



Research

Cite this article: Wooding AL, Wingfield MJ, Hurley BP, Gamas JR, de Groot P, Slippers B. 2013 Lack of fidelity revealed in an insect–fungal mutualism after invasion. *Biol Lett* 9: 20130342.
<http://dx.doi.org/10.1098/rsbl.2013.0342>

Received: 15 April 2013

Accepted: 13 May 2013

Subject Areas:

ecology, evolution

Keywords:

mutualism, insect–fungus symbiosis, symbiont fidelity, invasion, *Sirex* woodwasp

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2013.0342> or via <http://rsbl.royalsocietypublishing.org>.

Lack of fidelity revealed in an insect–fungal mutualism after invasion

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Symbiont fidelity is an important mechanism in the evolution and stability of mutualisms. Strict fidelity has been assumed for the obligate mutualism between *Sirex* woodwasps and their mutualistic *Amylostereum* fungi. This assumption has been challenged in North America where the European woodwasp, *Sirex noctilio*, and its fungal mutualist, *Amylostereum areolatum*, have recently been introduced. We investigate the specificity of the mutualism between *Sirex* and *Amylostereum* species in Canada, where *S. noctilio* co-infests *Pinus* with native *Sirex nigricornis* and its mutualist, *Amylostereum chailletii*. Using phylogenetic and culture methods, we show that extensive, reciprocal exchange of fungal species and strains is occurring, with 75.3 per cent of *S. nigricornis* carrying *A. areolatum* and 3.5 per cent of *S. noctilio* carrying *A. chailletii*. These findings show that the apparent specificity of the mutualism between *Sirex* spp. and their associated *Amylostereum* spp. is not the result of specific biological mechanisms that maintain symbiont fidelity. Rather, partner switching may be common when shifting geographical distributions driven by ecological or anthropogenic forces bring host and mutualist pairs into sympatry. Such novel associations have potentially profound consequences for fitness and virulence. Symbiont sharing, if it occurs commonly, may represent an important but overlooked mechanism of community change linked to biological invasions.

1. Introduction

A frequently cited consequence of globalization is the growing homogenization of biotic communities, commonly driven by biological invasions. Invasive species can have serious negative impacts on the ecosystems in which they become established. Such impacts include invasive species altering existing mutualisms among native species, or acquiring novel symbionts that affect virulence in one partner [1–3].

In the Southern Hemisphere, the invasive wood-boring wasp *Sirex noctilio* and its obligate nutritional fungal mutualist *Amylostereum areolatum* is a highly aggressive pest complex infesting and killing healthy plantation pines [4]. In its native range in Eurasia and North Africa, this complex is a secondary pest, infesting dead or dying conifers, primarily in the genus *Pinus* [5]. The complex has recently been introduced into eastern North America (ENA), where it poses a potential threat to planted and natural pine forests [6].

The introduction of *S. noctilio* into ENA provides an opportunity to study the specificity of the mutualism between *Sirex* and *Amylostereum* species. This mutualism was until recently assumed to be highly specific as a result of fungal mutualists being vertically transmitted as asexual spores [7]. This dogma has been questioned recently with the discovery of specimens of native *S. nigricornis* and *S. nitidus* carrying *A. areolatum* [8].

