 85% because of high mating activity of the non-melanic morphs. This decreased during the winter to c. 50% (Wang et al. 2009).

In contrast to the well studied variation of *H. axyridis* colour polymorphism in its native range, the pattern of variation in morph frequency in recently colonized areas has been studied only to a limited extent (Adriaens et al. 2008; Burgio et al. 2008; Pons et al. 2015; Jovicic et al. 2016). Thus here colour polymorphism in *H. axyridis* populations of recently invaded areas is investigated. Increased melanisation may confer fitness advantages in particular climatic conditions and/or at some times of year, potentially leading to differences in the relative survival of *H. axyridis* morphs. For example, heavily melanised morphs may have a thermal advantage in cooler conditions, since they are more able to absorb thermal radiation (Brakefield and Willmer 1985). The differing phenology of host plants may have an affect on phenotypic variation in *H. axyridis* morphs. For example, a 2 weeks difference was observed in leafing between *Acer* and *Tilia* in the Czech Republic (Honek et al. 2019). This differing host plant phenology, causing variation in micro-habitats, could potentially lead to the differential success of *H. axyridis* morphs between two host plants. Overall, the study of variation in frequency of colour morphs in invasive, non-native populations is important since (phenotypic) plasticity is a factor that may confer an advantage to an invasive species (Briolat et al. 2019).

Data on *H. axyridis* morphs were collected from the invaded range to: (1) investigate macro-geographic variation and seasonal and annual trends in morph variation; (2) investigate micro-geographic and
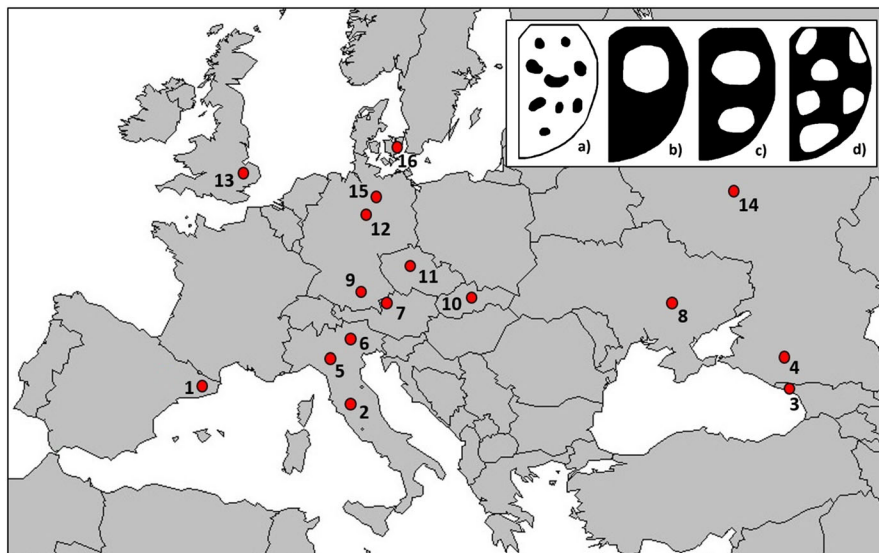
Fig. 1 The distribution of sampling localities of *H. axyridis* in Europe. The points indicate centres of particular areas where populations included in this study were collected, the areas are labelled serial numbers in the same order is as in Table 1. 1 Spain, 2 Italy 1, 3 Georgia, 4 Russia 1, 5 Italy 2, 6 Italy 3, 7 Austria, 8 Ukraine, 9 Germany 1, 10 Slovakia, 11 Czech Republic, 12 Germany 2, 13 UK, 14 Russia 2, 15 Germany 3, 16 Denmark. Insert: morphs of *H. axyridis*: a—*succinea*, b—*conspicua*, c—*spectabilis*, d—*axyridis*

