TESIS DOCTORAL

Towards food web engineering: A study of genetic variability and experimental evolution with the predatory mite *Amblyseius swirskii*.

Diego Serrano Carnero

Directores:
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Programa de Doctorado en Diversidad Biológica y Medio Ambiente
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Realizada bajo la tutorización de RAIMUNDO REAL GIMÉNEZ y dirección de MARTA MONTSERRAT LARROSA y JORDI MOYA LARAÑO

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Marta Montserrat Larrosa y **Jordi Moya Laraño**, Científicos Titulares del Consejo Superior de Investigaciones Científicas, en el Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC) y la Estación Experimental de Zonas Áridas (EEZA-CSIC) respectivamente, en calidad de **directores**, y **Raimundo Real Giménez**, Catedrático del Departamento de Biología Animal de la Universidad de Málaga (UMA) en calidad de **tutor**,

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D. **Diego Serrano Carnero**, doctorando del Programa de Doctorado en Diversidad Biológica y Medio Ambiente, ha realizado, en el Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), las investigaciones que han conducido a la redacción de la presente Memoria de Tesis Doctoral, titulada "**Towards food web engineering. A study of genetic variability and experimental evolution with the predatory mite *Amblyseius swirskii*"**.

La presente memoria, que recoge los resultados obtenidos y su interpretación, reúne los requisitos necesarios para ser sometida al juicio de la Comisión correspondiente. Por tanto, como directores y tutor de la tesis, autorizamos su exposición y defensa para optar al Grado de Doctor por la Universidad de Málaga.

Además, las publicaciones que avalan la mencionada tesis no han sido utilizadas en tesis doctorales anteriores.

Y para que así conste, el cumplimiento de las disposiciones vigentes, firmamos la presente acreditación en Málaga a 17 de Julio de 2023.

Marta Montserrat Larrosa   Jordi Moya Laraño   Raimundo Real Giménez

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Summary

One of the most challenging targets that modern agriculture faces today is the production of food by means of sustainable practices to minimize impacts on human health and the environment. At present, arthropod herbivore pests are responsible for approximately 20% of production losses in agriculture worldwide. The use of pesticides to manage these harmful species is questioned because they leave chemical residues on crops and may induce the appearance of resistance after few generations of herbivore exposure to the active component. Biological pest control (BPC), however, is a sustainable and environmentally-friendly alternative to pesticide use. Although the use of BPC in agricultural systems is gradually increasing worldwide, it is still not fully effective because in some cases biological control agents (BCAs) fail at establishing in the agroecosystems. In this thesis I address two potential causes contributing to the failure of BCAs in the crops: a biotic factor, that is, the presence of omnivorous or top-predator species in agroecosystems that can engage in predator-prey interactions with the BCA, and thus undermine the efficiency of the later at controlling the target pests; and an abiotic factor, temperature, which is key to the physiology of ectothermic species, such arthropod pests, and natural enemies. Because sensitivity to rising temperatures usually increases with trophic levels, environmental warming may provide herbivores with increasing chances of escaping predator control. Thus, it becomes crucial that BPC integrates potential effects of biotic and abiotic factors on their strategies to assess the real impacts of both factors on top-down pest control.

In **chapter I** of this thesis I present a brief introduction of BPC, pointing its strengths, weaknesses and limitations, and its different strategies. Next, I review the most recent experimental studies addressing the effects of biotic and abiotic factors, and their interaction, on augmentative pest control in agroecosystems, summarizing the qualitative impact and direction of such effects. The final conclusions of this introduction are that impacts from both warming (the most studied abiotic factor) and the presence of other natural enemies or alternative prey/food in agroecosystems, have little or no impact on augmentative pest control. However, considering that predation and competition, and abiotic factors such as warming, are strong selection forces, the detection of realized effects of these environments on the strength of trophic cascades needs species in the communities to be allowed to interact during several generations. This is necessary to capture potential effects from evolutionary responses to the environment under study. Therefore, I also

reviewed recent studies addressing BCAs' evolutionary responses to either or both, selection pressures. Most papers covering the range of this review were short-term lab or semi-field experiments, and, more importantly, only a few papers evaluated effects by exploring the dynamics, or community outputs after dynamics, of the whole community under study. Also, few studies provided estimates of heritability or genetic correlations between traits, which is crucial for the prediction of evolutionary responses/trajectories.

Until a few years ago, a very common practice was to import exotic natural enemies, usually from the same areas of origin of the pest species, to fight against them. However, with the implementation of the Nagoya protocol and other international restrictions on biological material trading, the access to allochthon BCAs has been severely restricted. Therefore, to enhance the effectiveness of autochthon BCAs, the genetic breeding of natural enemies could be a next level biological approach against pest populations in agricultural systems. However, despite there are extensive published lists of potential traits that can be improved, it is not straight forward to know what trait, or combination of traits, and in what direction, should they be selected. What we do know is that candidate traits should be both response and effect traits, that is, they should hold enough genetic variability to allow evolutionary responses, and they should influence the properties or functions of ecosystems, such as the strength of trophic cascades. To provide a potential solution, at the end of this chapter, I present an integrative approach for the genetic improvement of natural enemies, which has been coined food web engineering (FWE). FWE integrates ecology and evolution into the management of crops by combining experimental data with individual-based modelling, thus taking full advantage of the standing genetic variability in traits relevant to BPC.

In **Section 2** of this thesis, I carried out genetic studies on traits relevant to BPC. For a population to have the potential to adapt to hostile environments, it needs to hold enough intraspecific genetic variability to be able to respond evolutionarily to changes in the environment. Thus, a first necessary step to evaluate whether BCAs have the potential to evolve is to measure the genetic variability of relevant traits in the populations of the BCAs. Indeed, some commercial species may have undergone several bottlenecks during the process of mass-rearing, and may have been subjected to losses from genetic drift. Additionally, unwanted selection during the mass-rearing may have led to the fixation of some alleles, and to the loss of others. To explore this, my model species was the well-known predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae).

Amblyseius swirskii is a predatory mite widely used as BCA against the most important and damaging groups of pests in protected crops, (i.e., spider mites, whiteflies, and thrips). In addition, it is the most used BCA in augmentative biological pest control worldwide. It is, thus, a species of great relevance in the field of BPC. Furthermore, it is a species that is can be easily kept in captivity, it reaches high population numbers, and it has short generation times, what make it an ideal species for genetic and evolutionary experimental studies.

In **Chapter 2** I tested the hypothesis that commercial populations should have less genetic variability than wild ones due to the emergence of unwanted effects during their mass breeding. To this end, I created inbred isofemale lines in a commercial and a wild population of *A. swirskii* and explored the genetic variability of 23 traits, grouped into morphological, life-history, behavioural and physiological categories, through the estimate of their broad-sense heritability (H^2). The commercial population was obtained from one of the largest companies that produces BCAs; the wild population was collected by myself in the native range of occurrence of this species. To estimate H^2, I selected the most representative traits among the 23 while avoiding redundancies caused by correlations between related traits. Trait values and heritability were compared between populations to test for differences in their phenotypic values, and in their genetic variability, between the wild and the commercial populations. I found that the size of the eggs producing males in the commercial population were bigger than those in the wild population; that individuals of both sexes from the commercial population developed faster than those from the wild population; that pre-oviposition and oviposition time was shorter, early fecundity larger, and post-oviposition time longer, in the commercial than in the wild, populations; and that females from the wild population were more tolerant to fasting than those from the commercial population. Of all the representative traits for which H^2 was estimated (12 in females and 2 in males), only 3 traits for females (egg length, dorsal shield length and developmental time) and one for males (developmental time) presented significant H^2. In all cases, the wild population was the only one that presented significant values of H^2. Yet, when heritability was measured merging the two populations, significant values of H^2 emerged for other traits, namely early fecundity, tolerance to desiccation and tolerance to starvation in females, and egg length in males. When the genetic variability of both populations (the G-matrix) was compared, it revealed that the genetic multidimensional space of the commercial population was fully contained within that of the wild population.

In **Chapter 3**, I estimated the genetic correlations between assessed traits, to detect possible trade-offs. Specifically, I analysed the genetic correlations of traits assessed on the same individuals, which allows precise and accurate estimates of genetic correlations. In addition, I also estimated the genetic correlations of traits assessed on different individuals, using the mean values of each isofemale line. This was because measurements of some traits implied the death of the individuals (i.e., tolerance to desiccation, tolerance to starvation), or a change in their diet (predation rate on *Tetranychus* urticae Koch (Acari: Tetranychidae) eggs). Among the significant positive genetic correlations, I found a) the longer the time spent in the preadult motile stages, the longer the total development, as expected; b) the longer the egg, the longer the width of the egg, which indicates that the shape of the eggs does no change with size; c) the bigger the egg, the bigger the female, indicating that bigger eggs produce bigger females, and vice versa; d) the longer the developmental time, the longer the time females needed to lay their first egg; e) larger eggs took longer to develop; f) fecundity and oviposition time were positively correlated and both had a positive correlation to longevity; g) the three behavioural traits were highly positively correlated, that is, individuals that were more active walked faster and travelled longer distances; h) behavioural traits were correlated to traits related to fecundity, indicating that individuals with higher mobility were also more fertile; i) All traits related to tolerance to desiccation were positively correlated to each other. Furthermore, tolerance to 50% and 65% RH were positively correlated with starvation tolerance and with longevity, but negatively correlated to time spend egg (only at 50% RH). Among the significant negative genetic correlations, I found a) the well-known trade-off between fecundity and size; b) fecundity was also negatively correlated to developmental time; c) there seems to be a genetic trade-off between traits related to fecundity and the tolerance to desiccation and starvation; d) there was a negative correlation between developmental time and predation rate on *T. urticae* eggs.

In **Section 3** of this thesis, I used experimental evolution to evaluate whether traits responded to hostile environments, and whether processes of adaptation had any evolutionary cost**.** Firstly, in **chapter 4,** I performed experimental evolution using the wild population of *A. swirskii* (identified in chapter 2 as the population with the greatest genetic variability). I exposed subpopulations of the species to two selection pressures, one abiotic (high temperature) and another biotic (presence of a top-predator), for several generations, in a full-cross experimental design that allowed to disentangle evolutionary responses driven by either, or both selection forces. Next, I measured some traits in individuals from the resulting populations to assess whether they had evolved in response to the selection pressures, and to what degree and direction. The most remarkable result of this chapter is that I detected evolution towards smaller sizes only in the presence of both stressors. Also, individuals from populations long term exposed to high temperatures increased their developmental time.

In **Chapter 5**, I examined whether populations exposed to biotic or abiotic selection pressures had undergone any adaptation or had endured associated costs due to their long exposure to the new environment. To do so, I compared some traits in individuals from the evolved populations and from populations that did not experience stressful environments. Specifically, I measured the following traits: a) oviposition rate and egg size; b) survival and number of eggs laid when those populations were exposed or not to a heat shock; and c) survival, number of individuals escaping from the experimental arenas, and oviposition rates, in individuals exposed or not to the presence of higher order predators. Results showed no evidence of adaptation in any of the traits. However, a cost associated to the evolutionary response was found in the evolutionary lines exposed to high temperatures.

The most relevant conclusions that can be drawn from this thesis are: a) the wild population of *A swirskii* hold higher genetic variability than the commercial population; b) the commercial population might have undergone unknown processes of selection ; c) the trait holding higher heritability was developmental time, followed by fecundity, body size, egg size, and resistance to desiccation (35, 50 and 65% RH) and starvation; d) some of negative genetic correlations seem to emerge from strong past selection. However, some others may have emerged from genetical constrains, such as the negative correlation between developmental time and predation rate, or that between the tolerance to desiccation and starvation and traits related to fecundity; e) The positive correlation between behavioural traits, between egg size and egg developmental time, and between the developmental time and pre-oviposition time, may have been maintained by correlational selection (see Glossary), that is, when selection acts on a combination of trait values (statistical interaction) rather than additively on the traits; f) after experimental evolution, *A. swirskii* evolved towards smaller body sizes only when populations were exposed to both high temperatures and predation pressure; g) individuals from populations exposed to high temperatures lengthened their developmental time. h) Unexpected results obtained after experimental evolution highlights the relevance of considering potential evolutionary responses of populations over many generations, i) adaptation of individuals to the experienced hostile environment

was not detected; j) instead, an evolutionary cost related to the probability of survival was detected in individuals that evolved under hot conditions. Therefore, attention must be paid to the possible costs associated to long exposure to high temperatures, as it is currently occurring with global warming; k) These results highlight the need of approaching BPC strategies in a more realistic way that takes into account the existing abiotic and biotic factors and their interaction, as well as the evolutionary responses they may induce.

The results obtained in this thesis provide for the first-time valuable information about the genetics of a wide list of relevant traits for pest control, and the genetic correlations between them. It also shows that populations under hostile environments undergo evolutionary processes that can jeopardize the success of biological pest control in the future. Furthermore, the data obtained in this thesis will be used to parameterize biological control systems using next-generation individual-based models (NG-IBM) that incorporate genetic variability in traits to study emergent patterns of the dynamics of these agricultural systems, when exposed to hostile environments.

Resumen en español

Uno de los principales desafíos a los que los humanos nos enfrentamos en la actualidad es el de garantizar suficiente producción de comida para una cada vez mayor población humana a través de la implementación de prácticas agrícolas respetuosas con el medio ambiente. Estas prácticas, además de aumentar la producción de alimentos de forma que tengan un impacto mínimo en la salud humana, deben promover el uso sostenible de los ecosistemas terrestres. Además, deben prevenir la degradación de la tierra y la pérdida de biodiversidad, y preservar los ecosistemas que brindan servicios esenciales a los humanos, para cumplir con los principios para el desarrollo sostenible y el bienestar humano definidos por las Naciones Unidas (UN, 2015).

Resulta preocupante constatar que aproximadamente el 20% de las pérdidas sufridas en los ecosistemas agrícolas a nivel mundial se deben a la acción de plagas conformadas por especies de artrópodos herbívoros (Oerke, 2006). Dado que el uso de pesticidas para combatir estas especies dañinas esta siendo cada vez mas cuestionado, debido entre otras cosas al rápido desarrollo de resistencias de las plagas a los mismos, resulta imperativo buscar alternativas sostenibles y respetuosas con el medio ambiente. En este contexto, el manejo de las plagas en los agro ecosistemas a través del control biológico de plagas (CBP) se presenta como una alternativa sostenible y respetuosa con el medio ambiente. Es importante destacar que el objetivo de BPC en los sistemas agrícolas no busca la erradicación sino la regulación/reducción efectiva de las poblaciones de especies consideradas plagas hasta niveles que no supongan un daño significativo para los cultivos, es decir, hasta un umbral aceptable de pérdida económica (Stern et al., 1985), (Symondson et al., 2002). Sin embargo, aunque el CBP es cada día más utilizado como estrategia de manejo de plagas, no siempre es tan efectivo como se desearía (Cock et al., 2016; Collier & Van Steenwyk, 2004), lo que deja un margen considerable para mejorar. Una de las posibles razones que explicarían la falta de efectividad del CBP puede deberse a factores que limitan el establecimiento de los agentes de control biológico (ACB) en los ecosistemas agrícolas (Cock et al., 2016; Collier & Van Steenwyk, 2004). Estos sistemas no pueden ser considerados como entornos aislados en los que se desarrollan cadenas tróficas simples en las que solo existen un productor primario (es decir, nuestros cultivos), un consumidor primario (la especie plaga) y un consumidor secundario (el ACB utilizado contra la especie plaga). En realidad, estos ecosistemas son complejos y pueden estar compuestos por una amplia diversidad de especies que interactúan entre ellas (Jonsson et al., 2017), conformando intrincadas redes tróficas. En consecuencia, la presencia de especies omnívoras o súper depredadoras (consumidores secundarios) podría tener una gran relevancia en los resultados obtenidos al emplear el CBP (Letourneau et al., 2009; Rahman et al., 2011; Schmitz, 2007), ya que las interacciones entre depredadores y presas, es decir, entre los ACB y las plagas, pueden depender de la presencia o ausencia de otras especies en la red trófica. Además, es importante destacar que la mayoría de las plagas y sus depredadores naturales son organismos ectotérmicos, lo que implica que sus funciones fisiológicas, así como sus interacciones con otras especies, están altamente influenciadas por la temperatura ambiental (Beveridge et al., 2010; Dell et al., 2011). En este sentido, se ha observado que a medida que se asciende en los niveles tróficos, la sensibilidad a las temperaturas elevadas tiende a aumentar (Gilman et al., 2010; Vasseur et al., 2005; Voigt et al., 2003). Dado que el calentamiento global está provocando un incremento de las temperaturas, esto podría brindar a los herbívoros mayores oportunidades para evadir el control ejercido por sus depredadores naturales. Por lo tanto, resulta crucial que las estrategias de CBP tomen en consideración los efectos de los factores bióticos y abióticos presentes en las comunidades agrícolas, a fin de evaluar de manera integral los impactos reales de ambos factores en el control de plagas desde una perspectiva “top-down”.

Esta tesis se enmarca dentro del proyecto titulado "Hacia la ingeniería de redes tróficas: vinculando la variabilidad de rasgos con la función del ecosistema" y tiene, en última instancia, el objetivo de sentar las bases para un enfoque holístico del CBP y generar conocimiento sobre los procesos evolutivos que servirán para ofrecer soluciones "a la carta" para sistemas agrícolas específicos con problemas de plagas no resueltos, o con problemas futuros agravados por condiciones desfavorables como consecuencia del cambio climático, como el calentamiento global. El **principal objetivo** es el de generar datos experimentales que alimenten, en un futuro, a un modelo de nueva generación basado en individuos (Weaver). Específicamente, esta tesis abarca la evaluación de la arquitectura genética de rasgos relevantes en los ACB cuando los individuos están expuestos a presiones de selección bióticas y abióticas. Esto se realiza con el fin de determinar cuáles son los rasgos funcionales de respuesta, para posteriormente, y de forma computacional, poder parametrizar redes tróficas típicas de sistemas agrícolas y que, a través de simulaciones, podamos identificar cuales de los rasgos de respuesta lo son también de efecto, intentando maximizar la fuerza de la cascada trófica. Específicamente:

En el **capítulo I** de esta tesis, se presenta una breve introducción al CBP, donde se señalan sus fortalezas, debilidades y limitaciones. También se sintetizan sus diferentes estrategias. Entre las fortalezas del CBP se podrían destacar que, además de ser un método sostenible y respetuoso tanto con el medio ambiente como con la salud humana, al reducir el uso de pesticidas y evitar la presencia de residuos en los cultivos, no requiere de un intervalo de espera después de la liberación de los ACB para el consumo de los alimentos. Además, pueden tener una alta especificidad cuando se utilizan depredadores o parasitoides especializados, la probabilidad de desarrollo de resistencia por parte de las plagas a combatir es prácticamente nula, promueve la biodiversidad, reduce los brotes de plagas secundarias y puede contribuir a otros servicios ecosistémicos tales como la polinización (van Lenteren, 2012; van Lenteren et al., 2018). Por lo contrario, tiene algunas debilidades y limitaciones tales como el riesgo de efectos no deseados en el ecosistema, como interacciones con especies nativas que pueden causar su desplazamiento o incluso que los propios ACB se conviertan en plagas por sí mismos. También existen restricciones en la recolección y exportación de ACB debido a acuerdos internacionales, como el Protocolo de Nagoya sobre acceso a los recursos genéticos (Cock et al., 2010; Van Lenteren et al., 2011), además de las dificultades en la unificación de regulaciones entre países y regiones para el uso de los ACB (Mason et al., 2017). Además, no siempre es el método más rentable en comparación con el uso de pesticidas , dependiendo del caso específico (Collier & Van Steenwyk, 2004).

Los ACBs, al igual que todos los organismos vivos, están expuestos de forma permanente a estresores ambientales que pueden afectar su “fitness” (aptitud). Estos estresores incluyen la interacción con individuos conspecíficos o heteroespecíficos, la escasez de alimentos, temperaturas extremas, radiación ultravioleta, baja humedad relativa o exposición a pesticidas, entre otros (Ghazy et al., 2016). Por lo tanto, es muy importante estudiar los efectos de estos factores en el rendimiento de los ACB, con el fin de comprender cómo pueden afectar el resultado del manejo de plagas. Además, como estos factores de estrés pueden actuar como fuerzas de selección, los cambios en el entorno pueden conducir a una selección direccional y una evolución rápida en los organismos que habitan los sistemas agrícolas (Harmon et al., 2009). Estos procesos evolutivos pueden retroalimentarse y afectar las características ecológicas de las poblaciones y comunidades (Faillace et al., 2021; Fussmann et al., 2007; Moya-Laraño et al., 2014; Szűcs et al., 2019), con resultados desconocidos para el CBP. Por ello, en este capítulo también se identifican los distintos factores que pueden afectar al CBP, clasificados como factores abióticos y bióticos, y los procesos evolutivos desencadenados por ellos. Además, se hace una revisión los estudios experimentales más recientes que abordan los efectos de los factores bióticos y abióticos, así como su interacción, en el control de plagas en los sistemas agrícolas, resumiendo el impacto cualitativo y la dirección de dichos efectos. Las conclusiones finales son que los impactos de ambos factores, el calentamiento (el factor abiótico más estudiado) y la presencia de otros enemigos naturales o presas/alimentos alternativos en los sistemas agrícolas, tienen un impacto mínimo o nulo en el control de plagas. Sin embargo, considerando que la depredación y la competencia, y los factores abióticos como el calentamiento global, son fuertes fuerzas de selección, la detección de los efectos realizados de estos factores sobre la fuerza de las cascadas tróficas necesita que se permita a las especies de las comunidades interactuar durante varias generaciones. Esto es necesario para capturar los efectos potenciales de las respuestas evolutivas al entorno en estudio. Por lo tanto, también se revisan los estudios recientes (desde 2015 hasta 2021) que abordaron las respuestas evolutivas de los ACB ante una o ambas presiones de selección. La mayoría de los artículos que abarcaron el alcance de esta revisión fueron experimentos de laboratorio o semicampo a corto plazo, y, lo que es más importante, solo algunos de ellos evaluaron los efectos mediante el estudio de las dinámicas poblacionales o de las poblaciones resultantes de toda la comunidad bajo estudio. Además, solo un número limitado de estudios proporcionaron estimaciones de heredabilidad o correlaciones genéticas entre rasgos, lo cual es crucial para predecir respuestas evolutivas.

Hasta hace unos años, una práctica muy común era la importación de enemigos naturales exóticos, normalmente de los mismas areas de origen de las especies plaga, con el propósito de combatirlas. Además, con la implementación del Protocolo de Nagoya y otras restricciones internacionales sobre el comercio de material biológico (Cock et al., 2010; Deplazes-Zemp et al., 2018; Hunt et al., 2011), el acceso a ACB alóctonos, se ha visto gravemente restringido (Cock et al., 2010; Van Lenteren et al., 2011). En este escenario, la mejora genética de los ACB de los que actualmente disponemos se plantea como una herramienta prometedora para el manejo de las especies plagas en los sistemas agrícolas. Sin embargo, el problema que se plantea con la mejora genética de los ACB radica en que, a pesar de que se han publicado extensas listas de posibles rasgos susceptibles de poder ser mejorados (Bielza et al., 2020), es muy difícil saber que rasgos, o qué combinación de ellos, y en qué dirección deberían ser seleccionados. Lo que si sabemos es que los rasgos candidatos para su mejora genética deben ser tanto rasgos de respuesta como de efecto, es decir, deben otorgar al individuo la capacidad de adaptarse y sobrevivir en un entorno cambiante y, a su vez, deben tener la capacidad de producir cambios en las propiedades o funciones del ecosistema, como por ejemplo cambiar la fuerza de la cascada trófica. En un intento de proporcionar una potencial solución, al final de este capítulo, se presenta un enfoque holístico para la mejora genética de los enemigos naturales, denominada ingeniería de redes tróficas (IRT). La IRT integra la ecología y la evolución en la gestión de los cultivos mediante la combinación de datos experimentales junto con un modelado basado en individuos, aprovechando al máximo en nuestro beneficio la variabilidad genética existente en los rasgos relevantes para el CBP (Montserrat et al., 2021; Moya-Laraño et al., 2012, 2014).

En la **Sección 2** de esta tesis, realicé estudios genéticos sobre rasgos relevantes para BPC. Para que una población tenga el potencial de adaptarse a ambientes hostiles, necesita tener suficiente variabilidad genética intraespecífica para poder responder evolutivamente a los cambios en el ambiente (Lommen et al., 2017). Por lo tanto, un primer paso necesario para evaluar si los BCA tienen potencial para evolucionar es medir la variabilidad genética de rasgos relevantes para el CBP en las poblaciones de BCA. De hecho, algunas especies comerciales pueden haber sufrido varios obstáculos durante el proceso de cría masiva y pueden haber estado sujetas a pérdidas debido a la deriva genética (Rasmussen et al., 2018; Roderick & Navajas, 2003; Sørensen et al., 2012; E. Wajnberg, 2004). Además, la selección no deseada durante su cría masiva puede haber llevado a la fijación de algunos alelos y a la pérdida de otros. Para explorar esto, la especie modelo utilizada fue el conocido ácaro depredador *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae).

Amblyseius swirskii es un ácaro depredador ampliamente utilizado como BCA contra los grupos de plagas más importantes y dañinos, es decir, arañas rojas, moscas blancas y trips. Además, es el BCA más utilizado en control biológico aumentativo a nivel mundial (Calvo et al., 2015; van Lenteren, 2012). Se trata, pues, de una especie de gran relevancia en el campo del BPC. Además, es una especie que se puede mantener fácilmente en cautiverio, alcanza cifras poblacionales elevadas y tiene tiempos generacionales cortos, lo que la convierte en una especie ideal para estudios experimentales genéticos y evolutivos.

En el **Capítulo 2**, comprobé la hipótesis de que poblaciones comerciales debería tener menos variabilidad genética que las poblaciones salvajes debido a la aparición de efectos no deseados durante los procesos de cría en masa, ya que, debido a estas condiciones, las poblaciones comerciales podrían haber experimentado adaptaciones a las mismas (Hoffmann & Ross, 2018) y/o haber sufrido cuellos de botella. La población comercial estudiada fue proporcionada por una de las mayores empresas productoras y comercializadoras de *A. swirskii*. La población salvaje era proveniente de Israel, de donde es nativa. La recolección de la población salvaje la realicé muestreando parcelas de cítricos en dos localizaciones distintas al norte de Israel, obteniendo un total de 550 individuos aproximadamente, la mayoría de ellos hembras grávidas.

Para tal fin, creé líneas isogénicas de ambas poblaciones (22 líneas para la población comercial y 20 para la salvaje). Mediante esta técnica, lo que se hace es crear subpoblaciones (las llamadas isolineas) a partir de una población basal, que se lleva a cabo mediante un proceso de endogamia como consecuencia del cruzamiento entre hermanos, generación tras generación, aumentando de este modo el nivel de homocigosis. En este caso, realicé este proceso durante 10 generaciones para asegurar un alto nivel de consanguineidad. De esta manera, cada línea isogénica se obtuvo a partir de una única hembra fundadora. Esto dio como resultado la partición de la varianza fenotípica en dos componentes: la varianza fenotípica entre isolíneas y la varianza fenotípica dentro de las isolineas (Falconer & Mackay, 1996). De esta forma, utilizando un análisis de la varianza anidado, se pudieron obtener estimaciones de la proporción relativa de las varianzas genéticas (el componente entre las líneas isogénicas) y ambientales (el componente dentro de las líneas) en las poblaciones estudiadas (Hoffmann & Parsons, 1988).

