Ample genetic variation but no evidence for genotype-specificity in an all-parthenogenetic host-parasitoid interaction

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Abstract

Antagonistic coevolution between hosts and parasites can result in negative frequency-dependent selection and may thus be an important mechanism maintaining genetic variation in populations. Negative frequency-dependence emerges readily if interactions between hosts and parasites are genotype-specific such that no host genotype is most resistant to all parasite genotypes, and no parasite genotype is most infective on all hosts. While there is increasing evidence for genotype-specificity in interactions between hosts and pathogens or microparasites, the picture is less clear for insect host-parasitoid interactions. Here we addressed this question in the black bean aphid (*Aphis fabae*) and its most important parasitoid *Lysiphlebus fabarum*. Because both antagonists are capable of parthenogenetic reproduction, this system allows for powerful tests of genotype × genotype interactions. Our test consisted of exposing multiple host clones to different parthenogenetic lines of parasitoids in all combinations, and this experiment was repeated with animals from four different sites. All aphids were free of endosymbiotic bacteria known to increase resistance to parasitoids. We observed ample genetic variation for host resistance and parasitoid infectivity, but there was no significant host clone × parasitoid line interaction, and this result was consistent across the four sites. Thus, there is no evidence for genotype-specificity in the interaction between *A. fabae* and *L. fabarum*, suggesting that the observed variation is based on rather general mechanisms of defence and attack.

Keywords: *Aphis fabae*, genotype-by-genotype interactions, host-parasitoid coevolution, infectivity, *Lysiphlebus fabarum*, parthenogenesis, resistance

Introduction

Most organisms suffer from parasites, even many that are parasites themselves (Price, 1980). The ubiquity of parasitism and the fact that hosts and parasites are engaged in a coevolutionary arms race of adaptation and counter-adaptation led to the hypothesis that host-parasite interactions are an important mechanism maintaining genetic variation in populations (Judson, 1995), and may even select for sexual reproduction and recombination (Jaenike, 1978; Hamilton, 1980). This assumption hinges on a property that might be inherent in such interactions, namely that reciprocal selection between hosts and parasites is negative frequency-dependent, providing rare genotypes with a selective advantage that prevents their loss from the population. Negative frequency-dependence may arise either under high costs of resistance and infectivity, such that investment in defence is only favoured in a largely undefended poplation, for example, or if the interaction between hosts and parasites exhibits high genetic specificity (Hamilton *et al.*, 1990; Frank, 1996; Sasaki, 2000; Agrawal & Lively, 2002). If this specificity is such that no individual host genotype is most resistant to all parasite genotypes and no parasite genotype is most infective on all host genotypes, negative frequency-dependence emerges very readily (Frank, 1994; Parker, 1994). The recognition of the evolutionary importance of genotype-specificity has led to an increasing number of studies testing this assumption, many of which reported significant host genotype × parasite genotype (G × G) interactions (e.g. Webster & Woolhouse, 1998; Carius *et al.*, 2001; Schulenburg & Ewbank, 2004; Lambrechts *et al.*, 2005; Salvaudon *et al.*, 2007). This suggests that the potential for negative frequency-dependent selection is realised in many host-parasite systems.

It would appear that reciprocal selection is particularly intense in insect host-parasitoid interactions, because their outcome is always fatal for one of the antagonists. Parasitoids are either killed by their hosts' defences, or they kill their hosts if these defences fail. The term virulence, typically defined as the reduction in host fitness resulting from infection by a parasite (Read, 1994), is therefore of limited use for parasitoids. If a parasitoid is able to overcome host defences, the host