**Table 1** The distribution of colour morphs on main hostplant types, from 2010–2018

Hostplant	Total	*succinea* N (%)	*conspicua* N (%)	*spectabilis* N (%)	*axyridis* N (%)	Σ melanic N (%)
Crop	82	74 (90.2)	0 (0.0)	8 (9.8)	0 (0.0)	8 (9.8)
Herb	1870	1669 (89.3)	29 (1.6)	168 (9.0)	3 (0.2)	200 (10.7)
Tree	23,619	21,026 (89.0)	537 (2.3)	2031 (8.6)	25 (0.1)	2594 (11.0)
Total	25,571	22,769 (89.5)	566 (1.3)	2207 (9.1)	28 (0.1)	2802 (10.5)

temporal variation in morph frequency in relation to host plant and temperature. For the first hypothesis data was collected from throughout the invaded range in Europe and America, whilst for the second hypothesis a more detailed dataset was available, collected from one part of the European range (Czech Republic).

Materials and methods

Localities

Samples of invasive populations of *H. axyridis* adults were collected in 19 areas of North and South America and Europe (Table S1 in Supplementary Material, Fig. 2), between 2007 and 2018. The data, from sampling of coccinellid communities including *H. axyridis*, were mostly collected from small geographic areas determined by the different research programmes of the participating authors. As a consequence, the intensively searched and investigated areas were surrounded by large unexplored areas. Although the geographic pattern of collection sites and ladybird data accumulated in this way is irregular, the large total area covered by this sampling is likely to
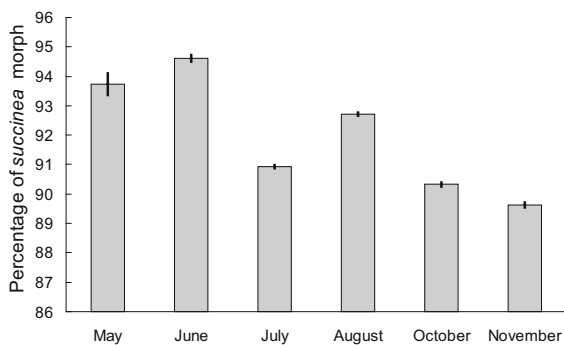
provide a clear insight into the general patterns of geographic variation of colour polymorphism of *H. axyridis*.

Sampling procedure

Harmonia axyridis populations were collected from trees, low growing herbaceous vegetation and crop stands. The sampling was performed by sweeping with an entomology net or beating the branches above sampling trays, during daylight hours, on dry days with low winds. More than 20 people participated in these sampling activities at 19 sites in 14 countries. It was impractical to compare differences in their sampling efficiency, but all participants were skilled entomologists with relevant fieldwork experience. This ensured that the composition of samples corresponded to the composition of natural populations and that colour morphs were determined correctly. Data on populations assembled at overwintering sites (buildings and shelters) were also included in this study where available (Czech Republic, Italy 2, Slovakia and Spain).

Data analysis

Macro-geographic variation in morph frequency was tested using the data of all sampling sites and samples collected at particular geographic areas. The "areas" are clusters of sampling sites situated close to each other (within maximum tens of km apart). The geographic areas are denoted by the political name of the country and a serial number where more than one cluster was sampled within a country (Table S1 in Supplementary Material). To assess variability in the morph frequency a logistic regression framework was used. The prevalence of the non-melanic *succinea* morph (among all morphs present) was used as the response variable in our models. To assess variability of the *succinea* proportion among geographic

Fig. 2 Seasonal trend in percentage of *succinea* morph on trees. The figure shows mean ± 95% CI calculated using angular transformed data

locations, a random country effect was used and fitted to the resulting model as a logistic generalized additive model (GAM) (Wood 2006). The geographic trends were further investigated in a GAM logistic model, allowing for a spatial trend with smooth additive latitude and longitude components.

