Physiological Entomology

Physiological Entomology (2019), DOI: 10.1111/phen.12286

The taste of origin in a lady beetle: do males discriminate between females based on cuticular hydrocarbons?

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Abstract. The Asian lady beetle *Harmonia axyridis* originates from Asia and has established invasive populations worldwide. Recent population genetic studies trace their invasion routes and demonstrate that bottlenecks in population size have reduced their genetic diversity. Consequently, phenotypical differences are highlighted between native and invasive populations. Among phenotypical traits, cuticular hydrocarbons (CHCs) might reflect the geographical origin of a lady beetle, especially because of their genetic basis. The present study investigates whether (i) the CHC profiles qualitatively and quantitatively differ between females of *H. axyridis* from native and invasive populations and (ii) males discriminate females from native and invasive populations using CHC profiles. CHCs are solvent-extracted before being quantified and identified by gas chromatography and mass spectrometry. In total, 17 CHCs are detected from female elytra, including six alkanes, three polyunsaturated and eight monounsaturated alkenes. The total quantity of CHCs differs among the populations, with lady beetles from Tai'an (China) displaying higher CHC concentrations than lady beetles from Gembloux (Belgium) and from Beijing (China) populations. Multivariate analyses detect differences in CHC qualitative profiles, with females from Tai'an being different from the two other populations. Finally, behavioural assays show that females originating from the native Tai'an population are less preferred by males, whereas females from the invasive population are mounted more often. The behavioural assays suggest that CHCs are not involved in discrimination of mating partners based on their origin.

Key words. *Harmonia axyridis*, inbreeding avoidance, mate selection, multicoloured Asian lady beetle, preferential mating.

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Introduction

The Asian lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is native to Asia and has been repeatedly introduced as a biological control agent into North America and then Europe. It has rapidly become an invasive pest on a worldwide scale. Lombaert *et al.* (2011,2014) showed that invasions of *H. axyridis* started in eastern North America, from where the lady beetle invaded Europe, South America and Africa. This invasion process has led the invasive populations to endure a genetic bottleneck of moderate severity that, rather than posing a barrier to invasion, has potentially enabled the evolution of invaders to a higher fitness level, including loss of inbreeding depression, lower generation time, higher male reproductive success and female reproductive investment (Facon *et al.*, 2011; Laugier *et al.*, 2013; Tayeh *et al.*, 2015). Included among the other traits potentially promoting the invasion success is the ability of *H. axyridis* to carry spores of obligate parasitic microsporidia that are lethal pathogens for native lady beetle species (Vilcinskas *et al.*, 2013; Verheggen *et al.*, 2017).

Cuticular hydrocarbons (CHCs) comprise linear *n*-alkanes, linear alkenes and ramified saturated hydrocarbons that cover the external layer (i.e. epicuticle) of the insect exoskeleton and serve as a waterproofing agent protecting against desiccation (Lockey 1988). However, evidence is accumulating regarding their major roles in intra- and interspecific chemical communication, including conspecific, nest and mate recognition (Alabi *et al.*, 2011; Durieux *et al.*, 2012; Snellings *et al.*, 2018). Because they are typically non- or semi-volatile long-chain hydrocarbons, when they serve communicative functions, they act as contact or short-range signals (Howard, 1993).

Lady beetles are no exception. An extensive literature documents the importance of CHC for guiding *Harmonia axyridis* individuals to overwintering sites, as well as their aggregation behaviour (Durieux *et al.*, 2012,2013,2015,2014). Lady beetles also rely on chemical cues to locate conspecifics and select mates, with long- and short-distance olfactory cues, respectively, being involved (Fassotte *et al.*, 2016). A volatile sex pheromone guides lady beetle males to calling females at a distance (Verheggen *et al.*, 2007; Fassotte *et al.*, 2014), whereas CHCs are involved in mate identification and selection (Keller & Passera, 1993; Singer, 1998; Ingleby, 2015; Legrand *et al.*, 2019). The qualitative and quantitative composition of the CHC blend covering a beetle cuticle is repeatedly reported to be affected by physiological (e.g. ageing, mating status) and climatic (e.g. temperature) factors (Menzel *et al.*, 2017). However, CHCs also have a genetic basis (Chung & Carroll, 2015) because they are synthesized in specialized secretory cells (oenocytes) situated in the abdominal epidermis (Millar, 2010). As a result of a combination of *in vivo* and *in vitro* studies using both radioisotope and stable isotope techniques, the biosynthetic pathways for the major types of hydrocarbons have been characterized (Howard & Blomquist, 2005), even if the enzymology and the molecular biology of hydrocarbon production remain to be clarified.

