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¿CÓMO AFECTARÁ EL CAMBIO CLIMÁTICO LAS COMMUNIDADES DE INSECTOS?

IMPACTO EN LOS SISTEMAS TRITRÓFICOS, COEVOLUCIÓN DIFUSA

Y PROBABILIDAD DE BROTE DE PLAGAS

TESIS

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« Science sans conscience n'est que ruine de l'âme »

François Rabelais (1483/1494 – 1532)

« Faire quelque-chose d'utile avec ce que les gens fichent en l'air »

Ma grand-mère Aline Schneider

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Contents

Resumen

En nuestro mundo en constante cambio, uno debe ser capaz de anticipar lo que podría suceder después. Según las peores predicciones de los modelos climáticos más recientes, durante los próximos 80 años estamos esperando un calentamiento global de 6ºC debido al incremento casi triplicado de la concentración atmosférica de CO_2. Por lo tanto, suponemos alteraciones drásticas en los ecosistemas y en los organismos, particularmente en los animales ectotermos. Con respecto al interés humano, los cultivos que nos alimentan son vulnerados por los organismos más diversos y eficientes que hay de tipo ectotérmico: los insectos. En consecuencia, es primordial de generar un conocimiento de la respuesta de los insectos al cambio climático actual y futuro.

En esta tesis, estudié el impacto de las condiciones del cambio climático sobre una plaga del frijol de distribución mundial: los escarabajos brúquidos. Empecé explorando los patrones de respuestas demográficas y morfológicas de estos insectos expuestos a un gradiente altitudinal que sirvió como un proxy térmico. Encontré que este gradiente de elevación/temperatura explicaba parcialmente las densidades de las poblaciones observadas, así como el tamaño de los individuos.

Con esta información en mente, pude diseñar una simulación experimental multigeneracional de las condiciones climáticas del año 2100 y monitoreé el tamaño, las proteínas totales, los lípidos totales, la fecundidad, y la tasa de sobrevivencia de desarrollo de huevo a adulto, a lo largo de 10 generaciones. Adicionalmente, evalué qué tanto la variación que estaba registrando podría ser atribuida a la plasticidad fenotípica o a una adaptación genética, usando un experimento de trasplante recíproco al final de la simulación. Los resultados demostraron que, a pesar de no haber observado una adaptación genética formal en todos los rasgos medidos, puedo afirmar prudentemente que la simulación de las condiciones atmosféricas de 2100 provocó un aumento y disminución del contenido de proteínas y de las reservas de lípidos de los brúquidos, respectivamente.

Además, la sobrevivencia incrementó aún cuando la fecundidad aumentó, lo cual es preocupante si reintroducimos esta información en el contexto del manejo de cultivos y almacenamiento de productos. Al fin de esta disertación, discuto la necesidad de estudiar las modalidades de la adaptación y la rápida evolución de las plagas en las condiciones del cambio climático.

Abstract

In our constantly changing world, one must be able to anticipate what might come next. In the next 80 years, the global climate is expected to warm up 6ºC due to a nearly three-fold increase of atmospheric CO_2, according to worst recent model predictions scenario. Therefore, we should expect drastic alterations in ecosystems and organisms as well, especially in ectotherm organisms. Regarding human interest, the crops feeding us are vulnerable to the most diverse and efficient ectotherm kind: the insects. Hence, the necessity of generating knowledge about insect response to current and future climate change conditions. In this thesis, I studied the impact under climate change conditions of a worldwide bean crop pest insect: the bruchid beetles. I started exploring the patterns of demographic and morphological response of these beetles to an altitudinal gradient that served as a thermal proxy. I found that the elevation/thermal gradient partially explained the observed population densities as well as individual body size. With this information in mind, I designed a multigenerational experimental simulation of climate change conditions in 2100 and monitored size, total protein, total lipids, fecundity, and egg to adult development survival during 10 generations. Additionally, I assessed the whether the variation I was recording could be attributed to phenotypic plasticity or genetic adaptation by performing a reciprocal transplant experiment at the end of the simulation. The results showed that despite not observing a formal genetic adaptation on all measured traits, I could safely affirm that the simulated atmospheric conditions of 2100 provoked and increase and decrease of protein content and lipid storage, respectively. Moreover, survival increased, even as fecundity raised which is concerning when this information is returned to the crop management and grain storage context. At the end of this dissertation, I discuss the necessity of studying the modalities of adaptation and the rapid evolution of pests under climate change conditions.

Introducción

El cambio climático contemporáneo está alterando los ecosistemas a un ritmo y escala sin precedente. Ciertamente, el aumento de la temperatura y concentración atmosférica de CO_2 tiene el potencial de modificar las distribuciones e interacciones de las especies, sus historias de vida y la conducta relacionada a la temperatura (Larson et al., 2019).

Esto aplica en particular a los insectos, debido a que la mayoría de sus historias de vida está literalmente bajo la influencia de la estacionalidad. El desempeño, la actividad y reproducción de los insectos están sincronizados con las fluctuaciones estacionales de luz, agua y disponibilidad de los recursos (Masaki, 1967; Tauber & Tauber, 1981).

Al principio de este proyecto, el peor escenario de predicción para el 2100 pronosticaba un aumento de 6°C principalmente asociado a un incremento mundial de la concentración atmosférica de CO_2 de los actuales 470ppm a 1000ppm(IPCC, 2014). De hecho, utilicé estos valores durante toda la tesis. De vuelta en los 80's, los primeros modelos climáticos ya predecían un aumento de 4.5°C y 850ppm [CO_2]. Un modelo en particular estaba sugiriendo una elevación de 1°C en 2020 en comparación con 1960, según un escenario moderadamente pesimista (Hansen et al., 1981).

Desafortunadamente, las predicciones fueron correctas. Nuevos modelos pronostican 7°C de aumento en 2100 (Eyring et al., 2016), y aparte del hecho de que sea extremamente preocupante, eso afectará ciertamente la dinámica de la biósfera a través de los efectos en los individuos y sus interacciones intra/interespecíficas. Por consiguiente, parece crucial generar conocimiento y datos relacionados a los efectos del cambio climático sobre los insectos, dada la condición ectodérmica de todos los Hexápodos y de sus impactos potenciales sobre la salud humana, la economía, y la predominancia que pueden ejercer en términos de diversidad y biomasa. Las plagas de insectos

reducen sustancialmente los rendimientos de cultivos, pudiendo también atacar los granos almacenados (Pimbert, 1985).

La literatura actual está dotada de estudios, que han modelado las relaciones entre la temperatura, el crecimiento poblacional y las tasas metabólicas de los insectos, permitiendo estimar cómo y en dónde el clima caliente estará aumentando las pérdidas de cultivos y/o rendimientos por los insectos (Zvereva & Kozlov, 2006; Deutsch et al., 2008). Se proyecta que las pérdidas de rendimiento al nivel mundial subirán de 10 a 25% por grado de calentamiento global en superficie (Battisti & Naylor, 2009; Liu et al., 2013). Además, los cultivos se perderán más en las zonas donde el calentamiento y la higrometría alterada aumenten el crecimiento poblacional y la tasa metabólica de los insectos (Deutsch et al., 2018).

Además de estas consecuencias para los humanos, los insectos, como parte de los ecosistemas, provocarán y soportarán las alteraciones, dado su rol omnipresente e inevitable en las biocenosis. Los cambios de temperatura podrían afectar los diferentes componentes biológicos de un sistema: las plantas, los herbívoros, sus enemigos naturales (parasitoides, depredadores y patógenos), así como también los niveles tróficos más altos (superdepredadores e hiperparasitoides). El cambio climático tiene un impacto de corto y largo plazo en los organismos y comunidades. Los de corto plazo involucran los efectos directos de la temperatura en los diferentes rasgos de historia de vida, como el tiempo de desarrollo, la tasa de sobrevivencia, la tasa metabólica, y hasta la asignación del sexo (van Baaren et al., 2010; Denis et al., 2012). La distribución de las especies y la sincronización de los fenómenos recurrentes entre niveles tróficos también son consecuencias directas de las alteraciones climáticas(Voigt et al., 2003; Both et al., 2009; Kharouba et al., 2018). Los efectos a largo plazos involucran cambios genéticos en las poblaciones que se asocian con adaptaciones al clima. En el caso de los insectos podría ocurrir a una velocidad suficiente como para ser detectada (Hoffmann, 2010; Kellermann & van Heerwaarden, 2019).

Un gran modelo biológico para evaluar los impactos de las alteraciones climáticas en el campo en condiciones de laboratorio son las interacciones entre plantas de frijol (*Phaseolus spp*.), los gorgojos del frijol (subfamilia Bruchinae) y sus avispas parasitoides (conjunto de Ichneumonoidae y Chacidoidae). Este sistema es extremadamente conveniente, ya que diferentes especies en distintos niveles tróficos pueden evaluarse experimentalmente e intercambiarse basándose en cuestionamientos científicos. Lo más importante es que los gorgojos del frijol, en particular *Acanthoscelide sobtectus* y *Zabrotes subfasciatus* (ambos Coleoptera: Chrysomelidae) son responsables de pérdidas sustanciales de cultivos y productos almacenados, a nivel de pequeñas comunidades humanas, así como a nivel agroindustrial a gran escala(Pimbert, 1985; Espinal et al., 2004).

El objetivo principal de esta tesis fue evaluar los impactos del cambio climático en un insecto plaga. Los objetivos especificos fueron (i) estudiar la influencia de factores bióticos y abióticos sobre los escarabajos brúquidos en la naturaleza, utilizando un gradiente geográfico como un proxy real del cambio climático; y, (ii) realizar un estudio de simulación multigeneracional de las condiciones atmosféricas pronosticadas para 2100 en el gorgojo mexicano del frijol, con el fin de detectar cambios genéticos y alteraciones fisiológicas / morfológicas asociadas. Las hipótesis generales de este trabajo son que: a) los efectos del cambio climático alterarán más intensamente a los escarabajos brúquidos si son especies nativas y adaptadas localmente o si son plagas recién llegadas; y b) el aumento de la temperatura y los niveles de CO_2 deberían generar adaptaciones genéticas detectables en una ventana de tiempo de 10 generaciones. Mis objetivos específicos e hipótesis fueron publicadas en las revistas *Journal of Agricultural and Urban Entomology* y *Ecology and Evolution*, cuyos pdfs se presentan a continuación. Finalmente, presento un último documento, en forma de apéndice, el cual contiene algunas innovaciones metodológicas en el análisis de los rasgos bioquímicos de mis muestras. Este documento se sometió a la revista Entomologia Experimentalis et Applicata.

Capítulo 1: La altitud, la temperatura y la presión de los parasitoides pueden prevenir la competencia entre dos especies de gorgojos atacando al frijol silvestre *Phaseolus vulgaris*

Altitude, temperature, and parasitoid pressure may prevent competition between two Mexican bruchid beetles attacking wild *Phaseolus vulgaris*[1]

David Schneider and Alex Córdoba-Aguilar

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ABSTRACT *Acanthoscelides obtectus* (Say) and *Acanthoscelides obvelatus* (Bridwell) (Coleoptera: Bruchidae) are two sympatric beetle species that infest bean seeds (*Phaseolus vulgaris* L.; Fabaceae). Using field-sampled wild *P. vulgaris* pods and data of population density, body size, and parasitoid pressure for both species across elevation and temperature gradients in the Mexican Altiplano, we explored whether interspecific competition occurs between the two bruchids. We expected that population density, body size, and parasitoid pressure of *A. obtectus* and *A. obvelatus* to be inversely related to one another. We found that population densities of the two species differed among the elevations, but their body sizes were independent of expected density patterns. Moreover, the parasitoid emergence rate was correlated to *A. obvelatus* abundance but not *A. obtectus* abundance. Our data suggest that niches of *A. obtectus* and *A. obvelatus* overlap only to such extent that interspecific competition seems unlikely due to a) alternative hosts are available for *A. obtectus*, and b) *P. vulgaris* seeds and alternative legumes are a fairly common resource in the studied area.

KEY WORDS *Acanthoscelides*, body size, competitive exclusion principle, geographical gradient, parasitoid, population density

Competitive exclusion principle states that competition will occur between two or more species sharing the same resources when the availability of these resources and/or demographic density reaches their respective carrying capacities (McPeek 2014). In natural ecosystems, ecological niche competition is generally the likeliest interactions between two sympatric species targeting the same resources (Dias and Rocha 2007, Ernst et al. 2011, Tarjuelo et al. 2017). Consequently, extinctions, exclusions, displacements, or specializations are the different possible results of such a scenario of competition (MacArthur & Levins 1964, MacArthur 1972, Holt et al. 1994).

Bruchid beetles are seed feeders found mostly on legumes. More than 300 species have been described, and about a quarter of them belong to the genus *Acanthoscelides* (Johnson 1983, 1990, Johnson & Siemens 1995). Two sibling species, *Acanthoscelides obtectus* (Say) and *Acanthoscelides obvelatus* (Bridwell), are strongly associated with their host plant *Phaseolus vulgaris* L. (Fabaceae).

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Female beetles drop their eggs inside the mature and dry bean pod. When the larvae hatch, they bore into the seeds and start consuming the nutritious tissues. An adult emerges the seed's testa 30 to 50 d later and is ready to disperse. Both species are sympatric and can be encountered as close to each other as on the same plant, inside the same pod, or even inside the same seed.

Acanthoscelides obvelatus is a univoltine species native to the Mexican Altiplano, whereas *A. obtectus* originates from the Peruvian mountains (Alvarez et al. 2005a) and is multivoltine. Both beetles are considered pests, with *A. obtectus* being economically important for stored products (Baier & Webster 1992, Paul et al. 2009). However, the evolutionary history of both species is different, as they come from distinct geographical regions. Their coexistence in Mexican populations of wild and cultivated *P. vulgaris* has been reported as a consequence of invasion by *A. obtectus* following domestication of beans and ancient grain exchanges between South and Central America (Alvarez et al. 2005a, 2006).