Before investigating macro-geographic variation of morph frequency we needed to estimate the extent of micro-geographic and temporal variation. To investigate the factors of small-scale variation in morph frequency, micro-geographic variation in morph frequency and variation associated with host plants were checked. Micro-geographic and temporal variation in morph frequency was tested using the extensive data of the Czech Republic. In this analysis, samples of ≥ 5 individuals of *H. axyridis* were used. Micro-geographic variation was investigated using data collected in stands of *Tilia* spp. at seven sites located along a 5 km longitudinal transect, between 50.0813N 14.2610E and 50.0936N 14.3331E. These data were not biased by seasonal variation in morph frequency because coccinellids were sampled at regular bi-weekly intervals through the growing season (May to October) of 2011–2016. Variation in morph frequency among host plants was established using cumulative data from trees (*Acer*, *Betula*, *Cerassus*, *Prunus*, *Salix*, *Tilia*), herbs (low growing herbaceous vegetation, *Artemisia*, *Tripleurospermum*, *Urtica*) and crops (*Avena*, *Hordeum*, *Medicago*) sampled over the period of 2010–2018. Seasonal variation in the frequency of morphs was analysed using data collected on trees (*Acer*, *Betula*, *Tilia*) in 2011–2018. The data were sampled in each of the years in weekly (2017–2018) or bi-weekly (2011–2016) intervals from May to October. Annual variation in morph frequency was tested using cumulative data of all sampling sessions from May to October 2011–2018.

Differences in morph frequencies between sampling sites and host plants and between seasonal and annual trends in morph proportions were tested using ANOVA, with the frequency of the *succinea* morph as the response variable and sampling site, host plant, month or year as factors. As the test of normality of distribution of morph percentage failed, in all analyses, the Kruskal–Wallis one-way Analysis of Variance on ranks was used. The trends in change of percentage of the non-melanic morphs were tested using linear regression, with the percentage of the *succinea* morphs as the response variable and time (month, year) as the explanatory variable. The frequency of recessive *succinea* alleles was calculated using the Hardy–Weinberg law as the square root of frequency of the *succinea* morphs. The calculations were made using the SigmaStat 3.5 software package (Systat Software 2006). Note that at best this provides only an approximate estimate, reliant on the invasive populations being in Hardy–Weinberg equilibrium, which in reality they are likely not to be.

The relationship between the frequency of the non-melanic morphs and climate at the place of origin of European populations was established using data of meteorology stations situated as close as possible to the centres of the geographic areas listed (Table S1 in Supplementary Material), using areas where N > 10. Climate data were obtained from the University of Indiana (https://webapp1.dlib.indiana.edu). Data for 20-year averages of monthly temperatures were available for all geographic areas. The regression of the percentage of the non-melanic morphs (raw data and arcsin transformed data) was based on mean temperatures of particular months and mean temperatures of all combinations of two and three successive month periods. The calculations were made using SigmaStat 3.5 (Systat Software 2006).

Results

Micro-geographic variation

Micro-geographic variation in morph frequency between closely positioned sampling sites of *Tilia* (N = 363, H = 10.998, $df = 6$, $P = 0.088$) was not significant (Table S2 in Supplementary Material). There was no significant difference in the frequency of morphs on particular hostplants, trees, herbs and crops (N = 860, H = 0.676, $df = 2$, $P = 0.713$) (Table 1). Also, no difference in the frequency of morphs was found among stands of *Tilia*, *Acer* and *Betula* (N = 777, H = 1.404, $df = 2$, $P = 0.496$) or when host plants were ranked according to growth form, i.e. low growing crop and herb vegetation vs. trees (N = 860, H = 0.665, $df = 2$, $P = 0.415$) (data not shown).

Temporal variation

Seasonal variation, i.e. in the percentage of the non-melanic morphs, in particular months from May to October in the Czech Republic (Table 2), significantly differed (N = 761, H = 20.584, df = 5, $P < 0.001$) and decreased from 90.9 ± 1.45% and 92.9 ± 0.89% in May and June to 88.8 ± 0.75% and 88.9 ± 0.74% in September and October. On trees, the frequency of light colour morph decreased significantly (a = 2.831, b = − 0.0347, R^2 = .0205, $F_{1,760}$ = 15.961, $P < 0.001$) (Fig. 2) over time.

