

Intrinsic pre-zygotic reproductive isolation of distantly related pea aphid host races

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Abstract

Human activities may weaken or destroy reproductive isolation between young taxa, leading to their fusion with consequences for population and community ecology. Pea aphid host races are adapted to different legume taxa, providing a degree of pre-mating isolation mediated by habitat choice. Yet, all races can feed and reproduce on the broad bean (*Vicia faba*), a major crop which represents a universal host plant, which can promote hybridization between races. Here, we ask if pea aphid host races have reproductive barriers which prevent or reduce gene flow when they co-occur on the universal host plant. We observed mating behaviour, female survival, number of eggs and egg fertilization rates for three types of crosses: among individuals of the same host race, between closely related host races, and between distantly related host races. We did not find significant differences in mating behaviour and female survival among the three types of crosses. However, we observed drastic reduction in the number of eggs laid, and in the number of fertilized eggs, in distant crosses. We conclude that widespread broad bean cultivation in agriculture may predispose closely related but not distantly related host races to hybridize, disrupting reproductive isolation between incipient species.

Introduction

Cases of speciation reversal (also known as fusion of lineages or despeciation) are increasingly being reported, often associated with human activities [1]. Human-mediated speciation reversal raises conservation concerns [2] and can have cascading ecological consequences [3]. Young taxa, mainly isolated by environmental-mediated reproductive barriers, are most likely to be affected.

The pea aphid complex consists of 15 host-plant races showing various levels of genetic divergence [4], with each race adapted to one or several legume species. Pea aphid host-race formation is normally interpreted as equivalent to the early stages of ecological speciation in the presence of gene flow. Differences between environments the host plants lead to divergent selection with adaptation to one host plant reducing fitness on another [4,5], and hybrids between host races underperforming on both parental host plants [6,7]. In addition, host races exhibit strong preferences for their host plant [8,9], and loci controlling performance on the host and host preference are linked [10], which may facilitate their diversification. Pea aphids are particularly suitable for studying speciation reversal due to the existence of a universal host plant: all races can feed and reproduce on broad bean (*Vicia faba*) [5,11] and this may affect both pre-mating and post-mating reproductive isolation. Pre-mating reproductive barriers may be weakened as different host races have similar preferences for, and performance on, *V. faba* [5,12,13]. Post-zygotic isolation can be weakened because hybrids do not suffer a disadvantage on broad bean [7]. Thus, the cultivation of broad bean may alter both pre-mating and post-mating reproductive isolation and result in fusion of the pea aphid host races. In this study, we ask whether pea aphid host races have intrinsic reproductive barriers which can keep them from fusing in the presence of the universal host. We performed experiments assessing behavioural pre-mating and post-mating pre-zygotic isolation barriers between two closely related host races of pea aphids from *Medicago lupulina* and *Vicia cracca* and between these and the more distantly related *Lathyrus pratensis* host race.

Materials and Methods

We constructed a pea aphid phylogeny based on approximately 500 chemosensory genes in aphids collected in the UK [14] and France ([File S1](#)). The pea

