RESEARCH ARTICLE

Jack, master or both? The invasive ladybird *Harmonia axyridis* performs better than a native coccinellid despite divergent trait plasticity

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Abstract

The plasticity of performance traits can promote the success of biological invasions and therefore, precisely estimating trait reaction norms can help to predict the establishment and persistence of introduced species in novel habitats. Most studies focus only on a reduced set of traits and rarely include trait variability that may be vital to predicting establishment success. Here, using a split-brood full-sib design, we acclimated the globally invasive ladybird *Harmonia axyridis* and a native co-occurring and competing species *Cheilomenes lunata* to cold, medium and warm temperature regimes, and measured critical thermal limits, life-history traits, and starvation resistance. We used the conceptual framework of "Jack, Master or both" to test predictions regarding performance differences of these two species. The native *C. lunata* had a higher thermal plasticity of starvation resistance and a higher upper thermal tolerance than *H. axyridis*. By contrast, *H. axyridis* had a higher performance than *C. lunata* for preoviposition period, fecundity and adult emergence from pupae. We combined trait responses, transport duration and propagule pressure to predict the size of the populations established in a novel site following cold, medium and warm scenarios. Although *C. lunata* initially had a higher performance than the invasive species during transport, more individuals of *H. axyridis* survived in all simulated environments due to the combined life-history responses, and in particular, higher fecundity. Despite an increased starvation mortality in the warm scenario, given a sufficient propagule size, *H. axyridis* successfully established. This study underscores how the combination and plasticity of multiple performance traits can strongly influence establishment potential of species introduced into novel environments.

Keywords

Introduction

The establishment and spread of invasive species in novel habitats have been attributed to factors pertaining to human dimensions such as propagule pressure and how and where these propagules have been introduced or moved, and to natural drivers, including the invasibility of the habitat and the specific traits of the invasive species (Hayes and Barry 2008; van Kleunen et al. 2010a; Blackburn et al. 2018; Enders et al. 2020). In particular, phenotypic plasticity, defined as the ability of a genotype to produce different phenotypes when exposed to varying conditions within an organism's lifetime, is often cited as enabling alien species to rapidly respond to novel or varying conditions, increasing their invasion potential (Baker 1965; Richards et al. 2006; Colautti et al. 2017; Torchyk and Jeschke 2018). Three patterns of response have been proposed to describe the reaction norms or performance curves that could benefit an invasive species compared to a native counterpart in the introduced range (Richards et al. 2006). First, the invasive species maintains fitness more consistently than the native species across environmental conditions, including stressful ones (also known as a "General-purpose" or "Jack-of-all-trades" phenotype). Second, the invasive species has a higher fitness in the most favourable conditions compared to the native one ("Master-of-some" phenotype). Finally, the invasive species has both a broader and a higher peak performance than the non-invasive species across varied environmental conditions ("Jack-and-master").

In plants, the extent of phenotypic plasticity can be greater in invasive alien species than native species, especially for key performance traits, but plasticity does not always translate into increased fitness (for a review see Davidson et al. 2011; Gallagher et al. 2015; Huang et al. 2015). Other studies, including meta-analyses, have found no support for increased plasticity in invasive species (Godoy et al. 2011; Palacio-López and Gianoli 2011). Although the study of plasticity in invasive insects is more limited than in plants, data suggest that life-history traits and temperature tolerance of invasive insects and terrestrial arthropods are generally more plastic than their native counterparts (e.g. Slabber et al. 2007; Janion et al. 2010; Nyamukondiwa et al. 2010; Weldon et al. 2011; Coccia et al. 2013; Mutamiswa et al. 2018). However, as for plants, a lack of support is also found in insects and springtails (Gibert et al. 2016; Janion-Scheepers et al. 2018; Phillips et al. 2020; Da Silva et al. 2021). The absence of a general pattern of increased plasticity in invasive species may stem from several factors: plasticity differences between native and alien insects can depend on the trait examined, the invasive and native species compared, the timing of the comparison during the invasion process, the experimental conditions and methodology used, as well as conditions and characteristics of the environment (Slabber et al. 2007; Terblanche et al. 2010; Nyamukondiwa et al. 2018; Weldon et al. 2018). Different combinations of plastic and non-plastic responses of traits, both morphological and physiological, can compound into flat or advantageous fitness reaction norms. Therefore, the extent of trait- and context-specific differences in reaction norms calls for examining multiple, rather than single, traits between closely-related or ecologically-equivalent species.

Furthermore, given the dependence of insects to surrounding conditions to maintain body temperature and the continuing change in temperature means and extremes associated with climate change (Arias et al. 2021), a focus on trait responses to different temperature exposures is crucial. This approach can highlight differences in the thermal plasticity of traits that underlie individual fitness and improve predictions of population persistence in new and changing conditions (Chun et al. 2007; Morris et al. 2011; Tomlinson et al. 2015; Da Silva et al 2021).

Several abiotic and biotic filters are encountered during and following colonization of species into a new environment (Catford et al. 2009). Typically, the invasion process is divided into several stages (transport, introduction, establishment and spread) with barriers that species need to overcome to move across stages (Blackburn et al. 2011). Some species possess characteristics that can enhance the successful transition across stages (Hayes and Barry 2008; Blackburn et al. 2011). For insects, most species are introduced as contaminants of imported products, or stowaways on airplanes and ships, as opposed to deliberate introductions, except for biocontrol agents (Kiritani and Yamamura 2003; Work et al. 2005; Hulme et al. 2008; Liebhold et al. 2006, 2012; Faulkner et al. 2016). Survival at the transport stage can rely heavily on the ability to tolerate conditions in the transport vessel, while the ability to cope with food shortages through starvation resistance will greatly increase the chances of survival (Renault et al. 2018). Once introduced, life-history traits such as developmental time and fecundity, and other performance-related traits including resource acquisition, competition ability and stress tolerance, can also assist species to establish and produce viable populations. Dispersal ability, and a wide tolerance and diet breadth can then promote spread into new environments (Hayes and Barry 2008; Blackburn et al. 2011). Thus, a combination of traits involved within and across the multi-stage invasion process is important for successful invasion, yet studies rarely consider how the combination of stress resistance, life-history and dispersal traits may impact species' overall survival and persistence in new environments (Renault et al. 2018).

Studies that model the dynamics of insect invasions often focus on the establishment and spread stages. Typically, these studies integrate population growth, population size, dispersal rate (e.g. Miller and Tenhumberg 2010; Hui and Richardson 2017; Lux 2018) and/or functional traits (such as thermal tolerance; e.g. Kearney et al. 2009; Hartley et al. 2010) to determine species' persistence and distribution. However, small numbers of starting propagules and trait responses to conditions along the pathway can result in failed invasions (Zenni and Nuñez 2013). Therefore, considering the sequence of events during an insect invasion rather than focusing on a single stage, and modelling the consequences of multiple trait's variation and plasticity on species survival or performance across invasion stages, can portray a more informative picture of a species' potential to establish.

