Experimental admixture among distinct lineages of *Deladenus siricidicola*, the biocontrol agent of *Sirex noctilio*

Katrin N.E. Fitza[a,*], Jeff Garnas[b,c] and Bernard Slippers[a]

[a]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa
[b]Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa
[c]Department of Natural Resources and the Environment, University of New Hampshire, NH, USA

*Corresponding author. katrin.fitza@fabi.up.ac.za

Highlights

- This study is the first to test experimental admixture among *Deladenus siricidicola* lineages.
- Lineages were shown to interbreed using microsatellites to confirm admixture in offspring.
- The offspring from one cross showed significantly higher growth rate compared to parental strains.
- An opportunity exists to introduce more genetic diversity into biological control programs using admixture between distinct lineages.

Abstract

Deladenus siricidicola is a principal biological control agent used to suppress populations of the globally invasive pine pest, the woodwasp *Sirex noctilio*. Previous studies have reported low genetic diversity in *D. siricidicola* populations in biological control programs in the Southern Hemisphere and identified two additional, distinct lineages in North America and Spain. In this study, we tested the ability of these three lineages to interbreed and produce viable offspring. We used microsatellite markers to confirm admixture in offspring. Secondly, we compared growth rates among parental and admixed replicates on four isolates of the *Amylostereum areolatum* fungus on which nematodes typically feed in their asexual, non-parasitic phase. We show that all the lineages were capable of interbreeding and that admixture was asymmetric (i.e., skewed towards one of the parents). The offspring from one of the crosses showed significant variation in growth rate on different isolates of *A. areolatum*, compared to the parental isolates, but specifically on the slowest growing fungal isolate. Our results pave the way for the strategic introduction of genetic diversity into biological control programs and also inform expectations of accidental introductions of *D. siricidicola* into new regions.

Keywords

Hymenoptera
Genetic diversity
Nematoda
Invasive pest
Population genetics

1. Introduction

Biological control is an important tool for the management of invasive pest insects (Heimpel and Mills, 2017). Given the increasingly global distribution of many pests of agriculture and forestry (Wingfield et al., 2008), the same biological control agents are frequently applied across regions or even continents as a management tool. During collection and rearing, biological control agent populations very likely undergo population bottlenecks, drift and inbreeding, as well as selection to laboratory conditions (Yek and Slippers, 2014). As a result, biological control agent populations are often likely to have reduced additive genetic variation, which can (but decidedly does not always, ”Garcia-Rossi et al., 2003, Garnas et al., 2016, Hufbauer, 2001, Mills, 2017, Rasmussen et al., 2018, Salt and van den Bosch, 1967″) result in reduced adaptive potential “Roderick and Navajas, 2003″. A lack of adaptive potential of the biological control agent could in turn limit fitness in novel environments or response to variable host phenology of defences, among myriad other selective pressures (Fowler et al., 2015).

Numerous authors have highlighted reduced genetic diversity in introduced natural enemy populations as a potential threat to biological control programs (Hufbauer, 2002, Phillips et al., 2008, Tomasetto et al., 2018, Tomasetto et al., 2017). Until recently, there was limited evidence for evolution of resistance to macroparasites (which develop but do not multiply within infected hosts) in biocontrol systems (Garcia-Rossi et al., 2003, Hufbauer, 2001, Mills, 2017). However, it has been documented that populations of the Argentine ryegrass stem weevil (*Listronotus bonariensis*) have evolved resistance to the South American braconid biocontrol parasitoid, *Microctonus hyperodae* (Tomasetto et al., 2018, Tomasetto et al., 2017). The authors speculated that reduced genetic diversity in biocontrol agent populations limit its responses to evolved resistance observed in the weevil pest, though definitive tests are lacking. In another example, the root-knot nematode *Meloidogyne javanica*, has apparently evolved resistance against the endospore-forming biocontrol bacterium (*Pasteuria penetrans*) resulting in decreased attachment ability over four generations (Tzortzakakis et al., 1996).

In cases where a lack of additive genetic variation is found to limit adaptive potential in biocontrol populations, a number of potential mitigation strategies exist. Proposed strategies include 1) collection across multiple biological control agent populations from a broader geographic range; 2) multiple introductions; and/or 3) maintenance of large or multiple populations in quarantine. Many of these strategies can be expensive so careful cost-benefit analysis is warranted (Yek and Slippers, 2014). Intentional genetic admixture (direct cross-breeding among multiple rearing lines) has also gained attention in the recent years as a method to introduce diversity in biological control programs (Myers and Cory, 2017, Rasmussen et al., 2018, Reed and Frankham, 2003, Reed et al., 2003, Rius and Darling, 2014). Genetic admixture results in gene flow between independent lineages (often from different parts of a geographic range) resulting in inter-lineage hybrid offspring (Benvenuto et al., 2012a, Rius and Darling, 2014). Interspecific hybrid individuals often show elevated fitness over parental sources at least in the short-term, via “hybrid advantage”, or heterosis (Crow, 1948, Lippman and Zamir, 2007). In principle, similar benefits could be conferred to admixed individuals of the same species, particularly where source populations represent evolutionarily distinct or isolated lineages.

The long-term positive (or negative) effects of admixture on adaptive potential via increased additive genetic diversity or through the creation of novel phenotypes are largely unknown

(Abbott et al., 2013, Barton, 2001). A few examples are emerging such as the improved establishment success (via enhanced reproduction, survival during periods of limited food availability, and cold tolerance) of the biocontrol agent, *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae), achieved through experimental admixture of two laboratory maintained populations (Li et al., 2018). In another biocontrol system, admixture between the invasive (North American) population of *Harmonia axyridis* and a local biocontrol strain used in Europe appears to have improved life-history traits (i.e., larval survival, fecundity, and age at first hatching), possibly facilitating the insect's subsequent invasion in Europe (Turgeon et al., 2011). Positive effects of admixture, however, can either be short-lived (Benvenuto et al., 2012b, Dlugosch et al., 2015, Lynch, 1991) or negative, resulting in local maladaptation (Burke and Arnold, 2001, Edmands, 2007, Johansen-Morris and Latta, 2006).

An important forestry biological control system is the entomoparasitic nematode, *Deladenus siricidicola* Bedding (Tylenchida: Neotylenchidae), deployed in annual augmentative release to control the woodwasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) in many parts of Southern Hemisphere (Hurley et al., 2008, Slippers et al., 2015). During its mycetophagous (fungus-feeding) phase nematodes feed and reproduce asexually on the mycelium of the basidiomycetous mycangial fungal symbiont (*Amylostereum areolatum),* inoculated into trees by ovipositing wasps. Upon encountering wasp larvae, the juvenile nematodes develop into a parasitic form which eventually enters the ovaries of the female or testes of the male wasp, sterilizing the female (Bedding, 1972, Bedding, 1974, Zondag, 1969). Nematodes reared too long on fungal cultures alone experience strong selection for rapid growth in laboratory rearing conditions and can lose the ability to convert to the parasitic form upon encountering a wasp host (Bedding and Iede, 2005).

Deladenus siricidicola is thought to be native to Eurasia (Bedding and Akhurst, 1978, Spradbery and Kirk, 1978), but today is distributed globally, having been moved both intentionally for biological control and accidentally with *S. noctilio* introductions. In countries such as Australia, Chile, Brazil, Argentina and South Africa, a single strain of *D. siricidicola* (known as the Kamona strain, forms the cornerstone of biological control program of *S. noctilio* (Hurley et al., 2007). Studies on biological control strains of the nematode have shown that the biological control agent has limited diversity (Mlonyeni et al., 2011, Fitza et al., 2019). The restricted range of the nematodes original collection and subsequent recollection, as well as the occurrence of bottlenecks and inbreeding, which are expected during mass rearing, could explain the limited genetic diversity of the biological control strain (Mlonyeni et al., 2011).