will invariably be killed and variation in virulence is restricted to more subtle differences such as time until killing or residual fecundity before death. Therefore, we will use the term infectivity for the ability of an insect parasitoid to parasitise its host. This is not completely satisfactory, as this term is normally used in the context of infectious diseases, but for lack of another term we will also apply it to parasitoids. While there is abundant evidence of genetic variation for resistance in hosts as well as infectivity in parasitoids (e.g. Henter, 1995; Henter & Via, 1995; Kraaijeveld & Godfray, 1999; Ferrari *et al.*, 2001; Kraaijeveld *et al.*, 2001; von Burg *et al.*, 2008), surprisingly little is known about the degree of specificity in insect host-parasitoid interactions. Maybe best addressed is this issue in *Drosophila melanogaster* and its hymenopteran parasitoids *Asobara tabida* and *Leptopilina boulardi*. There is evidence for G × G interactions based on major gene effects for *D. melanogaster* and *L. boulardi*, yet the interaction is such that universal infectivity is possible (Dupas *et al.*, 2003). Selection in *D. melanogaster* for resistance to a specific strain of *As. tabida* resulted in higher resistance to other strains, too (Kraaijeveld & Godfray, 1999), and this increased resistance even extended to other species of parasitoids (Fellowes *et al.*, 1999). Kraaijeveld & Godfray (1999) interpreted this as evidence for resistance and infectivity being quantitative traits that lack genetic specificity, although they acknowledged that the relevant experiments have yet to be done.

We have recently established the black bean aphid, *Aphis fabae*, and its most important parasitoid, *Lysiphlebus fabarum*, as a laboratory-based study system that is ideally suited to address the issue of genotype-specificity in insect host-parasitoid interactions. *Aphis fabae* is a cyclical parthenogen and can be maintained clonally for any period of time under suitable conditions. *Lysiphlebus fabarum*, exceptionally among aphid parasitoids, also reproduces by parthenogenesis in most populations (Mackauer & Starý, 1967; Starý, 1988; Belshaw & Quicke, 2003). It is therefore possible to work with genetically uniform lines of host and parasitoid, which allows for powerful tests of genotype-specificity in their interaction. Interestingly, aphids may harbour facultative endosymbiotic bacteria that are vertically transmitted and provide protection against

parasitoids (Oliver *et al.*, 2003). When Vorburger *et al.* (2009) exposed multiple clones of *A. fabae* with and without the defensive symbiont *Hamiltonella defensa* to two parthenogenetic lines of *L. fabarum*, they detected strongly increased resistance in clones harbouring *H. defensa* and a significant aphid clone × parasitoid line interaction on the proportion of aphids parasitised. However, this interaction was not observed when clones harbouring *H. defensa* were excluded from the analysis, suggesting that it may be due to specific interactions between symbiont and parasitoid genotypes, and that the direct interaction between aphids and parasitoids is characterised by a lack of genotype-specificity (Vorburger *et al.*, 2009). Yet with only two parasitoid lines tested, this result was far from conclusive. Here we present a more targeted and powerful test of G × G interactions between *A. fabae* and *L. fabarum*, yet we arrive at the same conclusion. If the aphids do not harbour any defensive endosymbionts, there is no evidence for genotype-specificity in the interaction between *A. fabae* and *L. fabarum*.

Materials and methods

Animals

All aphids and parasitoids were collected in June and July 2006 in the course of a Europe-wide sampling effort. The experiment reported here included animals from four different geographic origins, namely (i) the vicinity of Rennes in Britanny, France, (ii) the vicinity of Budweis in South Bohemia, Czech Republic, (iii) the Lower Rhine Valley in north-eastern Switzerland and (iv) the Lower Valais in south-western Switzerland.

In *Aphis fabae*, four different subspecies are described (Heie, 1986; Raymond *et al.*, 2001). They use the same primary hosts (mainly *Euonymus europaeus* and *Viburnum opulus*), where the sexual females mate and lay overwintering eggs, but they differ in the range of secondary host plants used by the parthenogenetic, viviparous summer generations. Here we focus exclusively on the nominal