The harlequin ladybird beetle, *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae), is a notorious invasive species whose establishment and spread has been associated with the rapid decline of native species in multiple countries, likely as a result of intraguild predation and competition for resources (Roy et al. 2016). Life-history traits

such as a large body mass, multiple generations per year, high fecundity and longevity, fast developmental rate, and an altered sex ratio in favour of females have been proposed to promote its invasiveness (Bazzocchi et al. 2004; Labrie et al. 2006; Hemptinne et al. 2011; Roy et al. 2016). A broad thermal tolerance range, mostly stemming from the beetle's high cold hardiness, is likely to favour its persistence in novel conditions (Berkvens et al. 2010; Barahona-Segovia et al. 2016). Moreover, thermal acclimation effects of multiple life-history traits have been reported in *H. axyridis*, such as development (Grill et al. 1997; Berkvens et al. 2008; Knapp 2014), fecundity (Castro et al. 2011; Zhang et al. 2014), body size (Grill et al. 1997; Knapp and Nedved 2013), quiescence or diapause (Sakurai et al. 1992; Lombaert et al. 2008), melanism (Michie et al. 2011; Knapp and Nedved 2013), foraging behaviour (Mondor and Warren 2000) and upper thermal tolerance (but see Boher et al. 2018; Shinner et al. 2020). Establishing whether this plasticity benefits the invasive species to a greater extent than a native or non-invasive ladybird beetle in the introduced range can be influenced by several factors, such as the site of origin of the species, period in the invasive range since introduction, and the genetic history and diversity of the invasive and native populations (Hulme 2008, van Kleunen et al 2010b). However, useful native and invasive insect comparisons stem from assessing co-occurring species from similar taxonomic families or ecological niches (e.g. Tomlinson et al 2015; Da Silva et al. 2021). Basal traits and their thermal plasticity can be used to simulate scenarios of establishment and spread in novel environments for both species, successful alien and native species, and predict their population dynamics and survival in different thermal conditions (van Kleunen et al. 2011; and see Brass et al. 2020 for complementary of stage-structured models to plasticity data).

In this study, we first examine trait responses of *H. axyridis* and a sympatric and often syntopic native species, *Cheilomenes lunata* (Fabricius, 1775) (Coleoptera: Coccinellidae), to three ecologically relevant temperature regimes (cold, medium and warm). We test if the patterns of phenotypic response to temperature in these species are in line with the "Jack-of-all-trades", "Master-of-some" or "Jack-and-Master" models as depicted in Fig. 1. While comparing two species has been highlighted as limiting the ability to make adaptive inferences of phenotypic variation (e.g. Felsenstein 1985; Garland and Adolph 1994), here, we do not aim to infer adaptation nor to describe the specific drivers of these differences. Instead, we test specific model predictions using an invasive and a co-occurring native coccinellid and assess the magnitude and direction of responses to temperature for multiple traits in these species (Kelley et al. 2014). We targeted traits likely to be important in determining successful transport, establishment and spread, including starvation resistance, thermal tolerance limits and life-history traits as fitness proxies. We then use a computer simulation to combine propagule size and trait responses measured in the study to determine the number of beetles per species that would establish after crossing multiple stages of invasion at three scenarios of temperature conditions and assess if these species' specific responses would facilitate their survival in these environments. Given the focus on temperature effects, our goals should also provide insights into potential responses of these species to contemporary climate warming, a facet that has been neglected for native coccinellid species (Slogget 2021).
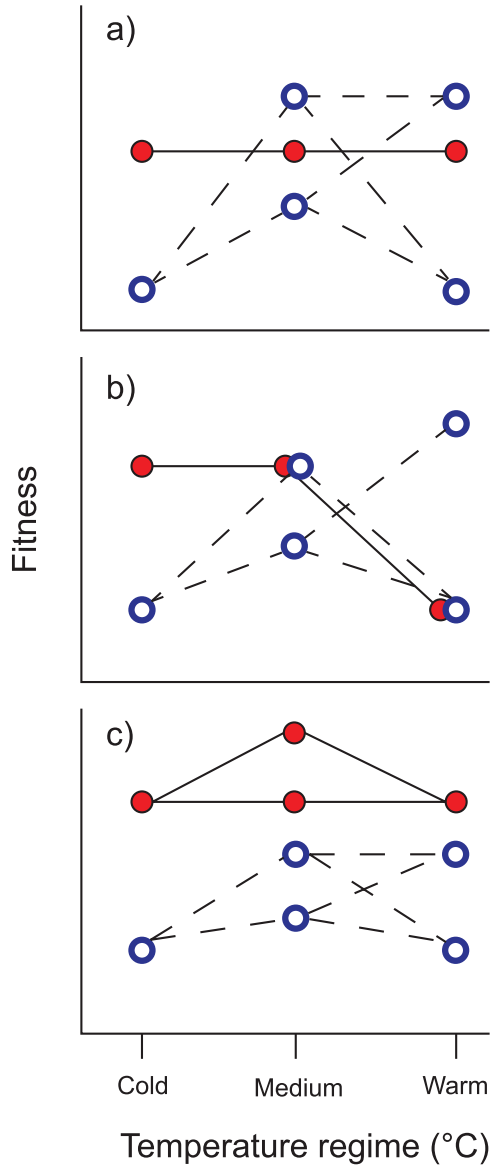
Figure 1. Theoretical expectations for the fitness responses of the study species at three temperatures. The invasive species *H. axyridis* (solid circles) follows one of three patterns **a** Jack-of-all-trades: *H. axyridis* is able to maintain fitness in all environmental conditions, including sub-optimal or stressful ones (cold and warm). The native *C. lunata* (empty circles) may outperform the invasive in some conditions **b** Master-of-some: *H. axyridis* is better able to increase fitness in favourable conditions and, typically, more so than the native **c** Jack-and-master: *H. axyridis* is able to maintain fitness and perform better than its native counterpart. The medium regime is assumed to be the most favourable environment for both species but given the lack of literature for the native species, optimal temperatures for fitness could correspond to the medium or the warm regime since information about its thermal optimum is limited (see text). Diverse lines of reaction norms within species means that multiple trajectories are possible.

Methods

Study species and field collections

Harmonia axyridis was first recorded in the south-western region of the Western Cape Province, South Africa, in 2001 (Stals 2010; Roy et al. 2016). It rapidly spread, occupying six of the seven biomes in the country, and extending from sea-level to > 1800m in elevation (Stals and Prinsloo 2007; Stals 2010). Although native to Asia, molecular data suggest that *H. axyridis* was introduced to South Africa from an invasive source population in eastern North America (Lombaert et al. 2010; Collop et al. in prep) and is now found in most urban and semi-urban habitats in the country. The native species, *Cheilomenes lunata*, is smaller than *H. axyridis* (from this study, mass (mg) = 16.3 ± 4.5 (n = 133) and 20.4 ± 4.7 (n = 112), for *C. lunata* and *H. axyridis*, respectively) and is ubiquitous in Southern Africa and some parts of Eastern and Central Africa (Picker et al. 2004; iNaturalist.org). There is currently limited information on its physiology and life-history. Both species feed primarily on aphids and are frequently found on the same plants. In South Africa, *H. axyridis* has a critical thermal maximum of ~44 °C (Shinner et al. 2020), and from data gathered in other regions, its development, fecundity and survival typically decline at temperatures above 30 °C (de Oliveira Ramos et al. 2014; Zhang et al. 2014; Barahona-Segovia et al. 2016; Roy et al. 2016). Most of this species' life-history traits have optimal temperatures between 20 and 25 °C (Suppl. material 1: table S1).

Individuals of *H. axyridis* and *C. lunata* were collected from 10 different locations around Stellenbosch, South Africa, between February and May in 2014 and 2015 (Suppl. material 1: table S2). Similar sample sizes were collected across sites to enhance genetic diversity and avoid inbreeding effects. Beetles were collected from rose plants in urban gardens where both species co-occur and feed mostly on rose aphids, *Macrosiphon rosae* (Linnaeus, 1758).

