Life-history adaptation under climate warming magnifies the agricultural footprint of a cosmopolitan insect pest

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Keywords: life history, trade-off, temperature, climate change, global warming, agriculture, ectotherm, insect, pest, crops, gene expression.

Abstract

Climate change is affecting the distributions and growth rates of cold-blooded pest species, with dire consequences for agriculture and global food security. To address this challenge, it is essential to forecast how pests will respond to changing climates. However, current projection models typically overlook the potential for climate change adaptation. By unifying life-history theory with thermodynamics based on first principles, we here show that pest species' climate adaptation can cause substantial increases in their agricultural impact via changes in temperature-dependent resource acquisition and allocation strategies. We test these predictions by studying thermal adaptation in life-history traits and gene expression in the cosmopolitan insect pest, *Callosobruchus maculatus*. Five years of adaptation to simulated warming caused an almost two-fold increase in the predicted agricultural footprint of *C. maculatus,* while adaptation to cold temperature had little effect. Consistent with the theoretical predictions, the magnified impact under warming was driven by synergistic increases in larval resource acquisition and adult reproductive potential, resulting in a predicted double blow on agricultural yields. This suggests that adaptation in insect life-history will significantly worsen agricultural loss under future climate change and emphasizes the need for integrating a mechanistic understanding of life-history evolution into ecological forecasts of pests.

Climate change is predicted to greatly impact growth rates and distribution ranges of cold-blooded pest species[1–7] with potentially severe outcomes for agricultural economics and world food supply[8–12]. To mitigate these events, forecasting of pests' responses to future climates is a fundamental task for biologists[13,14]. Current forecasts use deterministic models that rely on information about species niches and climate data. However, these projections typically do not incorporate the potential for genetic evolution of climate niches and key life-history traits that mediate the effect that pests have on their hosts[13,14]. Indeed, the evolution of ecological niches has been rapid in many cosmopolitan pests and not accounting for this adaptive potential may therefore provide inaccurate forecasts of their future agricultural impact[1,7,13–17].

Despite its importance, the evolution of pest species' climate niches is not well-understood[1,5,7,16]. Cold-blooded organisms are constrained by temperature-dependent biophysical properties of cells[18–22]. On the one hand, metabolism is fundamentally constrained by cold temperature, predicting that climate warming may increase growth rates[23,24] and thereby worsen pest impact in many parts of the world[2]. On the other hand, hot temperatures can jeopardize cellular homeostasis[25–27], leading to fertility decline[28,29] and increased mortality[22,30]. These thermodynamic constraints are expected to limit the distribution ranges of ectotherms. However, typical signatures of pest species are their broad climate niches coupled with fast growth rates[1,16]. One mechanism that can allow organisms to occupy broad climate niches is up-regulation of molecular chaperones that aid cellular homeostasis under harsh conditions[20,31]. Such responses are energetically costly and therefore need to be compensated by either increased resource intake[5,19], or by diverting resources away from reproduction in favour of cellular maintenance[20,31]. Such alternative strategies in pest species' life-history are predicted to impact both their invasion potential and the footprint left on their hosts[1,14,17,32–34], and thus, can have important consequences for pest management[1,3,35].

However, exactly how life-history evolution unfolds under climate change remains unclear[1,16,33]. Moreover, for adaptation to occur, genetic variation is a prerequisite. Geographically isolated populations can differ greatly in the abundance of both segregating and fixed genetic variation due to differences in their evolutionary past, which may affect future adaptive potential[36–40]. Such historical contingencies may be of particular importance in insect pests, as geographically isolated populations often originate from human-mediated long distance dispersal events of only a few individual genetic founders. Evolution under climate change may therefore proceed along different trajectories and rates across a species distribution range, even when climates change in parallel.

Here we explored the agricultural impact of life-history evolution under climate change by employing experimental evolution in the seed beetle, *Callosobruchus maculatus*, an economically important cosmopolitan pest on legume seeds, and a model organism for life-history research[41]. Life-history evolution over 5 years in response to warming caused a substantial increase in the agricultural footprint of *C. maculatus* via trait-specific responses, an effect not seen in populations adapting to cold conditions. This increase was also contingent on the genetic make-up of the founding populations. These findings suggest that equipping species distribution models with genetically explicit details accounting for life-history evolution can lead to a substantial improvement in predictive accuracy of pest impact under climate change, even over relatively short time scales.

Results

The agricultural impact of thermal adaptation via compensatory resource acquisition

To formalize predictions for how thermal adaptation of insect pests will impact agriculture, we combined a classic life-history framework of energy acquisition and allocation trade-offs[42–45] with the thermodynamic laws that govern enzyme kinetics[20,46]. Warm temperatures increase growth rates of ectotherms[19,23] because any biological rate is at some level governed by enzymatic reaction rates, *k*, that scale predictably with temperature according to[47,48]:

$$k \propto Ae^{-Ea/RT} \qquad \text{(Eq. 1)}$$

where *Ea* is the activation energy required for a reaction to occur, R is the universal gas constant, *T* is temperature in Kelvin, and *A* is a species and rate specific constant that may itself evolve to accommodate effects of temperature[49,50] (**Fig. 1A**). Complementing equation 1 with the power law describing how productivity scales with body mass, *M*, results in the well-known prediction of body mass and temperature-dependent population growth rate based on the metabolic theory of ecology[23,24]: $r \propto M^b k$, where *b* is the allometric exponent, often taken to be 0.75 based on the physics of distribution networks in plants and animals[23,51]. However, excessively hot temperatures lead to increased molecular failure rates[30] due to a range of detrimental processes that also scale exponentially with temperature, including protein unfolding[25,26], metabolic expenditure[52,53], production of reactive oxygen species[20], and oxygen depletion[54] (**Fig. 1A**). Thus, mortality rate can be described by equation 1 with its own activation energy (*Ea*) and rate-specific constant (*A*) (e.g.[30,53,55]). As follows, population growth rate is the result of temperature-dependent productivity and mortality rate[1,2] (**Fig. 1B**).

While there is variation in biological rates across organisms[49,56,57], inherent enzyme properties governing the temperature-dependence of reaction rate kinetics (*Ea* in eq. 1) evolve slowly[50,58]. Instead, thermal adaptation often involves rapid evolution of compensatory stress responses (*A* in eq. 1)[20,31,49,59]. Such responses involve upregulation of molecular chaperones, such as heat-shock proteins[20,60,61], that maintain cellular homeostasis at stressful temperatures but are costly to produce[31,60,62]. Here we therefore placed the predictions of equation 1 into a life-history framework incorporating acquisition and allocation trade-offs[42,43,62]. First, assume that body reserves, *M*, are accumulated by host feeding at a cost, *c*, so that survival to maturity equals: $(1-c)^M$. Further assume that body reserves can be allocated with fraction *p* to increase enzymatic reaction rate, and with fraction *q* to decrease molecular failure rates, leaving *M*(1-*p*-*q*) for reproduction, *B*, according to:

$$B \propto [M(1-p-q)]^b \;\; ; \quad A_B \propto pM \;\; ; \;\; A_m \propto 1/qM \qquad \text{(Eq. 2)}$$

where A_B and A_m are the rate-specific constant for productivity and mortality, respectively. Population growth rate, r', is then:

$$r'(T) \propto (1-c)^M * B * A_B\, e^{-\frac{Ea_B}{RT}} * (1 - A_m\, e^{-\frac{Ea_m}{RT}}) \qquad \text{(Eq. 3)}$$

We can find the optimal strategy of resource acquisition (increasing *M* at cost *c*) and allocation (of *M* to *p* and *q*) at different temperatures by numerically solving for the combination of *M*, *p* and *q* that maximize r'. The solution to this multidimensional trade-off depends on the specific form of equations 1 and 2 and parameters that govern temperature sensitivity (i.e. *Ea, A* and their implementation); mechanistic details which are empirically not well-defined. Indeed, equation 1 represents a phenomenological, rather than a mechanistic, relationship and studies have highlighted that the coefficients Ea_B and Ea_m can themselves be temperature-dependent (e.g. [30]), emphasizing the need for further experimental work quantifying these relationships.