Based on the elements listed above (i.e. the invasion history of *Harmonia axyridis* and the importance of cuticular hydrocarbons for this species), the present study investigates whether: (i) CHC profiles qualitatively and quantitatively differ between females of *H. axyridis* from native and invasive populations and (ii) males discriminate females from native and invasive populations using CHC profiles, giving preference to females originating from the same population.

Materials and methods

Biological material

Three populations of Asian lady beetles were included in the present study, comprising individuals collected in Gembloux (Belgium, Europe), Beijing and Tai'an (China, Asia). All individuals were collected in 2014 and kept under laboratory conditions before use for chemical extraction or behavioural assays. All individuals were grouped in an aerated plastic box ($36 \times 15 \times 8$ cm) according to their population and fed with sugar lumps (i.e. water-impregnated sponge) and bee-collected multifloral pollen. Pea aphids (*Acyrthosiphon pisum* Harris) were added *ad libitum* to stimulate mating and oviposition. Corrugated filter paper was used as egg laying material and was changed after egg deposition. The eggs were placed in separate plastic boxes to avoid cannibalism, and the larvae were fed *ad libitum* with pea aphids every day until pupation. Three days after emergence, lady beetles acquire their final pigmentation, and the gender is determined using morphological characteristics (McCornack *et al.*, 2007). Males and females were then kept in separate containers until bioassays. All insects were reared in controlled environment chambers, under a 16 : 8 h light/dark photocycle at 24 ± 1 °C and $45 \pm 15\%$ relative humidity. Because the profiles of cuticular compounds are sometimes age-specific (Everaerts *et al.*, 2010), only lady beetles of the same age were tested. The experiments were conducted on sexually mature adults that hatched from eggs approximately 30 days before the assays.

Cuticular profiles

The process of cuticular extraction was applied to non-melanic females from the Gembloux ($n = 20$), Beijing ($n = 20$) and Tai'an ($n = 18$) populations. Because CHCs could be influenced by insect diet (Chung & Carroll, 2015), all lady beetles were fed with pea aphids *ad libitum* during 4 days. On the fifth day, females were killed at −80 °C during 5 min. Then, the posterior half part of female elytra was cut off and directly immersed in 200 µL of *n*-hexane for 7 min under constant agitation (Durieux *et al.*, 2012). Samples were kept at −8 °C until gas chromatography analysis.

For quantification purposes, 1 µL of each sample was injected on a gas chromatograph (Trace GC Ultra; Thermo Fisher Scientific, Waltham, Massachusetts) with an OPTIMA 5MS ACCENT column (Macherey-Nagel, Germany) ($30\,m \times 0.25\,mm \times 0.25\,\mu m$) and a flame ionization detector set at 330 °C. The injector (AI 1310; Thermo Fisher Scientific) was set at 300 °C and in splitless mode. The carrier gas was helium (1.50 mL/min). The programmed temperature was 40 °C for 5 min, followed by a gradual increase of 10 °C/min to 330 °C,

which was held for 10 min. The limit of detection (LOD) was assumed to be reached when the peak height of the component was less than or equal to three times the ratio of signal height to noise height. The limit of quantification (LOQ) was assumed to be two times higher than the LOD. Each saturated compound was quantified in accordance with a calibration curve based on the solutions of their analytical standards (Alkanes Mix 10; Ehrenstorpher, Germany), which were injected at increasing concentrations (1, 5 10 and 25 μg/mL). Unsaturated compounds were quantified based on the calibration of the corresponding alkane. Because cuticular hydrocarbons were present at low concentrations, the samples from females belonging to the same population were pooled for identification purposes. Then, each pooled extract ($n = 4$) was concentrated (at ±200 μg/mL) under a gentle stream of nitrogen.

