Intraspecific variation in facultative symbiont infection among native and exotic pest populations: Potential implications for biological control

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ABSTRACT

Facultative bacterial symbionts can provide their host insects with protection from natural enemies. These symbionts are often found at low to intermediate frequencies among hosts in native populations, suggesting that fewer symbiont taxa (and their corresponding defensive properties) may be present in exotic populations, due to founder effects and drift in newly established populations. We tested this hypothesis by collecting aphid species from their exotic and native regions, and conducting diagnostic surveys for four facultative symbionts: Hamiltonella defensa, Regiella insecticola, Serratia symbiotica, and Arsenophonus nasoniae. We did not find fewer symbiont taxa in exotic host populations, but did find substantial intra- and interpopulation variation in symbiont infection. When we incorporated additional records from the literature, we found moderate support for the hypothesis, although few aphid species were sampled sufficiently to be conclusive. Finally, we tested whether laboratory colonies are prone to losing symbiont infection over time. We established four colonies of the cowpea aphid, Aphis craccivora, each initiated with a single aphid clone infected with H. defensa. Through repeated sampling, we found that all four colonies became uninfected over the course of one year. We suggest that symbiont surveys could aid importation biological control introductions by (1) establishing whether recently established exotic pest populations might have reduced symbiont complements and be particularly vulnerable to natural enemies, (2) providing clues on pest provenance, and (3) determining which native pest populations include the same defensive symbionts as the exotic target populations, as these may be the best sources for prospective agents. We also suggest that laboratory cultures of target and nontarget organisms be routinely monitored for symbiont composition, to ensure that laboratory trials produce field-relevant results.

1. Introduction

Invasive species represent ever-increasing ecological and economic threats (Hill et al., 2016). The globalization of human commerce provides extensive routes for accidental or deliberate movement of biological organisms into novel habitats across the planet (di Castri, 1989; Mack et al., 2000). While only a small percentage of these exotic species subsequently establish and spread within their new habitats (Williamson, 1996), those that do can be devastating. Pimentel et al. (2005) estimated that invasive species cost more than $100 billion per year in the US, of which ~$30 billion is attributed to exotic insects. Further ecological costs of invasive species include loss of biodiversity, threat of native species extinction, and alteration or loss of environmental services (Kenis et al., 2008). Some populations of invasive insects have been effectively neutralized by eradication efforts (Myers et al., 2000) or through introductions of predators, parasites and pathogens from the source region of the invasive species (reviewed in Heimpel and Mills, 2017; Van Driesche and Bellows, 1996). However, many invasive species continue to exert major detrimental effects despite substantial investment in control efforts (Kovacs et al., 2010; Simonsen et al., 2008). Given the magnitude of the problems caused by invasive species, it is not surprising that major effort has been invested to better understand invasion dynamics and identify key factors that promote the establishment and spread of introduced species (Hill et al.,...
One aspect of invasive species biology that has received little attention is the role of inherited bacterial symbionts. Maternally-inherited intracellular endosymbionts are common among arthropods (Douglas, 1989; Duron et al., 2008; Henry et al., 2015; Moran et al., 2008), including important pest and beneficial insect species (Floate et al., 2006). Many of these symbionts are facultative from the host’s perspective, infecting only a portion of a population. Facultative symbionts are capable of profoundly influencing their host’s ecology in ways that could affect invasive pest establishment, fitness, and susceptibility to biological control (Desneux et al., 2009; Floate et al., 2006; Zindel et al., 2011). For example, facultative symbionts have been shown to affect reproductive biology and sex ratios (Werren et al., 2008), dispersal (Leonardo and Mondor, 2006), dietary breadth (Tsuchida et al., 2004; Wagner et al., 2015), thermal tolerances (Harmon et al., 2009; Montillor et al., 2002; Russell and Moran, 2006), reproductive output and host survival (Himler et al., 2011; Wade and Chang, 1995; Vorburger and Gouskov, 2011; Weeks et al., 2007), and defense against natural enemies (Hamilton and Perlman, 2013; Oliver and Martinez, 2014). Examples of the latter include symbiont-based defense against potential biological control agents such as parasitoids (Asplen et al., 2014; McLean and Godfray, 2015; Oliver et al., 2003; Schmid et al., 2012; Xie et al., 2010), nematodes (Jaenike et al., 2010), fungi (Lukasik et al., 2013b; Scarborough et al., 2005) and viruses (Hedges et al., 2008; Teixeira et al., 2008). Symbionts can affect both physiological resistance to natural enemies (e.g., Oliver et al., 2003) and host defensive behaviors (Dion et al., 2011a).