Rearing and temperature regimes

For each species, stock populations (n = 200) were maintained in temperature-controlled chambers (SANYO MIR-254, SANYO Electric Co., Ltd; Osaka, Japan) with a summer photoperiod of 14L:10D, and a temperature cycle of 25.5 ± 0.1 °C for 18 h (day), 18.5 ± 0.1 °C for 6 h (night) (mean temperature of 23.8 ± 3.0 °C). This regime resembles microsite temperature profiles experienced in Stellenbosch during peak ladybird beetle abundance (i.e. March to June; Chidawanyika and Terblanche 2011 and field data), and is within the range of optimal temperatures reported for different traits of *H. axyridis* (Suppl. material 1: table S1).

Stock populations (F0) of each species were kept in 2-L plastic containers covered with mesh for ventilation (< 50 individuals per box to avoid crowding). Beetles were given live aphids *ad libitum* (~30 individuals/beetle from rose aphids *M. rosae*, oak aphids *Tuberculatus annulatus* (Hartig, 1841) and Russian wheat aphids *Diuraphis noxia* (Kurdjumov, 1913)) every three days and 1:10 honey:distilled water solution

ad libitum, and left to reproduce for one generation. F1 beetles were then reared in separate containers from field populations. Once mature, F1 females and males were chosen at random to form families (n = 50–60 unique pairs per species). Twenty-five to 30 F1 mating pairs (or families) were placed in individual 9-cm Petri dishes and fed 20 live aphids daily (as described above) with honey solution *ad lib*. When an egg clutch was laid, parents were moved to a different Petri dish to avoid cannibalism. At hatching (~3 days), F2 larvae were divided as < 10 individuals per Petri dish. Larvae were given frozen rose aphids and honey solution daily. Rose aphids were collected in the field but frozen at -80 °C prior to experiments to have high quantities of consistent food during development. Pilot trials showed that larvae from both species readily consume frozen aphids. Larvae were moved to individual Petri dishes when pre-pupae first appeared to prevent cannibalism. At emergence from pupae (~5 days), adults were placed in individual dishes and given honey solution *ad lib*. Adults were weighed 24 h after emergence and then fed 10 frozen rose aphids (see experimental design in Suppl. material 1: fig. S1).

F2 adults from each family (full-sibs) were then equally spread across three temperature treatments: cold (20.5 ± 0.1 °C for 18 h and 13.3 ± 0.1 °C for 6 h; mean temp = 18.7 ± 3.1 °C), medium (25.5 ± 0.2 °C for 18 h and 18.5 ± 0.1 °C for 6 h; mean temp = 23.9 ± 3.0 °C) and warm (30.5 ± 0.1 °C for 18 h and 23.3 ± 0.1 °C for 6 h; mean temp = 28.8 ± 3.1 °C) two days after pupal emergence, which is when beetles are capable of flight (Hodek et al. 2012). These temperature regimes were selected given field microsite data and the optimal temperatures of multiple traits found in other studies for *H. axyridis* (Suppl. material 1: table S1). Optimal performance temperatures are not available for the native species, but studies on the congener *C. sexmaculata* (Fabricius, 1781) have shown that fecundity, egg hatching success, successful pupal emergence and adult lifespan is highest between 30 and 35 °C (Wang et al. 2013). By contrast, Hodek et al. (2012) found that the net reproduction rate and lifetime egg production of 12 species of ladybird beetles peaked at 25 °C. We therefore chose optimal and warm temperature regimes within this range (20–30 °C). The photoperiod was kept as in the maintenance section, and 20 frozen aphids and honey solution were provided daily for each treatment.

Sex was determined for *H. axyridis* following McCornack et al. (2007), and using abdominal morphology and colouration for *C. lunata* (concave last posterior segment in males and convex in females, darker labrum pigmentation in females; pers. comm. Riaan Stals).

Starvation resistance

Two days post-emergence, distilled water was provided, all food removed and beetles assigned to temperature treatments by splitting siblings equally across treatments (*H. axyridis*: n = 36 (cold), n = 41 (medium), n = 35 (warm); *C. lunata*: n = 43 (cold), n = 37 (medium), n = 53 (warm)). Beetles remained in treatments until they succumbed to starvation. Mortalities were checked twice daily (09:00, 18:00) and when

found, body mass was measured within 30 min, and sex determined. Starvation resistance was measured as the number of days in the treatment until death, and the percentage of mass loss between the start and end of starvation trials recorded.

Critical temperature limits (CTLs)

Beetles were kept in temperature treatments for 7–9 days prior to thermal tolerance trials. This acclimation period was chosen as it induces plastic effects in most insects (Weldon et al. 2011). Food was removed and distilled water provided a day prior to trials. Individuals were first weighed (Avery Berkel, UK, $\pm$ 0.0001 mg) and then placed inside a double-jacketed chamber and left to equilibrate for 15 min to a set temperature of 25 $\pm$ 0.2 °C controlled by a programmable water bath (Grant GP200-R4; Grant Instruments, England). The experimental temperature was monitored inside an empty central chamber using a thermocouple connected to a logger (TC-08, Pico Technology) and ramped up (critical thermal maximum; CT_{max}) or down (critical thermal minimum; CT_{min}) at a rate of 0.1 °C·min^{-1}. This rate was chosen to avoid hardening effects induced by rates that are too slow and to avoid missing the end-point if rates are too fast. Beetles were checked every 30 min for the first 2 h, then every 10 min until vigorous movement (CT_{max}) or loss of righting response (CT_{min}), followed by 2–3 min checks until set thresholds. CT_{min} was reached when there was no visible movement of the head, antennae or legs after prodding (*H. axyridis*: n = 51 (cold), n = 44 (medium), n = 48 (warm); *C. lunata*: n = 40 (cold), n = 37 (medium), n = 37 (warm)). CT_{max} was reached when beetles no longer grabbed the end of a thin rod after gentle prodding, indicating a loss of muscle control (*H. axyridis*: n = 51 (cold), n = 51 (medium), n = 54 (warm); *C. lunata*: n = 38 (cold), n = 45 (medium), n = 45 (warm)). Beetles were always checked in the same order. Since some ladybird beetles are known to feign death and inactivity, individuals had to display physiological endpoints three times in a row with the first reading taken as the endpoint. At the end of the trials, beetles were weighed, placed in a -20 °C freezer and stored in 99% ethanol.

Life-history traits

After 7 days in respective temperature treatments, F2 females (*H. axyridis*: n = 21 (cold), n = 19 (medium), n = 24 (warm); *C. lunata*: n = 17 (cold), n = 19 (medium), n = 21 (warm)) were paired up with F2 males (that originated from different families but that had been exposed to the same temperature regime) to create mating pairs. The pre-oviposition period (i.e. number of days from pairing to first egg clutch) and number of eggs laid were recorded for 2 weeks.

Three randomly-chosen clutches per mating pair, maintained at the same temperature regimes as their respective parental F2 pairs, were checked twice daily for hatched larvae (fully emerged from egg casings) until a day after eggs started hatching. In addition, the number of yellow and black eggs were recorded. Yellow eggs are assumed to be trophic (unfertilised) eggs while black eggs are typically fertilised eggs that did not

hatch (Perry and Roitberg 2005). Hatching success was calculated as the percentage of total number of hatched larvae from the total number of viable eggs (which included hatched larvae and fertilised eggs).