Una vez obtenidas las isolineas, evalué 23 rasgos en individuos de cada una de las isolíneas de ambas poblaciones. Los rasgos fueron agrupados en distintas categorías (morfológicos, relativos al la historia de vida, comportamentales y fisiológicos), y estimé su heredabilidad en sentido amplio (H^2). Los rasgos morfológicos fueron el ancho y largo de la placa o escudo dorsal, siendo el último una medida indicativa del tamaño corporal. Los rasgos relativos a la historia de vida fueron el ancho y largo del huevo, tiempo de desarrollo de cada uno de los estadios de desarrollo, es decir, huevo, larva, protoninfa y deutoninfa, así como el tiempo de desarrollo total desde huevo hasta adulto, tiempos tanto de pre ovoposición, ovoposición, como de post ovoposición, fecundidad temprana -huevos puestos durante los 10 primeros días desde que pusieron el primer huevo- y total y longevidad. Los rasgos categorizados como comportamentales fueron la velocidad de crucero, la distancia recorrida y la fracción de tiempo en movimiento. Los rasgos fisiológicos evaluados fueron la tolerancia a la desecación a 3 humedades relativas distintas -35, 50 y 65%-, tolerancia al ayuno y la tasa de depredación sobre huevos de *Tetranychus urticae*.

Posteriormente se compararon los valores medios de los rasgos entre ambas poblaciones para detectar posibles diferencias entre ellas. Los resultados obtenidos mostraron que el tamaño de los huevos que dieron lugar a machos de la población comercial fue mayor que el de la población salvaje. En cuanto al tiempo de desarrollo de huevo a adulto, tanto en las hembras como en los machos de la población comercial fue más rápido que los de la población salvaje. Además, se encontró que tanto los tiempos de pre-ovoposición y ovoposición fueron más cortos en la población comercial. También presentó una mayor fecundidad temprana y un mayor tiempo de post-ovoposición la población comercial con respecto a la salvaje. Sin embargo, las hembras salvajes resistieron durante más tiempo que las comerciales bajo unas condiciones de ausencia de alimento.

Además, realicé un análisis de componentes principales (ACP) con el fin de seleccionar los rasgos más representativos, ya que muchos de ellos, especialmente los morfológicos, están altamente correlacionados entre sí, para posteriormente estimar H^2. Además, la representación del ACP mostró cómo la variación genética a través de los ejes de los primeros componentes principales fue mucho menor en la población comercial que en la población salvaje, y que de hecho la variación multidimensional de la primera está completamente incluida dentro de la segunda.

Del total de los 23 rasgos medidos, se estimó la H^2 en 12 de ellos para hembras (longitud del huevo, tiempo de desarrollo total de huevo a adulto, fecundidad temprana, velocidad de crucero, distancia recorrida, fracción de tiempo en movimiento (actividad), tamaño de la placa o escudo dorsal, tolerancia a la desecación -35, 50, y 65%-, tolerancia al ayuno y tasa de depredación sobre huevos de *T. urticae*) y dos para machos (longitud del huevo y tiempo de desarrollo desde huevo hasta adulto). La estimación de H^2 se realizó a través del calculo del coeficiente de correlación intraclase, el cual se utiliza como una aproximación a H^2. Entre todos los rasgos representativos para los cuales se evaluó H^2, tanto en hembras como en machos (12 y 2 respectivamente), solo 3 rasgos en hembras (longitud del huevo, longitud de la placa dorsal y tiempo de desarrollo) y uno en machos (tiempo de desarrollo) presentaron valores de H^2 significativos. En todos los casos, fue la población salvaje la que presentó valores significativos de H^2 mientras que la población comercial no presentó valores significativamente distintos de cero para ninguno de los rasgos evaluados. Sin embargo, cuando se tuvieron en cuenta ambas poblaciones conjuntamente, aparecieron valores positivos de H^2 tanto para la tolerancia a la desecación a las 3 humedades relativas como para la tolerancia al ayuno. Todos estos resultados son consistentes con la idea de que la población comercial podría haber estado sujeta a una fuerte selección.

En el **Capítulo 3**, estimé las correlaciones genéticas entre los rasgos previamente evaluados, con el fin de detectar posibles “trade-offs” genéticos, es decir, compensaciones genéticas que hagan que el aumento en el valor de un rasgo haga que el valor de otro rasgo disminuya. Específicamente, se analizaron las correlaciones genéticas entre rasgos evaluados en los mismos individuos, lo que permitió obtener estimaciones precisas y exactas, ya que se calcularon después de dividir la matriz de varianza-covarianza genética y ambiental de los dos rasgos comparados (Wilson et al., 2010). Además, también se analizaron las correlaciones genéticas entre rasgos evaluados en diferentes individuos, calculadas mediante medias de las líneas isogénicas. La razón detrás de la estimación de las correlaciones genéticas a través de las medias de las isolineas fue que algunos de los rasgos tuvieron que medirse en diferentes individuos dentro de cada isolinea, ya que algunas mediciones de rasgos implicaban la muerte de los individuos (por ejemplo, tolerancia a la desecación o tolerancia al ayuno) o un cambio en su dieta (tasa de depredación de huevos de *T. urticae*). Si bien este último método es menos preciso que el primero, la información obtenida pudo complementar los resultados obtenidos por el método más preciso.

En general la naturaleza de las correlaciones genéticas encontradas fue muy diversa. En lo relativo a las correlaciones genéticas obtenidas entre rasgos evaluados en los mismos individuos, algunos resultados sugirieron que el mantenimiento de algunas de ellas podría implicar una selección correlacional, es decir, combinaciones de valores de rasgos diferentes, en lugar de rasgos aislados, que son favorecidas por la selección natural. Uno de estos casos podría estar detrás de la correlación genética negativa entre el tiempo de desarrollo y la fecundidad. En general, esta relación negativa es contra intuitiva, ya que uno esperaría más bien un compromiso genético que surgiría de una correlación positiva, no negativa (es decir, desarrollarse más rápido y probablemente a un tamaño más pequeño, se compensaría con la cantidad de huevos puestos). De hecho, la selección correlacional podría estar detrás de la correlación genética, especialmente cuando es fuerte y crónica (Sinervo & Svensson, 2002). Esta relación negativa significa que la selección para un desarrollo más rápido implicaría fecundidades más altas, ambas propiedades deseables en programas de cría. De hecho, cuando se compararon ambas poblaciones para estos rasgos, la población comercial presentó tanto fecundidades más altas como tiempos de desarrollo más cortos que la población salvaje, lo que sugiere que se ha aplicado selección artificial a las poblaciones comerciales, ya sea enfocándose directamente en uno de estos dos rasgos o de manera sinérgica en ambos.

También puede haber restricciones fisiológicas que pueden estar detrás de algunas correlaciones como el caso de la correlación positiva entre la fecundidad y el tiempo de ovoposición, indicando que probablemente el tiempo que se tarda en producir huevos limita el tiempo total durante el que se ponen. Otros de los resultados más destacados mostraron que existe una relación positiva entre el tiempo que se pasa en las etapas móviles pre adultas y el tiempo total de desarrollo. Además, tanto el tiempo de desarrollo como el tiempo previo a la ovoposición se correlacionaron negativamente con la fecundidad en cualquiera de sus estimaciones, durante los primeros 10 días y a lo largo de la vida de las hembras. Sin embargo, no hubo correlación entre tener una determinación genética para desarrollarse más tarde y ser genéticamente más grande.

Los resultados también mostraron que los tres rasgos comportamentales están altamente correlacionados positivamente entre sí, es decir, los individuos que tendieron a moverse durante más tiempo, lo hicieron a mayor velocidad y por lo tanto también fueron capaces de recorrer mayores distancias.

Además, la longitud y el ancho del huevo estaban correlacionados positivamente y estos tenían una correlación positiva con la longitud de la placa dorsal de las hembras, indicando que los huevos mas largos son también mas anchos y que los huevos más grandes producirán hembras más grandes.

Por otro lado, los resultados obtenidos mediante las correlaciones genéticas calculadas a partir de las medias de las isolineas, independiente de si los rasgos fueron medidos en los mismos individuos o no, coincidieron en su mayoría con los obtenidos previamente. No obstante, se obtuvieron nuevos resultados entre los que destacan que tanto el tiempo de ovoposición como la fecundidad están relacionados positivamente con la longevidad. Del mismo modo, existe una correlación positiva entre el tamaño del huevo y el tiempo de desarrollo del huevo, lo que indica que los huevos genéticamente más grandes tardan más en desarrollarse. Una vez más, esto puede deberse a una limitación fisiológica ya que las limitaciones impuestas por una menor relación superficie-volumen en los huevos más grandes para la difusión de calor y los procesos enzimáticos podrían influir en esta correlación.

Igualmente, las correlaciones genéticas entre los rasgos comportamentales (velocidad de crucero, distancia recorrida y proporción de tiempo en movimiento) y los rasgos relacionados con la fecundidad (fecundidad temprana y total y tiempo de ovoposición) indicaron que los individuos genéticamente con una mayor capacidad de movimiento son a su vez más fértiles.

Todos los rasgos en los que se evaluó la tolerancia a distintos estreses (desecación al 35, 50 y 65% de humedad relativa y al ayuno) estuvieron altamente correlacionados entre sí. Además, la mayoría de ellos presentaron correlaciones genéticas negativas con la fecundidad temprana, indicando un posible “trade-off”. De hecho, la población comercial presento una mayor fecundidad temprana y una menor tolerancia al ayuno cuando se comparó con la población salvaje, lo que podría indicar de nuevo un proceso de selección de la misma. Tanto la tolerancia al 50% como al 65% de humedad relativa estaban correlacionadas positivamente con la longevidad, pero la primera de ellas negativamente con el tiempo pasado en el huevo. De la misma manera, los resultados revelaron una correlación negativa entre rasgos relativos a la fecundidad (fecundidad temprana y total y tiempo de ovoposición) con la tolerancia al ayuno. Por último, los resultados revelaron que parece haber una compensación genética entre los rasgos relacionados con la fecundidad y la tolerancia a la desecación y al ayuno, y una correlación negativa entre el tiempo de desarrollo y las tasas de depredación de huevos de *T. urticae*.

En la **Sección 3** de esta tesis, use la evolución experimental para evaluar si alguno de los rasgos que presentaron valores significativos de H^2 en el capitulo 2, es decir, con capacidad de poder cambiar bajo distintas presiones de selección, respondieron a ambientes hostiles y, además, si hubo algún proceso de adaptación y si éstos tuvieron algún costo evolutivo.

En primer lugar, en el **capítulo 4**, realicé un experimento de evolución experimental utilizando la población salvaje de *A. swirskii* (identificada en el capítulo 2 como la población con mayor variabilidad genética). Subpoblaciones de la población salvaje fueron expuestas durante 10 generaciones a dos presiones de selección concomitantes, una abiótica (alta temperatura) y otra biótica (presencia de un depredador). El diseño experimental fue factorial, es decir, todas las combinaciones posibles entre los dos factores fueron probados, con el fin de entender y desenredar las respuestas evolutivas impulsadas por cada una o ambas fuerzas de selección. El régimen de temperatura de las poblaciones expuestas fue de 34°C durante el día (5 horas) y 27°C durante la noche (8 horas), con un total de 11 horas de una rampa ascendente-descendente entre ambas temperaturas, con un promedio/día resultante de 30°C, mientras que el de las poblaciones expuestas a condiciones de temperatura no estresantes fue de 25°C. El tratamiento en el que se implementó una fuerza de selección biótica consistió en la adicción de 2 individuos semi adultos del chinche depredador *Orius laevigatus* a intervalos de 2 días de presencia y 2 de ausencia. El experimento se realizó en 2 bloques temporales y en cada bloque se llevaron a cabo 3 réplicas, es decir, un total de 6 réplicas en total. Una vez finalizado el experimento de evolución, las poblaciones resultantes se mantuvieron en un ambiente común y sin estrés (25±1°C y 70±10% de humedad relativa, con un ciclo de luz-oscuridad de 16:8 horas) y sin la presencia de depredadores, durante al menos 3 generaciones, para eliminar posibles efectos maternales o incluso relativos a las abuelas. Una vez que el experimento de evolución concluyó, utilicé los individuos de las poblaciones resultantes para evaluar los rasgos. Dicha evaluación se realizó bajo las mismas condiciones en las que se midieron sus heredabilidades en el capítulo 2, para ver si los rasgos evolucionaron en respuesta a las presiones de selección y en qué grado y dirección lo hicieron. Los resultados mostraron que hubo una evolución hacia un tamaño más pequeño tanto en las etapas de huevo como en adultos, pero solo en presencia de ambos factores estresantes. Estos resultados son consistentes con los resultados obtenidos por Siepielski et al. (2019), donde, tras realizar un meta análisis, concluyeron que no hay evidencias de que altas temperaturas seleccionen tamaños de cuerpo mas pequeños. De hecho, este es un descubrimiento importante y novedoso, y según mi conocimiento, es la primera vez que se documenta que el tamaño del cuerpo responde evolutivamente tanto a la temperatura como a la presión de la depredación conjuntamente. Además, bajo temperaturas altas, hubo un aumento significativo en el tiempo de desarrollo.

En el **Capítulo 5**, examiné si las poblaciones expuestas a las presiones selectivas (bióticas o abióticas) durante el experimento de evolución experimental habían experimentado alguna adaptación o si habían incurrido en costos asociados debido a su larga exposición al nuevo entorno, para lo cual las comparé con poblaciones que no habían experimentado tales condiciones estresantes. Concretamente, medí posibles adaptaciones y/o costos de las poblaciones previamente expuestas a altas temperaturas en: a) la tasa de ovoposición y el tamaño de los huevos; b) la supervivencia y el número de huevos depositados cuando estas poblaciones fueron posteriormente expuestas o no a un golpe de calor a altas temperaturas; y c) posibles adaptaciones y/o costos de la supervivencia, el número de individuos que huyeron de la arena y el número de huevos depositados ante la presencia o ausencia del factor biótico, el antocórido *O. laevigatus*. Los resultados no mostraron evidencia de adaptación en ninguno de los rasgos, ni para el factor abiótico ni para el biótico. Sin embargo, se encontró un costo asociado a la respuesta evolutiva en las líneas evolucionadas obtenidas bajo un régimen de altas temperaturas; es decir, la supervivencia fue más corta cuando los individuos fueron sometidos a una ola de calor.

Las conclusiones más relevantes que se pueden extraer de este trabajo son que las poblaciones salvajes de estos ácaros depredadores tienen una mayor variabilidad genética que las poblaciones comerciales. Además, es importante resaltar que, a pesar de que se observasen respuestas evolutivas en algunos rasgos bajo dos factores estresantes, tales como las altas temperaturas y la presencia de depredadores, no se observaron procesos de adaptación a dichos factores estresantes, lo cual podría afectar a la eficacia del CBP. Por otra parte, se debe prestar atención a los posibles costos asociados a una larga exposición a altas temperaturas, ya que actualmente ocurre con el calentamiento global. De hecho, las respuestas evolutivas a altas temperaturas pueden afectar negativamente la supervivencia de los agentes de control biológico en los episodios cada vez más frecuentes de eventos extremos de temperatura (olas de calor) derivados del cambio climático. Estos resultados demuestran la importancia de abordar las estrategias de CBP de manera más realista y completa, considerando los factores abióticos y bióticos existentes y su interacción, así como las respuestas evolutivas derivadas de ellos.

Los resultados obtenidos en esta tesis proporcionan información valiosa sobre los valores de heredabilidad de una amplia lista de rasgos clave para el control de plagas, junto con las correlaciones genéticas entre ellos. Además, proporciona información útil sobre los procesos evolutivos en entornos hostiles que pueden obstaculizar la efectividad de los agentes de control biológico en el futuro. Asimismo, esta información puede ser utilizada en investigaciones futuras para modelar redes alimentarias en agro sistemas mediante el uso de modelos basados en individuos, los cuales nos indican si estos rasgos de respuesta desencadenan cambios en la fuerza de las cascadas tróficas (rasgos de efecto), ayudándonos a evaluar qué rasgos mejorar y en qué combinaciones, para aumentar la efectividad del control biológico de plagas en diferentes condiciones ambientales.

SECTION 1: General introduction

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Biological control, a sustainable alternative.

One of the current challenges of society is to produce sufficient food production for a growing human population through the implementation of environmentally friendly agricultural practices. These practices should promote the sustainable use of terrestrial ecosystems, prevent land degradation and biodiversity loss, and in general preserve ecosystems that provide essential services to humans. This is to comply with the principles for sustainable development and human welfare defined by the United Nations (UN, 2015). In line with this, the current Common Agricultural Policy (CAP) of the European Union (EU) is designed to make a significant contribution to the ambitions of the European Green Deal, Farm to Fork Strategy and Biodiversity Strategy, which aligns with the sustainability of agriculture in the EU by tackling climate change, protecting natural resources, and enhancing biodiversity. Additionally, the Directive 2009/128/EC for the sustainable use of pesticides demands growers to drastically reduce their use to prevent damages to human health and the environment (European Parliament, 2009). Indeed, it is well known that the abuse of chemical products in agriculture during the XX century led to many pest species developing resistance to pesticides, turning the cure to be worse than the disease, which caused an even greater economic impact on growers (EPPO, 2022). Furthermore, the use of pesticides never led to a decrease in crop losses over time (Oerke, 2006). All of the above, together with an increase in the demand for healthy products of organic origin by end-users, encouraged the productive sectors to apply sustainable agricultural practices to fulfil the market demands (van Lenteren, 2012).

At present, the most realistic and feasible alternative to the use of chemicals is biocontrol, also called biological control (BC, hereafter). BC was defined as “the action of parasites, predators, and pathogens in maintaining another organism´s density at a lower average than would occur in their absence” (DeBach, 1964), always referring to the control of organisms considered harmful from a human perspective. BC has been applied in the framework of several disciplines, ranging from agriculture- to manage insect pests, weeds and pathogens of crops (Gerson, 2014)- to disciplines directly related to human health, such as the control of mosquitoes to reduce malaria, (Benelli et al., 2016), or as the direct treatment of patients with recurrent *Clostridium difficile* Hall & O’Toole (Clostridiales: Peptostreptococcaceae) infection with microbiota entire communities by duodenal infusion of faeces from healthy donors (van Nood et al., 2013). As a result of BC being adapted to these different disciplines, its meaning and terminology have been diluted and disintegrated (Stenberg et al., 2021). As a consequence, in an attempt to delimit and make the definition of BC of pests more precise and harmonizing the terminology, Stenberg et al. (2021) proposed three principles that should underpin the concept: i) biological control implies the use of living agents (Biological Control Agents, BCAs) or viruses; ii) the aim must be to reduce damage via the control of the abundance of harmful species directly or indirectly targeting a harmful organism to provide human benefits, rather than via general health improvement of the affected organisms; and iii) BC strategies can be classified into four main categories depending on whether native BCAs are used with or without human intervention (conservation and natural biological control, respectively), or whether agents are added for permanent or temporary establishment ("classical"-but see below- and augmentative biological control, respectively).

In this thesis, I specifically address biological pest control (BPC, hereafter), as the use of BCAs to control the populations of arthropod herbivore pests in agricultural systems. Also, I specifically refer to BCAs as arthropod organisms belonging to the higher trophic levels in food webs, such as predators and parasitoids. The aim of BPC in agro-systems does not seek the eradication but the effective regulation/reduction of pest populations down to levels that do not pose significant damage to the crops; that is, down to an acceptable threshold of economic loss (Stern et al., 1985), (Symondson et al., 2002).

BPC incorporates the top-down concept

BPC relays on one of the mechanisms by which herbivore populations are regulated in nature. The mechanism, top-down control, was first described by Hairston et al. (1960) in their "Green World Hypothesis", and it was later extended to include ecosystem productivity gradients by Oksanen et al. (1981) in their "Exploitation Ecosystem Hypothesis". In top-down control, populations of organisms in higher trophic levels (secondary consumers, such as BCAs) indirectly increase the abundance or biomass of organisms two trophic levels below (primary producers, such are crops) via indirect trophic or behavioural-mediated interactions through their adjacent trophic level (primary consumers, such as pests), a phenomenon known as trophic cascade (see Glossary) (Paine, 1980). BPC has been broadly used in pest management since the end of the nineteenth century. Yet, its practice may go back long before its definition in 1919 by Smith (H. S. Smith, 1919), when Egyptians started using cats to protect stored grain from damage by rodents (Waage et al., 1988), or when ancient China and medieval Arabs introduced colonies of ants in citrus groves to control lepidopteran pests (Gurr & Wratten, 2000; Konishi & Ito, 1973).

Types of Biological Pest Control

Although different classifications of the BPC have been proposed (Bale et al., 2008; Eilenberg et al., 2001), I will follow the one proposed by van Lenteren et al. (2018) and Stenberg et al. (2021) consisting of four classes (see above):

The first type is *natural* pest control, that is, the control of pests by their natural enemies without human intervention. Natural pest control has happened in the history of nature since the evolution of the first ecosystem and it continues acting in the present day across 55.5 billion hectares of the world´s terrestrial ecosystems (Bale et al., 2008). In economic terms, natural pest control is the greatest contribution of biological control to agriculture (van Lenteren et al., 2018).

The rest of the BPC methods entail human actions such as i) managing agricultural/natural systems to protect or stimulate the presence and performance of natural enemies (Bale et al., 2008), or ii) mass-rearing and releases of either macrobial (predatory insects and mites, parasitoids, and nematodes) or microbial (bacteria, viruses and fungi) BCAs (Lacey et al., 2015).

Conservation biological pest control entails direct human actions to boost BCA populations, but without their release. It is based on the deliberate manipulation of agroecosystems to mitigate detrimentally or enhance favourable, conditions for the BCA in the environment they inhabit (Barbosa, 1998). This is achieved by creating suitable ecological infrastructures within the agricultural landscapes or surroundings to provide resources that improve nesting, foraging, survival, fecundity, longevity and/or behaviour of native natural enemies, boosting their abundance and effectiveness in pest control (Landis et al., 2000). Some of these activities include appropriate plant selection and diversification, reduction in crop intensity and enhanced landscape composition or complexity (Begg et al., 2017). Conservation BPC is described as the biological control method with the greatest potential for use in agriculture within developing countries, and indeed it is being progressively introduced worldwide (Wyckhuys et al., 2013).

Classical biological control of pests consists of the release of natural enemies of exotic pests, collected in the areas of origin of the pest, to permanently control their populations (Bale et al., 2008; Eilenberg et al., 2001). However, Stenberg et al. (2021) argue that this term should be kept separate regardless of whether the target pest is exotic or native, and it should be focused only on the permanent control concept. This was the first, thereby “classical”, alternative method applied to avoid using pesticides. The first recognized and successful example of the application of Classical BPC dates back to 1888 in California, where the coccinellid Vedalia beetle, *Rodolia cardinalis* Mulsant

(Coleoptera: Coccinellidae) was imported from Australia and released to control the cottony-cushion scale insect, *Icerya purchasi* Maskell (Homoptera: Margarodidae), a severe pest of citrus groves (Doutt, 1958). Despite the existence of cases of great success, only about 5–10% of the classical BCAs introductions have had an impact on the target pest; thus, many releases did not benefit the receiving countries (Cock et al., 2010) (see Hokkanen and Sailer (1985) for a critical review of the application of classical BPC). Nonetheless, several cases of classical biological control have been reported when used against invasive species for ecosystem preservation and restoration (reviewed in Van Driesche et al. (2010).

Augmentative biological pest control consists of seasonal releases of BCAs, which are mass-reared in bio-factories, to artificially increase their numbers in the crops and control the pest temporarily. Under this concept of non-permanent biological control, some authors as Eilenberg et al. (2001) have tried to distinguish subcategories according to whether the effect on the pest is due to the release of BCA itself in large quantities, simulating the effect of pesticides in terms of rapid reduction of the pest in a very short time, and where no reproduction of the BCAs is expected (inundative biological control), or whether the release of BCAs is done periodically in smaller quantities and reproduction is expected to occur to provide long-term (but still non-permanent) control (inoculative biological control). However, these terms are not as widely used in the literature as it is the term “augmentative” (Stenberg et al., 2021). The history of commercial mass production of natural enemies spans a period of roughly 120 years. The first two documented cases are from 1902 when *Chilocorus circumdatus* Gyllenhall in Schonher (Coleoptera: Coccinellidae) and *Metaphycus lounsburyi* Howard (Hymenoptera: Encyrtidae) were mass-reared and commercially available against Diaspidids and Coccids pests, respectively (van Lenteren, 2012). In 2018, more than 440 species were commercially available as BCAs against several pests (van Lenteren et al., 2018). Examples of the most recent worldwide applications of augmentative biological control programs can be found in van Lenteren et al. (2018).

Strengths, weaknesses, and limitations of BPC

With globalization and the evolution of means of transport, the flow of goods and people throughout the world occurs to a great extent and speed. As a consequence, the introduction of non-native species into other ecosystems occurs with much ease, either intentionally or accidentally (Ricciardi, 2013). Furthermore, the risk of invasive species expanding their geographic range, and establishing and becoming a pest in new ecosystems dramatically increases with climate change (Skendžić et al., 2021). Partly, the success of colonization and disruption of ecosystems by exotic species that becomes a pest lies in the absence or the inefficiency of native natural enemies acting against them. Initially, the BPC of allochthonous species was implemented via the capture, in the area of origin, and release of their natural enemies, thus loading agroecosystems with new exotic species (Classical BPC). Despite all the cases where Classical BPC was reported to be successful, (Hokkanen & Sailer, 1985; R. G. Van Driesche et al., 2010), this strategy entailed an associated inherent risk. Indeed, the release of non-indigenous BCAs was usually done without prior knowledge of the range of dispersion of the exotic natural enemies, or how it could interact with the native species and its effects on the agroecosystems. Therefore, releases of exotic natural enemies had the potential to cause unintended effects on agrosystems and ecosystems around, and disrupt top-down pest control via indirect or non-target impacts (van Lenteren et al., 2006). Undesired effects include exotic species engaging in direct and indirect interactions with native species (predation, competition for prey, apparent competition), displacing native species, hybridizing with native species, and vectoring pathogens harmful to native natural enemies, among many others (Van Driesche & Hoddle, 2016; van Lenteren et al., 2006). One of the most famous cases of unsuccessful classical BPC is that of the multicoloured Asian ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), which was widely used for aphid and coccid control in Europe and North America (De Clercq & Bale, 2011). Nowadays, it is considered an invasive alien ladybird and a pest, threatening the diversity of native aphidophagous species through exploitative competition or intraguild predation, and producing serious damage in crops when it consumes soft fruits, having become a pest itself (P. M. J. Brown et al., 2008; R. L. Koch & Galvan, 2008). Another flagrant example of a BCA that turned into a pest was the introduction of cane toad *Rhinella (Bufo) marinus* L. (Amphibia: Anura) by the sugar cane industry against the cane beetle pest *Dermolepida albohirtum* Waterhouse (Coleoptera: Scarabaeidae) in Australia (Easteal, 1981). This toad, which is included in the list of the top 100 most harmful invasive alien species in the world by the International Union for Conservation of Nature (IUCN, 2022), has caused serious ecological problems due to predation or competition with many native species (Shine, 2010). In addition, this toad has highly toxic parotoid glands that can kill or injure many of their potential predators including carnivorous mammals [e.g. domestic dogs (Reeves, 2004)] and reptiles (Shine, 2010), causing the decline of varanid lizard populations by more than 90% (Doody et al., 2009).