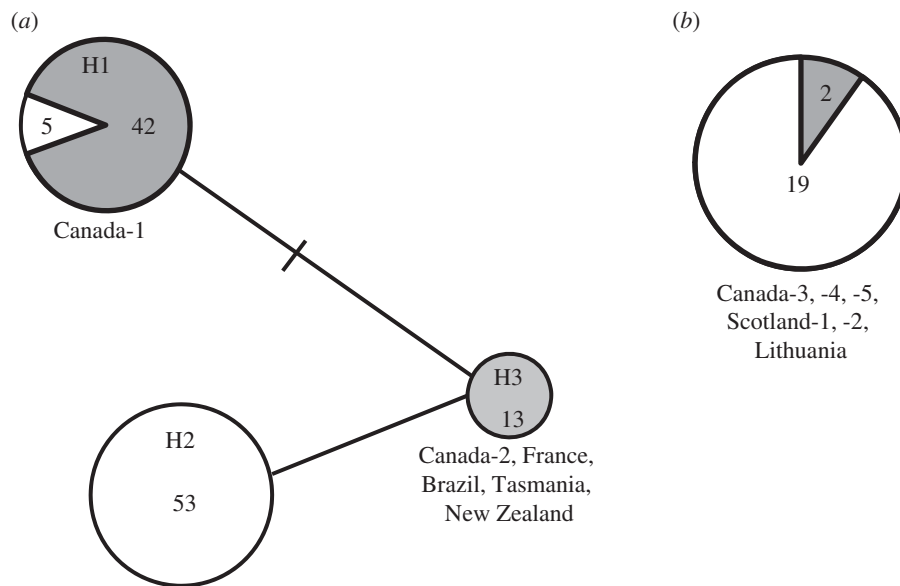


Figure 1. (a) Haplotype network of *Amylostereum areolatum* ($n = 113$). Numbers of isolates per wasp species are shown within haplotype nodes. Dark grey indicates samples isolated from *Sirex noctilio*, white from *S. nigricornis*. (b) Haplotype of *A. chailletii* isolates. Connecting lines specify one substitution, and the crossbar specifies an additional substitution separating haplotypes. Below the nodes are countries from which the haplotypes have previously been sampled (accession numbers in the electronic supplementary material, S11).

We investigate the specificity of the *Sirex*–*Amylostereum* mutualism in invasive and native populations in Canada. In this study, we question whether native *S. nigricornis* and invasive *S. noctilio* are strictly associated with their known symbionts, *A. chailletii* and *A. areolatum*, respectively. We further examine evidence for recent exchange of *A. areolatum* strains between *S. nigricornis* and *S. noctilio* by identifying shared clonal lineages of *Amylostereum* in native and invasive wasp populations.

2. Material and methods

A collection of 134 *Sirex* woodwasps and their mutualistic fungi, isolated from female mycangia (as described in [9]), was obtained from collaborators in Canada (see the electronic supplementary material, S1 and S2 for sampling locations and storage details). Wasp and fungal samples were identified to species using sequence data from the mitochondrial cytochrome *c* oxidase subunit I (COI) and mitochondrial small subunit (mtSSU) genes, respectively (see the electronic supplementary material, S3 and S4). The internal transcribed spacers (ITSs) and intergenomic spacer (IGS) regions of the rRNA locus were sequenced for representative samples of each *A. areolatum* mtSSU haplotype (electronic supplementary material, S5), to compare them with isolates from previous studies which produced multilocus genotypes (MLGs). Where cloning was necessary, the Promega pGEM-T Easy Vector System was used. PCR products were sequenced on an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems) at the sequencing facility of the University of Pretoria.

Bidirectional sequences were assembled and edited in CLC MAIN WORKBENCH v. 6.6.2 (CLC Bio Inc., Denmark) and aligned using MEGA v. 5 [10] and MAFFT [11]. Sequence evolution models were selected using AIC in jModelTest v. 0.1.1 [12]. Species identification was based on group membership in neighbour-joining trees constructed in PAUP v. 4.0 [13]. Haplotype analysis was carried out using SPLITSTREE4 [14]. Maximum-likelihood (PHYML [15]) and Bayesian (MRBAYES [16]) approaches were used for phylogenetic analysis of ITS sequence data from representative samples. Laboratory methodologies, sequence evolution models and program parameters can be found in the electronic supplementary material, S6–S8.

Vegetative incompatibility assays using randomly selected representative samples from each identified *A. areolatum* mtSSU

haplotype were performed using established methods to determine vegetative compatibility groups (VCGs; electronic supplementary material, S9; [17]). VCG richness was then used as an additional measure of genetic diversity.

3. Results

COI sequencing identified 77 *S. nigricornis* and 57 *S. noctilio* specimens. Fungal species switching has occurred in Canadian siricid populations with 75.3 per cent of *S. nigricornis* females carrying *A. areolatum* ($n = 58$), and 3.5 per cent of *S. noctilio* females carrying *A. chailletii* ($n = 2$; electronic supplementary material, S10). We detected a single mtSSU haplotype for *A. chailletii* and three mtSSU haplotypes for *A. areolatum*, which differed by a maximum of two base pairs (figure 1). Two of the *A. areolatum* haplotypes, H3 and H2, were uniquely detected in *S. noctilio* and *S. nigricornis*, respectively, whereas the third (H1) was carried by both species.