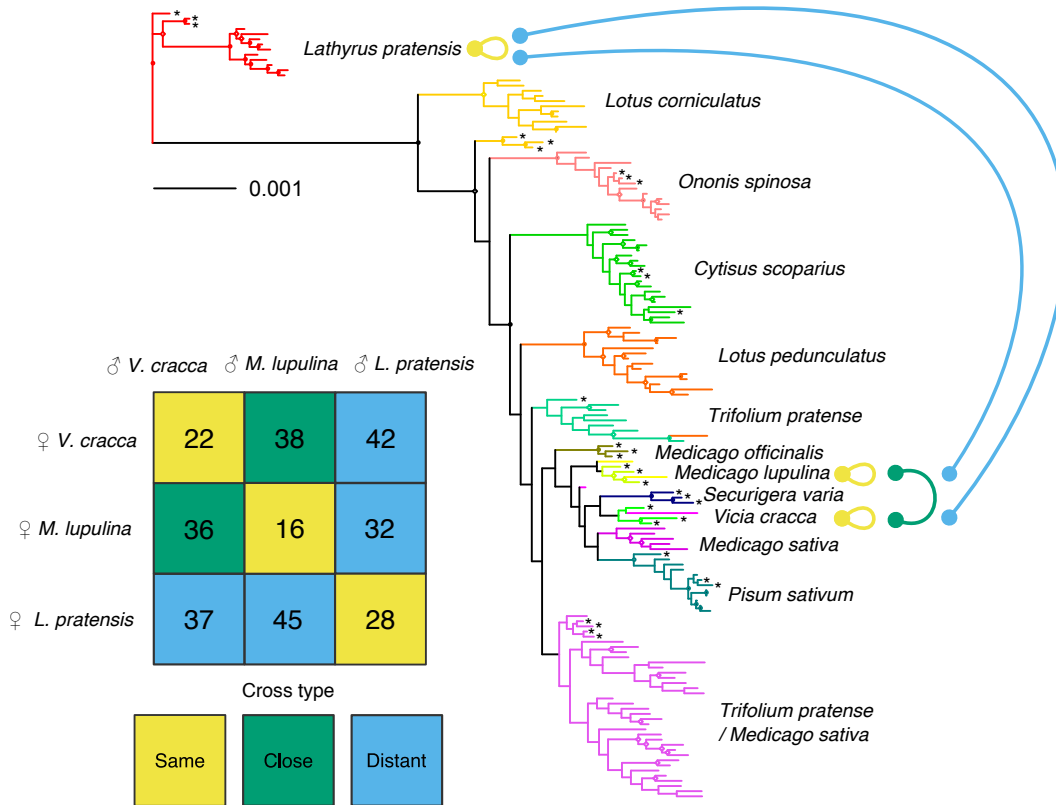


Figure 1: Crossing scheme, with the number of crosses (left). Pea aphid phylogeny based on approximately 500 chemosensory genes for clones collected in the UK (unmarked) and France (marked with asterisks) (right). Branch colours denote host-plant assignment, which is labelled in front of the respective clades.

aphids used for the mating experiment were collected within a 10km radius of Oxford, UK in 2015. They were genotyped with 14 microsatellite markers [4] to confirm host race assignment and clone identity ([File S2](#)). All the clonal lines were reared in Petri dishes on *V. faba* leaves at 16°C with a 16/8hrs light/dark photoperiod. Crosses were performed between different clones of all three host races ([File S3](#)) and classified as same (*Vicia* x *Vicia*, *Medicago* x *Medicago*, *Lathyrus* x *Lathyrus*), close (*Vicia* x *Medicago*, *Medicago* x *Vicia*) and distant (*Vicia* x *Lathyrus*, *Lathyrus* x *Vicia*, *Medicago* x *Lathyrus*, *Lathyrus* x *Medicago*) (**Figure 1**) where the female parent comes first. The production of sexually reproducing females and males was induced by shortening the photoperiod [15]. To ensure only virgin males and females were used in our experiment, we checked sexual offspring daily, and based on differences in body shape at the III or IV instar stage we identified males and females, and placed them in separate Petri dishes. We performed reciprocal no-choice mating experiments, by placing one female (5-10 days old) and one male (5-12 days old) in a 60mm x 15mm Petri dish and observing their mating behaviour for 90min. All mating experiments took place 4-6hrs after dawn, the peak time for mating activity [16]. After the observations were finished, we placed all observed mating pairs in 100mm x 15mm Petri dishes with *V. faba* leaves to feed, mate and oviposit for 5 weeks. Each week we recorded the number of eggs laid and the number of eggs with dark serosal cuticle, which forms only in fertilized eggs with normal early embryonic development [17]. Unfertilized eggs (or fertilized eggs with early embryonic mortality) remain green and decompose within days. For survival analysis, females which copulated during the observation window were checked weekly and followed until death. The variables measured in the mating experiment are shown in **Table 1**. Details of statistical analysis are shown in [File S4](#).