A recent study on the diversity of *D. siricidicola* identified three distinct lineages amongst 57 globally-collected nematode strains (Fitza et al., 2019). These lineages broadly conform to the accidental introduction of nematode populations in North America (lineage A), to biological control populations in the Southern Hemisphere (lineage B), and a population from the native range in Spain (lineage C). Additionally, Chilean samples appear to comprise of an admixed population between lineage A and an unintentionally introduced lineage B (Fitza et al., 2019). This latter discovery laid the foundation for the augmentation of diversity of *D. siricidicola* populations using inter-strain admixture since lineage interbreeding was possible and resulting progeny did not exhibit observable fitness disadvantage. The readily accessible admixed individuals in Chile suggest that fitness consequences of mixing were at least largely neutral, if not beneficial. Bittner et al. (2019) also demonstrated admixture between the USA (lineage A) and biological control (lineage B) strains of the nematode. The question

remained, however, whether all these nematode strains from evolutionary distinct lineages would readily interbreed and if so, how such admixture would influence fitness.

Variable levels of parasitism of *D. siricidicola* around the Southern Hemisphere have raised the question about its adaptive capacity and/or phenotypic plasticity (Hurley et al., 2007, Slippers and Wingfield, 2012, Villacide and Corley, 2008). To be successful, *D. siricidicola* populations must perform well under various environmental conditions including climate (e.g., in the summer and winter rainfall regions of South Africa), tree species, fungal isolates, and wasp hosts. Boissin et al. (2012) showed that the invasive populations of *S. noctilio* are highly variable and genetically distinct from each other across their invasive range and that global movement is complex. Variation in *A. areolatum* is also likely to be important – recent studies have shown significant variation in the growth of nematodes on different isolates of the fungus (Mlonyeni et al., 2018b, Morris et al., 2012, Morris et al., 2014).

The aim of this study was to investigate the ability of the different lineages of *D. siricidicola* identified by Fitza et al. (2019) to interbreed in culture. We considered whether such admixture would impact reproductive rates on different isolates of *A. areolatum*. We use previously designed microsatellite markers (Fitza et al., 2019, Mlonyeni et al., 2011) to type both parental and to identify admixed lineages.

2. Materials and methods

2.1. Nematode strains

We used "isolate" throughout the manuscript when referring to a specific isolate used in crosses, and "lineages/strain" when referring to a broader defined phylogenetic group (e.g. lineage A, B and C as defined by Fitza et al., 2019). Each strain of *D. siricidicola* used in this study has been isolated from a single female *S. noctilio*. All strains are maintained at the Biocontrol Centre of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria (Table 1). Two isolates from each of the three identified lineages in Fitza et al. (2019) (lineage A, B and C) and two Chilean isolates from an admixed population were selected for this study. The strains have been maintained on *A. areolatum* (CMW40871) using malt extract agar (MEA) in a 22 °C incubator.

Table 1. Isolate codes**,** country of origin, date of collection and CMW accession numbers (fungus only) for *D. siricidicola* and *A. areolatum* used in this study. Each strain was established as a culture from a single, field-collected *S. noctilio* female.

Isolate code	Organism	Origin	Collection date	Accession #
RSA410	*D. siricidicola*	South Africa, Mpumalanga	2013	
RSA450	*D. siricidicola*	South Africa, Mpumalanga	2013	
CHL1	*D. siricidicola*	Chile, Valparaíso	2013	
CHL3	*D. siricidicola*	Chile, BioBio	2013	
USA1	*D. siricidicola*	USA, Pennsylvania	2013	
USA6	*D. siricidicola*	USA, Pennsylvania	2013	
ESP264	*D. siricidicola*	Spain, Galicia	2014	
ESP313	*D. siricidicola*	Spain, Galicia	2014	
SA2013	*A. areolatum*	South Africa, KwaZulu Natal	2013	CMW46043
SA2012	*A. areolatum*	South Africa, KwaZulu Natal	2012	CMW47563
USA	*A. areolatum*	USA, Pennsylvania	2014	CMW40703
AUS	*A. areolatum*	Australia	2003	CMW40871

2.2. Fungal isolates

Four fungal isolates were selected based on data from a previous study (Mlonyeni, 2018; Table 1). Two isolates were collected in South Africa (CMW46043, CMW47563), one from the USA (CMW40703) and one from Australia (CMW40871). The two South African isolates represent one genotype (MLG6) and the Australian (MLG10) and USA (MLG11) isolates each are different genotypes, based on 11 microsatellite markers (codes are from Mlonyeni et al., 2018b). The Australian isolate is generally used for rearing in the *Sirex* biological control program in South Africa. Isolates differ in mean growth rates on PDA at 23 °C, where the South African isolate (CMW46043) has the slowest growth rate and the USA isolate (CMW40703) and the South African isolate (CMW47563) had the fastest growth rate in Mlonyeni (2018). The fungal isolate from Australia (CMW40871) had an intermediate growth rate (Mlonyeni et al., 2018b). These isolates have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria.

Table 2. Proportion of F_1 nematodes from hybrid crosses that were heterozygous (representing admixture) or homozygous for alleles of either parent. Expected proportions were calculate using the Hardy Weinberg equation (p2 + 2pq + q2 = 1) with lower and upper 95% confidence intervals depending on the number of nematodes screened. All parental nematodes were homozygous for all SSR alleles; the presence of two alleles at any of the two SSR loci screened were considered to be admixed. Numbers in red indicate statistically lower proportion of offspring in a given category (based on 95% Cis); blue indicated higher than expected proportion and black is undistinguishable from random. The numbers in the parentheses are lower and upper 95% CI values, respectively, and were derived using random resampling (10,000 iterations). Dashes represent the same expected frequency for parent 2 as in parent 1. Blue shading indicates two repeat crosses between the CHL1 and USA6 nematode isolates, representing that was the most successfully of the admixed crosses. These two repeat crosses were used for the growth assays where 40 nematodes were sampled to confirm admixture. Asterisk refers to the only asymmetric cross (USA1/RSA450) performed with 1000 and 500 eggs respectively (p = 0.67, q = 0.33).

Parent 1 (p)	**Parent 2 (q)**	**#single F_1 nematodes screened**	**Heterozygous (Admixed)**	**Homozygous for alleles of Parent 1**	**Homozygous for alleles of Parent 2**	**Expected Hz (2pq)**	**Expected homozygosity, Parent 1 (p^2)**	**Expected homozygosity, Parent 2 (q^2)**
ESP264	**RSA410**	31	**16.1%**	**0.0%**	**83.9%**	50.0% (32.3%, 67.7%)	25.0% (9.7%, 41.9%)	–
USA1	**RSA450**	224	**3.6%**	**0.0%**	**96.4%**	50.0% (43.3%, 56.7%)	25.0% (19.6%, 30.4%)	–
USA1*	**RSA450**	72	**19.4%**	**11.1%**	**69.4%**	44.4% (33.3%, 55.6%)	44.4% (33.3%, 55.6%)	11.1% (4.2%, 18.1%)
USA6	**ESP264**	40	**2.5%**	**0.0%**	**97.5%**	50.0% (35.0%, 65.0%)	25.0% (12.5%, 37.5%)	–
CHL1	**USA6**	79	**55.7%**	**26.6%**	**16.5%**	50.0% (39.2%, 60.8%)	25.0% (16.5%, 34.2%)	–
CHL1	**ESP313**	164	**43.3%**	**14.6%**	**42.1%**	50.0% (42.4%, 57.6%)	25.0% (18.8%, 31.5%)	–
CHL3	**RSA410**	77	**20.8%**	**0.0%**	**79.2%**	50.0% (39.0%, 61.0%)	25.0% (15.6%, 35.1%)	–
CHL1	**USA6**	36	**36.1%**	**27.8%**	**36.1%**	50.0% (42.4%, 57.6%)	25.0% (18.8%, 31.5%)	–
CHL1	**USA6**	35	**48.6%**	**37.1%**	**14.3%**	50.0% (39.0%, 61.0%)	25.0% (15.6%, 35.1%)	–