In the present study, we focus on two hypotheses regarding facultative symbionts in pest populations that could have potentially important implications for biological control efforts. First, we hypothesized that introduced insect species have fewer facultative symbiont species in their exotic than native ranges. Each host individual can be characterized by “symbiotype,” the presence or absence of one or more heritable facultative symbiont species. Analogous to allelic diversity in nuclear genes, a population bottleneck during colonization may mean that only a subset of the symbiotypes found in the source population are represented in an exotic population (Dlugosch and Parker, 2008; Shoemaker et al., 2000). Furthermore, many symbionts may provide protection against specific natural enemies (Asplen et al., 2014; Martinez et al., 2016) that may not be present in their introduced range. This elimination of benefits combined with fitness costs imposed by facultative symbionts (Fleury et al., 2000; Gwynn et al., 2005; Vorburger and Gouskov, 2011) may result in selection against infected individuals relative to uninfected individuals, given that the latter are able to invest more resources in growth, reproduction, or dispersal. Thus the introduction process may actively select for individuals without (or with fewer species of) facultative symbionts, and the diversity of symbiont species represented in exotic populations may be lower than native populations. Some high profile invasive pests (e.g., Argentine ant, red imported fire ant) have been documented to have a high prevalence of the ubiquitous endosymbiont Wolbachia in their native ranges, yet are almost completely lacking Wolbachia in their extensive invaded ranges (Bouwma et al., 2006; Reuter et al., 2005; Tsutsui et al., 2003). If such patterns of symbiont loss during introduction are widespread across the insects, then exotic pest populations may lose access to the suite of phenotypic traits conferred by facultative symbionts, including defense against natural enemies (Ferrari and Vavre, 2011). To test this hypothesis, we compared the number of symbiont taxa in native versus exotic populations of aphids, a taxon that is rife with both adventive populations and facultative bacterial symbionts (Footitt et al., 2006; Oliver et al., 2010), many of which are defensive (Oliver and Martinez, 2014).

Second, using a similar rationale, we hypothesized that laboratory populations of insects might lose their defensive symbionts under culture conditions. Such losses would be important when laboratory colonies are used to assess host range of potential biological control agents, because mismatch in the presence/absence of defensive symbionts between lab and field populations of the pest could lead to erroneous conclusions regarding the potential efficacy and safety of the agent. It has been established that defensive symbions, in particular, may carry a fitness cost (Dykstra et al., 2014; Schmid et al., 2012), such that symbiont-bearing individuals are at a selective disadvantage in laboratory cultures that are protected from natural enemies (Oliver et al., 2008). Thus colonies that were initially composed of a mixture of differentially infected individuals may eventually shift to be entirely uninfected through selection and drift. Indeed, in 2006, a laboratory colony of Aphis craccivora Koch had 100% prevalence for the symbiont Hamiltonella defensa (Desneux et al., 2009), but a screen one year later (MK Asplen and GE Heimpel, unpublished data) failed to detect this symbiont. It is possible that laboratory colonies initiated with a single clonal individual may be less prone to such an effect; however, vertical transmission of facultative symbionts can be imperfect (e.g., Currie et al., 2015; Dykstra et al., 2014) and even genetically uniform cultures of single aphid clones may eventually lose their symbionts. Here, we evaluated symbiont infection over the course of a year in laboratory colonies initiated with a single infected aphid clone, to validate and qualitatively document the time course of this phenomenon.