A complex assemblage of hymenopteran parasitoids of *Acanthoscelides* larvae has been described from Braconidae, Eulophidae, Eurytomidae, Pteromalidae, Eupelmidae and other chalcidoid wasps. These parasitoids exert strong mortality pressure on both beetle species (Aebi et al. 2008). Hence, *A. obtectus* and *A. obvelatus* are expected to compete for bean seeds, and parasitoid pressure may influence such competition.

We hypothesized that population density and body size (as proxies of interspecific competition outcome variables) patterns of *A. obtectus* and *A. obvelatus* on their common host (wild *P. vulgaris*) would co-vary across elevation and temperature gradients. Moreover, we integrated parasitoid pressure in our analysis to estimate the potential impact of parasitism. We investigated this hypothesis by sampling the beetle populations along an altitudinal gradient across the Mexican Altiplano.

Materials and Methods

A minimum of 50 wild *P. vulgaris* pods for each of the 29 sites located in three central Mexico states were collected and shelled (Table 1). The sites were chosen according to elevation and environmental variation, such as sun exposure, vegetation, and distance to areas of human activity. All GPS coordinates were recorded using the field GPS device Garmin eTrex 20x (Garmin Ltd., Schaffhausen, Switzerland). Annual mean temperatures (BIO1) were extracted from WorldClim Global Climate Dataset (Hijmans et al. 2005).

Each site was sampled twice to capture a realistic sample of the species' community along the dry season (December to April, which is also the breeding season of both bruchid species and the fruiting season of *P. vulgaris*). Sampling was conducted 10–24 January 2014 and 1–9 March 2014. It was impossible to exclude the possibility that insects collected during the second sampling might be offspring of insects active during the first sampling period, but we believed that the probability was low and similar across all sites, and therefore, should not make a difference in the results. At each site, and for each sampling session, pods were chosen based on the presence of the dehiscence hole opening at the base of the pod (allowing entry or egg dropping of the adult female), as well as the hardness as the seeds. All fully closed pods and soft seeds were not included in the samples. The pods were

Table 1. Sample field locations, geographic coordinates, and altitude values.

Abbreviation	Site name	Latitude (N)	Longitude (W)	Altitude (m)
ALEPV	Tepeojuma, Puebla	18.759687	−98.450752	1564
ANG	San Simon de Guerrero, Estado de Mexico	19.027113	−99.991258	2121
CAN	Valle de Bravo, Estado de Mexico	19.183244	−100.121521	1851
CAVE	Temascaltepec, Estado de Mexico	19.034728	−100.042149	1969
CUEVA	Meyuca de Morelos, Estado de Mexico	18.860715	−99.777944	1888
CVC2–3	Los Ramos, Morelos	18.978968	−99.216275	1843
CVC4	Alarcón, Morelos	18.980563	−99.213324	1871
FAC	Mina de Agua, Estado de Mexico	19.016961	−100.030046	1965
GOUI	Oaxtepec, Morelos	18.911642	−98.994331	1279
HOPE	Oacalco, Morelos	18.938479	−99.043886	1353
HUM	Santa Catarina, Morelos	18.975941	−99.170070	1676
HUY	Hueyapan, Puebla	18.874020	−98.713974	2039
IXT	Ixtapan de la Sal, Estado de Mexico	18.867241	−99.667943	1856
JBSS	Tochimizolco, Puebla	18.877303	−98.600183	2159
KAR	Totoltepec de la Paz, Estado de Mexico	18.851861	−99.843690	1847
LILA	San Pedro, Morelos	18.990981	−99.114051	1860
MAL	Malinalco, Estado de Mexico	18.953325	−99.504476	1880
MARTH	Llano de las Casas, Estado de Mexico	18.823325	−99.782712	1804
PRES	Tecomátlan, Estado de Mexico	18.965876	−99.507043	2108
SJSA6	San Jose de los Laureles, Morelos	18.981695	−99.004819	1863
SUL2	Sultepec, Estado de Mexico	18.846700	−99.969319	2368
TECO	Chalchihuitepetl, Morelos	18.976478	−99.117044	1743
TEJ14	Tejupilco, Estado de Mexico	18.945207	−100.133964	1714
TEJS	Ricón de Ugarte, Estado de Mexico	18.931054	−100.150280	1391
TEM	Albarradas, Estado de Mexico	19.057581	−100.051710	1817
TENE1	Teneria, Estado de Mexico	18.967705	−100.079362	1773
VUL3	Huilotepec, Morelos	18.970738	−99.076617	1612
VUL5	Santiago Tepetlapa, Morelos	18.958158	−99.064841	1502
YAUS	Jacarandas, Morelos	18.916285	−99.041516	1227

hand-shelled, and the seeds were placed in plastic containers (7 cm diameter × 3 cm height) at room temperature (15–26°C) and local photoperiod (L:D) of 11:13 to 13:11 h. All seeds from each sampling site were separated in batches (10 g per container) for better control of collections and to minimize the probability of additional oviposition in the sampled seeds. Seeds damaged by curculionid beetles, as well as atrophied seeds, were discarded.

For a period of 60 d (during which all insects should have emerged), all insects emerged from the harvested seeds were isolated, identified (based on voucher specimens deposited at Universidad Nacional Autónoma de México (UNAM) Insect Collection and stored in 70% ethanol at 4°C. The parasitoids collected in this study were not identified. The seed containers were checked daily, and the insects were removed to minimize the probability of an insect ovipositing in the seeds inside the containers. The length (from the anterior end of pronotum to the posterior end of pygidium) and width (between the most distal part of the metacoxae) of each bruchid beetle was measured by digital photography and pixel based measurement using Image J (Schneider et al. 2012).

Simple scatter plots were used to show the pattern of insect density along the elevational gradient, temperature, and parasitoid density. When necessary, the

response variable was log-transformed to adjust for the normality of residuals to respect model assumptions. The variation in insect densities (numbers of insects per 10 g of seeds) as well as the body length along elevation gradient were tested using regression models. We used a quadratic term (elevation^2) in our models to optimize the fit of the response variables to elevation (Hoiss et al. 2012, Read et al. 2014, Slatyer and Schoville 2016, Rolhauser et al. 2018). We compared *F*-statistic values to determine whether the linear or quadratic model best accounted for variation in each of the tested response variables. All analysis were conducted in *R* (R Development Core Team 2014).

Results and Discussion

We collected a total of 16,953 insects emerging from a total of 4.2 kg of wild *P. vulgaris* seeds. We identified 10,399 bruchid beetles (97% were *Acanthoscelides* spp.), 3799 hymenopteran parasitoids, and 2755 other arthropods associated with *P. vulgaris*. Although we intended to sample to the greatest altitude range possible, we did not find any plant population that fulfilled our sampling requirement below 1227 m or above 2368 m. Additional challenges to sampling and collecting enough material were that the plant populations at the sampling sites had been destroyed before the second field sampling (fire, cutting, or pesticide spray). Insect densities varied greatly among sampled sites with 0 to 12.5 *A. obtectus* and 0 to 111.6 *A. obvelatus* emerged from 10 g of seeds.

Altitude, temperature, or parasitoid abundance did not explain *A. obtectus* population density distribution (altitude: $R^2 = 0.01, F_{2,38} = 0.22, P = 0.8$; temperature: $R^2 = 0.03, F_{2,38} = 0.78, P = 0.46$; parasitoid: $R^2 = 0.1, F_{2,38} = 2.18, P = 0.12$). On the other hand, density distribution of *A. obvelatus* was correlated with altitude ($R^2 = 0.688, F_{2,46} = 50.92, P < 0.0001$) and temperature ($R^2 = 0.438, F_{2,46} = 17.95, P < 0.0001$). Density of *A. obvelatus* reached a maximum around 1900 m before decreasing at higher altitudes (Fig. 1D). Temperature optimum was not as evident, but the data showed that the greatest density occurred at 19°C (Fig. 1E). Parasitoid abundance was positively correlated to *A. obvelatus* population density ($R^2 = 0.171, F_{1,46} = 50.92, P = 0.007$) (Fig. 1F).

Abiotic factors had significant influences on the body sizes of both bruchid species (Fig. 2). Body size of *A. obtectus* appeared to correlate negatively to altitude ($R^2 = 0.325, F_{1,25} = 12.05, P = 0.002$) (Fig. 2A) and positively to temperature ($R^2 = 0.301, F_{2,25} = 5.185, P = 0.013$) (Fig. 2B) but did not correlate with parasitoid abundance ($R^2 = -0.067, F_{2,24} = 0.1734, P = 0.842$) (Fig. 2C). Body size of *A. obvelatus* showed no correlation with altitude ($R^2 = 0.06, F_{1,38} = 2.65, P = 0.11$) (Fig. 2D) nor temperature ($R^2 = 0.14, F_{1,38} = 3.04, P = 0.06$) (Fig. 2E), but was negatively correlated with parasitoid density ($R^2 = 0.171, F_{1,38} = 7.856, P = 0.007$) (Fig. 2F).

Acanthoscelides obvelatus density showed a relation with elevation and temperature, which was not the case for *A. obtectus* (Fig. 1). This difference is unexpected but nonetheless relevant to the biology of both species and their recent evolutionary history. Distribution and lower genetic variability of *A. obtectus* has been explained by the numerous anthropogenic seed exchanges between local markets as well as its recent arrival to the Mexican Altiplano from South America about 7000 yr ago (Alvarez et al. 2005b). Such exchanges would enhance gene

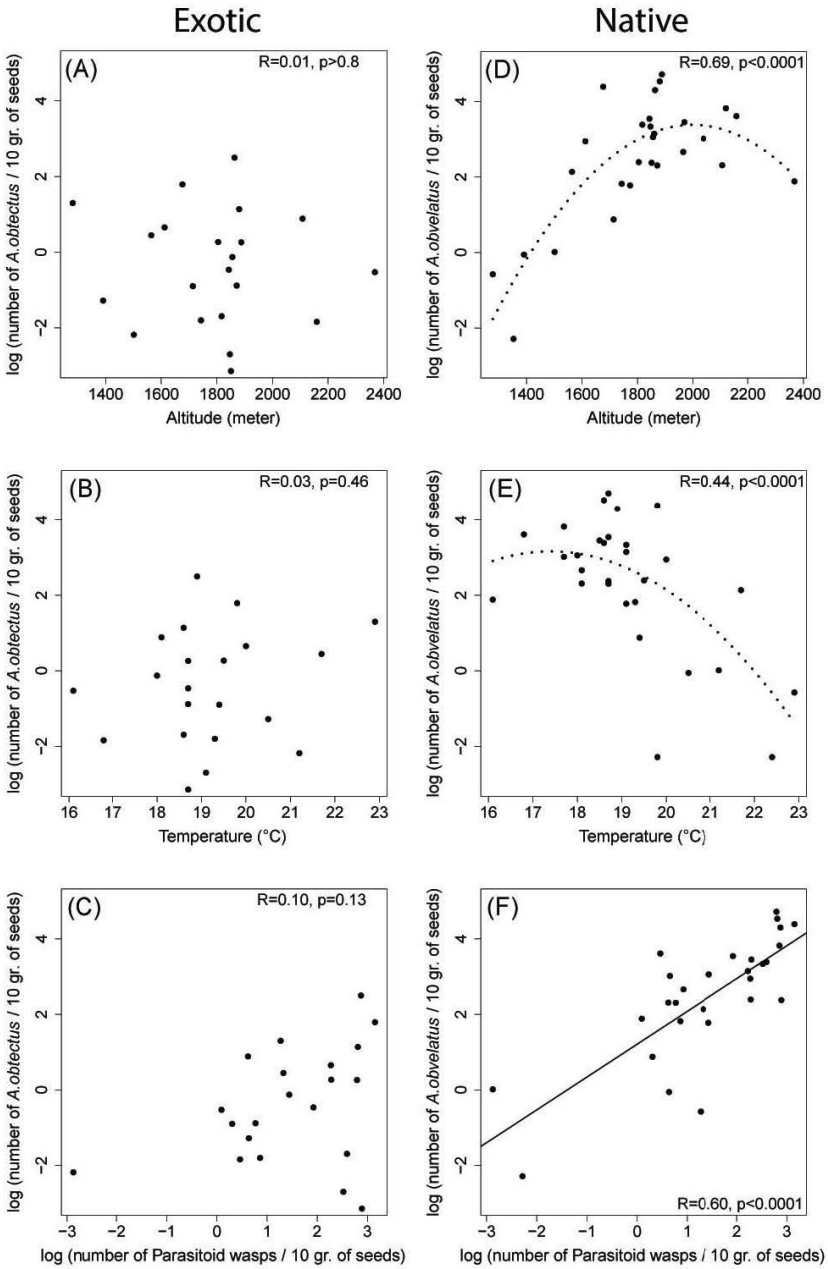

Fig. 1. Relationship between altitude, temperature, parasitoid abundance and the densities of *Acanthoscelides obtectus* (A–C) and *Acanthoscelides obvelatus* (D–F). Dashed lines show quadratic regression and solid lines show linear regression.