Larvae (F3) were placed in individual Petri dishes and monitored until emergence from pupae with developmental time set as the number of days from egg (Day 0) to successful emergence from pupae. Mating pairs had to at least produce three live larvae to be included in analyses which resulted in the exclusion of a single pair. Beetles (F3) were given honey solution, weighed 24–36 h after eclosion, and sex determined five days after eclosion to allow for hardened elytra. The percentage of adult beetles that successfully emerged from pupae was calculated per mating pair as the total number of successfully emerged adults over the total number of pupae monitored.

Larvae that died within a day of being transferred to Petri dishes were assumed to have died from the transfer process and were discounted from calculations (<3%). Mating pairs and larvae were fed 20 live aphids daily (rose, oak or Russian wheat aphids) and provided with honey solution. To ensure that mating adults had not lost body condition, body mass was recorded before and after mating periods.

Intrinsic rate of population increase (r) was determined as $r=(\ln R_0)/T_g$, where the net reproductive rate (R_0) (Birch 1948) was defined as

$$R_0 = \sum l_x m_x$$

where x is the age of the female in days from the day of emergence from pupa until the end of data collection at $x = 23$ days, l_x is the probability (0 to 1) of being alive at age x, and m_x the number of females produced by each female at age x. Since only those females that survived the trial were used in the analysis, l_x was set to 1 for all values of x (Dillon et al. 2007). Therefore, the net reproductive rate was the expected number of F3 females produced by an F2 female and reflects the maximum reproductive rate (since mortality was not included in the calculation). We used the mean development time of F3 larvae as a proxy for the generation time T_g of F2 females.

Statistical analysis

For the analysis of starvation resistance, we first drew Kaplan-Meier survival curves to illustrate survival probability over time (in days) (survival package; Therneau and Lumley 2018) using R Statistical software v. 3.6.3 (R Core Team 2017). We then used a Cox-proportional hazards model to test for the effects of percentage of mass loss, sex, species and temperature treatment on survival (coxme package; Therneau 2018). Family ID of individuals used in trials was included as a random effect.

For all other traits (CT_{min}, CT_{max}, preoviposition period, total eggs produced, hatching success, developmental time, pupal emergence success, and intrinsic rate of increase), we constructed full general or generalized linear mixed effects models comprising mass, sex, species, treatment and their interactions as predictors, and a random effect of family

ID. We compared each full model with a model without the random effect using the nlme package (Pinheiro et al. 2016) following Zuur et al. (2009). As body mass and sex can predict life-history, starvation resistance and tolerance traits of beetles and other insects (e.g. Weldon et al. 2011; Lancaster et al. 2015; Barahona-Segovia et al. 2016; Raak-van den Berg et al. 2017; Nyamukondiwa et al. 2018), we included these variables in our statistical models. An appropriate variance structure was used for critical thermal limits to improve model fit (Zuur et al. 2009). For preoviposition period, total eggs laid and developmental time, we used a Poisson error distribution while for egg hatching success and pupal emergence success, we fitted the model using a binomial error distribution. The mass of the female was included in models of preoviposition, total eggs, and intrinsic rate of increase, while the mass prior to the trial was included as predictor for critical limits and adult mass was included in models predicting developmental time. Model outputs were also compared to those of mixed-effects models constructed using the lme4 package (Bates et al. 2015). Backward selection of non-significant terms was used until a minimum adequate model was obtained using maximum likelihood. Interpretations were based on the parameter estimates of the most parsimonious model. Plots of fitted vs standardised residuals and histograms of standardised residuals were checked for violations of model assumptions. Full model structure and best model outputs for all traits are provided in Suppl. material 1: table S4. Interpretations of significant interactions were made by plotting model estimates (effects package; Fox 2003). Pairwise differences of trait means between species and/or sexes within treatments were assessed using least squares means (lsmeans package; Lenth 2016). Trait original means ± SD and sample sizes are provided in Suppl. material 1: table S5.

In addition, for each trait, candidate models that had a ΔAICc value of < 2 were used in model averaging (Burnham and Anderson 2002; Grueber et al. 2011). We used the full-average method to determine parameter coefficients of the averaged model (Burnham and Anderson 2002), and the 'model.avg' function in the MuMln package (Barton 2018). Model averaging was performed for CT_{min}, CT_{max}, developmental time and egg hatching success. Averaged model outputs did not change results and are therefore only provided in supplementary materials (Suppl. material 1: table S6).

Modelling population size across invasion stages

Using R, we simulated the effect of trait combination on population size for each species using an invasion framework that includes the transport and establishment stages. We assumed that starvation resistance, life-history traits and upper thermal tolerance shaped individual persistence through transport and establishment. The model simulated the case where a number of individuals were transported to a new habitat with starvation resistance shaping the number of survivors through time. These survivors could establish in the new habitat and produce a new generation. Temperature scenarios (cold, medium or warm) were assumed to be fixed across transport and establishment stages.

We used experimental data to model survival numbers at each stage of the journey. We calculated the proportion of individuals that survived transportation based on the

starvation resistance data. Specifically, we counted the number of individuals that died each day from 1 to 20 days at 0.5-day increments (the resolution of the starvation resistance data), with all individuals surviving on day 1 and all succumbing to starvation in 20 days. We fitted a Gompertz sigmoid growth function (Gompertz 1825; Tjørve and Tjørve 2017) to cumulative mortality count data for each species within each treatment: Mortality count = $a \cdot \text{Exp}(-\text{Exp}(-c(t-b/c)))$, where a represents the total number of beetles measured in each treatment per species, t time (in 0.5-day increments), c is the mortality change rate, and b/c is the time when mortality peaks. The simulation started with a randomly-chosen propagule size (between 1 and 100 adult beetles) transported to a new area with no access to food, and a randomly-selected period of travelling time between 1 and 20 days (at 0.5-day increments). Propagule size was multiplied by the survival proportion to determine the total number of beetles that survived transportation. Upon arrival in the novel environment, we assumed a 50% sex ratio of surviving and reproducing beetles (the sex ratio of both species in our experiments varied from 45.1 to 53.6% across treatments, with an average of 51.5%). To determine the number of beetles that successfully established in the new habitat, we calculated the product of the number of surviving females × (i) the number of eggs laid × (ii) the probability of viable eggs × (iii) the probability of a viable egg that successfully hatched × (iv) the probability of a larva that survived to adulthood. Parameters (i-iv) were randomly sampled from the corresponding probability density distributions based on the experimental data for each temperature treatment. The model ran for 1000 iterations for each species and each temperature scenario.

Data resources

Supplementary material is available at https://doi.org/10.3897/neobiota.@@.91402. suppl1 and original data can be requested from the corresponding author.

Results

Parental family affiliation was important for traits of temperature tolerance, starvation resistance and life history (ΔAICc > 2 for models with versus without Family ID as a random effect) except for hatching success, pupal emergence success and intrinsic rate of increase.