subspecies *A. f. fabae*, which mainly uses broad bean (*Vicia faba*) and several Chenopodiaceae such as sugar beet (*Beta vulgaris*) or *Chenopodium album* as secondary hosts. Aphids were collected by clipping infested leaves or shoots of suitable secondary host plants, from which a single parthenogenetic female was used to establish a clonal line in the laboratory. We maintained clones on caged seedlings of broad bean (*V. faba*, Var. 'Scirocco') at 20°C and a 16 h photoperiod. Under these conditions, *A. fabae* reproduces by continuous apomictic parthenogenesis. We genotyped all clones at eight microsatellite loci (Coeur d'Acier *et al.*, 2004), and we screened them for the presence of facultative symbiotic bacteria as described in Vorburger *et al.* (2009). From the clones testing negative for facultative symbionts, we selected six from Rennes and five from each of the other three sites, all with different multilocus microsatellite genotypes. These genotypes and detailed collection information for all test clones are available in Table S1.

Parasitised colonies of aphids are easily recognised by the presence of 'mummies', i.e. dead aphids containing the parasitoid's pupa. We sampled parasitoids by collecting colonies with mummies of known host species of *L. fabarum* into air-permeable containers, where we allowed the adult wasps to emerge. Single parthenogenetic females were then isolated from each sample and allowed to attack colonies of *A. fabae* on broad bean to found parthenogenetic isofemale lines. These lines are since maintained in the laboratory as mass cultures on a highly susceptible clone of *A. fabae* that was not included in the experiment. All females founding an isofemale line were genotyped at 11 microsatellite loci (Fauvergue *et al.*, 2005; Sandrock *et al.*, 2007). Unlike aphids, in which parthenogenetic reproduction only includes mitotic cell divisions (apomixis), parthenogenetic females of *L. fabarum* undergo a modified meiosis in which diploidy is restored by central fusion automixis (Belshaw & Quicke, 2003), which is why parthenogenetic isofemale lines should not be termed clones. Despite that, these lines can be regarded as genetically uniform, because central fusion automixis rapidly leads to homozygosity distal to chiasmata and leaves nonrecombining areas of the genome unaffected. This was evidenced by the fact that when re-genotyped before the experiment in spring 2007, the microsatellite genotypes of parthenogenetic

isofemale lines were still identical to those of the founding individuals in June/Juli 2006. We included three lines from Rennes and two lines from each of the other three sites in the experiment. Their microsatellite genotypes and collection details are provided in Table S2.

Experiment

Our general assay to estimate host susceptibility and parasitoid infectivity, respectively, was to expose known numbers of aphids to wasps for a fixed period of time, and determine the proportion of individuals successfully parasitised (Henter & Via, 1995). If this is done with several host clones and parasitoid lines in a fully crossed factorial design, a G × G interaction is detectable as a statistical interaction between host clone and parasitoid line on the proportion of aphids parasitised. The advantages of this approach are that we can determine the outcome of host-parasitoid encounters under realistic conditions in a reasonably complex environment, and that the simplicity of the assay allows for good replication. The disadvantage is that the approach is essentially blind to mechanism and cannot distinguish between pre-and postovipositional defences of hosts. For example, aphids may show behavioural avoidance of parasitoids (Foster *et al.*, 2007), which is unlikely to be genotype-specific. However, Henter & Via (1995) have shown that a resistant and a susceptible clone of the pea aphid did not differ in the number of parasitoid ovipositions they suffered, and Vorburger *et al*. (in press) found that in *A. fabae*, up to three quarters of individuals on which parasitoid attacks have been observed may survive. This suggests that physiological defences of aphids can be quite effective. We therefore assume that the variation observed in our experiment will largely (but not exclusively) reflect the interaction between host and parasitoid after oviposition.

Because this experiment was concerned with the potential presence of G × G interactions as a prerequisite for negative frequency-dependent selection and not with local adaptation, we only exposed host and parasitoids from the same site to each other, i.e. we only worked with

host/parasitoid combinations that could have occurred in the field. Thus we had a six host clones × three parasitoid lines infection matrix for Rennes and a five aphid clone × two parasitoid lines matrix for each of the other three sites, resulting in a total of 48 different combinations of host and parasitoid genotypes. Each combination was replicated ten times. We used more aphid clones than parasitoid lines in this cross-infection experiment because space constraints in the laboratory prevented us from keeping a larger number of parasitoid lines as mass cages.