Annual variation in the percentage of the non-melanic morphs increased significantly (N = 784, H = 31.650, df = 8, $P < 0.001$) from 2011 to 2018 (a = − 58.675, b = 0.0304, R^2 = 0.0348, $F_{1,745}$ = 26.834, $P < 0.001$) (Table 3). Calculated from these data, the frequency of the recessive *succinea* allele (mean = 95.8 ± 0.36%) varied between 94.5% (in 2013) and 97.4% (2017) (Fig. 3). The mean absolute difference in frequency of the *succinea* allele between successive years was 0.6 ± 0.22% (c.i. 0.84%). The mean annual difference
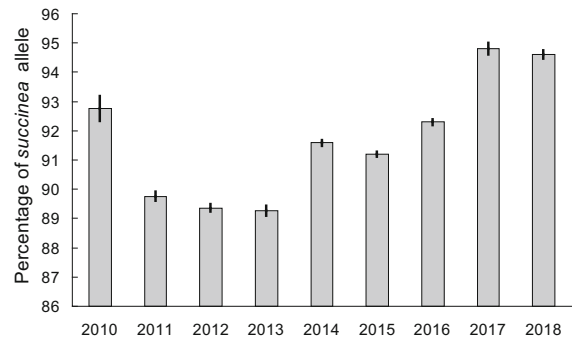
Fig. 3 Annual variation in frequency (%) of *succinea* allele in total annual samples of 2010–2018. Mean ± 5% CI calculated using angular transformed data

calculated from the difference between minimum and maximum frequency of the *succinea* allele was 0.72%.

Macro-geographic variation

At a macro-geographical scale, the largest difference in colour polymorphism distribution of invasive, non-native *H. axyridis* populations is between America,

Table 2 The distribution of colour morphs of *H. axyridis* through the season, from May to October

Month	Total	*succinea* N (%)	*conspicua* N (%)	*spectabilis* N (%)	*axyridis* N (%)	Σ melanic N (%)
May	774	695 (89.8)	15 (1.9)	62 (8.0)	2 (0.3)	79 (10.2)
June	2687	2450 (91.2)	58 (2.2)	175 (6.5)	3 (0.1)	236 (8.8)
July	7142	6347 (88.9)	152 (2.1)	637 (8.9)	6 (0.1)	795 (11.1)
August	6124	5475 (89.4)	138 (2.3)	507 (8.3)	4 (0.1)	649 (10.6)
September	5740	5050 (88.0)	138 (2.4)	543 (9.5)	10 (0.2)	691 (12.0)
October	2813	2498 (88.8)	57 (2.0)	255 (9.1)	3 (0.1)	315 (11.2)
Total	25,280	22,515 (89.3)	558 (2.2)	2179 (8.4)	28 (0.1)	2765 (10.7)

Table 3 Annual variation in distribution of colour morphs of *H. axyridis* in central Czech Republic, from 2010–2018

Year	Total N	*succinea* N (%)	*conspicua* N (%)	*spectabilis* N (%)	*axyridis* N (%)	Σ melanic N (%)
2010	897	796 (88.7)	15 (1.7)	86 (9.6)	0 (0.0)	101 (11.3)
2011	3930	3449 (87.8)	102 (2.6)	373 (9.5)	6 (0.2)	481 (12.2)
2012	2551	2241 (87.8)	75 (2.9)	233 (9.1)	2 (0.1)	310 (12.2)
2013	1744	1532 (87.8)	33 (1.9)	176 (10.1)	3 (0.2)	212 (12.2)
2014	3832	3391 (88.5)	99 (2.6)	339 (8.8)	3 (0.1)	441 (11.5)
2015	5008	4451 (88.9)	107 (2.1)	443 (8.8)	7 (0.1)	557 (11.1)
2016	4189	3767 (89.9)	89 (2.1)	327 (7.8)	5 (0.1)	422 (10.1)
2017	1301	1194 (91.8)	21 (1.6)	85 (6.5)	1 (0.1)	107 (8.2)
2018	2119	1948 (91.9)	25 (1.2)	145 (6.8)	1 (0.0)	171 (8.1)
Total	25,571	22,769 (89.2)	566 (2.1)	2207 (8.6)	28 (0.1)	2802 (10.8)