2. Methods

2.1. Symbionts in native versus exotic aphid populations

To test whether exotic populations of aphids are less likely to be infected by facultative bacterial symbionts than their native counterparts, we first examined the symbiont infection of paired populations of the same species (“paired populations comparison”), collected from native versus exotic portions of the species’ range. We field-collected aphids directly into 95% ethanol, primarily in the US and Western Europe (Tables S1–S2). We subsequently identified aphids using a combination of morphological keys (Blackman et al., 1994; Blackman and Eastop, 2006; Moran, 1984; Smith and Dillery, 1968) and molecular barcoding using mitochondrial COI sequences (see below). We classified each population as native or exotic based on published distributions (Blackman et al., 1994; Blackman and Eastop, 2006; Foottit et al., 2006). Populations that could not be unambiguously identified to species level were not considered further, except for one instance in which a generic designation was sufficient to classify the population as native or exotic (Monellia genus). In total, we had paired populations from 13 species. As much as possible, we used paired populations that originated from the same host plant species, although for two aphid species (Aphis spiraecola Patch and Rhopalosiphum padi (L.)) only populations collected from different plant species were available. Additionally, two species (Aphis fabae Scopoli and Aphis gossypii Glover) each had two exotic and two native populations that were paired based on host plant. Additional aphid species that had no paired population for comparison (Table S2) were considered in a separate analysis (“non-paired species comparison”) to evaluate whether native species in their native range generally had more symbiont taxa than exotic species within their introduced range.

For each population, we used diagnostic PCR to screen aphids for four facultative symbionts that commonly infect aphids: Hamiltonella defensa, Regiella insecticola, Serratia symbiotica, and Arsenophonus nasoiae, all of which are common in aphids (Henry et al., 2015; Jousselin et al., 2013; Oliver et al., 2010; Russell et al., 2003). Strains of the first three symbionts are known to provide defense to some aphid species (Lukasik et al., 2013b; Oliver et al., 2003; Vorburger et al., 2010), whereas the function of Arsenophonus is unknown in most aphids (Jousselin et al., 2013; Wulf et al., 2013; Wulf and White, 2015; but see Wagner et al., 2015). We randomly chose 8–10 individual aphids per population, surface sterilized with 95% ethanol, and extracted DNA
from each aphid using DNeasy tissue kits (Qiagen, Valencia, CA). As described in Brady et al. (2014) we used diagnostic PCR to determine whether each aphid was infected with each symbiont (primer sets and annealing temperatures in Table S3). PCR products were visualized on a 1% agarose gel stained with Gel-Red (Biotium, Hayward, CA). All PCR sets included DNA from infected specimens as positive controls, as well as negative controls that used extra water rather than specimen DNA in the reaction. All extractions were verified for quality by screening samples for the obligate nutritional symbiont Buchnera aphidicola (Table S3). Samples testing negative for Buchnera were discarded from analysis.

At least one positive individual per population per symbiont was sequenced to verify symbiont identity. PCR products were purified using QIAquick PCR Purification Spin Column Kits (Qiagen, Valencia, CA) and sent to the University of Kentucky AGTC sequencing facility for Sanger sequencing. Similarly, we purified and sequenced aphid mitochondrial COI for molecular confirmation of taxonomic identity. Identification of symbionts and aphid species was based on comparison to NCBI and/or barcode of life (www.boldsystems.org). For endosymbiont verification, sequences with greater than 95% similarity to known insect endosymbiont taxa were considered positive at the generic level. Identification by sequence comparison has been deposited in GenBank under accession numbers KT336569-KT336597. In a few instances, we identified symbionts other than the diagnostic target using this procedure; these additional symbionts were included in subsequent analyses because the probability of detecting them would have been equivalent in all samples. For populations where symbiont infection of the initially-sequenced specimen could not be validated, we sequenced additional individuals and/or used different primer sets (Table S3) until we could classify each individual as infected or uninfected. This diagnostic-based protocol may underestimate symbiont diversity, but enables making statistical comparisons between native and exotic populations.