At the start of the experiment, aphid stock cultures were split into the required number of colonies by placing two adult females on seedlings of broad bean grown in 0.07 l plastic pots and covered with a small cage. These colonies were then distributed to random positions in 10 plastic trays such that each tray contained one replicate of all host clone/parasitoid line combinations (randomised complete blocks). To avoid any inflation of among-clone differences by environmental maternal or grand-maternal effects carried over from the stock cultures, the replicated aphid colonies were maintained for two generations before exposure to parasitoids in the third generation. The test generation was started by placing seven second-generation adults from each aphid colony on new seedlings, where they reproduced for 24 h before being discarded. When their offspring were 48-72 h old, we counted them (mean colony size = 51.9 ± 17.1 SD) and added two female parasitoids of the required lines to each cage. We removed the wasps again after 6 h and replaced the cage with a cellophane bag. Although the two wasps might interfere in the same cage and even superparasitise each other, preliminary trials showed that using two rather than a single wasps reduces the variation in mummification rates among replicates with the same combination of genotypes, possibly because this limits the influence of wasps that are unmotivated to sting. Nine days post exposure to wasps, successfully parasitised aphids were recognisable as mummies and counted. To keep the daily work doable, we had to temporally stagger the experiment such that two complete blocks were handled per day over five consecutive days.

179

180 **Statistical analyses**

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182 All analyses were carried out with the open source statistical software R 2.7.1 (R Development
183 Core Team, 2008). Substantial overdispersion prevented us from analysing our proportion data as a
184 success-failure vector using a generalised linear model with binomial errors. Instead, we arcsin-
185 square root transformed the proportions of aphids exposed to wasps that were mummified by
186 parasitoids and analysed them with a linear mixed model, using the LMER procedure of LME4, a
187 contributed library to R. We tested for the effects of site (fixed), block (random), host clone
188 (random), parasitoid line (random) and the host clone × parasitoid line interaction (random). Site
189 was treated as a fixed effect because with four levels only, the corresponding variance component
190 would be estimated poorly. As the number of aphid nymphs exposed to parasitoids varied
191 somewhat among replicates, we also included colony size as a covariate in the analysis. Tests of
192 fixed effects were carried out with the PVALS.FNC function of the LANGUAGER library. The function
193 employs Markov Chain Monte Carlo sampling to obtain the highest posterior density (HPD)
194 intervals and associated *P*-values for fixed effect parameters (Baayen, 2008). This offers a modern
195 alternative to conventional significance tests of fixed effects in mixed models based on *t* or *F*
196 statistics, which remain a contentious issue for ongoing disagreement about appropriate degrees of
197 freedom (Baayen *et al.*, 2008). To get an overall test of a fixed effect with more than two levels (i.e.
198 site), the AOVLMER.FNC function was used. The PVALS.FNC function also provides 95% HPD
199 intervals for random effects. We report these intervals with the estimates of variance components,
200 but because they are constrained to never contain zero, the intervals cannot be used to infer
201 statistical significance (Baayen, 2008; Baayen *et al.*, 2008). Random effects were therefore tested
202 by comparing models with and without the effects using likelihood ratio tests, i.e. by comparing the
203 increase in scaled deviance resulting from removal of the term to a χ^2-distribution with df = 1.

