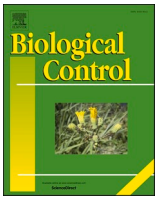
Biological Control 187 (2023) 105403

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon



# Reproductive compatibility of a newly imported Australian population of the biocontrol agent *Anaphes nitens* with an existing South African population

Harm Barten [a], Michelle L. Schröder [a], Bernard Slippers [b], Andy G. Howe [c], Simon A. Lawson [c], Brett P. Hurley [a,*]

[a] Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa
[b] Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa
[c] Forest Research Institute, University of the Sunshine Coast, Sippy Downs, Queensland, Australia

## HIGHLIGHTS

- Compatibility of Australian and South African *Anaphes nitens* populations was tested.
- No signs of reproductive isolation were found.
- No signs of cytoplasmic incompatibility were found.
- Positive or negative effects on fitness were not observed.
- No detrimental effect expected on the South African population after release.

## ARTICLE INFO

*Keywords:*
Intra-specific variation
Hybrid vigour
Inbreeding
Cytoplasmic incompatibility

## ABSTRACT

Introductions of natural enemies in classical biocontrol programs potentially cause genetic bottlenecks which can be detrimental for biocontrol. This can be mitigated by introducing multiple populations of a natural enemy, but thorough pre-release testing is needed to ensure compatibility. In this study compatibility between an established population of *Anaphes nitens* in South Africa and a newly imported *A. nitens* population from Australia was tested. *Anaphes nitens* is an egg parasitoid of *Gonipterus* sp. n. 2, an important pest in *Eucalyptus* plantations. South African and Australian *A. nitens* lineages were compared to two admixed lineages, which were reared from the F0 to the F2 generation. No differences were found in the proportion of replicates producing offspring overall, or female offspring specifically, indicating there was no sexual isolation between the populations. The typical symptoms of cytoplasmic incompatibility in haplodiploids, namely male biased sex ratios, were not observed. The lack of significant differences in fecundity and development time between the lineages suggested that there was no hybrid vigour or outbreeding depression. We conclude that a field release of the imported *A. nitens* population poses a low risk of disrupting the existing biocontrol program due to reproductive barriers or outbreeding depression. Whether there is a benefit of adding the newly imported Australian *A. nitens* population to the existing biocontrol system in South Africa needs to be studied further, for example by performing a field release combined with post-release assessments for determining establishment and spread.

## 1. Introduction

In classical biological control programs natural enemy species are artificially introduced into a new area. Establishment of this species depends on whether it can survive and reproduce in the target area. Traits associated with survival and reproduction can exhibit considerable intraspecific variation which might affect performance of a natural enemy (Liu et al., 2002; Lommen et al., 2017). Intraspecific variation is

* Corresponding author.
*E-mail address:* brett.hurley@fabi.up.ac.za (B.P. Hurley).

https://doi.org/10.1016/j.biocontrol.2023.105403
Received 6 May 2023; Received in revised form 15 November 2023; Accepted 21 November 2023
Available online 23 November 2023

the component natural selection will act upon to select the best performing individuals in a certain area; individuals not suited to the conditions there will go extinct. For a biocontrol program it is therefore important to capture enough of the variation present in the native range to achieve establishment of a natural enemy. Additionally, intraspecific variation in specific traits, such as host adaptation or climate tolerance, might be present in the native range, the absence or presence of which could determine whether an established natural enemy is effective in an introduced range.

A genetic bottleneck during introduction and subsequent mass rearing can cause the loss of genetic variation and high degrees of inbreeding. This can negatively affect biocontrol performance in some cases (Andersen and Mills, 2016; Bueno et al., 2017), although not necessarily in others (Quaglietti et al., 2017). Negative effects of genetic bottlenecks could be mitigated by importing additional populations of the natural enemy from the native range. Intraspecific variation in relevant life-history traits exists within natural enemies and the potential of exploiting this variation for augmentative biocontrol has been recognized (Lommen et al., 2017). Naturally occurring variation has been observed for a range of traits such as behavior (Colazza and Rosi, 2001; Lartigue et al., 2022), climate response (Scotta et al., 2021), fecundity (Andreazza and Rosenheim, 2015) and various other reproductive traits (Jervis et al., 2008). An example of utilizing this intraspecific variation is the practice of climate matching of parasitoid populations to a target area. Climate matching has been found to be useful for determining establishment success of some parasitoid species (Fischbein et al., 2019), but not for different populations of a single, asexually reproducing parasitoid species (Phillips et al., 2008). Thus, the usefulness of climate matching for guiding the exploitation of intraspecific variation of natural enemies needs more study, especially in parasitoids with different reproductive systems.

The biological control of the Eucalyptus snout beetle, *Gonipterus* sp. n. 2 (Coleoptera: Curculionidae), by *Anaphes nitens* Girault (Hymenoptera: Mymaridae) in South Africa is an example of a historically successful biocontrol program that recently started to show signs of failure. The beetle is native to the southeastern states of Australia (Schröder et al., 2021) and was first found in South Africa in 1916. It soon spread throughout the country and became a serious pest in *Eucalyptus* plantations (Mally, 1924). The beetle oviposits egg capsules constructed of excrement containing on average nine eggs, which hatch in about 15 days depending on temperature (Tooke, 1955). The four larval stages take about four to six weeks to become fully grown, after which they pupate in the soil, from where the beetles emerge after 30 to 40 days (Tooke, 1955). Both the adult beetles and larvae cause damage by feeding on *Eucalyptus* foliage. Control methods such as chemical or cultural control were not feasible, which expedited the implementation of a biocontrol program (Tooke, 1955).

In 1926 the egg parasitoid *A. nitens* was discovered in Penola, South Australia, and was imported into South Africa. *Anaphes nitens* has a haplodiploid sex determination system and is weakly synovigenic (Santolamazza-Carbone and Cordero-Rivera, 2002) with age-specific realized fecundity resembling a Type 1 curve (Santolamazza-Carbone et al., 2007; Jervis et al., 2008). The parasitoid prefers ovipositing in young host eggs (Santolamazza-Carbone, 2002), and has been observed to parasitize multiple eggs in one egg capsule successively (Mally, 1924), indicating it is semi-gregarious. Male parasitoids emerge before females and stick around on the egg capsules to mate with emerging females (Tooke, 1955). In the field, male *A. nitens* have been observed to chase each other away from the egg capsules (personal observation), suggesting there is competition between males for mating opportunities. Females mate several times during their lifetime, with different males (Tooke, 1955). Although not exhaustively tested, a lifetime fecundity of about 25 was found in South Africa (Tooke, 1955), close to the numbers found for *A. nitens* reared on *Gonipterus platensis* Marelli (Coleoptera: Curculionidae) (Santolamazza-Carbone et al., 2007). Parasitoids kept without food live for a maximum of three to four days, while honey fed individuals can live for over three weeks (Tooke, 1955).

The number of *A. nitens* parasitoids that was imported into South Africa is not well documented. Four shipments were sent over in 1926 with parasitized egg capsules collected from *Eucalyptus viminalis* near Penola, in South Australia. The second and third shipment contained 315 and 339 parasitized egg capsules, respectively (Tooke, 1955), while the information is lacking for the other shipments. After mass rearing, 736 395 parasitoids were released all over South Africa (Tooke, 1955). *Anaphes nitens* successfully established and became an example of the success of classical biological control (Tooke, 1955). However, recent outbreaks of *Gonipterus* sp. n. 2 indicate that the biological control by *A. nitens* is at times suboptimal, even in areas where biocontrol had previously been effective (Schröder et al., 2020). Various reasons have been hypothesized to explain failing biocontrol of *Gonipterus* spp. by *A. nitens* worldwide. Climate has been recognized as a key factor in this biocontrol system early on (Reis et al., 2012; Tooke, 1955), and is expected to become increasingly important with ongoing global warming. Furthermore, the *Eucalyptus* species compositions of South African plantations have been changing over the past decades (Morris, 2022), which could affect this biocontrol system through tri-trophic interactions. Subsequently a loss of genetic diversity of *A. nitens* at introduction has been hypothesized (Schröder et al., 2020), which may have impaired the adaptation potential of the parasitoid to these variable environments.

The geographic origin and the tree species from which the South African *A. nitens* population was collected in the native range were reported (Tooke, 1955), but the beetle species it was collected from is unknown. Recently it was discovered that *Gonipterus* sp. n. 2 belongs to a species complex of which multiple species are native to Australia (Mapondera et al., 2012), which makes it impossible to conclude from which species the parasitoid was originally collected in 1926. This uncertainty is magnified by the recent discoveries that *A. nitens* and *Gonipterus* sp. n. 2 natively occur over a wide geographic range in Australia (Schröder et al., 2021). This could mean that native parasitoid populations adapted to certain climates better resembling the climate in the main *Eucalyptus* growing areas of South Africa exist in Australia. Furthermore, as we currently do not know from which area the invasive beetle population in South Africa originates, there could be mismatches between the parasitoid and host populations.