For the paired populations, we tallied the number of different facultative endosymbiont taxa detected per population, and compared the number of symbionts in paired native vs. exotic populations using a sign test (SPSS v. 20). For the non-paired populations, we counted the number of facultative symbiont taxa detected per aphid species. Three aphid species had multiple populations, but we only used a single population per species in the analysis (in all three species, populations were invariant in symbiont distribution). We used a generalized linear model to compare the number of symbiont taxa in native versus introduced species by assuming a Poisson distribution of symbiont counts per species for each region, and designating region of collection (native vs. exotic) as a fixed factor (SPSS v. 20).

Finally, we conducted an extensive literature search to find additional native and exotic aphid populations that had been surveyed for symbionts, to increase the size of the dataset. For this comparison, we considered only the three most commonly screened aphid symbionts: H. defensa, R. inseictola, and S. symbiotica. To be included in the dataset, aphids had to be collected from a known location that could be classified as native or exotic. We identified 13 additional aphid species that had at least one aphid screened from both native and exotic locales, and then repeated the sign test with the expanded dataset. It should be explicitly recognized that this is an extremely simple analysis for a very complicated dataset, which does not incorporate differences in sample size, population structure, detection methodology, etc. However, our intent is not to provide a definitive analysis based on current, disjointed data, but rather to provide a qualitative overview of available data to inform and focus future research efforts on this topic.

2.2. Laboratory loss of symbionts

To test whether a clonally uniform colony of aphids would lose its symbiont infection over time under laboratory conditions, we collected wild Aphis craccivora clones from the Spindletop Research Farm (Lexington, Fayette Co., KY) in September 2010. Individual foundress clones were propagated on leaf disks of Vicia faba L. embedded in 1% agar in 35 mm petri dishes. For each dish, three offspring aphids were chosen for DNA extraction. We used a Chelex DNA extraction protocol as described in Wulff et al. (2013), and screened the clones for H. defensa using diagnostic primers (Brady and White, 2013). We identified 4 aphid sub-clones that were infected with H. defensa and initiated a separate colony for each; three of these originated from the same foundress and were identical with respect to host and symbiont genomes (Line 17), whereas the fourth colony originated from a separate foundress (Line 17). In North America, alfalfa-infesting A. craccivora shows remarkably little genetic variation over wide geographic regions (Angelella, 2015; Brady et al., 2014; Dykstra et al., 2014; Wagner et al., 2015), thus all four colonies may have been identical, but representative of a widespread and important “superclone” (Harrison and Mondor, 2011; Vorburger et al., 2003). The replicate aphid colonies were reared on V. faba plants in microcosm plastic cages that had mesh panels for ventilation (Wulff et al., 2013). The four clones were maintained separately on single plants for one year in laboratory conditions (22 ± 1 °C, LD 16:8). A subsample of ~100 aphids (mixed instars) was transferred to a fresh plant every 3 weeks. A total of 10 samples of eight individual aphids per clone were taken at approximately monthly intervals over the year. We extracted DNA and tested the aphids for the presence of H. defensa as described above.