204

205 ## Results

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207 Colony size, i.e. the number of aphid nymphs exposed to parasitoids, did not significantly affect
208 the proportion of individuals that were mummified, but there was a significant block effect (Table
209 1). While the mean rates of successful parasitism did not differ significantly among the four sites
210 included in the experiment, the variation in susceptibility among aphid clones within sites was large
211 and highly significant (Table 1). This was most obvious in aphids from the Rennes area, where
212 there was a more than five-fold difference in susceptibility between the least and the most resistant
213 clone (Fig. 1a). We also found significant variation in infectivity among parasitoid lines (Table 1),
214 but this variation appeared to be general rather than specific to the host clone they attacked. More
215 infective lines tended to mummify a higher proportion of individuals in most aphid clones than less
216 infective lines or, from the host's perspective, more resistant aphid clones generally had a lower
217 proportion of individuals mummified by all parasitoids (Fig. 1). Accordingly, there are only few
218 crossing lines in the interaction plots (Fig. 1), and the host clone × parasitoid line interaction is not
219 significant in the analysis (Table 1). We also ran separate analyses for each of the four sites and
220 found this interaction to be non-significant in all cases (all $P > 0.1$), suggesting that the lack of
221 evidence for G × G interactions is consistent across four widely separated sites.

222

223 ## Discussion

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225 In this study, we used the black bean aphid and parthenogenetic lines of its parasitoid *L.*
226 *fabarum* to test if aphid host-parasitoid interactions exhibit genotype-specific interactions. We
227 found no evidence for genotype-specificity, but we cannot rule out that genotype-specificity would
228 be detected if additional host clones and parasitoid lines were tested. Yet considering that we tested
229 48 different combinations of host and parasitoid genotypes with ten replicates per combination, we

feel confident in concluding that if present at all, such interactions have a weak influence on the outcome of host-parasitoid encounters compared to the substantial genetic variation for general resistance and infectivity we observed. However, this conclusion is limited to the 'hard' outcome of infection, i.e. whether a parasitoid is able to establish in the host and kill it. This is undoubtedly the trait with the most direct effect on fitness. But we cannot exclude that there may be genotype-by-genotype interactions on fitness-relevant traits of surviving parasitoids that were not studied here, such as the time until mummification, the rate of emergence from mummies, or the body size of emerging parasitoids. In fact, there is some evidence for genotype-by-genotype interactions on parasitoid body size (Vorburger *et al.*, in press). Similarly, we cannot rule out that there may be genotype-by-genotype interactions on traits of surviving aphids, e.g. fecundity. We only know that aphid fecundity is generally reduced upon resisting a parasitoid attack (Vorburger *et al.*, 2008).

A lack of specificity in the defences of aphids that are not protected by endosymbionts was also suggested by the results of a previous study on the same system (Vorburger *et al.*, 2009), as well as by a study on green peach aphids by von Burg et al. (2008), who exposed many aphid clones to two different species of parasitoids and detected no significant host clone × parasitoid species interaction. A caveat is in order here, however. Since these studies used similar assays as in the present experiment, it is also possible that part of the variation was due to behavioural mechanisms that are unlikely to be specific, such as differences in avoidance behaviour among host clones or variation in the motivation to sting among parasitoids.

Although based on somewhat different lines of evidence, Kraaijeveld & Godfray (1999) arrived at the similar conclusions for *Drosophila*: variation for resistance is general rather than specific to certain strains or species of parasitoids (but see Dupas *et al.*, 2003). Thus it seems that in contrast to interactions between hosts and pathogens or microparasites, in which specificity is frequently observed (see Introduction), insect host-parasitoid interactions may be characterized by a low degree of genotype-specificity. The similar conclusions from flies and aphids are also interesting because the mechanisms of defence against parasitoids are almost certainly different in the two

systems. *Drosophila* resists parasitoids by encapsulation, a well-understood defence mechanism that is widespread in insects (Strand, 2008), but typically not observed in aphids (Henter & Via, 1995; Kraaijeveld *et al.*, 2002). Very recent work by Oliver et al. (2009) has shown that aphid resistance conferred by the bacterial endosymbiont *H. defensa* is due to phage-encoded toxins, but the mechanistic basis of genetic variation in the aphids' own resistance is still largely unknown.