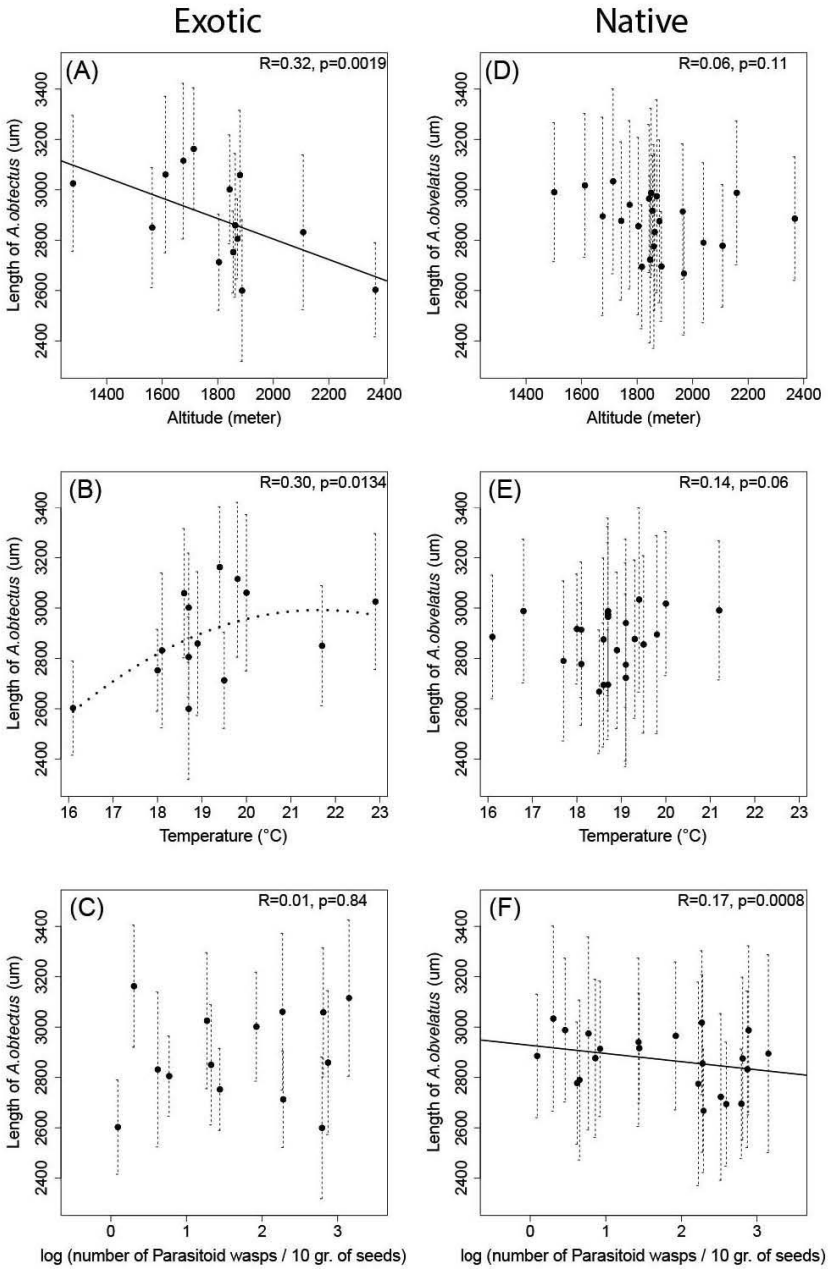

Fig. 2. Relationship between altitude, temperature, parasitoid abundance (C–F) and the body length (head to pygidium) of *Acanthoscelides obtectus* (A–C) and *Acanthoscelides obvelatus* (D–F). Dashed lines show quadratic regression and solid lines show linear regression.

flow and mitigate local adaptation leading to a reduced ability to exploit local resources by *A. obtectus*. The ability of *A. obtectus* to utilize other host plants when *P. vulgaris* is not available (Johnson & Siemens 1995) and its multivoltinism (*A. obvelatus* is univoltine) might also explain the absence of correlation between demographic density and elevation. In other words, polyphagous *A. obtectus* has the potential to avoid competition with *A. obvelatus* by simply ignoring wild plants if less chemically resistant (absence of phaseolins and cyanogenic compounds) and more nutritious plants are nearby. Beetle density can be easily influenced by factors such as host plant distribution and the polyphagous habit of *A. obtectus*; therefore, another proxy such as body length (a more suitable parameter for influence of plant quality on beetle development) may be needed to detect species competition and niche partition reliably and accurately. A performance trait such as body size would allow adjustment to wild *P. vulgaris* only.

Interestingly, the body size of *A. obvelatus* was not correlated with altitude or temperature, whereas the body size of *A. obtectus* was negatively affected by altitude and positively correlated to temperature (Fig. 2). Since the body size of *A. obtectus* restricts its distribution at high altitudes, this species is generally more common at lower altitudes and on cultivated beans (Aebi et al. 2008). Because our sampling focused on wild *P. vulgaris* only, we might have just observed a fraction of a larger picture. Interestingly, *A. obvelatus* has never been recorded from plant species other than beans of the *P. vulgaris* group (Alvarez et al. 2005b) and must be tightly connected to the spatial distribution of its host plant that is not particularly well suited for low altitude (Debouck et al. 1993) or high temperatures. Observed population density and body size patterns of *A. obvelatus* distributed along geographical and temperature gradients seemed consistent with its reported biology and ecology. Indeed, this univoltine beetle should be tightly attached to the geographic distribution of its primary host and seasonality, particularly because this species is specialized on few hosts such as the *P. vulgaris* group.

Our data suggested that *A. obvelatus* population density and body size distribution may also be explained by biotic factors, given its relationship with parasitoid density. On the one hand, this result may have implications for a situation of interspecific competition. The fact that both bruchid species experience parasitoid threats in distinct manners may reduce significantly the similarity of their niches. In this extent, it is known that parasitoids limit their hosts at a level which resource competition becomes virtually insignificant (Holt & Lawton 1993). Further studies should look at this potential using the study system we describe here. There are implications for the ecology of *A. obvelatus*. It is more beneficial for the parasitoids to utilize a stable and predictable host (Ringel et al. 1998), such as *A. obvelatus*, than constantly evolve to utilize new invasive bruchid species, such as *A. obtectus*.

Alvarez et al. (2006) showed that *A. obvelatus* originated from Central America, whereas *A. obtectus* originated from the Andean mountains and reached the Mexican Altiplano through ancient human exchanges of early domesticated beans. Therefore, *A. obtectus* can be considered as an alien species even though its introduction in Central Mexico is several thousand years old. Despite that both beetles have been sharing resources for many years, it seems that their niches overlap. Two explanations for this are that alternative hosts are available for *A. obtectus*, and even when such alternative hosts are not present, *P. vulgaris* seeds are fairly common.

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ORIGINAL RESEARCH

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Multigenerational experimental simulation of climate change on an economically important insect pest

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Abstract

Long-term multigenerational experimental simulations of climate change on insect pests of economically and socially important crops are crucial to anticipate challenges for feeding humanity in the not-so-far future. Mexican bean weevil *Zabrotes subfasciatus*, is a worldwide pest that attacks the common bean *Phaseolus vulgaris* seeds, in crops and storage. We designed a long term (i.e., over 10 generations), experimental simulation of climate change by increasing temperature and CO_2 air concentration in controlled conditions according to model predictions for 2100. Higher temperature and CO_2 concentrations favored pest's egg-to-adult development survival, even at high female fecundity. It also induced a reduction of fat storage and increase of protein content but did not alter body size. After 10 generations of simulation, genetic adaptation was detected for total lipid content only, however, other traits showed signs of such process. Future experimental designs and methods similar to ours, are key for studying long-term effects of climate change through multigenerational experimental designs.

KEYWORDS

climate change, experimental simulation, insect pest, life history, multigenerational, *Phaseolus vulgaris*, reciprocal transplant, *Zabrotes subfasciatus*

1 | INTRODUCTION

Current models of human activity and climate change predictions, according to the most pessimistic scenarios, foresee an atmospheric concentration of 1000 ppm of CO_2 and an associated global temperature increase of 6°C by 2100 (IPCC, 2007, 2014). Assuming that human population growth and food consumption follow its current trend (Bajželj et al., 2014), global crop production will require a 60% increase by mid-century to respond to food demands (Godfray et al., 2010). Notwithstanding, climate change will hinder this achievement in two ways: (a) crop production will encounter constraints due to plant productivity itself (Olesen et al., 2011; Waha et al., 2013), and (b) pest population dynamics and physiology will be altered (Bale et al., 2002; Harrington et al., 2001). While there is no easy way to deal with future plant productivity constrains, pest management can be adjusted (Estay et al., 2009, 2014). Notwithstanding, we are still far away from understanding how pests will deal with realistic climate change scenarios (Bannerman & Roitberg, 2014; Gillespie et al., 2012; Haridas et al., 2016).

Typical variables associated to global change scenarios, namely increased temperature, and CO_2, affect pest survival and/or fecundity. For example, development survival (measured from egg-to-adult) of *Heliothis viriplaca* (Cui et al., 2018) and *Thrips palmi* (Yadav & Chang, 2014) increased with elevated temperature until reaching a certain threshold. Similarly, development survival, fecundity and parasitizing activity of *Trichogramma buesi*, increased with

temperature until a maximal value was reached (Reznik et al., 2009). Additionally, when the bean weevil *Acanthoscelides obtectus* grows at lower altitude, hence at higher temperature, fecundity increased, as well as egg hatching rate and ovarian production (Huignard & Biemont, 1978). These studies have also suggested that although an increase of temperature favors survival and fecundity, there is a temperature threshold that led trait expression start plateauing or collapsing, most likely because a maximum of metabolic rate was reached.

Resource allocation theory assumes that organisms have a limited amount of resources, which will be traded off among life history traits (Boggs, 2009; Deas & Hunter, 2014; Parker & Courtney, 1984; Pianka, 1981; Roff, 2002). For the case of females, a large proportion of their resources must be allocated to producing successful offspring. However, insect oviposition opportunities are often coerced to assign offspring to a limited patch of resources, such as seeds or insect hosts (Díaz-Fleischer & Aluja, 2003). Egg load, or the number of mature eggs a female is carrying (Ellers & Jervis, 2003; Harvey et al., 2001), is expected to shape the temporal (and spatial) variability in choices related to these oviposition resources. However, given that temperature is a major driver of insect lifespan, one expects oviposition strategy to covary with temperature. Such alteration will take place, for example, if death or loss of ability to reproduce is imminent (Sevenster et al., 1998). In this case, females will produce and lay eggs as soon as possible.

Selection and evolution of thermal reactions imply that environmental temperature and adult body size are linked in different geographical populations (i.e., Bergmann's rule). This relationship predicts that species living in colder conditions reach a larger adult size than species living in hotter climates (Bergmann, 1847). Alternatively, the temperature–size rule stipulates that the plastic phenotypic response to increased temperatures can produce smaller insects by increasing developmental rate (Atkinson, 1994) as increased temperatures shorten insect life span (Papanikolaou et al., 2013). On the other hand, Bergmann's rule implies that environmental temperature and adult body size are linked in different geographical populations: species living in colder conditions reach a larger adult size than species living in hotter climates (Bergmann, 1847). In this regard, the same temperature–size rule, interestingly, stipulates that the plastic phenotypic response to increased temperatures can produce smaller insects by increasing developmental rate (Atkinson, 1994). Body size and temperature relationship rules have not been corroborated and are consequently not as straightforward as theory predicts (Angilletta & Dunham, 2003). To solve this, it has been suggested that a better approach might be to generate and test theories that are tailored specifically to organisms with similar behavior and physiology (Angilletta & Dunham, 2003; DeLucia et al., 2012). Indeed, thermal response is rather the expression of the coevolution of thermal reaction norms for growth rate and size at maturity than a simplistic response that focuses on one or two mechanisms influencing life history (Angilletta et al., 2004).

Temperature has been described as a factor altering insect's body lipid and protein levels (Gligorescu et al., 2018; McCue et al., 2015). One illustrating case is that of the beetle *Ophraellla communa* whose lipid and glycogen storages decrease and increase respectively when the insect was exposed to daily phasic high temperatures (Chen et al., 2019). Changes in metabolic rates leading to anatomical and physiological alterations are the most evident expected consequences of global warming on insects (González-Tokman et al., 2020; Sheridan & Bickford, 2011). However, combined effects of elevated temperature and carbon dioxide have been described to mitigate each other (Zvereva & Kozlov, 2006). Hence, insect body size and lipid reserves are expected to diminish (Atkinson, 1994) due to a higher metabolic rate as well as a higher total protein content produced by hydric stress and development time reduction (Papanikolaou et al., 2013). Simultaneously, fecundity is expected to increase (Huignard & Biemont, 1978), and larval development survival to decrease because of oviposition time compensation and lesser per—egg investment as females experiencing elevated temperature dispose of a shorter time window to lay eggs and harsher conditions are more likely to affect survival.

Insect responses to future climatic conditions are usually explored using the following approaches: assessment of current impacts of climatic changes based on accumulated data from the past (Andrew et al., 2013), bioassays testing climate drivers on a short-term scale (Dyer et al., 2013), field monitoring using a geographical gradient (Hodkinson, 2005; Read et al., 2014; Slatyer & Schoville, 2016), meta-analyses (Saban et al., 2019), and computer models predicting future scenarios (Estay et al., 2009; Northfield & Ives, 2013). Besides purely in silico models, most approaches tend to compile data to produce some predictions based on present or past conditions, which is relevant for extrapolations or climate change simulations on a short-term scale. Despite these reasons, only a handful of investigations have used experimental designs lasting longer than 3–5 generations or explored the impacts of climate change using multigenerational experimental designs. Most of these studies have concerned marine organisms and focused on a single climate driver such as temperature (Munday et al., 2017; Shama et al., 2016), acidification or water pCO_2 (Rodríguez-Romero et al., 2016). One exception to these studies where temperature and pCO_2 have been integrated is that with the marine polychaeta *Ophryotrocha labronica* (Gibbin et al., 2017). As a matter of fact, the various climate change drivers tend to offset each other's effects (Gibbin et al., 2017; Kroeker et al., 2013). Consequently, it seems reasonable to consider "climate change", that is, increased CO_2 concentration and temperature as a single factor.