Results

We performed 296 crosses, and observed mating in 143 of them ([Files S3, S4](#)). We consistently observed that males were active and capable of multiple copulations, and found no evidence for pre-mating behavioural isolation. The number of copulations (Kruskal-Wallis Test, $H=0.69$, 2 d.f., $p = 0.71$) and the total copulation time (Kruskal-Wallis Test, $H=0.58$, 2 d.f., $p = 0.75$) did not vary significantly between crosses ([Figure 2A,B](#), [File S4](#)). The number of weeks females survived after the mating experiment (Kruskal-Wallis Test,

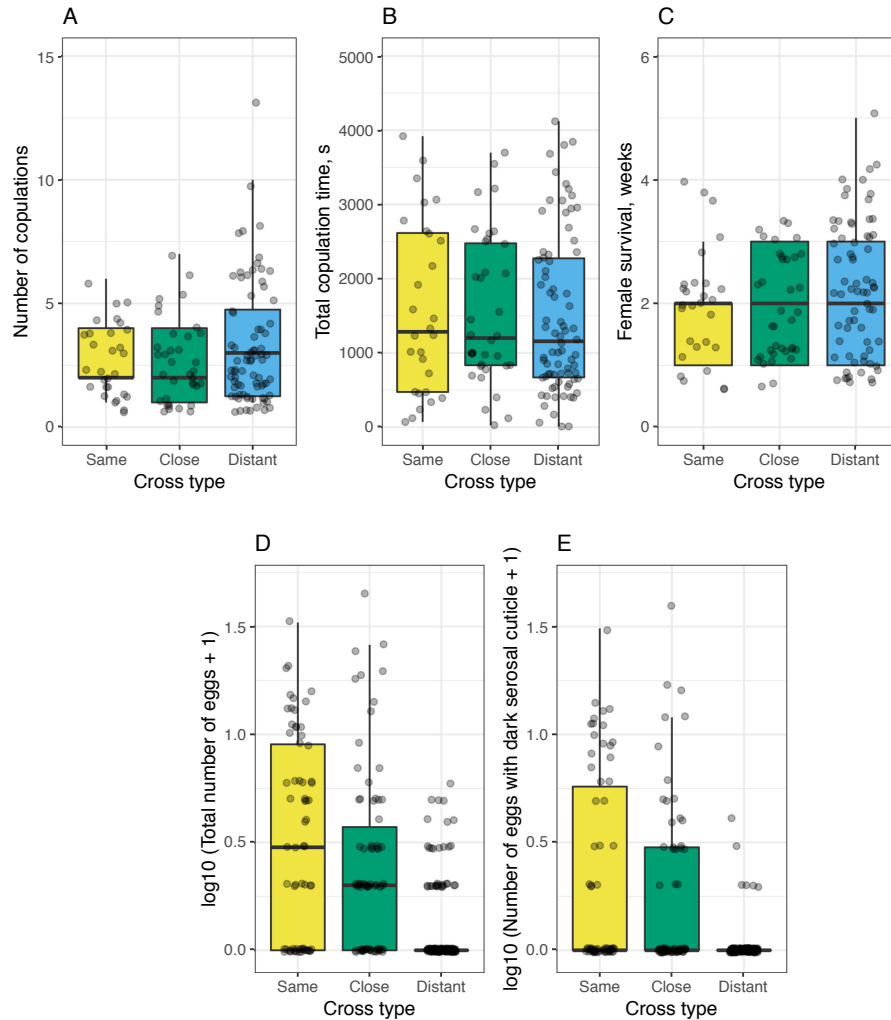


Figure 2: Graphical summary of the mating experiment. Number of copulations (A); Total copulation time (sum of all copulations) (B); The number of weeks females survived after the start of the mating experiment (C); Total number of eggs per female on Day 35 (D); Number of eggs with dark serosal cuticle per female on Day 35 (E). The boxes denote interquartile range (IQR). Lower whiskers extend to lowest data points \geq first quartile $- 1.5 \times \text{IQR}$. Upper whiskers extend to largest observation \leq third quartile $+ 1.5 \times \text{IQR}$. Gray dots represent individual data points

Table 1: Variables measured in the experiment and potential reproductive isolation mechanisms involved

Variable measured	Reproductive isolation mechanism	Type of barrier
Number of copulations	Females/males not performing copulations	Pre-mating pre-zygotic
Total copulation time		
Female survival time after mating	Females injured during copulation	Post-mating pre-zygotic
Total number of eggs laid	Sperm transfer unsuccessful / female control of fertilization	Post-mating pre-zygotic
Number of eggs with dark serosal cuticle (fertilized eggs with normal early embryonic development)	Sperm transfer unsuccessful / female control of fertilization	Post-mating pre-zygotic
	Early embryonic death	Post-zygotic

$H=2.68$, 2 d.f., $p = 0.26$) did not vary significantly between the crosses (**Figure 2C**).