2.3. Crosses between *D. siricidicola* isolates in culture

Six crosses were made between the three *D. siricidicola* isolates belonging to three lineages (A, B, C; Fitza et al. 2019) and two Chilean isolates that represent admixture between two lineages (A, B, Fitza et al., 2019) (Fig. S1, Table 2). Cultures of the individual nematode strains were grown on 90 mm diameter $^1/_2$ potato dextrose agar Petri dishes (PDA, Difco™ Potato Dextrose Agar, LOT 2347578) (19.5 g/L potato dextrose extract, 17.5 g/L agar) on the CMW40781 fungal isolate. Petri dishes were left for 25 days, after which eggs from the F_0 generation were collected. To remove nematodes and eggs from agar plates, Petri dishes were flooded with 2 ml sterile water and decanted into a small, excavated glass block. After gently swirling, the eggs were separated from the nematodes by sedimentation. Ten µl of the sediment with eggs for each strain was pipetted with a Gilson pipette, and all eggs were counted using a counting slide with a 3 mm^2 grid under a dissecting microscope.

Nematode concentration (individuals ml^{-1}) was estimated as the average of three counts of 10 ul of well-mixed nematode-water suspension and a volume containing approximately 500 eggs was collected. For each cross, approximately 500 eggs per strain from two parental strains were put together on one spot on the plate. The nematode isolates were maintained on an *A. areolatum* (CMW40871) isolate, which is used in the Sirex Biocontrol Programme in Australia, before the start of this study, and were maintained for this experiment on this fungal isolate. Plugs (5 mm diameter) of *A. areolatum* CMW40871 were placed on the agar surface, approximately 70 mm from the nematode eggs. Cultures were left to develop for 20 days after which the eggs representing the F_1 generation were harvested and transferred onto a new Petri dish. An additional cross (2xUSA1/RSA450; Table 2) was carried out with approximately 1000 eggs from USA1 with approximately 500 eggs of RSA450 to investigate if doubling the number of eggs of USA1 would increase the presence of this parental genotype in the offspring.

2.4. Molecular characterization of interbreeding between lineages

For each cross, single nematodes were picked up using a micro-dissection needle from the F_1 generation for DNA extraction. In the first set of crosses between 150 and 250 nematodes were screened, while between 30 and 80 nematodes were considered adequate in subsequent crosses. The DNA extraction protocol used by Barstead et al., 1991, Williams et al., 1992 was adapted for this study. A single nematode was placed in 15 µl of lysis buffer (5 µg proteinase K, 1 µl of 10x PCR buffer and 9 µl of SABAX). The sample was briefly spun down at 2000 rpm for 2 min using a centrifuge (Eppendorf Centrifuge 5417C) and then frozen at −80 °C for 10 min. After freezing, the sample was placed into the PCR machine for proteinase K activation at 65 °C for 60 min and deactivation at 95 °C for 15 min.

Fragment analysis from microsatellite markers was used to determine potential admixed offspring. The microsatellite genotype profile of each parental strain was known from previous work (Fitza et al., 2019) and all were known to be polymorphic across populations. As single nematode DNA was limited, only one PCR could be done for individual collected offspring. For each cross between 31 and 224 individual nematodes were screened, using two microsatellite markers for each cross and dividing the collected individual nematodes roughly equally between the two markers (Table 3; Fig. S1A). that were homozygous for different alleles in each parental strain, such that any heterozygosity could be uniquely interpreted as admixture.

Table 3. Microsatellite markers and their primer sequence used for the screening of the various *D. siricidicola* crosses.

Cross (Parent 1 / Parent 2)	Primer 1	Primer 2
USA1/RSA450	DS105 F5′ TGGTAGCAATCGATCGAAAA 3′ R5′ CGTGTCCACTTGTCCCTCTC 3′	DS325 F5′ ACGCTTATGTGTGCCACTTG 3′ R5′ GGGTCTCTTGATGATGTTTCG 3′
USA6/CHL1	DS105	DS325
RSA410/CHL3	DS316 F5′ TGCGGATATCTTCTCATTGTAA 3′ R5′ TCAAATGTTATGCGAAATTCTG 3′	DS388 F5 ‘AAGTCAGCTGAAAGGCGAAG 3′ R5′ TGTGTGCATGAAAACGGAAC 3′
CHL1/ESP31	DS105	DS325
ESP264/RSA410	DS105	DS325
ESP264/USA6	DS105	DS325

PCR reactions were conducted in a total volume of 21.5 µl made up with 5 µl 5x MyTaq™ buffer (Bioline USA, Taunton, Massachusetts), 5 µM of each primer, 2.5 units of MyTaq™ DNA Polymerase (Bioline USA, Taunton, Massachusetts) and 15 µl of the DNA template. PCR cycling conditions were a 4 min initial denaturation step at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 58 °C for 30 sec, extension at 72 °C for 1 min, and a final extension of 72 °C for 45 min, followed by a cooling step of 4 °C for 10 min. The products were then visualized on 2% (w/v) agarose gels using Gel Doc™ EZ Imager (Bio-Rad, USA). PCR products were diluted to a ratio 1:100 using SABAX water. One µl of this diluted DNA was added to 10 µl of HiDi™ Formamide and 0.2 µl GeneScan-500 Liz size standard (Applied Biosystems). These samples were run on the ABI PRISM™ 3500xI DNA Analyser (Applied Biosystems, USA) to determine product size (DNA sequencing facility, University of Pretoria). The software GeneMapper® v4.1 (Life Technologies, Foster City, CA) was applied on the GeneScan data to score allele fragment sizes.

2.5. Nematode productivity assay

To assess the consequence of admixture on one component of fitness, population growth assays were performed with one admixed *D. siricidicola* culture (USA6/CHL1) and its two parental isolates (USA6, CHL1; Fig. 1). Of course, even the admixed culture contained the offspring from within-lineage mating, as genotyping individuals is destructive and could not be performed. The USA6/CHL1 was chosen because the proportion of admixed offspring was the highest among all of our experimental crosses (56%), as expected under random mating.