Nevertheless, the qualitative predictions from this heuristic model are robust (see also [53]). We provide a parameterized example in **Figure 1** grounded in knowledge of how temperature affects protein catalysis and stability[25,27,58,63] (details in **Suppl. 1)**. First, and as pointed out previously[2], warm temperatures increase growth rates and agricultural impact by reducing thermodynamic constraints on reaction rates. Second, when thermodynamic constraints jeopardize survival, hot temperatures favour increased resource acquisition and more body reserves (*M*) that can be allocated to maintenance[53] (**Fig. S1A, Fig. 1B**). When increased resource acquisition comes at low cost, this life-history strategy is particularly favourable and

results in increased thermal niche breadth and population growth rates (**Fig. 1C**). Third, this has an even greater agricultural impact, given by the product of population growth rate (*r'*) and the per capita host consumption (assumed proportional to *M)* (**Fig. 1D**). In fact, the strategy to increase resource acquisition at stressful temperatures causes populations experiencing temperatures slightly hotter than those which maximize growth rate to have the greatest agricultural impact (**Fig. S1**).

Experimental evolution of life-history traits and agricultural impact

To evaluate the prediction that trait and temperature-specific responses in pest species' life-history can influence their agricultural impact under climate change, we compared twelve experimental evolution lines of the seed beetle *C. maculatus*; six adapted to 35°C (*Hot*) and six adapted to 23°C (*Cold*). All lines were reared on seeds from their preferred and economically important host, black-eyed bean (*Vigna unguiculata)* (**Fig. 2**). The lines were derived from three geographically isolated founding populations (*Ancestors*, kept at 29°C) originating from Brazil, California (USA) and Yemen[64], allowing us to assess the importance of geographic differences and evolutionary history for future responses to climate change.

Molecular evidence for a temperature-dependent trade-off between reproduction and maintenance

To provide evidence for the central assumption of a trade-off between investment in reproduction and cellular maintenance under thermal stress (Eq. 2), we analysed data on gene expression from two separate experiments on *C. maculatus*. These datasets contained genes that were significantly differentially expressed in female abdomens in response to heat shock, and between virgin and reproducing females. We identified 1269 reproduction-related genes and 765 heat stress genes. We found 137 genes present in both datasets, which is significantly more than expected by chance (exp: 83.4 genes, $\chi^2 = 37.5$, df = 1, $p < 0.001$, **Fig. 3A**). Moreover, only 3 of the 137 genes showed concordant responses to mating and heat-shock (all three decreased in expression), whereas 134 genes showed antagonistic responses (binomial test: $P < 0.001$, **Fig. 3B**), providing strong evidence for a trade-off between reproduction and the heat stress response. Out of these genes, 21 were up-regulated in response to reproduction and down-regulated in response to heat. These genes included common ribosomal genes that are crucial for protein translation, signifying reproductive investment. The 113 antagonistic genes that were down-regulated in response to mating and up-regulated in response to heat stress included common insect heat-shock proteins (**Suppl. Table 2a**). GO enrichment analysis showed that the antagonistic genes that overlapped both gene sets were enriched for biological process involved in protein folding and degradation of

unfolded proteins, which are essential components of cellular maintenance under thermal stress (**Fig. 3C, Suppl. Table 2b**).

To confirm that the revealed trade-off also was at play in the thermal conditions used during experimental evolution, we analysed gene expression data from all Cold and Hot lines, as well as their Ancestors, reared in a common garden design including 23, 29 and 35°C assay temperature. Each evolution line was represented by a single mRNA library per temperature, based on a pool of 10 abdomens from mated females. The ancestors were represented by two such replicates. To evaluate investment into reproduction versus maintenance, we calculated a score for each of these mRNA libraries along the axis describing the trade-off based on the expression of the 134 antagonistic genes (**Fig. 3B**). We then analysed differences between assay temperatures and evolution regimes using nested ANOVAS (**Suppl. Table 2c**). Beetles reared at 35°C showing a strong change in allocation towards cellular maintenance and away from reproduction compared to beetles reared at the other two temperatures (assay temperature: $F = 263.0$, $P < 0.001$, **Fig. 3D**). There were no significant differences between evolution regimes overall ($F = 0.12$, $P = 0.89$), although the differences between the evolution regimes and their ancestors showed a dependence on geographic origin (interaction: $F = 11.0$, $P = 0.002$). Qualitatively similar results were obtained when analysing all 765 heat stress genes and 1269 reproduction genes (**Suppl. Tables 2d, e**). While statistical power was low due to the small number of mRNA libraries, this implies that there has been little evolutionary change in resource allocation overall (i.e. q in Eq. 2). This result further predicts that if hot-adapted lines have evolved a means to deal with the increased cellular stress of hot temperature (35°C), they must have done so mainly via increased resource acquisition (M in Eq. 2), which should be apparent as an evolved increase in adult body mass.

Thermal adaptation in life-history traits

We assessed the predicted agricultural impact of life-history evolution by assaying three classic traits; juvenile development time, female adult body mass, and lifetime reproductive success (LRS: number of offspring produced), and four rate-dependent traits assayed over the first 16h of female reproduction; early fecundity, weight loss, water loss and metabolic rate (**Fig. 4**). This also allowed us to evaluate if Hot lines had adapted to their temperature regime by increased energy acquisition (*M;* captured by adult body mass).

To characterize trait-values at the start of experimental evolution, we assayed the three ancestors reared at 23, 29 and 35°C. This allowed us to quantify both the direction and magnitude of adaptation during experimental evolution, as well as its subsequent agricultural impact (*see below*). Temperature affected the expression of all seven life-history traits. For

development time, body mass, early fecundity and LRS, there were also main effects of the geographic origin of the ancestors. Body mass, early fecundity and LRS also showed a significant interaction between geographic origin and assay temperature, indicating differences in thermal plasticity between ancestors (**Fig. 4A-G, Suppl. 3**).

To assess temperature-dependent life-history evolution we assayed the 12 evolved lines when reared in a common garden experiment including 23°C and 35°C. All traits except metabolic rate and weight loss showed significant differentiation between the Hot and Cold regime. In particular, the Hot regime had indeed evolved larger adult body mass and higher reproductive output (**Fig. 4A-G**), in accordance with model predictions (**Fig. 1C**). All traits except metabolic rate and LRS showed a significant interaction between regime and assay temperature, demonstrating thermal adaptation *sensu stricto* (**Fig. 4B-G**). Several alternative estimates of laboratory fitness also showed clear signs of temperature-specific adaptation, with Hot lines outperforming Cold lines at 35°C, while there were only small differences at 23°C (**Suppl. 4**). In addition, for metabolic there was an interaction between evolution regime, assay temperature and geographic origin, indicating that thermal adaptation was contingent on founding genetic variation (**Fig. 4B, E, Suppl. 3**). Analysing all traits together using a non-parametric MANOVA[65] confirmed main effects of assay temperature ($F = 205.5$, $P < 0.001$), evolution regime ($F = 57.1$, $P < 0.001$) and the interaction between evolution regime and assay temperature ($F = 6.8$, $P < 0.001$).

Thermal adaptation in life-history shapes the agricultural footprint

To predict how the observed life-history evolution may affect agricultural yields, we estimated the relationship between life-history traits and the amount of host consumption by *C. maculatus.* We reared Cold, Hot and Ancestral lines at 23, 29 and 35°C. At each assay temperature, we let females lay eggs in a petri-dish containing ad libitum black-eyed beans. The number of eggs laid, the number of individuals surviving to pupation, the mass of adult beetles, and the seed mass consumed, was calculated from each dish.

The Hot regime consumed significantly more of the host than the Cold regime ($F_{1, 5.97} = 22.8$, $P = 0.003$), demonstrating that the agricultural footprint indeed had evolved. The difference between the Hot and Cold regime tended to be greater at 35°C, although the two-way interaction between regime and assay temperature was marginally non-significant ($F_{2, 11.9} = 3.25$, $P = 0.075$, **Fig. 5A**). To assess the importance of life-history traits in affecting food consumption, we performed analyses while sequentially including information on the number of eggs laid, juvenile survival, and body mass so that the final model predicted the amount of host consumed as a function of the cumulative beetle mass produced. Adding life-history traits

to the models reduced the differences between evolution regimes and in the final analysis including all traits we no longer found a significant effect of regime ($F_{1, 5.73} = 0.60$, $P = 0.47$), demonstrating that life-history adaptation alone seems responsible for the evolved differences in host consumption. Indeed, a model containing only the main effects of eggs laid, adult body mass and juvenile survival to pupation explained 93% ($P < 0.001$) of all the observed variance in bean consumption across the entire experiment (**Fig. 5A**). The amount of host mass lost was 3-4 times greater than the mass of beetle produced, which is similar to what has been reported in a previous study[66], underlining the severe impact that *C. maculatus* can have on host crops (**Suppl. 5**).