Table 4 Frequency of colour morphs in macro-geographic areas of America and Europe

	Total N	succinea N (%)	conspicua N (%)	spectabilis N (%)	axyridis N (%)
America					
Chile	780	780 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
USA	86	86 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Canada	1812	1812 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	2678	2678 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Europe					
Spain	1618	1256 (77.6)	125 (7.7)	237 (14.6)	0 (0.0)
Italy 1	4	2 (50.0)	1 (25.0)	1 (25.0)	0 (0.0)
Georgia	4	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Russia 1	544	497 (91.4)	0 (0.0)	47 (8.6)	0 (0.0)
Italy 2	1152	1139 (98.9)	4 (0.3)	9 (0.8)	0 (0.0)
Italy 3	327	256 (78.3)	13 (4.0)	58 (17.7)	0 (0.0)
Austria	51	48 (94.1)	1 (2.0)	2 (3.9)	0 (0.0)
Ukraine	45	39 (86.7)	0 (0.0)	6 (13.3)	0 (0.0)
Germany 1	362	347 (95.9)	4 (1.1)	11 (3.0)	0 (0.0)
Slovakia	19,197	17,451 (90.9)	365 (1.9)	1371 (7.1)	7 (0.0)
Czech Republic	22,105	19,585 (88.6)	518 (2.3)	1974 (8.9)	25 (0.1)
Germany 2	133	125 (94.0)	0 (0.0)	8 (6.0)	0 (0.0)
United Kingdom	3904	3101 (79.4)	238 (6.1)	565 (14.5)	0 (0.0)
Russia 2	3	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)
Germany 3	239	232 (97.1)	2 (0.8)	5 (2.1)	0 (0.0)
Denmark	100	91 (91.0)	0 (0.0)	8 (8.0)	1 (1.0)
Total	49,788	44,175 (88.7)	1271 (2.6)	4303 (8.6)	33 (0.1)

The areas are ranked according to mean geographic latitude, from south to north

which consists entirely of the non-melanic morphs, and Europe, which consists of a mixture of several colour morphs (Table 4). The morph composition of populations in America was the same, despite the fact that the species' distribution spans more than 30 degrees of latitude in the Northern (Nearctic region) and Southern (Neotropical region) hemispheres. Consequently, neither local or temporal trends were identified, nor any macro-geographic variation in morph distribution in American populations.

Throughout the area of Europe already invaded by invasive, non-native *H. axyridis* populations, the non-melanic morphs dominated in local populations. In particular, in well-sampled areas (Table 4), the frequency of the non-melanic morphs varied between 77.7% (Spain) and 98.7% (Italy 2). Melanic morphs were present in all areas, including Georgia, where they were collected after 2016 (data not shown). Of the melanic morphs, *spectabilis* was the most frequent, with proportions varying between 0.8% (Italy 2) and 17.7% (Italy 3). The *conspicua* morph was scarce: it

was absent from four areas (Russia 1, Ukraine, Germany 2, Denmark), and in other areas represented 0.3% (Italy 2) to 7.7% (Spain) of totals. The *axyridis* morph was found only in the Czech Republic, Slovakia and Denmark and in all cases it represented ≤ 1% of local populations. One individual of the morph *aulica* was found in Czech Republic populations.

In Europe, there were no monotonic latitudinal or longitudinal trends in distribution of the non-melanic morphs. The frequency of the non-melanic morphs increased in general from north to south (Fig. 4a), with a maximum at c. 44°N. The longitudinal trend was flat hill-shaped (Fig. 4b), with maxima of the non-melanic morphs between 10 and 30°E.