Representative *A. areolatum* isolates for which we obtained MLGs grouped into two clades (A and B), based on ITS sequence data (see table 1 and electronic supplementary material, S12). Both MLG1 and MLG3 corresponded with previously isolated samples (MLG3 and MLG2 in Bergeron *et al.* [18], respectively). MLG2 was unique in this study, although ITS (MLG2a and MLG2b) and IGS sequences (MLG2a) have been previously isolated [8,19].

Multiple VCGs were identified within each mtSSU haplotype. VCG richness was high; 14 VCGs were identified from 27 isolates of *A. areolatum*. Ten isolates were incompatible with all others, and up to five distinct VCGs were isolated from wasps emerging from the same tree. One VCG was shared between *S. noctilio* and *S. nigricornis*, confirming recent lateral transfer of strains between invasive and native Canadian *Sirex* populations.

4. Discussion

The identification of a shared MLG and VCG of *A. areolatum* between newly sympatric *Sirex* species strongly supports

Table 1. Multilocus genotypes (MLGs) of representative *Amylostereum areolatum* samples in comparison to previously sampled *A. areolatum* isolates.

genotype	mtSSU – ITS profile	IGS profile	mtSSU previously sampled	ITS previously sampled	IGS previously sampled	region
MLG1	H1-A	D	Bergeron <i>et al.</i> [18]	Bergeron <i>et al.</i> [18]	Bergeron <i>et al.</i> [18] and Hayek <i>et al.</i> [19]	ENA, Southern Hemisphere
MLG2a ^a	H2-B	BE	no	Nielsen <i>et al.</i> [8]	Nielsen <i>et al.</i> [8] and Hayek <i>et al.</i> [19]	USA
MLG2b ^a	H2-B	E	no	Nielsen <i>et al.</i> [8]	no	USA
MLG3a ^a	H3-A	BD	Bergeron <i>et al.</i> [18]	Bergeron <i>et al.</i> [18]	Bergeron <i>et al.</i> [18] and Hayek <i>et al.</i> [19]	ENA
MLG3b ^a	H3-A	D	Bergeron <i>et al.</i> [18]	Bergeron <i>et al.</i> [18]	Bergeron <i>et al.</i> [18] and Hayek <i>et al.</i> [19]	ENA

^aa and b represent IGS profiles.

direct lateral transfer of symbionts. This transfer is bidirectional, as approximately 3 per cent of *S. noctilio* females carried *A. chailletii*, but is skewed towards *A. areolatum* transfer to *S. nigricornis*. This skewed directionality of transfer could result from temporal patterns in oviposition and emergence, or disproportionate utilization of *S. noctilio*-weakened trees by *S. nigricornis*.

The lack of host–symbiont fidelity detected in this study, also shown in an independent concurrent study [19], calls into question the mechanisms maintaining the apparent fidelity of symbiont associations in the native range of *S. noctilio*. It is possible that geographical, host or temporal segregation among native siricids may be sufficient to maintain the low rates of transfer observed in Europe and elsewhere [5]. Alternatively, fungal and or wasp misidentification and sparse sampling across Europe could have led to underestimates of symbiont switching [6]. We propose that the process of invasion of *S. noctilio* into Canada has facilitated symbiont switching among *S. noctilio* and *S. nigricornis*. The sequence data produced in this study (GenBank; electronic supplementary material, S3–S5) will serve as an important reference for further studies examining the mechanism of horizontal symbiont transfer among siricid woodwasps.

Symbiont transfer is stable over at least one generation; 54 of 58 *S. nigricornis* females carrying *A. areolatum* emerged from trees where no *S. noctilio* emerged in the sampling season. Similarly, *S. noctilio* specimens carrying *A. chailletii* emerged from logs that were not infested with *S. nigricornis*. However, these wasps could have entered trees pre-infected via wind dispersed spores (as can occur with *A. chailletii* in Europe [20]), un-emerged woodwasp infestation or aborted woodwasp attacks. Important questions which should be addressed by further studies are whether carrying the ‘wrong’ symbiont influences wasp or fungal fitness, and whether novel symbiont associations are stable over longer time periods. It is evident, however, that fungal switching increases opportunities for the fungi to spread by increasing the pool of potential vectors.