Starvation resistance

Starvation resistance of both species increased as the temperature of treatments decreased (p < 0.0001; Fig. 2). However, starvation resistance of the native *C. lunata* increased more between the warm and cold treatments than for the alien *H. axyridis* (from 4 days to 11 days and from 4.5 days to 9 days, respectively; species × treatment interaction, Suppl. material 1: table S3). Overall, the relationship between the percentage mass loss during the starvation trial differed between sexes and across treatments
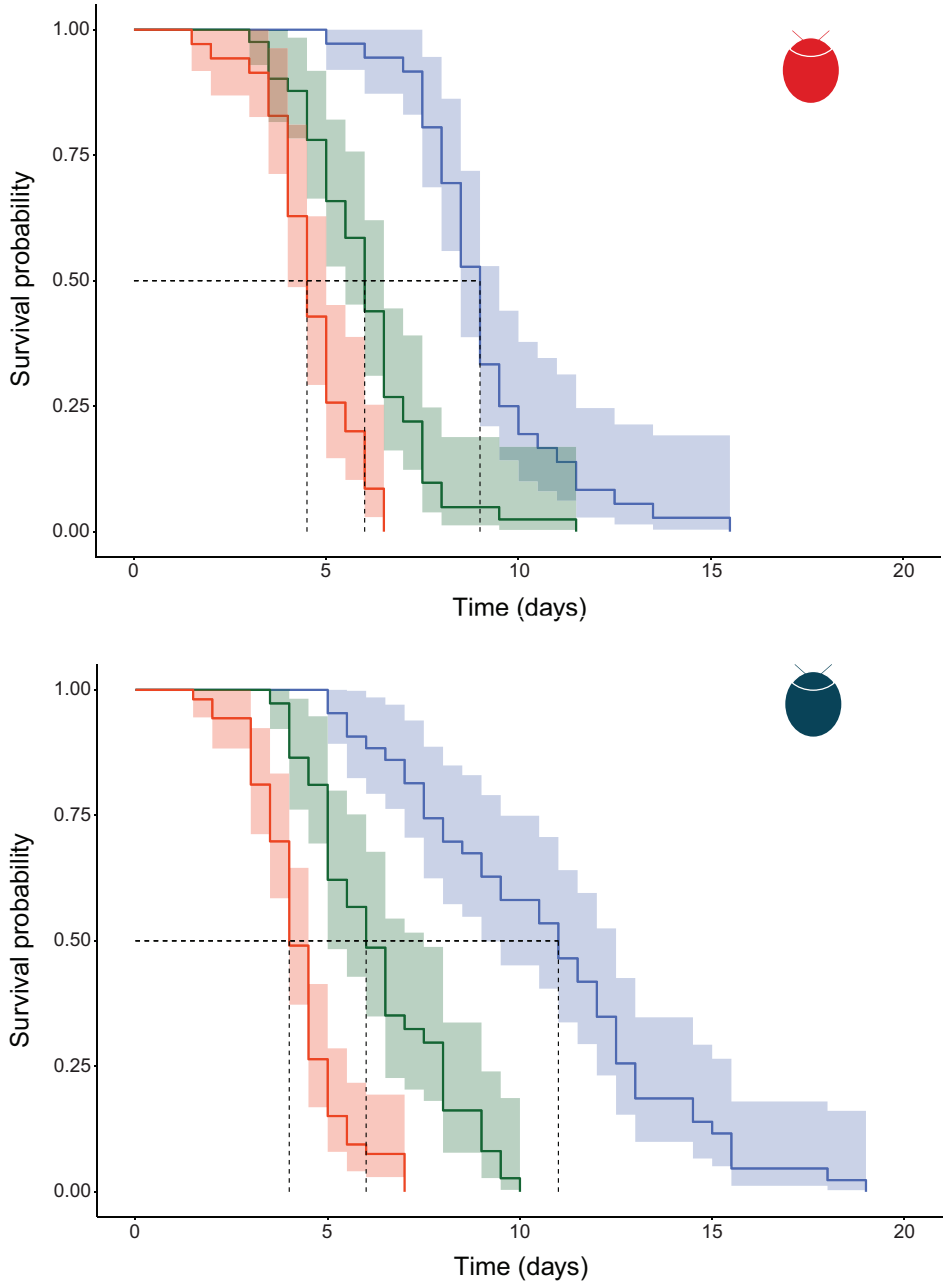
Figure 2. Starvation resistance of the invasive *Harmonia axyridis* and native *Cheilomenes lunata* kept at three temperatures. Kaplan-Meier survival curves were used to plot survival times of *H. axyridis* (top) and *C. lunata* (bottom) maintained at the cold (right - blue), medium (middle - green) and warm (left - orange) temperature treatments. Dotted lines represent 50% survival probability per treatment and corresponding numbers of days.

(significant three-way interaction among % mass loss × sex × treatment; Suppl. material 1: table S3, fig. S2), irrespective of species. In beetles that lost less body mass, females survived longer than males in the cold (10.5 days and 8.5 days, respectively) and medium treatments (6.5 days and 6 days, respectively), although both sexes survived for the same amount of time in the warm treatment (i.e. 4 days). For beetles that lost more body mass, females survived longer than males in the cold (11 days and 8.5 days, respectively) and medium treatments (7 days and 5.5 days, respectively), but for a shorter period in the warm treatment (females: 4 days and males: 5 days) (Suppl. material 1: fig. S2).

Thermal tolerance

The critical thermal minimum, CT_{min}, did not differ between species. Overall, CT_{min} was higher in beetles maintained in the warm (1.0 ± 1.4°C, n = 85) than cold treatment (0.3 ± 1.6°C, n = 91) (p < 0.001), and larger beetles had a lower CT_{min} (p = 0.004).

For CT_{max}, there was a significant interaction between mass, sex, and species. In *H. axyridis*, the CT_{max} of males and females increased with body mass, whereas, for *C. lunata*, CT_{max} of females did not vary with mass, but larger males had a higher CT_{max} than smaller males (mass × sex × species interaction, Fig. 3a). CT_{max} of females across treatments varied more than CT_{max} of males (sex × treatment interaction; Suppl. material 1: fig. S3); for females, CT_{max} increased across all temperature treatments (p < 0.01 for all treatment comparisons), whereas CT_{max} of males only increased between cold and warm (p < 0.001), and between medium and warm treatments (p = 0.003). Overall, *C. lunata* had a higher CT_{max} than *H. axyridis* (45.4 ± 0.8 °C (n = 128) and 44.8 ± 0.5 °C (n = 156), respectively; p = 0.02), and males had a higher CT_{max} than females in both species (p < 0.01). In both species, CT_{max} increased with temperature (p < 0.01).

Life-history traits

For both species, the preoviposition period decreased with temperature treatment (p < 0.05) but *H. axyridis* (3.9 ± 2.0 days, n = 64) had a shorter preoviposition period than *C. lunata* (5.5 ± 4.7 days, n = 57) (p < 0.0001; Suppl. material 1: table S3).
The relationship between total number of eggs and the mass of females differed across treatments and between species, which explains some of the variation in the total number of eggs produced (species × treatment × female mass interaction; Suppl. material 1: fig. S4). *Harmonia axyridis* laid consistently more eggs as temperature increased (p < 0.0001 for all comparisons), whereas *C. lunata* only had a lower number of eggs in the cold treatment (p < 0.0001; species × temperature interaction; Fig. 3b). *Harmonia axyridis* laid more eggs than *C. lunata* in all treatments (p < 0.0001 for all comparisons).