3. Results

3.1. Symbionts in native versus exotic aphid populations

Among the aphid species included in the paired populations comparison, we found that 7/13 (54%) were infected with one or more of the four symbionts for which we screened (Table 1). However, we did not identify consistent patterns of symbiont species loss in exotic versus native locales within this set of paired populations (Fig. 1A; sign test P = 0.18). Only a single species (Aphis spiraecola) showed the predicted pattern of having a symbiont in the native population that was lacking in the exotic population. The other six infected species either had the same symbiont in both native and exotic populations (Macroisiphum rosae and one paired population of Aphis gossypii), had a symbiont in the exotic population but not the native (Aphis pomi, Brevicoryne brassicae, and the other paired population of A. gossypii) or had different symbionts in native versus exotic populations (Macroisiphum euphorbiae and one paired population of Aphis fabae). Most infected populations (9/12 = 75%) had variation in infection status; i.e., some aphids from the same population were infected with a facultative symbiont and some were not. Only a single aphid species (Macroisiphum rosae (L.)) had all individuals in both populations uniformly infected with the same symbiont (Serratia).

In the non-paired populations comparison, we detected two symbionts in addition to the four bacteria we specifically screened for (Table 2), due to false-positive diagnostic screens that were subsequently allocated to the proper symbiont upon sequence validation. Two aphid species (Cinara strobi (Fitch) and Monellipis caryae (Monelli)) were infected with a symbiont in the genus Sodalis, which has previously been reported in aphids (Burke et al., 2009). One aphid species, Drepanaphis carolensis Smith, was infected with a bacterium that was aligned to a variety of bacterial endosymbionts of insects at 93% sequence similarity (e.g., AB576932, AY334375, DQ115535), suggesting that the symbiont in D. carolensis is a novel endosymbiotic lineage that has not previously been described for aphids. We included these unexpected symbionts within the native/exotic comparison, because they were discovered using the diagnostic primer sets, and we were equally likely to detect them in all surveyed specimens.

We did not find any difference in number of symbiont taxa between native and introduced species in the non-paired population comparison (Fig. 1B; Wald = 1.42, P = 0.23). Fifty-eight percent of native species were infected with at least one endosymbiont, versus 35% of exotic
species. Populations of two native species (*Periphyllus negundinis* (Thomas) and *M. caryae*) and one exotic species (*Prociphilus fraxinifolii* (Riley)) had multiple symbionts represented; *Pe. negundinis* and *Pr. fraxinifolii* had individual aphids infected with more than one symbiont, whereas *M. caryae* had a mixture of individuals with different symbiont infections. Of the 18 species that were symbiont-infected, half of the species were uniformly infected with the same symbionts across all sampled aphids, and half showed within-population variation in symbiont infection.

When we expanded the paired population comparison to include

<table>
<thead>
<tr>
<th>Species</th>
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<td><em>Aphis fabae</em> (Cirsium)</td>
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<td><em>Dysaphis plantaginea</em> (Malus)</td>
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<td><em>Eriosoma lanigerum</em> (Malus)</td>
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<td><em>Eucallipterus tiliae</em> (Tilia)</td>
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<tr>
<td><em>Macrosiphum rosae</em> (Rosa)</td>
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<tr>
<td><em>Therioaphis trifolii</em> (Medicago)</td>
<td>KT336573-4</td>
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</table>

N = Aphids screened per population; all passed extraction quality control screen for the obligate symbiont *Buchnera*. 
* Ars = *Arsenophonus*, Ham = *Hamiltonella*, Reg = *Regiella*, Ser = *Serratia*, Un = uninfected. Because sequences were confirmed at 95% similarity, symbiont strains are identified to bacterial genus, not species.

Native and exotic populations on the same line were collected from the same host plant (indicated in parentheses); native and exotic populations on adjacent lines were collected from different host plants.