The problem of non-target effects caused by exotic natural enemies is, in fact, so serious that it has been addressed in several books (Follet & Duan, 2000; K. R. Wajnberg et al., 2001) and publications (Howarth, 1991; Van Driesche & Hoddle, 2016). Importantly, the Food and Agriculture Organization (FAO) of the United Nations adopted in 2005 their third International Standards for Phytosanitary Measures guideline about the export, shipping, import, and release of biological control agents. This guideline, which was introduced in the International Plant Protection Convention (IPPC), has as its main aim to protect the world´s plant resources from the spread and introduction of pests, and promote safe trade and demands a critical evaluation of imported species concerning the potential risks of releasing exotic natural enemies (FAO, 2005). In addition, other regulatory agencies from the European and Mediterranean Plant Protection Organization (EPPO) and the North American Plant Protection Organization (NAPPO) regions have established similar regulations concerning the import and release of non-native organisms. However, these have not been implemented in all countries, and there is no unification in the regulations of the countries of the EPPO region, although this could change soon, at least in the countries of the EU [see European Parliament (2021)]. Actually, many of its countries have their own regulatory systems (Mason et al., 2017). These regulations demand, among other things, carrying out environmental risk assessments (ERA) before the release of exotic natural enemies for their use in classical or augmentative pest control (Mason et al., 2017; van Lenteren et al., 2006). This legislation results in increased costs of using exotic natural enemies (Cock et al., 2010). Furthermore, in 2014 it became mandatory to comply with the Nagoya protocol on access to genetic resources and the fair and equitable sharing of the benefits derived from their utilization, which was previously accepted in 2010 by the Convention on Biological Diversity of the United Nations (CBD) (Secretariat of the Convention on Biological Diversity, 2011). This is a supplementary agreement which has already been ratified by 131 countries worldwide (https://absch.cbd.int). This agreement provides a transparent legal framework granting sovereign rights to countries over their genetic resources, it dictates a fair and equitable distribution of benefits within their limits, and it demands that both the governing access to genetic resources and the distribution of the benefits derived from its use must be established and agreed upon between the parties involved (Kariyawasam & Tsai, 2018). With the agreement of the Nagoya Protocol, some researchers believed that BPC would be negatively compromised because it severally restricts the access to potential new BCAs (Cock et al., 2010; Deplazes-Zemp et al., 2018; Mason et al., 2018). Indeed, the Nagoya protocol has made it difficult, if not impossible, to collect and export exotic natural enemies for biological control research in several countries (Cock et al., 2010; Van Lenteren et al., 2011) due to the conflict of interest between the parties involved. In addition, some countries restrict the use of exotic BCAs and allow only the use of species or strains native to their area (Hunt et al., 2011).

As explained above (see "Types of Biological Pest Control") not all types of BPC involve the importation and release of exotic BCAs and therefore do not imply the aforementioned risks. BPC is considered by some authors as the most sustainable, environmentally safe, and economically profitable pest management method. The list of advantages and benefits of BPC over the use of chemicals is extensive. From a human health point of view, a) BPC is healthier for farm workers and persons living in farming communities; b) crop products are healthier for humans because they do not have traces of pesticide residues; c) it does not require a harvesting interval, or waiting period, after the release of biological control agents (BCAs); and d) BPC lacks harmful side effects. From the economical point of view; e) BPC has the highest rate of success(1:10 *vs* 1:140,000) when compared to the use of chemicals; e) the lowest cost of development, estimated to be less than 100 times the cost of developing a new pesticide [(van Lenteren, 2012), but see Collier and Van Steenwyk (2004) where the authors detect that BPC is not always the best cost-effective method]. Therefore, BPC has f) the highest benefit/cost ratio because g) BCAs' large-scale mass production is inexpensive, and h) there is an absence or low risk of emergence of resistance against BCAs as commonly happens with some pests after pesticide applications. From an ecological perspective, BPC can promote biodiversity as i) it has no phytotoxic damage to plants, j) it can reduce secondary pest outbreaks that commonly occur when pesticide use cause mortality to a wide range of non-target species, including natural enemies that in the absence of pesticides keep secondary pests at bay, due to k) its large specificity when specialist predators or parasitoids are used, as there is a wide availability of different BCAs against numerous pests. Moreover, l) by reducing or eliminating the use of chemicals, BPC enhances other ecosystem services such as pollination (van Lenteren, 2012; van Lenteren et al., 2018). Altogether the above arguments emphasize the relevant role of BPC for crop protection under the present social circumstances.

Factors affecting the outcomes of BPC

BCAs, as all living organisms, are permanently exposed to environmental stresses with the potential to affect their fitness. For example, interacting with other con- or heterospecific individuals, or being exposed to periods of food shortage, extreme temperatures, ultraviolet radiation, low relative humidity or exposure to pesticides, can undermine the chance of their survival and that of their offspring (Ghazy et al., 2016). Thus, to understanding how BCAs determine the outcome of pest control it becomes crucial to study what are the effects of such environmental stressors on their performance. In addition, as these stressors can act as selective pressures, changes in the environment can lead to directional selection and rapid evolution in organisms inhabiting agroecosystems (Harmon et al., 2009), and these evolutionary processes can in turn feedback to affect the ecological characteristics of populations and communities (Faillace et al., 2021; Fussmann et al., 2007; Moya-Laraño et al., 2014; Szűcs et al., 2019), with unknown results for the outcomes of BPC. For example, a literature review on studies evaluating the effectiveness of augmentative BPC and studies that compared it with pesticide applications mainly in the United States agricultural production (Collier & Van Steenwyk, 2004), reported only 15% of success in the biological method, being usually less effective than the use of chemicals, though not always. Another study by Cock et al. (2016) on classical BPC reported that the failure of the establishment of all BCA introductions for BPC is as high as 68%, and only 10% of the introductions resulted in a satisfactory control against insect pests. These results could be the consequence of pest control strategies traditionally ignoring the effects of abiotic (climate) and biotic (community contexts) factors on the dynamics and persistence of agricultural communities under BPC, and, importantly, ignoring the inherent evolutionary processes these stressors produce [recently reviewed in Montserrat et al. (2021)]. See also Sentis et al. (2022), where they highlight the importance of including evolutionary perspectives in BPC.

Biotic factors

Although agricultural systems are much simpler than natural systems, they still hold complex communities. Upon introduction into crops, BCAs are embedded in pre-existing food webs composed of several interacting species (Jonsson et al., 2017); thus, one can expect that single link trophic interactions between BCAs and pests will be likely rare. Increasing the diversity of natural enemies may have different outcomes for BPC (Cloyd, 2020; Jonsson et al., 2017), from top-down pest control being strengthened when natural enemies complement each other (Rahman et al., 2011; Schmitz, 2007), to being weakened when natural enemies engage in negative interactions such as competition for resources (Letourneau et al., 2009). Furthermore, BCAs may not only compete for resources with other predators but also engage in predatory-prey interactions [intraguild predation, IGP (Polis et al., 1989)]. Finke and Denno found that increasing predator species richness in salt-marsh food webs where predators engaged in antagonist interactions such as IGP, dampened the strength of trophic cascades by diminishing the predator's ability to suppress herbivore populations, which then led to decreased plant productivity (Finke & Denno, 2005). In fact, in communities with IGP, if the BCA is preyed upon by an IG-predator that is competitively inferior to the BCA at exploiting the shared pest species, it may lead to a decrease in the strength of the trophic cascade, and consequently, a decrease in pest control (Mylius et al., 2001; Polis et al., 1989; Rosenheim et al., 1995). On the one hand, if the IG-predator were competitively superior, it would simply exclude the BCA from the agrosystem (Janssen et al., 2006; Mylius et al., 2001). Also, the success of BPC can be reduced when natural enemies are exposed to their predators. For example, the natural biological pest control of the invasive species Cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) in India was severely hampered by indigenous parasitoid species actively parasitizing the potential native predators (Gupta et al., 2021). This is an indication that to improve the predictions of BPC prior experimental who-eats-whom assessments need to be done accounting for the community contexts where BCAs are to be embedded. This is because trophic links occurring when species are confronted in pairs are not always realised when other species are present (Torres-Campos et al., 2020).

Similarly, cannibalism (predation on conspecifics) and the availability of other food sources for BCAs can also affect the outcome of BPC. On the one hand, cannibalism entails risks for cannibals, such as being injured or killed by conspecifics, or the possibility of contracting diseases (Polis, 1981). Also, intense cannibalistic behaviour may decrease reproductive success because of the tendency to attack and eat (potential) mates, and it has the added cost of a net decrease in inclusive fitness if the cannibal and victim are relatives (Polis, 1981). On the other hand, it can help species to persist when resources are scarce via life-boat mechanisms (van den Bosch et al., 1988), and it has the benefit of eliminating potential competitors or possible future predators of their offspring (Polis, 1981). In addition, cannibalism implies not only direct lethal effects but also indirect non-lethal effects on potential prey, such as changing activity levels, changing residence sites, retarding development, etc., and it may thus result in stress-related individual phenotypic alterations (Lima, 1998) that can affect population dynamics and alter the strength of trophic cascades, mediated by non-consumptive cannibalistic effects such as behavioural avoidance of cannibals. Therefore, cannibalism is a major factor in the biology of many species that may influence population structure, the life history of individuals, or their behaviour (Polis, 1981), as well as affecting the population dynamics and the strength of trophic cascades (Rudolf, 2007).

A way to reduce the frequency of cannibalism is to provide additional food sources in the agroecosystem (Durga Prasad & Prasad, 2018; Marcossi et al., 2020), although the impact of alternative food on rates of cannibalism, and thus on the outcomes of pest control, depends on its quality (Marcossi et al., 2020). For example, the dynamics of a cannibalistic predator-prey system when predators were provided with additional food showed different scenarios, depending on the quality and amount of the additional food, ranging from limiting and controlling the pest to eradicating predators (Durga Prasad & Prasad, 2018; Polis, 1981; Prasad et al., 2013). Indeed, the availability of alternative food, provided either naturally by plants or introduced artificially, can affect differently the control of pests by their predators (Sabelis & van Rijn, 2006): On the one hand, some works report benefits for BPC when predators and alternative food are introduced into the crop before pests occur or in periods when prey is scarce (aka “predator-in-first” strategy, a concept coined by (Ramakers, 1990)), because it promotes predator survival and population growth, thus assuring the establishment of predators in the crops before pest numbers start to grow dangerously (Delisle, Shipp, et al., 2015; Duarte et al., 2015; Ghasemzadeh et al., 2017; Montserrat, Guzmán, et al., 2013; Seko et al., 2019). This is because predators usually perform better on mixed diets than on single diets [(Marques et al., 2015, 2021; Messelink et al., 2008, 2010; Muñoz-Cárdenas et al., 2014, 2017); but see Cañarte et al. (2017) where it is reported that the performance as BCA of a predatory mite fed on a mixed diet was lower than when it was fed on a single diet]. On the other hand, the availability of alternative food can lead, mainly at the beginning of the predator-pest dynamics, to apparent mutualism between pest and alternative food, caused by a reduction in the per capita predation rate of predators on the pest due to predator satiation (Abrams & Matsuda, 1996; Holt & Lawton, 1994), with negative consequences for BPC (Musser & Shelton, 2003; Skirvin et al., 2006, 2007; Spellman et al., 2006; Van Maanen et al., 2012). Alternative food reduces in some cases the dispersion of the BCAs, which tend to remain in the plants where additional food is present (Skirvin et al., 2006, 2007). Also, caution should be taken with the type of food provided as it could be suitable not only for the BCA but also for the pest. For example, Leman and Messelink (2015) showed that the addition of supplemental food increased the reproduction rate of thrips, which compromised the success of BPC in the short term. Yet, they reported a positive effect on pest control in the long term due to a strong numerical response of the predators. Therefore, the addition of supplemental food should be taken with caution. In addition, its impact on the outcome of biological pest control depends also on other factors, such as the initial predator–prey abundance ratio, the type of dynamics (e.g., equilibrium *vs*. transient dynamics), the type of predator (e.g., stage-related consumption and life history effects of alternative food), the spatial structure of the environment (e.g., source-sink, metapopulation), and the structure of the food web (e.g., presence of hyper-predators or intraguild predators) (Sabelis & van Rijn, 2006).

BCAs can also prey on herbivore species other than the targeted pest. In this case, the two prey species may engage in apparent competition (Holt, 1977) mediated by their shared predator. In apparent competition, the presence of a second prey species may favour a stronger numerical response in the predator due to the higher availability of food and lead to better pest control (C. Z. Liu et al., 2006; Marques et al., 2015; Messelink et al., 2008, 2010; Muñoz-Cárdenas et al., 2017). Indeed, the increase in predator abundances due to the presence of a second prey species usually results in higher overall predation on the target pest species as a consequence of the higher numeric response of predators, and thus to lower pest densities at equilibrium [e.g., (C. Z. Liu et al., 2006; Messelink et al., 2008)]. However, the occurrence of apparent competition between pest species does not always implies a reduction of yield loss (Jaworski et al., 2015). In their work, the population growth of the mirid bug *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) was higher in the presence of two major pest species of tomato plants [*Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) and *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae)] than in treatments with the two prey alone, and the population densities of the two pests were lower when they were together. Yet, tomato plant and fruit damage caused by *B. tabaci* was reduced in the presence of *T. absoluta*, but damage due to *T. absoluta* was not reduced in the presence of *B. tabaci*, resulting in similar levels of total damage when both pests co-occurred, an output similar to that expected in apparent mutualism.

For all the above, due to the importance that biotic factors can have in the outputs of BPC, we reviewed (Montserrat et al., 2021) the most recent studies (from 2015 to 2021) looking for top-down pest control being hampered/promoted by biotic factors in agroecosystems, focusing exclusively on invertebrate predatory macro-BCAs (Table 1.1.1). Most of the recent papers addressing the effects of the presence of other predator species that engage in predatory and/or competitive interactions with the BCA (i.e. top-predators or intraguild predators) conclude either positive or no effects on pest control (Bouagga et al., 2018; Dib et al., 2020; Frizzo et al., 2020; Krey et al., 2020; Ostandie et al., 2021; Roubinet et al., 2015, 2017). Similarly, when other biotic factors such as true omnivory (predators that feed also on plants) or the presence of alternative food or prey for the BCA is evaluated, there is a generally positive effect on pest control (Delisle, Shipp, et al., 2015; Duarte et al., 2015; Ghasemzadeh et al., 2017; Leman & Messelink, 2015; Maselou et al., 2015; Pappas et al., 2015; Seko et al., 2019; Torres-Campos et al., 2020; G. H. Zhang et al., 2018) (Table 1.1.1).

Summarizing, altogether our literature review indicates that before releasing BCAs into agroecosystems more attention should be paid to the community contexts and assess whether these can cascade up or down through the food web.

Table 1.1.1. Experimental studies (from 2015 to 2021) addressing a) top-down pest control being hampered (Negative “ - “), not affected (Neutral “ =”), or promoted (Positive “ +”) by biotic factors, in agroecosystems. The table also provides information on b) the type of experiment performed (either Laboratory or Field/semi-field), and on c) whether individuals/species could interact during several generations (Population/community dynamics, No or Yes). Table extracted and modified from Montserrat et al. (2021).

Table 1.1	**Biotic**							
Driver	**a) Pest control effect**			**b) Type of experiment**		**c) Population/ community dynamics**		**References**
	-	**=**	**+**	**Laboratory**	**Field/ Semi-field**	**No**	**Yes**	
Presence of IG/Top predator	x			x		x		(Torres-Campos et al., 2020)
	x				x	x		(Blubaugh et al., 2021; Paredes et al., 2015)
			x	x		x		(Dib et al., 2020)
			x		x	x		(Frizzo et al., 2020; Krey et al., 2020; Roubinet et al., 2017)
	x		x		x		x	(Bouagga et al., 2018)
	x		x		x	x		(Ostandie et al., 2021)
		x			x	x		(Roubinet ete al., 2015)
	x	x		x			x	(Maoz et al., 2016)
Presence of alternative food/prey			x	x		x		(Torres-Campos et al., 2020)
			x	x			x	(Duarte et al., 2015)
			x		x		x	(Leman & Messelink, 2015; Seko et al., 2019)
		x	x		x		x	(Delisle, Shipp et al., 2015; Ghasemzadeh et al., 2017)
	x	x	x	x		x		(Maselou et al., 2015)
True omnivory		x	x		x	x		(Pappas et al., 2015; N. X. Zhang et al., 2018)

Abiotic factors

Other factors of the environment that may affect the efficiency of BCAs and disrupt BPC are related to climate, especially to temperature and water availability (Collier & Van Steenwyk, 2004), among others (Ghazy et al., 2016). Most pests and their natural enemies are arthropods, and as ectothermic organisms, many of their physiological and fitness-related processes are highly dependent on the ambient temperature (Beveridge et al., 2010; Dell et al., 2011). In addition, the body surface of terrestrial arthropods scales to the 2/3 power of their mass, and thus smaller bodies have a relatively large surface through which they can lose water (Gibbs, 2002). However, although in arthropods water loss through the surfaces´ body is reduced by epicuticular lipids, in a context of global warming in which temperatures are estimated to increase (IPCC, 2021), the epicuticular lipids could melt, increasing the cuticular permeability and, thus, water loss (Gibbs, 2002). Actually, a recent field experiment found that smaller soil arthropods are more sensitive to water availability (Melguizo-Ruiz et al., 2016). Furthermore, considering that the sensitivity of individuals to rising temperatures increases with trophic level (Gilman et al., 2010; Vasseur et al., 2005; Voigt et al., 2003), natural enemies are expected to suffer a higher impact from warming than herbivores. In fact, experimental approaches in laboratory environments show that higher-order consumers are more prompt to extinction than primary producers and consumers (Kalinkat & Rall, 2015). This, together with the hypothesis that increased herbivory should follow rising temperatures according to the distribution and behaviour of contemporary insects (Delucia et al., 2008), undesired heat-driven effects on natural enemies could cascade down destabilizing agricultural communities (Gilman et al., 2010) and disrupting BPC. Likewise, rising temperatures due to climate change are expected to increase the pest population growth and their severity to crops worldwide, with the exception of the tropics (Deutsch et al., 2008). In a review of the effects of climate warming on several herbivore insect pests, Lehmann et al. (2020) reported that 41% of herbivores presented responses to warming that increased the damage to crops despite more than 50% showing mixed effects ranging from an increase to a decrease of damage. Also, considering the influence of rising temperatures on the metabolic rates of insects, Deutsch et al. (2018) estimated 10-25% global yield losses per Celsius degree of the global mean surface warming on the three most widely cultivated crops in the world (rice, maize, and wheat) Deutsch et al., 2018. For example, although rising temperatures typically strengthen the predator's functional response via increasing attack rates and decreasing handling times, feeding rates of predators usually increase at a slower pace than metabolic rates, producing in the long term a deficit of nutrients and death by starvation (Vucic-Pestic et al., 2011).

In the literature, many studies are reporting the negative effects of extreme climate conditions on arthropod BCAs' life history, survival, development, reproduction, or on their capacity to control the target pest (Evans et al., 2013; Gillespie et al., 2000; Ji et al., 2013; Meineke et al., 2014; Montserrat, Guzmán, et al., 2013; Montserrat, Sahún, et al., 2013; Stenseth, 1979; Urbaneja-Bernat, Ibáñez-Gual, et al., 2019). For example, Evans et al. (2013) reported a decrease in BPC of the cereal leaf beetle *Oulema melanopus* L. (Coleoptera: Chrysomelidae) by its natural enemy, the parasitoid wasp *Tetrastichus julis* Walker (Hymenoptera: Eulophidae), as a consequence of a phenological mismatch between the two species caused by an increase in the occurrence of warmer springs. Nevertheless, other studies report no negative effects of increasing temperatures on predator efficiency in controlling pests (El-Danasoury & Iglesias-Piñeiro, 2018; Frank & Bramböck, 2016; Y. J. Wang et al., 2017).

Climate change data-fed models predict an increase in the frequency, intensity, and duration of extreme weather events (IPCC, 2023), such as heat waves or periods of drought. One of the most studied is the occurrence of heat weaves. For example, Y. B. Zhang et al, (2019) showed that in the parasitoid *Eretmocerus hayati* Zolnerowich & Rose (Hymenoptera: Aphelinidae), an increase in the frequency of heat waves affected life history traits negatively at the individual level, as well as the intrinsic rate of increase (*r*) at the population level, decreasing its efficiency at controlling the populations of *B. tabaci* (Y. B. Zhang et al., 2019), a key pest in many horticultural crops (De Barro et al., 2011).

Abiotic conditions not only have direct but also indirect effects on individuals, which can also have important consequences on food-webs (Gilman et al., 2010; Tylianakis et al., 2008), for example, triggering natural enemy-prey phenological mismatches (Damien & Tougeron, 2019; Furlong & Zalucki, 2017; Hance et al., 2007), or changes on species interaction strength, under climate change conditions. For instance, heat waves triggered a phenological mismatch between the specialist endoparasitoid *Diadegma semiclausum* Héllen (Hymenoptera: Ichneumonidae) and its host, the plant feeding diamondback cabbage moth *Plutella xylostella* L. (Lepidoptera: Plutellidae). When the intensity of the heat wave increased, a delay in the parasitoid development occurred, disrupting the host-parasitoid synchrony (Schreven et al., 2017). Similarly, heat waves can affect trophic interaction strength. Sentis et al. (2013) showed a tri-trophic system composed of sweet pepper *Capsicum annuum* L., the green peach aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) and the predatory ladybeetle *Coleomegilla maculata lengi* Timberlake (Coleoptera: Coccinellidae), in which an increase in the frequency and intensity of heat waves reduced the impact of ladybeetles on the aphid populations due to the direct effect of heat waves on the predator's fecundity (Sentis et al., 2013). Hence, understanding how environmental drivers impact trophic interactions is key to predicting food web responses to global climate change (Rosenblatt & Schmitz, 2016). Regarding an increase in temperatures without fluctuations, Guzmán et al. (2016) showed an example of the implications of temperature-dependent competitive dominance between two congeneric species of predatory mites, *Euseius scutalis* Athias-Henriot (Acari: Phytoseiidae) and *Euseius stipulatus* Athias-Henriot (Acari: Phytoseiidae), that co-occur in avocado orchards in south-eastern Spain coast and compete for the same resources. Whereas the former excluded its competitor at hot temperatures, the opposite occurred at mild temperatures (Guzmán et al., 2016). In a similar system, Torres-Campos (2017) found that the trophic structure of a simple community drastically changed with increasing temperatures. Therefore, the role of temperature in shaping the structure of agricultural communities through changes in the strength and direction of competitive and predation interactions could have implications for the failure of BPC.

Environmental stressors cannot be studied in isolation as they may act in combination. For example, high temperatures and low relative humidity decrease the control effectiveness of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) on *Tetranychus urticae* Koch (Acari: Tetranychidae) in indoor tomato crops because development and feeding rates of spider mites increase with temperatures whereas the performance of *P. persimilis* is negatively affected by heat and drought (Nihoul, 1993). In addition, under high temperatures and limited access to prey, relative humidity may play an important role in the survival of predators. When relative humidity is low, predators may lose water, dehydrate, and eventually die, whereas high humidity may alleviate and compensate partially for the severity of both high temperatures and food shortages (Ghazy et al., 2016). Alternatively, the occurrence of low relative humidity conditions may have little effect on predators when food is abundant because water loss can be compensated via liquid intake from their prey (Ghazy et al., 2016). Another potential effect in the population dynamics with possible implications in the BPC is the interaction between temperature and Ultraviolet-B radiation (UV-B) in plant-dwelling mites (Ghazy et al., 2016). Studies such as the one conducted by Barcelo (1981) suggested that the major component of solar ultraviolet radiation that damages *T. urticae* is UV-B. Although the highest rate of UV-B solar irradiation is estimated to occur during summer, more recent studies showed that it is in spring and autumn when it has more deleterious biological effects, as in the case of *T. urticae* eggs survival (Sakai et al., 2012). This is because the UV-B damage in organisms does not depend on the intensity but on the cumulative exposure time (Murata & Osakabe, 2013). For this reason, lower temperatures in spring and autumn increase developmental time compared to those in summer, because of the increasing cumulative UV-B irradiance and, therefore, the negative effects (Sakai et al., 2012). Thus, it is important emphasizing how covariation in environmental factors may lead to counterintuitive trait responses.

Furthermore, beyond covariation, environmental factors can interact in different ways and result in overshadow, additive, subtractive, or synergistic effects (Ghazy et al., 2016). For example, Miller et al. (2017) studied the combined effects of warming during night-time and light pollution in an agro-system composed of alfalfa, *Medicago sativa* L., one of its most common pests, the pea aphid *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), and two of its natural enemies, the ladybeetles *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae). Both ladybugs can hunt at night but *C. maculata* is less effective in the dark because it does not use visual cues, whereas *C. septempunctata* does. The authors showed different interactive effects between the two stressors on the two predators. When these stressors acted alone neither night-time warming nor light pollution influenced ladybeetle control of aphids because warming increased both the aphid growth and predation rates, thus balancing each other out and because both predators hunted effectively in darkness. However, in the more-visual predator *C. septempunctata*, the presence of both stressors caused a synergistic effect on aphid mortality that led to much lower aphid abundances than those when stressors acted alone. These results show us that the non-additive effects of multiple environmental drivers may vary among species, depending on their natural history (Table 1.1.2).

According to the review conducted by Montserrat et al (2021) on recent studies of invertebrate predatory macro-BCAs and the effect of abiotic factors (Table 1.1.2), or the interaction between biotic and abiotic factors (Table 1.1.3), on top-down pest control, the most studied climate change driver in agricultural systems is warming, or its interaction with biotic factors [(Drieu & Rusch, 2017; El-Danasoury & Iglesias-Piñeiro, 2018; Frank & Bramböck, 2016; Miller et al., 2017; Urbaneja-Bernat, Ibáñez-Gual, et al., 2019; Y. J. Wang et al., 2017), except for one study (Jensen et al., 2019)] (Tables 1.1.2 and 1.1.3). As with the biotic factors, the trend of recent works addressing the effects of warming on a predator's ability to suppress pests is that of positive effects on top-down control with increasing temperatures, or no effect at all (Tables 1.1.2 and 1.1.3).

Most papers from Tables 1.1.1, 1.1.2 and 1.1.3 were short-term lab or semi-field experiments, and, more importantly, only a few papers evaluated outputs after dynamics of the whole community under study. This is relevant if we are to estimate impacts from both abiotic factors such as warming and biotic factors such as the presence of other natural enemies or alternative prey/food, among others, in agroecosystems. Considering that predation and competition, and abiotic factors (e.g., warming) are strong selective forces, in order to capture potential evolutionary responses to the environment under study the detection of realized effects of these environments on the strength of trophic cascades needs species in the communities to be allowed to interact during several generations (Montserrat et al., 2021).

Tables 1.1.2 and 1.3: Experimental studies (from 2015 to 2021) addressing: a) whether abiotic (Table 1.2) or the interaction between biotic and abiotic (Table 1.3) factors influenced negatively (" - "), not affected (" ="), or promoted (" +") the top-down pest control; b) the type of experiment performed (" Laboratory" or " Field/semi-field"), and c) whether Population/community dynamics could occur (" No" or " Yes"). Table extracted and modified from Montserrat et al. (2021).