The idea of a multigenerational selection experiment is to test the magnitude of rapid evolution. Hence, in order to discriminate whether a given phenotype is explained by plasticity or a genetic basis, a reciprocal transplant appears to be a powerful tool (Ågren & Schemske, 2012; Svensson et al., 2018). This technique was originally designed for detecting local adaptation between geographically distant populations or within a metapopulation pooling demes

sharing geneflow (Blanquart et al., 2013; Kawecki & Ebert, 2004). Interestingly, some recent studies used reciprocal transplants to measure the adaptive change in a multigenerational simulation of climate change on marine species (Gibbin et al., 2017; Rodríguez-Romero et al., 2016).

In this study, we investigated the impact of global change conditions on an insect pest's life history traits, physiology, and phenotypic plasticity. Our work is novel for the following reasons: (a) only few studies have been focused on pests' evolutionary responses to climate change; (b) we simultaneously manipulated the two driving factors of climate change; temperature and CO_2 concentration, and (c), a multigenerational approach is used. We used the Mexican bean weevil *Zabrotes subfasciatus* Boheman as a study subject and had the following specific aims: (i) to estimate and project the modulation of the insect´s fecundity and development survival, (ii) to measure the impact of 2100 predicted climatic conditions on body size and total protein and lipid content, (iii) and to detect whether 10+ generations settles genetic adaptation or whether phenotypical plasticity is solely responsible for any measured effect on insects. Hence, we expected insects' fecundity to increase (Huignard & Biemont, 1978), and larval development survival to decrease because of oviposition time compensation and lesser per—egg investment as females experiencing elevated temperature dispose of a shorter time window to lay eggs and harsher conditions are likelier to affect survival. Simultaneously, we predicted that insect body size and lipid reserves would diminish (Atkinson, 1994) due to a higher metabolic rate as well as a higher total protein content produced by hydric stress and development time reduction (Papanikolaou et al., 2013). Finally, we also expected that genetic adaptations would be measurable by the end of the experiment and that more than phenotypic plasticity would be observed as over 10 generations have been described as more than sufficient to trigger adaptative responses (Christie et al., 2012; Laukkanen et al., 2018).

2 | MATERIAL AND METHODS

2.1 | Study system

The Mexican bean weevil *Zabrotes subfasciatus* Boheman (Coleoptera; Chrysomelidae; Bruchinae; Amblycerini) is a worldwide pest that affects crops and stored products of the common bean *Phaseolus vulgaris* L. This weevil is responsible for substantial agricultural damage, mostly in the New World as well as in Africa and Asia where the common bean is massively produced. The insect is sexually mature and ready for copulation immediately after emergence. Indeed, females typically lay their eggs at the very beginning of their imago life, with a peak of oviposition reached within few days (Sperandio & Zucoloto, 2004). As a capital breeding animal, *Zabrotes* adults do not feed and instead use the resources accumulated during larval development (Teixeira et al., 2009; Teixeira & Zucoloto, 2002). Consequently, females mature eggs from a limited amount of reserves and then stick them on the bean seed coat. The first instar larva will hatch and bore into the cotyledon where it will establish a larval chamber for its 30–50 days long juvenile life. A fully developed imago will emerge by cutting its way out of the seed coat.

2.2 | Insect collection and rearing

Wild *Z. subfasciatus* were obtained from *Phaseolus lunatus* (L.) seeds collected along the South Mexican Pacific coast (Figure 1). Four locations were selected based on their relative distance (more than 15 km apart from each other) and on the number of emerging beetles (more than 15 individuals per 10 gr of wild seeds): Las Salinas (lat: 17.435301980003715, lon:-101.19412103667855), Acapulco (lat:16.860116589814425, lon:-99.870241926982999), Vista Hermosa (lat:16.609215969219804, lon: -98.483678000047803) and the experimental station of Universidad del Mar (lat: 15.922161927446723, lon: -97.152206227183342). The emerged wild beetles were reared in controlled environmental chambers (LD 10/14, 28°C/18°C) with random mating and no artificial selection during 10 generations prior to this study. From approximately 1,500–2,000 individuals collected from the field, 5 colonies were started by splitting the founding population in equal proportions. Furthermore, colonies were split another 2 times as the populations expanded and kept in 15 cm long side cubic glass jars each containing 2 kg of organic black bean seeds (*Phaseolus vulgaris* variety Negro Queretaro). Every 2 months, beans from all colonies were sieved, dead adults were discarded, as well as 500 g of infested seeds, and living adults were all randomly redistributed to all 20 jars with 500 g of fresh bean seeds. Despite this control of seeds, population size for all stages of our experiment cannot be calculated as beetles often hide inside the seeds.

2.3 | Experimental colonies settings and climate change simulation

Based on the IPCC model predictions (IPCC, 2007, 2014) from 2007 to 2014 (scenario A1F1 and more recently RCP8.5), an increase of global mean temperature of 6°C and a shift of atmospheric CO_2 concentration from 370 ppm to 1,000 ppm was selected. Since these values correspond to the worse scenario of the IPCC (2014), we decided to adopt them as the predictions from the 80's were fairly optimistic regarding the current climatic situation (Hansen et al., 1981). Two incubators (Precision Model 818, Thermo Fisher Scientific Inc) were used for the climate change simulation, a first as control (L/D: 10 hr/14 hr, 26°C/16°C with ambient air; "2017" or "present"), and a second as treatment (L/D 10 hr/14 hr 32°C/22°C mounted with an Atlas 8 digital CO_2 controller and its flowmeter regulator [Titan controls, Vancouver, WA, USA] maintaining a constant CO_2 air concentration of 1000 ppm [±6%], 2100" or "future"). The homogenous gas mixture inside the chambers were maintained by two 12 cm diameter fans (Essendant, Inc.). To minimize genetic drift (Rich et al., 1979), 40 replicas (starting populations) of 200 individuals (1:1 approximate

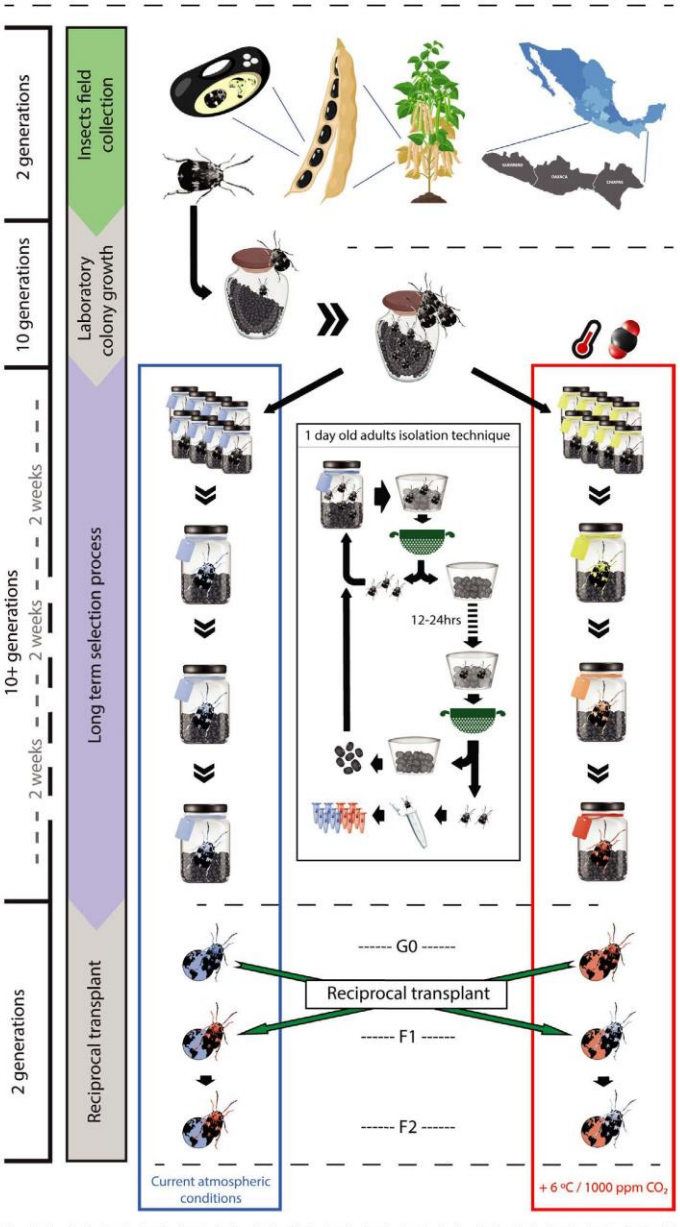

FIGURE 1 From top to bottom: Field collection of wild Zabrotes subfaciatus in the Mexican South West pacific coast, laboratory colony creation and growth during 10 generations, long-term selection experiment with climate change simulation (2100) lines in the red box, and control lines (2017) in blue box, followed by the reciprocal experiment allowing the assessment of the genetic component of the multigenerational climate change simulation. The central black box described the technique permitting the isolation of less than one-day-old adult beetles for subsequent analysis and/or bioassays. The left side chronogram resumes the different phase of the whole experimental process as well as the time range in terms of generations and sampling frequency

sex ratio) were introduced in 15 cm long side cubic glass jars each containing 1 kg of organic black bean seeds (*Phaseolus vulgaris* variety Negro Queretaro; same variety used for the laboratory colony rearing). Each month 200 g of extra beans were added to discard competition for resources and minimizing inbreeding. Replica jars colonies were started sequentially in pairs (one replica control and one replica treatment) following the availability of freshly emerged beetles from the laboratory rearing. To minimize any internal incubator effect, all jars were randomly rearranged inside each chamber on a weekly basis.

2.4 | Selection process monitoring

For 18 months, the colonies were monitored during the multigenerational exposure to simulated climate change and control conditions (Figure 1). Every week, a volume of 60 cm^3 of seeds from every single jar was sieved to remove adult beetles and isolated in 5 × 5 × 4 cm plastic containers. 12 hr after, less than 1-day-old new imagoes emerged. This simple procedure of sieving–waiting–collecting provides younger than 1 day old, newly emerged adult beetles (Figure 1) and was used repeatedly in this study. A maximum of 10 individuals per sieved samples were randomly collected in order not to affect the population dynamics of the colonies. Every sampled insect was frozen killed and kept at −20°C until the end of the experiment. Any replica jar that would fail to provide adult insects for 3 consecutive weeks was permanently discarded.

2.5 | Life history traits measurements

After 180 days of experimental simulation and using the same process of sieving and sampling as previously described, 1-day-old adult beetles were collected from each experimental jar. Males and females from the same experimental jar were randomly grouped by pairs before being deposited in 5 × 5 × 4 cm plastic boxes containing 10 bean seeds using the set.seed() function in R (R Development Core Team, 2013). Each pair was allowed to mate and lay eggs for 7 days. Each box was checked daily and any seed with eggs was removed and isolated after counting the eggs. Consequently, an emerging offspring individual could share siblings in the same seed, as well as in another seed (maximum 9 other seeds) oviposited by the same parents. Given this, replicates are nested by seed, parents and colony jar (c.f. statistical analysis). The larval development took place inside a modified 1.5 ml Eppendorf tube that was perforated 10 times with a Ø 0.7 mm needle to allow gas exchange with the controlled chamber's environment. This process was repeated every two weeks for each replica jar from day 200 to day 460. All tube-isolated seeds were checked daily for emerging adults, which were immediately collected, frozen killed and kept at −20°C for the subsequent measurements. This protocol allowed to record larval development time, fecundity (number of eggs per pair i.e. female) and survival (number of emerging offspring/number of eggs laid per pair), and prevented the young adults from using body-stored energy resources such as lipids before being collected.

2.6 | Reciprocal transplant experiment

After 400 days of experimental simulation, we performed a reciprocal transplant between both 2017 and 2100 chambers. Using the same protocol for measuring life history traits described above, four experimental combinations of insect and chamber were set. However, to minimize maternal effects, F2 offspring were used to assess all measured traits. In other words, the sons and daughters of the insects emerging after the sieving process were used to provide the experimental individuals on which we performed all measurements. Our experimental groups were set as follows: (a) two control groups were arranged by introducing 2017 and 2100 insects into 2017 and 2100 chambers respectively (Figure 1); and, (b) two experimental groups. For these, a 1 day old, freshly emerged pair of adult beetles from 2017 were offered 10 seeds to oviposit and were then introduced into the 2100 chamber, whereas 2100 insects were introduced into the 2017 chamber. Each pair could oviposit for 7 days and was discarded afterward. Daily, all seeds were examined for fresh eggs, if one or more was found, then the seed presenting the egg(s) would be isolated into an individual 1.5 ml Eppendorf tube and kept in the same chamber until emergence. The remaining eggs were kept in the box until the end of the 7-day oviposition phase. This process was repeated every two weeks until the end of the experiment (when the chambers were shut down, i.e. 240 days after the start of the reciprocal transplants). In the same manner as previously described, seeds were checked daily for emerging adults, which were immediately frozen killed and stored at −20°C for further analysis. For convention purposes and to simplify interpretation, the following terminology will be used: the 2017 chamber is referred to as "home" while the 2100 chamber is referred to as "away" for 2017 insects, and reciprocally for 2100 insects.

2.7 | Body size, weight, total protein, and lipid measurements

The length (from the anterior end of pronotum to the posterior end of pygidium) of each bruchid beetle was measured by digital photography and pixel-based measurement using Image J (Schneider et al., 2012) and weighted using a digital Cahn microbalance (Thermo Fisher Scientific Inc). Protein and lipid contents were assessed using a shortened version of a sequential colorimetric measurement protocol adapted for 96 well microplate assays and ELISA–type absorbance readers (Foray et al., 2012). Individuals were crushed into a single 2 ml Eppendorf tube using a steel bead and a Tissue Lyser II device (Qiagen) at 25 Hz for 30 s in 180 μl of aqueous lysis buffer solution (100 mM KH_2PO_4, 1 mM dithiothreitol and 1 mM ethylenediaminetetraacetic acid). After a low-spin centrifugation of 180 g for 30 s, protein contents were measured using a simple Bradford essay (Bradford, 1976) having bovine serum albumin as standard. Absorbance at 595 nm was subsequently recorded with an Absorbance Reader ELx 800 spectrophotometer (BioTek, Inc.). Secondly, lipids were solubilized with 1,000 μl of chloroform–methanol solution (1:2 v/v) and their concentration was measured with the classic vanillin assay procedure (Van Handel, 1985). Triolein was used as standard, and absorbances were read at 540 nm using the same spectrophotometry equipment.