CHC identification was performed on a gas chromatograph equipped with a HP-5 column (Agilent Technologies Inc., Santa Clara, California) (30 m × 0.25 mm × 0.25 μm) coupled with a mass spectrometer. The injector was set at 300 °C and injections were made in splitless mode using 1 μL of each sample. The carrier gas was helium (1.5 mL min^{-1}). The initial temperature was held at 40 °C for 2 min, followed by a gradual increase of 10 °C min^{-1}, with a final hold of 10 min at 320 °C. The mass spectra were recorded in the electron impact mode at 70 eV (source temperature of 230 °C, scanned mass range from 30 to 600 amu). Saturated compounds were identified by their characteristic molecular ion and the injection of pure *n*-alkane standards (from nC8 to nC40). To localize the double bound monounsaturated alkenes, the molecules of interest were subjected to epoxydation (Mallet *et al.*, 1985). Epoxy derivatives of monounsaturated CHC show fragmentations in the α position of the epoxy-moiety leading to typical diagnostic ions for which the *m*/*z* clearly indicates the double bound position. Epoxydation was performed on 400 μL of extract from which the *n*-hexane was evaporated under a gentle stream of nitrogen. Then, 40 μL of chloroform was added, followed by 40 μL of chloroformic solution of *m*-chloroperbenzoic acid (25 mg mL^{-1}). The resulting blend was gently agitated for 2-h. Next, 400 μL of an aqueous solution containing sodium bisulfite (50 mg mL^{-1}) and sodium bicarbonate (50 mg mL^{-1}) was added, followed by 200 μL of chloroform. With a syringe, we extracted the chloroformic phase that was evaporated under a gentle stream of nitrogen. Finally, the hydrocarbons were solubilized in 25 μL of *n*-hexane, and the resulting sample was stored at −18 °C until gas chromatography-mass spectrometry analysis.

Total concentrations of CHCs (i.e. absolute abundances, ng per individual) were compared among the three populations using a Kruskall–Wallis test (i.e. nonparametric equivalent for one-way analysis of variance). When the result was significant ($P < 0.05$), we performed multiple pairwise comparisons. Differences in CHC profiles (i.e. relative abundances, percentage of total CHCs) among populations were then assessed using permutational multivariate analysis of variance (Bray–Curtis dissimilarity index, 999 permutations, 'adonis' command) and multiple pairwise comparisons with Bonferroni's adjustment after testing for multivariate homogeneity ('betadisper' command) (R-package vegan) (Oksanen *et al.*, 2016). Differences were visually assessed on a non-metric multidimensional scaling ordination using a Bray–Curtis similarity matrix, two or three dimensions and 50 runs. A stress value is given to calculate how well the particular configuration produces the observed distance matrix (conventional cut-off of < 0.2). Indicator compound analyses on absolute abundances were also performed to identify CHCs that were indicative of particular population ('indval' command) (R-package labdsv) (Roberts, 2016). All univariate and multivariate statistics were conducted in R, version 3.4.0 (R Core Team, 2017) using the seven CHCs that were above the LOQ.

Mating bioassays

All individuals were fed exclusively with pea aphids during 4 days to stimulate sexual behaviour (Fassotte *et al.*, 2014). On the fifth day, three females (one from each population) were placed together into a Petri dish (diameter 5.5 cm, height 1.5 cm). The females were identified by means of coloured dots painted onto the upper left side of the elytra. The colour code was made 1 day before the stimulation by aphids and was randomized across populations to prevent side effects of marking. A male from one of the three populations was then introduced in the Petri dish for 10 min and we recorded the identity of the first female with which the male attempted to copulate. The interaction was considered to be a mating attempt when the male was bending the tip of the abdomen downwards to penetrate the female (Obata, 1987).

We conducted a general linear model (GLM) using Poisson probability distribution for modelling count data including both male origin and mating choice (i.e. female population) as crossed factors to assess differences in the number of males mating with females from their own versus foreign populations [GLM model: glm (copulation number ~ male population * mating choice, family = 'Poisson'], using the 'glm' function from the R-package 'stats'; R Core Team, 2017). After checking for overdispersion in variance of the data ($P > 0.05$), a type II analysis of variance (ANOVA) was performed to determine whether males were more likely to mate with local versus foreign females ('ANOVA' function, R-package 'car') (Fox & Weisberg, 2011). To assess the incidence of the amount of CHCs (i.e. individual compounds tested separately) and also the total CHC quantification on the number of copulations, we tested for intercorrelations using Pearson's *r* correlation coefficients ('rcorr' function, R-package 'Hmisc') (Harrell, 2015).

We also counted the number of trials (i.e. interactions before copulation) until a male accepted to mate with female to assign a 'trial latency score' for each male. The impact of both male population and mating choice on trial latency was assessed using a GLM with Poisson probability distribution for modelling count data, including male origin and mating choice as crossed factors in the model [GLM model: glm (number of trials ~ male population * mating choice, family = 'Poisson')]. After checking for overdispersion ($P > 0.05$), a one-way ANOVA followed by a pairwise post-hoc Tukey's honestly significant difference test were performed to determine the effect of male population and mating choice on trial latency.