Hatching success did not differ between species or treatments. The developmental time (from egg to pupal emergence) decreased with temperature (p < 0.0001 for all comparisons), but *H. axyridis* had a steeper change in developmental time
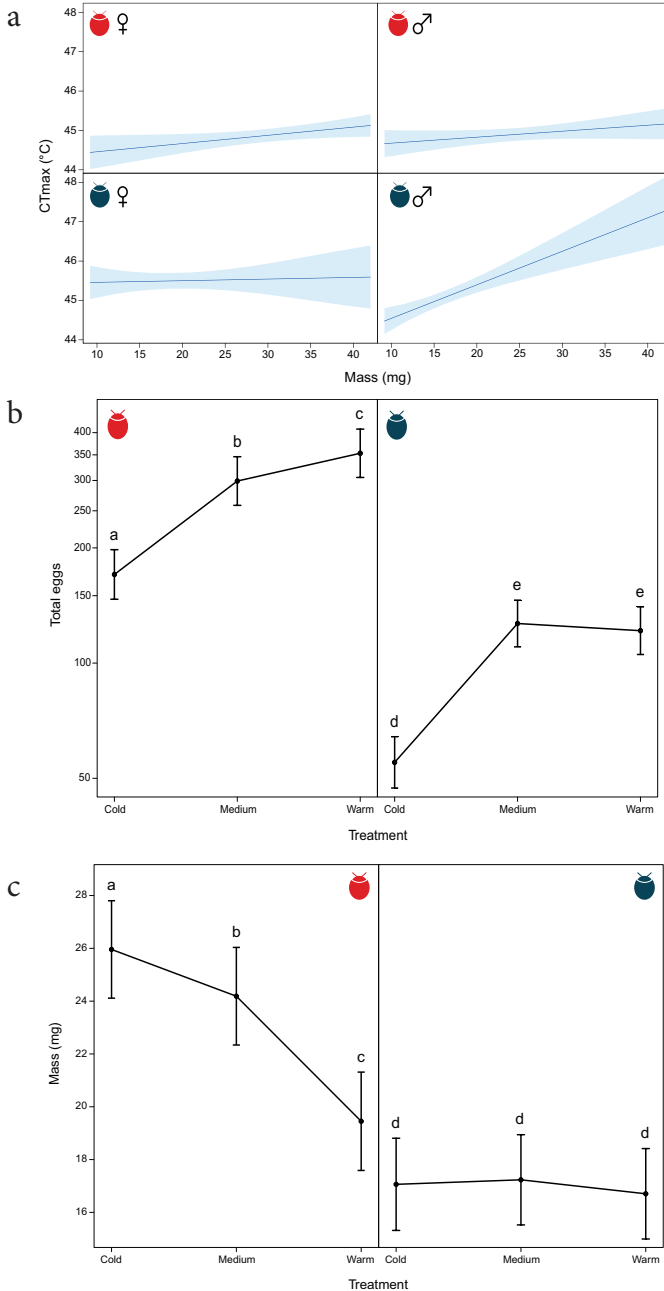
Figure 3. Contrasting responses of *Harmonia axyridis* and *Cheilomenes lunata* maintained at three temperature treatments. **a** critical thermal maxima (CT_{max}) of female and male *H. axyridis* (top row) and *C. lunata* (bottom row) as a function of body mass. Slopes are based on the best model parameter estimates **b** total number of eggs laid by *H. axyridis* (left) and *C. lunata* (right) at each temperature treatment **c** adult mass (mg) of *H. axyridis* (left) and *C. lunata* (right) in each treatment. In **b** and **c** different letters indicate significant differences between groups. Values are based on the best model parameter estimates and standard errors.

Table 1. Estimates of population size of *Cheilomenes lunata* and *Harmonia axyridis* across stages of invasion. Last column presents the number of individuals after a heatwave event in the novel site (see Suppl. material 1: fig. S9 for more details). Results include simulations in cold, medium and warm temperature regimes after 1000 repeats. *Cheilomenes lunata* is indicated by 'CL' and *Harmonia axyridis* 'HA'. Values are mean number of individuals ± SD.

Species	Scenario	Adults surviving transport	Females surviving transport	Total eggs laid in novel site	Viable eggs in novel site
CL	Cold	26.7 ± 26.6	13.6 ± 13.3	815.2 ± 813.4	747.9 ± 745.3
CL	Medium	15.5 ± 23.8	8.1 ± 11.9	999.4 ± 1469.5	877.1 ± 1291.4
CL	Warm	9.0 ± 19.0	4.9 ± 9.4	671.0 ± 1298.4	479.2 ± 931.3
HA	Cold	20.7 ± 28.2	10.7 ± 14.0	1752.6 ± 2311.6	1510.7 ± 1993.9
HA	Medium	14.1 ± 23.5	7.4 ± 11.7	2401.0 ± 3800.1	2176.2 ± 3442.5
HA	Warm	10.6 ± 21.6	5.7 ± 10.7	2219.5 ± 4191.7	1831.1 ± 3467.1
Species	Scenario	Larvae in novel site	Adult offspring in novel site	Adults after a heatwave event	
CL	Cold	705.6 ± 702.9	514.9 ± 516.0	287.5 ± 288.2	
CL	Medium	800.1 ± 1177.7	596.0 ± 875.7	364.4 ± 535.1	
CL	Warm	418.2 ± 810.9	322.0 ± 625.1	273.1 ± 529.7	
HA	Cold	1364.2 ± 1799.2	1298.4 ± 1712.1	225.0 ± 297.3	
HA	Medium	2024.2 ± 3201.8	1887.3 ± 2982.2	502.2 ± 794.2	
HA	Warm	1606.6 ± 3041.3	1320.7 ± 2497.7	669.0 ± 1264.4	

between the cold and medium, and between the cold and warm treatments than the native species (species × treatment interaction; Suppl. material 1: fig. S5). Overall, males had a faster developmental time than females ($p < 0.001$). Larger adults were associated with slower developmental times and this relationship slightly differed across treatments (mass × treatment interaction; Suppl. material 1: fig. S6). *Harmonia axyridis* had a higher percentage of successful pupal emergence than *C. lunata* (90.4 ± 10.1% (n = 40) and 75.6 ± 21.7% (n = 37), respectively; $p = 0.02$), but there were no differences between temperature treatments.

The mass of adults of *C. lunata* remained constant across treatments ($p > 0.28$ for all comparisons), while that of *H. axyridis* decreased with the treatment temperature ($p < 0.0001$ for all comparisons; species × treatment interaction; Fig. 3c). Similar patterns emerged when examining sexes separately (sex × species × treatment interaction; Suppl. material 1: fig. S7). For both species, and across all treatments, males were smaller than females ($p < 0.001$ for all comparisons). *Harmonia axyridis* males and females were heavier than *C. lunata* males and females in the cold and medium treatments ($p < 0.001$ for all comparisons), but not in the warm treatment ($p > 0.14$ for all comparisons) (Suppl. material 1: fig. S7).