Previous studies have shown that diversity of *A. areolatum* in the Southern Hemisphere is low, with only two VCGs identified [17]. We identified 14 VCGs demonstrating higher than expected diversity in an invaded area, even higher than in the putative native range in Northern Europe [20]. This

diversity could reflect more than one introduction of the fungus into ENA, including the possibility of introduction prior to the *S. noctilio* invasion, together with *S. juvencus* [21]. The high amount of VCG diversity could also be influenced by sexual reproduction of the fungus in ENA. However, fruiting bodies of *A. areolatum* have not been reported in North America, and are rare in the native European range [17].

The detection of an *A. areolatum* MLG unique to Canada supports the results of Nielsen *et al.* [8] and Bergeron *et al.* [18], who identified *A. areolatum* isolates unique to ENA. These findings suggest that this genotype could have been introduced from a previously unsampled *S. noctilio* source population, as identified by a recent analysis of a global *S. noctilio* collection [22], or that ENA harbours an unsampled native *A. areolatum* population. A concurrent study of woodwasp–fungal fidelity in eastern USA revealed that two native woodwasps, *S. nigricornis* and *S. nitidus*, carried *A. areolatum* in their mycangia [19]. The majority of these fungal isolates were shown to contain IGS type BE, which was identified in MLG2 in this study. MLG2 was associated with 55 per cent of *A. areolatum* carrying *S. nigricornis* specimens. This IGS type and IGS type E unique in the present study have been identified exclusively from ENA. This lends further support to the hypothesis of a native population *A. areolatum* in ENA.

The ecological and evolutionary consequences of symbiont switching in the *Sirex*–*Amylostereum* mutualism are not known but could be significant [1]. One dramatic possibility is that symbiont switching could induce changes in wasp virulence with respect to their ability to attack and kill healthy host trees [3]. Undoubtedly, the potential threat of the *S. noctilio*–*A. areolatum* complex to native and commercial forest ecosystems in ENA is more complex than might previously have been anticipated. Given the specificity of interactions between native siricids and their parasites, these relationships could also be altered by symbiont switching [6]. The discovery of symbiont switching at considerable frequency also calls into question the wisdom of importing foreign strains of the nematode *Deladenus siricidicola*, which feeds on *A. areolatum* during part of its life cycle, as a biological control agent [6], as the nematode could easily escape into native siricid populations. The lack of specificity observed between *Sirex* and *Amylostereum* species after the invasion of *S. noctilio* into ENA highlights a

need to reassess the specificity of the mutualism in Eurasia and North Africa.

We thank members of the Tree Protection Cooperative Programme (TPCP), the Department of Trade and Industry (DTI), THRIP

programme and the National Research Foundation (NRF) of South Africa for support. We also thank our collaborators, Mr Chuck Davis (Natural Resources Canada, Canadian Forest Service) for providing the samples used in this study, and Sarah Drabble (Ontario Ministry of Natural Resources) for monitoring emergence of *Sirex* specimens. The authors declare no competing interests.

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Correction

Cite this article: Wooding AL, Wingfield MJ, Hurley BP, Garnas JR, de Groot P, Slippers B. 2013 Lack of fidelity revealed in an insect–fungal mutualism after invasion. *Biol Lett* 9: 20130609.
<http://dx.doi.org/10.1098/rsbl.2013.0609>

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Biol. Lett. 9, 20130342 (23 August 2013; Published online 5 June 2013) (doi:10.1098/rsbl.2013.0342)

The name of the first author of reference [19] from this article was incorrectly listed as Hayek AE. The reference should read:

Hajek AE, Nielsen C, Kepler RM, Long SJ, Castrillo L. 2013 Fidelity among *Sirex* woodwasps and their fungal symbionts. *Invert. Microbiol.* 65, 753–762. (doi:10.1007/s00248-013-0218-z).

Table 1 should therefore read as follows:

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^aa and b represent IGS profiles.