Considering this, there is a strong case to import additional *A. nitens* populations from Australia. Several issues need to be addressed in order to do so safely. Non-target effects were not considered to be a concern for the importation in this study, as *A. nitens* has been present in South Africa for almost a century with no records existing of it attacking other species than *Gonipterus* sp. n. 2. Furthermore, testing for non-target effects in South Africa is only needed per imported species and not per population. Morphological and molecular identification, as well as testing of reproductive compatibility confirmed that the imported population was indeed *A. nitens*.

Assessing reproductive compatibility by comparing performance of South African, Australian and admixed *A. nitens* populations was further needed to address other concerns, as crossing genetically distant populations of parasitoids can have various outcomes. In a study on *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae), crosses between seven different populations resulted in outcomes ranging from inbreeding depression, to heterosis, to sexual isolation (Benvenuto et al., 2012). Inbreeding happens when there is a high degree of sib-mating and it can easily occur in biocontrol programs because of the inherent bottleneck at introduction and subsequent lab-rearing. It can result in inbreeding depression, which negatively affects fitness (Bueno et al., 2017). One way to reduce homozygosity of inbred genes in an introduced natural enemy population is to introduce a more distantly related population of the same species. Crossing genetically distant parents results in heterosis which can lead to hybrid vigour in the offspring (Das et al., 2021). However, care should be taken as inbreeding can have fitness costs, but so does outbreeding. Inbreeding costs decrease with

increasing genetic distance between parents, while outbreeding costs increase. Crossing parents that are genetically too distantly related can result in sexual isolation (Benvenuto et al., 2012). The interaction of inbreeding and outbreeding costs varies between species, depending on their specific life-histories. While *Mastrus ridens* Horstmann (Hymenoptera: Ichneumonidae), parasitoid of *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae), was found to suffer fitness costs when subjected to inbreeding (Bueno et al., 2017), for *Allotropa burrelli* Muesebeck (Hymenoptera: Platygastridae), parasitoid of *Pseudococcus comstocki* Kuwana (Hemiptera: Pseudococcidae), no detrimental effects of inbreeding on fitness were found (Quaglietti et al., 2017). The specific response of a population to genetic bottlenecks and inbreeding depends on the demographic and evolutionary processes to which it is subjected (Szűcs et al., 2019). In the context of supplementing biological control with additional intraspecific genetic variation, newly imported populations should be tested to see if hybrid vigour outweighs the inbreeding as well as outbreeding costs. If this is not the case the imported population will have a disadvantage compared to the existing population and establishment will be unlikely.

Cytoplasmic incompatibility (CI) can be induced by endosymbionts and could affect compatibility between insect populations (Ma et al., 2013). This phenomenon has been related to reduced reproductive potential when a differently infected parasitoid population is introduced into an existing population (Mochiah et al., 2002). CI is induced when an infected male mates with either an uninfected female or with a female harbouring a different endosymbiont strain. In haplodiploid species the most common symptom is a biased sex ratio towards haploid males as the paternal chromosomes are removed (Ma et al., 2013). In insects, the endosymbiont genera *Wolbachia*, *Cardinium* (Ma et al., 2013) and, more recently discovered, *Spiroplasma* (Pollmann et al., 2022) are known to be able to cause CI. Endosymbiont diversity of *A. nitens* has only been studied in Brazil, where none of the endosymbiont species encountered seemed to be involved in CI (Ribeiro et al., 2022). The Brazilian *A. nitens* population is an introduced population, and endosymbionts can potentially be lost at introduction (Nguyen et al., 2016). This means that endosymbiont diversity in *A. nitens* might be higher in the native range in Australia and could include CI inducing species. This may pose a risk when differentially infected *A. nitens* are introduced into South Africa and reproductive potential decreases due to CI. Pre-release testing is essential and can be performed by creating crosses between the populations and testing for the expected symptoms of CI in haplodiploids, namely male biased sex ratios.

In consideration of the above, a crossing experiment was conducted between the existing *A. nitens* population in South Africa and a newly imported *A. nitens* population from Queensland, Australia, to investigate the effect of admixture on this parasitoid. The first aim of this study was to assess possible sexual isolation between the South African and Australian *A. nitens* populations, which could happen either when cryptic species are involved or when endosymbiont induced CI occurs. The second aim was to see if the admixed parasitoids showed hybrid vigour (beneficial) or outbreeding depression (detrimental). The outcomes of the experiment are discussed in reference to the planned release of the Australian *A. nitens* into the field in South Africa.

## 2. Materials and methods

### 2.1. Populations

Crosses were performed between South African and Australian *A. nitens* parasitoids. A lab population was set up from parasitoids collected close to Boonah, Queensland, Australia (GPS: 27.974°S, 152.720°E) on 29 October and 4 November in 2021. A total of 1422 *Gonipterus* spp. egg capsules were collected from *Eucalyptus robusta*, *E. camaldulensis* and *E. propinqua*. Each egg capsule was placed individually in gelatin vials which were stored at 12 °C. After shipment to South Africa the egg capsules were transferred into the quarantine section of the biocontrol and insect rearing facilities, Innovation Africa campus, University of Pretoria. Egg capsules were kept inside an incubator set to 23 °C and 95 %RH with a light:dark cycle of 14:10 h. All emerging insects were sorted, the *Gonipterus* spp. larvae and other parasitoid species were killed and stored in 99.9 % ethanol, while emerging *A. nitens* parasitoids were used to establish a lab population. On average four male and female parasitoids were placed in a 6 x 1.5 cm glass vial covered with gauze and a plastic lid and supplied with a 3 x 3 mm piece of paper towel saturated with honey. A total of 25 *Gonipterus* sp. n. 2 egg capsules were offered to the parasitoids for the duration of one week, after which the parasitoids were killed and stored in 99.9 % ethanol. The parasitized egg capsules were stored in the same incubator until parasitoids emerged and the cycle was repeated. Generations F1 to F4 from the Australian lab population were used for the experiment. To increase the chances of finding virgin parasitoids for the experiment, egg capsules were stored individually in vials.

Since rearing of two lab populations of *A. nitens* simultaneously was not feasible due to limited availability of *Gonipterus* sp. n. 2 egg capsules, field collected *A. nitens* were used for the South African population. Egg capsules of *Gonipterus* sp. n. 2 were collected on 2 and 14 December 2021 and 7 and 19 January 2022 in the Rooihoogte plantation close to Carolina, Mpumalanga (GPS: 26.063°S, 30.270°E), planted with *Eucalyptus benthamii*. The collected egg capsules were placed individually in 1.5 mL Eppendorf tubes to increase chances of finding virgin parasitoids. All vials were kept at room temperature at 25 °C ± 2 °C and 95 %RH.

To obtain 0–1 day old virgin parasitoids for the experiment, the vials and Eppendorf tubes with parasitized egg capsules were checked daily for emergence. Each day the vials and Eppendorf tubes were cleared, the unused Australian parasitoids were set aside for rearing of the lab population and the unused South African parasitoids were killed and stored in 99.9 % ethanol. Only virgin, 0–1 day old female parasitoids were used for the experiment, but for males this was not always possible. Due to rearing constraints 1–3 day old males had to be used in some cases (Supplementary Table S1). When male parasitoids were used that had emerged together with females it was assumed that the male had mated, and the mating status was noted for later analysis.

*Gonipterus* sp. n. 2 egg capsules were used for rearing the Australian *A. nitens* lab population and for the experiment. The egg capsules were obtained from a culture which was kept at the same facility. This population consists of predominantly field collected beetles which were fed three times a week on *Eucalyptus dunnii* foliage. For the duration of the experiment the containers with beetles were checked daily for egg capsules to provide 0–1 day old egg capsules for the experiment. Egg capsules were punched from the leaf on a ± 5 mm diameter leaf disc.