The large amounts of genetic variation for resistance and infectivity we detected indicate ample scope for directional selection, yet it would be premature to conclude from the lack of G × G interactions that reciprocal selection between host and parasitoid cannot be frequency-dependent in the *A. fabae*/*L. fabarum* system. Models show that negative frequency-dependence may also emerge in host-parasite systems that lack strong specificity as long as increased resistance or infectivity come at a cost (Sasaki & Godfray, 1999; Sasaki, 2000; Agrawal & Lively, 2002). Nothing is known yet about costs of infectivity in aphid parasitoids, but there are studies looking for costs of resistance in aphids, and they provide only limited support for such costs. Gwynn *et al.* (2005) found more resistant clones of the pea aphid to be less fecund on average, but several other studies using larger numbers of aphid clones did not observe this correlation (Ferrari *et al.*, 2001; von Burg *et al.*, 2008; Vorburger *et al.*, 2009). In the *Drosophila*/*Asobara* system, on the other hand, selection experiments provided evidence for evolutionary costs of resistance as well as costs of infectivity (Kraaijeveld & Godfray, 1997; Kraaijeveld *et al.*, 2001).

Another factor to consider in aphids are defensive endosymbionts like *H. defensa*. Here we only used clones without *H. defensa*, but this bacterium infects a fraction of individuals in many species of aphids. The percentage of infected individuals may vary widely, with estimates ranging from 3 to almost 80% (Darby *et al.*, 2001; Sandström *et al.*, 2001; Darby *et al.*, 2003; Haynes *et al.*, 2003; Leonardo & Muiru, 2003; Russell *et al.*, 2003; Ferrari *et al.*, 2004; Vorburger *et al.*, 2009), although populations uninfected with *H. defensa* have also been reported (Tsuchida *et al.*, 2002; von Burg *et al.*, 2008; Wille & Hartman, 2009). In the present study species, *A. fabae*, about one fourth of the individuals seem to be infected on average (Vorburger *et al.*, 2009). Although this

estimate is still based on rather limited sampling, it certainly indicates that *L. fabarum* is typically confronted with a mixture of hosts with and without *H. defensa* in the field. We have recently found preliminary evidence that *H. defensa* may increase not only the overall level but also the specificity of aphid resistance to parasitoids, possibly through symbiont × parasitoid genotype interactions (Vorburger *et al.*, 2009, R. Rouchet & C. Vorburger, unpubl. data). As *H. defensa* is vertically transmitted, this would modify how reciprocal selection between hosts and parasitoids acts and therefore affect their coevolution. This interesting possibility deserves further research, for which it is important to know that the direct interaction between host and parasitoid genotypes in our study system is characterised by very limited specificity, as is shown here.

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420

Table 1 Results of linear mixed effects models for the proportion of aphids mummified by parasitoids. Proportions were arcsin square-root transformed before analysis. *P*-values of random effects are based on likelihood ratio tests, *P*-values of fixed effects on the HPD intervals obtained from MCMC sampling as implemented in the LANGUAGER library of R (Baayen, 2008).

Source	Variance components for random effects/ coefficient for covariate (95% HPD)	LR χ^2_1	*P*
Colony size	-0.0009 (-0.0024, 0.0006)		0.215
Block	0.0025 (0.0004, 0.0095)	8.893	0.003
Site	-		0.237
Host clone (site)	0.0195 (0.0065, 0.0253)	104.070	< 0.001
Parasitoid line (site)	0.0071 (0.0015, 0.0272)	23.408	< 0.001
Host clone × parasitoid line (site)	0.0013 (0.0000, 0.0034)	0.585	0.445
Residual	0.0518 (0.0462, 0.0604)		

Figure captions

Fig. 1 Interaction plots depicting the susceptibility of clones of *Aphis fabae* from four geographic origins to syntopic lines of the parthenogenetic parasitoid *Lysiphlebus fabarum*. Aphid clones are ordered by increasing mean susceptibility. Each point represents the mean of 10 replicate assays of the same combination.