Table 1. Composition of cuticular hydrocarbons (CHCs) on the posterior half of a female elytra pair (ng/individual) according to *H. axyridis* population (mean ± SD).

					Quantity (ng/ladybeetle)		
Name	CHC[a]	RT[b]	RI[c]	M + [d]	Gembloux	Beijing	Tai'an
n-Tricosane	C23	21.173	2300	324	495 ± 152	286 ± 78	807 ± 276
9-Tetracosene	9C24:1	21.717	2375	336	< LOQ	< LOQ	< LOQ
n-Tetracosene	C24	21.947	2400	338	< LOQ	< LOQ	< LOQ
9-Pentacosene	9C25:1	22.471	2475	350	1647 ± 598	862 ± 376	3518 ± 978
n-Pentacosane	C25	22.687	2500	352	391 ± 142	329 ± 90	558 ± 208
9-Hexacosene	9C26:1	23.197	2575	364	< LOQ	< LOQ	< LOQ
n-Hexacosane	C26	23.402	2600	366	< LOQ	< LOQ	< LOQ
9-Heptacosene	9C27:1	23.901	2675	378	2520 ± 732	1517 ± 353	3943 ± 1123
n-Heptacosane	C27	24.087	2700	380	217 ± 124	205 ± 72	296 ± 139
9-Octacosene	9C28:1	24.573	2775	394	< LOQ	< LOQ	< LOQ
9-Nonacosene	9C29:1	25.235	2875	406	1505 ± 701	1138 ± 442	1566 ± 641
n-Nonacosane	C29	25.4	2900	408	< LOQ	< LOQ	< LOQ
9-Triacontene	9C30:1	25.863	2975	420	< LOQ	< LOQ	< LOQ
Hentriacontadiene	C31:2	26.302	3045	432	< LOQ	< LOQ	< LOQ
Hentriacontadiene	C31:2	26.347	3055	432	< LOQ	< LOQ	< LOQ
Hentriacontadiene	C31:2	26.408	3065	432	< LOQ	< LOQ	< LOQ
9-Hentriacontene	9C31:1	26.473	3075	434	753 ± 271	298 ± 95	873 ± 261
Total					7528 ± 1954	4635 ± 939	11 562 ± 2997

[a]Abbreviation of hydrocarbon name.
[b]Retention time.
[c]Retention index.
[d]Molecular ion.
LOQ, limit of quantification.

Among the males that mated, the time from initiation of the trial until mounting the female was scored as the 'copulation latency.' A GLM was used to assess the incidence of both male population and mating choice (i.e. female population) on copulation latency, including male origin and mating choice as crossed factors in the model [GLM model: glm (copulation latency ~ male population * mating choice)]. A one-way ANOVA was then conducted to detect significant effect of either factor. Prior to these analyses, data were log-transformed to achieve normality of the residuals. Overdispersion in variance of the data was also checked ($P > 0.05$). All analyses were performed in R, version 3.4.0 (R Core Team, 2017)

Results

Chemical analysis

In total, 17 cuticular compounds were detected from the posterior half of female elytra pairs, regardless of the population. Based on retention data (i.e. comparison of retention times with pure standards) and mass fragmentations, we identified six alkanes, three polyunsaturated alkenes and eight monounsaturated alkenes (Table 1). Seven of them were present in quantifiable amounts and considered for statistical analyses: *n*-tricosane, 9-pentacosene, *n*-pentacosane, 9-heptacosene, *n*-heptacosane, 9-nonacosene and 9-hentriacontene.

The total amount of CHCs from a female elytra pair significantly differed among the populations of *H. axyridis* ($H = 21.57$,