2.8 | Statistical analysis

To test for an effect of climate change simulation on body size, protein content, lipid content as well as development time data over colonies age, we used linear mixed-effects model (LMM) via restricted maximum likelihood (REML with Satterthwaite-approximated degrees of freedom for the fixed effect), with replica jar as a random factor (Kuznetsova et al., 2017).

Regarding the life history trait approach, firstly, a GLMM (general linear mixed model) allowed us to fit by maximum likelihood (Laplace approximation) the variable development survival rate. Secondly, additional GLMM models fitted body size, protein content, lipid content, and development survival with fecundity and the number of generations, using replica jars as a random factor. The latter models were visualized as planes into a 3 days graphic representations when the models were significant.

Reciprocal transplants data were analyzed using a different approach. Primarily, for each measured trait, that is, body length, protein and lipid contents, time to emergence and fecundity, a linear mixed model including the original "home" treatment as factor for genotype (G) and the destination "away" treatment as environment factor (E) were designed in order to determine whether the variance can be attributed to genetic adaptation and assess how each trait evolved. These models were fitted by restricted maximum likelihood using Satterthwaite's method with jar replica, mother ID, and seed ID as random factors. Secondly, internal multiple comparisons were performed on least square means of the models. Regarding the predictor survival, a GLMM was performed as the data had a binomial distribution, and a Tukey (Contrasts) test permitted multiple comparisons of means in this specific case.

3 | RESULTS

3.1 | Life history traits

Fecundity alone significantly explained development survival rate ($z = -3.175$, $p = .001496$), as well as when interacting with the treatment ($z = 3.564$, $p = .000365$). Indeed, development survival decreased as fecundity increased, however, the 2100 model seemed to maintain higher probability of completing development than the control group as fecundity increased (Figure 2a).

Overall development survival rate decreased over generations in the control chamber while the treatment showed a mild increase independently of fecundity (Figure 2b). Nonetheless, when fecundity is considered, the control plane shows a stronger negative inclination as fecundity increases throughout the experiment. In other words, survival decreases strongly and significantly (Table 1) at higher fecundity for later generations. More generally, survival rate in the 2100 chamber is homogenous relatively to the control survival rates, regarding fecundity and/or the number of generations. However, neither body size, total protein, nor lipid content provided a significant model predicting development survival rate when fitted with fecundity (respectively $z = -0.764$, $p = .44$; $z = -0.667$, $p = .505$; $z = 0.46$, $p = .65$).

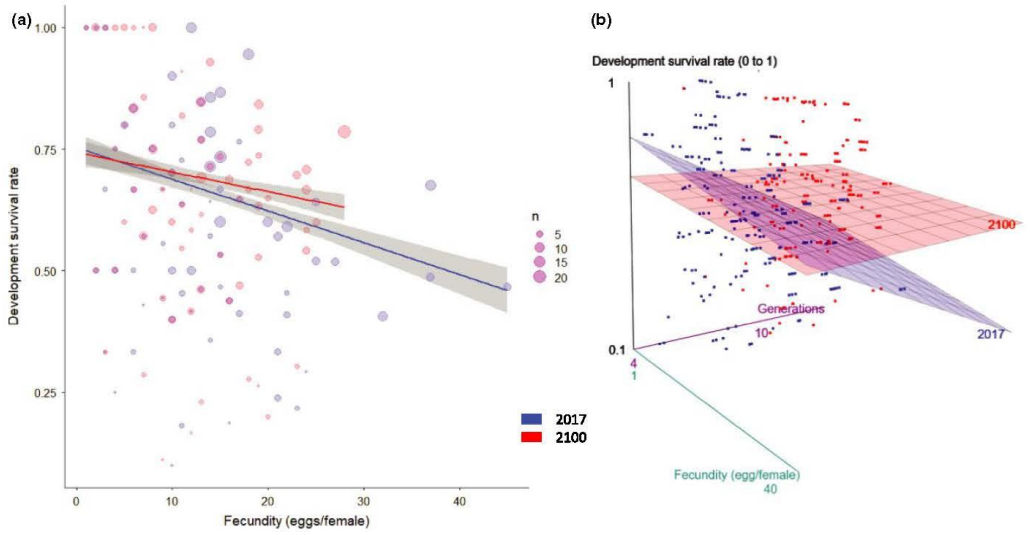

FIGURE 2 (a) GLMM model fitting survival with fecundity, dot size represents the number of overlapping data values (n), shaded gray area displays 95% confidence interval. (b) 3D surface plane representations of GLMM model fitting survival with fecundity over generations, blue dots and red dotes are observed values of 2017 and 2100 respectively that allowed to generate the model and their associated regression surface planes calculated from the GLMM predicted values

TABLE 1 Model outputs of GLMM fitting 1

Pooled data model				
Variables and interactions	**Estimate**	***SE***	***z* value**	***p***
Intercept	1.54835	0.23903	6.478	<.0001
treatment	−0.57051	0.39597	−1.441	.149
fecundity	−0.03875	0.0122	−3.175	<.01
treatment fecundity	0.09042	0.02537	3.564	<.001
Model including generations				
intercept	2.756	0.646	4.264	<.0001
treatment	−2.41	1.12	−2.152	<.05
generation	−0.185	0.091	−2.046	<.05
fecundity	−0.035	0.012	−2.921	<.01
treatment generation	0.241	0.118	2.034	<.05
treatment fecundity	0.092	0.025	3.53	<.001

Note: Development survival with fecundity 2. Development survival with fecundity and generations.

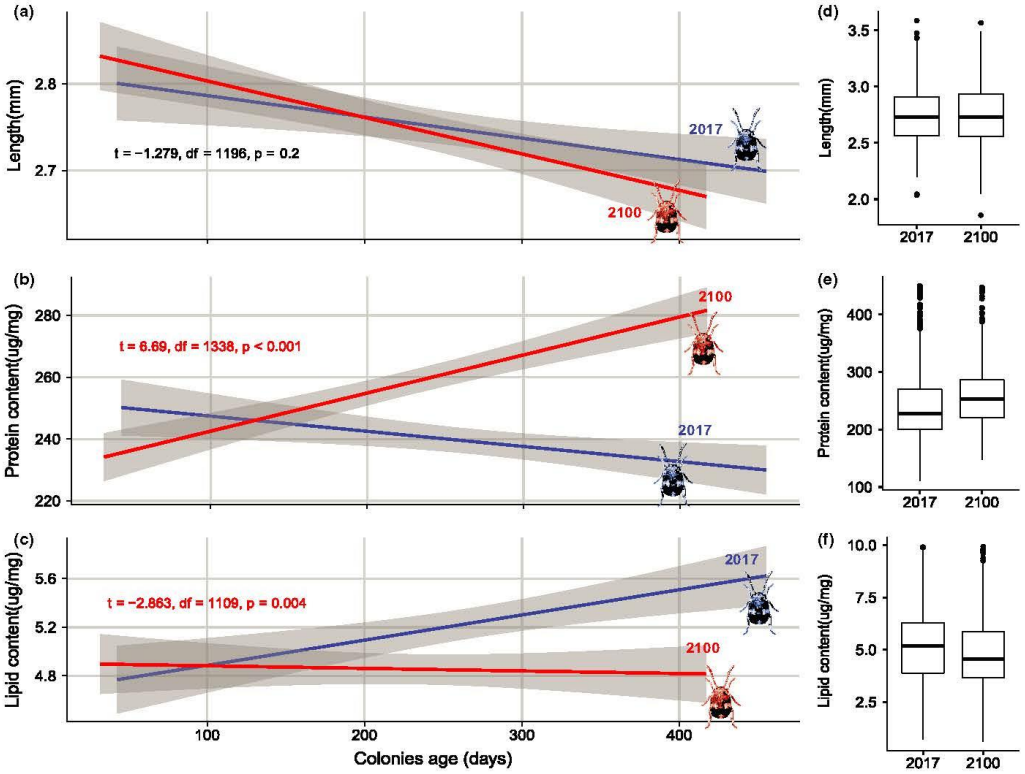

FIGURE 3 Body size (a), protein content (b), and lipid (c) content of Z. subfasciatus over colonies age, blue lines:2017 control environmental chamber group, red lines: 2100 climate change atmospheric simulation group. Shaded gray areas display 95% confidence interval bands, and (d), (e), and (f) boxplots display actual data range and distribution without the time component for plots (a), (b), and (c) respectively

3.2 | Growth and physiological traits monitoring

Body size did not show any significant variation during the experimental simulation (Figure 3a) despite a trend to decrease over time ($t = -1.279$, $df = 1,196$, $p = .2$). However, protein content of 2100 insects (Figure 3b) clearly increased while the control group seemed to show a mild negative slope ($t = 6.69$, $df = 1,338$, $p < .001$). Contrarily, total lipids content of the control beetles increased significantly ($t = -2.863$, $df = 1,109$, $p = .004$) in comparison to the individuals in the 2100 chamber (Figure 3c). Development time was shorter in the 2100 chamber by a factor 2 (Figure 4), and this difference was maintained during the entire experiment ($t = -13.67$, $df = 634$, $p < .0001$). An overall negative slope trend can be observed in both groups. Moreover, the variance of development time is greater in the control group (82.19) than in the 2100 group (37.45). Estimates of replica convergence show that variation of replicas variance was significant for body size and total protein but not for total lipids and the development survival rate versus fecundity data (Table 2).

3.3 | Reciprocal transplant experiment

All statistical values and details are listed in Table 3. For all bioassays performed in this study, 5.4 individuals in average depending on the fecundity of the mother, and an average of 2.7 seeds per mother. Body size content shows the same pattern of increment when insects are exposed to the alternative chamber conditions (Figure 5a): In both cases (2017 and 2100 chambers), insect size is greater in the "away" conditions than in the "home" conditions. Both protein contents of insects from 2017 and 2100 are statistically similar in 2017 conditions, and 2100 insects show a higher content in 2100 home conditions (Figure 5b). However, insects of 2100 are richer in protein by a clear 20 μg/mg in average than insects of 2017. Lipid content dropped drastically when beetles of 2017 were exposed to 2100 conditions, but no difference was observed regarding insects of 2100 (Figure 5c). Development time from both 2017 and 2100 was similar in 2017 conditions. Similarly, both insect lines developed at the same speed in the 2100 chamber (Figure 5d). Fecundity and survival of beetles from 2017 did not show any change when transferred to the 2100 chamber, however, 2100 insects displayed a strong increase in fecundity and survival when exposed to 2017 conditions (Figure 5e,f). Regarding the variance contribution (Table 4), body size, total protein, and development time variance were explained by the environment factor only. Fecundity phenotype could not be attributed to neither genotype nor environment, while survival rate showed a significant result on genotype variance only. However, total lipid content pattern can be attributed to both genotype and environment.

4 | DISCUSSION

In general, our results suggest that climate change alters life history strategies. For example, higher temperature and CO_2 concentrations favored egg-to-adult development survival of *Z. subfasciatus*. Despite this, one would expect a higher mortality due to lesser per-egg investment which remains true even at high female fecundity (Figure 2a). Indeed, when the component of evolutionary time is added to the model (Figure 2b), developmental survival maintains itself across generations independently of fecundity. However, it is necessary to mention that the fecundity decrease observed in the control group may be the response of laboratory selection for increased fecundity but also resulting in decreased survival.

As *Z. subfasciatus* development survival appears to increase, even in the case of high maternal fecundity, several potential explanations can be put forward. First, egg quality is greater in 2100 conditions, meaning that the ovipositing females are capable to provide more viable eggs despite the cost of laying more eggs. Second, since *Z. subfasciatus* is a capital breeder, the physiological assignment of resources during larval development is shifted from egg number to egg quality. Third, eggs and larvae simply develop better in the conditions we simulated as this species has a relatively wide temperature tolerance but a thermal optimum of 27–30°C (Sperandio & Zucoloto, 2009). In other words, our data suggest that augmented temperature and CO_2 have the potential to lead to an increased fitness as the females seem to change their ovipositing strategy by laying fewer eggs and the developing larvae show a higher probability to reach the imago stage. Before moving on to the next section, it is important to mention that formally estimating the absence of drift is difficult as we had no total knowledge of the genetic diversity of the founder individuals used to start each colonies.

Insect body size for the 2100 simulation did not diminish throughout the experiment which is contrary to what we predicted. Rather, there was a tendency of a reduction in body size of both control and treatment groups which is likely an effect of the artificial environment. In this regard, distinct climate change drivers tend to offset each other's effects (Gibbin et al., 2017; Kroeker et al., 2013), also, body size is usually poorly described by general rules (Angilletta & Dunham, 2003; DeLucia et al., 2012) Given that our study includes CO_2, is interesting yet not surprising to observe no body size difference between the control group and the 2100 treatment. We predict that using identical experimental designs involving either increased temperature or CO_2 would, however, provide different outcomes. Worth mentioning is the fact that body size and temperature relationship rules are being repeatedly broken and are consequently not as straightforward as theory predicts. To fully clarify these body size responses, a better approach might be to generate and test theories using organisms with similar behavior and physiology (Angilletta & Dunham, 2003; DeLucia et al., 2012).