### 2.2. Identification of parasitoids

Morphological and molecular identification was performed on the imported Australian *A. nitens* parasitoids. Representative samples of all parasitoid species from the F0 generation, as well as *A. nitens* samples from the F1, F2 and F3 generations of the lab culture were sent to the Agricultural Research Council (ARC), Pretoria, South Africa, where they were identified by Dr. Gerhard L. Prinsloo. Furthermore, three female and two male *A. nitens* specimens from the F1 generation were sequenced for molecular identification. An 881 bp fragment of the mitochondrial CO1 gene region was sequenced using the primers C1-J-2183 (5′-CAA CAT TTA TTT TGA TTT TTT GG) and TL2-N-3014 (5′-TCC AAT GCA CTA ATC TGC CAT ATT A) (Simon et al., 1994). DNA was extracted from whole specimens which were ground up with a pipette tip, using the ZyGEM PrepGEM® Universal kit and following the manufacturer's protocol for insect DNA extraction. Incubation was performed for 15 min at 75 °C, followed by an inactivation step at 95 °C for 5 min. From this extract 0.5 μL was added as template to a PCR reaction with a total volume of 12.5 μL, consisting of 1.25 μL 10x PCR buffer (ROCHE, Roche Diagnostics), 1.25 μL magnesium chloride (ROCHE) (25 mM), 0.1 μL FastStart Taq Polymerase (ROCHE) (5U/μl), 1.25 μL

DNTPs (New England Biolabs) (2.5 μM each), 0.5 μL of the forward and reverse primer (Whitehead Scientific (Pty) Ltd.) (10 mM each) and 7.15 μL distilled water. Initial denaturation was performed at 95 °C for 7 min, followed by a touch-down step consisting of 11 cycles of denaturation at 95° for 30 s, annealing for 40 s with temperature decreasing from 55 °C to 45 °C with −1°C per cycle, and extension at 66 °C for 1 min. This was followed by 29 cycles of denaturation at 95 °C for 30 s, annealing at 51 °C for 40 s and extension at 66 °C for 1 min followed by a final extension step at 66 °C for 7 min. Successfully amplified PCR products were purified with the Zymo DNA Clean & Concentrator kit (Zymo Research) according to the manufacturer's protocol and diluted to 25 ng/μL. Duplicate cycle sequencing reaction were run for each primer separately. The reactions consisted of 1.875 μL sequencing buffer (Applied Biosystems, Thermo Fischer Scientific), 0.25 μL BigDye (Applied Biosystems™), 1 μL primer (10 mM), 5.875 μL distilled water and 1 μL purified PCR product. Cycle sequencing was performed with initial denaturation at 96 °C for 2 min followed by 30 cycles of denaturation at 96 °C for 30 s and a combined annealing and extension step at 60 °C for 4 min and 15 s. Cycle sequencing products were cleaned up by running them through columns loaded with hydrated G-50 Sephadex (Sigma-Aldrich). Sequencing was performed on an ABI3500xL Genetic analyser (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA).

### 2.3. Lineages

Four lineages were set up, which are indicated with a code describing the origin on the F0 parents. The pure South African and Australian lineages served as the controls, indicated with the codes ZA♂xZA♀ and AU♂xAU♀ respectively. Two admixed lineages were set up, the first one was initiated by crossing a South African male with an Australian female, and the second one by pairing an Australian male and South African female. These lineages are indicated with the codes ZA♂xAU♀ and AU♂xZA♀ respectively. All four lineages were reared up to the F2 generation according to the crossing scheme shown in Fig. 1.

For the F0 crosses one male and female from the respective populations were placed in a glass vial and mating was confirmed visually. A mated parasitoid pair was supplied with three host egg capsules. The

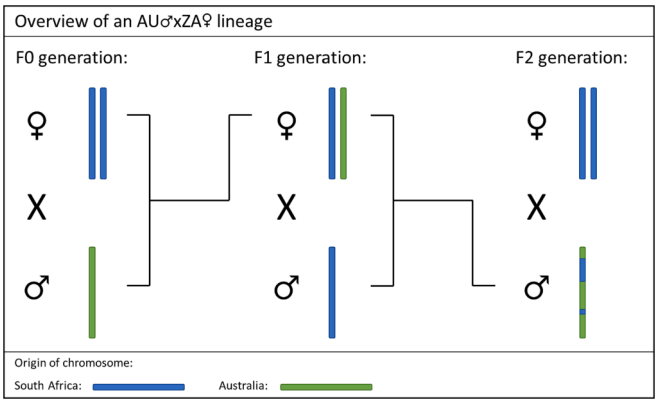



**Fig. 1.** An example crossing scheme of an AU♂xZA♀ lineage which was started with an Australian male and South African female in the F0 generation. The bars represent an arbitrary chromosome, while the colours indicate the geographical origin of this chromosome. In the F1 generation only the female offspring is a mixed individual; it received one chromosome set from the mother and one from the father. This individual was crossed in the F1 cross with an unrelated South African male parasitoid. Due to recombination, the F2 offspring contains males with chromosomes with mixed South African and Australian origin (as well as females with one mixed chromosome and one South African chromosome, but they were not tested). The mixed male was crossed with an unrelated, South African female in an F2 cross to test its performance.

vials were supplemented with a piece of paper towel saturated with honey as described above, and kept in an incubator at 23 °C at 95 %RH with a 14:10 light:dark cycle. On day three the egg capsules were removed, and three new egg capsules were offered, and this procedure was repeated on day five. Supply of *Gonipterus* sp. n. 2 egg capsules was generally unpredictable during the experiment, with significant fluctuations occurring over consecutive days. To increase chances of finalizing a replicate, the replicates were terminated after one week and on day seven the parent parasitoids were killed and stored in 99.9 % ethanol. The parasitized egg capsules were kept in the incubator and checked daily for offspring emergence. For each batch of three egg capsules the date and number of parasitoids emerging was recorded. The total number of male and female offspring per replicate was counted and the sex ratio was calculated by dividing the number of male offspring by the total number of offspring. For each parent pair, the median development time of the male and female offspring was calculated separately. After 45 days the replicates were ended.

For the F1 crosses a virgin female offspring from the F0 cross was taken and paired with a male parasitoid. The offspring of the AU♂xAU♀ lineages were paired with a male from the Australian lab population while the offspring of the other three lineages were paired with a South African male. In this way the control lineages remained pure South African and Australian respectively, while for the admixed lineages the most likely scenario after a release in the field was mimicked, namely mating with a South African male. Mating was confirmed visually, and supply of egg capsules and termination of the experiment followed the procedure of the F0 crosses.

From each F1 cross one male offspring was taken to perform the F2 crosses. Each male offspring from the AU♂xAU♀ lineages was paired with an Australian female, while for the other lineages South African females were used, for the same reason described above. Mating was confirmed, and supply of hosts and termination of the experiment was performed in the same way as for the F0 and F1 crosses. Only replicates where the mother survived until the 7th day were used for further data analysis.

Setting up replicates for the F0 crosses of the experiment was dependent on timing of parasitoid emergence from the South African field collected population and the Australian lab population, as well as host availability. Additionally, F1 and F2 replicates depended on emergence of suitable parasitoids from the previous generations. The constraint that only 0–1 day old female parasitoids could be used, and the requirement of using virgin females, led to a declining number of replicates which could be performed for the later generations; the total number of successful replicates are shown in Supplementary Table S2. In five instances a replicate was disqualified for further analyses because the mother died before day seven. The higher number of replicates of the ZA♂xAU♀ lineage in the F1 generation compared to the F0 generation is because two replicates of the F0 crosses were disqualified, but still produced viable daughters which could be used to set up the subsequent F1 crosses.

### 2.4. Maternal size and paternal mating status

Considerable variation was observed in the number of offspring produced between individuals, even within treatments. Fecundity of parasitoids is generally positively related to the maternal body size (Durocher-Granger et al., 2011; Ellers et al., 2001; Gao et al., 2016). For *A. nitens* wing length was found to be positively correlated to egg load (Santolamazza-Carbone et al., 2008) and negative to sex ratio (Santolamazza-Carbone et al., 2007). To test if size played a role in determining fecundity and sex ratio in our experiment, femur size was measured for each mother in the experiment. The right hind leg was removed from each individual and mounted on a glass slide with a droplet of 70 % ethanol and a cover slide. Photos were taken using a Nikon SMZ1500 microscope equipped with an Olympus DP21 camera at a 112.5x magnification. Measurements were performed using the

Olympus Stream Basic 1.9.4 software. Besides maternal size, another potential source of variation was the paternal mating status. Due to the semi-gregarious lifestyle of the parasitoid, sometimes males had to be used that had potentially already mated when they emerged together with a female, which was taken into account in some of the analyses, as indicated below.

### 2.5. Data analysis

To assess sexual isolation, the proportion of replicates failing to produce either any offspring, or female offspring were compared. First the proportion of replicates producing no offspring was compared between the four lineages within each generation using Fisher's exact tests. Significant results were followed by a pairwise Fisher's post-hoc test with Holm correction. Subsequently, from the replicates that did produce at least one offspring, the proportion of replicates producing only males versus replicates producing at least one female was compared in the same way.

Sex ratios were compared between the lineages within each generation. The proportion of males from the total number of offspring was used as a measure for sex ratio and analysed in a GLM with binomial distribution and logit link function. A separate GLM was run for each generation, and lineage was used as an explanatory variable, and maternal femur length and paternal mating status were used as covariates. Chi-square tests were performed to test the significance of the explanatory variables, and in case a significant effect of lineage was found, a post-hoc analysis was done using the 'emmeans' package in R to calculate Tukey adjusted p-values and (back-transformed) estimated marginal means.