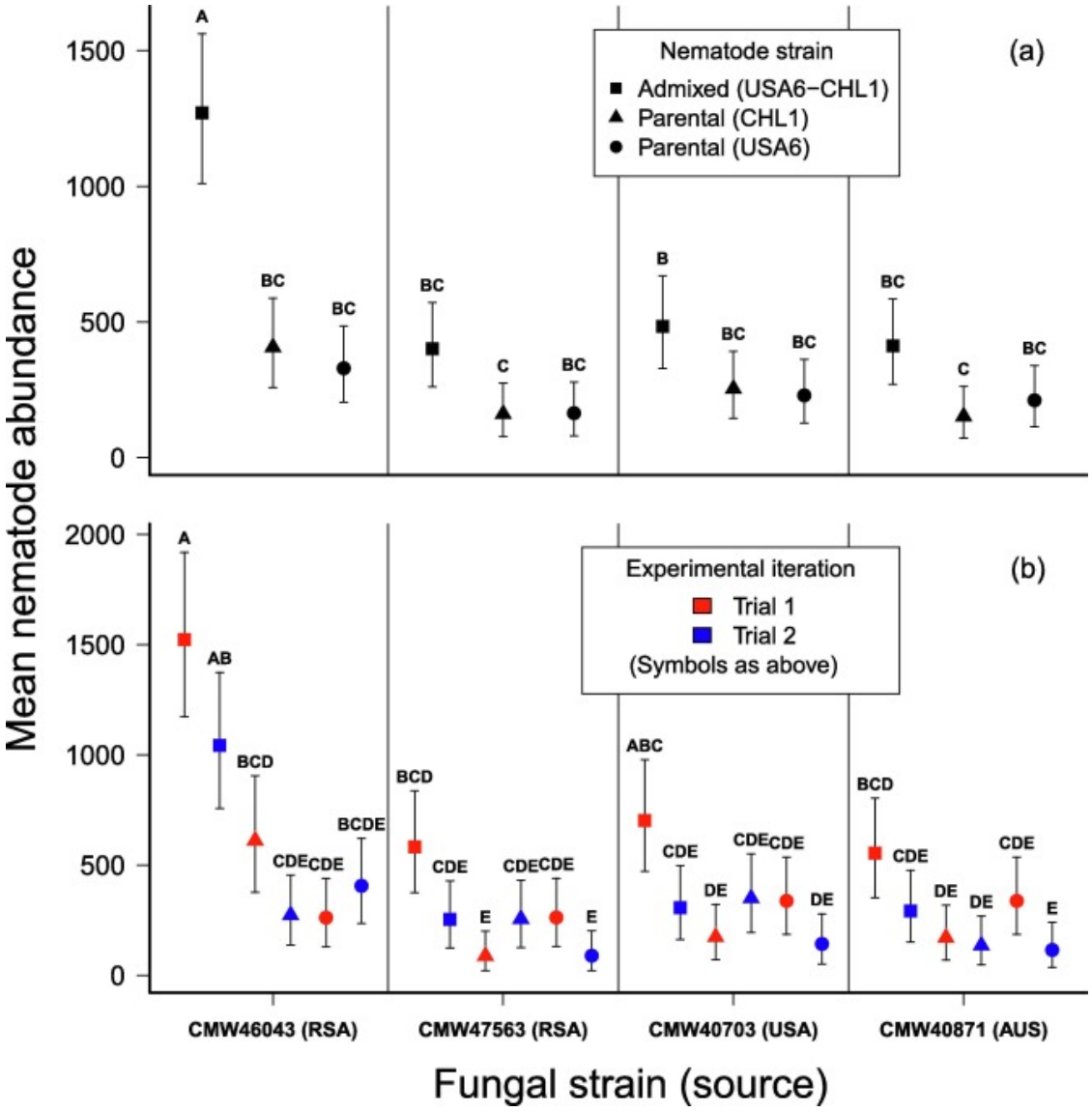


Fig. 1. Mean nematode abundance of parental and hybrid *D. siricidicola* nematodes in 100 µl (±SE) grown per Petri dish after 25 days on four strains of *A. areolatum*. One-way ANOVA Tukey HSD at $P < 0.05$ was used to determine statistical differences of the nematode strains reproducing on different fungal isolates. Different letters represent significant difference between the nematode isolates. The various nematode isolates are represented through different shapes and the error bars show standard error. (A) Mean productivity of nematodes of both trials combined with the three nematode sources and the four fungal isolates (CMW46043 = RSA2013; CMW47563 = RSA2012; CMW40703 = USA; CMW40871 = AUS). (B) Mean productivity for the first and second trial.

Nematode population growth rate assays, as reflected by differences in nematode abundance in culture, were conducted on 19.5 g/L PDA in 90 mm diameter Petri dishes using the four isolates of *A. areolatum* (Table 1). A five mm diameter plug was transferred from the growing edge of an *A. areolatum* culture to the edge of a new Petri dish and left to grow for five days at 23 °C. Eggs from the two parental strains, reared on the CMW40871 fungal isolate were collected as described above. Approximately 1000 eggs per parental strain were placed together on different plates with the four fungal isolates. After 20 days F_1 eggs were collected and approximately 300 eggs were placed on a new plate with the respective fungal

isolate. Only the first generation of admixture is expected, since the F1 offspring did not have sufficient time to mature and reproduce (mean generation is = 14 days). For the parental control 300 eggs of each parental strain were placed separately on the four fungal isolates.

A total of 20 Petri dishes (5 replicates for each of the four *A. areolatum* isolates) were incubated at 23 °C in the dark for 25 days. Subsequently, the nematodes were harvested by washing with 2 ml sterile water and decanting into the glass excavator block. A second wash was done to ensure the collection of all nematodes. Nematodes were allowed to settle and water was removed. One ml sterile water was added to the sediment and mixed well, after which a 100 µl subsample was taken and all nematodes were counted using a counting slide with a 3 mm^2 grid under a dissection microscope. The whole trial was then repeated to ensure consistency of results. To confirm the success of interbreeding between isolates from different strains, 40 single nematodes were screened using a microsatellite marker that was homozygous within, but different between, the parental strains, as described above (Fig. S1B).

2.6. Statistical analysis

Analysis of Variance (ANOVA) comparing the interaction between the independent variables (fungal isolate and nematode strain) on the dependent variable (nematode abundance), was performed. The data were square root transformed to conform to the assumption of constant variance and normality of the ANOVA test. Tukey's Honestly Significant Difference (HSD) post-hoc tests were performed to compare pairwise treatment combinations. These analyses were run using the statistical programme JMP® Statistical Software (SAS, USA).

3. Results

3.1. Characterization of crosses of *D. siricidicola* lineages in culture

All seven *D. siricidicola* lineage crosses produced viable offspring, and all contained at least some heterozygous individuals (indicating admixture), with the proportion ranging from 3 to 56% (unshaded rows, Table 2). Heterozygotes were rarer than expected by chance (2.5%-28.8%) in five of these seven crosses. Both crosses using CHL1 (with USA6 and ESP313) produced an expected proportion of heterozygotes (55.7% and 43.3%, respectively). One of two subsequently repeated crosses USA6/CHL1 likewise yielded the expected proportion of heterozygotes (48.6%; Table 2, shaded rows), with the other still yielding more heterozygotes than any other cross (36.1%). Most crosses resulted in significant asymmetry in the representation of parental strain among the homozygous (non-admixed) nematodes. With the exception of USA6/CHL1 cross, all showed over-representation of one parent ranging from 42.1% to 96.4%, with the lowest percentage coming from the CHL1/ESP313 cross (which yielded the expected number of heterozygotes).

3.2. Nematode productivity assay

Looking first at pooled results across the two experimental iterations performed (Fig. 1a), a two-way ANOVA model including fungal isolate, nematode strain, and their interaction accounted for over 52% of variation in square root-transformed nematode abundance after 25 days of incubation ($F = 10.8$; df = 11,107, $P < 0.0001$; $R^2 = 0.522$ [adjusted-$R^2 = 0.476$]). Both main effects were highly significant (fungal isolate: $F = 14.6$; df = 3,107; $P < 0.0001$; nematode strain: $F = 29.9$; df = 2,107; $P < 0.0001$) and the nematode × fungal interaction was

moderately significant (F = 2.2; df = 6,107; P = 0.0461). The USA6/CHL1 admixed culture showed a significantly higher population growth rate on all fungal isolates (Tukey's HSD post-hoc test on the nematode strain main effect, alpha = 0.05). When all pairwise comparisons between treatment levels were considered (Fig. 1a) the growth rate was only statistically significant on the CMW46043 (RSA) fungus, driving the interaction effect. This admixed culture had an average abundance that was 256% (±51% SD) and 259% (±87% SD) greater than its parental Chilean and USA isolate, respectively (maximum of 386%). Abundance did not differ significantly among the two parental strains overall or on specific fungal isolates.