We predicted the agricultural impact of *C. maculatus* at cold and hot temperature based on the observed life-history evolution by calculating the agricultural footprint as: $\varphi 1 = C_T \omega M / d_1$, where C_T is the temperature-specific constant converting beetle mass produced to host mass consumed (**Fig. 5A**), ω is lifetime offspring production of a single female (**Fig. 4A**), M is body mass at adult emergence (**Fig. 4B**) and d_1 is mean development time in days (**Fig. 4C**). Hence, this measure of the footprint captures the magnitude of agricultural loss per time unit as a function of an organism's life-history strategy[67]. To assess the sensitivity of our predictions to both estimation error and choice of life-history variables we calculated a second independent estimate of the footprint, based on measurements of a different set of individuals: $\varphi 2 = \psi C_E / d_2$, where ψ is the number of eggs laid by a single female over her first 16h of reproduction (**Fig. 4D**), C_E is the amount of host consumed per laid egg, and d_2 is the mean development time in days of the fastest developing individual in each petri-dish (both variables estimated from the host consumption experiment presented in **Fig. 5A**). These two metrics were highly correlated ($r = 0.88$, $t_{31} = 10.4$, $P < 0.001$, **Fig. 5B**) suggesting high predictive accuracy.

To provide a generalisable metric of the agricultural impact of life-history evolution under climate change, we scaled the predicted footprint for all lines relative to their respective ancestor reared at 29°C (**Fig. 5C**). This metric allowed us to assess both the direct effect of temperature on the footprint in the ancestors, and how evolution, relative to this direct effect of temperature, impacted the footprint. Cold (23°C) and hot (35°C) assay temperature reduced the footprint by 35-45% and 0-25% respectively, relative to benign 29°C in the ancestors (**Fig. 5C**). However, evolution had a fundamental impact on how temperature affected the footprint. While cold-adapted lines showed very little difference to ancestral lines at 23°C (Δfootprint = 7.6%; CI: 2.0 – 15%), Hot-adapted lines left almost twice as large a footprint as the ancestral lines at 35°C (Δfootprint = 80%; CI: 70 – 97%). Moreover, these effects were contingent on the geographic origin of the founders. At 23°C, cold-adapted lines from California left a slightly larger footprint compared to the ancestor (Δfootprint = 29%; CI: 13 – 42%) while the footprint

had not evolved on the Brazil (Δfootprint = -3.1%; CI: -11 – 6.3%) and Yemen (Δfootprint = 1.0%; CI: -11 – 11%) background. At 35°C, the footprint evolved substantially on all backgrounds, but the increase relative to the ancestor tended to be greater for Brazil (Δfootprint = 84%; CI: 64 – 110%) and California (Δfootprint = 93%; CI: 71 – 120%) compared to Yemen (Δfootprint = 62%; CI: 44 – 87%) (**Fig. 5C**).

Discussion

Climate change is predicted to fundamentally impact growth rates and distribution ranges of ectotherms[5,30,37,68–71], many of which are pests on economically important crops[1,2,7,16]. Current predictions of increased agricultural impacts of pests typically rely on deterministic statistical projections of how temperature affects ectotherm metabolism and population growth, but do not incorporate evolution[13,14,67,72]. However, rapid evolutionary responses are characteristic of many pest species[1,5,7,16]. Here we therefore asked to what extent the incorporation of evolution can alter current projections of climate-induced changes in pest impact. We show three features that emphasize that future projections need to account for thermal adaptation in pest species' life-history. First, adaptation affected the agricultural footprint left by *C. maculatus,* but the effect was dependent on temperature; with little or no effect of cold adaptation while hot-adapted populations caused an almost doubling of the footprint relative to ancestors (**Fig. 5**). Second, these effects depended on the ancestral founder of the adapting populations, suggesting that geographic differences in segregating genetic variation in life-history traits may influence future agricultural loss under climate warming. Third, attempts to incorporate niche evolution in species' distribution models may need to do so by relatively simple means in the absence of empirical data that allow parametrisation and evaluation of more complex mechanistic models[13]. However, our study echoes concerns that such predictions may be off the mark because the mechanisms and type of traits that mediate niche evolution have central influence on pest impact[14,17,34,35].

Indeed, the observed temperature-dependent effect of evolution on the agricultural footprint can be understood by considering trait-specific responses. Even though cold-adapted populations did show evolved responses in traits such as development time, weight loss and water loss, these traits are predicted to have small to no effect on the agricultural footprint compared to lifetime reproductive success, early fecundity and adult body mass, that evolved more readily in hot-adapted populations (**Fig. 4**). The responses of hot-adapted populations are in line with two predictions from biophysical theory. First, warmer temperatures are predicted to ease thermodynamic constraints on metabolic rate[52], which can increase selection

for larger size via effects on fecundity[73,74]. Second, evolution at stressful hot temperature may favour compensatory feeding and sequestering of resources devoted to maintaining cellular homeostasis[5,19,20,31,42,53,62] (**Fig. 1, Suppl. 1**). Indeed, increased larval feeding and larger adult body mass in *C. maculatus* is associated with extended adult lifespan and greater fecundity under aphagous conditions corresponding to laboratory and grain storage environments (e.g.[75,76]). These effects are likely magnified at hot temperatures that increase metabolism and water loss ([23,77] and **Fig. 4**), and the need for upregulation of molecular chaperones to secure cellular homeostasis ([20,60,61] and **Fig. 3**). In accordance with this hypothesis, previous studies on *C. maculatus* also found that evolution at hot temperature increased body mass[78,79], an adaptive genetic response that paralleled the observed thermal plasticity in body mass[78]. To further support this hypothesis, we used the estimates of female life-history to calculate selection differentials on body size at hot and cold assay temperature. For all three estimates, selection was stronger at the hot temperature (**Suppl. 6**). Thus, the evolved increase in the agricultural footprint at hot temperature observed here is likely mediated by two synergistic effects of life-history adaptation, *i)* increased larval feeding enabling larger size at maturity, and *ii)* the positive effects of a large adult size on survival and reproduction at hot and aphagous conditions.

These observations agree broadly with the model prediction that stressful temperature can favour increased host consumption and energy allocation to maintenance to mitigate thermal constraints on growth and reproduction (**Fig. 1, Suppl. 1**). Note, however, that this prediction does not necessarily equate to the evolution of a large size at maturity, as resources might be spent on higher metabolic expenditure and maintenance at warm temperature prior to the organism reaching adult maturity, and our simple model makes no explicit predictions in this regard (see also[80,81]). Moreover, increased energy intake necessitates access to host plants. If food availability becomes limiting under climate warming, this mechanism could instead severely limit thermal tolerance and population growth rates of heat exposed ectotherms[53,55]. Thus, while host abundance is already acknowledged as a key factor regulating pest outbreaks[1,7,67,82], the interdependency between heat stress and the benefits of energy acquisition may magnify the effects of host accessibility on pest outbreaks under future climate warming (**Fig. 1C, D**).

Early findings suggested that most ectotherms follow the “temperature-size rule”[83,84], stating that body size shows plastic reductions with increasing temperature. However, later results have shown that these patterns are far from general. In particular, terrestrial ectotherms, the group to which many insect pests belong, show no consistent plastic response to temperature, and genetic adaptation along latitude shows a trend towards larger genetic body sizes at

warmer latitudes[85], which is consistent with the evolved response observed here. Moreover, thermal responses in body size tend to vary even within and between closely related species[71], as for *C. maculatus*[78,86] and other Chrysomelid beetles[87]. Understanding whether taxonomic variation represent constraints rooted in thermodynamics or adaptive plasticity may offer insights into the future routes of evolution under climate warming but has proven a major challenge for the field of eco-physiological adaptation[5,19,71,80,84]. Moreover, as discussed above, the effect of heat on energy expenditure suggests that the temperature scaling of adult body mass may not follow a one-to-one relationship with that of host consumption. Controlled experimental approaches as used here may provide further clues to what extent patterns of thermal plasticity are indicative of future genetic responses to climate and how the evolution of classic life-history traits affects host consumption. Such general information could be used to increase accuracy of future projections for species where thermal plasticity can be accessed, but where evolution is hard to study directly.