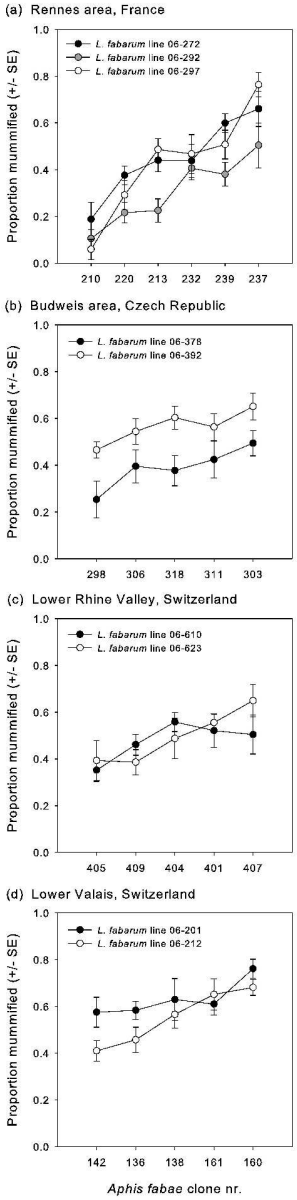

Fig. 1. Interaction plots depicting the susceptibility of clones of Aphis fabae from four geographic origins to syntopic lines of the parthenogenetic parasitoid Lysiphlebus fabarum. Aphid clones are ordered by increasing mean susceptibility. Each point represents the mean of 10 replicate assays of the same combination.
86x326mm (600 x 600 DPI)

Table S1. Collection information and genotypes at eight microsatellite loci (Coeur d'Acier *et al.*, 2004) for the 21 clones of *Aphis fabae* used in this study.

				Microsatellite locus							
Sample ID	Collection site	Latitude, longitude	Host plant	AF-48	AF-50	AF-82	AF-85	AF-86	AF-181	AF-beta	AF-F
Lower Valais, Switzerland											
136	Martigny	46°06'N, 7°04'E	*Vicia faba*	313 315	257 272	179 188	220 220	219 219	311 311	280 282	132 136
138	Martigny	46°06'N, 7°04'E	*Vicia faba*	315 319	257 272	167 177	220 220	219 219	309 309	280 282	127 127
142	Fully	46°08'N, 7°07'E	*Vicia faba*	315 319	257 272	177 177	220 224	219 219	311 325	280 282	127 136
160	Sion	46°14'N, 7°21'E	*Vicia faba*	315 315	272 272	177 188	220 220	215 219	311 311	280 297	127 134
161	Sion	46°14'N, 7°21'E	*Vicia faba*	321 321	255 257	167 177	220 220	215 219	309 309	282 282	129 136
Rennes area, France											
210	Le Rheu	48°06'N, 1°47'W	*Vicia faba*	313 315	257 257	165 171	220 222	219 219	309 309	280 318	127 132
213	Le Rheu	48°06'N, 1°47'W	*Vicia faba*	313 315	257 272	167 177	220 220	219 219	311 311	295 301	127 129
220	Le Rheu	48°06'N, 1°47'W	*Vicia faba*	313 315	272 272	177 177	220 224	219 219	309 313	280 280	127 134
232	Vezin le Coquet	48°07'N, 1°45'W	*Vicia faba*	313 313	257 272	177 177	220 222	219 219	311 325	280 280	123 127
237	Le Verger	48°04'N, 1°56'W	*Vicia faba*	313 319	272 272	173 182	222 222	217 219	311 311	266 280	117 127
239	Le Verger	48°04'N, 1°56'W	*Vicia faba*	315 315	257 272	177 192	220 222	217 219	309 313	280 280	127 127
Budweis area, Czech Republic											
298	Stráž nad Nežárkou	49°04'N, 14°54'E	*Vicia faba*	307 315	272 272	171 177	220 220	219 219	309 313	280 282	127 127
303	Stráž nad Nežárkou	49°04'N, 14°54'E	*Vicia faba*	315 321	272 276	175 177	220 224	219 219	309 313	266 297	127 134
306	Stráž nad Nežárkou	49°04'N, 14°54'E	*Vicia faba*	313 319	257 272	167 177	220 220	215 219	309 311	280 282	129 132
311	Stráž nad Nežárkou	49°04'N, 14°54'E	*Vicia faba*	313 321	272 274	177 204	220 220	217 219	311 311	282 282	127 127
318	Stráž nad Nežárkou	49°04'N, 14°54'E	*Vicia faba*	313 313	255 257	177 177	222 224	217 217	309 313	282 291	127 129
Lower Rhine Valley, Switzerland											
401	St. Margrethen	47°27'N, 9°38'E	*Chenopodium album*	307 321	257 274	177 198	220 220	219 219	311 311	282 282	127 134
404	St. Margrethen	47°27'N, 9°38'E	*Chenopodium album*	307 313	272 272	177 177	220 220	217 219	313 313	280 280	129 129
405	St. Margrethen	47°27'N, 9°38'E	*Chenopodium album*	315 317	257 257	167 177	220 220	217 219	311 311	280 282	127 127
407	St. Margrethen	47°27'N, 9°38'E	*Chenopodium album*	315 315	272 272	177 177	218 220	215 215	309 309	280 282	127 127
409	St. Margrethen	47°27'N, 9°38'E	*Chenopodium album*	313 315	257 257	177 177	220 224	219 219	309 309	280 282	127 134