When included as a blocking factor, experimental iteration resulted in a stronger model overall based on AIC (F = 8.8; df = 23,95; P < 0.0001; R^2 = 0.68 [adjusted-R^2 = 0.603]); Experimental iteration was significant as a main effect and in all its two and three-way interactions, with the exception of the two-way interaction with fungal isolates. Fig. 2b shows all pairwise treatment comparisons based on Tukey's HSD. While current sample size and the number of comparisons under consideration made it difficult to discriminate among treatment levels, overall, there was a high degree of qualitative agreement among trials. Nematode abundance was higher in Trial 1 relative to Trial 2 (F = 14.3; df = 1,95; P = 0.003). In both iterations, the USA6/CHL1 cross performed best, particularly when grown on the CMW46043 (RSA) fungus, though the performance of this admixed culture was more strongly differentiated in Trial 1.

4. Discussion

In this study, *D. siricidicola* strains representing three distinct lineages (from the US, South Africa, and Spain) plus two Chilean strains, which appear to be admixed between North America and the Southern Hemisphere lineage, were shown to be capable of interbreeding. SSR genotyping of F_1 offspring of multiple individuals, however, revealed substantial asymmetries in the proportion of both parental strains and hybrid strains resulting from these crosses.

The frequency of heterozygotes (admixed individuals) present in the F_1 generation varied from 3% to 56% in the different crosses. The absence of one parental (homozygous) genotype in sampled offspring in 4 of 9 crosses was surprising – in all but one cross (one of the three USA6/CHL1 crosses) there was a significant over-/under-representation of one of the parental strains in the resulting offspring. In two-thirds of the crosses (6 of 9), there were significantly fewer hybrid individuals than would be predicted under Hardy-Weinberg equilibrium. The reasons we detected fewer admixed individuals than would be expected under a random mating model is unclear. Pre- or post-zygotic barriers to admixture (i.e., reduced mating or offspring success) in some of our crosses is possible, although alternatives (such as assortative mating, cannot be ruled out (Coyne and Orr, 2004). Caetano et al., 2016, Morris et al., 2012, Mlonyeni et al., 2018a all indicated that the reproductive performance of the nematode strain was depended on the fungal isolate on which the nematode strain was grown.

All of the crosses where the proportion of hybrids conformed to random expectations included CHL1 as one of the parental isolates. This is particularly interesting given that CHL1 is hypothesized to itself have undergone one or more admixture events (Fitza et al., 2019). This suggests the possibility that the barriers to admixture are lower in this isolate, though whether this is likely to be a cause or a consequence of admixture in Chilean

populations is unknown. For example, in the cereal rust mite *Abacarus hystrix* intraspecific hybrid inviability in crosses seems to be host species dependent. If crosses were done on the host on which the males developed, no progeny was found. Crosses conducted on hosts on which females developed, only male progeny was obtained. One possible mechanism could be incapability of the mite sperm from the one host to fertilize mite eggs from the other host (Skoracka, 2008). A postzygotic barrier was observed during interbreeding of strains of the nematode *Haemonchus contortus* from different geographic regions, as there were less than the mendelian expectation of L1 offspring, even though egg genotyping indicated no inter-strain hybrid genotype deficit (Sargison et al., 2019).

Reproductive fitness of the resulting strains varied both by nematode strain and the fungal isolate on which they were grown. Additionally, clear, though comparatively weaker, nematode × fungal isolate interaction effects were observed. Interestingly, in both trials, the hybrid USA6/CHL1 culture grew better than either parental strain, suggesting some form of hybrid advantage. This advantage was to some degree context dependent, as the differences in reproductive output were mostly on one fungal isolate (CMW46043, RSA). CMW 46,043 is a fungal isolate obtained in 2013 and identified by a previous study to be the slowest growing isolate of the four fungal isolates tested (Mlonyeni, 2018). All nematodes and fungi for this experiment were collected from wasps that were obtained from plantation or naturally growing trees, so none have lost its ability to persist under field conditions. Still, why this isolate of fungus was particularly good for nematode growth, and disproportionately good for the admixed culture, is not clear.

Development of a method to conduct crosses with various nematode strains, as well as the modification of a single nematode DNA extraction protocol, allowed for the screening of offspring using microsatellite markers to indicate admixture. These crossbreeding experiments showed clear interbreeding potential of all three lineages. Recent evidence has shown that lineages interbreeding in *D. siricidicola* also occurs in nature, as a Chilean population appear to represent admixture between nematodes of North American and Southern Hemisphere origins (Fitza et al. 2019). Akhurst (1975) likewise showed that intraspecific crosses of *D. siricidicola* produced viable eggs with a hatching success rate of 75%, however the genotypes of the parental *D. siricidicola* cultures used were not known. Akhurst (1975) further reported that egg deposition and hatching success varied substantially among interspecific crosses of *Deladenus* spp. These studies suggest a high likelihood of interbreeding between the different lineages.

Interbreeding capabilities of various nematode strains has implications for the introduction of the biological control nematode strain. Admixture has the potential advantage of increased hybrid fitness due to selection of favourable phenotypes (Facon et al., 2008, Szűcs et al., 2012, Szűcs et al., 2019). However, studies of the long-term impact of admixture on population fitness are lacking, and where they exist, results are equivocal (Garnas et al., 2016, Garnas, 2018). In many cases, short-term hybrid advantage (heterosis) is a phenomenon that disappears with further generations and with back-crossing (Johansen-Morris and Latta, 2006, Lippman and Zamir, 2007).

The opportunity to use controlled lineage crossing to improve aspects of biological control efficacy is rooted in following two fundamental ideas: first, that increasing additive genetic diversity is generally positive for populations (Hahn and Rieseberg, 2017, Lommen et al., 2017); and second, that desirable traits could be intentionally imported into otherwise well-adapted and established populations (Lirakis and Magalhães, 2019, Shi et al., 2018). On the

first point, admixture clearly has the potential to increase additive genetic diversity in otherwise low-diversity, often inbred strains, assuming that survivorship of hybrid offspring is equivalent to or greater than parental stains. Whether such diversity would result in novel desirable traits or trait combinations that could be selected for (either in the laboratory or in the field), is largely unknown. However, many native and invasive populations are highly tolerant to inbreeding and sustain low levels of diversity (Frankham, 2005, Roman and Darling, 2007, Simberloff, 2009). In fact, it has been challenging to demonstrate a strong positive correlation between additive genetic diversity and population fitness (Estoup et al., 2016, Johansen-Morris and Latta, 2006, Rius and Darling, 2014).

The potential to strategically import desirable traits into local populations via admixture is an intriguing possibility. This approach requires considerably more knowledge of strain fitness values and/or traits that are variable across populations, and also assumes some degree of local adaptation (otherwise strain replacement would be preferred). Additionally, desirable traits could be distributed among lineages such that admixture could combine such traits. In the *D. siricidicola* system, traits such as conversion efficiency (from the mycetophagous to the parasitic form) are important (Mlonyeni et al., 2018a), as well as more obvious traits such as population growth rate or tolerance to different environmental conditions. The inability to sterilize *S. noctilio* by entering eggs has clear negative consequences for the control of wasp populations (Kroll et al., 2013, Yu et al., 2009).