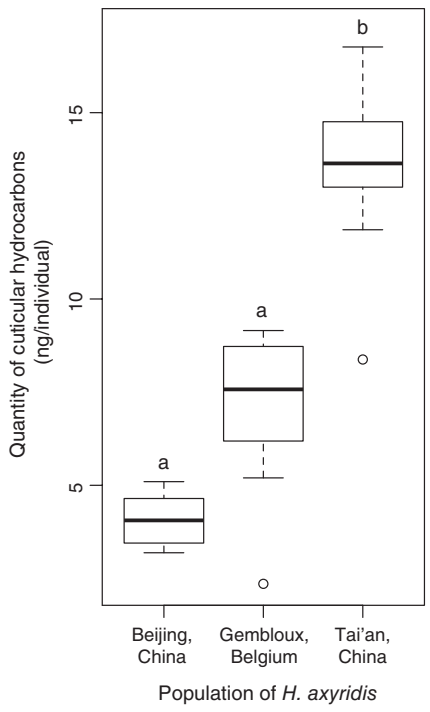

Fig. 1. Absolute quantity of cuticular hydrocarbons (CHCs) on the posterior half of a female elytra pair (ng/individual) according to *H. axyridis* population. Populations differing significantly from each other are marked with different lowercase letters (i.e. multiple pairwise comparisons).

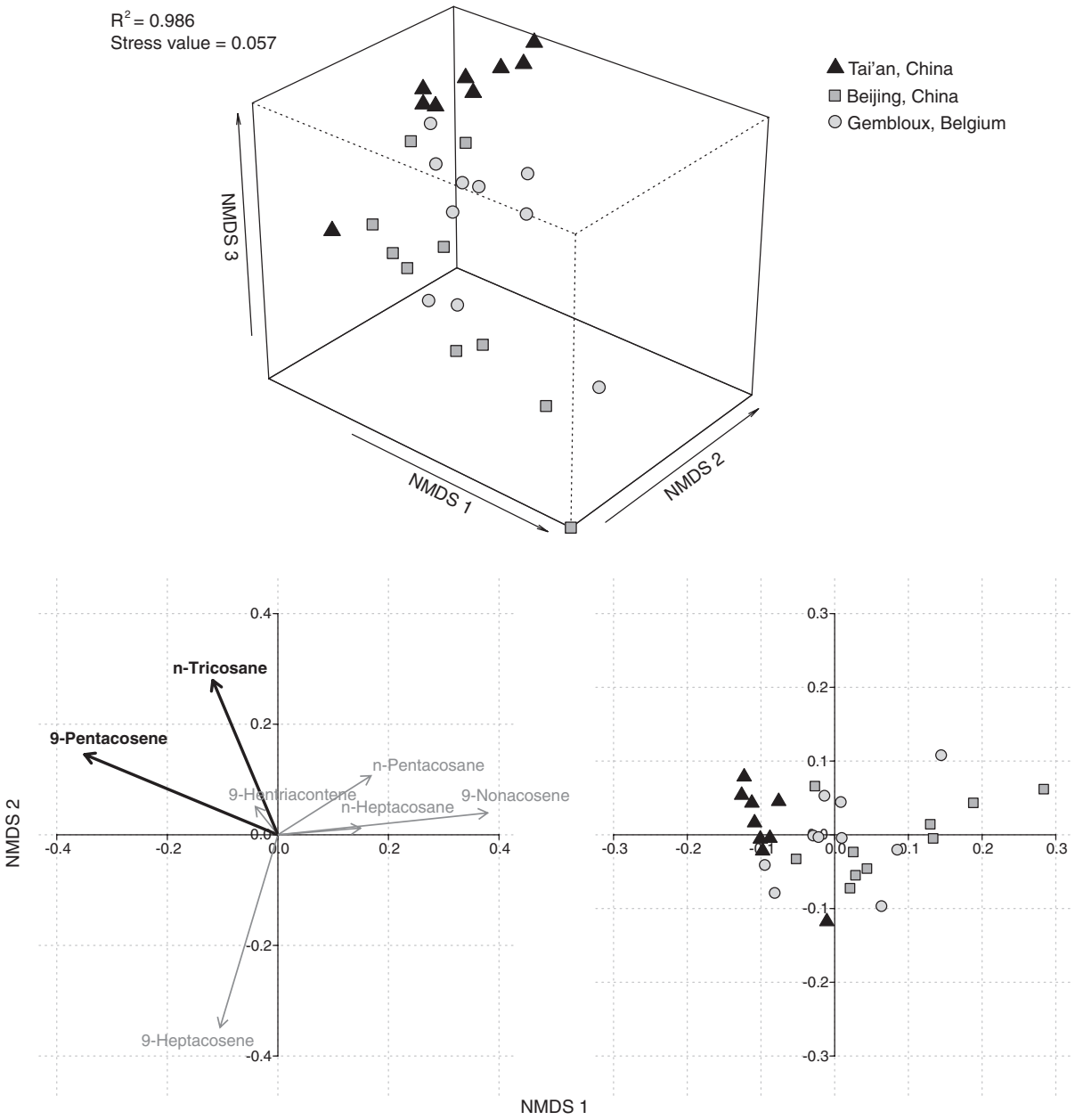

Fig. 2. Non-metric multidimensional scaling ordination plots based on Bray–Curtis distances calculated on relative abundances of cuticular hydrocarbons (CHCs) from the posterior half of a female elytra pair showing 3D and 2D configurations, as well as CHC vectors.