Table S2. Collection information and genotypes at 11 microsatellite loci (Fauvergue *et al.*, 2005; Sandrock *et al.*, 2007) for the two thelytokous lines of *Lysiphlebus fabarum* used in this study.

				Microsatellite locus										
Sample ID	Collection site	Lat., long.	Collected from	Lysi02	Lysi03	Lysi05	Lysi06	Lysi07	Lysi08	Lysi10	Lysi13	Lysi15	Lysi16	Lysi5a12
Lower Valais, Switzerland														
06-201	Sion	46°14'N, 7°21'E	*A. f. cirsiiacanthoides* on *Cirsium arvense*	092 115	167 167	112 122	197 197	183 183	092 094	098 098	119 121	105 105	125 125	176 176
06-212	Sion	46°14'N, 7°21'E	*A. f. fabae* on *Vicia faba*	108 119	167 167	110 110	197 201	183 183	092 094	096 171	121 125	103 103	117 117	176 176
Rennes area, France														
06-272	Vezin le Coquet	48°07'N, 1°45'W	*A. hederae* on *Hedera helix*	098 098	165 167	112 112	195 199	183 183	094 107	123 139	123 125	103 103	117 117	176 176
06-292	Le Rheu	48°06'N, 1°47'W	*A. ruborum* on *Rubus fruticosus*	082 236	165 170	110 112	203 203	183 183	094 094	107 107	121 123	101 101	109 109	176 176
06-297	Le Rheu	48°06'N, 1°47'W	*A. f. cirsiiacanthoides* on *Cirsium arvense*	084 096	165 167	110 120	197 203	183 183	092 109	111 165	119 119	103 103	125 125	174 174
Budweis area, Czech Republic														
06-378	Stráž nad Nežárkou	49°04'N, 14°54'E	*A. f. fabae* on *Vicia faba*	086 113	167 167	110 110	197 201	183 183	092 094	096 181	121 125	103 103	117 117	176 176
06-392	Stráž nad Nežárkou	49°04'N, 14°54'E	*A. hederae* on *Hedera helix*	092 229	161 165	110 112	197 201	183 183	092 107	096 139	125 125	109 109	125 125	176 176
Lower Rhine Valley, Switzerland														
06-610	St. Margrethen	47°27'N, 9°38'E	*A. ruborum* on *Rubus fruticosus*	086 262	165 170	110 112	203 203	183 183	094 094	107 107	121 123	103 103	109 109	174 174
06-623	St. Margrethen	47°27'N, 9°38'E	*A. hederae* on *Hedera helix*	238 238	165 169	112 112	195 195	183 183	094 098	111 135	123 123	107 107	135 135	174 174

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