**Table 1.** Facultative symbiont distribution and prevalence in aphids from native versus exotic populations.
data extracted from the published literature, we found modest support for the hypothesis that exotic populations of aphids are infected with fewer symbiont taxa than native populations (Fig. 1C; sign test \( P = 0.03 \)). Of the 29 species included in the analysis (Table 3), 20 (69%) were infected by at least one of the three symbionts. Eleven aphid species (38%) had more symbiont species represented in the native locales, five (17%) had more in the exotic locales, four (14%) had the same symbionts represented in both locales, and the remaining nine species (31%) were uninfected in both locales. Thus, the majority of aphids showed different symbiont profiles between native and exotic regions. However, sampling effort was often low, and sometimes unequal between the native and exotic ranges, suggesting that greater similarity between native and exotic populations may be evident with greater sampling effort. For example, in Aphis fabae, approximately 500 native-range aphid specimens were surveyed, versus only 18 exotic-range specimens (Table 3). For this species, it is unsurprising that Serratia symbionts were not detected in the exotic range, particularly given that they were only present in 10% of native-range specimens. In contrast, Serratia appears to have been lost from exotic Sitobion avenae (Fabricius), at least within the screened Chilean populations (Sepúlveda et al., 2016). Serratia was not detected in more than 200 exotic-range aphids, despite being found in greater than 50% of aphids screened from the native range.

### 3.2. Laboratory loss of symbionts

Within a year, all four A. craccivora colonies, each of which had been initiated with a single Hamiltonella-infected aphid clone, were uniformly uninfected (Fig. 2). The three clones that originated from the same foundress (line 21) each remained infected for at least 6 months, with only one of the three showing less than 100% infection at any point within this timeframe. Then, at 6, 10, and 11 months respectively, each of the replicate colonies completely lost infection within the next 1–2 months. The fourth colony, initiated with a different but likely very similar clone (17A), had less than 100% Hamiltonella infection starting in the first month, but did not completely lose the infection from the colony until the 9th month.

### 4. Discussion

In the present study we have substantially contributed to the body of knowledge regarding intraspecific variation in facultative endosymbiont infection in aphids on a global scale (Vorburger, 2017). In screening over 50 aphid species, we found that nearly half were infected with one or more facultative symbionts, a percentage that is consistent with previous studies (e.g., Henry et al., 2015; Jousselin et al., 2013; Russell et al., 2013). Our study further establishes that...
Table 3
Facultative symbiont distribution and prevalence in aphids from native versus exotic populations, including data gathered from published literature.

<table>
<thead>
<tr>
<th>Aphid Species</th>
<th>Native</th>
<th>Exotic</th>
<th>References</th>
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<tr>
<td>Uroleucon sonchi</td>
<td>1/12</td>
<td>1/2</td>
<td>2, 5, 7, 14</td>
</tr>
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</table>

References: 1 = Chen and Purcell (1997); 2 = Sandstrom et al. (2001); 3 = Tsuchida et al. (2002); 4 = Haynes et al. (2003); 5 = Russell et al. (2003); 6 = Carletto et al. (2008); 7 = Degnan and Moran (2008); 8 = Burke et al. (2009); 9 = Frantz et al. (2009); 10 = Ferrari et al. (2012); 11 = Bilodeau et al. (2013); 12 = Peccoud et al. (2014); 13 = Russell et al. (2013); 14 = Henry et al. (2015); 15 = Sepúlveda et al. (2016); 16 = Tsuchida et al. (2005); 17 = Tsuchida et al. (2006); 18 = Brady et al. (2014); 19 = Chandler et al. (2008); 20 = Vorburger et al. (2009); 21 = Jones et al. (2011); 22 = Lukasik et al. (2011b); 23 = Luo et al. (2016); 24 = Telesnicki et al. (2012).

* ps = includes data from present study.

** Bold represents a symbiont detection in one locale (native/exotic) but not the other.

*** In some instances, symbiont screens did not include all three symbionts in both locales. Only symbionts that were screened in both locales were included in table and corresponding analyses.

Fig. 2. Proportion infection by Hamiltonella defensa in four replicate Aphis craccivora laboratory colonies over time. Each colony originated from a single infected foundress. Three of these (21A, 21B, and 21C) were genetically identical to one another whereas the fourth colony (17A) originated from a different field collected aphid, which may or may not have genetically differed from the other three.