In contrast to the measured life-history traits, feeding efficiency was not strongly affected by assay temperature and did not evolve in our experiment, suggesting that differences in the agricultural footprint of insect pests may be accurately predicted from life-history variables alone. Yet, our study also highlights the important role of founding genetic variation in dictating life-history adaptation. This suggests that evolution under climate change may proceed along different trajectories across a species distribution range, even when regional climates change in parallel. When such divergent evolutionary responses involve key life-history traits, as observed here, this could lead to regional differences in pest impact[1,2,35].

The impact of insect pests on agriculture is paramount[11,67], with severe repercussions in human societies; 2.6 billion people (26% of the world's population) are currently directly dependent on agriculture for a secure living[88]. In African countries alone, the financial loss from pests was estimated to at least 1 billion USD for 2019, with agriculture accounting for 99% of this loss in central regions[9,10]. Clearly, the redistribution of species has already caused massive ramifications for the Earth's ecosystems[89], and the fear is now that future climate warming will allow insect pests to colonize new habitats[1,2,4,7,11,12]. To mitigate these effects, management strategies need to be equipped with accurate predictive models[13,14,72,90–92], as well as an understanding of the limits to their accuracy[38,93–95]. It is our hope that our study can contribute towards this development by *i)* providing general insights into the repeatability and mode of thermal adaptation in life-history of insect pests, which can inform on the general accuracy of ecological forecasting of species responses to climate change, and *ii)* by emphasizing the need for incorporating a mechanistic and genetic basis of thermal niche evolution into eco-evolutionary forecasts, on which improved management decision can be based[6,13,17,35,72,90,96,97].

Methods

Experimental evolution

C. maculatus is a pest on legume seeds and is common in tropical and sub-tropical regions[98]. Females lay eggs on the surface of seeds. After the egg hatches, the larva burrows into the seed to feed until it pupates. Adults emerge from seeds after ca. three weeks at standard temperatures around 29°C and are reproductively mature almost instantly and do not require food or water to reproduce[64,78,99]. These characteristics have likely contributed to *C. maculatus* being a cosmopolitan pest that invades seed storages[12,41]. Indeed, *C. maculatus* has become a model organism for research in both evolutionary ecology and pest management because laboratory and grain storage environments are very similar.

The experimental evolution protocol exposed six lines to 35°C (*Hot* regime) and six lines to 23°C (*Cold* regime), for 85 and 130 generations, respectively (difference due to effects of temperature on generation time). The lines were derived from three ancestors that were collected from different geographic regions (Brazil, Yemen and California (USA)), with two replicate lines per thermal regime created per region. The three founders were kept at ancestral 29°C throughout experimental evolution (**Fig. 2A**). A humidity of 50-55% was used throughout this study. Ancestors were reared, both before and during experimental evolution, by placing 300-400 newly emerged adults on 150 ml black-eyed beans, whereas evolution lines were reared by placing 600 adults on 250 ml black eyed beans[64], resulting in similar densities of adult beetles per bean. To reduce direct selection on development time, all lines were transferred to a new jar with fresh beans around the peak of emergence of adult beetles at each generation. This occurred approximately three days after the first beetle emerged at for ancestors and the Hot regime, and five days for the Cold regime.

Gene expression

To provide evidence for a temperature-dependent trade-off between reproduction and maintenance, we leveraged transcriptomic data from three separate experiments. The first two experiments estimated gene expression responses in female abdomens to heat shock and mating, respectively, in a separate lab population collected from Lomé, Togo[100]. The third dataset estimated expression in female abdomens in response to rearing temperature in our experimental evolution lines.

To identify heat stress responsive genes, we compared reproductively active control and heat shocked females. Individuals were collected as virgins and kept individually. On day 2, females were heat shocked in individual 60 mm petri dishes at 55° C for 20 minutes, which is stressful and reduces female fertility[101,102]. After a 2.5 h at 29° C and 55% rh, all females were mated to males. Females were then kept in individual 60 mm dishes until being flash frozen in liquid nitrogen 2-3 h, 6-7 h or 24-25 h after mating. The experiment was carried out on three consecutive days. Each replicate sample consisted of 5 pooled female abdomens from the same treatment, time-point and day, resulting in a total of seven replicate samples per time-point for the control treatment and five replicate samples per time-point for the heat shock treatment. Preliminary analyses showed that there were only three differentially expressed genes at the last time-point, which therefore was removed in the final analyses (although results did not qualitatively change if including them), resulting in 24 RNA libraries.

To identify reproductive genes, we compared virgin and mated females 24 h after a single mating. We mated females to males from 8 different genetic lines. The lines had undergone 53 generations of experimental evolution under one of three mating regimes manipulating the relative strength of natural and sexual selection[102]. Accordingly, we had 8 samples of mated females, with 2 or 3 samples per male mating regime. Additionally, we had 3 samples of virgin control females. Flash freezing of all females occurred 24 h after mating, with females kept singly and allowed to lay eggs on fresh beans during the 24h period. The final 11 RNA libraries each consisted of 12 female abdomens.

To quantify expression of reproductive and heat stress responsive genes (identified in the first two datasets) in our experimental evolution lines and their ancestors, we reared all lines at 23, 29 and 35°C. All lines were first propagated for one generation at 29°C to remove potential difference between evolution regimes stemming from temperature-induced (non-genetic) parental effects. Once adults emerged at respective rearing temperature, 5 males and 5 females were placed together in a 90mm petri dish with access to fresh beans. Beetles were allowed to reproduce for 23h (for 29 and 35°C) or 46h (for 23°C) after which females were flash frozen. The longer time allowed for reproduction at cold assay temperature was given to make a more direct comparison across females from different temperatures; reproductive output (Fig. 4B), metabolism (Fig. 4E) and weight loss (Fig. 4F) is about twice as fast at 35°C compared to 23°C. Two petri dishes of females were pooled into one sample (i.e. 10 female abdomens pooled in total) for extractions and further analysis, resulting in one sample per line and temperature for the Hot and Cold regime. Two samples were taken per temperature for each of the three ancestors. This resulted in a total of 54 RNA libraries.

RNA from all samples was extracted using the Qiagen RNeasy Mini kit with beta-mercapto-Ethanol added to the lysis buffer and an on-column DNase treatment with the Qiagen RNase-free DNase kit. Tissue lysis was done in a bead mill with two stainless steel beads at 28 Hz for 90 s and RNA was eluted in two times 30-50 µl water. Some samples went through an additional clean-up step with the Qiagen RNeasy Mini Kit after the RNA extraction. Next generation sequencing of the samples was done at the SNP&SEQ Technology Platform in Uppsala. Libraries were prepared using the TruSeq stranded mRNA library preparation kit with polyA selection. Libraries were sequenced in one flowcell as paired-end 150 bp reads on a NovaSeq 600 system.

Raw reads were quality checked with FastQC 0.11.9[103] and MultiQC 1.11[104] and afterwards cleaned up using Trimmomatic 0.39[105]. Adapters were trimmed from the raw reads and ends below an average accuracy of 99% over a five base pair sliding window were clipped. Finally, reads shorter than 20 bp were discarded. Resulting read pairs were mapped to the *Callosobruchus maculatus* genome[106] using HISAT2 2.2.1[107] with default settings for stranded libraries and sensitivity set to "--very-sensitive". Number of reads per gene was then determined using HTSeq 2.0.2[108] with default settings for stranded libraries. This resulted in 10-27M (heat stress), 13-30M (reproduction) and 9-35M (rearing temperature during experimental evolution) uniquely mapped reads per library going into the final downstream analyses.