To test if crossing the Australian and South African *A. nitens* populations had an effect on fitness, offspring development time and realized fecundity were used as a measure of fitness. Because development time of male offspring was shorter compared to female offspring, they were analysed separately. For development time, GLMs with a Gamma error distribution and log link were run with lineage as the explanatory variable and maternal femur length as covariate. Total, male and female offspring production was compared between lineages using linear models. Lineage was used as the explanatory variable and maternal femur was used as covariate. Additionally, the mating status of the father was used as a covariate in the analyses for male and female offspring production, but not for total offspring production. For each analysis, when a GLM or linear model indicated a significant effect of lineage, post-hoc analyses were performed as described for sex ratio. All analyses were run separately for each generation.

All statistical analyses were performed in R4.2.1 (R Core Team 2022).

## 3. Results

### 3.1. Identification

All specimens from the Australian lab population which were sent to the ARC for morphological identification were confirmed to be *A. nitens* (ID report nr.: 2022-DSI-017). Molecular identification performed by the FABI Diagnostic Clinic further confirmed the identity of the parasitoids. A portion of the mitochondrial CO1 region was successfully amplified, from which an 821 bp part was aligned and compared. The sequences from the Australian specimens (GenBank acc. nr.: OR797879) proved to be 100 % identical to the one *A. nitens* sequence already present on GenBank (acc. nr.: AY642452.1) as well as to South African *A. nitens* sequences (GenBank acc. nr.: OR797880).

### 3.2. Maternal size and paternal mating status

Some variation in maternal femur length was observed between lineages (Supplementary Fig. S1). In the F0 crosses the mothers of the ZA♂xAU♀ lineages were significantly smaller compared to the ZA♂xZA♀ and AU♂xZA♀ lineages (Kruskal-Wallis test; $\chi^2 = 10.2986$, df = 3, p = 0.02; see Supplementary Table S3 for post-hoc results), while no differences were observed in the F1 generation (Kruskal-Wallis test; $\chi^2 = 0.2602$, df = 3, p = 0.97). In the F2 generation the female parasitoids of the AU♂xZA♀ lineages were significantly larger compared to the AU♂xAU♀ lineages (Kruskal-Wallis test; $\chi^2 = 8.6234$, df = 3, p = 0.03; see Supplementary Table S4 for post-hoc results). The proportion of replicates with fathers which had potentially mated prior to the experiment is shown in Supplementary Fig. S2.

### 3.3. Potential reproductive isolation

The Fisher's exact test indicated there was a marginally significant difference in the proportion of replicates producing no offspring at all within the F1 generation (p = 0.0497, Supplementary Table S2), but subsequent post-hoc tests showed no differences between any pair of lineages (Supplementary Table S5). For the F0 and F2 crosses no significant differences were found. Most replicates that did produce at least one offspring, also gave female offspring. In the F0 generation one AU♂xZA♀ and two AU♂xAU♀ replicates failed to produce females, as well as one ZA♂xZA♀ replicate in the F1 generation. The Fisher's exact tests showed no significant differences in the numbers of replicates failing to produce female offspring between the lineages in the F0 and F1 generation (p = 0.322 and 0.714, respectively).

Sex ratios differed slightly between lineages (Fig. 2). The lineages were compared within each generation and no significant effect of lineage was found in the F0 and F2 generation (see Supplementary Table S6, and Supplementary Fig. S4), but a significant effect was detected in the F1 generation (Chi-square test; $\chi^2 = 9.521$, df = 3 (72), p = 0.023). However, the Tukey adjusted p-values in a post-hoc analysis indicated that there were no significant differences when specific lineages were compared in a pairwise comparison (Supplementary Table S7). No significant effect of maternal femur length or mating status of the father on sex ratio was detected in any generation (see Supplementary Table S6).

### 3.4. Hybrid vigour and outbreeding depression

To assess admixed performance compared to the control lineages, median development time and fecundity were compared. Male and female development time differed significantly, with male parasitoids developing faster than females (Wilcoxon signed rank test; V = 4944.5, p < 0.001 and effect size = 0.304; Supplementary Fig. S3). Thus, the development time between lineages within generations were compared for males and females separately. Minor differences in offspring development time were observed between lineages (Fig. 3), and only for male development time in the F0 generation this difference was significant (Chi-square test; $\chi^2 = 0.1089$, df = 3 (74), p = 0.0143; see Supplementary Table S8 and Supplementary Fig. S5). Here, male offspring developed faster in the ZA♂xAU♀ compared to the ZA♂xZA♀ lineages (t = 2.663, df = 73, p = 0.0459, see Supplementary Table S9 and Fig. 4). No significant difference in female development time was found between lineages in any generation (Supplementary Table S10 and Supplementary Fig. S5). Maternal size did not affect either male or female development time in any generation (Supplementary Table S8 and Supplementary Table S10).

Fecundity was compared between lineages within each generation. Some variation was observed (Fig. 5), but no significant differences were found between lineages (Supplementary Table S11 to Supplementary Table S13 and Supplementary Fig. S6). In F1 a significant effect of maternal femur length was found on total and female offspring production (Supplementary Table S11 and Supplementary Table S13, respectively), in both cases larger females produced more offspring (Supplementary Fig. S7). No effect of the mating status of the father was observed in any lineage or generation.

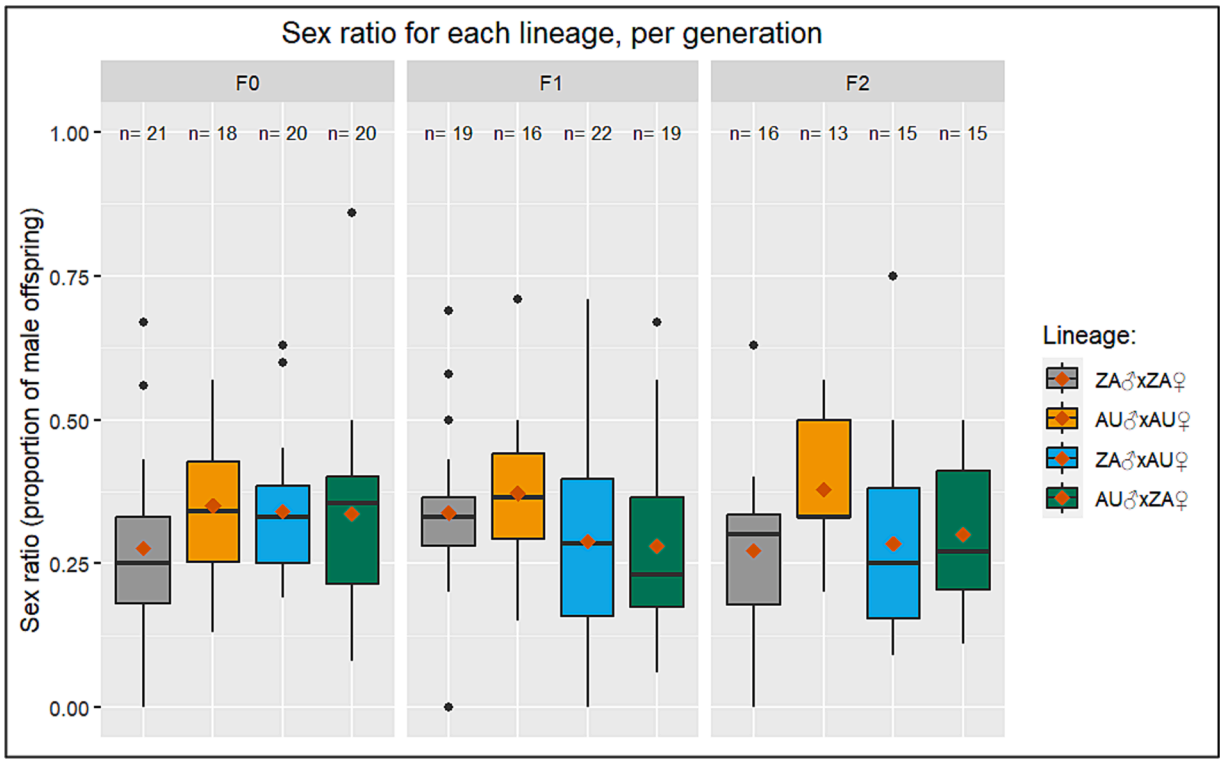



**Fig. 2.** Box plots showing the data distributions for sex-ratio of each lineage within each generation. The red diamonds indicate the mean sex ratio for each lineage, and the number of replicates is shown on top of the graphs. A significant effect of 'lineage' was detected in the F1 generation (Chi-square test; $\chi 2 = 9.521$, df = 3 (72), $p = 0.023$), but the Tukey adjusted p-values from the post-hoc analysis indicated that there were no significant differences between any specific lineages.