Intrapolation variation in symbiont infection status is common; in species infected by facultative symbionts, we usually found a mixture of aphids with different symbiotype, even within the same population. Where symbionts are defensive, such a pattern would suggest that a population can respond rapidly to the selection pressure imposed by the presence or absence of relevant natural enemies (Oliver et al., 2008; Zytenska and Weisser, 2016).

We found only modest support for our hypothesis that exotic populations of introduced species will have fewer facultative endosymbiont taxa than their native counterparts. Indeed, our sampling of paired populations of the same aphid species found no significant association between status as native versus exotic and number of facultative symbiont taxa. Our methodology limited the number of populations surveyed per species, and the number individual aphids (8–10) sampled per population. While the intent was to control for sampling depth, specimen identification, and symbiont detection, this strategy limited our power to characterize heterogeneously infected species, particularly when some endosymbionts are typically found at low frequency, spatially structured, and/or associated with particular biotypes of the host aphid species (Brady et al., 2014; Peccoud et al., 2015; Wagner et al., 2015; Sepúlveda et al., 2016). When we incorporated data from the literature, a pattern of fewer symbionts in exotic populations emerged, but this result should be viewed with caution for several reasons. This analysis compared all native versus all exotic locales per aphid species, masking underlying heterogeneity within each region, and did not account for differential sampling effort between ranges. In a number of species (e.g., A. craccivora, Eucaliptus tiliae (Linnaeus), Macrosiphum euphorbiae (Thomas)) the missing symbiont(s) from one range showed a low frequency of infection in the other range. More sampling may have detected the symbiont, or the low frequency of infection in the native habitat may have increased the likelihood of loss during the process of introduction, similar to founder effects seen with rare alleles for Mendelian traits (Dlugosch and Parker, 2018).
2008; Nei et al., 1975). Thus these low-frequency symbionts are the most likely to be absent from exotic range populations. Unfortunately, very few species have been sampled deeply enough to draw reasonably confident conclusions regarding symbiont absence. As also discussed by Vorburger (2017), additional efforts in characterizing symbiont diversity among global aphid populations would be of great utility for addressing this and other hypotheses of relevance to biological control.

Rather than look for broad patterns across many shallowly sampled species, it is perhaps more informative to look more closely at a few species that have been sampled deeply. *Acyrthosiphon pisum* (Harris) is the most widely surveyed aphid, in which facultative symbiont infection frequency varies dramatically among host-plant-associated biotypes (Ferrari et al., 2012). When viewed globally, frequency of symbiont infection is roughly equivalent between exotic and native locales (Table 3), but specific exotic locations have reduced frequency of some symbionts (e.g., S. America, Sepúlveda et al., 2016), likely reflecting the fact that only a subset of aphid biotypes are represented in these locales (Peccoud et al., 2008). Similarly, in *A. craccivora* the symbiont *H. defensa* is particularly associated with alfalfa-feeding clones of the aphid (Brady and White, 2013; Brady et al., 2014); it is suspected that a cryptic introduction of alfalfa-feeding *A. craccivora* occurred in North America in the 1980s (Foottit et al., 2006), which presumably also is when *H. defensa*-infected aphids first appeared in the region. Recent developments in invasion genetics suggest that the genetic bottlenecks experienced by introduced populations likely become eroded over time through the action of gene flow, thanks to complex and cryptic movement of individuals among global populations (Dlugosch and Parker, 2008; Garnas et al., 2016). It seems likely that symbiont diversity in introduced populations may follow a similar trajectory, with founder effects winnowing symbiont diversity shortly after introduction, but subsequent cryptic introductions adding facultative symbionts to exotic populations over time. Thus, relatively recent introductions, such as S. avenae in S. America, may still have fewer symbiont species relative to native populations (Henry et al., 2015; Lukasik et al., 2013a; Sepúlveda et al., 2016), but more long-established exotic populations have had a greater time period to have acquired differentially infected individuals from the native range, even individuals infected with rare symbionts (e.g., *A. pismum* on alfalfa; Ferrari et al., 2012; Russell et al., 2013).