Table 1.2	**Abiotic**							
Driver	**a) Pest control effect**			**b) Type of experiment**		**c) Population/ community dynamics**		**References**
	-	**=**	**+**	**Laboratory**	**Field/ Semi-field**	**No**	**Yes**	
Warming			x	x	x		x	(Y. J. Wang et al., 2017)
			x	x	x	x		(El-Danasoury & Iglesias-Piñeiro, 2018)
			x	x		x		(Frank & Brambock, 2016)
	x	x			x		x	(Millet et al., 2017)
Warming + Light pollution		x	x		x		x	(Miller et al., 2017)

Table 1.3	**Biotic x Abiotic**							
Driver	**a) Pest control effect**			**b) Type of experiment**		**c) Population/ community dynamics**		**References**
	-	**=**	**+**	**Laboratory**	**Field/ Semi-field**	**No**	**Yes**	
Warming/ Interspecific competition			x	x	x		x	(Urbaneja-Bernat, Ibañez-Gual et al., 2019)
Warming/ Predator diversity			x	x		x		(Drieu & Rusch, 2017)
Cooling/Apparent competition	x		x	x		x		(Jensen et al., 2019)

Evolutionary processes

When it comes to evolution, the common wisdom is to think of processes that occur during long periods of time, in the order of millions of years, and that imply the origin of new taxa diverging from other taxa. These processes are framed within the so-called macroevolution. Macroevolution describes patterns on the tree of life at a grand scale across vast periods (Hautmann, 2020). These processes are easily spotted as big events in the history of life, for example, the case of appearance and divergence of the primate group to which we humans belong (Sesink Clee & Gonder, 2012). However, evolutionary processes also occur in ecological time scales (Carroll et al., 2007), and they imply changes in the frequency of single or multiple genes in populations within species over short periods, or a few generations, which are known as microevolution. Such microevolutionary processes may occur via mutation, but also via other processes such as genetic drift, selection, or gene flow, as long as populations hold enough genetic variation in the traits that characterize them, which allows adaptive processes or neutral evolution to occur without the need for new mutations to appear (Hufbauer & Roderick, 2005). Contrary to macroevolution, microevolution is associated with rapid or contemporary evolutionary processes, which occur at ecological time scales (Carroll et al., 2007; Reznick & Ghalambor, 2001).

One can find in the literature many examples of populations undergoing evolutionary processes that have been observed and measured within ecological time scales (Carroll et al., 2007; Szűcs et al., 2019), after such populations have been exposed to biotic (Lawrence et al., 2012; Yoshida et al., 2003), and/or abiotic selection pressures (Berg et al., 2010; Gilman et al., 2010; Hoffmann & Sgrò, 2011; Maloney et al., 2009; Peralta-Maraver & Rezende, 2021). For example, Lawrence et al. (2012) showed in prokaryotes that interspecific competition among 5 species of bacteria exposed to a new environment accelerated the evolution rates of the whole pool of bacteria, compared to when they were left to evolve in isolation. In addition, species from polycultures diverged in the use of resources and evolved to use the waste products generated by heterospecific bacteria. Also, interspecific competition led to a decrease in the growth rates of the whole bacteria community in comparison with those bacteria cultured in isolation, suggesting that there was a trade-off between adapting to the abiotic and biotic selection pressures. Microevolution can also occur in the vertebrate species. Maloney et al. (2009) suggested that the occurrence of two phenotypes (one bigger and dark, the other smaller and lighter-coloured) in the Soay sheep *Ovis aries* L. (Artiodactila: Bovidae), a rare breed of domesticated sheep from the St. Kilda archipelago (United Kingdom), was driven by temperature. Whereas in cold environments the dark and bigger coat phenotype prevailed, in a context of climate change where temperatures were increased, the smaller and light-coloured phenotype prevailed. This was because the frequency of the two phenotypes was driven by the metabolic costs of thermoregulation associated with the absorbing of solar radiation and the selective advantage of body size due to Bergmann´s rule (Bergmann, 1847).

Rapid (micro)evolutionary processes also occur in invertebrates (Garnas, 2018; Hoffmann & Ross, 2018), as one may expect given their relatively short generation times. In fact, this type of evolution can even occur in communities of arthropods that inhabit agricultural ecosystems, despite most of them being short-termed (e.g., horticultural crops) (Belliure et al., 2010; Hoffmann, 2017). This is possible because arthropod pests and their natural enemies typically have extremely short generation times (e.g., less than a week in many mites, both pests and predators) (Belliure et al., 2010). For example, in a review of experimental evolution studies, Belliure et al. (2010) compiled several publications reporting the rapid evolution of mites acquiring resistance to pesticides, and the evolution of several behavioural and life history traits, and sex-ratio, not only in herbivore pest species but also on their predators. Indeed, most responses occurred within the first 10 generations of selection. Some of these papers reported rapid evolution in just two generations, as is the case for resistance to the pesticide hexythiazox in the pest *Brevipalpus phoenicis* Geijskes (Acari: Tenuipalpidade) (Campos & Omoto, 2002), or rapid response (within 2-7 generations) under artificial selection to several foraging traits in the predatory mite *P. persimilis* (Nachappa et al., 2010).

Several studies have demonstrated that rapid evolution in traits can influence population dynamics, species interactions, and ecosystem functioning. In addition, such effects can feed back to affect further evolutionary changes (H. Koch et al., 2014) possibly engaging in eco-evolutionary feedback loops (Schoener, 2011). Therefore, given all these effects, ignoring the occurrence of evolutionary processes (rapid evolution) may dramatically limit the predictive power of most research topics in ecology.

Ecology and evolution to improve BPC: a holistic approach

BPC beyond simple food chains and a benign climate

Uncertainty of successes in BPC might be curtailed if the designs of strategies integrate the ecological and evolutionary processes that agricultural communities undergo, even including a multitrophic perspective (Abdala-Roberts et al., 2019). The presence of omnivorous or top-predator species in agroecosystems can affect the outputs of the BPC strategies, as realized predator–prey interactions may depend on whether other interacting species are present (Blubaugh et al., 2021; Torres-Campos et al., 2020). Indeed, undesired unaccounted competitive or predatory interactions among carnivorous or omnivorous species can counteract and benefit herbivore populations (Rosenheim, 1998; Rosenheim et al., 2004) (see "Biotic factors", p. 12). For example, predation of the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) on the aphidophagous gall midge *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) eggs, reduced the control of aphid pests in peppers when compared to plants without the top predator (Messelink et al., 2011).

Considering that predation and competition, and abiotic factors such as warming, are strong selective forces (see "Evolutionary processes", p. 24), the detection of realized effects of these environments on the strength of trophic cascades intrinsically needs species in the communities to be allowed to interact during several generations to capture potential evolutionary responses to the environment under study. For example, a two-year community dynamics experiment done in avocado orchards revealed that supplying alternative food to predators contributed to pest control in spring, when temperatures were mild, but not in summer when high temperatures reduced the numerical response of predators (Montserrat, Guzmán, et al., 2013).

It is important to know how and to what extent changes in abiotic and biotic conditions affect communities and the evolutionary processes these factors trigger. In addition, this evolution can in turn affect the ecological interactions that produce new biotic conditions that alter the functioning of food webs, leading to eco-evolutionary feedback loops (H. Koch et al., 2014; Moya-Laraño et al., 2012), therefore being able to affect BPC outcomes (Szűcs et al., 2019). Consequently, to reduce uncertainty in BPC caused by selection forces and the evolutionary processes therein, a holistic approach (Figure 1.1.1) of BPC is necessary, one that integrates the effects of both abiotic and biotic factors, as well as the potential evolutionary processes of species that integrate the agricultural systems.

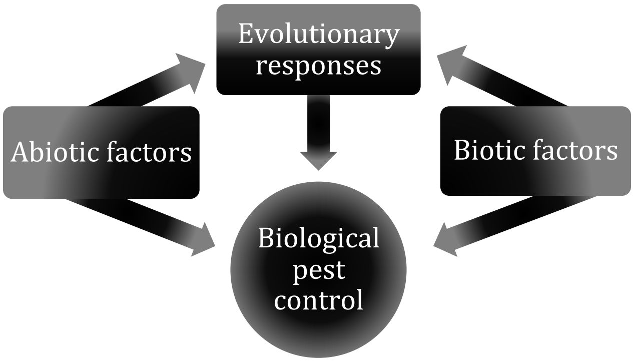

Figure 1.1.1: Proposal of a holistic approach to BPC where biotic, abiotic, and evolutionary processes must be considered to increase BPC success in agroecosystems.

Exploiting intraspecific variability to improve the performance of the BCAs

Negative effects on trophic cascade strength influenced by biotic (see “Biotic factors”, p. 12) and abiotic selection pressures (see “Abiotic factors”, p. 18) offer the opportunity to exploit the inherent intraspecific variability in the populations of BCAs as a tool to improve their performance when they are exposed to either or both types of selection forces. On the one hand, precedent studies verify that ecological interactions have major effects on evolutionary responses (Lawrence et al., 2012; Yoshida et al., 2003) (see “Evolutionary processes”, p. 24). On the other hand, prolonged exposure to extreme temperatures may have severe consequences for an individual’s fitness (Roux et al., 2010) or performance (Dell et al., 2011), even leading protein denaturalization and death of individuals (Hazell et al., 2010). Therefore, increasing periods of biotic and abiotic stress may originate directional selection for ‘tolerance’ to such stresses (Berg et al., 2010; Gilman et al., 2010; Hoffmann & Sgrò, 2011) and exploiting the potential for rapid evolution in natural enemies might become crucial successful BPC-managed agricultural communities.

To exploit the potential for evolutionary adaptation in BCAs it is necessary to firstly identify its <u>functional traits</u> that in addition to holding genetic variation are both <u>response</u> and <u>effect traits</u> (de Bello et al., 2010; Harrington et al., 2010). These traits are defined as morphological, physiological, behavioural, and even phenological traits which impact fitness indirectly influencing growth, reproduction, and survival (Violle et al., 2007). Whereas the list of potential functional traits for BCA genetic improvement is quite extensive [see Bielza et al. (2020)], in Montserrat et al. (2021), we were able to find only one paper evaluating genetically selected populations based on their trophic cascade strength in the trophic chain (Dumont et al., 2019). This is likely because experiments to assess whether particular traits hold genetic variability and are both response and effect are time-consuming. On the one side, measuring the genetic variability of traits needs complex quantitative genetic designs (David et al., 2005; Lynch & Walsh, 1998), which demand a lot of human effort and time. On the other side, measuring the effects of adapted BCAs on trophic cascade strength would require field or semi-field long-term experiments permitting community dynamics. However, work overload can potentially be significantly reduced with the aid of realistic computer simulations (see next section).

Recent works have evaluated the genetic variability of single traits to address specific problems, namely, adaptation of BCAs to lab and mass-rearing conditions (Lommen et al., 2019; Rasmussen et al., 2018), the genetic background of being zoophagous versus phytophagous in zoophytophagous BCA's species (Chinchilla-Ramírez et al., 2020; Dumont et al., 2016, 2019; Dumont, Lucas, et al., 2017; Dumont, Réale, et al., 2017), adaptation to drought (Le Hesran et al., 2019), adaptation to high temperatures (G. H. Zhang et al., 2018), enhanced fitness feeding on suboptimal foods (Mendoza et al., 2020a), or faster developmental time (Siddiqui et al., 2015). Others applied artificial selection towards specific phenotypes, for example, wingless predators (Lommen et al., 2019; Seko & Miura, 2020; Takahashi et al., 2019) or larger body size (Mendoza et al., 2020b) (Table 1.1.4). Negatively correlated responses with other functional traits, however, were mostly not evaluated, and thus potential genetic trade-offs hampering target evolutionary responses were ignored (Table 1.1.4). Furthermore, some of the works suffered from methodological issues detected also in the recent review by Lirakis and Magalhães (2019), which may lead to questioning the robustness of the results: poor replication, base populations being started from way too few individuals, or collected from single sites, or the number of isolines/families to estimate the heritability of traits being too low [e.g., below 20 for isolines, (David et al., 2005)] (Table 1.1.4). Altogether, these poor results may explain why the genetic breeding of BCAs is a methodology still in its infancy; yet, a recent increase in reviews (Bielza et al., 2020; Leung et al., 2020; Lirakis & Magalhães, 2019; Lommen et al., 2017) and experimental works [e.g., (Lartigue et al., 2021)] addressing it, enlighten the need for setting the genetic improvement of BCAs in motion in a very near future.

Tables 1.1.4: Experimental studies (from 2015 to 2021) on BCAs´ evolutionary responses, including a) context of the studies, b) n° of sites and/or individuals used to obtain the initial BP, c) n° of replicated populations tested, d) the implemented methodology, e) whether heritability was measured, f) target traits, and e) whether genetic correlations with other traits were assessed. Table from Montserrat et al. (2021).

Genetic variability of BCAs traits / Experimental evolution using BCAs.							
a) Context	b) # Sites/BP (# indiv. /site	c) # Replicated populations	d) Experimental method	e) Heritability (#IFL or IGL)	f) Target trait	g) Genetic Correlations with other traits	References
Predator evolutio	1 (10000)	1	EE	No	Heat shock, LH (T, F, Lo, OR)	No	(Zhang et al., 2018)
Mass rearing	3 (500)	n/a	CB	No	LH (PR, ST) Body size, optimal Tª	No	(Rasmussen et al., 2018)
	Several (>100)	2	POR + AS	Yes (41)	Wing truncation	No	(Lommen et al., 2019)
Predator performance	14 (NG)	n/a	None	No	LH (HR)	No	(Le Hesran et al., 2019)
	30 (50-100- <100)	1	AS + FS	No	LH (F)	No	(Mendoza et al., 2020a)
	NG (NG)	1	AS	No	LH (DT)	No	(Siddiqui et al., 2015)
	3 (NG)	n/a	None	No	Overwintering	No	(Takahasi et al., 2019)
Zoo-phytophagous performance	6 (NG	n/a	IGL	Yes (11)	Zoophagy	Yes	(Dumont et al., 2019)
	2 (9, 16)	n/a	IFL/IGL	Yes (8-9)	Zoo-*vs* phytophagy	Yes	(Chinchilla-Ramírez et al., 2020)
	2 (300-NG)	n/a	IGL	Yes (12)	LH (PR, DT)	Yes	(Dumont et al., 2016)
	Several (NG)	n/a	IGL	No	Zoo *vs* phytophagy	No	(Dumont, Lucas, et al., 2017)
	6 (NG)	n/a	IGL	No	Cannibalism rate	No	(Dumont, Réale, et al., 2017)
Predator trait	2 (80/150)	n/a	GS	Mendelian inheritance	Wingless	No	(Seko & Miura, 2020)
Predator	30 (NG)	1	AS + FS	No	Body size	No	(Mendoza et al., 2020b)

*Abbreviations: BP (Base population) NG (Not Given), AS (Artificial Selection), FS (Full-sib design), CB (Cross breeding (reciprocal cross)), IFL (Isofemale line), IGL (Isogroup line), POR (Parent-offspring regression), GS (Genetic segregation). LH (Life-history traits): F (Fecundity), PR (predation rate), DT (development time), Lo (Longevity) ST (starvation tolerance), Hatching rate (HR), Oviposition rate (OR), n/a: Not applicable.

Future prospects: food web engineering as an integrative tool to approach BPC.

Food web engineering (FWE hereafter) was defined as an extension of biological pest control that integrates ecology and evolution into the management of agricultural systems potentially exposed to biotic/abiotic stressors (Montserrat et al., 2021; Moya-Laraño et al., 2012, 2014). In FWE, one seeks to combine knowledge in evolutionary biology with that of food web theory in order to engineer webs that are efficient at keeping crop pests at bay, thereby increasing trophic cascade strength. In the context of global warming, for instance, researchers should improve the performance of natural enemies at warm temperatures over that of their potential prey. To that end, one could use artificial selection targeting optimal performance temperature in a trait that improves predator top-down control, such as attack rate (Figure 1.1.2.a), and an evolutionary response will be obtained if, and only if, there is genetic variation for that trait and no major genetic trade-offs. This would successfully bring the predator evolutionarily ahead of its target pest prey, as the latter would, in principle, evolve more slowly from natural selection occurring at the pace of global warming in the wild (Figure 1.1.2.c). Alternatively, as it may not always be obvious which trait to target in artificial selection directed at improving BPC, one can use experimental evolution at high temperatures (e.g., that predicted from climate change, T_{cc}), which would act as the selective pressure (Figure 1.1.2.b). After several generations, one should test the performance of the predator on keeping prey numbers down at different temperatures. Additionally, since even in agricultural environments predators and prey usually coexist with other species, in experimental evolution one should consider the food web or community context (Figure 1.1.2.d). This can be accomplished by crossing temperature (T_{cc}) with treatments including community modules (Holt, 1997) in which both direct and indirect ecological and selection effects are considered. Top-down predator performance should be then tested for improvement in the temperature-context combination closer to the agricultural setup. Unfortunately, the combination of community contexts and temperature treatments may soon grow extremely large and make this sort of evolutionary experiments unfeasible. One potential solution is to run simulations in Next-Generation Individual-Based Models (NG-IBM- (Grimm et al., 2017)) which may be used as a link between the researcher and the real agroecosystem (Moya-Laraño et al., 2012, 2014), helping engineering food webs that maximize the strength of trophic cascades from target predators (Figure 1.1.3).

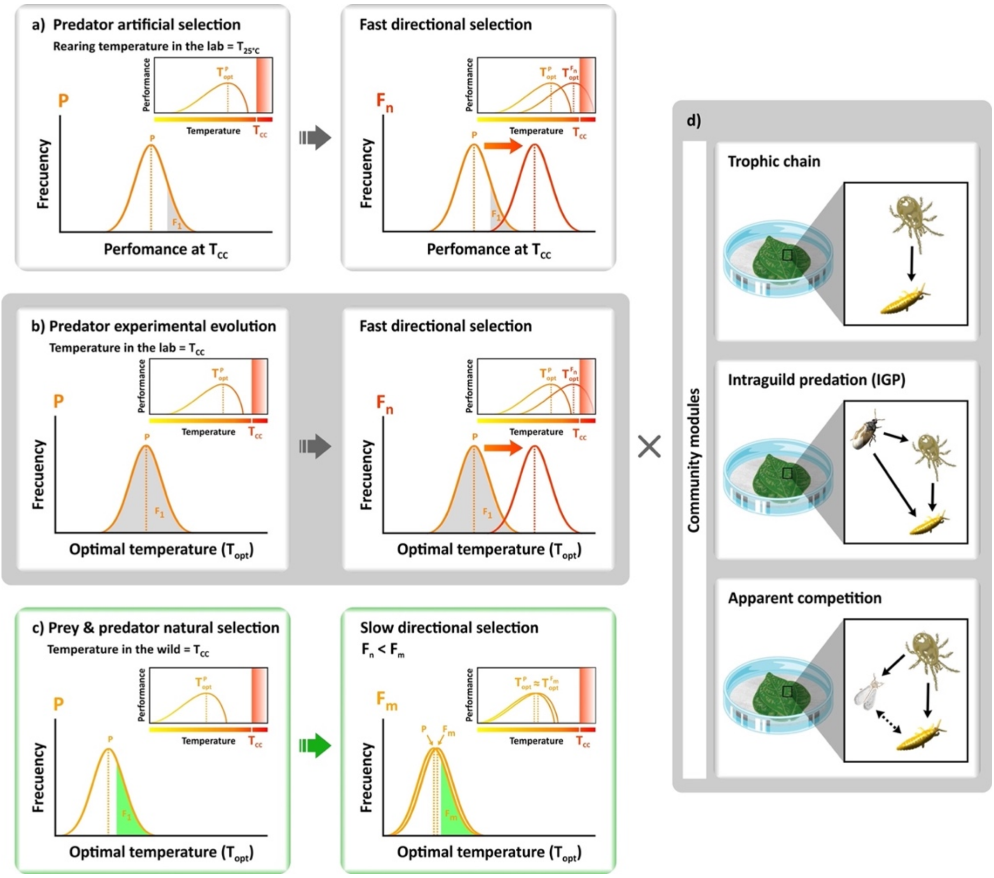

Figure 1.1.2. Different approaches to obtain BPC strains adapted to climate change. **a)** Artificial selection, in which at each generation all individuals (in replicated populations reared at conventional lab temperature) are tested for performance at target climate change temperature (T_{cc}). Only those that perform better (e.g., have higher attack rates), are allowed to breed (grey shadow). The offspring are tested again the following generation and so on. **b)** Experimental evolution, in which replicated populations are reared at T_{cc} and natural selection let to occur in the lab. In both a) and b) the response to selection (middle panel) is tested after *n* generations. After selection, the temperature of optimal performance (T_{opt}) should increase and approach T_{cc} (insets). Natural selection occurring in both the target BCA and the target pests occurs at a much slower pace in the wild **(c),** both because the number of generations is much less (*m*) and because selection is less intense. **d)** Because natural selection occurs in a community context in the wild, crossing abiotic factors with different community modules during experimental evolution may better grant the procurement of climate change adapted strains of BCAs. In d) solid arrows account for direct negative interactions (predation) and dashed arrows for indirect negative interactions (apparent competition). Figure from Montserrat et al. (2021).

In particular, the software Weaver (Moya-Laraño et al., 2012, 2014) combines information on the biology of the species involved in relatively small communities with different genetic and food web architectures. This along with the inclusion of the principles of the metabolic theory of ecology (J. H. Brown et al., 2004; Dell et al., 2011) allows testing for the responses of many context-temperature combinations, which helps, for instance, understanding what is the top-down strength of predators with different traits (e.g.; body size, foraging activity, temperature-dependent performance). The researcher can then select a few more promising combinations (e.g.; more persistent and which maximize effects cascading down) to be used for experimental evolution assays. Feeding these IBMs with parameters as accurately as possible (e.g., real genetic architectures) will help matching the simulation results with those of experimental evolution. Fortunately, the biology of pests and their natural enemies is much better known than, for instance, the biology of species that integrate soil food webs, for which we have nevertheless been successful at generating NG-IBM simulations including up to 20 predator and prey species with distinct genetic architectures (Moya-Laraño et al., 2014). FWE is an example of a Feedback Research Program (Moya-Laraño et al., 2014), in which the data from the real system serves to feed the IBM, and the IBM results serve to collect further data that may be subsequently used to feed further IBM simulations. This feedback process should make predictions more realistic and accurate at each step (Figure 1.1.3), approaching the *in-silico* world to the *in-vivo* world to eventually improve BPC. Since we expect novel emerging properties to arise and find explanations during this process; for instance, the fact that pairwise interactions cannot predict emerging simple food chains (Torres-Campos et al., 2020), this approach fits well into the field of eco-evolutionary systems biology by analogy to Friedman & Gore, 2017, as a model and real data are combined to improve our knowledge about the emerging properties of agroecosystems. Therefore, gathering information on 1) trait quantitative genetics, and 2) the responses to bio-abiotic selective pressures are important steps, in addition to running simulations, in the FWE loop to improve BPC. This PhD Thesis focuses on these two aspects of FWE.

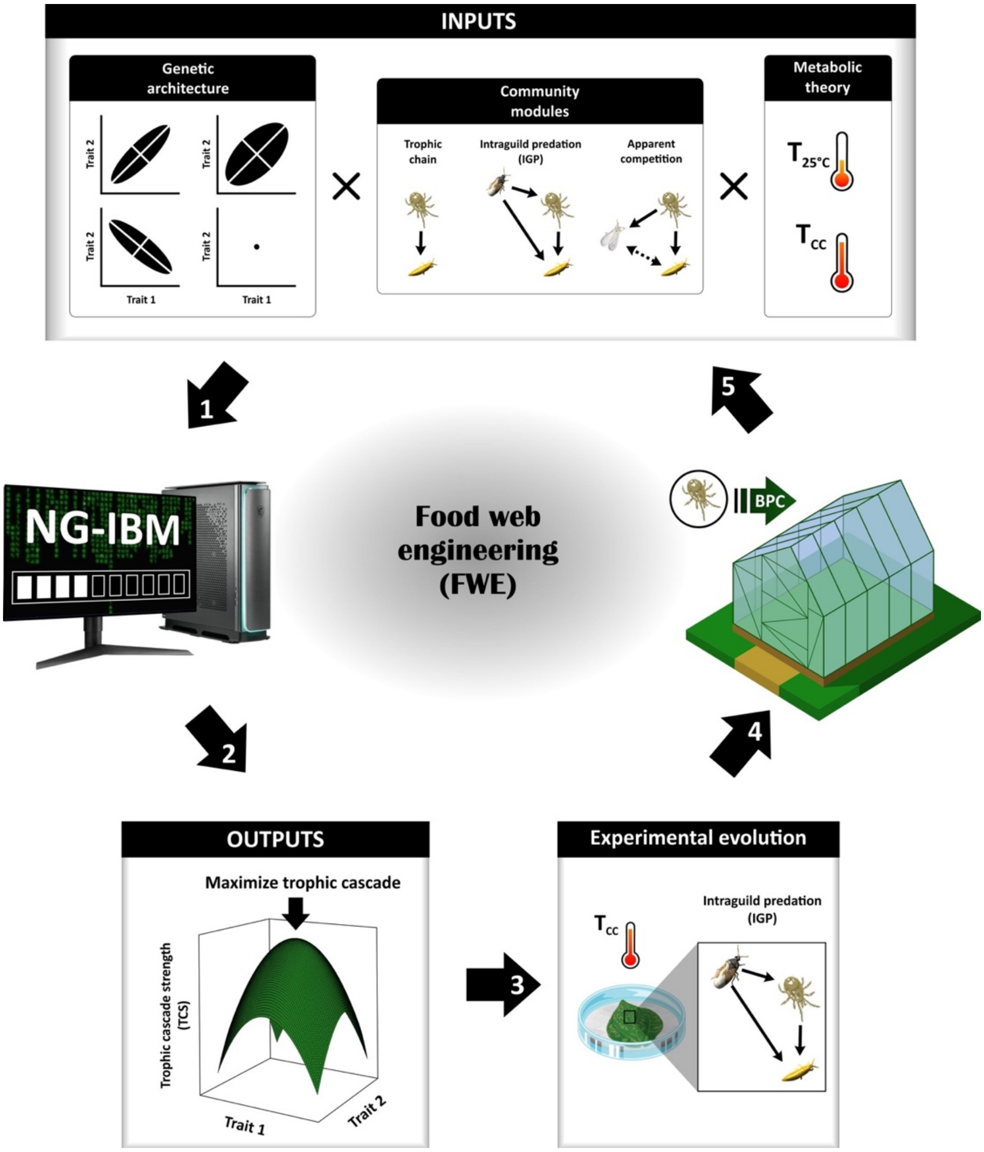

Figure 1.1.3: Food web engineering feedback loop. In step 1 (starting counterclockwise at the top panel), different combinations of potential genetic architectures (left top panel) for all the species involved, are tested against different food web architectures (community modules) and temperatures, by feeding a NG -IBM (left panel). Step 2 is to analyse the outputs of the simulations and to obtain the predator trait combination that maximizes the trophic cascade under climate change conditions. If there is a candidate predator whose genetic architecture is similar to that in the simulations, experimental evolution is conducted in step 3. The new strains are then tested for efficacy in BPC at step 4. The new data obtained during steps 3 and 4, including the details of the genetic architecture of several predator species, are used to feed further –now more realistic- simulations, starting a new iteration of the loop, thereby increasing the efficiency of BPC. Figure from Montserrat et al. (2021).

The model species: the predatory mite *Amblyseius swirskii*

Predatory mites of the family Phytoseiidae are widely used as biocontrol agents against some of the most important and damaging groups of pests, (i.e., phytophagous mites such as spider mites, whiteflies, and thrips among others). More than 60% of the market of BCAs is composed of phytoseiid predatory mites, with about 20 species commercially available worldwide (Knapp et al., 2018). In addition, in the list of the 10 most important invertebrate BCAs used in augmentative biological control, ranked by the number of countries in which they are used, four species of phytoseiid mites are included (Knapp et al., 2018), with the species *A. swirskii* taking the lead (Calvo et al., 2015; van Lenteren, 2012) (Table 1.1.5).

Table 1.1.5: List of the most important BCAs (by turnover) used in augmentative biological pest control (Knapp et al., 2018).