d.f. = 2, $P < 0.001$) with lady beetles from the Tai'an displaying a significantly higher CHC concentrations on their elytra than those from the Beijing ($P < 0.001$) and Gembloux ($P < 0.05$) populations (Fig. 1). Multivariate analyses also detected a significant difference in CHC profiles from female elytra pair among populations ($F_{2,26} = 8.10$, $P = 0.001$), with females from Tai'an being significantly different from the females from Beijing ($P = 0.001$) and Gembloux ($P = 0.002$). The non-metric multidimensional scaling ordination highlighted this difference, with the Tai'an population clearly being found to cluster apart from the other ones (Fig. 2). CHC vectors, as well as indicator compound analysis, showed a significant association of *n*-tricosane ($P = 0.007$, indicator value = 0.600) and 9-pentacosene ($P = 0.007$, indicator value = 0.643) with females of *H. axyridis* from Tai'an population (Fig. 2).

Mating bioassays

Although there was no significant difference in copulation number for either male population with local versus foreign females (male population $\chi^2 = 0$, $P = 1$; mating

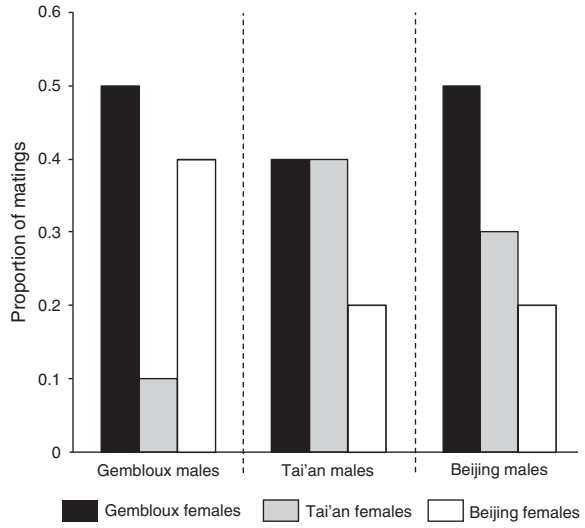

Fig. 3. The proportion of males that copulated with females from the different populations.

choice $\chi^2 = 2.281$, $P = 0.320$; male population: mating choice $\chi^2 = 3.078$, $P = 0.545$), females from the invasive population (i.e. Gembloux, Belgium) tended to be preferred by males from whatever origin (Fig. 3 and Table 2). Using a Pearson correlation test, no CHC amount (i.e. individual compounds tested separately), nor total quantification was correlated with the number of copulations ($P > 0.05$).

An analysis using trial latency score (i.e. the number of trials each male interacted with a female before mating) revealed that male response was not significantly different according to its population ($F_{2,21} = 0.023$, $P = 0.977$) but depended on its mating choice ($F_{2,21} = 12.412$, $P < 0.001$), with a significant interaction between male and female populations ($F_{4,21} = 15.349$, $P < 0.001$). Multiple pairwise analyses revealed that this response difference in the trial latency was the result of one Gembloux male that interacted 12 times before mating (Fig. 4A and Table 2).

When analyzing the latency to copulation (in seconds), males showed no difference in either their population ($F_{2,21} = 1.876$, $P = 0.178$) or mating choice ($F_{2,21} = 1.342$, $P = 0.283$), indicating no preference for the female population (Fig. 4B and Table 2).