Protein content increased and lipid content decreased as predicted for 2100 insects. This may be a consequence of mechanisms for coping with dehydration and a different assignment of energy reserves and metabolic water. Indeed, increased metabolic flow due to higher temperature and enhanced uncoupling of mitochondrial respiration from oxidative phosphorylation, allow for greater production of metabolic water in insects in "dry environments" (Jindra & Sehnal, 1990). A similar pattern has been previously observed in

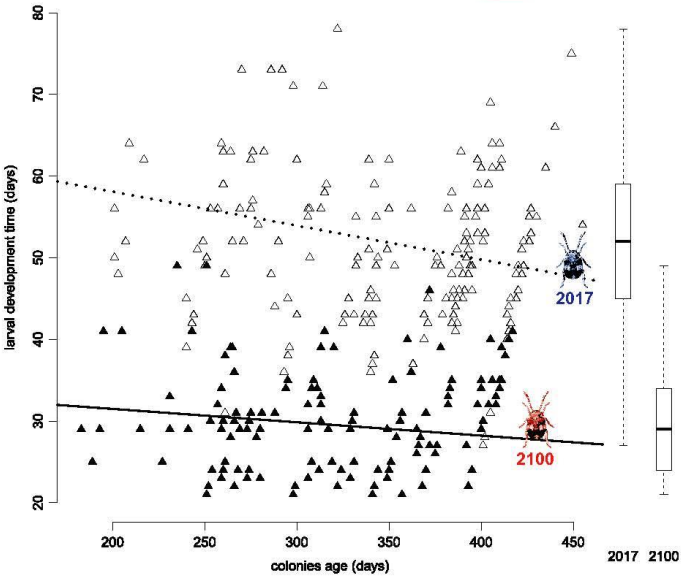

FIGURE 4 Development time of Z. subfasciatus over colonies age, empty triangles;2017 control environmental chamber group, full triangles; 2100 climate change atmospheric simulation. Dashed line is linear regression fit for 2017 data and full line is regression for 2100 data. Boxplots on the right represent distribution range of the whole dataset for both 2017 and 2100 groups

TABLE 2 GLMMs Random factors output values of measured traits (Estimate of adaptation convergence)

Variance component	Variance	*p*
Body size		
Replica jar	0.00149	.01451
Residual	0.0679	
Total proteins content		
Replica jar	116.8	.00116
Residual	2,816.4	
Total lipids content		
Replica jar	0.03384	.09805
Residual	2.80623	
Development survival rate versus fecundity		
Replica jar	4.01 E−10	1

a short-term experiment using *Acanthoscelides obtectus*, a bruchid beetle species close to *Z. subfasciatus*, when temperature increased from 20 to 30°C (Sönmez & Gülel, 2008), but no relevant explanation was found regarding the context of our multigenerational simulation. Consequently, as triglycerides yield almost two times more metabolic water than glycogen (Arrese & Soulages, 2010), fat storage would have been compromised in a dryer environment during larval development. Hence, the pupation process might have further reduced the fat storage through metabolic water extraction as the insect stops feeding and experiences costly transformations in dryer air conditions. This is coherent with findings in other insects. For example, the tsetse fly *Glossina* spp. uses lipid storage during pupation for water balance control depending on the ambient humidity and temperature. Moreover, fat consumption increases with the temperature while the pupal period reduces (Bursell, 1958, 1960; Kleynhans & Terblanche, 2009). Regarding the clear positive slope of protein content in 2100 group, one explanation is that it shows desiccation and increasing tolerance to desiccation. As the experiment progressed, it is likely that individuals from the 2100 group achieved a greater tolerance to warmer and dryer conditions. Possibly these animals afforded to be functional with less water in their environment and body as the lipid content stabilizes over time. This is in agreement with studies in *Drosophila melanogaster* which were selected for increased desiccation resistance (Telonis-Scott et al., 2006),

While studies on heat shock proteins and other temperature stress-related processes are common (Adamo, 2012; Sørensen & Loeschcke, 2007; Sørensen et al., 2005; Wang et al., 2014), very scarce data are available on total protein and lipid contents. In a similar way, numerous studies have provided evidence of, for example, the indirect role of CO_2 through plant tissue alteration (Cornelissen, 2011; Knepp et al., 2005; Murray et al., 2013; Xu et al., 2019) or behavioral approaches on hematophagous insects using CO_2 as a pointer (Guerenstein & Hildebrand, 2008; Jones, 2013; Lazzari et al., 2013; McPhatter & Gerry, 2017). Despite this, literature on direct effects of CO_2 is scarce, aside from studies testing extreme cases such as over 20% of CO_2 (Mitcham et al., 2006), as it has been considered that direct metabolic effects of CO_2 on herbivorous insects are insignificant when the effect on the plant is removed (Coviella & Trumble, 1999). However, laboratory studies using CO_2 anesthesia on *Drosophila melanogaster* show that metabolic changes persist 14 hr after acute CO_2 exposure (Colinet & Renault, 2012; Nilson et al., 2006). This fact should be considered to understand that a

TABLE 3 Models output of multiple comparisons from the reciprocal transplant experiment

Fixed effects				Random effects		
Multiple comparisons (line-chamber versus line-chamber)	***df***	***t* value**	**Pr(>|*t*|)**	**Variance component**	**Variance**	***p***
Body length						
2017–2017 versus 2017–2100	273.8	−2.4138	0.016446	Common seed	0.007005	.04613
2017–2017 versus 2100–2017	28.1	−1.9257	0.064326	Common mother	0.001306	.60885
2017–2017 versus 2100–2100	30.1	1.0246	0.313736	Replica jar	0.002223	.24604
2017–2100 versus 2100–2017	29.5	−0.2693	0.789597	Residual	0.063005	
2017–2100 versus 2100–2100	32.1	2.614	0.01352			
2100–2017 versus 2100–2100	170	2.9291	0.003866			
Protein content						
2017–2017 versus 2017–2100	251.8	−3.6565	0.0003113	Common seed	150.28	.139624
2017–2017 versus 2100–2017	24.7	0.5288	0.6016974	Common mother	309.5	.000255
2017–2017 versus 2100–2100	22.9	−4.355	0.0002335	Replica jar	96.67	.276748
2017–2100 versus 2100–2017	25.9	2.5274	0.0179403	Residual	1789.05	
2017–2100 versus 2100–2100	24.3	−2.2448	0.0341501			
2100–2017 versus 2100–2100	233.8	−5.0507	<0.0001			
lipid content						
2017–2017 versus 2017–2100	291.6	5.3709	<0.0001	Common seed	1.71E−01	.1422
2017–2017 versus 2100–2017	337.1	4.1886	<0.0001	Common mother	1.36E−09	1
2017–2017 versus 2100–2100	333.6	4.5424	<0.0001	Replica jar	3.50E−09	.987
2017–2100 versus 2100–2017	372.2	−0.4555	0.649	Residual	2.52E+00	
2017–2100 versus 2100–2100	328.3	0.1104	0.9121			
2100–2017 versus 2100–2100	361.3	0.4924	0.6227			
Time to emergence						
2017–2017 versus 2017–2100	327	24.8865	<0.0001	Common seed	17.51	<.0001
2017–2017 versus 2100–2017	29.7	0.7307	0.4707	Common mother	4.29	.009633
2017–2017 versus 2100–2100	28.7	8.1946	<0.0001	Replica jar	18.63	1.42E−11
2017–2100 versus 2100–2017	29.9	−8.0573	<0.0001	Residual	17.09	
2017–2100 versus 2100–2100	29.1	−0.7865	0.4379			
2100–2017 versus 2100–2100	248	12.0237	<0.0001			
Fecundity						
2017–2017 versus 2017–2100	656.9	0.409	0.682657	Replica jar	35.69	<.0001
2017–2017 versus 2100–2017	29.2	0.765	0.450402	Residual	47.19	
2017–2017 versus 2100–2100	28.8	2.2334	0.033461			
2017–2100 versus 2100–2017	29.4	0.6571	0.516231			
2017–2100 versus 2100–2100	29	2.1232	0.0424			
2100–2017 versus 2100–2100	663.9	3.2521	0.001203			
Fixed effects				Random effects		
	z value	Pr(>|*t*|)		Variance component	Variance	*p*
Survival						
2017–2100 versus 2017–2017	1.445	0.46124		glass.jar.number	4.85E−10	1
2100–2017 versus 2017–2017	4.826	<0.0001				
2100–2100 versus 2017–2017	3.586	0.00181				
2100–2017 versus 2017–2100	3.772	<0.0001				
2100–2100 versus 2017–2100	2.492	0.05822				
2100–2100 versus 2100–2017	−1.194	0.62197				

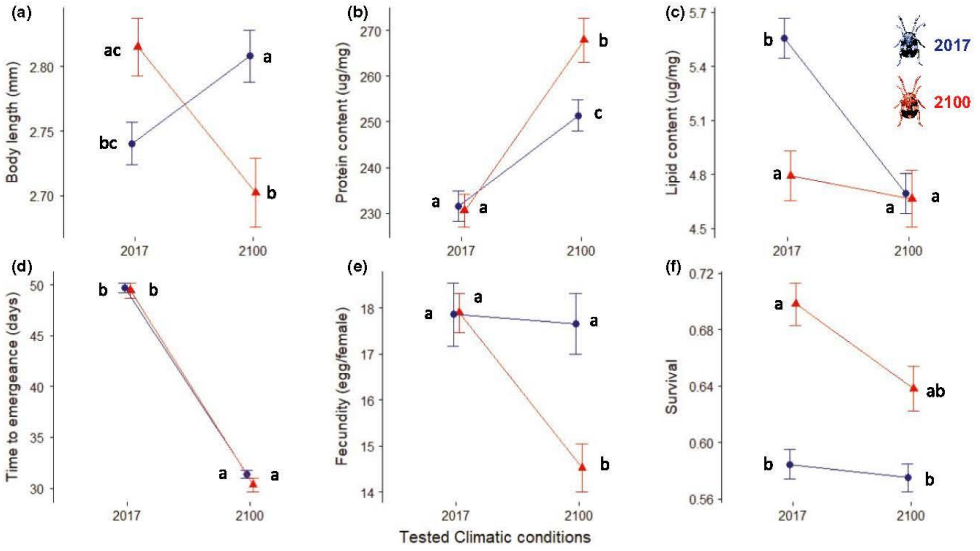

FIGURE 5 Reciprocal transplants of 2017 (blue lines and circles) and 2100 (red lines and triangles) groups bars show standard error. Blue circles in 2017 columns and red triangles in 2100 columns are controls. Small letters allow visualization of least square means multiple comparisons of Satterthwaite´s REML LMM models inside each subgraph. Exception for subgraph (f) where a GLMM was performed and a Tukey (Contrasts) test permitted multiple comparisons of means in this specific case

prolonged exposure to doubled or tripled CO_2 air concentration may impact insect physiology.

The multigenerational simulation of climate change conditions provided a clear contrasting pattern between anatomical and physiological data. As suggested by literature (Angilletta & Dunham, 2003), body size and temperature were not tightly associated by the temperature–size rule (Atkinson, 1994) as no difference was observed between 2017 and 2100. This would fit with a recent metanalysis that explored the species-specificity of the temperature–size rule (Klok & Harrison, 2013). This study indicated that large interspecific variation is either explained by strong interactions with nutrition, or selection based on microclimatic or seasonal variation not captured in classic macro-environmental variables. Indeed, the clear patterns of protein content increase and lipid decrease (relatively to control) imply that metabolic changes are undergoing while the overall exoskeleton size remained unchanged. Interpreting the body size and total protein data must be done with the knowledge that not all replicas converged toward the same outcome as they did for the other measured traits. A small initial population size could have generated such a phenomenon, but some traits converged while others did not, suggesting a greater starting effective population for future experimental designs.

Besides the anticipated faster growth in elevated temperature, an interesting pattern of reduced variance was observed in the development time data. This pattern is actually coherent with previous bioassays (Marinho et al., 2015) and models (Régnière et al., 2012) addressing the impact of temperature on this parameter. Indeed, development time variance tends to shrink at warmer temperatures only if a metabolic and/or physiological threshold is not reached or verged upon (Régnière, 1987). A narrower temporal phenology might reduce variability in ecosystems and agrosystems and is also prone to desynchronize plants–herbivores–predators. Another aspect that should be considered for further investigation is that development time can be offset by temperature fluctuation range (Xing et al., 2015) and a greater climatic, hence, temperature stochasticity is expected with global warming predictions (IPCC, 2007, 2014). Therefore, longer development time and larger variances are expected under a more realistic climate variability simulation.

The overall pattern leads to hypothesize that a pest insect such as *Z. subfasciatus* could in fact be advantaged when facing elevated temperature and CO_2 levels. Bean seeds providing a micro-environment, the growing larvae are virtually affected by a highly similar number of factors in the field and in a laboratory colony, aside from, of course, parasitoid attacks which are common predators in this system (Schneider & Córdoba-Aguilar, 2019a). Given that the young beetles spend over one month encapsulated into their seed, larvae are protected from most physical factors such as atmospheric and mechanical damages. However, Chacidoids and Braconids parasitoid wasps would obviously be able to attack the protected larvae inside the bruchid larval chamber and then constrain population growth (Aebi et al., 2008; Schneider & Córdoba-Aguilar, 2019b).