Read count data was pre-processed and analysed in R 4.3.1[109] using edgeR[110,111]. For the heat shock response, only genes with at least 3 counts per million in at least 5 samples were further analysed, resulting in 10246 genes for final analysis. For the mating response, only genes with at least 1 count per million in at least 2 samples were further analysed, resulting in 11640 genes for final analysis. The last approach was also used for the genes expressed in the evolution regimes, resulting in 11292 genes for analysis. Counts were normalized using the 'Trimmed Mean of M-values' method via edgeR's NormLibSizes() function. Count data for the heat shock and mating response was further analysed with edgeR[110,111] and limma[112] using linear models on the normalized log2 transformed counts per million to determine differentially expressed genes. All analyses used a false discovery cut-off of 5 %.

For reproductive genes, effect sizes (i.e. Log-fold changes) were averaged across the three male selection regimes. However, we only considered genes that were significantly differentially expressed in all three separate contrasts between virgin females and females mated to males from the three mating regimes. For heat stress genes, effect sizes and significance was calculated by averaging across the two time-points. Significant genes were

further subject to gene ontology analysis, using the R package GOstats (2.66.0)[113] and the HyperGTest function. Results were visualised using clusterProfiler (4.10)[114].

To provide a powerful, yet, unbiased estimate of temperature-dependent allocation between reproduction (mating response) and maintenance (heat stress response) in the experimental evolution lines, we focused on the 134 genes that displayed significant antagonistic differential expression patterns in response to heat shock and mating (Fig. 3B). We first multiplied the estimated log fold changes of the antagonistic genes for both mating and heat shock responses by the vector of normalized read counts of the same 134 antagonistic genes found for each of the 54 RNA libraries from the experimental evolution lines. The resulting values were then summed to compute a score for the heat stress and reproduction response separately. As expected, the scores for heat stress and reproduction, based on only the antagonistic genes, were tightly negatively correlated (r = -0.999) across all combinations of evolved lines and assay temperatures and fell along a singular linear trade-off axis (Supplementary Fig. 2). The response along the allocation trade-off for each sample was taken by projecting samples along this axis (quantified as the first eigenvector of the data). We repeated the same analyses using all differentially expressed genes in response to heat shock (765) and mating (1269) with qualitatively similar results (Suppl. Tables 2d, e).

Life-history traits

We quantified thermal adaptation in female life-history by measuring three core traits: lifetime reproductive success (LRS), juvenile development time and adult body mass, and four rate-dependent traits: early fecundity, weight loss, water loss, and mass-specific metabolic rate (ml CO^2/mass/min) over the first 16h of female reproduction. All life-history traits were collected at generations 40 for cold-adapted lines and 60 for hot-adapted lines, in a large common garden experiment including the two assay temperatures corresponding to the experimental evolution treatments (23°C and 35°C). Ancestral lines were scored in the same experimental conditions with the addition of the ancestral 29°C assay temperature, but on a later occasion following ca. 125 generations of experimental evolution. Note that the ancestors had been kept at the ancestral conditions, to which they had already adapted for more than 300 generations prior to the start of experimental evolution. It can therefore be assumed that the measured trait values correspond well with the trait values at the start of experimental evolution. To control for potential differences in the separate experiments on ancestors and evolved lines stemming from unknown sources, we reared an independent laboratory adapted reference population in both experiments. This indicated that differences in rearing had affected the life-history traits scored over the first 16h of reproduction. We therefore standardized the traits scored during respirometry of the three founding ancestors by this estimated amount (adult mass: increased

by 6.4%, metabolic rate: reduced by 12%; early fecundity: reduced by 18%; water loss: reduced by 15%, and weight loss: reduced by 25%) in order not to erroneously assign these differences to evolutionary divergence between ancestors and evolved lines. Note that this was done averaged across the three assay temperatures and geographic origins. Therefore, our approach to provide more accurate measures of evolutionary divergence between evolved lines and ancestors did not affect the estimated temperature-dependence of adaptation or the importance of geographic differences.

Before assays of life-history traits, non-genetic parental effects were removed by moving F0 grandparents of the assayed individuals into a common temperature of 29°C to lay eggs. The emerging beetles in the next (parental) F1 generation were allowed to mate and lay eggs on beans provided *ad libitum*. Following 48 hours of egg laying, the beans were split and assigned to one of the two (for ancestors, three) assay temperatures. The emerging adult F2 offspring were phenotyped for their life-history (**Fig. 4**). Newly emerged (0-48 hours old) virgin females were mated to males by placing three males and females together in a petri dish over night at the assay temperature. In the following morning, the three females were weighed for their body mass and then placed together inside a glass vial filled with black eyed beans to be measured for their metabolic rate, water loss and early fecundity at their designated assay temperature. The glass vials were placed in a Sable Systems (Las Vegas, NV, USA) high-throughput respirometry system. Briefly, the respirometry was set up in stop-flow mode, and CO^2 production and water-loss was measured for up to 23 vials on a given experimental day. The first vial was left empty and served as a baseline to control for any drift of the gas analysers during each session. Vials were measured over 17 cycles, each of a length of 57.5 minutes. Mean metabolic rate and water loss for each vial was calculated across cycles 2-17, with the readings from the first cycle discarded (as it contains human-produced water and CO_2). After respirometry, females were weighed again to record their weight loss and beans with eggs were isolated and counted to record early fecundity. In total we followed 386 triads of females for the evolved lines and another 115 triads from their ancestors.

From the same rearing we measured egg-to-adult development time for two technical replicates per line and assay temperature, each consisting of 40-120 individuals. We calculated a mean development time per technical replicate and used this in analysis. We also collected virgin males and females and placed three males and three females together in a petri dish with ad libitum beans to record lifetime reproductive output (LRS) at each assay temperature. In total we recorded LRS for 258 couple triplets for evolved lines, and another 115 couple triplets for the ancestors. These data were complemented with additional data from both evolved and ancestral lines reared in a common garden design in two consecutive years

(corresponding to generation 120/135 for ancestors, 115/130 for hot-adapted lines, and 80/90 for cold-adapted lines). In these rearings, a single male and female were put together in a petri dish with ad libitum host seeds, with otherwise the same conditions. For ancestors we scored 396 couples, and for evolved lines 789 couples, across both experimental years. LRS was analysed per female, hence we divided all offspring counts from female triads by three before analysis.

We carried out all statistical analyses using the statistical and programming software R (v. 3.6.1). All seven traits were first analysed separately using linear mixed effect models available in the lme4 package[115]. When analysing differences between hot- and cold-adapted lines, evolution regime, geographic origin and assay temperature were added as fully crossed fixed effects. Line replicate crossed with assay temperature and nested within geographic origin were added as random terms to assure correct level of replication when estimating significance of fixed terms including evolution regime. For analyses on metabolic rate, water loss and weight loss, the date of the respirometry run was added as an additional random effect. Metabolic rate and weight loss were corrected for body mass of the measured female triad by taking an average of the weight measures before and after respirometry and adding it as a covariate in analysis. For water loss measured from evolved lines, measures from vial 2 (the first vial in the measuring sequence containing beetles) and measurements from three entire experimental days were discarded following an outlier analysis as these measures were magnitudes greater than other measures and clearly represented water vapour from other sources than the beetles. For LRS, experiment (triads and two separate rearings of single couples) and its interaction with assay temperature were added as additional fixed effects to account for possible block effects. The data from ancestral lines were analyzed in similar fashion but without the fixed effect of evolution regime and random effect of population replicate.

P-values were calculated using the car package and type II sums of squares with the Kenward-Roger approximation for the degrees of freedom. To analyse evolutionary responses while taking all traits into consideration at once, we also performed a non-parametric MANOVA using residual randomization[65] with the same structure for fixed and random effects (**Fig. 4H**).

The agricultural footprint

We set up both evolved and ancestral lines in a common garden experiment including the 23, 29 and 35°C assay temperature, following approximately 85, 120 and 125 generations of experimental evolution for cold-adapted, hot-adapted and ancestral lines, respectively. We removed parental effects by moving all lines to 29°C two generations prior to the start of the

experiment. Newly laid F2 eggs were split among the three assay temperatures and resulting virgin adults were collected. Three males and three females were placed together in petri-dishes with ad libitum *V. unguiculata* seeds for 24h at 29°C and 35°C, and 34h at 23°C (as reproductive rate is slower at cold temperature; **Fig. 4B**). Three dishes were prepared per line and assay temperature. Note that the experiment was aimed at linking life-history variation to host consumption, and the sample size was low for detecting more fine-scaled differences between evolution regimes due to the imprecise measures of fecundity (6-9 females per line and temperature measured over 24-34h), which were much better estimated in the previous experiments (~80 females per line and temperature across the entire adult stage).