The distribution of morph frequencies in the invaded area of Europe is concentric. In the central parts of its recent (2018) distribution, the non-melanic morphs were most frequent, representing more than 90% of the population in Slovakia, the Czech Republic, Germany, Denmark and Sweden (2018 data of
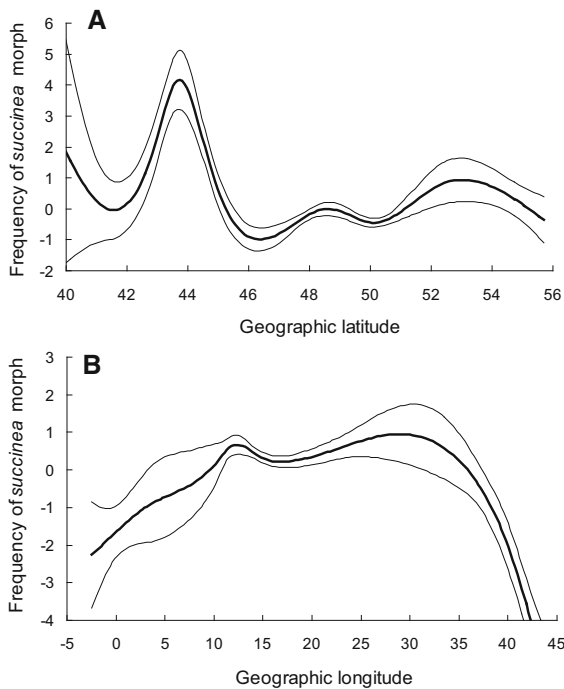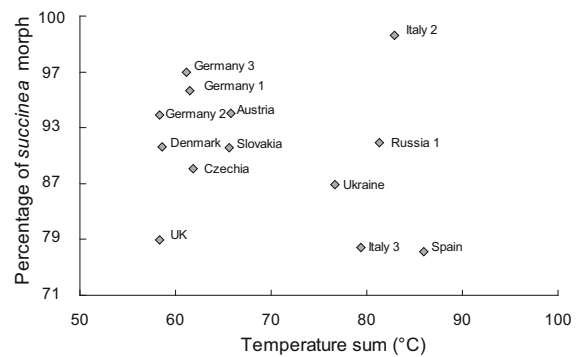
Fig. 4 Geographic trends in frequency of *succinea* morphs (logit(p) = log(p/1 − p) where p is proportion of *succinea* morphs in the populations modelled via GAM logistic regression)

Fig. 5 The regression of the percentage of *succinea* morphs (angular transformation) in the geographic areas of Europe on sum of average May–August temperatures in these areas ($R^2 = 0.0457$, a = 2.858, b = − 0.00492, $F_{1,12} = 0.527$, $P = 0.483$)

Göteborg and Stockholm: N = 22 individuals, 95.5% non-melanic morphs, not included in the analysis). This morph was also frequent in the Po Valley of northern Italy. In contrast, in the margins of the current distribution (Spain and the United Kingdom in the west, and southern Russia (Russia 1) in the east) the percentage of the non-melanic morphs was under 80% (Fig. 4). Populations with a low proportion of the non-melanic morphs also occurred in Alpine regions of northern Italy (Italy 3). Melanic morphs contributed to the amount of melanic individuals in similar ratios (Table 4), with the rarer *conspicua* morph representing about a quarter of melanic individuals (weighted average 3.0 ± 1.47%), and the more common *spectabilis* morph representing about three quarters of melanic individuals (weighted average 9.0 ± 2.14%) (Table 4).

The frequency of the non-melanic morphs was not correlated with the meteorological data from the investigated areas. There was no significant correlation between the frequency of the non-melanic morphs and the average temperature of particular months of the growing season (April–October) or with periods

combining the average temperature over two or three months. The absence of a significant relationship is shown in Fig. 5 using a non-significant regression of arcsin percentage of the non-melanic morphs on sums of average temperatures over the May–August period in particular areas. The regressions calculated using the data for particular months and their combinations have nearly identical patterns of distribution of the data (not shown). This was because seasonal variation of temperatures at different individual localities were correlated, i.e. monthly temperatures were consistently high or low, with similar patterns of seasonal variation at multiple locations.