Our reciprocal transplant experiment produced interesting patterns of phenotypic variation. However, genetic adaptation can be attributed to the variance of the total lipid content only, despite our

TABLE 4 Models output of reciprocal transplant experiment (Genotype × Environment)

Variables and interactions	Estimate	*SE*	*df*	*t* value	*P*
Body length					
Intercept	2.74072	0.02367	20.701	111.77	<.0001
Genotype	0.07681	0.03989	28.066	1.926	.064
Environment	0.06597	0.02733	273.814	2.414	.016
Genotype × Environment	−0.18448	0.04893	238.219	−3.771	<.001
Total proteins					
Intercept	234.617	4.769	15.497	49.199	<.0001
Genotype	−4.477	8.466	24.687	−0.529	.602
Environment	17.094	4.675	251.841	3.656	<.001
Genotype × Environment	23.027	9.224	279.856	2.496	.013
Total lipids					
Intercept	5.5642	0.1122	271.874	49.579	<.0001
Genotype	−0.7892	0.1884	337.143	−4.189	<.0001
Environment	−0.8765	0.1632	291.607	−5.371	<.0001
Genotype × Environment	0.7669	0.2767	316.837	2.771	.006
Larval development time					
Intercept	50.8982	1.2839	24.352	39.643	<.0001
Genotype	−1.4987	2.051	29.7356	−0.731	.47
Environment	−18.029	0.7245	326.975	−24.886	<.0001
Genotype × Environment	1.4426	1.4426	301.985	2.135	.034
Fecundity					
Intercept	18.2566	1.6479	26.198	11.079	<.0001
Genotype	−1.9454	2.543	29.178	−0.765	.451
Environment	−0.273	0.6673	656.941	−0.409	6.83E−01
Genotype × Environment	−3.4124	1.3151	673.855	−2.595	.009
Variables and interactions	Estimate	*SE*		*z* value	*P*
Survival rate					
Intercept	0.4839	0.134	-	3.611	<.001
Genotype	1.4441	0.2992	-	4.826	<.0001
Environment	0.2894	0.2003	-	1.445	.148
Genotype × Environment	−0.7244	0.4158		−1.742	.082

expectations that 10+ generations would generate such adaptation in most measured traits. Body size is the only trait displaying an expected pattern of "local adaptation". However, this pattern indicates an increase of body size when the insects are transplanted, independently of their origin (2017 or 2100). Nonetheless, this is consistent with the aforementioned theory and literature conjecturing that both elevated temperature and CO_2 concentration might mitigate their effects reciprocally (Zvereva & Kozlov, 2006). On one hand, it seems extremely counterintuitive that body size when transplanted, especially in the case of 2017 insects that are supposedly more constrained in the challenging 2100 conditions. On the other hand, one would expect the 2100 acclimated beetles to have a better fitness in more supposedly optimal conditions.

Unfortunately, body size variation as well as protein, time to emergence, and fecundity variances are not explained by a genetic component. Moreover, the development time data confirms that the thermal difference amplitude between both chambers is such an extent that the metabolic rate is irrelevant for detecting genetic change. Indeed, the phenological response to temperature is literally masking off any potentially measurable difference between transplants and control groups in each conditions (Figure 5), due to the fact that insect's development time is tightly connected to temperature (Damos & Savopoulou-Soultani, 2012). The total lipid phenotypes recorded in the reciprocal transplant experiment is the only case in our data where the measured trait variance can be properly attributed to genetic adaptation. Unfortunately, one would expect the lipid levels of 2100 insects to be higher when exposed to their home conditions. Typically, insects experimenting their optimal environmental conditions present optimal energy storage levels (Arrese & Soulages, 2010; Klepsatel et al., 2019). Consequently, this pattern cannot be associated to the idea that the selected lines are fitter under home conditions and then forbid affirming that *Z. subsfasciatus*

as an organism is adapted to 2100 conditions. Nonetheless, it is well known that different organs, pathways, and genes evolve at different speeds (Gillespie, 1986; Wilke, 2004; Zhang & Townsend, 2009), therefore, it is safe to hypothesize that lipid metabolism is under selection.

The puzzling finding that fecundity and development survival of 2100 beetles are lower under their home conditions could be explained by the cost of thermal tolerance plasticity. When an organism is being thermally challenged, either by colder, warmer or highly variable temperatures, thermal tolerance plasticity tends to vary whether this organism is adapted to cold, warm, stable or instable temperatures (Angilletta, 2009; Brahim et al., 2019; MacLean et al., 2019). In our study, it is premature to attribute higher thermal tolerance plasticity to the resilience of 2017 insects or to the adaptability of 2100 insect's fecundity and survival when exposed to different conditions. Therefore, if the lipid data is added to the interpretation, we could hypothesize that 2017 and 2100 insects present different cost when handling strategies for maintaining fitness in "away" conditions. On one hand, the 2100 insects adjust fecundity and survival to their lower fat storage: colder conditions are less costly in terms of heat resistance. Additionally, their metabolism is already able to handle high temperatures with minimal energy requirements. On the other hand, 2017 insects respond to heat with high energy coping mechanisms using their greater stock of lipids (González-Tokman et al., 2020).

Plasticity is usually expected to enable organisms to cope with fast-changing environments (Gienapp et al., 2008). However, even though plasticity mostly occurs within a generation, it has been reported that the conditions experienced by one generation could interact with the conditions experienced by the subsequent generations (Donelson et al., 2018). This phenomenon is known as transgenerational plasticity (TGP), which is likely to take place in a reciprocal transplant experiment as logistics do not fully discard maternal and paternal effects as well as epigenetic transmission (Donelson et al., 2018; Shama et al., 2016). In fact, we cannot discard maternal effects due to the ovipositing behavior (several eggs per seed and on several seeds) of *Z. subfasciatus*, we should discuss our results consistently with this fact. Hence, we cannot exclude TGP as the outcome of our study. It is true that *Zabrotes*, being a worldwide spread multivoltine pest, should have a great potential for phenotypic plasticity (Aebi et al., 2008; Alvarez et al., 2006; Cuny et al., 2017). Consequently, the experiment should be performed for a greater number of generations. Ideally a similar design could be easily implemented using faster developing insects such as *Drosophila spp*. or sister genders causing economic damage such as *Rhagoletis*. Another approach would be to use full genome transcriptomics, as mentioned previously, or even more modern tools such as "Evolve and resequence" (Schlötterer et al., 2015) to pinpoint which genes or gene cluster regulations are affected by increased temperature and CO_2.

5 | CONCLUSION

The role of life history traits plasticity and evolution has been overlooked in climate change ecology (Donelson et al., 2018; Lancaster et al., 2017). This fact is probably explained by the lack of multigenerational experimental data. Our study provides such data and helps guiding the way to more realistic predictions in climate change biology. Moreover, it informs that elevated temperature and CO_2 together affect the physiology, life history traits and the evolutionary direction of a laboratory raised colony of *Zabrotes subfaciatus*. So far, it seems that this pest will deal with climate change by adjusting mainly survival and physiological traits. Future research should look at whether such changes imply higher costs for plant productivity and thus risks for food security.

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CONFLICTS OF INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

David Schneider: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing–original draft (lead); writing–review and editing (equal). **Alejandra G. Ramos:** Formal analysis (supporting); software (equal); validation (equal); writing–original draft (supporting); writing–review and editing (equal). **Alex Córdoba-Aguilar:** Conceptualization (equal); funding acquisition (lead); investigation (equal); supervision (lead); validation (equal); writing–original draft (equal); writing–review and editing (equal).

DATA AVAILABILITY STATEMENT

Multigenerational experimental simulation data & reciprocal experiment data: Dryad https://doi.org/10.5061/dryad.h44j0zph5.

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Discusión

Los estudios de esta tesis permitieron evaluar el impacto de las condiciones de cambio climático previstas para el 2100 en una plaga de insectos de las semillas de frijol. Encontré que los patrones de densidad de población y tamaño corporal en la naturaleza siguen un gradiente altitudinal, y que esta distribución evita la competencia entre dos especies hermanas de escarabajos brúquidos. Lo más importante es que llevé a cabo una simulación experimental multigeneracional del cambio climático sin precedentes en una plaga de insectos, con el fin de llenar la brecha de conocimiento/datos empíricos que existía entre los bioensayos a corto plazo y los modelos *in silico* en la literatura. Este diseño permitió descubrir que *Zabrotes subfasciatus*, una plaga importante de los cultivos de frijol y productos almacenados, mostrará una mayor fecundidad y tasa de supervivencia de huevo a adulto en las condiciones atmosféricas de 2100. Además, estos insectos tendrán un mayor contenido de proteínas y una menor reserva de lípidos, mientras que sus tamaños corporales se mantendrán sin cambios.

Para la primera parte de mi proyecto, planteé la hipótesis de que los efectos del cambio climático alterarían a los escarabajos brúquidos de manera diferente si son especies nativas y adaptadas localmente o si son plagas exóticas recién llegadas. Para la segunda, predije que el aumento de la temperatura y los niveles de CO_2 deberían generar adaptaciones genéticas detectables en una ventana de tiempo de 10 generaciones.

Los datos del gradiente geográfico nos permitieron observar diferentes patrones de distribución en las densidades de población y tamaños corporales individuales a lo largo de la elevación, es decir, el gradiente de temperatura promedio anual. De hecho, *Acanthoscelides obtectus*

(llegada recientemente, muestra comportamiento similar a una plaga) y *Acanthoscelides obvelatus* (especie nativa, adaptada localmente) mostraron patrones opuestos de distribución de densidad y tamaño corporal, sin embargo, los parasitoides tuvieron una presión constante y significativa sobre *A. obvelatus* solamente. Curiosamente, estos resultados me llevaron a una interpretación centrada más en la relación interespecífica entre las dos especies hermanas que en un proxy del cambio climático, ya que los valores de correlación explicaban marginalmente la variación observada en el campo. En consecuencia, los resultados me llevan a concluir que las especies de brúquidos estudiadas no compiten entre sí a pesar de vivir en la misma región geográfica. De ahí que este hecho respalde el principio de exclusión competitiva, que estipula que la competencia ocurrirá entre dos o más especies que comparten los mismos recursos cuando la disponibilidad de estos recursos y/o la densidad demográfica alcance sus respectivas capacidades de carga (McPeek, 2014). No obstante, los datos sugieren que la elevación/gradiente térmico explica parcialmente las densidades de población y los patrones de tamaño corporal, lo que me motivó a diseñar el experimento del siguiente capítulo.

Para la segunda parte de esta tesis, tengo que mencionar que este diseño experimental nunca se había hecho antes mientras escribo estas palabras. La simulación experimental multigeneracional de las condiciones del cambio climático en un insecto plaga me permitió descubrir algunos de los medios fisiológicos, morfológicos y genéticos de los efectos a largo plazo del aumento de la temperatura y los niveles de CO_2. Indiscutiblemente, las condiciones atmosféricas simuladas del 2100 provocaron un aumento y una disminución del contenido de proteínas y el almacenamiento de lípidos respectivamente. No obstante, sorprendentemente, no se registró ninguna variación en el tamaño corporal a pesar de nuestras expectativas al observar insectos más pequeños (Horne et al., 2015). Estos resultados me sorprendieron, dado que los datos del capítulo anterior sugirieron algo diferente. Afortunadamente, una exploración más profunda de la literatura sobre la relación entre el

tamaño corporal y la temperatura me permitió comprender que la modalidad de las adaptaciones térmicas y sus respuestas deben tratarse especie por especie cuando se trata de insectos (Angilletta, Jr., & Dunham, 2003; Angilletta et al., 2004; Klok & Harrison, 2013), idea preconcebida que tenía sobre la relación temperatura-tamaño basada principalmente en la ley de Bergmann (Bergmann, 1847; Atkinson, 1994). De hecho, la regla del tamaño-temperatura sugiere que la respuesta fenotípica plástica a temperaturas elevadas debe producir insectos más pequeños al aumentar la tasa de desarrollo, a medida que las temperaturas más altas acortan la vida de los insectos (Atkinson, 1994; Papanikolaou et al., 2013).

Otro hecho sorprendente producido por este experimento multigeneracional fue que la tasa de supervivencia de huevo a adulto aumenta en las condiciones del 2100, incluso con una fecundidad más alta de la madre, lo que sería adecuadamente inesperado ya que se supone que la inversión por huevo disminuirá debido al costo de división de producir más huevos (Stearns, 1989; Viney, 2012). Este resultado es de alguna manera perturbador, ya que un aumento de la tasa de natalidad junto con una tasa de desarrollo más rápida sin duda suscitaría importantes preocupaciones para los agrosistemas y el almacenamiento de cultivos. Es decir, predecimos un efecto más devastador para el frijol desde la perspectiva de la plaga.

Finalmente, el resultado más inesperado es que no se detectó formalmente ningún cambio genético al final del experimento a pesar de los resultados de estudios publicados anteriormente (Christie et al., 2012; Laukkanen et al., 2018). De hecho, más de 10 generaciones es un período de tiempo razonable para observar suficientes alteraciones genéticas. Sin embargo, solo se registraron los cambios fenotípicos, que seguramente conducen a tales alteraciones genéticas. Esto agrega una nueva perspectiva en la discusión del cambio climático y la rápida evolución. Una de las principales explicaciones posibles de este desconcertante resultado es que en realidad observamos plasticidad transgeneracional (Shama et al., 2016; Donelson et al., 2018), ya que no pudimos controlar

completamente los efectos maternos durante el experimento. La segunda posible explicación es simplemente la resiliencia genética de un insecto plaga multivoltino como *Zabrotes subfasciatus*.

Este trabajo destacó la necesidad de generar datos empíricos sobre la respuesta real de los insectos a la exposición prolongada a las condiciones futuras de cambio climático previstas, especialmente en lo que respecta a plagas, especies sensibles e insectos de importancia médica. Recomiendo utilizar un modelo con un tiempo de generación más corto, como los pulgones, para llegar a un mayor número de generaciones en un tiempo más corto, así como para beneficiarse del conjunto de herramientas potentes que se están desarrollando actualmente para este taxón. La ontogenia, la fisiología y el comportamiento de los áfidos están muy bien estudiados y se dispone de datos genómicos completamente secuenciados para unas pocas especies. Además de que estos genomas todavía están mal anotados, la identificación de genes, rutas y grupos genéticos bajo selección durante la exposición al cambio climático abriría las puertas a un nivel completamente nuevo de precisión en las predicciones evolutivas.