To quantify the amount of host seed consumed by beetles, each petri dish with beans was weighed prior to the addition of beetles, to record a starting weight of each assay. After beetles had emerged and been removed, assays were placed in standard conditions for 6 weeks during which they were weighed on several occasions to record the weight of the infested beans and to check for potential inconsistencies and time-dependencies in estimates of bean consumption. However, the measures of the beans' weight loss following infestation were highly repeatable (r^2 = 94%) and we chose the final (fourth) measure to calculate our estimate of host consumption. To account for potential weight loss of beans that was independent of the beetle infestation, we also set up 5 assays without beetles at each temperature and measured them in the exact same way. The host consumption was then corrected based on the weight changes in these control assays; this correction was very small and only corresponded to 1-2% of the total bean weight. For each assay, we also recorded the number of hatched eggs laid on beans (i.e. fecundity), the number of those that resulted in the adult pupating (i.e. juvenile survival), and the mean body mass of emerged adult beetles.

Differences in food consumption were first analyzed using linear mixed effect models in the lme4 package with the same structure for fixed and random terms as described for life-history traits. We excluded the ancestors from formal analysis since these had only half the samples compared to the evolution regimes, but we included them in **Fig. 5A** to show the direction of evolution of host consumption. We sequentially added information on fecundity, juvenile survival, and mean body mass for each assay to explore how life-history traits accounted for host consumption. We finally fitted a linear model including only the three traits (removing the fixed terms of assay temperature, evolution regime and geographic background) to estimate how much of the variation in host consumption that could be predicted by life-history alone.

Motivated by the observation that the life-history traits accounted for more than 93% of the observed variation in host consumption (see **Fig. 5A** and[66]), we calculated the two independent

measures of the agricultural footprint (see main text) from our measured life-history traits in the main experiment, as this data was estimated with high accuracy for all lines. To provide 95% confidence limits and P-values, we performed parametric bootstrap on all calculations using the means and standard errors for each trait per line and assay temperature derived from the univariate mixed models (**Fig. 4A-G, Suppl. 3**).

Acknowledgements

We thank Johanna Liljestrand-Rönn, Elina Immonen and Göran Arnqvist for help in the lab as well as for helpful input on our study design and results. Sequencing was performed by the SNP&SEQ Technology Platform in Uppsala. The facility is part of the National Genomics Infrastructure (NGI) Sweden and Science for Life Laboratory. The SNP&SEQ Platform is also supported by the Swedish Research Council and the Knut and Alice Wallenberg Foundation. This work was supported by grant no. 2019-05023 from the Swedish Research Council (VR), grant no. 2022-01117 from Formas, and grant no. CTS22:2101 from Carl Tryggers Stiftelse, to DB.

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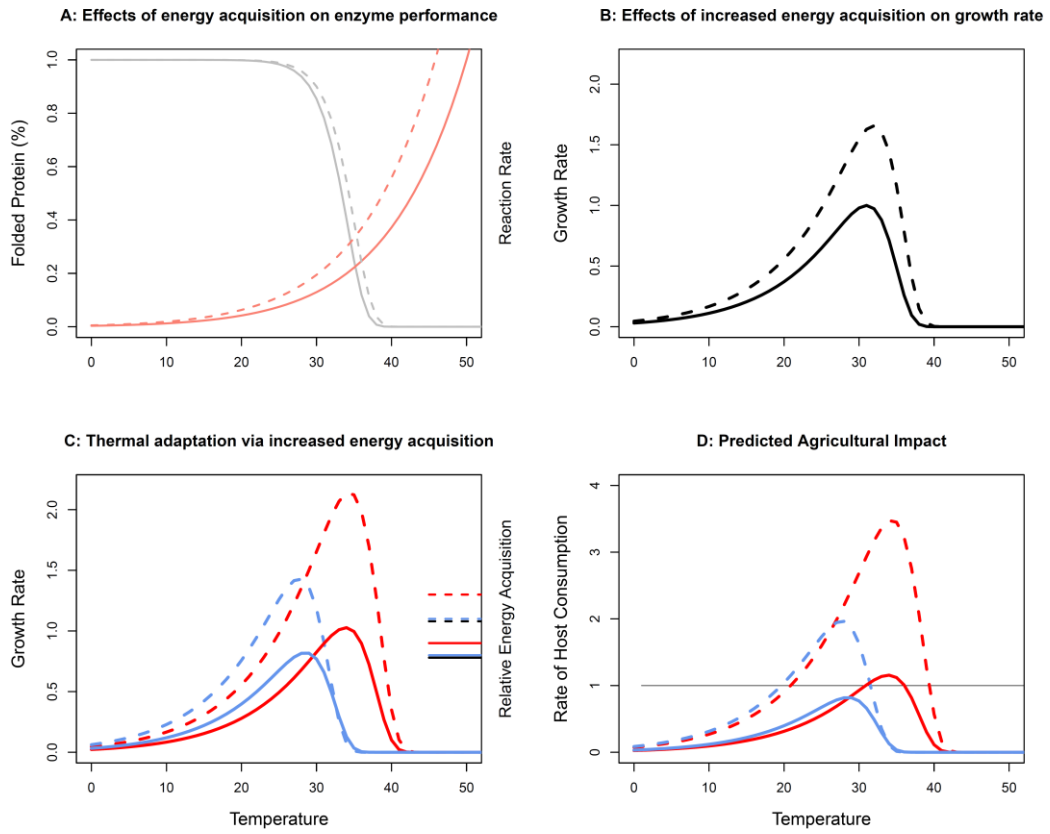
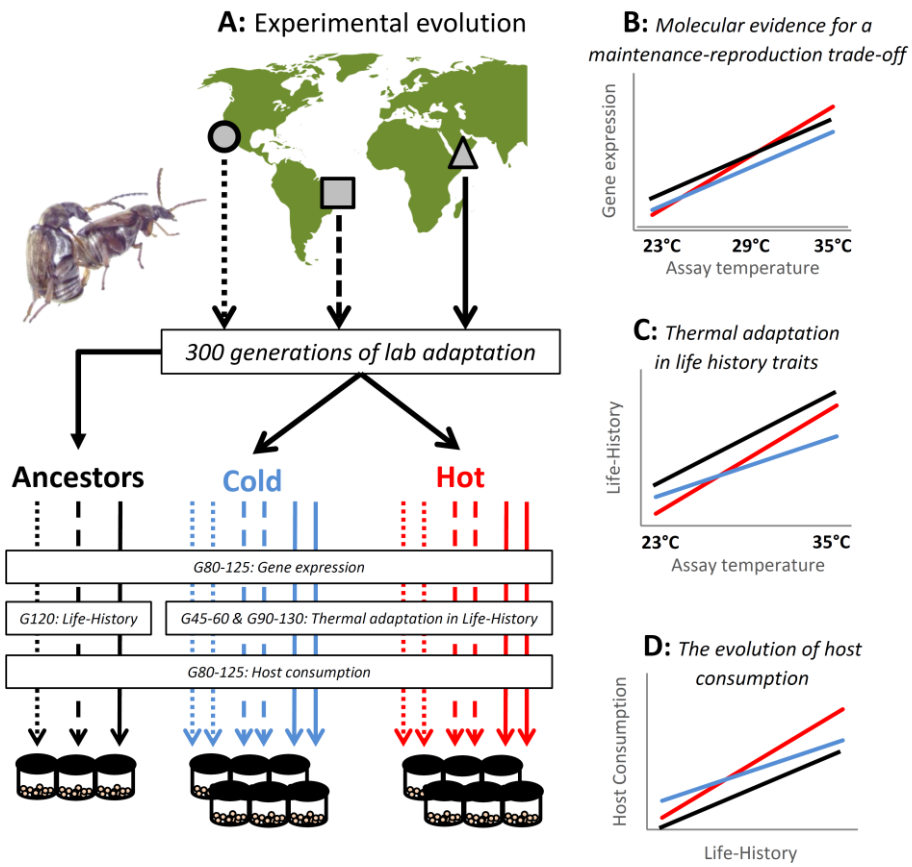