The capability of the USA isolate from our study to interbreed with strains from other parts of the world raises possibilities to breed for the sterilizing ability in the North American population. A previous study provided evidence of hybridization between the non – sterilizing North America strain (lineage A) and the sterilizing Kamona strain (lineage B) due to tree host co – infection, influencing the sterilizing ability in the North American population (Bittner et al., 2019). Alternatively, this trait could also be exported from North American populations given the unintentional movement among global populations of this nematode and the propensity to hybridize (Fitza et al., 2019). While non-sterilization of the wasp host is highly undesirable from a biocontrol perspective, this trait may enhance nematode fitness considerably, since female wasps still act as vectors and the lack of sterilization would mean more wasps in the tree serving as host for offspring. If this trait were to escape the North American population, it therefore has the potential to spread.

Admixture among independently evolving lineages can also break up co-evolved allele combinations resulting in negative fitness consequences. In a biocontrol context, this could manifest as a reduction in the infectivity rate of the Kamona strain or dilution of desirable characteristics (Hajek and Morris, 2014, Williams and Hajek, 2017, Williams et al., 2012). Interbreeding as a strategy to improve control efficacy, therefore, needs to be carefully considered for future development of biological control programs in the USA and elsewhere.

The USA6/CHL1 hybrid offspring showed a higher reproductive rate compared to the parental nematode strains, indicating the potential to increase fitness through admixture, at least in the short term. This likely represents hybrid vigour (heterosis), often attributed to elevated heterozygosity at loci were deleterious, often recessive alleles were present. The Chilean populations had higher genetic diversity compared to other Southern Hemisphere populations, perhaps as a consequence of an earlier admixture event (Fitza et al., 2019), but did not perform better than the USA6, non-admixed parent.

5. Conclusion

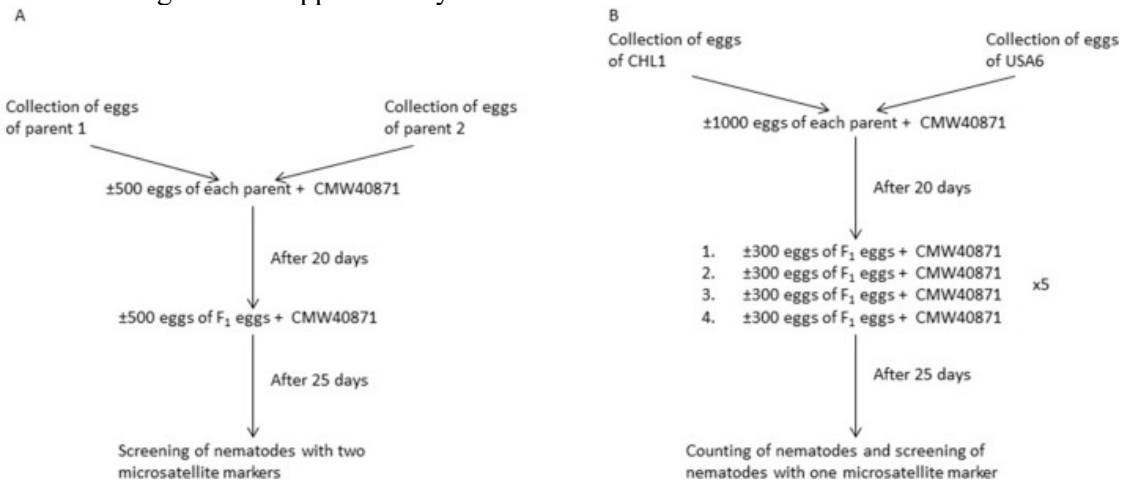
Biological control programs rely on effective parasitism, successful establishment and spread, high fecundity and efficient mass rearing capabilities of the control agent. The current study demonstrated the interbreeding potential amongst various lineages of *D. siricidicola.* This provides a means to introduce more genetic diversity to the *Sirex* biological control programs, and potentially the opportunity to introduce and subsequently select for desirable traits or trait combinations. Increased genetic diversity could be especially valuable in the Southern Hemisphere, where previous studies have identified high homozygosity in the nematode population (in contrast to comparatively high diversity in the *S. noctilio* populations). However, high variability and systematic asymmetries in the success and fitness consequences of admixture demonstrate that careful study is warranted prior to its implementation as a biocontrol enhancement strategy.

Acknowledgement

The University of Pretoria, members of the Tree Protection Co-operative Programme (TPCP), South Africa, and USDA Forest Service International Programs are acknowledged for providing funding for this research. We also acknowledge the support by the Sequencing Facility of the University of Pretoria for their services related to the fragment analyses.

Appendix A. Supplementary data

The following are the Supplementary data to this article:



Supplementary Fig. 1.

References

R. Abbott, D. Albach, S. Ansell, J. Arntzen, S. Baird, N. Bierne, J. Boughman, A. Brelsford, C. Buerkle, R. Buggs. **Hybridization and speciation.** J. Evol. Biol., 26 (2013), pp. 229-246

R.J. Akhurst. **Cross-breeding to facilitate the identification of *Deladenus* spp., nematode parasites of woodwasps.** Nematologica, 21 (3) (1975), pp. 267-272

R.J. Barstead, L. Kleiman, R.H. Waterston. **Cloning, sequencing, and mapping of an α-actinin gene from the nematode *Caenorhabditis elegans.*** Cell Motility Cytoskeleton, 20 (1991), pp. 69-78

N. Barton. **The role of hybridization in evolution.** Mol. Ecol., 10 (2001), pp. 551-568

R.A. Bedding. **Biology of *Deladenus siricidicola* (Neotylenchidae) an entomophagous-mycetophagous nematode parasitic in siricid woodwasps.** Nematologica, 18 (4) (1972), pp. 482-493

R.A. Bedding. **Five new species of *Deladenus* (Neotylenchidae), entomophagous-mycetophagous nematodes parasitic in siricid woodwasps.** Nematologica, 20 (2) (1974), pp. 204-225

R.A. Bedding, R.J. Akhurst. **Geographical distribution and host preferences of *Deladenus* species (Nematoda: Neotylenchidae) parasitic in siricid woodwasps and associated hymenopterous parasitoids.** Nematologica, 24 (3) (1978), pp. 286-294

Bedding, R., Iede, E., 2005. Application of *Beddingia siricidicola* for *Sirex* woodwasp control. Nematodes as biocontrol agents. Wallingford, UK, CAB Internation, pp. 385–399.

C. Benvenuto, S. Cheyppe-Buchmann, G. Bermond, N. Ris, X. Fauvergue. **Intraspecific hybridization, life history strategies and potential invasion success in a parasitoid wasp.** Evol. Ecol., 26 (6) (2012), pp. 1311-1329

C. Benvenuto, E. Tabone, E. Vercken, N. Sorbier, E. Colombel, S. Warot, X. Fauvergue, N. Ris. **Intraspecific variability in the parasitoid wasp *Trichogramma chilonis*: can we predict the outcome of hybridization?** Evol. Appl., 5 (5) (2012), pp. 498-510

T.D. Bittner, N. Havill, I.A. Caetano, A.E. Hajek. **Efficacy of Kamona strain *Deladenus siricidicola* nematodes for biological control of *Sirex noctilio* in North America and hybridisation with invasive conspecifics.** NeoBiota, 44 (2019), pp. 39-55

E. Boissin, B. Hurley, M.J. Wingfield, R. Vasaitis, J. Stenlid, C. Davis, P. de Groot, R. Ahumada, A. Carnegie, A. Goldarazena, P. Klasmer, B. Wermelinger, B. Slippers. **Retracing the routes of introduction of invasive species: the case of the *Sirex noctilio* woodwasp.** Mol. Ecol., 21 (23) (2012), pp. 5728-5744

J.M. Burke, M.L. Arnold. **Genetics and the fitness of hybrids.** Ann. Rev. Genet., 35 (1) (2001), pp. 31-52

I.A.L. Caetano, E.E. Morris, A.E. Hajek. **Growth of the *Sirex*-parasitic nematode *Deladenus siricidicola* on the white rot fungus *Amylostereum.*** J. Invertebrate Pathol.gy, 134 (2016), pp. 12-14

Coyne, J., Orr, H., 2004. Speciation Sinauer, Sunderland, MA.