Discussion

Native and invasive populations

We found low variation in *H. axyridis* morph frequency in recently colonized areas compared to native areas (Dobzhansky [1933]; Komai [1956]; Zakharov and Blekhman [2001]; Noriyuki and Osawa [2015]). However, whilst the populations in America were monomorphic, this was not the case in Europe. There, 11.4% of melanic morphs (Table S2 in Supplementary Material) was found, similar to that in east continental Asia, with a similar prevalence of the non-melanic morphs, but absence of a clear clinal trend in morph frequency (typical of populations from Japan (Komai [1956])). Macro-geographic variation in the percentage of melanic morphs is likely to be greater

than in native populations of eastern continental Asia. Similar variation was also observed at the micro-geographic scale: studies of populations in the Czech Republic revealed the absence of micro-geographic variation among sampling sites spaced by tens of kilometres (Komai and Hosino 1951). Whilst we found no variation in morph frequency on particular host plants, our work on this question was only from one region, so it may justify further study.

Origin of polymorphism in recently colonised areas

The history of introduction of American *H. axyridis* populations from 1916 involved several intentional attempts and unintentional introductions, but the species failed to establish until the late 1980s (Lombaert et al. 2010; Roy et al. 2016), whilst the morphs of the introduced population(s) were not recorded. The probability of introducing a genetically recessive pure *succinea* population is greater than that of introducing a mixed population if the source population originated from east continental Asia (a large area including eastern China, Korea, eastern Russia, north-eastern Kazakhstan, Mongolia and northern Vietnam). Despite a restrictive political situation, transport in the late 1980s was at least possible from some open countries, e.g. South Korea. In contrast, transport of inoculum populations from Japan seems less probable because of the prevalence of dominant melanic morphs in this area (e.g. Noriyuki and Osawa 2015). Furthermore, in the case of South America, the presence of only the non-melanic morphs is explained by the most probable source of populations being eastern North America (Lombaert et al. 2010).

Presence of melanic morphs in Europe points to co-founders, inocula of populations containing dominant melanic allelomorphs, likely escapees of commercial laboratory reared populations sold for biological control in glasshouses or, less likely, accidental introductions of native populations from Japan. By admixture with populations from eastern North America, this inoculum is likely to have contributed to the establishment of invasive, non-native European populations. The far-reaching agreement between molecular and morphological data confirms the outcrossing event as a probable source of European populations (Lombaert et al. 2010, 2011).

In determining factors and mechanisms of origin of different morph composition in populations of particular areas, we should distinguish two processes: (1) maintenance of colour polymorphism within a population and (2) processes which lead to differentiation of geographic populations (Gray and McKinnon 2006; White and Kemp 2016).

Maintenance of morph frequency in local populations

The mechanisms of the maintenance of colour polymorphism in *H. axyridis* are very likely similar in native (east Asia) and invaded (Europe) areas. Seasonal change in morph frequencies in populations of *H. axyridis* in central Europe was smaller (fractions of percent) and took place in the opposite direction. Decreasing frequency of the non-melanic morphs from late spring to early autumn appears to have been reset by greater mortality of melanic morphs during the winter. Overwintering experiments (Honek et al. 2018) observed greater mortality in melanic than non-melanic morphs during the winter (Zdenka Martinkova and Alois Honek, unpubl.). However, this observation was made in an artificial hibernation site (Honek et al. 2018) and should be confirmed in naturally assembled overwintering aggregations. Raak-van den Berg et al. (2012) did not observe an effect of colour morph on overwintering survival of *H. axyridis* in the Netherlands.