## 4. Discussion

In this study, we assessed compatibility of a newly imported *A. nitens* population from Australia with the existing population in South Africa. No signs of sexual isolation were found as most replicates produced offspring, most of which also yielded female offspring. Similarly, the results suggest that there were no signs of CI as sex ratios were similar between admixed and control lineages. Furthermore, no detrimental or beneficial effects of crossing were observed on fitness, except for faster development time of male offspring of the ZA♂xAU♀ lineage in the F0 generation. Together, this points towards a mostly neutral effect of admixture of the South African and Australian *A. nitens* populations, as has been found before in other parasitoid species (Bertin et al., 2018).

Both morphological and molecular identification confirmed the imported Australian parasitoid population consisted of the same species already present in South Africa, namely *A. nitens*. Sequencing of an 881 bp region of the mitochondrial COI region revealed that amplicons of South African and Australian specimens were 100 % identical, indicating that genetic distance between the populations is small. The COI gene is one of the most conserved mitochondrial genes (Simon et al., 1994), and is commonly used for species identification of insects. Other genetic markers could reveal genetic differentiation between these two *A. nitens* populations, e.g. alternative genes in the mitochondrial genome with higher evolutionary rates (Dong et al., 2021). In addition, since one aspect of utilizing intraspecific variation for improving biological control involves climate matching, the mitochondrial climatic adaptation hypothesis (Camus et al., 2017; Chung and Schulte, 2020) could be used to choose suitable mitochondrial marker genes for further investigation. Ideally, such analyses would be paired with nuclear markers such as microsatellites to improve understanding of the population genetics (Andersen and Mills, 2016; Zepeda-Paulo et al., 2015).

Sexual isolation has been found between geographically distant populations of some parasitoid species (Joyce et al., 2010; Takada and Tada, 2000), and the possibility of sexual isolation occurring between the South African and Australian *A. nitens* populations needed to be excluded. If no offspring was produced at all, this would mean that the mother parasitoids did not produce or oviposit viable eggs. Failure to produce specifically female offspring would indicate the presence of other factors such as male sterility (Clark et al., 2010) or the presence of post-mating prezygotic or postzygotic barriers, for example cytoplasmic incompatibility (König et al., 2019). From the successful replicates performed in this study, most also yielded female offspring indicating successful admixture occurred between the populations.

A closer inspection of the replicates failing to produce female offspring revealed that in all three cases in the F0 generation, 1–3 day old males were used that had already been exposed to females before the experiment. Two of those replicates were AU♂xAU♀ lineages, and one was an AU♂xZA♀ lineage. The replicates produced 17, 12 and 16 male offspring respectively, which was close to the average total offspring production in the F0 generation. Since older and mated fathers were used in those replicates, sperm depletion rather than reproductive isolation likely happened in those cases. Furthermore, two of the replicates were pure Australian making reproductive isolation unlikely. The one replicate in the F1 generation failing to produce female offspring was from a pure South African lineage, which produced only three male offspring in total. Also, in this case reproductive isolation was unlikely because it concerned a pure South African lineage. Those four datapoints were excluded from the other analyses since a different process such as sperm depletion likely played a role instead of reproductive isolation, and they significantly skewed the results. The absence of reproductive isolation found between the *A. nitens* populations agrees with findings from other studies where different parasitoid populations were crossed without signs of reproductive incompatibility (Bertin et al., 2018; Liu et al., 2002; Vallina et al., 2020).

Cytoplasmic incompatibility (CI) can occur when populations of a same parasitoid with a different endosymbiont infection status are crossed (Branca et al., 2019; Ma et al., 2013; Werren et al., 2008). When complete CI happens in a haplodiploid organism either all fertilized eggs

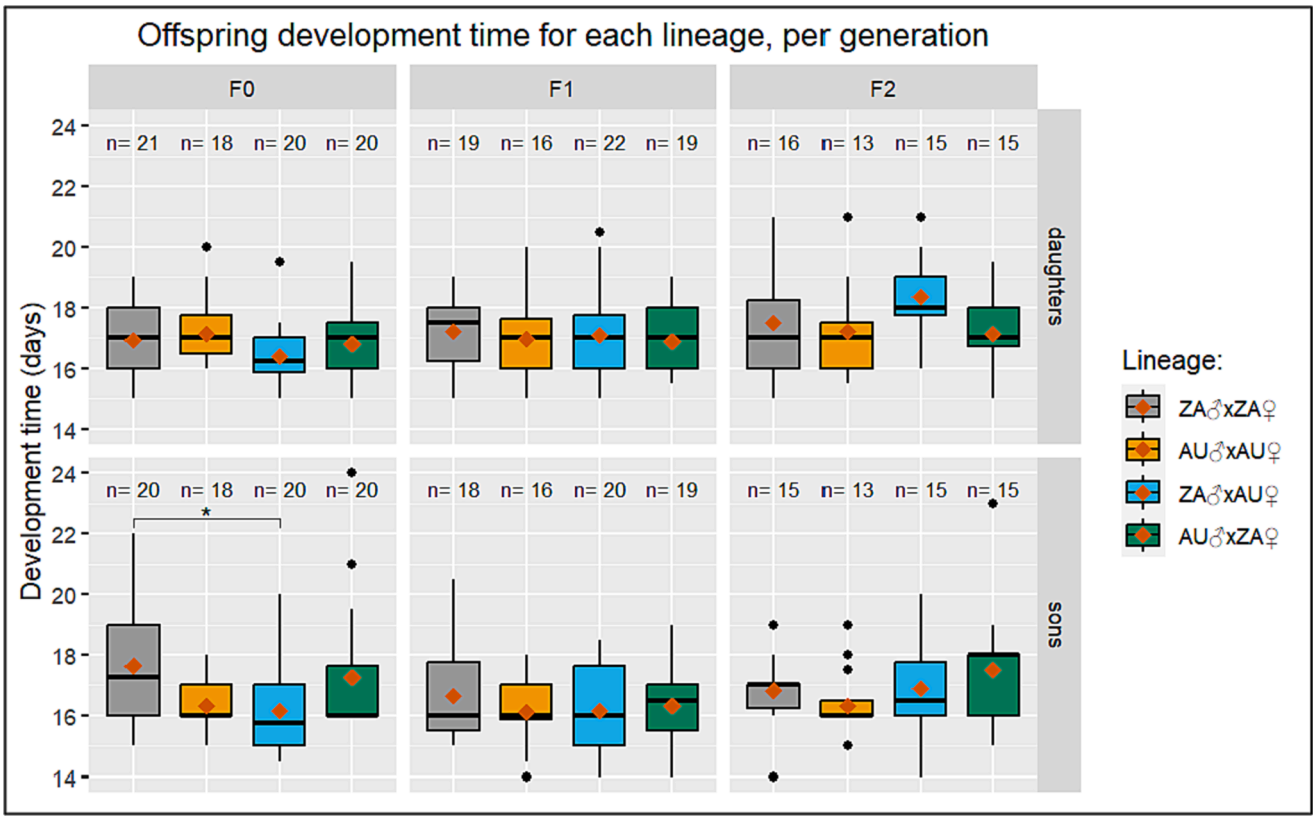



**Fig. 3.** Boxplots depicting the data distributions of development time of the male and female offspring of each lineage, per generation. The red diamonds indicate the mean development time for each lineage, and the number of replicates is given at the top of the graphs. A significant effect of 'lineage' was found on development time of the sons in the F0 generation (Chi-square test; $\chi 2 = 0.1089$, df = 3 (74), p = 0.0143; see Table 2). They developed faster in the ZA♂xAU♀ compared to the ZA♂xZA♀ lineage (t = 2.663, df = 73, p = 0.0459), as indicated with the asterisk.


**Fig. 4.** Estimated marginal means of male development time per lineage for the different generations. Comparisons were made within a generation, and only in the F0 generation a significant difference was found between lineages. Lineages with different letters are significantly different, and the letters only apply within a generation. Sons developed faster in the ZA♂xAU♀ compared to the ZA♂xZA♀ lineage (t = 2.663, df = 73, p = 0.0459). The error bars show the 95 % confidence intervals.