El cambio climático es definitivamente un impulsor de alteraciones en la interacción de ecosistemas y organismos. Esta tesis confirma ese hecho, debido a que el trabajo mostró que las distribuciones actuales de densidades de población y tamaños corporales en el campo sugieren claramente que un cambio en las condiciones climáticas conduciría a la reorganización de funciones complejas del ecosistema, como las interacciones entre especies. Además, el experimento de selección a largo plazo arroja luz sobre una brecha preocupante de datos empíricos entre los bioensayos a corto plazo y los modelos *in silico,* que simulan las condiciones climáticas futuras esperadas. Necesitamos desarrollar una mayor comprensión de la evolución rápida de las plagas de insectos, así como una mejor capacidad para anticipar la amenaza potencial de la agresividad aumentada de las plagas por el clima y sus consecuencias sobre la seguridad alimentaria. El siguiente paso de esta investigación es repetir un diseño de experimento multigeneracional similar utilizando

insectos de desarrollo más rápido, como pulgones o moscas drosofílidas, en períodos de tiempo de selección más largos para lograr una alteración genética real. Así, será posible identificar y aumentar el enfoque en los rasgos afectados por las condiciones climáticas futuras.

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Apéndice: Dos adaptaciones del protocolo unificado de medición del presupuesto energético de Foray para insectos altamente quitinizados y determinación más rápida de glucógeno.

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Two adaptations of Foray's unified energetic budget measurement protocol for highly chitinized insects and faster glycogen determination

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Two adaptations of Foray's unified energetic budget measurement protocol for highly chitinized insects and faster glycogen determination

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Running head: Energetic measurement of large insects

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Introduction

The methodological study "A handbook for uncovering the complete energetic budget in insects: the van Handel's method (1985) revisited" (Foray et al., 2012) allows detecting and measuring energy levels in the different components of lightweight insects. The main advantage of this technique is its easy implementation to measure sequentially total proteins, total lipids, total carbohydrates, and glycogen concentrations from a single individual. Hence, it allows a general approach of the physiological condition of the insects, avoiding the necessity of a large number of redundant samples. Despite the ample utility of this technique, two issues have hampered its application in some cases. First, Foray´s technique was originally described for lightly chitinized insects weighting no more than few milligrams, but this could render some problems when the methodology has been applied to larger animals that grow thicker cuticula. Second, to measure glycogen concentration, Foray's unified method stipulates low-protein binding membranes filtration after an anthrone reaction with a precipitated pellet of sodium sulfate containing the glycogen. "Low-protein binding" refers to the membrane´s property of binding a low quantity of proteins per unit surface area compared to other material membranes, maintaining the sample in terms of molecular compounds content as close as possible to the original. In the classic procedure the membrane filtration was recommended as it reduces anthrone reagent turbidity (personal communication with authors). However, when processing large batches, this filtration step appears to be fairly expensive and time consuming.

Given the above shortcomings, here we propose to use: (i) liquid nitrogen to process large and highly chitinized insects without affecting the measured concentrations of each energetic compartment (ii) simple centrifugation rather than low-protein binding membrane filtration for glycogen concentration measurement. We reached these aims using two beetle pests, *Zabrotes subfasciatus* (Coleoptera; Chrysomelidae; Bruchinae; Amblycerini) and *Tenebrio molitor* (Coleoptera; Tenebrionidae). Both insects differ in size and weight as the former enters in the mass range required by Foray's protocol (<15mg) while the latter is greatly oversized (130 to 160mg). Moreover, as all coleopterans, both species present heavily chitinized cuticula.

Material and methods

Study system

The Mexican bean weevil *Z. subfasciatus* attacks seeds of the common bean *Phaseolus vulgaris* L, as well as closely related Fabaceae. This pest is responsible for substantial agricultural damage on crops and stored products, mostly in the New World as well as in Africa and Asia where the common bean is massively produced. *T. molitor* (Coleoptera, Tenebrionidae), the mealworm beetle, is a worldwide spread insect originating from the Mediterranean basin, its natural habitat is forest litter or decaying material but is mostly found in granaries and stored cereal, especially if moisture has accumulated.

Insect collection and rearing

Wild *Z. subfasciatus* were obtained from *Phaseolus lunatus* (L.) seeds collected from the South Mexican Pacific coast near the city of Acapulco (lat:16.86011, lon: -99.87024), and were reared for 20 generations at 27°C 12/12 LD. *T. molitor* larvae were obtained from four different commercial suppliers in Mexico City and the State of Mexico, and were reared for 15 generations at 25°C 12/12 LD. Both study organisms used female adults.

We randomly assigned 52 *Z. subfasciatus* and 52 *T. molitor* in four groups: 26 insects of each species were processed using strictly Foray's protocol (from now on ZF for *Z. subfasciatus* Foray and TF for *T. molitor* Foray) and 26 remaining insects of each species were processed using our technique modifications (respectively group ZN for *Z. subfasciatus* New and TN for *T. molitor* New).

Crushing and grinding process

Individuals from the ZN group were first crushed in liquid nitrogen with a standard ceramic mortar before being transferred into 2 mL Eppendorf tubes with 180 µl of phosphate buffer (100 mM KH_2PO_4, 1 mM dithiothreitol, (DTT) and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4). A second crushing stage was performed using a Tissue Lyser (Tissue Lyser; Qiagen, Valencia, California) at 25Hz

for 30 seconds. The groups ZF and TF were processed following accurately Foray's protocol (Foray et al., 2012), modifying the buffer quantity to maintain the corresponding Foray´s concentrations for TF group. Animals from the TN group were crushed in liquid nitrogen in a larger ceramic mortar before being transferred into a 2 mL Eppendorf tubes with 1000 µl of the same phosphate buffer and vigorously vortexed for 30 seconds. A second crushing stage for ZN was performed using a Tissue Lyser at 25Hz for 30 seconds. In the case of *T. molitor*, because the organism was big and consequently the samples concentrated, an aliquot of 90 µl was taken from the homogenized mix and transferred into a new tube where an additional 90 µl of buffer were added in order to reach the final volume of 180 µl required by Foray's protocol. This crushing method allows to process large insects that would jam the Tissue Lyser step. Simultaneously the vortex + aliquot + 1:2 dilution allows to extract a maximum of material without dealing with the issues of large cuticle fragments expected from oversized hard-shelled insects.

Main energetic compartment quantification

Total proteins, total lipids, and total carbohydrates were measured following rigorously the original "handbook" (Foray et al., 2012). We decided to skip neutral lipids and fructose quantifications as we considered these subsections to be facultative options for responding to specific research questions only. However, we focused on testing whether the use of low-protein binding membranes required for glycogen optical reading could be omitted as it requires a significant investment of time for batches greater than 100 samples. Hence, we extended the glycogen quantification protocol.

After washing the sodium sulfate pellets bounded with glycogen (c.f. p. 298 of Foray's method), a milliliter of anthrone reagent was added to the pellet, then incubated 15min at 90 °C in water. The reaction was stopped on ice, as recommended, and the reacted anthrone reagent milliliters were divided into 3 separated 240 µl volumes. The first volume was filtered using low-protein binding membranes (polyvinylidene fluoride; d = 0.45 µm, Durapore; Millipore, Billerica, Massachusetts), the second was centrifugated 1 min at 10,000 g, and the third volume was introduced directly in the microplate wells with any post processing. Finally, the glycogen content of all three

split-samples volumes was measured using an Absorbance Reader ELx 800 spectrophotometer (BioTek, Inc., Winooski, Vermont) at 625 nm with glucose as the standard.

Statistical analysis

Proteins, total lipids, total carbohydrates and glycogen concentrations were analyzed with general linear mixed models (GLMM) fit with maximum likelihood (Laplace Approximation) (Kuznetsova et al., 2017) assuming a gamma distribution of the residuals and with the species as random factor in order to test the effect of liquid nitrogen based crushing method. Secondly, a similar GLMM was used to determine the effectiveness of centrifugation and membrane filtration of the anthrone reagent, with species and nitrogen-based crushing method as random factors. Thirdly, we checked the model's validity using a simulation-based residual diagnostic tool (R package DHARMa) (Hartig, 2016) for hierarchical regression models prior to utilizing the outputs for subsequent analysis. Finally, pair comparisons were performed with post-hoc multiple comparisons of means (Tukey Contrasts) in order to pinpoint potential flow in the new methods (R package multcomp) (Bretz et al., 2011). We used the R environment, version 3.6.2 (R Development Core Team; http://www.r-project.org/) to generate all graphics and statistical analyses.

Results and Discussion

First, crushing the insects in liquid nitrogen did not affect the detection sensitivity of proteins, total lipids, and total carbohydrates (Table 1, Fig. 1). Moreover, note that the process permitted to measure about 30% more glycogen from *T. molitor*, but no change was observed regarding *Z. subfasciatus*.It seems likely that the cold crushing permitted an improvement of the glycogen extraction rate, but we do not discard the possibility of a protective effect of the polymer by the inactivation of glycogen degrading enzymes, given that glycogen phosphorylase is inactive at low temperatures (Graves et al., 1965). Since using liquid nitrogen crushing did not provoke any significant difference in *Z.subfasciatus* glycogen determination, the most likely explanation must be purely mechanical. Given their greater mass and cuticula

hardness, larger insects require a well-adapted grinding process, in order to provide an adequate emulsion when fine crushed into the Tissue Lyser tubes. Surprisingly, no increased extraction rate was observed in the other energetic components (Table 1, Fig. 1), which is a fact that remains to be investigated.

Second our results did not show any significant difference at detecting sensitivity of glycogen (Table 1, Fig. 1) neither by using membranes, nor centrifuging nor by direct reading of the anthrone reagent without any further processing. Consequently, our results indicate that reacted anthrone reagent can be directly loaded into the microplate for absorbance reading. This observation would reduce considerably the time frame of large-scale studies requiring a great number of samples or replicates, as the filtration process is the most time-consuming highly expensive step of the whole original protocol.

In conclusion, our first modification regarding the grinding of large sized and highly chitinized insects with liquid nitrogen assures a proper homogenization of the entire organism. We consider this step essential, since it subsequently allows a better extraction of glycogen for this kind of samples. With respect to the low-protein binding membrane filtration step, this could be substituted by a simple centrifugation of the samples. Hence, this is a time and resource saving improvement that makes the technique easier to implement and more economically accessible.

Acknowledgment

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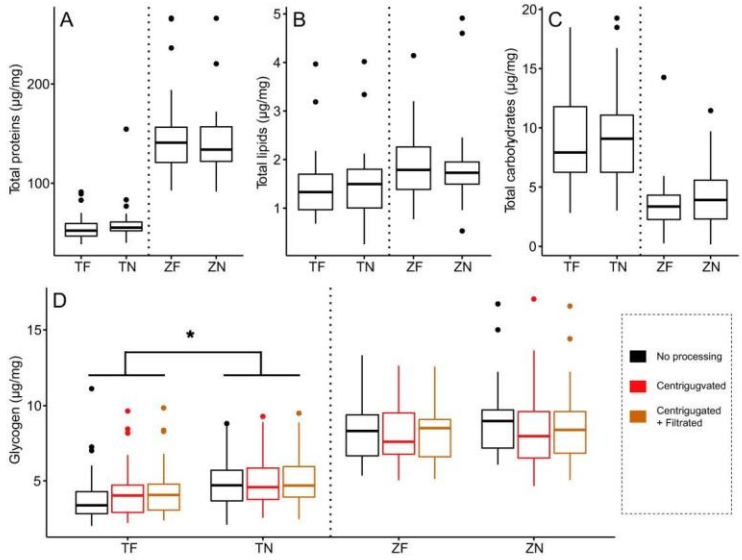
Table and figures legends

Figure 1: Boxplots of proteins (A), total lipids (B), total sugar (C) and glycogen (D) concentrations in µg/mg of total insect mass. ZN: Z. subfasciatus + liquid nitrogen, ZF: Z. subfasciatus processed with original technique, TN: T. molitor + liquid nitrogen, TF: T. molitor processed with original unmodified protocol. Glycogen techniques; in black boxes: no centrifugation + no membrane filtration, in red boxes: 1 min centrifugation at 10,000 g + no membrane filtration, in orange boxes: 1 min centrifugation at 10,000g + low-protein binding membrane filtration.

Table 1: Results from the general linear mixed models post-hoc multiple comparisons. ZN: Z. subfasciatus + liquid nitrogen, ZF: Z. subfasciatus processed with original technique, TN: T. molitor + liquid nitrogen, TF: T. molitor processed with original technique. NFNS ("No Filter, No Spin"): no centrifugation + no membrane filtration, NFYS ("No Filter, Yes Spin"): 1 min centrifugation at 10,000g + no membrane filtration, YFYS ("Yes Filter, Yes Spin"): 1 min centrifugation at 10,000g + low- protein binding membrane filtration.

	Liquid nitrogen pair comparisons		**Estimate**	**Standard Error**	**z value**	**Pr(>|z|)**
Proteins	TN	TF	0.0763	0.0705	1.081	0.701
	ZN	ZF	-0.0385	0.0719	-0.536	0.950
Lipids	TN	TF	0.0506	0.1195	0.424	0.974
	ZN	ZF	0.0305	0.1218	0.251	0.994
Carbohydrates	TN	TF	0.0126	0.1583	0.079	0.998
	ZN	ZF	0.1922	0.1613	1.191	0.632
Glycogen	TN	TF	**-0.0518**	**0.0199**	**-2.600**	**0.045**
	ZN	ZF	-0.0055	0.0276	-0.198	0.997

Fixed effects	**Techniques combinations comparisons**		**Estimate**	**Standard Error**	**z value**	**Pr(>|z|)**
Glycogen techniques	NFYS	NFNS	0.0028	0.0030	0.952	0.607
	YFYS	NFNS	0.0001	0.0029	0.024	0.997
	YFYS	NFYS	-0.0028	0.0030	-0.928	0.623



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