For five weeks after mating, we recorded the total number of eggs and the number of eggs with dark serosal cuticle produced every week by each female (**Figure 2D,E**). We found that the total number of eggs varied significantly among crosses (Kruskal-Wallis Test, $H=66.33$, 2 d.f., $p < 10^{-5}$), and was significantly smaller in distant compared to same (Dunns test; $p < 10^{-5}$) or close (Dunns test; $p < 10^{-5}$ crosses, but not between same and close crosses (Dunns test; $p = 0.28$). We also found that the number of eggs with dark serosal cuticle varied significantly among crosses (Kruskal-Wallis Test, $H=51.53$, 2 d.f., $p < 10^{-5}$, and was significantly smaller in comparisons between the distant and same (Dunns test; $p < 10^{-5}$ and distant and close (Dunns test; $p < 10^{-5}$ but not same and close crosses (Dunns test; $p = 0.13$).

Discussion

The universal host, *V. faba*, may act as a bridge for gene flow between pea aphid host races [5]. Our results show that fusion between closely related host races is possible as they do not exhibit pre-zygotic non-ecological isolation. Conversely, for distantly related host races we find strong non-ecological iso-

lation with a significant reduction in the number of eggs laid (**Figure 2D**). This may be caused by a post-mating pre-zygotic barrier related to sperm transfer or to female control of fertilization, possibly similar to the cryptic female choice to avoid inbreeding previously described in pea aphids [16]. The finding of fewer eggs with dark serosal cuticle (**Figure 2E**) could also be explained by post-mating prezygotic barriers, although additional post-zygotic barriers (e.g. early embryonic death) could also be present. Our results suggest very strong reproductive isolation of the *L. pratensis* host race, which agrees well with the observation of lack of admixture in nature [18]. It is also the most genetically differentiated host race [14,19] leading to suggestions that it should be given species status [20].

Viewing the pea aphid complex as a speciation continuum allows us to infer how reproductive isolation barriers accumulate during speciation. Pea aphid host-plant races are assumed to have diverged in the presence of gene flow, and exhibit pre-zygotic isolation due to habitat choice. The finding of additional pre-zygotic barriers in distantly related host races can be explained in a number of ways. First, mating between host races may be harmful for females, prompting evolution of mating avoidance [21]. Second, genetic drift may lead to fixation of incompatible alleles leading to post-mating pre-zygotic isolation (through mechanisms described above). And third, selection may drive fixation of genes responsible for reproductive isolation, either via reinforcement against formation of maladapted hybrids, or via genomic hitchhiking if loci responsible for reproductive isolation and divergent ecological selection are linked [22]. We find the first explanation unlikely, because our crossing experiment did not find any reduction in lifespan of females nor evidence for female avoidance of inter-host males. Random fixation of incompatible alleles is also unlikely in the presence of gene flow between host races. Instead, selection, via reinforcement or genomic hitchhiking, seems a more likely candidate mechanism for the evolution of additional prezygotic reproductive barriers in the pea aphid complex.

Overall, our results show that, within the pea aphid complex, closely related host races are more likely to be affected by widespread agricultural cultivation of broad bean than distantly related ones. Whether these results affect patterns of connectivity between host races in nature, and whether timing of gene flow between host races coincides with onset of human agriculture, remains to be tested and is a promising direction for future research.

Ethics

The research follows the ASPA /ASAB guidelines for use of animals in research.

Data accessibility

Raw sequencing data is available from GenBank, Bioprojects PRJEB6325 and PRJNA255937.

Authors contributions

VF and CG conceived the study. VF and BN collected aphids and performed the analysis. VF, BN, AM, CG designed the mating experiment. VF and AM performed the mating experiment. VF wrote the manuscript, BN, AM and CG provided comments and contributed to the manuscript. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Competing interests

We declare we do not have competing interest.

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Footnotes

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.4297670>.

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