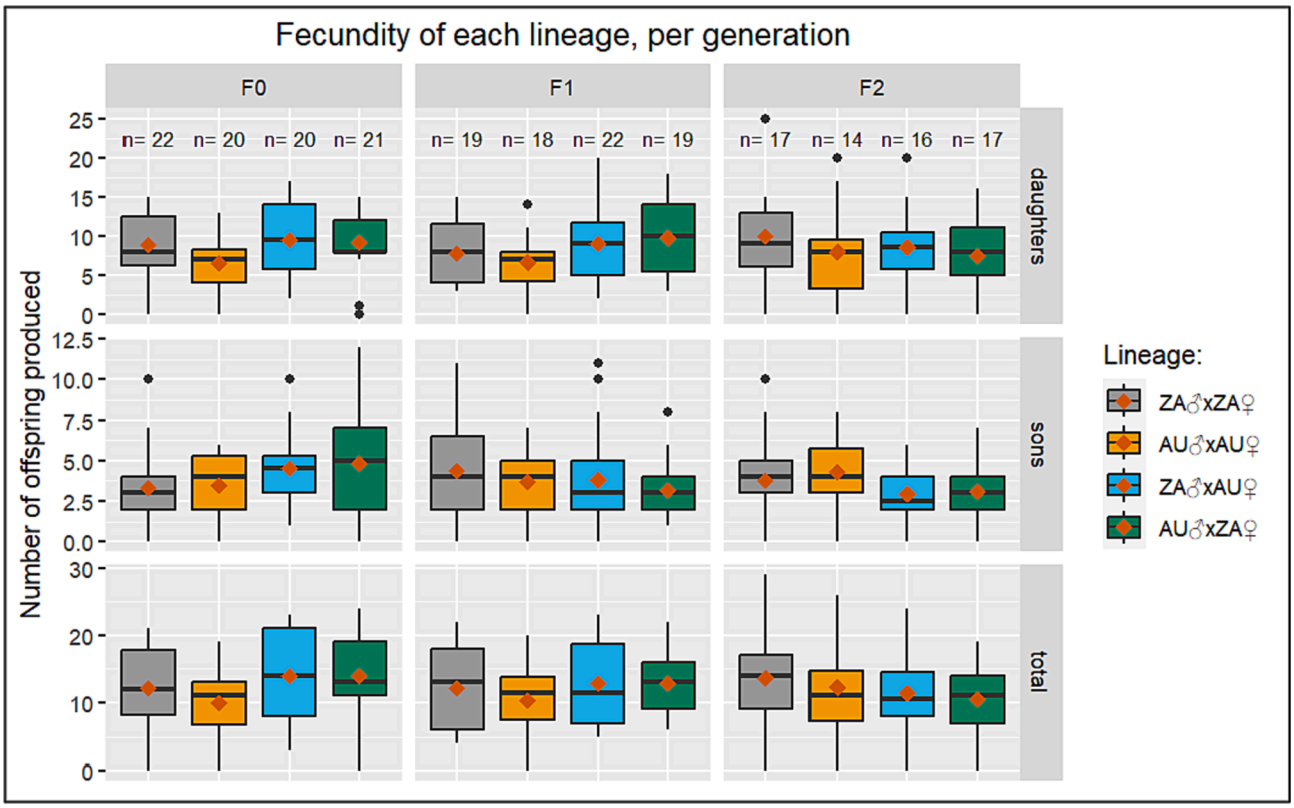



**Fig. 5.** Boxplots showing fecundity per lineage for each generation, shown separately for female, male and total offspring production. The red diamond indicates the mean number of offspring produced for each lineage. The number of replicates is given at the top of the graph. No significant differences in fecundity were found between lineages within any generation.

fail to develop, or they develop as males (Ma et al., 2013). Incomplete CI can happen when endosymbiont infection prevents some, but not all female offspring to develop, which also leads to male-biased sex ratios (Betelman et al., 2017). In the context of biological control, CI has the potential to create hybrid zones where parasitoid population growth and therefore biocontrol efficiency is reduced (Mochiah et al., 2002). In this study most replicates produced both male and female offspring in the F0, F1 and F2 crosses, and sex ratios did not differ significantly between the South African and Australian control lineages or between the control and admixed lineages. While our findings do not rule out the possibility of endosymbionts being present in *A. nitens*, if present, they do not cause reproductive barriers between the South African and Australian populations. In the context of a field release this means that there is no risk of hybrid zones being formed because of CI, where differently infected parasitoids are incompatible and biocontrol efficiency is reduced. Future studies are needed to elucidate the diversity of endosymbionts occurring in *A. nitens* in populations in the native and introduced range, which should ideally be combined with general population genetic studies.

No clear signs of hybrid vigour or outbreeding depression were observed when comparing fecundity and offspring development time. Fecundity has been used as a primary proxy of parasitoid fitness (Benvenuto et al., 2012; Roitberg et al., 2001). Usually lifetime fecundity is measured, but determining lifetime fecundity of *A. nitens* was not feasible in this experimental setup due to limited host egg supply. Considering that *A. nitens* prefers ovipositing in young host eggs (Santolamazza-Carbone, 2002), and can live up to three weeks (Mossop, 1929; Tooke, 1955), this would have meant that egg supply needed to be constant during this whole period. Instead, a standardized approach was taken where parasitoids were allowed to parasitize during one week while fresh egg capsules were offered every second day, and realized weekly fecundity was recorded. Various patterns of age-specific fecundity have been reported for different parasitoid species (Jervis et al., 2008). For *A. nitens* daily fecundity peaks on the first day after emergence and decreases with age (Santolamazza-Carbone et al., 2007), indicating it follows a Type 1 curve as described in (Jervis et al., 2008). Therefore, fecundity of the first week should be a good measure of fitness of *A. nitens* and any significant deviations in fecundity are expected to show up during this period. Development time is another important metric for assessing parasitoid fitness (Roitberg et al., 2001; Valente et al., 2017; Zhou et al., 2014), and together with fecundity it is tightly linked to the intrinsic rate of increase of a population (Laughlin, 1965), which can show intra-specific variation in parasitoids (Liu et al., 2002). For *A. nitens* no difference in fecundity was found between the South African and Australian *A. nitens* populations and their crosses, and only in the F0 generation a significantly shorter development time was observed for male offspring of the ZA♂xAU♀ lineage. However, this difference was not carried over to the next generations and is likely not heritable. Together this indicates that admixture between the populations had a neutral effect on those fitness measures, as was found for other parasitoid species (Benvenuto et al., 2012; Bertin et al., 2018). This was also to be expected from the small genetic distance between the populations as discussed earlier (Benvenuto et al., 2012).

The absence of sexual isolation, outbreeding depression, and incompatibility between the Australian and South African populations, indicates that the admixture between the populations will not incur detrimental effects on the fitness of admixed individuals. While no fitness advantages were found which could increase chances of establishment, not all possible fitness metrics were considered in this study. Assessing other important fitness metrics such as longevity and lifetime fecundity (Roitberg et al., 2001) could reveal differences between the populations. Besides, in the current experiment *A. nitens* was reared at a constant temperature of 23 °C and 95 %RH, and thus the effect of climate on fitness of the different populations was not considered. Climate data retrieved from the WorldClim 2 dataset (Fick and Hijmans,

2017) show that temperatures in Boonah are generally higher compared to Penola, where the original *A. nitens* import originates from. Boonah better matches the climate in Kwambonambi, South Africa, which is the intended target release site of the newly imported *A. nitens* population (Supplementary Table S14). If climate adaptation has taken place in *A. nitens*, the population collected in Boonah is expected to perform better at higher temperatures, as has been found for different populations of *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), a pupal parasitoid of *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae) (Liu et al., 2002). This could mean that fitness benefits of the newly imported *A. nitens* population only show up in warmer areas, such as the tropical parts of KwaZulu-Natal.

Increasing the number of fitness traits tested in the laboratory might not be feasible, especially considering the difficulties of rearing *A. nitens* and its host *Gonipterus* sp. n. 2. Instead, it could be more practical to release the Australian population into the field and track its establishment and spread, for example using microsatellite markers. Combining a release with further experiments will present an opportunity to use this study system to address various applied ecological questions, such as whether importing additional intraspecific variation into an existing biocontrol program is beneficial, which has historically been found to be seldom the case (Clarke and Walter, 1995). Furthermore, additional lab experiments are needed to compare climatic tolerances of the Australian and South African *A. nitens* populations. If *A. nitens* has the capability to adapt to climate, it is hypothesized that the newly imported population will perform better in the warmer *Eucalyptus* growing areas in South Africa. If this is the case this study system could be used to validate the use of intra-specific climate matching for improving biological control (Phillips et al., 2008).

## 5. Conclusion

This study is a first foray into the exploration of utilizing intraspecific variation in *A. nitens* for the improvement of biological control of *Gonipterus* sp. n. 2 in South African *Eucalyptus* plantations. We found that a newly imported *A. nitens* population from Australia was able to breed with the South African population, despite that it was imported nearly 100 years earlier from a different state in Australia. No negative effects of admixture between the populations were found, but no improved fitness was observed under the current experimental conditions either. Nevertheless, this result paves the way for a field release and sets up a framework for using this parasitoid to study key ecological questions. It can be used to investigate the usefulness of addition of intra-specific variation to existing biocontrol programs and the practicality of intra-species climate matching.