Species	Family	Target(s)	Year of first use
Amblyseius swirskii	Phytoseiidae	Whiteflies, thrips, mites	2005
Phytoseiulus persimilis	Phytoseiidae	Spider mites	1968
Neoseiulus californicus	Phytoseiidae	Mites	1985
Macrolophys pigmaeus	Miridae	Whiteflies	1994
Encarsia formosa	Aphelinidae	Whiteflies	1925
Orius laevigatus	Anthocoridae	Thrips	1993
Nesidiocoris tenuis	Miridae	Whiteflies, *Tuta absoluta*	2003
Neoseiulus cucumeris	Phytoseiidae	Thrips	1985
Eretmocerus eremicus	Aphelinidae	Whiteflies	1995
Aphidius colemani	Braconidae	Aphids	1991

At present, *A. swirskii* is released in more than 50 countries all over the world (Calvo et al., 2015), and it is one of the most successful BCA. Some of the key features of its success are: 1) it is a generalist predator that forages on many soft-bodied arthropod pest species in the groups of whiteflies, thrips and herbivore mites, 2) it can develop and reproduce on other food sources, such as pollen, and thus it can persist when pest densities are low and be released preventively before the pests are present in many crops; 3) it establishes with no problem in many vegetable crops of economic importance, such as pepper, cucumber, and eggplant, as well as in ornamentals and fruit tree crops, 4) it is an efficient predator at a wide range of temperatures, and 5) it is easily mass-produced (Calvo et al., 2015). Furthermore, *A. swirskii* is a free-living leaf-dwelling predatory mite that has been reported on a surprisingly wide variety of plants, from trees to grasses (Swirski & Amitai, 1997). All these features make *A. swirskii* a very good model to study which traits in predatory mites make them good as biological control agents, and what is the potential for predatory mites to undergo rapid evolution and adapt to a rapidly changing climate.

Biology of Amblyseius swirskii

Kingdom: Animalia
Phyllum: Arthropoda
Subphyllum: Chelicerata
Class: Arachnida
Subclass: Micrura
Infraclass: Acari
Superorder: Anactinotrichidae
Order: Mesostigmata
Suborder: Dermanyssina
Superfamily: Ascoidea
Family: Phytoseiidae
Subfamily: Amblyseiinae
Genus: *Amblyseius* Berlese, 1904
Species: *swirskii* Athias-Henriot,1962

Amblyseius swirskii is a predatory mite of the family Phytoseiidae. This family is composed of more than 2500 species distributed in 95 genera (Demite et al., 2023). It was described for the first time in Israel (Athias-Henriot, 1962) in almond trees (*Prunus amygdalus* Miller). Synonyms of the species are *Amblyseius capsicum*, *Amblyseius enab,* and *Amblyseius rykei* (Demite et al., 2023), and it has been given other names such as *Typhlodromips swirskii* or *Amblyseius swerski* (Demite et al., 2023).

According to its world distribution, before it was commercially available in 2005, it has been reported mainly in the Middle East (Israel, Gaza-strip and Egypt) although some individuals have been reported in Azerbaijan, Cape Verde, Georgia, Italy, Turkey and Yemen (for specimens mentioned as *A. swirskii*), and Benin, Burundi, Cuba, Democratic Republic of Congo, Ghana, Kenya, Malawi, Nigeria, Tanzania and Zimbabwe (for specimens mentioned as *A. rykei*) (Demite et al., 2023) (Figure 1.1.4). As previously mentioned, all individuals firstly reported as *A. rykei* are from Africa, with the exception of Cuba (Martínez et al., 2004) which is quite doubtful and a misidentification can be suspected, (Tixier et al., 2022). Despite it was synonymised with *A. swirskii* (Zannou et al., 2007; Zannou & Hanna, 2011) two hypothesis to this strange distribution appear: i) *A. rykei* is not a synonym of *A. swirskii* and the African specimens considered by (Zannou & Hanna, 2011) were actually issued from commercial releases of *A. swirskii*, or (ii) *A. rykei* is a synonym of *A. swirskii* and gaps in the geographical distribution are explained by particular climatic conditions (Tixier et al., 2022). Nowadays, it is widely spread around the world as it is used as BCA in many European countries, as well as North America, North Africa, Japan and Argentina (Cédola & Polack, 2011; EPPO, 2021; Sato & Mochizuki, 2011) (Figure 1.1.4).

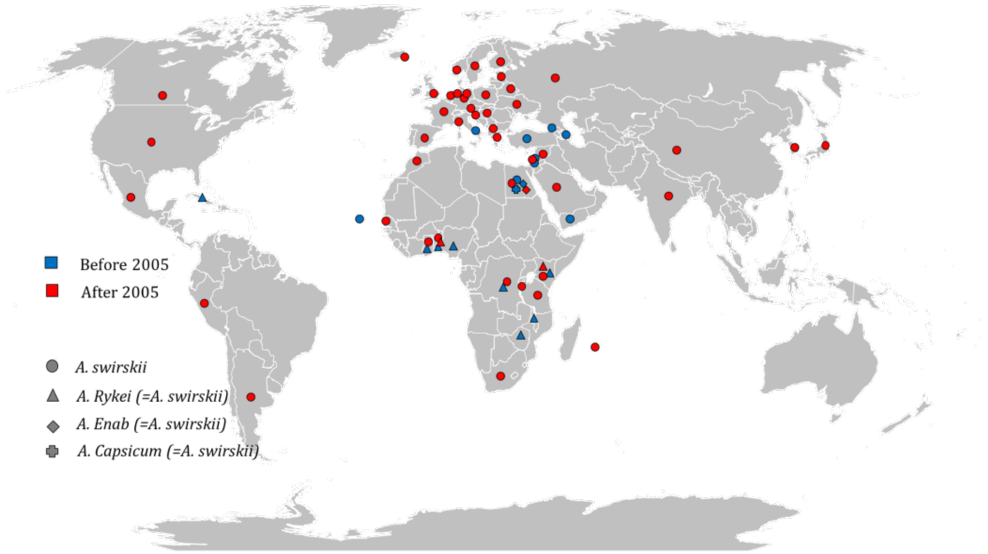

Figure 1.1.4: Distribution of *A. swirskii* (including synonyms) before (blue dots) and after (red dots) its commercial availability, from 2005 on, as BCA. Red dots include locations where it has been reported or locations where it is sold. Sources: (Demite et al 2023) and www.koppert.com.

The morphology of *A. swirskii* is pearl shaped with a maximum body size of 500µm. As an arachnid, it has 4 pairs of legs (except egg and the larval stages, which has 3 pairs). The first pair of legs usually points forward as it is mostly used with a sensory rather than a locomotor purpose. As with most phytoseiids, *A. swirskii* cannot be distinguished to the naked eye from most species of the same family. To be identified it is necessary to examine different characteristics of taxonomic interest under the microscope, such as the length and position of setae on the dorsal shield, the shape of the annal shield, and the shape of the spermatheca, among others (Z.-Q. Zhang, 2003). Its colour depends on the diet and it varies from reddish to brownish, although the most common colour is amber or pale yellow.

Its life cycle consists of five developmental stages: Egg, larva, protonymph, deutonymph and adult (Figure 1.1.5). Eggs are c.a. 200µm in size, oval-shaped, and have a pale white colour. Larvae are slightly bigger than eggs, with a dorsal shield of 180-220µm, have 3 pairs of legs and have a pale white to nearly transparent colour. They are facultative feeders, that is, they can moult to protonymph without the need to eat (Schausberger & Croft, 1999). Protonymphs and deutonymphs have four pairs of legs and are much more mobile than larvae, with a dorsal shield size of 216-266µm and 281-315µm respectively. They are mandatory feeders and darker than larvae, from beige to pale yellow. A detailed description of immature stages can be found in (Swirski et al., 1973). As most ectotherms, adults have sexual size dimorphism, with females being bigger than males (Fairbairn, 1997).

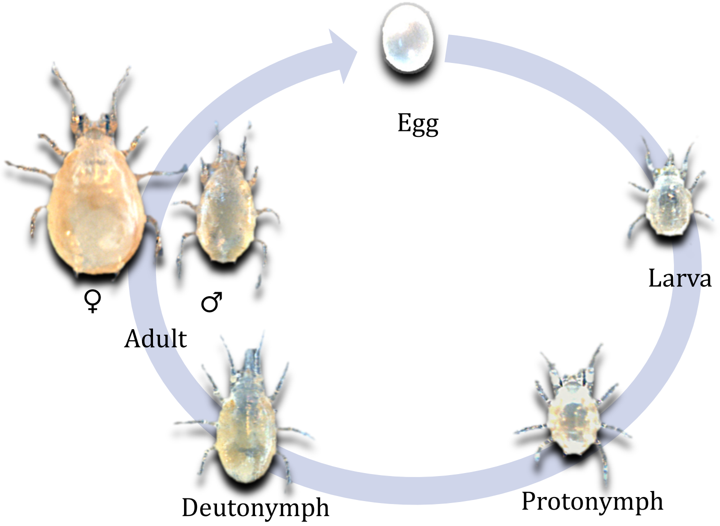

Figure 1.1.5: Life cycle of phytoseiids. Pictures of developmental stages of *A. swirskii*

Females need to mate to be able to oviposit. They are pseudo-arrhenotokous; that is, both males and females develop from fertilized eggs but males lose their paternal part of the genome at an early stage of their development (Sabelis & Nagelkerke, 1988). Consequently, males are haploids (n) and females diploids (2n) (Wysoki & Swirski, 1968). Females produce one egg at a time that when fully developed, occupies a very large proportion of the body cavity (Chant, 1985). They preferentially oviposit on hair tufts or other types of domatia (Figure 1.1.6.a), on top or along non-glandular trichomes (Figure 1.1.6.b), and prefer to oviposit in clusters (Figure 1.1.6.c) (Faraji et al., 2002a, 2002b), to minimize egg predation from conspecifics and heterospecific predators (Walter, 1996), or from counter-attacking prey (de Almeida & Janssen, 2013; Faraji et al., 2001, 2002a), and to oviposit near food sources to ensure their offspring have sufficient food after hatching (de Almeida & Janssen, 2013).

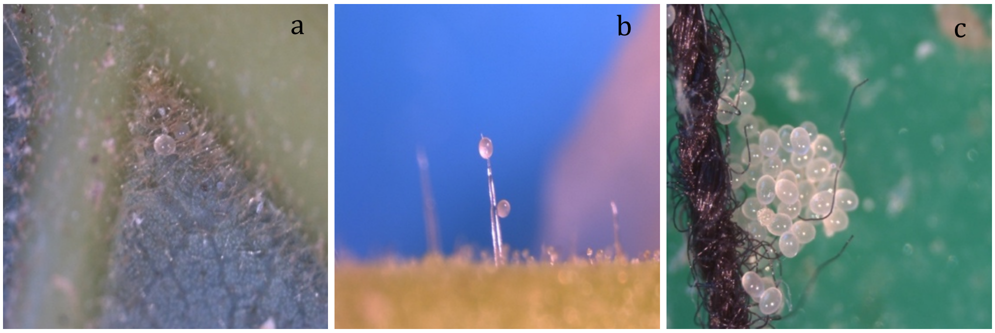

Figure 1.1.6: Pictures of *A. swirskii* egg slayed on **a)** in hair tufts (domatia) **b)** on top of non-glandular trichomes, and **c)** in clusters, to minimize egg predation.

Life history traits (developmental rate, survival, longevity, fecundity) usually depend on the type of food, on its availability, and on abiotic conditions such as temperature and relative humidity (Figure 1.1.7, for developmental time *vs* temperature when feeding on different food sources). When individuals are fed on *Typha latifolia* L., and at 60% RH, the lower and upper-temperature thresholds wherein development occurs are 8°C and 37,5°C, respectively, with the optimum temperature at 31,5°C (Lee & Gillespie, 2011) which were similar to those reported by Farazman et al. (2020) when fed with *T. urticae*. However, other studies report no development beyond the larval stage at 35°C (Allen, 2009). The intrinsic rate of increase (*r*) is above 0 leading to population increase between 15,5 and 37°C, with its maximum at 30°C (Lee & Gillespie, 2011). It is estimated that *A. swiskii* populations will optimally respond numerically to food availability (pollen or prey) between 20 and 32°C (Lee & Gillespie, 2011). *Amblyseius swirskii* is a non-diapausing species (Allen, 2009; Veerman, 1992). Yet, Dicke et al. 1989 were able to induce a low diapause incidence (25-30% approx.) under L:D 8:16 and 17°C on individuals fed with pollen of *Vicia faba* L..

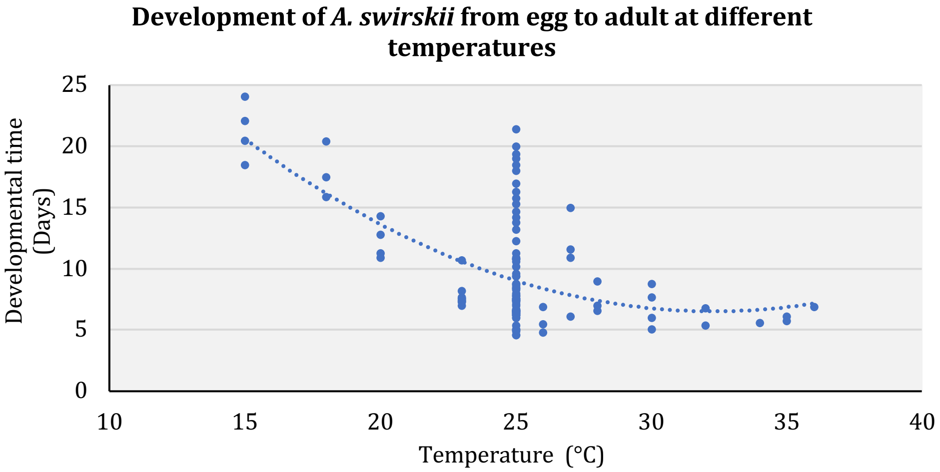

Figure 1.1.7: Developmental time of *A. swirskii* from egg to adult on different diets at each temperature. The relative humidity was never below 60%. Notice that most of the research has been conducted at 25°C. Data compiled from (Ali & Zaher, 2007; Allen, 2009; Chen et al., 2011; Delisle, Brodeur, et al., 2015; El-Laithy & Fouly, 1992; Goleva & Zebitz, 2013; Lee & Gillespie, 2011; Midthassel et al., 2013; Momen, 2009; Momen & Abdel-Khalek, 2008; Momen & EL-Saway, 1993; Nguyen et al., 2013, 2014; Nomikou et al., 2001; Onzo et al., 2012; Park et al., 2011; Riahi et al., 2017b, 2017a; Seiedy et al., 2017; Wimmer et al., 2008; Zannou & Hanna, 2011)

Phytoseiid mites are typically classified depending on their lifestyle, based on their food habits and related biological and morphological traits (McMurtry et al., 2013; McMurtry & Croft, 1997). Specifically, in Type I (Specialized mite predators) predatory mites are categorized into three subtypes depending on their degree of specificity to mite prey: subtype I-a: Specialized predators of the genus *Tetranychus* (Tetranychidae); subtype I-b: Specialized predators of web-nest producing mites (Tetranychidae); subtype I-c: Specialized predators of tydeoids (Tydeoidea); in Type II (Broadly specific mite predators), predatory mites are often associated with dense web-producing species of tetranychid mites. Type III (Generalist predators) includes a diverse group of generalist feeders of pollen, mites, and small insects. This group is subdivided into 5 subgroups, based on the microhabitat they occupy: subtype III-a: Generalist predators living on pubescent leaves; subtype III-b: Generalist predators living on glabrous leaves; III-c: Generalist predators living in confined spaces on dicotyledonous plants; III-d: Generalist predators living in confined spaces on monocotyledonous plants; III-e: Generalist predators from soil/litter habitats. Type IV (Generalist predators of polyphagous plant mites but specialist feeders on pollen). Another classification is based on the morphology of the gnathosoma (S. Liu et al., 2017). Group I include specialized and selective predators of *Tetranychus* species, Group II includes generalist predators, and Group III includes specialized pollen feeders. *Amblyseius swirskii* is categorized as Type III and Group II, respectively. In both categories the species is defined as a generalist predator; that is, in addition to animal prey the species can also survive and reproduce on different types of pollen. It develops to adulthood, and it oviposits, when feeding on several mite and insect species (Calvo et al., 2015), and on the pollen of various plants and trees (Delisle, Brodeur, et al., 2015; Goleva & Zebitz, 2013; Ragusa & Swirski, 1975), and on artificial diets (Abou-Awad et al., 1992; Nguyen et al., 2013). Yet, it is not able to feed on structural plant tissues (Adar et al., 2012, 2015; Nomikou et al., 2003b).

Amblyseius swirskii, as with most phytoseiid mites, is able to engage in cannibalism and intraguild predation with other predator species (Bohloolzadeh et al., 2018; Ji et al., 2015; Momen & Abdel-Khalek, 2009; Rasmy et al., 2004). They do cannibalise, but their fecundity and longevity decline (Ji et al., 2015; Rasmy et al., 2004). This practice allows the species to survive in periods of prey shortage, yet *A. swirskii* can survive up to a week without any food source (Buitenhuis et al., 2010a). Its ability to forage on other BCAs may have negative implications on BPC, as in the case of the control of the western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) by the predatory mites *A. swirskii* and *Neoseiulus cucumeris* Oudemans (Acari: Phytoseiidae) on yellow chrysanthemum *Dendranthema grandiflora* Tzvelev. In this system, *A. swirskii* exerted great predation pressure on *N. cucumeris* with no detrimental effects on the life-history traits in the former species, as the intra-guild prey (*N. cucumeris*) was as good as, or even better, food source for *A. swirskii* than the shared prey (thrips) (Buitenhuis et al., 2010b).

As BCA, *A. swirskii* performs well when it preys on the two-spotted spider mite, *T. urticae* (El-Laithy & Fouly, 1992) -despite it does not deal satisfactorily with the webbing of the herbivore (Messelink et al., 2010)-, eriophyoid mites such the tomato russet mite *Aculops lycopersici* Massee (Acari: Eriophydae) (Park et al., 2010), the broad mite *Polyphagotarsonemus latus* Banks (Acari: Tarsonemidae) -a serious pest of ornamentals and vegetables (Onzo et al., 2012; Tal et al., 2007; van Maanen et al., 2010)-, the western flower thrips, *F. occidentalis* (Messelink et al., 2005) and the poinsettia thrips, *Echinothrips americanus* (Morgan) (Thysanoptera: Thripidae) (Ghasemzadeh et al., 2017)-, the two most damaging whitefly pests in horticultural crops, *Bemisia tabaci* Gennadius (Nomikou et al., 2002) and *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) (Messelink et al., 2008, 2010)-, and the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Juan-Blasco et al., 2012). *Amblyseius swirskii* is used as BCA in a wide variety of vegetable crops as well as in ornamentals and fruit trees (Calvo et al., 2015). In fact, it has been reported on 132 plant species, belonging to 97 genera and 48 families and these reports mainly correspond to cultivated plants (Tixier et al., 2022). However, *A. swirskii* does not perform well as BCA in crops that express glandular and non-glandular trichomes as constitutive defences (Buitenhuis et al., 2014), such as tomatoes (Paspati et al., 2021).

Objectives

This thesis is framed within the project entitled "Towards food web engineering: linking trait variability to ecosystem function", founded by the Spanish Ministry of Economy and Competitiveness (CGL2015-66192-R), and it is embedded within the FWE framework as a first step to generate experimental data with which to feed the NG-IBM Weaver in the future (Figure 1.1.3 -inputs, p. 33). Specifically, it covers the evaluation of the genetic architecture of relevant traits in BCAs when individuals are exposed to biotic and abiotic selection pressures, to determine which ones are response functional traits. Ultimately, the main objective of this thesis is to set the groundwork for a holistic approach to the BPC and generate knowledge about evolutionary processes that will serve to offer solutions "à la carte" for specific agricultural systems with unresolved pest problems, or with expected aggravated future problems caused by unfavourable future climate change conditions, such as warming. This main objective is addressed specifically in the following 2 objectives:

Objective 1: To estimate the broad sense Heritability (H^2) of a wide number of life-history, morphological, physiological, and behavioural traits in the predatory mite *A. swirskii*, to define those with potential to be response traits, and to assess genetic correlations among traits to detect potential trade-offs. To achieve this goal, I created isofemale lines by means of full-sib designs and measured different traits of individuals from these isogenic lines. Furthermore, because commercial populations are expected to harbour less genetic variability, I compared the heritability of traits between two populations, a commercial strain -obtained from a well-known international company- and a wild strain -captured by myself in the area where this species is native. This objective is addressed in Section 2 of this thesis.

Objective 2: Firstly, to disentangle evolutionary processes driven by either, or both abiotic and biotic factors through exposing a population holding enough genetic variability in several traits to selection pressures accounting for both the community context (presence of a top-predator) and climate change (high temperature), in a full-crossed factorial design. In this objective, I evaluated whether traits holding heritable genetic variability can evolve, and towards what direction. Secondly, to determine whether populations exposed to biotic and/or abiotic selection pressures have adapted to the new environment, compared to populations that did not undergo prolonged exposure to stressful conditions. This objective is addressed in the Section 3 of this thesis.

SECTION 2: Heritability and genetic correlations

Introduction common to Chapters 2 and 3

Restrictions for sources of new potential BCAs

Biological pest control (BPC) is a crop management practice that fully complies with the demands of the Directive 2009/128/EC (European Parliament, 2009) and the current Common Agricultural Policy (CAP) of the European Union. Since the application of this Directive, the number of growers applying BPC has steadily increased in the EU (van Lenteren et al., 2018).

It is not long ago that BCAs to be used against invasive exotic pest species were sought, and imported from the areas where both natural enemies and pests naturally co-occurred. However, with the implementation of the Nagoya protocol and other governmental regulations (Cock et al., 2010; Deplazes-Zemp et al., 2018; Hunt et al., 2011), the importation of exotic BCAs into the EU has been severely restricted, but see recent successful examples, like *Tamarixia dryi* Waterson (Hymenoptera: Eulophidae) against *Trioza erytreae* Del Guercio (Hemiptera: Psyllidae) (Urbaneja-Bernat, Pérez-Rodríguez, et al., 2019), or *Anagyrus aberiae* Howard (Hymenoptera: Encyrtidae) (Guerrieri & Cascone, 2018) and *Allotropa delotococci* Förster (Hymenoptera: Platygastridae) (Buhl, 2019) against *Delottococcus aberiae* De Lotto (Hemiptera: Pseudococcidae).. As consequence, the screening for new species of BCAs is strongly limited to native species (Cock et al., 2010; Lommen et al., 2017; Van Lenteren et al., 2011) (see “Strengths, weaknesses, and limitations”, p. 8), or commercialized species that were already available before the implementation of the protocol.

As reported in section “Factors affecting the outcomes of BPC” of this thesis (p. 12), in the literature there are numerous examples where BCAs were not effective enough and failed at controlling target pests, probably because commercial BCAs did not adapt satisfactorily to the field conditions where they were released (Cock et al., 2016; Collier & Van Steenwyk, 2004). Indeed, BCAs might be exposed to environmental stressors (e.g., extreme temperatures, presence of antagonistic species) that can affect negatively their efficiency at controlling pests (see “Factors affecting the outcomes of BPC”, p. 12). Furthermore, the potential of populations to respond rapidly to changes in the environment is only possible when populations hold enough intraspecific genetic variability (Lommen et al., 2017). Thus, commercial populations of BCAs, which likely have undergone several bottlenecks or genetic drift during the process of mass rearing, in addition to unwanted, and mostly unmeasured selection, might have limited capability for adaptation to new environments.

The problem of mass rearing of BCAs

Biological control agents are mass-reared in bio-factories for their release in large numbers (van Lenteren, 2012). Typically, in the process of mass rearing, there is a trade-off between quantity (that is, the number of individuals produced per unit of time) and quality (that is, the ability of populations to perform as well as wild populations after their release) of BCAs (Nunney, 2003). Furthermore, BCAs are usually cultured in environments that differ from those where they will be released, for many generations (Dicke et al., 1989). This can lead to populations undergoing undesired selection to the mass-rearing conditions, and to maladaptation of BCAs to the environments of release (Postic et al., 2021; Sørensen et al., 2012). Indeed, the adaptation rates of arthropods to lab conditions can be extremely high (Hoffmann & Ross, 2018). This unintended selection can rapidly reduce the genetic variability of biological traits relevant to pest control (E. Wajnberg, 2004). In addition, inbreeding due to bottlenecks or genetic drift during mass production can also lead to rapid loss of genetic variability in commercial populations of BCAs (Rasmussen et al., 2018; Roderick & Navajas, 2003; Sørensen et al., 2012). In fact, Postic et al. (2021), Rasmussen et al. (2018) and Paspati et al. (2019) reported higher levels of genetic variability in wild than in commercial populations of the generalist aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae), the pirate bug *Orius majusculus* Reuter (Hemiptera: Anthocoridae), and the predatory mite *Amblyseius swirskii*, respectively. In the latter study, the authors found that the commercial strain was 2.5 times less heterozygous than the wild strain.

To solve the problem of genetic variability loss in captive populations of BCAs, Nunney (2003) proposed some recommendations: i) Usage of more than one source of founding populations, in large numbers, ii) maintaining stocks as a large number of inbred lines (isolines) thereby minimizing their capacity to evolve, iii) if captive populations are not maintained as a large number of inbred subpopulations, regularly augmenting or replacing them with wild individuals after a defined number of generations. Whether biocontrol companies follow these recommendations is not known.

Genetic breeding of natural enemies to increase their efficiency as BCAs

Although the idea of breeding BCAs is not new (E. Wajnberg, 2004), the interest in this approach has recently increased (Bielza et al., 2020; Kruitwagen et al., 2018; Leung et al., 2020; Lirakis & Magalhães, 2019; Lommen, 2013; Lommen et al., 2017; Montserrat et al., 2021; Plouvier & Wajnberg, 2018; Thompson et al., 2022) due to *i*) a significant increase in the use of BPC worldwide. Indeed, augmentative pest control is applied on more than 30 Mha in the world, and even some crops are 100% managed under BPC, for example, the greenhouse production of sweet peppers in South-eastern Spain (Acebedo et al., 2022), which has a sales growth rate greater than 15% per year (van Lenteren et al., 2018); *ii*) a decrease in the availability of BCAs, mainly due to legal restrictions imposed by the Nagoya Protocol; *iii*) the negative effects of climate change on pest management: warming can affect negatively the performance of BCAs, increase pest populations and its severity on crops (Deutsch et al., 2008), and increase the risk of invasion of exotic pest species (Skendžić et al., 2021); and lastly, *iv*) increased availability of genetic information on non-model species such as BCAs as a result of cheaper molecular methods due to the continuous development of the genetic and genomic fields. Consequently, this boosts the study of their genetic variation, which may be used for selective breeding to increase their efficiency (Lommen et al., 2017).

The processes of adaptation (i.e., natural selection) to unfavourable conditions can be artificially accelerated in organisms via artificial selection or experimental evolution (see Chapter 1 -Figure 1.1.2, p. 31). However, the genetic breeding of BCAs is not an easy task and it can be very time-consuming in addition to requiring extensive biological and ecological knowledge (Hoy, 1986; Lirakis & Magalhães, 2019). This is likely the reason why this discipline is so much in its infancy. For example, it is necessary to know if traits to be genetically improved are codified by few or multiple *loci* and which these are, what is the role of epigenetic effects, and of genetic correlations between traits among others (Lommen et al., 2017). Additionally, we need to know whether these traits are eco-evolutionary functional; that is, besides being evolutionary responding traits (response traits), they should act as ecological effect traits for BPC. In fact, Messenger and van der Bosch (1971) stated more than 50 years ago that "Artificial selection of natural enemies..., has been considered by many, attempted by few, and, unfortunately, proved practicable in terms of improving biological control by no one (sic)."