If populations of recently introduced invasive species have diminished symbiont diversity, then this would provide a window of opportunity for importation biological control. Given that many facultative symbiont strains have been shown to provide protection against subsets of potential enemies (Asplen et al., 2014; Jaenike et al., 2010; Lukasik et al., 2013b; Martinez et al., 2016; Mateos et al., 2016; McLean and Godfray, 2015; Rouchet and Vorburger, 2014), then these populations would have a reduced arsenal available for defense against enemies, increasing the likelihood of successful control. Widespread symbiont screening of both exotic and native pest populations as early as possible in the invasion process could therefore identify and potentially exploit transient reductions in symbiont complement, and correlated defensive ability. Such an effort might also provide clues as to pest provenance through presence or absence of facultative symbiont strains relative to various source populations (von Dohlen et al., 2013). When defensive symbionts are detected in the invading population, prospective agents should be sought in association with native populations that include the same symbionts, because these enemies with prior exposure may have evolved countermeasures to these microbially mediated defenses (Dion et al., 2011b; Oliver et al., 2012; Rouchet and Vorburger, 2014).

Similarly, symbionts should be taken into consideration during laboratory screening of potential biological control agents. There is already literature on inadvertent laboratory selection and the potential pitfalls of using laboratory cultures as analogs for field populations (Hopper et al., 1993). In the present study, the defensive symbiont *H. defensa* was lost very quickly from laboratory culture of *A. craccivora*, even when the colonies were initiated with genetically uniform clones. In pea aphids, while *H. defensa* can be stably maintained for long periods, a bacteriophage (APSE) that is required for symbiont protection is repeatedly lost leading to the breakdown of the defensive mutualism (Oliver et al., 2009; Weldon et al., 2013). In general, vertical transmission rates and constitutive costs of infection determine how quickly symbionts are removed from the population. Even slightly less-than-perfect symbiont transmission, as seen in *A. craccivora* (Dykstra et al., 2014), may result in rapid loss of symbiont-infected individuals from the population, if uninfected individuals are more fit than their infected counterparts (Oliver et al., 2008). Colonies initiated with populations of mixed infection status are even more likely to quickly shift composition, potentially diverging in a substantial way from their field counterparts (Luo et al., 2016).

From the perspective of biological control introductions, such divergence between field and laboratory populations is worrisome. If laboratory populations lose defensive symbionts, then host suitability tests conducted with lab populations may yield false positives: a prospective agent is able to successfully attack a host in a laboratory trial (because the host has lost its defensive symbionts), but would be less effective in the field (where defensive symbionts are present in some members of the population). When the host in question is a non-target species that is incorporated in host range testing experiments, a false positive lab assay might suggest greater non-target effects than would actually occur in the field. Perceived danger of non-target effects in such cases could lead to rejecting a potentially viable agent, even though defended field populations may actually be in little danger from the prospective agent.

If possible, we recommend that populations of target invasive pests should be evaluated for the presence of defensive endosymbionts in association with foreign exploration for potential biological control agents. Next-generation sequencing and indexed barcodes that allow extensive multiplexing have made it simple and relatively inexpensive to quickly screen bacterial diversity from hundreds of insect specimens (Fadrosh et al., 2014; Jousselin et al., 2016; Koizich et al., 2013). If endosymbiotic bacteria are detected, then an inexpensive diagnostic PCR protocol could be adopted to determine the distribution of symbionts across pest populations in the exotic and native ranges. Symbiont status of laboratory colonies could also be monitored, and colony composition could be adjusted to match various target populations. Once a prospective agent has been selected and host range testing commences, then symbiont screens of field versus laboratory populations of non-target species would also be appropriate.
References


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physiologist who do not walk alone. BMC Biol. 6, 27.


1803–1807.