## CRediT authorship contribution statement

**Harm Barten:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Michelle L. Schröder:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Bernard Slippers:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Andy G. Howe:** Writing – review & editing. **Simon A. Lawson:** Writing – review & editing. **Brett P. Hurley:** Conceptualization, Methodology, Writing – review & editing, Supervision.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bernard Slippers reports financial support was provided by Tree Protection Co-operative Programme (TPCP).

## Acknowledgement

We thank Tim Phelps and Frank Mikula from the Lone Pine Koala Sanctuary, Brisbane for access to the eucalypt plantations to collect the Australian *A. nitens* population. We thank SAPPI for access to the Rooihoogte plantation to collect the South African parasitoids used in this experiment. We thank Sandisiwe Jali and Josias Letaoana for the field collections, as well as Amy Collop, Idea Makowe and Samantha Bush for maintaining the lab colony. We thank Dr. Gerhard L. Prinsloo for the morphological identifications of the imported material. We acknowledge the DNA Sanger sequencing facility, Faculty of Natural and Agricultural Sciences, University of Pretoria, for performing the DNA sequencing.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2023.105403.

## References

Andersen, J.C., Mills, N.J., 2016. Geographic origins and post-introduction hybridization between strains of *Trioxys pallidus* introduced to western North America for the biological control of walnut and filbert aphids. Biol. Control 103, 218–229. https://doi.org/10.1016/j.biocontrol.2016.09.011.

Andreazza, F., Rosenheim, J.A., 2015. Absence of transgenerational phenotypic plasticity in fecundity in the parasitoid *Anagrus erythroneurae* (Hymenoptera: Mymaridae). J. Insect Sci. 15 (1), 138. https://doi.org/10.1093/jisesa/iev122.

Benvenuto, C., Tabone, E., Vercken, E., Sorbier, N., Colombel, E., Warot, S., Fauvergue, X., Ris, N., 2012. Intraspecific variability in the parasitoid wasp *Trichogramma chilonis*: can we predict the outcome of hybridization? Evol. Appl. 5 (5), 498–510. https://doi.org/10.1111/j.1752-4571.2012.00279.x.

Bertin, A., Pavinato, V.A.C., Parra, J.R.P., 2018. Effects of intraspecific hybridization on the fitness of the egg parasitoid *Trichogramma galloi*. BioControl 63 (4), 555–563. https://doi.org/10.1007/s10526-018-9883-7.

Betelman, K., Caspi-Fluger, A., Shamir, M., Chiel, E., 2017. Identification and characterization of bacterial symbionts in three species of filth fly parasitoids. FEMS Microbiol. Ecol. 93 (9) https://doi.org/10.1093/femsec/fix107.

Branca, A., Le Ru, B., Calatayud, P.-A., Obonyo, J., Musyoka, B., Capdevielle-Dulac, C., Kaiser-Arnauld, L., Silvain, J.-F., Gauthier, J., Paillusson, C., Gayral, P., Herniou, E. A., Dupas, S., 2019. Relative influence of host, *Wolbachia*, geography and climate on the genetic structure of the Sub-saharan parasitic wasp *Cotesia sesamiae*. Front. Ecol. Evol. 7, 309. https://doi.org/10.3389/fevo.2019.00309.

Bueno, E., Romero, A., Osorio, I., Zaviezo, T., 2017. Laboratory rearing methods that promote inbreeding have a negative impact on the fitness of *Mastrus ridens* Horstmann (Hymenoptera: Ichneumonidae), a parasitoid used to control the codling moth. Chilean Journal of Agricultural Research 77, 413–419. https://doi.org/10.4067/S0718-58392017000400413.

Camus, M.F., Wolff, J.N., Sgrò, C.M., Dowling, D.K., 2017. Experimental support that natural selection has shaped the latitudinal distribution of mitochondrial haplotypes in Australian *Drosophila melanogaster*. Mol. Biol. Evol. 34 (10), 2600–2612. https://doi.org/10.1093/molbev/msx184.

Chung, D.J., Schulte, P.M., 2020. Mitochondria and the thermal limits of ectotherms. J. Exp. Biol. 223 (20) https://doi.org/10.1242/jeb.227801.

Clark, M.E., O'Hara, F.P., Chawla, A., Werren, J.H., 2010. Behavioral and spermatogenic hybrid male breakdown in *Nasonia*. Heredity 104 (3), 289–301. https://doi.org/10.1038/hdy.2009.152.

Clarke, A.R., Walter, G.H., 1995. "Strains" and the classical biological control of insect pests. Can. J. Zool. 73 (10), 1777–1790. https://doi.org/10.1139/z95-210.

Colazza, S., Rosi, M.C., 2001. Differences in the searching behaviour of two strains of the egg parasitoid *Telenomus busseolae* (Hymenoptera : Scelionidae). European Journal of Entomology 98 (1), 47–52. https://doi.org/10.14411/eje.2001.009.

Das, A.K., Choudhary, M., Kumar, P., Karjagi, C.G., Kr, Y., Kumar, R., Singh, A., Kumar, S., Rakshit, S., 2021. Heterosis in genomic era: advances in the molecular understanding and techniques for rapid exploitation. Crit. Rev. Plant Sci. 40 (3), 218–242. https://doi.org/10.1080/07352689.2021.1923185.

Dong, Z., Wang, Y., Li, C., Li, L., Men, X., Reddy, G.V.P., 2021. Mitochondrial DNA as a molecular marker in insect ecology: current status and future prospects. Ann. Entomol. Soc. Am. 114 (4), 470–476. https://doi.org/10.1093/aesa/saab020.

Durocher-Granger, L., Martel, V., Boivin, G., 2011. Gamete number and size correlate with adult size in the egg parasitoid *Trichogramma euproctidis*. Entomol. Exp. Appl. 140 (3), 262–268. https://doi.org/10.1111/j.1570-7458.2011.01158.x.

Ellers, J., Bax, M., Van Alphen, J.J.M., 2001. Seasonal changes in female size and its relation to reproduction in the parasitoid *Asobara tabida*. Oikos 92 (2), 209–314. https://doi.org/10.1034/j.1600-0706.2001.920213.x.

Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37 (12), 4302–4315. https://doi.org/10.1002/joc.5086.

Fischbein, D., Lantschner, M.V., Corley, J.C., 2019. Modelling the distribution of forest pest natural enemies across invaded areas: Towards understanding the influence of