If we are to exploit intraspecific genetic variability of BCAs to address the potential to improve their performance in hostile environments, it is necessary to characterize the standing intraspecific genetic variability in traits of interest, to assess whether genetic breeding can be applied to specific strains or populations of BCAs (Lommen et al., 2017). The selection of traits should be directed not only to adaptation to the environment to which BCAs will be exposed, but also to maximize their response to the pest species against which they will be used (Lommen et al., 2017).

Intraspecific genetic variability

Intraspecific genetic variability is essential for the persistence of populations. It gives them the ability to adapt and respond to environmental changes via evolutionary processes (Barrett & Schluter, 2008). Although the study of genetic variability has been addressed in the disciplines of population genetics and evolutionary biology for nearly 100 years (Godhe & Rynearson, 2017), this field is still in its early stages of development in Applied Sciences, remaining poorly described and studied (E. Wajnberg, 2004). Indeed, an extensive review of published studies addressing the genetic variability of arthropod BCAs reported studies on only 29 different species of BCA, all focussing on a limited number of traits (Ferguson et al., 2020). Also, the authors reported a lack of papers estimating heritability or assessing the genetic and environmental variance from which to estimate it (only 19).

Most of the functional traits related to the efficiency of BCAs are polygenic, that is, influenced by many loci, each with a small effect (E. Wajnberg, 2004). In polygenic (or quantitative) traits, phenotypic variability is continuously distributed among all the individuals that compose a natural population, usually following a normal statistic distribution (Mackay, 2009). The existence of genetic variability in these traits is crucial for phenotypic evolution in natural populations, as well as for selective breeding of domesticated plant and animal species (Mackay, 2009). Phenotypic variation results from the combined effect of the genotype of individuals and the environment where they live; that is, all the non-genetic circumstances that influence the phenotypic value (Falconer & Mackay, 1996). Variation across individuals can be quantified by estimating the phenotypic variance (V_P); that is, the square of the deviation of each individual value from the population mean. Thus, phenotypic variance is composed of two sources of variation: a heritable component [genetic variance (V_G)] and a non-heritable component [environmental variance (V_E)] (see [1]). Thus, the genetic variance of quantitative traits can be calculated from the study of the phenotypic variability and the partitioning of V_p into its two components. The V_G component represents the fraction of the V_p that determines the potential of populations to adapt and evolve when these are exposed to environmental stressors, and this is achieved by changes in gene frequencies that imply transmission to the offspring by the parental generation; whereas the V_E estimates the extent to which environmental variability affects the phenotype beyond the genotype. Furthermore, V_G can be subdivided into three components: i) genetic additive variance (V_{ad}), which refers to the deviation from the population mean phenotype due to the mendelian inheritance of a large set of alleles and their additive effect of inherited alleles on the phenotype; ii) genetic dominance variance (V_d) that involves deviation due to interactions between alternative alleles at a specific locus, and iii) genetic interaction variance or epistasis (V_i) which refers to the interaction between alleles associated to different *loci* affecting the expression of a trait (see [1.1]), (Byers, 2008; Falconer & Mackay, 1996; E. Wajnberg, 2004). Hence, to explore the possibility of genetic improvement of BCAs', the first step is to assess whether traits with potential interest for biocontrol hold sufficient genetic variability.

$$V_P = V_G + V_E \qquad [1]$$

$$V_P = V_{ad} + V_d + V_i + V_E \qquad [1.1]$$

The heritability of a quantitative trait is a term that indicates the level of similarity among relatives (Falconer & Mackay, 1996). It is a relevant parameter because is a predictor of the degree by which a population can respond to artificial or natural selection (Byers, 2008). Because heritability depends on all the components of the phenotypic variance, its value depends not only on the measured quantitative trait but also on the environmental conditions under which it has been measured (the denominator in equation 2 below) and on the particular population under study (Brakefield, 2003; Falconer & Mackay, 1996). For example, populations that have undergone inbreeding processes, which have a higher proportion of fixed genes (that is, a higher proportion of homozygosity), will have lower heritability values than other populations that have not undergone these processes. This is because high homozygosity (i.e.; in diploid organisms the proportion of loci that have two identical alleles) is an indication of poor allele availability in the population. Also, in populations in which genetic variation is estimated under more homogeneous environmental conditions heritability is expected to be greater than in those in which it is estimated in more variable environments (Falconer & Mackay, 1996).

There are two types of heritability: the so-called heritability in the broad sense, H^2 [2], which is an estimate of the proportion of V_P attributable to both additive (V_{ad}) and non-additive genetic variance (V_d, V_i), and the narrow-sense heritability, h^2 [3], which indicates the proportion of the total V_P that is attributable to additive genetic variance (Brakefield, 2003; Roff, 1997).

$$H^2 = \frac{V_G}{V_P} \qquad [2]$$

$$h^2 = \frac{V_{ad}}{V_P} \qquad [3]$$

Note that since $V_P = V_G + V_E$, an increase in the environmental variance under which H^2 is measured will necessarily lead to lower estimates. The same applies to h^2.

Correlations between traits

Two traits are correlated when a change in the value of one of the traits involves a change in the value of the other, without implying cause-effect. In fact, it can be caused by a third unmeasured variable. This correlation can be either positive (when the phenotypic value of one trait increases, the other does too) or negative (when the phenotypic value of one trait increases, the value of the other decreases).

Phenotypic correlations are estimated from the phenotypic values measured in different individuals of the same population. These correlations capture the genetic and environmental influence on two traits simultaneously. In contrast, when the variance and covariance genetic components are obtained and the environmental component is controlled for, either because it is considered to be constant or because it is explicitly modelled, one estimates *genetic correlations*. Genetic correlations capture the pleiotropic action of genes; that is, genes that are involved in the expression of more than one trait, and this explains in large part the genetic component of the phenotypic correlation (Falconer & Mackay, 1996). Also, linkage disequilibrium is involved in genetic correlations but to a lesser extent, that is, when certain alleles of different genes tend to be present together in a population to a greater extent than would be expected if the genes segregated independently during sexual reproduction, for example, due to the proximity between the loci in which they are found. (Roff, 1997).

When dealing with artificial selection or experimental evolution, negative genetic correlations between traits can be of great importance when they involve genetic trade-offs, as one trait cannot increase in response to selection without a decrease in the response of the other (and *vice versa*)(Garland, 2014; Garland et al., 2022). Usually, trade-offs between traits are fitness-related. A very common example is the classic trade-off between the number and size of eggs (C. C. Smith & Fretwell, 1974; Winkler & Wallin, 1987) reported in different groups of organisms such as beetles (Czesak & Fox, 2003), fish (Snyder, 1991), hens (Francesch et al., 1997) or planktonic crustaceans (Ebert, 1993; Lynch, 1946). Another example of trade-off was reported by Kraaijeveld and Godfray, which via artificial selection experiments detected a trade-off between the resistance of *Drosophila melanogaster* Fallen (Diptera: Drosophilidae) against an endoparasitoid, *Asobara tabida* Nees (Hymenoptera:

Braconidae) and its larval competitive ability. Once they selected lines with improved resistance against the endoparasitoid, they compared the performance of both the control and selected flies under conditions of weak to strong intraspecific competition, seeing that under weak competition treatments, survival of both selected and non-selected lines was high and with no differences between the two sets of lines. However, survival was reduced and there was significantly greater mortality among selected than control flies under more severe competition conditions (Kraaijeveld & Godfray, 1997). Thus, if two traits are genetically negatively correlated, the intentional selection of a target trait that should improve the efficacy of pest control may unintentionally select a trait that is not of interest and may act against it. For example, the selection of wingless morphs of *Adalia bipunctata* to reduce its dispersal is correlated negatively with relevant life-history traits (longer developmental time, reduced life span, and lower lifetime reproduction) compared to their winged conspecifics (Ueno et al., 2004). Similarly, if the target trait is negatively correlated with another trait that is at its evolutionary minimum, and therefore cannot longer evolve to be smaller, the trait of interest would not be able to evolve to a larger value either. Therefore, the rate and direction of the response to selection can be driven, or limited, by genetic correlations.

Isofemale line technique as a tool to estimate genetic variability, heritability, and genetic correlations between traits

The isofemale line technique (ILT) is used not only to investigate the genetic variability of natural populations but to analyse also ecological and evolutionary genetics. It consists of creating sub-populations (i.e., isolines) from a basal population, which are carried out through an inbreeding process by full or half-sib crossings, increasing therefore their homozygosity (Figure 2.0.1.A). This inbreeding produces a purge of deleterious genes as they usually are recessive and as such they only express as homozygotic. In this way, each isoline is obtained from a single founding female. This results in the partition of the whole phenotypic variance into two components: the phenotypic variance between lines (V_b) and the phenotypic variance within lines (V_w) (Falconer & Mackay, 1996) (Figure 2.0.1.B). Using nested analysis of variance, estimates of the relative proportion of genetic (the between isofemale line component) and environmental (the within isofemale line component) variances in populations can be obtained (Hoffmann & Parsons, 1988). V_b is a good estimate of the genetic variance (V_G) when n (that is, the number of isofemale lines) is high enough [i.e. n>20, (David et al., 2005)]. This occurs because as being founded by a single female, each isoline harbours a small portion of the total genetic variability of the population due to founder effect and genetic drift as each founding female is, as in all sexually reproducing species, genetically different. In the process of isoline creation, high consanguinity is achieved within isolines via inbreeding, generation after generation. After a certain number of generations, V_w is expected to harbour very little or no genetic variance. Yet, quantitative genetic models (Falconer & Mackay, 1996) show that V_w still harbours a significant genetic component. Nevertheless, this technique decreases the V_w whereas increasing the V_b, which allows the characterization of the original population (Moreteau et al., 1995). In other words, the greater the degree of similarity among individuals within isolines, the greater the degree of difference among isolines. This method provides valuable genetic information about populations when the details of their genome are not well known. Using this procedures it is possible to study and compare the nature of the phenotypic variation among different populations (Hoffmann & Parsons, 1988) via the estimation of the genetic variability of quantitative traits, correlations between traits, or even the analysis of phenotypic plasticity (David et al., 2005; Moreteau et al., 1995).

David et al. (2005) provided a formula to calculate the so-called intraclass correlation (t) [4] as a way to approximate H^2 while correcting by the number of isolines:

$$t = \frac{nV_b - V_w}{nV_b + (n-1)V_w} \tag{4}$$

where $t=H^2$ as n approaches infinity.

Despite the increasing awareness of the need for research exploring the potential of genetic breeding in BCAs [see reviews: (Bielza et al., 2020; Kruitwagen et al., 2018; Leung et al., 2020; Lirakis & Magalhães, 2019; Lommen, 2013; Lommen et al., 2017; Montserrat et al., 2021; Plouvier & Wajnberg, 2018; Thompson et al., 2022)], phenotypic studies addressing the genetic variability and heritability of relevant traits in BCAs, and potential genetic trade-offs, are still lacking (Ferguson et al., 2020). Although there are some studies where the genetic variability of some populations of the predatory mite *A. swirskii* was evaluated (Paspati et al., 2019; Ramadan et al., 2004; Tixier et al., 2022), these studies were carried out at a molecular level, not at the phenotypic level, and none of them reported heritability values or possible trade-offs among traits. In this section, I fill this gap and provide extensive data on genetic variation of multiple traits, many of them measured in the same individuals, for which I use the predatory mite *A swirskii*, one of the most used BCA at present.

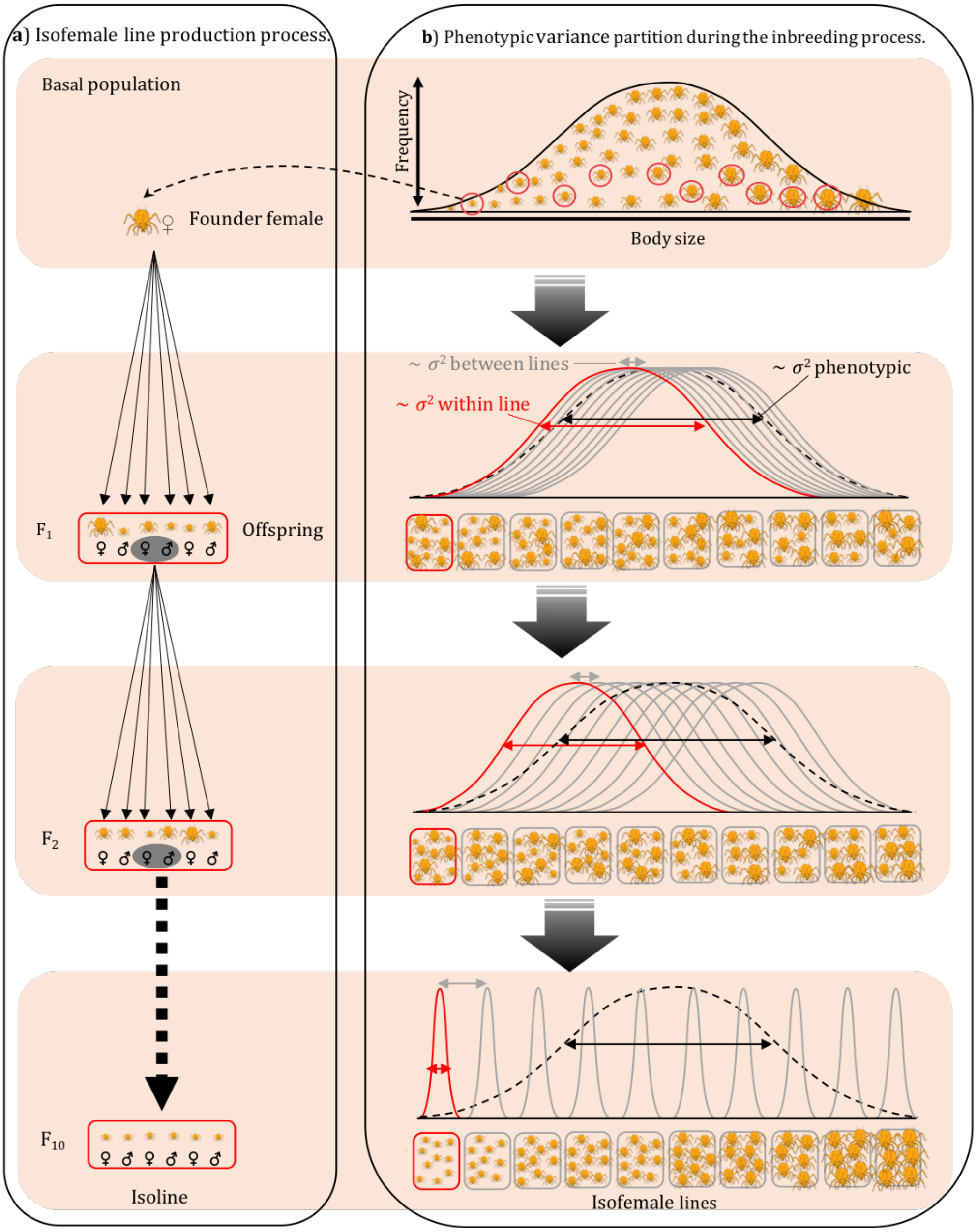

Figure 2.0.1.a) shows the isofemale line process, until reaching F_{10}, by inbreeding through full sib crossing. **b)** represents the total phenotypic population variance (black arrow) fragmentation throughout the generations using the body size trait as an example. Inbreeding increases the genetic similarity among individuals within isoline, decreasing thus the within-line genetic variance (red arrow) and increasing the between-line genetic variance (grey arrow). By F_{10}, differences within lines would be mainly attributable to environmental variation because of inbreeding. Along this process, the genetic differences among isolines increase due to the founder effect.

Objectives of Section 2

This section addresses the main Objective 1 of this thesis (p. 41). In short, the objective was to evaluate the genetic variability of a wide number of traits to identify potential response traits, and to evaluate the genetic correlations among them, addressed in chapters 2 and 3, respectively.

In Chapter 2 I estimated the broad sense heritability of a wide number of traits in two populations of the phytoseiid mite *A. swirskii*, one of them (the wild population) hypothesized to hold higher variability that the other (commercial). To this end I used Isofemale lines that I originated for this purpose, to identify traits that could respond to selection. Specifically:

1. I compared the phenotypic value of the traits between the two populations.
2. I used Principal Component Analyses (PCA) for dimensionality reduction and choose the more representative traits within each category (life history, morphological, behavioural, and physiological).
3. I estimated the H^2 of the chosen traits to identify those with the potential to respond to selection.

In Chapter 3, I estimated the genetic correlations among traits with the potential to respond to selection, in order to detect potential trade-offs. Specifically:

4. I analysed the genetic correlations of traits assessed on the same individuals.
5. I analysed genetic correlations of traits assessed on different individuals, calculated from the means of the isofemale lines, to test for genetic correlations when traits were not estimated in the same individuals.

Material and Methods common to Chapters 2 and 3

This part of the material and methods of Section 2 applies to both Chapters 2 and 3.

Collection of a wild population of A. swirskii

Amblyseius swirskii is native to the eastern Mediterranean region and his first description was reported from Israel (Athias-Henriot, 1962). In the summer of 2017, I carried out field samplings in citrus orchards at two different locations in Israel, to broaden the genetic variability of the captured population. In the chosen locations, *A. swirskii* occurs naturally and it is the dominant phytoseiid species in citrus orchards [(Porath & Swirski, 1965) and Palevsky -personal information-]. The two locations, separated between them by 52km approx., were: (1) (32°56'36.43"N; 35° 6'1.26" E), near Acre at the northern Israeli coast; and (2) (33° 9'54.57"N; 35°35'17.43"E), at the region of Hula Valley in the northeast (Figure 2.0.2). In location 1, samplings were carried out on red grapefruit *Citrus* × *paradisi* Macfad. orchards with a cover crop of *Chloris gayana* Kunth (Poales: Poaceae) (Rhodes grass) planted between tree laneways. Rhodes grass, which is a serious invasive alien species in the Mediterranean area, provides wind-borne pollen to the crop that serves as additional food for predatory mites and promotes their establishment on citrus and avocado crops (Maoz et al., 2011; D. Smith & Papacek, 1991; Warburg et al., 2019). In location 2, two different orchards, separated 100m from each other, were sampled. One had *Citrus×tangelo* J.W. Ingram & H.E. Moore and the other had red grapefruit *Citrus×paradisi*. These two orchards did not have cover crops. In all the sampled orchards *A. swirskii* had never been released before (Palevsky, personal information). Samplings were done by softly beating citrus branches

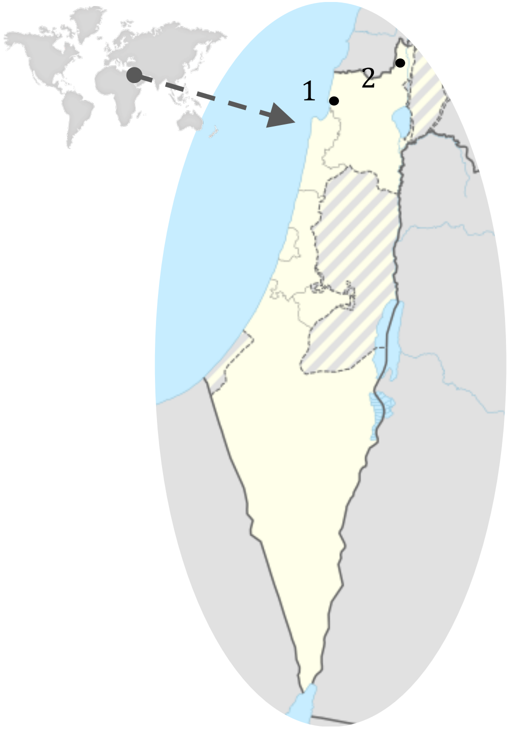

Figure 2.0.2. Map of sampling locations of *A. swirskii* in Israel

with a plastic stick, and predatory mites falling from the vegetation to a collection tray, which was blue to provide optimal background contrast. Individuals were collected using a mouth-aspirator into a 200 µl micropipette tips (McCravy, 2018; Z.-Q. Zhang, 2003). In total, 600 approx. individuals were captured (550 from location 1 and 50 from location 2). At Dr Erik Palevsky's laboratory at Newe-Ya'ar Research Center, I firstly recognized under the stereoscope that all individuals from location 1 seemed to belong to the same species whereas individuals from location 2 seemed to belong to two different species. Secondly, 20 individuals from location 1 were randomly chosen and identified to the species level under a phase contrast microscope at 40x, using taxonomic keys (Zannou et al., 2007). All the identified individuals were *A. swirskii.* Mites from location 2 were separated according to morphological characteristics into two groups. Approx. 50% of individuals of each group were identified. All the identified individuals from one of the groups belonged to the Typhlodromus genus whereas all identified individuals from the other group were *A. swirskii*. Once back at the laboratory of the IHSM "La Mayora" only six gravid females from location 2 remained alive. After two generations approx., both populations were mixed.

Mite Cultures

Amblyseius swirskii

Two populations of the predatory mite *A. swirskii* were cultured at the laboratory facilities of the IHSM "La Mayora". One was of commercial origin, started from > 1000 individuals kindly provided by Koppert Biological Systems. The other was of wild origin, started from ca. 500 individuals collected in Israel. Rearing units consisted of a plastic sheet, placed on top of a water-saturated foam covered by a wet layer of cotton, and placed in a plastic tray half-filled with water (Overmeer, 1985). The margins of the plastic sheet were surrounded by strips of moistened filter paper which served as a source of water and a physical barrier that helped to dissuade and prevent predators from escaping. Cotton threads were provided as oviposition substrates to enhance the oviposition of predatory mites on flat surfaces (Lougher et al., 2011), and small pieces of roof-shape plastic were placed on top of them, to provide shelter to the mites. New cultures of similarly-aged individuals (egg waves) were made weekly by transferring the cotton threads filled with eggs to the new rearing units. Mites were fed 3 times per week *ad libitum* with pollen of *Carpobrotus edulis* L. (Caryophyllales: Aizoaceae). This type of pollen is known to be a good food source for *A. swirskii* (Ragusa & Swirski, 1975, 1977). Colonies were cultured in a climate chamber at 25±2°C, 70±10% RH, and 16:8h L:D (Light: Dark) photoperiod.

Pollen of *C. edulis* was obtained from the pink-petal flower phenotype. When flowers were about to open, sepals and petals were taken to the lab, stamens were removed and dried on a stove at 37°C for 48h, and sieved through 0.25mm and 0.075mm sieves, consecutively, to obtain pollen free of impurities. Lastly, pollen was stored in a freeze at -20° until it was needed.

Tetranychus urticae

Tetranychus urticae Koch (Acari: Tetranychidae) (strain London) was reared on potted common bean *Phaseolus vulgaris* L. (Fabales: Fabaceae) var. Oriola. Three times a week new clean plants were added to the *T. urticae* rearing and the old and dried plants were removed. The colonies were kept in a climate room at 25±2°C, 70±10% RH and 16:8h L:D photoperiod. Clean bean plants were grown in a separate climate room at 23±3 °C 70±5% RH and 16:8h L:D photoperiod.

Creation of isofemale lines

Isofemale lines of both commercial and wild populations of *A. swirskii* were created in the laboratory to estimate the genetic variability and heritability of quantitative traits, as well as genetic correlations between them, in both the commercial and wild populations of *A. swirskii*. The ultimate goal of creating isofemale lines is to redistribute the total genetic variance in a population across a gradient of subpopulations (isofemale lines), increasing the genetic variance between isolines whereas decreasing the genetic variance within isolines (David et al., 2005). This process of inbreeding leads to genetic differentiation between isolines and genetic uniformity within the isolines (Falconer & Mackay, 1996).

During the process of an isofemale line formation, some isolines might be lost due to the accumulation of deleterious genes caused by high degrees of consanguinity (Tien et al., 2015). Indeed, the degree of recessiveness of deleterious alleles and their impact on fitness will determine the intensity of their purge, therefore during strong inbreeding processes, recessive, strongly deleterious alleles will be quickly eliminated (Hedrick, 1994; J. Wang et al., 1999). To minimize isofemale loss whereas obtaining a high enough level of consanguinity within lines, I used a model created by Prof. Dr Vítor Sousa, leader of the Evolutionary Genomics and Bioinformatics group at the Centre for Ecology and Evolution and Environmental Changes of the University of Lisbon. Prof. Dr Sousa simulated the dynamics of 10.000 alleles when mimicking a more relaxed process of obtention of isofemale lines via a) initially isolating a brother and a sister for *N* generations, followed by b) isolating 10 juvenile siblings at each run, and randomly choosing a female (who had mated with sibling males) to start the next run, and c) continue the later procedure till reaching the overall 10th generation. The simulation revealed that starting each run with one brother and one sister for 4 generations, then grouping 10 siblings and choosing a female from the group to start the next run for six more generations, the proportion of the genome where individuals of each isoline are either 90% or 95% similar is at least 79 and 76 %, respectively (Table 2.0.1).

Table 2.0.1: Results of the simulation of the dynamics of 10.000 alleles when mimicking a process for isofemale production minimizing losses of isolines. For each generation, which started with 2 individuals (brother and sister) for the first 4 generations, it is given the proportion of the genome where individuals of each isoline are 90% or 95 % similar when the procedure is continued until the 10th generation with the more relaxed method of isofemale lines creation since the fifth generation onwards (groups of a maximum of 10 siblings mating randomly to each other).

N° generations		% of similar genome at F_{10}	
(Full sibling)	(Groups of max. 10 siblings)	90%	95%
1	9	0.72	0.69
2	8	0.77	0.73
3	7	0.75	0.74
4	6	0.79	0.76

Following the results of the simulation, isofemale lines were created as follows: Gravid females, randomly chosen from both commercial and wild populations, were isolated in experimental arenas (see below), and were allowed to oviposit for at least 5 days. These females were to be the founders of each isoline. Their offspring [i.e., the first generation (F_1)] was isolated individually and upon reaching adulthood a virgin sister and a brother were allowed to mate. This procedure continued until reaching the fourth generation. From F_4 on, groups of a maximum of 10 sibling eggs were isolated and left to undergo development together till reaching adulthood. Then, a gravid female (who mated with an unknown brother from the group of 10 siblings) was chosen at random to start the next generation using a maximum of 10 of her eggs, when possible. This group selection continued up to the 10th generation. This procedure was done with various females from the same lineage as the generations went forward, so if some of them were unable to produce viable offspring, another female from the same mother was available as a backup. When one female from each isoline reached F_{10}, it could oviposit indefinitely until a small sibling population was created. After that, each isofemale population was nurtured in bigger rearing units like those used for the cultures (see “Mite cultures”, p. 55). A total of 20 and 22 isofemale lines from the wild and commercial populations respectively were created. David et al. (2005) estimated that to have the whole genetic variability of a population contained in isolines, the minimum recommended number of lines is 20 per population.

Experimental arenas consisted of a *P. vulgaris* leaf disc (∅ 1,5cm) floating on water-soaked cotton wool and placed upside down in a ∅ 5cm plastic glass and maintained in a climatic chamber at 25±2°C, 70±10% RH, 16:8 L-D photoperiod. Mites were fed *ad libitum* with pollen of *C. edulis.*

Measurements of Traits

A total of 23 traits were assessed on individuals from the 22 and 20 isolines of both the commercial and wild populations, respectively. When possible, different traits were measured on the same individuals (e.g., Life-history, morphological, and behavioural traits) (see Figure 2.0.3). Other traits, however, had to be measured on different individuals within isolines, because trait measurement implied the death of the individual (i.e., tolerance to desiccation, tolerance to starvation) or a change in their diet (predation rate on *T. urticae* eggs). Measurement of traits was made in climate chambers (FitoClima 600PHL, Aralab) under controlled and benevolent rearing conditions (25±1°C, 70±5% RH, 16:8 L-D photoperiod and pollen of *C. edulis* as a source of food *ad libitum*). This was to favour high genetic repeatability as non-optimal conditions always result in an increase of the variability at the isoline level (David et al., 2005). In addition, tested individuals were of similar age, to minimize effects of developmental stage on the estimates of phenotypic variation (E. Wajnberg, 2004).