Harmonia axyridis had a higher intrinsic rate of increase (i.e. per capita change in the population per generation) than *C. lunata* overall (0.10 ± 0.03 (n = 40) and 0.09 ± 0.04 (n = 37), respectively; $p = 0.02$), but there was no significant effect of treatment (see Suppl. material 1: table S5 for means ± SD and sample sizes for the net reproductive rate (R_0) and generation time (T_g) of both species for each temperature treatment). Larger reproductive females of *H. axyridis* had a higher intrinsic rate than smaller ones, while this relationship was not found for *C. lunata* (female mass × species interaction; Suppl. material 1: fig. S8).
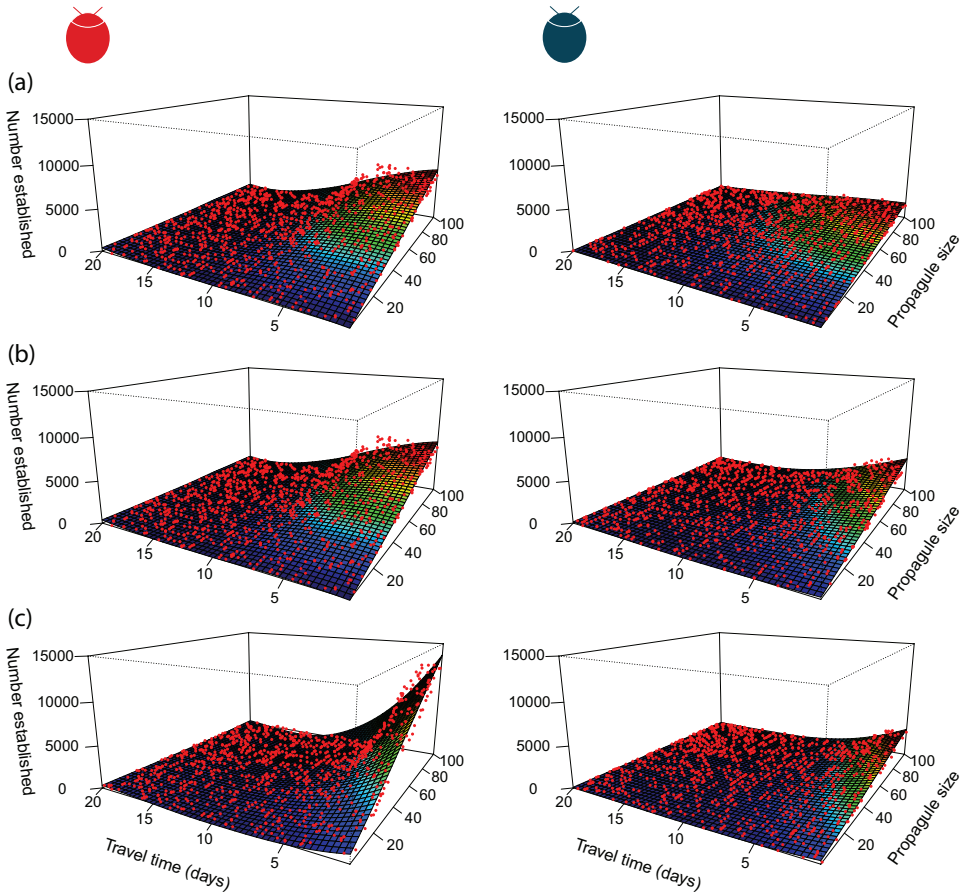
Figure 4. Predicted number of beetles established as a function of propagule size and traveling time. Plots present the number of individuals of *Harmonia axyridis* (left column) and *Cheilomenes lunata* (right column) that would survive the transport and establishment stages in **a** cold **b** medium and **c** warm environments (see assumptions, and starvation and fitness data collection in text). One thousand repeats were used and starting propagule size (1 to 100) and travel time (1 to 20 days) were randomly selected for each run. Outcomes of the iterations are shown as red points. Surface regression planes were obtained using locally estimated scatterplot smoothi.

Modelling population size

Once individuals survived transport and successfully arrived in the new area, more offspring of *H. axyridis* established than *C. lunata* in all temperature scenarios (Table 1). The average established population size (number of adult offspring) was highest in the medium scenario for both species (Table 1). In warm environments, high propagule pressure resulted in high levels of establishment in *H. axyridis*, while this was less notable for *C. lunata* (Fig. 4).

Discussion

This study showed that the native *C. lunata* had a greater upper temperature tolerance than the invasive *H. axyridis*, but *H. axyridis* had a higher performance than *C. lunata* for several life-history traits, in particular, fecundity and intrinsic rate of population increase. Despite the native species being more plastic for some traits (e.g. starvation resistance), *H. axyridis* displayed consistently higher performance over the range of temperature conditions compared to the native species (preoviposition period, number of eggs, % pupal emergence), fitting the model of a general purpose or Jack of-all-trades phenotype (Richard et al. 2006). The combination of traits, such as those linked to fitness, resulted in a consistently higher intrinsic rate of increase of the invasive species compared to the native one.

Studies that examine the plasticity of starvation resistance in invasive insects have found no thermal acclimation effects (e.g. Weldon et al. 2018), or that starvation resistance increases or decreases with temperature (e.g. Hoffmann et al. 2005). Knapp and Řeřicha (2020) exposed laboratory-reared introduced populations of *H. axyridis* to several months of cold, average and warm over-wintering temperature regimes and then scored starvation resistance at 22 °C. They found that after the cold winter-regime, beetles had reduced body mass loss and increased starvation resistance. However, the physiological mechanisms underlying starvation resistance after quiescence or winter diapause are likely to be very distinct from those of an acute food shortage as in our current study. We found that both *H. axyridis* and *C. lunata* survived longer without food in colder conditions, but *C. lunata* was more plastic for this trait, counter to the expectation of higher plasticity in the invasive species. This finding was not due to species' differences in the percentage of body mass loss as the native species lost a higher percentage of body mass than the invasive (Suppl. material 1: table S5), and there was a lack of a significant interaction between body mass lost, treatment and species explaining starvation resistance. Increased plasticity in the native species could result from an increased ability to store energy reserves, a reduction in their usage, or a higher tolerance for low body energy content (Rion and Kawecki 2007). However, we cannot confirm that the extent of food shortage stress imposed in our experiments was equivalent for both species due to unknown aspects that influence starvation resistance, especially for the native species (e.g. life span). Moreover, differences between sexes in starvation resistance and mass loss across temperature treatments for both species could be explained by different energy use and energy storage strategies, and their sensitivity to temperature change (Dmitriew et al. 2009; Hodek et al. 2012; Knapp and Nedvěd 2013; Knapp 2014). Since starvation resistance impacts dispersal ability, females may be better dispersers than males in cold and optimal environments, but males may cope better in warm environments.

Thermal tolerance limits of insects are often plastic and thermal acclimation effects are typically more notable for CT_{min} than CT_{max} (e.g. Terblanche et al. 2010; Lancaster et al. 2015; Weldon et al. 2018). A few studies have also reported increased plasticity or faster time course of acclimation in invasive compared to native species (e.g. Kellet

et al. 2005; Nyamukondiwa et al. 2010; Mutamiswa et al. 2018). Our results are in line with thermal acclimation of temperature limits in insects. For both species, acclimation to colder temperatures resulted in lower CT_{min}. However, for CT_{max}, complex interactions with sex and mass differentiated the species. Overall, basal upper tolerance was slightly higher in the native species, and these differences, albeit their small effect size, can elicit negative effects on survival when facing extreme warm events. For example, by simulating a stochastic heatwave event for established beetles of either species in the form of a temperature exposure to 45 °C, we find that the percentage mortality of *H. axyridis* is higher than *C. lunata* in all temperature scenarios (Cold: 83% vs 44%, Medium: 73% vs. 39%, Warm: 49% vs 15% mortality for *H. axyridis* and *C. lunata*, respectively; see Suppl. material 1: fig. S9). Despite this high mortality however, the population size of *H. axyridis* would remain larger than the native species even when both species are transported and introduced with the same propagule size, except for the cold scenario (Table 1). This simple simulation suggests that predictions of global warming effects on successful invasive species and native species require examining performance beyond the plasticity of critical limits alone and need to incorporate simulations of population fluctuations that are mediated by other factors (e.g. initial propagule size), traits (life-history) and associated adaptive processes (e.g. plasticity and evolutionary potential of thermal traits; Garnas 2018; Logan et al 2019). While this may seem obvious, the direction and consequences of these effects on population size can often be counterintuitive (Sgrò et al. 2016) as for example, plastic effects such as those reported here, can be of small magnitude.