climate on parasitoid establishment success. Biol. Control 132, 177–188. https://doi.org/10.1016/j.biocontrol.2019.02.016.
Gao, S., Tang, Y., Wei, K., Wang, X., Yang, Z., Zhang, Y., 2016. Relationships between body size and parasitic fitness and offspring performance of *Sclerodermus pupariae* Yang et Yao (Hymenoptera: Bethylidae). PLoS One 11 (7), e0156831. https://doi.org/10.1371/journal.pone.0156831.
Jervis, M., Ellers, J., Harvey, J., 2008. Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. Annu. Rev. Entomol. 53, 361–385. https://doi.org/10.1146/annurev.ento.53.103106.093433.
Joyce, A.L., Bernal, J.S., Vinson, S.B., Hunt, R.E., Schulthess, F., Medina, R.F., 2010. Geographic variation in male courtship acoustics and genetic divergence of populations of the *Cotesia flavipes* species complex. Entomol. Exp. Appl. 137 (2), 153–164. https://doi.org/10.1111/j.1570-7458.2010.01048.x.
König, K., Zundel, P., Krimmer, E., König, C., Pollmann, M., Gottlieb, Y., Steidle, J.L.M., 2019. Reproductive isolation due to prezygotic isolation and postzygotic cytoplasmic incompatibility in parasitoid wasps. Ecol. Evol. 9 (18), 10694–10706. https://doi.org/10.1002/ece3.5588.
Lartigue, S., Yalaoui, M., Belliard, J., Caravel, C., Jeandroz, L., Groussier, G., Calcagno, V., Louâpre, P., Dechaume-Moncharmont, F.-X., Malausa, T., Moreau, J., 2022. Consistent variations in personality traits and their potential for genetic improvement in biocontrol agents: *Trichogramma evanescens* as a case study. Evolutionary Applications 15, 1565–1579. https://doi.org/10.1111/eva.13329.
Laughlin, R., 1965. Capacity for increase: a useful population statistic. J. Anim. Ecol. 34 (1), 77–91. https://doi.org/10.2307/2370.
Liu, S.-S., Gebremeskel, F.B., Shi, Z.-H., 2002. Reproductive compatibility and variation in survival and sex ratio between two geographic populations of *Diadromus collaris*, a pupal parasitoid of the diamondback moth. *Plutella Xylostella*. Biocontrol 47 (6), 625–643. https://doi.org/10.1023/A:1020576128920.
Lommen, S.T.E., de Jong, P.W., Pannebakker, B.A., 2017. 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. Entomol. Exp. Appl. 162 (2), 108–123. https://doi.org/10.1111/eea.12510.
Ma, W.-J., Vavre, F., Beukeboom, L.W., 2013. Manipulation of arthropod sex determination by endosymbionts: diversity and molecular mechanisms. Sex. Dev. 8 (1–3), 59–73. https://doi.org/10.1159/000357024.
Mally, C.W., 1924. The eucalyptus snout-beetle. J. Department of Agriculture 9 (5), 415–442.
Mapondera, T.S., Burgess, T., Matsuki, M., Oberprieler, R.G., 2012. Identification and molecular phylogenetics of the cryptic species of the *Gonipterus scutellatus* complex (Coleoptera: Curculionidae: Gonipterini). Aust. J. Entomol. 51 (3), 175–188. https://doi.org/10.1111/j.1440-6055.2011.00853.x.
Mochiah, M.B., Ngi-Song, A.J., Overholt, W.A., Stouthamer, R., 2002. *Wolbachia* infection in *Cotesia sesamiae* (Hymenoptera: Braconidae) causes cytoplasmic incompatibility: implications for biological control. Biol. Control 25 (1), 74–80. https://doi.org/10.1016/S1049-9644(02)00045-2.
Morris, A.R., 2022. Changing use of species and hybrids in South African forest plantations. Southern Forests: a J. Forest Sci. 84 (3), 193–205. https://doi.org/10.2989/20702620.2022.2110538.
Mossop, M.C., 1929. A Mymarid parasite of the Eucalyptus snout beetle (*Gonipterus scutellatus*, Ghl) and its introduction into South Africa. Sci. Bull. 81 (81), 1–19.
Nguyen, D.T., Spooner-Hart, R.N., Riegler, M., 2016. Loss of *Wolbachia* but not *Cardinium* in the invasive range of the Australian thrips species, *Pezothrips Kellyanus*. Biol. Invasions 18 (1), 197–214. https://doi.org/10.1007/s10530-015-1002-4.
Phillips, C.B., Baird, D.B., Iline, I.I., McNeill, M.R., Proffitt, J.R., Goldson, S.L., Kean, J. M., 2008. East meets west: adaptive evolution of an insect introduced for biological control. J. Appl. Ecol. 45 (3), 948–956. https://doi.org/10.1111/j.1365-2664.2008.01461.x.
Pollmann, M., Moore, L.D., Krimmer, E., D'Alvise, P., Hasselmann, M., Perlman, S.J., Ballinger, M.J., Steidle, J.L.M., Gottlieb, Y., 2022. Highly transmissible cytoplasmic incompatibility by the extracellular insect symbiont *Spiroplasma*. iScience 25 (5), 104335. https://doi.org/10.1016/j.isci.2022.104335.
Quaglietti, B., Tamisier, L., Groussier, G., Fleisch, A., Le Goff, I., Ris, N., Kreiter, P., Fauvergue, X., Malausa, T., 2017. No inbreeding depression in laboratory-reared individuals of the parasitoid wasp *Allotropa burrelli*. Ecol. Evol. 7 (3), 964–973. https://doi.org/10.1002/ece3.2643.
Reis, A.R., Ferreira, L., Tomé, M., Araujo, C., Branco, M., 2012. Efficiency of biological control of *Gonipterus platensis* (Coleoptera: Curculionidae) by *Anaphes nitens* (Hymenoptera: Mymaridae) in cold areas of the Iberian Peninsula: Implications for defoliation and wood production in *Eucalyptus globulus*. For. Ecol. Manage. 270, 216–222. https://doi.org/10.1016/j.foreco.2012.01.038.
Ribeiro, M.F., Carvalho, V.R., Favoreto, A.L., de Marchi, B.R., Bello, V.H., Jordan, C., Soliman, E.P., Zanuncio, J.C., Sabattini, J.A., Wilcken, C.F., 2022. Symbiotic bacteria in the relationship between *Anaphes nitens* (Hymenoptera: Mymaridae) and *Gonipterus platensis* (Coleoptera: Curculionidae). Austral Ecol. 48 (1), 182–196. https://doi.org/10.1111/aec.13259.
Roitberg, B., Boivin, G., Vet, L., 2001. Fitness, parasitoids, and biological control: An opinion. Can. Entomol. 133 (3), 429–438. https://doi.org/10.4039/Ent133429-3.
Santolamazza-Carbone, S., 2002. Ecología del comportamiento del gorgojo del eucalipto *Gonipterus scutellatus* Gyllenhal y de su parasitoide *Anaphes nitens* Girault. Universidade de Vigo.
Santolamazza-Carbone, S., Cordero-Rivera, A., 2002. Egg load and adaptive superparasitism in *Anaphes nitens*, an egg parasitoid of the Eucalyptus snout-beetle *Gonipterus scutellatus*. Entomol. Exp. Appl. 106, 127–134. https://doi.org/10.1046/j.1570-7458.2003.00011.x.
Santolamazza-Carbone, S., Nieto, M.P., Rivera, A.C., 2007. Maternal size and age affect offspring sex ratio in the solitary egg parasitoid *Anaphes nitens*. Entomol. Exp. Appl. 125 (1), 23–32. https://doi.org/10.1111/j.1570-7458.2007.00595.x.
Santolamazza-Carbone, S., Nieto, M.P., Rivera, A.C., 2008. Egg resorption behaviour by the solitary egg parasitoid *Anaphes nitens* under natural conditions. Entomol. Exp. Appl. 127 (3), 191–198. https://doi.org/10.1111/j.1570-7458.2008.00699.x.
Schröder, M.L., Slippers, B., Wingfield, M.J., Hurley, B.P., 2020. Invasion history and management of Eucalyptus snout beetles in the *Gonipterus scutellatus* species complex. J. Pest. Sci. 93 (1), 11–25. https://doi.org/10.1007/s10340-019-01156-y.
Schröder, M.L., Nahrung, H.F., de Souza, N.M., Lawson, S.A., Slippers, B., Wingfield, M. J., Hurley, B.P., 2021. Distribution of *Gonipterus* species and their egg parasitoids in Australia: implications for biological control. Forests 12 (8), 969. https://doi.org/10.3390/f12080969.
Scotta, M.I., Margris, L., Sellier, N., Warot, S., Gatti, F., Siccardi, F., Gibert, P., Vercken, E., Ris, N., 2021. Genetic variability, population differentiation, and correlations for thermal tolerance indices in the minute wasp. *Trichogramma Cacoeciae*. Insects 12 (11), 1013. https://doi.org/10.3390/insects12111013.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87 (6), 651–701. https://doi.org/10.1093/aesa/87.6.651.
Szűcs, M., Vercken, E., Bitume, E.V., Hufbauer, R.A., 2019. The implications of rapid eco-evolutionary processes for biological control - a review. Entomol. Exp. Appl. 167 (7), 598–615. https://doi.org/10.1111/eea.12807.
Takada, H., Tada, E., 2000. A comparison between two strains from Japan and Europe of *Aphidius ervi*. Entomol. Exp. Appl. 97 (1), 11–20. https://doi.org/10.1046/j.1570-7458.2000.00711.x.
Tooke, F.G.C., 1955. The Eucalyptus snout beetle, *Gonipterus scutellatus* Gyll. A study of its ecology and control by biological means. Department of Agriculture and Forestry, Union of South Africa.
Valente, C., Gonçalves, C.I., Reis, A., Branco, M., 2017. Pre-selection and biological potential of the egg parasitoid *Anaphes inexpectatus* for the control of the Eucalyptus snout beetle, *Gonipterus platensis*. J. Pest. Sci. 90, 911–923. https://doi.org/10.1007/s10340-017-0839-y.
Vallina, C., Aquino, D.A., Minardi, G.M., Puch, L.I., Garrido, S.A., Sánchez, N.E., Luna, M. G., 2020. Reproductive compatibility and morphological characterization of local populations of the larval endoparasitoid *Pseudapanteles dignus* in Argentina. BioControl 65 (3), 273–284. https://doi.org/10.1007/s10526-020-10014-1.
Werren, J.H., Baldo, L., Clark, M.E., 2008. *Wolbachia*: master manipulators of invertebrate biology. Nat. Rev. Microbiol. 6 (10), 741–751. https://doi.org/10.1038/nrmicro1969.
Zepeda-Paulo, F., Dion, E., Lavandero, B., Mahéo, F., Outreman, Y., Simon, J.C., Figueroa, C.C., 2015. Signatures of genetic bottleneck and differentiation after the introduction of an exotic parasitoid for classical biological control. Biol. Invasions 18 (2), 565–581. https://doi.org/10.1007/s10530-015-1029-6.
Zhou, Y., Abram, P.K., Boivin, G., Brodeur, J., 2014. Increasing host age does not have the expected negative effects on the fitness parameters of an egg parasitoid. Entomol. Exp. Appl. 151 (2), 106–111. https://doi.org/10.1111/eea.12173.