Figure 1: Thermal adaptation via compensatory energy acquisition can increase pest species' impact on agriculture.
Shown are predictions based on protein kinetics (details in Supplementary 1). **A**) Temperature increases enzymatic reaction rates[20,51] (pink lines) while too hot temperatures lead to (reversable) protein unfolding (grey lines), leaving fewer enzymes available to do work[25,26]. Reduced costs of feeding (broken lines) leads to more body reserves acquired (*M*), of which a fraction (*p*) can be devoted to increased production of catalytic enzymes that speed up constrained reaction rates at cold temperature[20], and another fraction (*q*) that can be devoted to molecular chaperones that stabilize proteins at hot temperature[31]. **B**) The resulting growth rate is the product of the remaining body reserves that can be devoted to growth and reproduction, *M* (1-*p*-*q*), the reaction rate, and the proportion of properly folded protein. **C**) The resulting growth rates for populations adapting to cold (23°C; blue) and hot (35°C; red) temperature via compensatory responses (increased *M*, and allocation to *p* and *q*) are shown for high (full lines) and low (broken lines) costs of feeding. While the cold-adapted population outcompetes the hot-adapted population at cold temperature, growth rates are greater at warm temperatures and for the hot-adapted population due to thermodynamic constraints on reaction rates[23,51]. Horizontal lines on the right-hand y-axis show *M* at the optimal strategy; lower costs of feeding favour increased energy acquisition, which improves thermal performance and increases thermal niche breadth. In the depicted scenario, increased energy acquisition is particularly beneficial at hot temperature that puts increased demands on cellular maintenance to ensure protein stability. **D)** The possibility to evolve thermal performance via compensatory energy acquisition leads to an even greater agricultural impact (the product of *M* and population growth rate; compare difference between full and hatched lines in panels **C** and **D**). All panels show responses relative to the maximum value for ancestors reared at benign 29°C at high costs of feeding (black full line in panel B).



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965 **Figure 2: Experimental evolution of thermal niches and life history in seed beetles.**
966 Dryland legumes are nutritionally rich, drought tolerant and resilient to harsh weather[11,116] and
967 provide subsistence for more than half a billion people in the driest regions of the world[11].
968 However, these crops suffer substantial losses from seed beetles[116]. To investigate how
969 genetic adaptation to climate change might shape the impact of seed beetles on legume crops,
970 we performed large-scale phenotyping of long-term experimental evolution lines of *C.*
971 *maculatus*. **A**) Populations from three geographic locations (Brazil, California and Yemen)
972 were sampled and allowed to adapt to common laboratory conditions for ca. 300 generations
973 before experimental evolution. Four replicate populations were created per genetic
974 background and split between the two evolution regimes (23°C and 35°C), while the original
975 ancestor remained at the benign temperature (29°C). Experimental evolution proceeded over
976 90 and 130 generations for the Cold (blue) and Hot (red) adapted populations. During this time,
977 the ancestors went through 130 generations of evolution. **B**) To provide molecular evidence
978 for a temperature-dependent trade-off between maintenance and reproduction, gene
979 expression was measured in both evolved and ancestral lines after 80-125 generations of
980 evolution. **C**) After 45 (Cold adapted) and 60 (Hot adapted) generations, life-history adaptation
981 was assessed in a common garden experiment. This data was complemented in generations
982 90 (Cold lines) and 130 (Hot Lines and Ancestors). Life-history traits were measured in the
983 three ancestors after 120 generations of evolution. **D**) Following 80-125 generations of
984 experimental evolution, the host consumption of all lines and ancestors was scored in a
985 common garden to assess how life-history evolution under simulated climate change had
986 shaped the agricultural footprint left by *C. maculatus*.

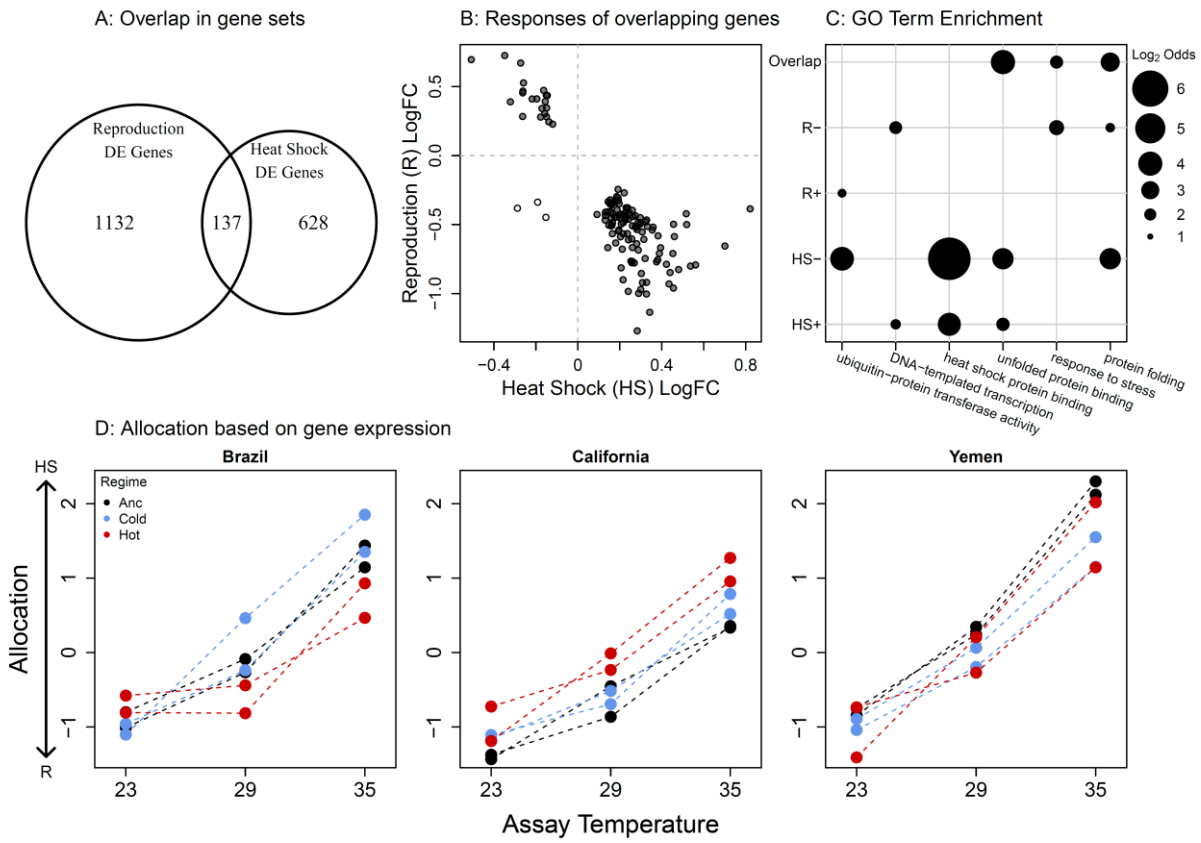

Figure 3. Molecular evidence for a temperature-dependent trade-off between maintenance and reproduction.
A) Differentially expressed genes in response to heat shock (HS) and mating (R); 137 genes responded to both treatments, which was more than expected by chance. **B**) Of these genes, all but three showed antagonistic responses to the two treatments, signifying a trade-off between investment in reproduction and maintenance under heat stress. **C)** GO term enrichments for genes upregulated (+) or downregulated (-) in response to mating (R) and heat stress (HS), as well as for the overlapping antagonistic genes. **D)** The expression of the 134 antagonistic genes in the experimental evolution lines and ancestors indicate a clear increase in allocation towards cellular maintenance at the cost of reduced reproduction when beetles were reared at 35°C (the thermal regime of hot-adapted lines). However, there were no consistent differences in expression between evolution regimes.