J.F. Crow. **Alternative hypotheses of hybrid vigor.** Genetics, 33 (5) (1948), pp. 477-487

K.M. Dlugosch, S.R. Anderson, J. Braasch, F.A. Cang, H.D. Gillette. **The devil is in the details: genetic variation in introduced populations and its contributions to invasion.** Mol. Ecol., 24 (9) (2015), pp. 2095-2111

Suzanne Edmands. **Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management.** Mol. Ecol., 16 (3) (2007), pp. 463-475

A. Estoup, V. Ravigné, R. Hufbauer, R. Vitalis, M. Gautier, B. Facon. **Is there a genetic paradox of biological invasion?** Ann. Rev. Ecol., Evol., Systemat., 47 (1) (2016), pp. 51-72

B. Facon, J.-P. Pointier, P. Jarne, V. Sarda, P. David. **High genetic variance in life-history strategies within invasive populations by way of multiple introductions.** Curr. Biol., 18 (5) (2008), pp. 363-367

K.N.E. Fitza, J.R. Garnas, M.J. Lombardero, M.P. Ayres, F.E. Krivak-Tetley, R. Ahumada, B.P. Hurley, M.J. Wingfield, B. Slippers. **The global diversity of *Deladenus siricidicola* in native and non-native populations.** Biol. Control, 132 (2019), pp. 57-65

S.V. Fowler, P. Peterson, D.P. Barrett, S. Forgie, D.M. Gleeson, H. Harman, G.J. Houliston, L. Smith. **Investigating the poor performance of heather beetle, *Lochmaea suturalis* (Thompson)(Coleoptera: Chrysomelidae), as a weed biocontrol agent in New Zealand: has genetic bottlenecking resulted in small body size and poor winter survival?** Biol. Control, 87 (2015), pp. 32-38

R. Frankham. **Resolving the genetic paradox in invasive species.** Heredity, 94 (4) (2005) 385–385

D. Garcia-Rossi, N. Rank, D.R. Strong. **Potential for self-defeating biological control? Variation in herbivore vulnerability among invasive *Spartina* genotypes.** Ecol. Appl., 13 (6) (2003), pp. 1640-1649

J.R. Garnas. **Rapid evolution of insects to global environmental change: conceptual issues andempirical gaps.** Current Opinion in Insect Science, 29 (2018), pp. 93-101

J.R. Garnas, M.-A. Auger-Rozenberg, A. Roques, C. Bertelsmeier, M.J. Wingfield, D.L. Saccaggi, H.E. Roy, B. Slippers. **Complex patterns of global spread in invasive insects: eco-evolutionary and management consequences.** Biol. Invasions, 18 (4) (2016), pp. 935-952

M.A. Hahn, L.H. Rieseberg. **Genetic admixture and heterosis may enhance the invasiveness of common ragweed.** Evol. Appl., 10 (3) (2017), pp. 241-250

Hajek, A., Morris, E., 2014. Biological control of *Sirex noctilio*. In: Van Driesche, R.G. and Reardon, R. (Editors), The Use of Classical Biological Control to Preserve Forests in North America. FHTET-2013-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia**,** 331–346.

G.E. Heimpel, N.J. Mills. **Biological control: ecology and applications.** Cambridge University Press (2017)

R.A. Hufbauer. **Pea aphid–parasitoid interactions: have parasitoidsadapted to differential resistance?** Ecology, 82 (3) (2001), pp. 717-725

R.A. Hufbauer. **Evidence for nonadaptive evolution in parasitoid virulence following a biological control introduction.** Ecol. Appl., 12 (1) (2002), pp. 66-78

B.P. Hurley, B. Slippers, P.K. Croft, H.J. Hatting, M. van der Linde, A.R. Morris, C. Dyer, M.J. Wingfield. **Factors influencing parasitism of *Sirex noctilio* (Hymenoptera: Siricidae) by the nematode *Deladenus siricidicola* (Nematoda: Neotylenchidae) in summer rainfall areas of South Africa.** Biol. Control, 45 (3) (2008), pp. 450-459

B.P. Hurley, B. Slippers, M.J. Wingfield. **A comparison of control results for the alien invasive woodwasp, *Sirex noctilio*, in the Southern Hemisphere.** Agric. For. Entomol., 9 (3) (2007), pp. 159-171

A.D. Johansen-Morris, R.G. Latta. **Fitness consequences of hybridization between ecotypes of *Avena barbata*: hybrid breakdown, hybrid vigor, and transgressive segregation.** Evolution, 60 (8) (2006), pp. 1585-1595

S.A. Kroll, A.E. Hajek, E. Erin Morris, S.J. Long. **Parasitism of *Sirex noctilio* by non-sterilizing *Deladenus siricidicola* in northeastern North America.** Biol. Control, 67 (2) (2013), pp. 203-211

H.-S. Li, S.-J. Zou, P. De Clercq, H. Pang. **Population admixture can enhance establishment success of the introduced biological control agent *Cryptolaemus montrouzieri.*** BMC Evol. Biol., 18 (2018), p. 36

Z.B. Lippman, D. Zamir. **Heterosis: revisiting the magic.** Trends Genet., 23 (2) (2007), pp. 60-66

M. Lirakis, S. Magalhães. **Does experimental evolution produce better biological control agents? A critical review of the evidence.** Entomologia Experimentalis et Applicata, 167 (7) (2019), pp. 584-597

S.T.E. Lommen, P.W. de Jong, B.A. Pannebakker. **It is time to bridge the gap between exploring and exploiting: prospects for utilizing intraspecific genetic variation to optimize arthropods for augmentative pest control–A review.** Entomologia Experimentalis et Applicata, 162 (2) (2017), pp. 108-123

M. Lynch. **The genetic interpretation of inbreeding depression and outbreeding depression.** Evolution, 45 (3) (1991), pp. 622-629

N.J. Mills. **Rapid evolution of resistance to parasitism in biological control.** Proceed. Natl. Acad. Sci., 114 (15) (2017), pp. 3792-3794

Mlonyeni, X.O., 2018. Characterization of genetic variability in the *Sirex-Amylostereum-Deladenus* symbioses. Department of Biochemistry, Genetics and Microbiology. University of Pretoria, South Africa.