Differentiation in morph frequency among geographic populations

The origin of differences in morph frequency of *H. axyridis* populations inhabiting different geographic areas implies a mechanism overcoming the stabilising effect of recurrent seasonal variation in morph proportions (Wang et al. 2011). The area-specific differences in morph frequencies may arise by (1) long-term directed selection of particular morph composition, or (2) short-term processes, most probably random changes in small populations, e.g. founder effects or bottleneck effects.

Consider first the selection of particular morph proportions in local populations which may imply long term gradual changes in morph proportions. Indeed, long-term variation in *H. axyridis* morph proportions was found in native populations (Komai

and Chino 1969; Bogdanov and Gagalchij 1986). The extent of these changes, e.g. more than 25% decrease in the percentage of phenotypic non-melanic morphs during a 50-year period in central Japan (Komai and Chino 1969), is greater than the differences observed among invasive non-native populations in Europe. This may be because the data from native populations are available for a longer period than the data of invaded populations in Europe. However, only c. 7% increase in the frequency of the phenotypic non-melanic morphs was observed over a 55-year period in Vladivostok, Russia (Bogdanov and Gagalchij 1986), which is similar to that observed in Europe.

A likely factor important for selection is climatic difference between areas. Local climate, temperature and humidity have proved the most important correlates of morph frequencies in several species of coccinellid (Dobzhansky 1933; Kryltzov 1956; Honek 1996; Sloggett and Honek 2012; Kawakami et al. 2015). Different thermoregulation capacities of colour morphs have been advocated as a basis of the maintenance of geographic differences in morph frequency by climatic selection (Brakefield and Willmer 1985). In *H. axyridis*, the likely effect of climate is different in native and invaded populations. Climate is likely a driving factor of geographic variation in Japan (Komai 1956). However, in Europe the effect of this factor is unclear because of the limited variation in morph proportions observed and the absence of a correlation between climatic conditions and morph frequency.

We may consider a possibility that geographic differences originated by selection enforced through affiliation to local complexes of Müllerian mimicry. As with some other coccinellid species (Rothschild 1961; Frazer and Rothschild 1962; Pasteels et al. 1973), *H. axyridis* hemolymph contains distasteful and poisonous alkaloids advertised by bright "warning" coloration (aposematism) (Bezzerides et al. 2007; Pruchova et al. 2014; Vesely et al. 2017). The species may belong to a "Müllerian mimicry" complex of species, with similar warning coloration and unpalatability to predators. Here polymorphism presents a problem if the species as a whole should be protected by deceptive mimicry (Briolat et al. 2019). As in *Adalia bipunctata* (L.) and *Adalia decempunctata* (L.), polymorphic species composed of non-melanic and melanic morphs may belong to different "circles" of Müllerian mimicry (Brakefield 1985). Since warning

coloration does not confer full protection against predation (Heikertinger 1932; Krištín 1988, 1991), predators may exert different pressures on particular mimicry complexes. The morph composition of a species may thus be influenced by variation in local pressures against particular circles of Müllerian mimics. This scenario could be further investigated.

Due to the temporal stability of *H. axyridis* morph frequency in local populations, it was assumed that the populations are free of, or resistant to, selective pressures and the differences may have originated by founder effects, i.e. a random combination of morph frequency in the initial populations. Spreading populations may have occasionally been reduced to small migrant groups with randomly changed morph frequencies which were subsequently maintained in local populations. Alternatively, local populations may have originated from several founder populations whose morph compositions differed from each other. The eastern North America population, which is likely one of the genetic sources of European populations (Lombaert et al. 2010), was certainly monomorphic *succinea*. Neither origin or genetic composition explaining colour morphs of these inocula populations are known. The explanation of macro-geographic variation due to possible different origins of source populations thus remains speculative.

Assembling data on world morph variation of invasive, non-native *H. axyridis* populations confirmed a clear difference between the populations of America and Europe. The results demonstrate insular distribution of morph frequencies in local populations of Europe, and pose an unsolved problem of the origin of these differences. Further research is necessary to elucidate (1) origin of this variation, (2) ecological significance and (3) consequences of this variation for applied problems of nature conservation and agriculture.

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