Given the cold origin of *H. axyridis*, and its recent introduction to South Africa (early 2000s; Stals 2010; Roy et al. 2016), the native *C. lunata* may be better adapted to local conditions compared to *H. axyridis*. However, exploring the biogeography and invasion history of *H. axyridis* in South Africa, and the microclimates used by both species in the field, should be paramount to address this question, as environmental heterogeneity and associated costs and risks of using the environment could underlie plastic responses in these species (Hoffmann and Sgrò 2018; Shinner et al. 2020).

Previous studies on life-history traits have found mixed results regarding consistent plastic responses of traits in invasive insects as fecundity, offspring survival and developmental time to temperature (e.g. Kingsolver et al. 2007; Terblanche et al. 2010; Ferrer et al. 2014; Fält-Nardmann et al. 2018). Invasive *H. axyridis* performed better over a broader range of conditions than *C. lunata* for the majority of life-history traits (with the exception of hatching success and developmental time) but we found no notable differences in plasticity of these traits between species. A meta-analysis that compared life-history traits of *H. axyridis* in its introduced range (i.e. Europe and North America) and native range showed that invasive beetles had higher mean fecundity (Raak-van den Berg et al. 2017), perhaps highlighting the role of this trait in driving the invasiveness of *H. axyridis*. Several studies reported that its fecundity was highest at intermediate temperatures (24–27 °C) compared to colder (18–20 °C) and warmer (30 °C) temperatures (Lombaert et al. 2008; de Oliveira Ramos et al. 2014; Barahona-Segovia et al. 2016). In our study, fecundity increased consistently with temperature

(Fig. 3b), but the warm regime (fluctuations between day-time ~30 °C and night-time ~23 °C) was likely more benign compared to the typical chronic exposures used in these other studies. Fluctuating temperatures may increase performance as the overall temperature regime remains within the permissive range, and night-time temperatures can allow repair of potential damage incurred during daytime (Colinet et al. 2015).

Combining species' traits and their plasticity in an invasion framework demonstrated that, given the same variation in propagule pressure and stages of invasion, a larger number of invasive beetles will establish compared to the native, but medium temperature conditions will maintain the highest numbers for both (Table 1). Several patterns emerged when assessing how species' traits collectively affected population size at the novel site, depending on the temperature scenario. First, in all temperature scenarios, travel or transportation time drastically reduced establishment, especially at warm temperatures (Fig. 4). Second, for both species, at least 30 propagules were required at all temperatures for a few individuals to survive transportation and establish, but the patterns that emerged from the transport stage onwards deviated substantially between species. Third, both species were able to resist starvation for longer during transport in the colder scenario, but a greater number of eggs laid in the medium scenario meant that both species were able to compensate for losses incurred during transport and establish the highest number of individuals. Simulations showed that, despite the constraining effects of lower starvation resistance at warm temperatures, high numbers of *H. axyridis* were able to establish given the high fecundity at the warm regime and a sufficiently large propagule size. By contrast, for the native *C. lunata*, the number of individuals that established in the warm scenario was compromised due to the low number of individuals that survived the transport stage coupled with reduced fitness.

Our results should be interpreted with caution for several reasons. While there is a fair amount of information about *H. axyridis* thermal biology and life-history, we have less knowledge of the native species' general biology and limited understanding of microclimates experienced by coccinellids in general (Roy et al. 2016; Sloggett et al. 2021). Therefore, the temperature regimes and protocols used may be more ecologically relevant to one of the species; nevertheless, from our own observations, both species frequently co-occur on the same plant microsites and are observed feeding on the same aphid prey. In addition, differences in the shape of performance curves between species combined with the fluctuating environmental conditions could result in different realized fitness outcomes (Colinet et al. 2015; Denny 2017). This is not problematic if the fluctuating regimes used here are relevant to both species. Finally, more facets of these species' ecology are needed to refine the predictions on their colonization and establishment potential in new thermal environments. Plasticity in phenological, behavioral and developmental responses that allow persistence or avoidance of stressful conditions, as well as dispersal and competitive abilities, would be essential (Hulme 2008; Turcotte and Levine 2016). For example, although both species are primarily aphidophagous, *H. axyridis* can be polyphagous and cannibalistic during periods of its life cycle (Wagner et al. 1999; Snyder et al. 2000). These characteristics are unknown for the native species.

Regardless of these potential limitations, we show that for a set of key traits, the invasive species had higher performance than a co-occurring native species, mostly resulting from higher mean effects and despite both species having some plasticity for different traits. This study demonstrates that making interpretations from a reduced set of performance traits or invasion stages would present an inaccurate estimation of these species' potential establishment into new thermally-distinct areas. Baseline knowledge of traits, plus their variability in different thermal environments as examined here, is essential if we aim to predict the response of successful invasive and native species to future climate scenarios. Disentangling between 'active' and 'passive' thermal plasticity (Havird et al. 2019) will be a useful next research focus to improve predictions of these species' responses to unprecedented temperature variations, both acute and long-term changes, associated to climate change.

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Supplementary material 1

Tables and figures

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Data type: Docx file.

Explanation note: **Tables: S1.** Incubator temperatures (IT in °C) at which *Harmonia axyridis* performed best in terms of reproduction and population growth characteristics. **S2.** *Harmonia axyridis* and *Cheilomenes lunata* collection sites (GPS coordinates) in Stellenbosch (Western Cape Province, South Africa). **S3.** Best model outputs for each trait: (a) Starvation resistance, (b) CTmin, (c) CTmax, (d) preoviposition period, (e) total eggs laid, (f) hatching success, (g) developmental time, (h) pupal emergence success, (i) adult mass, and (j) intrinsic rate of increase. **S5.** Summary statistics (mean, standard deviation and sample size) for each trait and temperature treatment: (a) starvation resistance, (b) thermal tolerance, (c) life-history traits. **S6.** Averaged model summary outputs: (a) CTmin, (b) CTmax, (c) developmental time, and (d) hatching success. HA = *Harmonia axyridis*, CL: *Cheilomenes lunata*. **Figures: S1.** Study experimental design for rearing and determining physiological and life-history traits of the two beetle species. T1 to 3: treatments 1 to 3. CTL = Critical Thermal Limits. Ladybird illustration by Corneile Minnaar. **S2.** Kaplan-Meier survival curves used to plot starvation resistance data of *Harmonia axyridis* and *Cheilomenes lunata* for the cold (right - blue), medium (middle - green) and warm (left - orange) temperature treatments for beetles that had a) low mass loss (≤16.43%, the median mass loss percentage for all beetles in all treatments) and b) high mass loss (>16.43%) groups. **S3.** Critical thermal maximum (CTmax, °C) of male and female beetles of *Harmonia axyridis* and *Cheilomenes lunata* for each temperature treatment. **S4.** Values are model parameter estimates. Total number of eggs laid by *Harmonia axyridis* (top row) and *Cheilomenes lunata*. **S5.** Developmental time (days) from egg to pupal emergence of *Cheilomenes lunata* (left) and *Harmonia axyridis* (right) in each temperature treatment. Values are model parameter estimates. **S6.** Developmental time (days) of *Harmonia axyridis* and *Cheilomenes lunata*. **S7.** Adult (F3) mass (mg) of male and female *Harmonia axyridis* (top row) and *Cheilomenes lunata*. **S8.** Intrinsic rate of increase of *Cheilomenes lunata* (left) and for *Harmonia axyridis* (right) as a function of female mass (mg). **S9.** Density plots of measured critical thermal maximum (CTmax) data for *Harmonia axyridis*.

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