X.O. Mlonyeni, B.D. Wingfield, J.M. Greeff, B.P. Hurley, M.J. Wingfield, B. Slippers. **Population variation in traits of *Deladenus siricidicola* that could influence the biocontrol of *Sirex noctilio* in South Africa.** Int. J. Pest Manage., 59 (2018), pp. 348-353

X.O. Mlonyeni, B.D. Wingfield, M.J. Wingfield, R. Ahumada, P. Klasmer, I. Leal, P. de Groot, B. Slippers. **Extreme homozygosity in Southern Hemisphere populations of *Deladenus siricidicola*, a biological control agent of *Sirex noctilio.*** Biol. Control, 59 (3) (2011), pp. 348-353

X.O. Mlonyeni, M.J. Wingfield, J.M. Greeff, B.D. Wingfield, B. Slippers. **Genetic diversity of *Amylostereum areolatum*, the fungal symbiont of the invasive woodwasp *Sirex noctilio* in South Africa.** For. Pathol., 48 (6) (2018), p. e12449, 10.1111/efp.2018.48.issue-610.1111/efp.12449

E. Morris, A.E. Hajek, E. Zieman, D.W. Williams. ***Deladenus* (Tylenchida: Neotylenchidae) reproduction on species and strains of the white rot fungus *Amylostereum.*** Biol. Control, 73 (2014), pp. 50-58

E.E. Morris, A. Jimenez, S.J. Long, D.W. Williams, A.E. Hajek. **Variability in growth of *Deladenus siricidicola* on strains of the white rot fungus *Amylostereum areolatum.*** BioControl, 57 (5) (2012), pp. 677-686

J.H. Myers, J.S. Cory. **Biological control agents: invasive species or valuable solutions?, Impact of Biological Invasions on Ecosystem Services.** M. Vilà, P.E. Hulme (Eds.), Impact of Biological Invasions on Ecosystem Services, Springer International Publishing, Cham (2017), pp. 191-202, 10.1007/978-3-319-45121-3_12

C. Phillips, D. Baird, I. Iline, M. McNeill, J. Proffitt, S. Goldson, J. Kean. **East meets west: adaptive evolution of an insect introduced for biological control.** J. Appl. Ecol., 45 (2008), pp. 948-956

L.B. Rasmussen, K. Jensen, J.G. Sørensen, E. Sverrisdóttir, K.L. Nielsen, J. Overgaard, M. Holmstrup, T.N. Kristensen. **Are commercial stocks of biological control agents genetically depauperate?–A case study on the pirate bug *Orius majusculus* Reuter.** Biol. Control, 127 (2018), pp. 31-38

D.H. Reed, R. Frankham. **Correlation between fitness and genetic diversity.** Conserv. Biol., 17 (1) (2003), pp. 230-237

D.H. Reed, E.H. Lowe, D.A. Briscoe, R. Frankham. **Fitness and adaptation in a novel environment: effect of inbreeding, prior environment, and lineage.** Evolution, 57 (8) (2003), pp. 1822-1828

M. Rius, J.A. Darling. **How important is intraspecific genetic admixture to the success of colonising populations?** Trends Ecol. Evol., 29 (4) (2014), pp. 233-242

G.K. Roderick, M. Navajas. **Genes in new environments: genetics and evolution in biological control.** Nat. Rev. Genet., 4 (11) (2003), pp. 889-899

J. Roman, J. Darling. **Paradox lost: genetic diversity and the success of aquatic invasions.** Trends Ecol. Evol., 22 (9) (2007), pp. 454-464

G. Salt, R. van den Bosch. **The defense reactions of three species of *Hypera* (Coleoptera, Curculionidae) to an ichneumon wasp.** J. Invertebrate Pathol., 9 (2) (1967), pp. 164-177

N.D. Sargison, E. Redman, A.A. Morrison, D.J. Bartley, F. Jackson, E. Hoberg, J.S. Gilleard. **Mating barriers between genetically divergent strains of the parasitic nematode *Haemonchus contortus* suggest incipient speciation.** Int. J. Parasitol. (2019)

J. Shi, M. Macel, K. Tielbörger, K.J. Verhoeven. **Effects of admixture in native and invasive populations of *Lythrum salicaria.*** Biol. Invasions, 20 (2018), pp. 2381-2393

D. Simberloff. **The role of propagule pressure in biological invasions.** Ann. Rev. Ecol., Evol., Systemat., 40 (2009), pp. 81-102

A. Skoracka. **Reproductive barriers between populations of the cereal rust mite *Abacarus hystrix* confirm their host specialization.** Evol. Ecol., 22 (2008), pp. 607-616

B. Slippers, B.P. Hurley, M.J. Wingfield. ***Sirex* woodwasp: a model for evolving management paradigms of invasive forest pests.** Ann. Rev. Entomol., 60 (2015), pp. 601-619

B. Slippers, M.J. Wingfield. ***Sirex* research and management: future prospects. The *Sirex* Woodwasp and its fungal symbiont: research and management of a worldwide invasive pest.** Springer (2012), pp. 287-295

J. Spradbery, A. Kirk. **Aspects of the ecology of siricid woodwasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of *Sirex noctilio* F. in Australia.** Bull. Entomol. Res., 68 (1978), pp. 341-359

M. Szűcs, S.D. Eigenbrode, M. Schwarzländer, U. Schaffner. **Hybrid vigor in the biological control agent, *Longitarsus jacobaeae.*** Evol. Appl., 5 (2012), pp. 489-497

M. Szűcs, P.E. Salerno, B.J. Teller, U. Schaffner, J.L. Littlefield, R.A. Hufbauer. **The effects of agent hybridization on the efficacy of biological control of tansy ragwort at high elevations.** Evol. Appl., 12 (2019), pp. 470-481

F. Tomasetto, S. Cianciullo, M. Reale, F. Attorre, O. Olaniyan, S.L. Goldson. **Breakdown in classical biological control of Argentine stem weevil: a matter of time.** BioControl, 63 (2018), pp. 521-531

F. Tomasetto, J.M. Tylianakis, M. Reale, S. Wratten, S.L. Goldson. **Intensified agriculture favors evolved resistance to biological control.** Proceed. Natl. Acad. Sci., 114 (2017), pp. 3885-3890

J. Turgeon, A. Tayeh, B. Facon, E. Lombaert, P. De Clercq, N. Berkvens, J. Lundgren, A. Estoup. **Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion.** J. Evol. Biol., 24 (2011), pp. 1044-1052

E. Tzortzakakis, S. Gowen, D. Goumas. **Decreased ability of Pasteuria penetrans spores to attach to successive generations of *Meloidogyne javanica.*** Fund. Appl. Nematol., 19 (1996), pp. 201-204

J.M. Villacide, J.C. Corley. **Parasitism and dispersal potential of *Sirex noctilio*: implications for biological control.** Agric. For. Entomol., 10 (2008), pp. 341-345

B. Williams, B. Schrank, C. Huynh, R. Shownkeen, R. Waterston. **A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites.** Genetics, 131 (1992), pp. 609-624

D.W. Williams, A.E. Hajek. **Biological control of *Sirex noctilio* (Hymenoptera: Siricidae) in the northeastern United States using an exotic parasitic nematode.** Biol. Control, 107 (2017), pp. 77-86

Williams, D.W., Zylstra, K.E., Mastro, V.C., 2012. Ecological considerations in using *Deladenus* (= Beddingia) *siricidicola* for the biological control of *Sirex noctilio* in North America. The Sirex woodwasp and its fungal symbiont: Research and mangement of a worlwide invasive pest. Springer Science-Business Media, pp. 135–148.

M.J. Wingfield, B. Slippers, B.P. Hurley, T.A. Coutinho, B.D. Wingfield, J. Roux. **Eucalypt pests and diseases: growing threats to plantation productivity.** Southern For.: J. For. Sci., 70 (2008), pp. 139-144

S.H. Yek, B. Slippers. **Biocontrol opportunities to study microevolution in invasive populations.** Trends Ecol. Evol., 20 (2014), pp. 1-2

Q. Yu, P.d. Groot, I. Leal, C. Davis, W. Ye, B. Foord. **Characterization of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) associated with *Sirex noctilio* (Hymenoptera: Siricidae) in Canada.** Int. J. Nematol., 19 (2009), pp. 23-32

R. Zondag. **A nematode infection of *Sirex noctilio* (F.) in New Zealand .**New Zealand J. Sci., 12 (1969), pp. 732-747