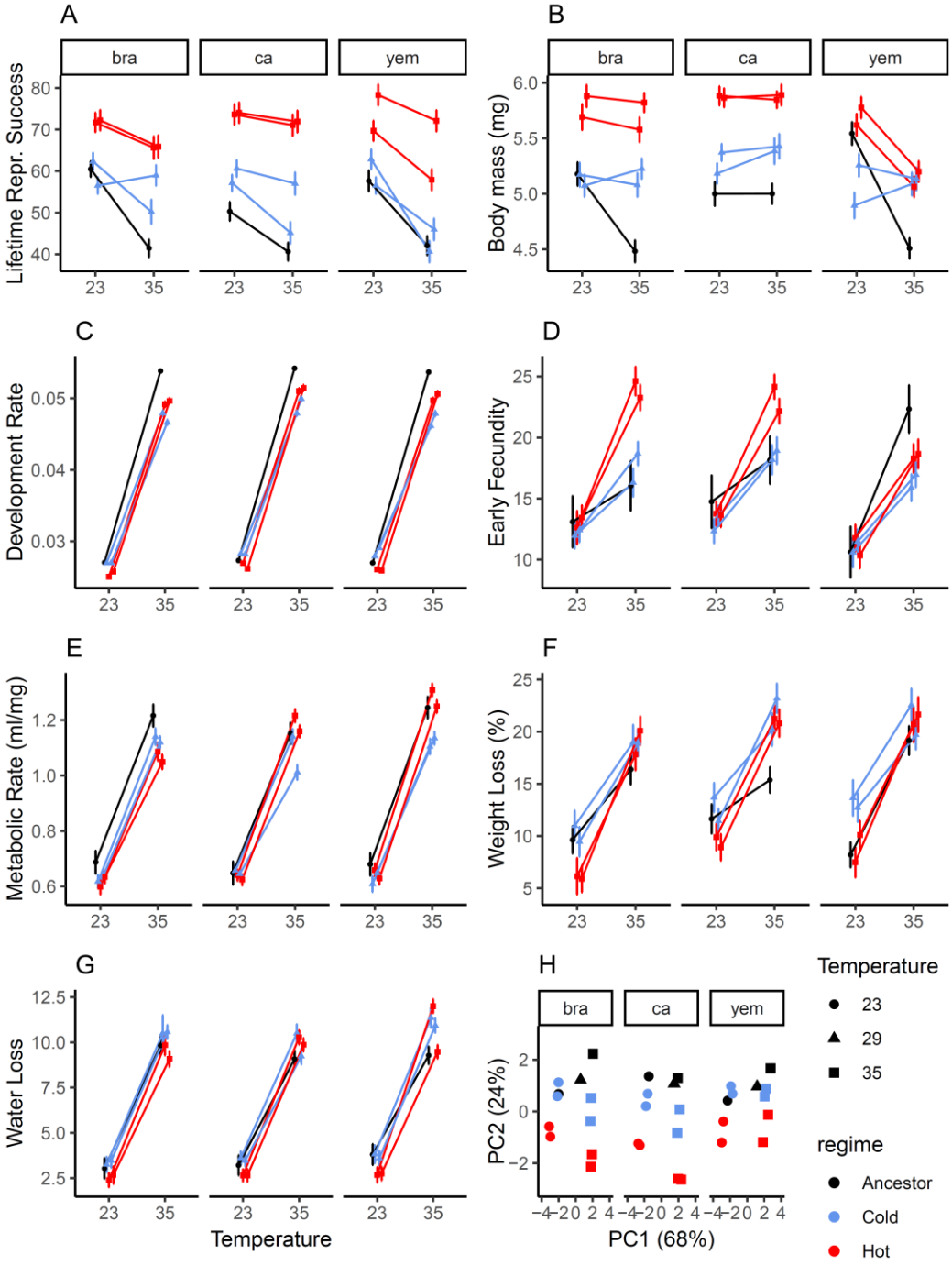

Figure 4. Thermal adaptation in life-history traits.
Single traits in (**A-G**) for Hot (red) and Cold (blue) adapted lines, as well as their Ancestors (black). Development time is presented as a rate (1/development time in days) for consistency with other traits. Means ± 1SE. **H**) Ordination using principal component analysis showing that multivariate divergence occurs along both a temperature-sensitive axis (PC1) and along a general life-history axis describing overall variation in body mass and LRS (PC2), with more divergence from the ancestor in Hot-adapted lines.

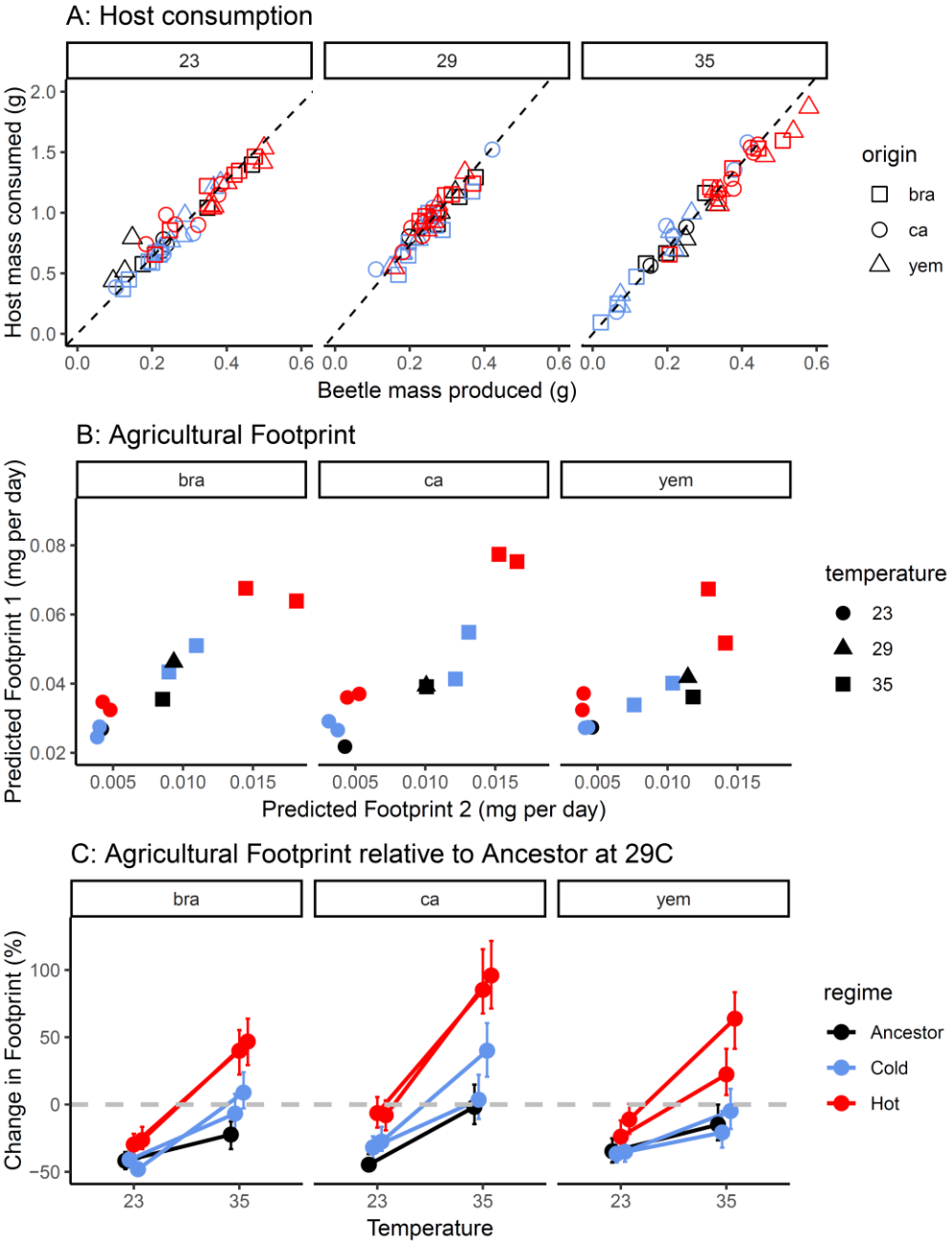

Figure 5: Temperature-dependent evolution of the agricultural footprint.
A) The relationship between beetle mass produced and host bean consumed. Each assay is represented by an individual datapoint. The slopes of the dashed lines correspond to the temperature-specific constant (C_T) giving the amount of host consumed per beetle produced (23°C: 3.17, 29°C: 3.66, 35°C: 3.55). **B**) The two independent metrics used to predict the agricultural footprint. **C**) The predicted footprint of each line (using the first metric) relative to respective ancestor raised at 29°C (grey hatched line). Means ± 95% CIs. The effect of life-history adaptation at cold temperature is small, but life-history adaptation at hot temperature almost doubles the agricultural footprint relative to the ancestral impact.