Discussion

Evidence is accumulating regarding the important roles that hydrocarbons play in lady beetles (Fassotte *et al.*, 2016). Hydrocarbons are found in larval tracks, adult footprints and elytra, and are also involved in the location of overwintering sites or the detection of competitors (Hemptinne *et al.*, 1998; Nakashima *et al.*, 2006; Magro *et al.*, 2010; Durieux *et al.*, 2012,2013; Susset *et al.*, 2013; Wheeler & Cardé, 2014; Verheggen *et al.*, 2017). Furthermore, there is no doubt that elytra hydrocarbons are involved in mate selection. Behavioural experiments performed on *A. bipunctata* demonstrate the presence of active compounds on the elytral surface of females (Hemptinne *et al.*, 1996). When encountering a female, the male first palpates the female elytra with its maxillary palps. Elytra washed in chloroform fail to trigger mating. The chloroform extracts from elytra reveal that both male and female *Adalia bipunctata* are coated with the same blend of hydrocarbons. Even though hydrocarbon profiles tend to be species specific in lady beetles (Howard, 1993), the existing literature is contradictory regarding whether hydrocarbons allow gender identification (Nelson & Carlson, 1986; Hemptinne *et al.*, 1998; Ginzel & Hanks, 2003).

The results of the present study show that geographical origins are reflected by both the CHC profile (i.e. relative abundances) and the total amount in *H. axyridis* females. Especially, our statistical analyses indicate that females from Tai'an clearly differ from the two other populations by displaying higher CHC concentrations, as well as higher proportions of 9-pentacosene and *n*-tricosane, which were indicative of this geographical origin in the present study. The causes for high interspecific and intraspecific CHC variations are scarcely understood. In ants, physiological constraints and climatic and biotic selection pressures can be causes of variations: warm-acclimated individuals have higher proportions of linear alkanes and less methyl-branched or unsaturated CHCs (Sprenger *et al.*, 2018), whereas individuals living under wet climates have more alkenes and fewer dimethyl alkanes than those from drier habitats (Menzel *et al.*, 2017). The presence of mutualistic partners in specific areas can also select a population of individuals with a specific CHC profile. Because *H. axyridis* are spread over a wide native and invasive area, such variations are likely to occur. Two geographically distinct genetic clusters of *H. axyridis* are already reported in Asia (Lombaert *et al.*, 2011). Because of the likely bottleneck effect

Table 2. Behavioural parameters of conspecific (indicated in bold) and heterospecific matings (mean ± SD).

Male population	Mating choice	Proportion of copulations	Trial latency score (number of trials)	Copulation latency (s)
Gembloux ($n = 10$)	**Gembloux**	**0.5**	**1.8 ± 1.3**	**10.0 ± 6.4**
	Tai'an	0.1	12.0	17.2
	Beijing	0.4	1 ± 0	5.1 ± 2.4
Tai'an ($n = 10$)	Gembloux	0.4	1.75 ± 1.5	4 ± 1.9
	Tai'an	**0.4**	**4.0 ± 0.8**	**7.1 ± 2.9**
	Beijing	0.2	1.5 ± 0.7	3.6 ± 3.5
Beijing ($n = 10$)	Gembloux	0.5	3.6 ± 1.8	8.1 ± 5.1
	Tai'an	0.3	1.7 ± 0.6	3.6 ± 1.4
	Beijing	**0.2**	**1.5 ± 0.7**	**7.3 ± 2.6**