There were two exceptions imposed by the trait measured itself, for the predation rate, only *T. urticae* eggs were supplied as a unique source of food and for tolerance to desiccation and starvation no food was provided and different %RHs were tested.

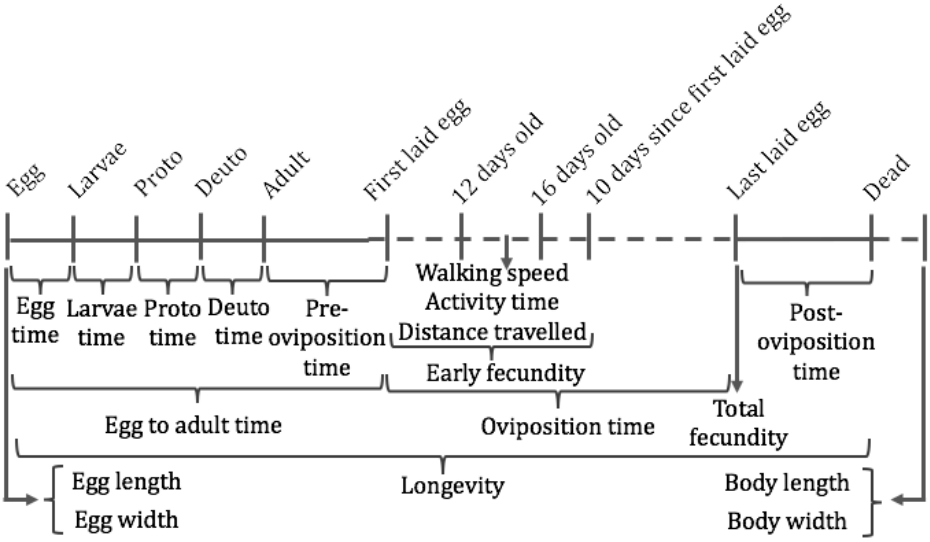

Figure 2.0.3. Chronological timeline of traits measured on the same individuals.

Life-history traits

-*Egg length and width:* Forty-five eggs (maximum 12 h old) per isoline and population, chosen at random, were isolated individually in experimental arenas consisting of 1,8cm ∅ leaf discs of *P. vulgaris* placed upside down on top of water-soaked cotton wool inside 5,5cm ∅ plastic containers. Eggs were photographed using a Leica S8APO stereoscope with a Leica EC4 camera and LAS EZ software, from a perpendicular plane. However, the egg chorion contains a sticky substance to increase their adherence on the surface where mothers lay them (Sabelis, 1985). Thus, there was the risk of depositing the eggs on the leaf discs in a non-horizontal position. This would underestimate their real length, as photos were taken from the zenith point, perpendicular to the eggs. To minimize this experimental error, eggs were positioned as horizontally as possible on the leaf disc before taking a photo 3 consecutive times. That is, after a picture was taken, the egg was collected and repositioned on top of the leaf disc, again in the flattest position possible, using a fine brush, and a new picture was taken. Egg length and width were measured in the three photos using the software Leica LAS EZ. The longest value of length and width among the three measurements was the value assigned to the individual (Figure 2.0.4).

-*Developmental Time*: Thirty of the eggs used above, per isoline and population, were used to assess developmental time. Eggs were kept isolated in experimental arenas inside a climate chamber (FitoClima 600PHL, Aralab) at 25±1°C, 70±5%RH; 16L:8D hours. After hatching, individuals were fed *ad* *libitum* with *C. edulis* pollen every two days. The developmental stage of individuals was recorded every 12h until they reached adulthood. Development to each successive stage was recognized by identifying moulted cast skins. Subsequently, the sex of the individuals was recorded. Thus, I recorded the time (h) male and female individuals spent at stages egg, larva, protonymph, deutonymph, and from egg to adult.

-*Fecundity and Longevity*: Because sex cannot be determined until adulthood when individuals reached the deutonymph stage, an adult male of the same isoline was added to all the experimental arenas to facilitate mating when the deutonymph became an adult female. Arenas with deutonymphs moulting into males were discarded. Recently emerged females were allowed to mate with the male for one day, and then sires were removed from the arenas. Females were kept in the experimental arenas and every 24h I recorded a) the number of eggs laid, and b) whether females were alive or dead. Measurements lasted till the death of individuals. When the death of females was due to external causes unrelated to the experiment (i.e., drowning, being rescued from the water and found dead after 24h., or just disappearing from the arena) data were recorded as censored. Thus, the following traits were obtained: pre-oviposition time (that is the total time between observations where females becoming adults and they laid their first egg), oviposition time, Ovi_t, (that is, the number of days between the first and the last egg laid by females, before dying), early fecundity (that is, the number of eggs laid during the first 10 days since the first egg was laid), ensuring to evaluate the maximum oviposition peak, which is correlated with the intrinsic rate of increase (*r*) in phytoseiids (Janssen & Sabelis, 1992), fecundity (total number of eggs during life-time), surviving time after oviposition, Post_ovi, (that is, the number of days between the day females laid their last egg and they died), and female longevity (time to death).

Morphological traits

-*Female body length and width*: Upon death, tested females were cleared in lactic acid 65% at 40°C for 48 hours approx. and then mounted on microscope slides with a drop of Hoyer's medium (Z.-Q. Zhang, 2003). When the medium dried, slides were observed under a Leica DM LB2 upright phase-contrast microscope equipped with a Leica DM Camera and LAS software and a photo of their dorsal shields was taken at x200 magnification. Subsequently, I used the ImageJ software to measure the length of the dorsal shield of females from each photo (Figure 2.0.5). I used the dorsal shield as it is considered a good indicator of the body size in phytoseiid mites (Croft et al., 1999; Walzer & Schausberger, 2011). Indeed, the idiosomal size is sensitive to variations depending on the physiological state of individuals (e.g., starving or full-fed, gravid or not, and if gravid, degree of development of the egg, degree of dehydration, etc), which results in a contraction or expansion of the lateral integument. On the contrary, dorsal shields have a fixed size during adulthood. Thus, I obtained measures for the following traits: length and width (at S2-S2 setae level, which is the most common maximum width point -personal observation-) of the females' dorsal shield.

Summarizing, I measured 13 traits within the life-history category, of which 2 traits were related to egg size (male and female egg length and egg width), 5 traits related to the developmental time of individuals in females and males (egg, larva, protonymph, deutonymph, and total developmental time), 5 traits related to fecundity (pre-oviposition time, oviposition time, early fecundity, fecundity, post-oviposition time,), and the female longevity. In addition, I measured 2 morphological traits related to the size of adult individuals (female dorsal shield length and width). All of them were measured on the same individuals.

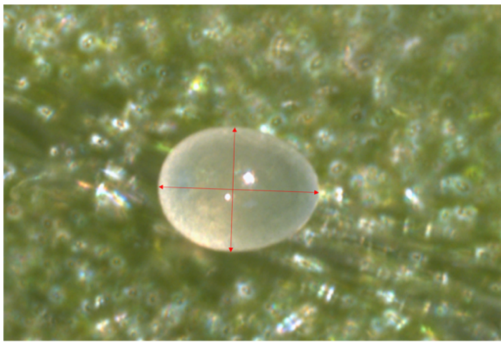

Figure 2.0.4. Image of the egg length and width measurements.

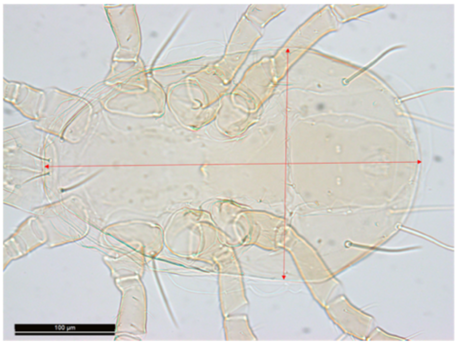

Figure 2.0.5. Image of the female body size (dorsal shield length and width).

Behavioural traits

Behavioural traits were measured using a robot built for this purpose. The robot's hardware and the software to extract behavioural data were designed and built by Alberto Ruiz Moreno and Ramón Ordiales Plaza, computer scientists from the Estación Experimental de Zonas Áridas (EEZA).

The set up consisted of an armed robot that handled 2 cameras (one camera per row) over 2 rows of 6 plates each, to be able to process a total of 12 Petri dishes, sequentially, in a single bout (Figure 2.0.6). It was programmed to record a set of 12 recordings of 60 seconds each per individual, taken with an interval of 12 minutes between them, per session. Recordings performed by the robot were analysed with specific software that was able to track the path walked by the mites (Figure 2.0.7), giving the walking speed (cruise velocity) and activity time (proportion of time that the animal was moving in the entire recording).

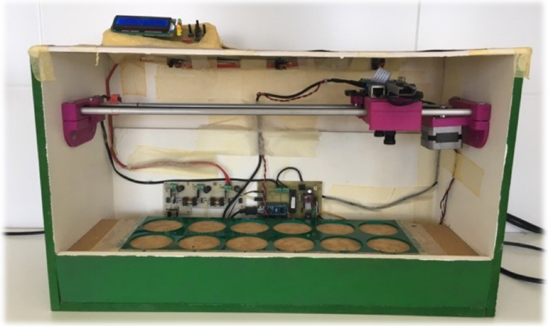

Figure 2.0.6: Photo of the robot used, which is able to record *A. swirskii* mites in order to assess cruise walking speed, activity time and distance travelled.

Figure 2.0.7: Processed image recorded by the robot from an *A. swirskii* individual path tracked during 1 min.

-*Walking Speed, Activity time, and Distance travelled:* Depending on the availability of individuals, subsets ranging between 6 and 24 females per isoline, ranging between 12 and 16 days of age (see Figure 2.0.3), were used to measure behavioural traits. Prior to the recordings, females were isolated individually in the experimental arenas for 30 min for acclimatization. Arenas consisted of small Petri dishes (5,5cm Ø) with the bottom coated with black EVA (Ethylene Vinyl Acetate) foam. To prevent mites from scaping, a ring of Tanglefoot® glue was applied along the outer margin of the EVA foam, along the joint with the Petri dish is. The reason to use black EVA foam is that it provides a standardized flat surface to assess the mobility of individuals and a dark background that provides enough contrast for the robot´s cameras to be able to detect the mites' movement on the surface. Recordings were carried out at 25±2°C, 60±10%RH and in semi-darkness, that is, under a tiny infra-red light so that the robot could discriminate the mites from the black background. Using the software described above, I obtained for each individual a measure of a) cruise walking speed, defined as the average speed at which individuals walked in full activity paths; i.e., in paths in which they were moving the entire time; b) activity time, defined as the time spent moving from the total recording time (% time walking), and c)

Distance travelled as the total sum of the distances travelled during the recording period across the 12 recordings.

Physiological traits

-Tolerance to desiccation: Gravid females (12-14 days old) from each of the isolines were individually isolated in test cylinders and placed in desiccation chambers that were kept at different relative humidity. Test cylinders consisted of 1.5 ml Eppendorfs cut from the bottom and with a hole on the lid (Figure 2.0.8). Both holes were covered with a 70μm ∅ fine-mesh nylon gauze, which allowed the cylinders´ relative humidity to become equal to that of the chambers, exposing mites to the different relative humidity treatments. Desiccation chambers consisted of hermetic plastic recipients (14x14x9cm) with 330ml of different glycerol-water solutions to maintain the desired relative humidity in the ambient where mites were to be exposed (see below). A 1cm ∅ light rack (where test cylinders were to be placed) was attached at 2/3 of the container´s height, 4cm above the glycerol-water solution to avoid contact with the test cylinders (Figure 2.0.9).

Figure 2.0.8: Test cylinder (modified Eppendorf) where *A. swirskii* females from each of the isofemale lines were isolated individually.

Figure 2.0.9: Desiccation chamber where test cylinders were placed. Each chamber had a different relative humidity (35, 50, 65%).

Different relative humidity values inside climate chambers were achieved by using a glycerol solution at different concentrations (Table 2.0.2) as described in (Forney & Brandl, 1992). Before the experiment, mites were starved for 12h at 25°C and 70% RH to standardize their starting physiological conditions. During the experiment, females were deprived of food and water. Mites were checked every 12h until they died. Eggs that were laid by females during the experiment were removed at each check to avoid egg cannibalism, and therefore, a potential source of food and water. Desiccation chambers were kept in a climatic chamber (FitoClima S600PHL) at 25 ± 1°C, 16L: 8D h. There were 18 replicates per population, isoline, and relative humidity.

Table 2.0.2.: Volume of Glycerol and water to get a total of 330ml of solution at 35, 50 and 65 ±2% of Relative Humidity (RH) at 24°C

RH (%)	**Volume (ml)**		
	Glycerol	H_2O	Solution
35	285	45	330
50	250	80	330
65	207	123	330

-Tolerance to starvation: The same experimental set-up and methods as above were used to assess resistance to starvation. In this case, however, experimental chambers contained only water, which provided 100% RH in the environment, and thus removed an effect of relative humidity on the ability to resist food deprival. There were 18 replicates per population and treatment.

-Predation rate: Predation rates on *T. urticae* eggs by females from all isolines of both populations of *A. swirskii* were assessed on experimental arenas consisting of leaf discs (1,8cm ∅) of *P. vulgaris,* placed upside down on cotton wool water-soaked, inside plastic glasses. Five *T. urticae* gravid females per arena were placed and allowed to oviposit for 48h. Next, they were removed carefully with a fine brush without damaging the spiderweb they produced. Then, 12-14 days-old gravid *A. swirskii* females, previously starved for 24h, were placed individually in the arenas containing *T. urticae* eggs and webbing, and they were allowed to forage for 24h. After that, the number of eaten eggs was assessed from the difference between the initial and the final number of eggs in the arenas. Note that predation rate may be considered a physiological trait because it affects, and is affected by the physiological state of the individual, but also a behavioural trait, as it is part of the foraging behaviour repertoire.

During the experiment, mites were kept in a climate chamber (FitoClima S600PHL) at 25±1°C, 70±5%RH and 16L:8D h photoperiod.

CHAPTER 2: Genetic variability in populations

Material and Methods: Statistical Analysis

All the statistical analyses were done using the computer environment R (v. 4.0.2) (R Core Team, 2022).

Differences in trait values between the commercial and wild populations

In general, all the models fitted to identify differences in trait values at the population level included “Population” (commercial or wild) as a fixed factor, and “Isoline” as a random factor (to reflect the experimental design) and nested within Population. In traits that were measured in both males and females (that is, egg length and width, and egg, larva, protonymph, deutonymph and egg to adult developmental time), the variable "Sex" (female or male) was included as a fixed factor, as well as its interaction with “Population”. To determine the significance of the random (Isoline) effect, I used the “*ranova*” function from the package “lmerTest” (Kuznetsova et al., 2017). When the interaction between main factors was significant, Tukey’s *post hoc* tests were performed to compare trait combinations using the “*emmeans*” function from the “emmeans” package (Searle et al., 2012).

For morphological, life-history, and behavioural traits, Linear Mix Models (LMM) were adjusted, using the function “*lmer*”(package “lme4”) (Bates et al., 2015). Particularly, models to analyse the traits “oviposition time”, “post-oviposition time” and “fecundity”, included "longevity" as a co-variable. Only females that remained alive during the first 10 days of oviposition were considered in the analyses of the trait “early fecundity”, and only females that died naturally were included in the analyses of the traits “oviposition time”, “post-oviposition time”, “total fecundity” and “longevity”. Additionally, due to the high variability of results related to oviposition traits, outliers were identified using the Rosner test ("EnvStats" package) (Millard, 2013), and they were removed if necessary. Outliers identified for the pre-oviposition time were data from either non-gravid females that never laid an egg or females with extremely high pre-oviposition times. All the data measured in these individuals (pre-oviposition trait and the rest of the traits related to oviposition and fecundity) were excluded from the analyses.

The analyses of behavioural traits, that is, distance travelled, walking speed, and activity time, included the covariable "recording time" to correct for differences in the total time of recordings among the individuals tested, which was caused by a technical problem with the hardware of the robot, consisting of very short but unpredictable stops during recording. In addition, because not all the isolines could be tested simultaneously in each recording (the robot only could assess 12 individuals at a time), the variable "Recording ID" (i.e., each recording block of 12 Petri dishes) was included in the models as a random effect. For the trait “activity time” values lower than 0.52 (n=5) and 0.55 (n=7) in the commercial and wild populations, respectively, were identified as outliers by the Rosner test and consequently excluded from the analysis.

Physiological traits related to tolerance to desiccation and starvation were analysed by means of a mixed effects survival Cox proportional hazards regression model, using the function “*coxme*” from the package “coxme” (Therneau, 2022), which is based on the hazard rate; that is, the dependent variable is interpreted as the probability that an event occurs in a given time interval provided that it has not occurred in the previous time interval. Thus, it is proportional to the inverse of survival time, and as such the signs of coefficients must be interpreted backwards in how they affect survival time. Thus, a positive estimate in the regression (or mixed model) means a negative association to survival time (Allison, 1995). In addition to including “Population” as a fixed factor and “Isoline” as a random effect, “Number of eggs laid” during the experiment was included as a covariate. The aim was to control for female reproductive effort prior to death, as this trades off with body water and energy, and thus with the ability to survive to either stressor. Predation rates were analysed with generalized linear mixed models (GLMM) using the Package “*glmmTMB”* version 1.1.3 (Brooks et al., 2017), with a negative binomial family “nbinom2” (link = "log") as error distribution. Additionally, the model included the “initial number of eggs” as covariate, which was log-transformed to make it linear to the link function.

Dimensionality reduction

In total, I measured 23 traits categorized as life-history, morphological, physiological, and behavioural traits. Within each category, some traits might be highly correlated and thus be redundant. Here I applied PCAs to choose the most representative traits within each category. The criteria followed for the selection of traits to estimate their heritability was based on the results of the PCA. When traits grouped under the same category were loading at the same principal component, with eigenvectors having similar length and direction. In addition, biological criteria were also considered to choose the traits.

Principal component analysis

Principal component analyses were done with the function “*PCA*” (package “FactoMineR”) (Sebastien et al., 2008) using isofemale line means for each of the traits measured, discriminating by sex when appropriate and by population.

Graphs were implemented with the function "*fviz_pca*" from the "factoextra" package, adding 95% level ellipses around the pre-defined grouping factors (Kassambara & Mundt, 2020).

Heritability

Heritabilities were estimated in representative traits within each category that were chosen using the criteria explained above.

The heritability of traits, from the wild, commercial, and a combination of both populations, was estimated using the formula [4], (p. 51) from David et al. (2005). GLMM were implemented through the "*MCMCglmm*" function ("MCMCglmm" package) (Hadfield, 2010) in a Bayesian framework using Markov chain Monte Carlo (MCMC) sampling following Wilson et al. (2010). The models included the covariates explained above as fixed factors, and "Isoline" as a random factor. Each model had a total run length of 300,000 iterations with a "burn-in" period of 5,000 and a thinning interval every 10 interactions, ensuring more than 6,000 efficient samples in each test. I took a prior variance of 1 (implying standardization of the dependent variable to mean 0 and variance of 1) and a low degree of believe (*nu*) parameter of 0.002. Two instances (chains) with a different seed were run for each trait and chain convergence tested by means of Gelman Rubin diagnostic (Gelman & Rubin, 1992), which is acceptable when the value is close to one.

Results

Differences in trait values between populations

Life-history traits (except those related to oviposition and fecundity)

Regarding the results obtained for the traits "Egg length" and "Egg width", both the main factor "Sex" and the interaction "Population*Sex" were significant (Table 2.2.1.a). Indeed, *post-hoc* tests revealed that both egg length and width were not different between populations for the eggs that became females (Tukey $Z_{51.1}$=-0.261, P=0.795 and Tukey $Z_{46.9}$=0.987, P=0.329), respectively, but both traits were larger in the commercial than in the wild population for the eggs that became males (Tukey, $Z_{82.8}$=2.927, P=0.004 and Tukey, $Z_{82.8}$=2.927, P=0.004), respectively (Table 2.2.1.b). The random effect "Isoline" was significant (P<0.001), suggesting genetic effects.

Table 2.2.1.a. Results of the LMM applied to life-history traits (except those related to oviposition and fecundity).

Traits	**Source of variation**	***F***	**df1;df2**	***P***
Egg Length	Intercept	204570	1; 49	<0.001
	Population	0.068	1; 51	0.795
	Sex	99.721	1; 1457	<0.001
	Population*Sex	13.619	1;1458	<0.001
Egg Width	Intercept	143440	1; 45	<0.001
	Population	0.974	1; 47	0.329
	Sex	191.66	1; 1451	<0.001
	Population*Sex	8.163	1; 1452	0.004
Egg	Intercept	12714.695	1; 58	<0.001
	Population	0.232	1; 61	0.632
	Sex	0.731	1; 1126	0.393
	Population*Sex	6.345	1; 1125	0.012
Larva	Intercept	3002.274	1; 77	<0.001
	Population	0.570	1; 82	0.500
	Sex	3.541	1; 1129	0.060
	Population*Sex	2.486	1; 1130	0.115
Protonymph	Intercept	2484.624	1; 55	<0.001
	Population	28.110	1; 57	<0.001
	Sex	25.217	1; 1122	<0.001
	Population*Sex	6.254	1; 1122	0.013
Deutonymph	Intercept	1972.498	1; 52	<0.001
	Population	31.389	1; 53	<0.001
	Sex	67.274	1; 1117	<0.001
	Population*Sex	8.006	1; 1116	0.005
Egg-Adult	Intercept	8997.851	1; 45	<0.001
	Population	32.923	1; 46	<0.001
	Sex	77.660	1; 1105	<0.001
	Population*Sex	12.313	1; 1105	<0.001
Longevity	Intercept	1191.515	1; 30	<0.001
	Population	0.255	1; 36	0.619

Table 2.2.1.b: Differences in life-history trait values (Mean ± SD) between both commercial and wild populations for each sex. Units: All values related to length and developmental times are expressed in µm and Days respectively.

Females			
Traits	**Population**		**Sig.**
	Commercial	**Wild**	
Egg Length	(*543*) 208.71 ± 5.81	(*426*) 208.99 ± 6.48	ns
Egg Width	(*543*) 159.08 ± 4.19	(*426*) 158.51 ± 5.43	ns
Egg	(*401*) 2.22 ± 0.28	(*341*) 2.20 ± 0.29	ns
Larva	(*401*) 0.72 ± 0.26	(*341*) 0.74 ± 0.25	ns
Protonymph	(*401*) 1.50 ± 0.38	(*341*) 1.74 ± 0.50	***
Deutonymph	(*401*) 1.53 ± 0.38	(*341*) 1.80 ± 0.52	***
Egg to adult	(*401*) 5.98 ± 0.46	(*341*) 6.48 ± 0.80	***
Longevity	*(157)* 47.37 ± 14.95	*(104)* 46.17 ± 16.32	ns
Males			
Traits	**Population**		**Sig.**
	Commercial	**Wild**	
Egg Length	(*255*) 204.13 ± 5.88	(*260*) 201.85 ± 6.65	**
Egg Width	(*255*) 154.47 ± 3.93	(*260*) 152.40 ± 5.06	**
Egg	(*218*) 2.23 ± 0.31	(*177*) 2.31 ± 0.31	*
Larva	(*218*) 0.77 ± 0.26	(*177*) 0.73 ± 0.25	ns
Protonymph	(*218*) 1.34 ± 0.36	(*177*) 1.43 ± 0.40	ns
Deutonymph	(*218*) 1.24 ± 0.36	(*177*) 1.37 ± 0.44	**
Egg to adult	(*218*) 5.56 ± 0.44	(*177*) 5.84 ± 0.54	**

ns = non-significant differences. *, **, *** means significant differences where p-value is < 0,05, <0,01 and <0,001 respectively.

The only significant source of variation for the time spent in the egg stage was the interaction "Population*Sex" (Table 2.2.1.a). Indeed, the duration of the egg stage of eggs that became females did not differ between populations (Tukey, $Z_{60.6}$=-0.482, P=0.631), but that of eggs that became males was longer in the wild than in the commercial population (Tukey, $Z_{129.4}$=-2.221, P=0.028) (Table 2.2.1.b).

A similar pattern was found for the time spent in protonymph, but in this case the wild females were the ones that last longer in this stage [Tukey for females: (Tukey, Z_{57}=-5.302, P<0.001); Tukey for males (Tukey, Z_{112}=-1.977, P=0.051)]. For time spend in the deutonymph stage, both wild females and males, it was longer than those from commercial individuals [Tukey for females: (Tukey Z_{53}=-5.602, P<0.001); Tukey for males: (Tukey $Z_{93.4}$=-2.267, P=0.025)]. This deutonymph developmental time pattern led to a similar one when the trait was the total developmental time (from egg to adult) [Tukey for females: (Tukey $Z_{45.4}$=-5.738, P<0.001); Tukey for males: (Tukey $Z_{60.5}$=-2.881, P=0.005)]. On the contrary, this did not occur when the time spent in larva was considered, where no differences were found between populations neither for females nor males. In the case of the total longevity of individuals (which was only measured for females), no differences were found between both populations (Table 2.2.1.a and b). Except for larva and longevity (P=630 and P=0.375) respectively, the random effect “Isoline” was significant (P<0.001), suggesting genetic effects on these traits.

Life-history traits related to oviposition and fecundity

The traits pre-oviposition time, oviposition time and post-oviposition time were all significantly different between the wild and the commercial population (Tables 2.2.2.a and b). Indeed, it took wild females 17 hours more than commercial females to lay their first egg after they reached adulthood (Table 2.2.2.b), but the oviposition time was longer, and the post-oviposition time shorter, in the wild than in the commercial population (Table 2.2.2.b), resulting in no differences between populations in the total number of eggs laid during their life-span (Table 2.2.2.b). Early fecundity, that is the number of eggs laid during the first 10 days since the first laid egg, was slightly higher in the commercial than in the wild population. The covariate “Longevity” in the oviposition, post-oviposition and fecundity models was significant and positive. The random effect “Isoline”, and thus the potential for genetic determinism, was only significant for pre-oviposition, and early fecundity (P=0.006 and P=0.003 respectively) whereas for oviposition, post-oviposition, and fecundity it was not (P=0.689, P=0.274 and P=0.375).

Table 2.2.2.a. Results of the LMM applied to life-history traits related to oviposition and fecundity.

Traits	Source of variation	*F*	Estimate	SE	df1;df2	*P*
Preoviposition time	Intercept	929.2			1; 30	<0.001
	Population	26.9			1; 36	<0.001
Oviposition time	Intercept	37.370			1; 200	<0.001
	Population	4.404			1; 35	0.043
	Longevity	83.641	0.275	0.03	1; 262	<0.001
Postoviposition time	Intercept	130.1			1; 202	<0.001
	Population	9.811			1; 36	<0.001
	Longevity	566.89	0.729	0.03	1; 263	0.003
Early fecundity	Intercept	2939.9			1; 35	<0.001
	Population	16.16			1; 39	<0.001
Fecundity	Intercept	65.448			1; 202	<0.001
	Population	0.03			1; 36	0.864
	Longevity	75.512	0.328	0.04	1; 263	<0.001

Table 2.2.2.b: Differences in life-history trait values (Mean ± SD) between both commercial and wild populations. Time is expressed in days and oviposition in the number of eggs.