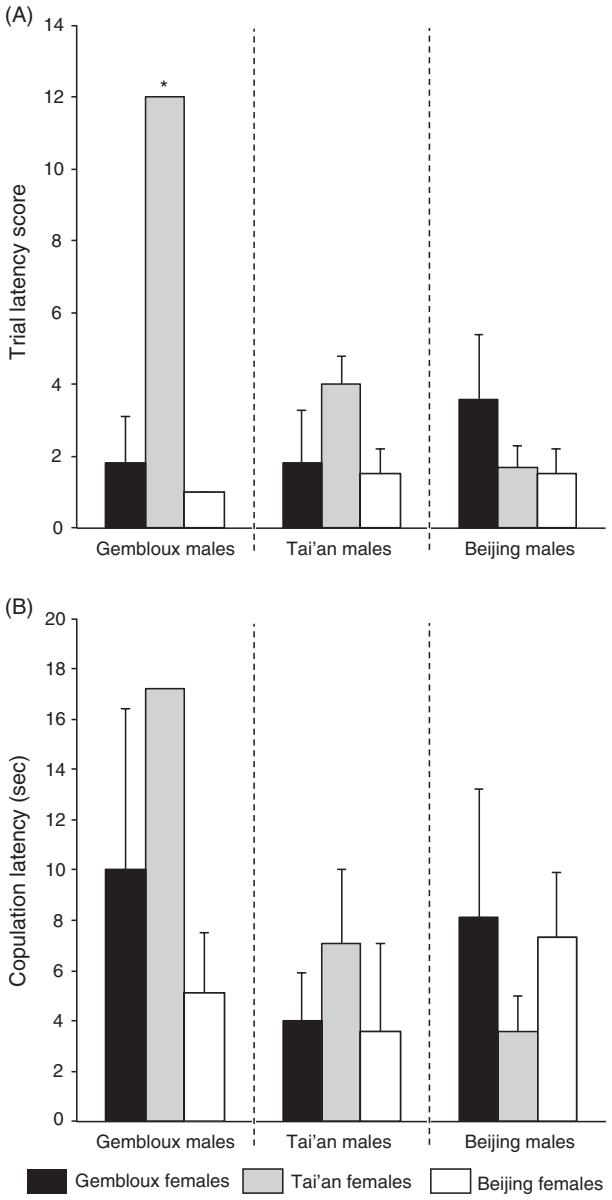

Fig. 4. (A) Male response as measured by the trial number before mating occurred ('trial latency score') according to the final mating pair. (B) Latency (s) to copulation according to the final mating pair.

occurring in invasive populations (Facon *et al.*, 2011), it is possible that the invasion process has led to changes in gene allele frequencies without necessitating selective processes (Brown *et al.*, 2011). Even though the present study is based on a limited number of population samples, the results obtained suggest that the invasive population established in Europe exhibits a CHC profile remaining similar, to a large extent, to that observed in the native range, both in terms of composition and concentration. As a result of the wide geographical dispersion of *H. axyridis* and its wide range of habitats (Brown *et al.*, 2011), the cuticular profile could be affected by the climate, or the adaptation to cold or humidity level (Boullis *et al.*, 2016). Such changes could lead to potential reproductive isolation (Chung & Carroll, 2015). Future studies should investigate the variation of cuticular profile in accordance with the different environmental conditions encountered by invasive and native *H. axyridis* populations.

Being less inbreeding sensitive than expected (Facon *et al.*, 2011), *H. axyridis* males from invasive populations show no mating preference according to the population of their sexual partner. The results obtained in the present study confirm that invasive males from Gembloux copulate with females from any origin, although there is no evidence of any pre-copulatory mechanisms being used by *H. axyridis* males from native areas to avoid mating with related populations. In agreement with this, Laugier *et al.* (2013) does not report any inbreeding avoidance in native populations of *H. axyridis* when mixed groups of four males from invasive and native populations are allowed to mate freely with one female of either type. However, Laugier *et al.* (2013) also show that males from invasive areas have a higher probability of being the first to copulate with both native and invasive females. The results suggest that the rapidity to initiate the first copulation attempt is independent of the population of both mating partners. At this point, it should be highlighted that the behavioural set-up used in the present study, consisting of a Petri dish containing four individuals (three females and one male), could be small and stressful for the tested insects. Because males are also kept separated from females before being tested, it might be hypothesized that males are eager to mate and thus choosing is not their priority.

To limit the risks of inbreeding depression, both sexual partners could use pre- and post-copulatory mechanisms (Hosken & Blanckenhorn, 1999). The results obtained in the present study do not suggest that the chemical profile of the cuticle could be involved in sexual interactions and the discrimination of mating partner based on their origin. However, some post-copulatory strategies could also lower the risk of inbreeding. Most of these adaptations involve sperm manipulation such as (i) favouring sperm of unrelated males; (ii) discarding or controlling sperm storage or (iii) the transfer of smaller ejaculates to related females (Saxena *et al.*, 2016). The existence of such post-copulatory strategies remains to be investigated. Some Czech populations of *H.axyridis* are capable of storing the sperm of multiple males to fertilize their eggs (Awad *et al.*, 2015). Invasive males also sire a greater proportion of the offspring than native ones, even if they are not the first to copulate (Laugier *et al.*, 2013). On the one hand, females of *H. axyridis* might store the ejaculates of several males and control the sperm precedence according to relatedness of the male or his population. On the other hand, males might allocate ejaculate according to the relatedness of the female or her population. These hypotheses also remain to be tested and they might explain the differences in inbreeding sensitivity and reproductive fitness observed in native and invasive *H. axyridis* populations (Facon *et al.*, 2011; Laugier *et al.*, 2013).

Acknowledgements

This work is funded by a BiodivERsA (Horizon 2020 ERA-NET COFUND scheme) grant. The present paper is dedicated to the

memory of Istvan Marko, who could not read the final published version of this manuscript. The authors declare that they have no conflicts of interest.

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Accepted 9 March 2019