Traits	Population		Sig.
	Commercial	Wild	
Pre-Oviposition	*(346)* 2.69 ± 0.79	*(256)* 3.41 ± 1.47	***
Oviposition time	*(157)* 22.64 ± 7.95	*(104)* 24.28 ± 9.50	**
Post-Oviposition	*(157)* 16.18 ± 14.73	*(104)* 12.07 ± 12.30	**
Early Fecundity	*(302)* 16.23 ± 3.42	*(211)* 14.47 ± 4.28	***
Total Fecundity	*(157)* 31.82 ± 10.09	*(104)* 31.54 ± 11.59	ns

Units: Pre-oviposition, Oviposition and Post-oviposition time (days). Early and Total fecundity (n° eggs).

ns = non-significant differences. *, **, *** means significant differences where p-value is < 0,05, <0,01 and <0,001 respectively.

Morphological traits

The population factor was marginally significant, indicating a tendency of the females from the commercial population to be smaller than those in the wild population (Table 2.2.3.a), the difference, however, is of only 2 µm.

Table 2.2.3.a. Results of the LMM applied to morphological traits.

Traits	Source of variation	*F*	df1;df2	*P*
Dorsal shield length	Intercept	278940	1; 39	<0.001
	Population	3.803	1; 40	0.058
Dorsal shield width	Intercept	133060	1; 38	<0.001
	Population	0.271	1; 40	0.605

Table 2.2.3.b: Differences in morphological trait values (Mean ± SD) between both commercial and wild populations for female's Dorsal shield length. Units: all the values are expressed in µm.

Traits	Population		Sig
	Commercial	Wild	
Dorsal Shield Length	(*420*) 378.44 ± 7.30	(*346*) 380.67 ± 8.27	ns
Dorsal Shield Width	(*420*) 233.45 ± 7.75	(*344*) 233.18 ± 9.56	ns

ns = non-significant differences. *, **, *** means significant differences where p-value is < 0,05, <0,01 and <0,001 respectively.

Behavioural traits

None of the behavioural traits measured in individuals; that is, distance travelled, walking speed, and activity time, were statistically different between the wild and the commercial populations (Table 2.2.4.a and b). The covariate "recording time" significantly and positively influenced all three behavioural traits (Table 2.2.4.a). The random effect "Recording ID" influenced significantly the Distance travelled, Walking speed and Activity time (P<0.001) whereas the random effect "Isoline" was not significant in none of the three cases (P=0.1447, P=0.2099 and P=0.960 respectively), suggesting a lack of genetic effects.

Table 2.2.4.a. Results of the LMM applied to behavioural traits.

Traits	Source of variation	*F*	Estimate	SE	df1;df2	*P*
Distance travelled	Intercept	0.553			1; 180	0.458
	Population	1.719			1; 63	0.195
	Recording time	1091.274	0.158	0.005	1; 532	<0.001
Walking speed	Intercept	952.186			1; 180	<0.001
	Population	2.538			1; 62	0.107
	Recording time	10.035	<0.001	<0.001	1; 531	0.002
Activity time	Intercept	4882.459			1; 181	<0.001
	Population	2.819			1; 47	0.1
	Recording time	18.627	<0.001	<0.001	1; 457	<0.001

Table 2.2.4.b: Differences in behavioural trait values (Mean ± SD) between females of both commercial and wild populations.

Traits	Population		Sig.
	Commercial	Wild	
Distance travelled	(*298*) 58.98 ± 28.99	(*249*) 68.75 ± 28.35	ns
Walking speed	(*298*) 0.17 ± 0.03	(*249*) 0.18 ± 0.03	ns
Activity time	(*293*) 84 ± 8	(*242*) 86 ± 7	ns

Units: Distance travelled (cm), Walking speed average (cm/s), Activity time (%).
ns = non-significant differences. *, **, *** means significant differences where p-value is < 0,05, <0,01 and <0,001 respectively.

Physiological traits

There were no significant differences in tolerance to desiccation between populations for any of the relative humidities tested. However, the number of eggs laid positively influenced the hazard rate of desiccation in the linear mixed model, having more influence the higher the relative humidity except for the lowest relative humidity (35%) (Tables 2.2.5.a and b). This means that more reproductive effort shortened survival time more so at higher relative humidities. On the contrary, there was a difference between populations for tolerance to starvation, with the wild population lasting longer than the commercial. In addition, the number of eggs laid during the experiment positively and significantly affected the hazard rate from starvation, meaning that reproductive effort decreased starvation tolerance.

Table 2.2.5.a. Results of the Survival Mixed Effects Cox Models and GLMM using Template Model Builder for Tolerance to desiccation and starvation and predation rate traits respectively.

Traits		**Source of variation**	**χ^2**	**Estimate**	**SE**	**df**	***P***
Tolerance to desiccation	35%	Nº eggs laid	4.668	0.175	0.081	1	0.031
		Population	0.061			1	0.805
	50%	Nº eggs laid	10.22	0.231	0.072	1	0.001
		Population	1.995			1	0.158
	65%	Nº eggs laid	22.64	0.329	0.069	1	<0.001
		Population	0.413			1	0.520
Tolerance to starvation		Nº eggs laid	13.96	0.230	0.062	1	0.001
		Population	4.764			1	0.029
Predation rate		Intercept	44.63			1	<0.001
		Population	2.20			1	0.138
		Log (Nº initial of eggs)	1.699	-0.190	0.145	1	0.192

Table 2.2.5.b: Differences in physiological trait values (Mean ± SD) between females of both commercial and wild populations.

Traits		**Population**		**Sig.**
		Commercial	**Wild**	
Tolerance to Desiccation	35% RH	(391) 2.38 ± 0.68	(356) 2.38 ± 0.67	ns
	50% RH	(391) 2.81 ± 0.83	(351) 2.92 ± 0.93	ns
	65% RH	(386) 3.35 ± 1.02	(347) 3.49 ± 1.06	ns
Tolerance to starvation		(388) 5.20 ± 1.27	(345) 5.61 ± 1.49	*
Predation rate		(340) 16.77 ± 7.32	(308) 15.92 ± 7.17	ns

Units: Desiccation and starvation tolerance(days). Predation rate (nº prayed eggs/day). ns = non-significant differences. *, **, *** means significant differences where p-value is < 0,05, <0,01 and <0,001 respectively.

Regarding the predation rate on *T. urticae* eggs, there were no differences between populations nor did the initial number of eggs offered (Tables 2.2.5.a and 2.2.5.b).The test applied for predation rate did not provide significance of the random factor.

Dimensionality reduction

Tables 2.2.6. and 2.2.7 display the results obtained from the PCA of the means by isoline of all the traits assessed on females or in both females and males of both populations for the 6 first PCs. In addition, graphic representations of these results are shown in figures 2.2.1. and 2.2.2 respectively. See Appendix I, for detailed information on the correlations between variables and dimensions, the contribution of each variable (ctr) and the cosinus square (cos2), the latter indicating the quality of each variable in the PCA (also indicated by the length of the arrows in Figure 2.2.1).

When the PCA was performed for the isoline means of all the traits measured on the female individuals, the first two principal components explained 42,1% of the variance, being the 25% of the variance explained by the Principal Component 1 (PC1) and 17,1% by the PC2 (Table 2.2.6).

The PCA of the trait means by isoline, measured on both males and females of both populations, the % of variance explained by the PC1 (50,1%) and PC2 (18,6%) was almost 69% (Table 2.2.7).

The above analyses were performed as a preliminary way to visualize the relationships among variables, and to reduce the dimensionality of the analysis. However, note that the results of these analyses reflect multidimensionally what is obvious in the genetic correlation analyses performed in the next chapter. This is particularly so, since these PCAs are based on mean isoline values. One could potentially re-run the PCA analysis from the bivariate genetic correlation matrix obtained in the following chapters, the results would not likely be very different. However, obtaining bivariate genetic correlations is necessary to understand whether genetic constraints and trade-offs are truly meaningful (i.e., estimate their effect size and whether the estimates overlap with zero – see below).

Table 2.2.6: Results of the PCA for the variance explained by each PC, its proportion with respect to the total and the accumulated variance on traits assessed on females of commercial and wild populations.

PCA of Females						
	Dim.1	**Dim.2**	**Dim.3**	**Dim.4**	**Dim.5**	**Dim.6**
Variance	5.754	3.937	2.283	2.169	1.655	1.223
% of Var.	25.017	17.119	9.925	9.431	7.194	5.316
Cumulative % of var.	25.017	42.136	52.061	61.492	68.686	74.002

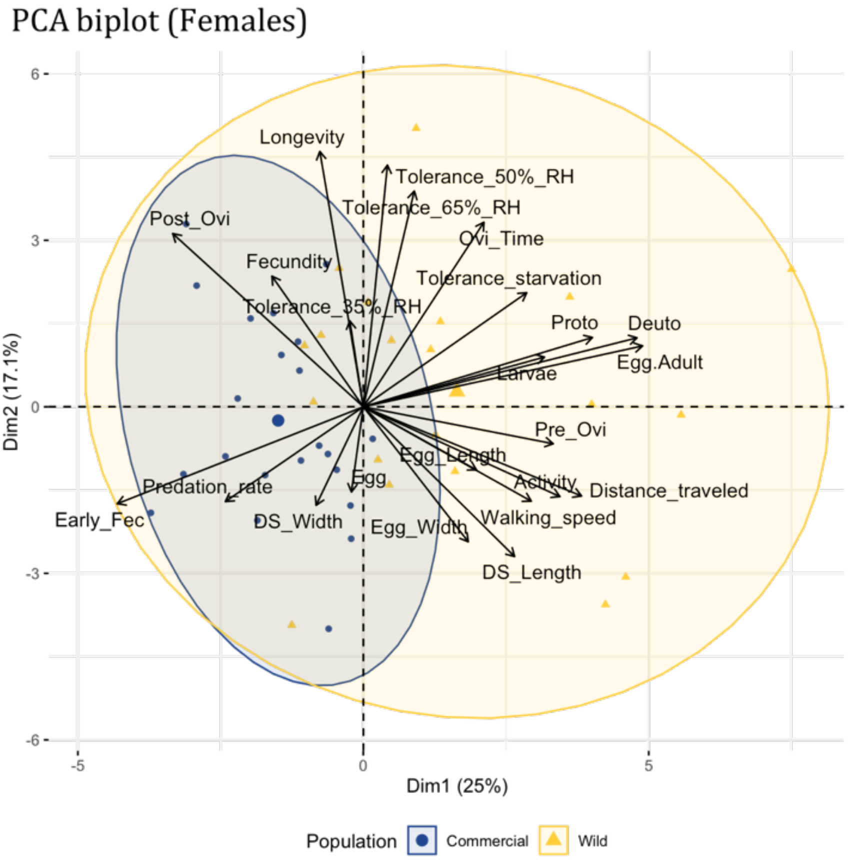

Figure 2.2.1: PCA plot of traits measured on females of commercial and wild populations.

Table 2.2.7: Results of the PCA for the variance explained by each PC, its proportion with respect to the total and the accumulated variance on traits assessed on both males and females of commercial and wild populations.

PCA of Females and males						
	Dim.1	**Dim.2**	**Dim.3**	**Dim.4**	**Dim.5**	**Dim.6**
Variance	3.509	1.301	1.006	0.793	0.303	0.089
% of Var.	50.129	18.581	14.367	11.324	4.325	1.270
Cumulative % of var.	50.129	68.711	83.078	94.402	98.727	100.00

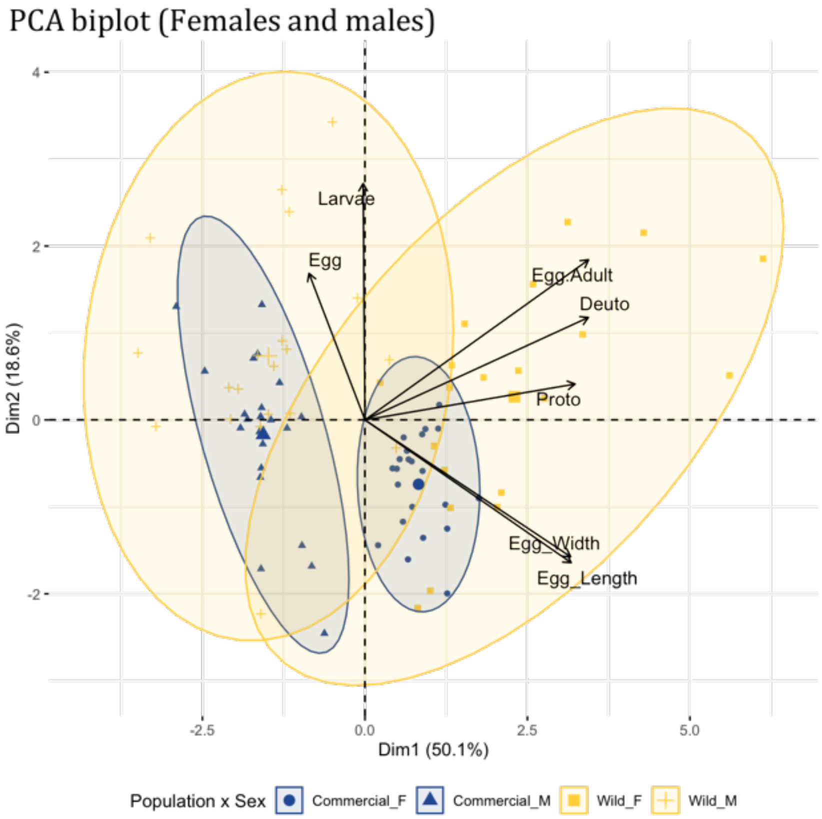

Figure 2.2.2: PCA plot of traits measured on both males and females of commercial and wild populations.

Regarding the reduction of traits for the calculation of H^2:

Life-history traits (except those related to oviposition and fecundity)

Both “*eigenvectors*” related to egg size had very similar lengths and directions. In addition, any of them had similar eigenvectors with the rest of life-history traits. Therefore, at least one of them should be included as a candidate for further H^2 calculation due to its relevance for the fitness of individuals. Since I consider that the measurement of the egg length was easier than the egg width despite having to deal with the resting position of the egg, and because both eigenvectors are very similar, I chose egg length as trait related to the egg size for further H^2 calculation. The figures show how the eigenvectors for most traits related to developmental time have practically the same size and direction, except for the time spent in the egg stage. Therefore, total developmental time from egg to adult was chosen as representative of the traits related to developmental time.

Life-history traits related to oviposition and fecundity

Regarding the traits related to fecundity, their eigenvectors have very different directions, each of them occupying one of the quarters made up of the first 2 dimensions, apart from the fecundity and post-oviposition period, whose vectors shared the same quarter. Due to the disparity in the direction of the vectors, I choose early fecundity as representative of the traits related to fecundity, based on a) methodological criteria, since it is easier and faster to evaluate than total fecundity, oviposition time or post-oviposition time, and b) the number of eggs laid during the first 10 days can be a good proxy for total fecundity since the maximum oviposition peak occurs during within this period.

Morphological traits

Body size is a trait that has many ecological implications, being key to determining the structure of communities and the dynamics of food webs (Peters, 1983; Woodward et al., 2005), and which helps to predict who eats whom (Brose et al., 2006). Therefore, a trait related to body size should be included. However, even though dorsal shield length and width eigenvectors did not have the same direction, both were in the negative part of the second dimension. Thus, between both traits, I choose the dorsal shield length as the trait for further H^2 calculation because i) the measurements obtained from the dorsal shield length showed less error among measures than those obtained for the dorsal shield width and ii) the dorsal shield is considered a good indicator of the body size in phytoseiid mites (Croft et al., 1999; Walzer & Schausberger, 2011).

Even though the egg dimensions and the dorsal shield of females had similar eigenvectors, I decided to estimate the heritability of both traits, as the former is a life-history trait highly related to early survival.

Behavioural traits

Although the "*eigenvectors*" for the behavioural traits were similar in the PCA (similar length and direction), I decided not to follow the criterion (see section "Dimensionality reduction", p. 77) and include all of them despite they were grouped under the same category. I took this decision because they are very important traits for BPC because they are related to both dispersal and foraging, and thus they are likely to be relevant effect traits.

Physiological traits

Within this category, traits related to tolerance to desiccation at different relative humidities and to starvation, all share a very similar direction of the eigenvectors, although with different lengths. Despite this, I included all of them to estimate their heritability as I consider their heritabilities at different RH conditions to be relevant, as they could provide valuable information for further research such as genetic improvement programs for low RH conditions. In addition, the Predation rate trait was also included as pointed to a very divergent direction from as compared to the other physiological or behavioural traits.

Thus, the traits chosen for the further analyses of H^2, according to the criterion for the reduction of variables, except for behavioural traits, were: egg length, dorsal shield length, developmental time from egg to adult, early fecundity, distance travelled, walking speed, activity time, desiccation to 35, 50 and 65% RH, starvation tolerance and predation rate (Tables 2.2.7 a and b).

In addition to the objective of reduction of the number of traits to calculate H^2, it can be seen in figures 2.2.1 and 2.2.2 that the variance of the wild population was much higher than that of the commercial one, which was always found within the wild population variance, that is, the genetic variance of the commercial population was held within the wild population.

Heritability of representative traits

Life-history traits (except those related to oviposition and fecundity)

H^2 of egg length of females was not significant for the commercial population, but it was for the wild population (H^2=0.11; Table 2.2.7.a). Significant H^2 was also detected when data from both populations were analysed together. The H^2 of Egg length of males was only significant when data from both populations were analysed together (H^2=0.08).

Developmental time from egg to adult did not present heritability for the commercial population neither for females nor males. On the contrary, females and males from the wild population showed substantial heritability (H^2=0.28 and H^2=0.22 respectively). When both populations were considered as one, there were significant H^2 values for both female and male sexes [H^2=0.32 and H^2=0.18 respectively; (Tables 2.2.7.a and b)].

Life-history traits related to oviposition and fecundity

None of the two populations showed heritability for early fecundity. However, when analysed together a significant H^2 value was found (H^2=0.13).

Morphological traits

H^2 of dorsal shield length of females was not significant for the commercial population. On the contrary, significant H^2 was detected for the wild population (H^2=0.17) and when data from both populations were analysed together (H^2=0.12).

Behavioural traits

None of the behavioural traits considered presented values other than zero for either of the two populations, not even when it was calculated by joining both populations.

Physiological traits

No significant H^2 values for Desiccation tolerance to 35, 50 or 65% RH nor Tolerance to starvation were found. Only when considering both populations together, significant values of heritability, despite being low, were shown for desiccation tolerance to 35, 50 or 65% RH (H^2=0.08 H^2=0.06 and H^2=0.06 respectively) and Tolerance to starvation (H^2=0.04). In addition, there was no heritability for predation rate on *T. urticae* eggs for either of the two populations or even when they were considered together.

For more extensive information on the results of the statistical analyses for the calculation of heritability, see Appendix I. Additionally, Appendix I shows graphs with the mean values and variation for all traits in each of the isolines from both populations.

Table 2.2.7 a and b: Broad-sense heritability (H^2) of traits measured on a) females and b) males when calculated by population (Commercial, Wild) or jointly (Total). LCI = Lower Credible Interval, UCI = Upper Credible Interval. Estimates were obtained from equation [4] (p. 51) as implemented in the outputs of the library MCMCglmm.

a) Females

Traits		Population	H^2	LCI	UCI	Eff. Size	Sig.
Egg Length		Commercial	-0.05	-0.05	0.04	14449.48	ns
		Wild	0.11	0.03	0.27	29500.00	*
		Total	0.07	0.03	0.14	29500.00	*
Egg-adult time		Commercial	-0.02	-0.05	0.07	17670.42	ns
		Wild	0.28	0.13	0.47	29500.00	*
		Total	0.32	0.23	0.46	29500.00	*
Early Fec.		Commercial	-0.05	-0.05	0.02	11857.96	ns
		Wild	0.09	-0.01	0.29	28934.37	ns
		Total	0.13	0.06	0.24	29500.00	*
DS Length		Commercial	-0.01	-0.05	0.09	19993.04	ns
		Wild	0.17	0.05	0.35	29500.00	*
		Total	0.12	0.06	0.22	29500.00	*
Distance Travelled		Commercial	-0.04	-0.05	0.02	16246.18	ns
		Wild	-0.05	-0.05	0.07	15319.75	ns
		Total	-0.02	-0.02	0.04	10073.08	ns
Walking speed		Commercial	-0.05	-0.05	0.04	10891.46	ns
		Wild	-0.05	-0.05	0.03	11949.53	ns
		Total	-0.02	-0.02	0.03	6653.80	ns
Activity		Commercial	-0.05	-0.05	-0.01	13821.04	ns
		Wild	-0.05	-0.05	-0.01	14349.52	ns
		Total	-0.02	-0.02	0.00	8452.45	ns
Desiccation tolerance	35% RH	Commercial	0.04	-0.02	0.17	28212.04	ns
		Wild	0.07	-0.02	0.21	27462.26	ns
		Total	0.08	0.03	0.16	29500.00	*
	50% RH	Commercial	0.04	-0.03	0.15	27576.01	ns
		Wild	0.01	-0.05	0.13	24210.87	ns
		Total	0.06	0.01	0.13	28490.38	*
	65% RH	Commercial	-0.01	-0.05	0.09	17749.42	ns
		Wild	0.06	-0.03	0.18	28723.01	ns
		Total	0.06	0.01	0.13	27680.12	*
Starvation tolerance		Commercial	-0.05	-0.05	0.07	14635.77	ns
		Wild	-0.01	-0.05	0.09	18140.73	ns
		Total	0.04	0.00	0.11	27348.40	*
Pred. Rate		Commercial	-0.05	-0.05	0.04	12495.99	ns
		Wild	-0.05	-0.05	0.03	11669.58	ns
		Total	-0.02	-0.02	0.04	7666.30	ns

b) Males						
Traits	**Population**	H^2	**LCI**	**UCI**	**Eff. Size**	**Sig.**
Egg Length	Commercial	0.03	-0.05	0.16	16131.35	ns
	Wild	-0.05	-0.05	0.12	13188.82	ns
	Total	0.08	0.02	0.17	26198.53	*
Egg-adult time	Commercial	-0.05	-0.05	0.01	12075.08	ns
	Wild	0.22	0.07	0.46	29500.00	*
	Total	0.18	0.09	0.31	29634.47	*

CHAPTER 3: Genetic correlations

Material and Methods: Statistical Analysis

All the statistical analyses were done using the computer environment R (v. 4.0.2) (R Core Team, 2022).

Genetic correlations were calculated differently depending on whether calculations were done from traits measured in the same individuals or whether they came from different individuals. The former, by including data at the individual level, allows precise accurate estimates as these are calculated after partitioning the genetic and environmental variance-covariance matrix of the two traits (Wilson et al., 2010). The second approach will provide less accurate estimates; i.e., the estimates more distant from the true population value than in the first approach. This is because the isoline means are directly used as genetic estimates, implicitly assuming that their value has no environmental component. However, if there is environmental variation, it will add noise (error variance) to the estimates. Thus, traits measured in the same individuals are preferable because individual variation can be used to tease apart environmental from genetic variance-covariances. However, when logistics for the measurement of very disparate traits does not allow this path, using the isoline means is still an option (David et al., 2005).

Genetic correlations of traits measured in the same individuals were estimated by running bivariate "MCMCglmm" models (Wilson et al., 2010). Prior covariances were set at zero and isoline and residual variances at 0.5, after dependent variables were all standardized to zero mean and unit variance. Therefore, 1:1 variances were assumed in the prior specification. The degree of believe (*nu*) was set at 2. For correlations I chose a burning of 500, a thinning interval of 10 and a number of iterations of 30000, resulting in more than 2200 effective samples for all correlations. Posterior probabilities; i.e., the degree to which the hypothesis of a relationship (either positive or negative) was supported, were calculated by dividing the number of times that posterior estimates were higher (if the posterior mean of r_G was >0) or lower (if the posterior mean of r_G was < 0) than zero, divided by the number of posterior samples (Korner-Nievergelt et al., 2015).

When correlations were estimated from different individuals, I calculated Pearson correlations between the mean isoline values using Bayesian robust estimation assuming Student t instead of normal distributions in the function "robust_correlation" (Baez-Ortega, 2019) written for "stan" and which I ran in "Rstan" package (Stan Development Team, 2023). I ran 2000 interactions and 500 to warmup, in each of four MCMC chains. Posterior probabilities were calculated from the posterior values in the MCMC chains as explained above for MCMCglmm models.

Graphs were done using the ggplot2 function (package "ggplot2") (Wickham, 2008).

Results

Genetic correlations

Figures 2.3.1 and 2.3.2 show the significant genetic correlations between traits measured in the same individuals, in females and males, respectively. The results (genetic correlation coefficients (r_g), 95% credible intervals, and posterior probabilities) of all pairwise relationships, including also those not significant, are provided in Appendix II.

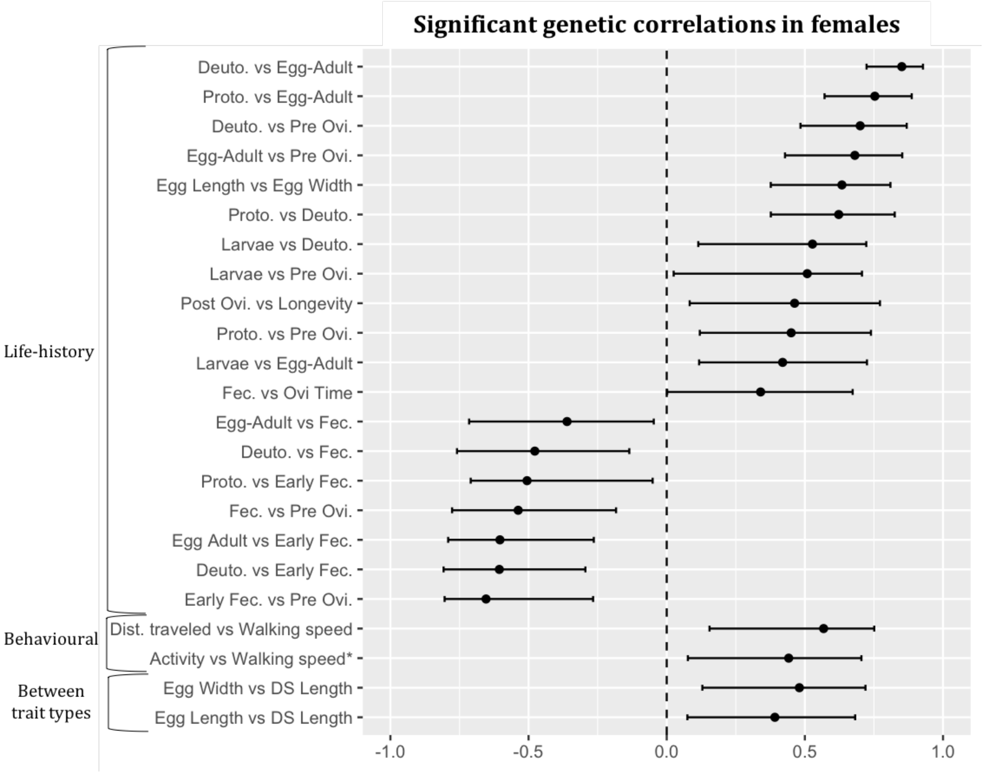

Figure 2.3.1: Significant genetic correlations (r_g +/- credible intervals) between traits assessed in the same female individuals. Behavioural traits were corrected for the number of recordings by including this variable as a covariate in the models.

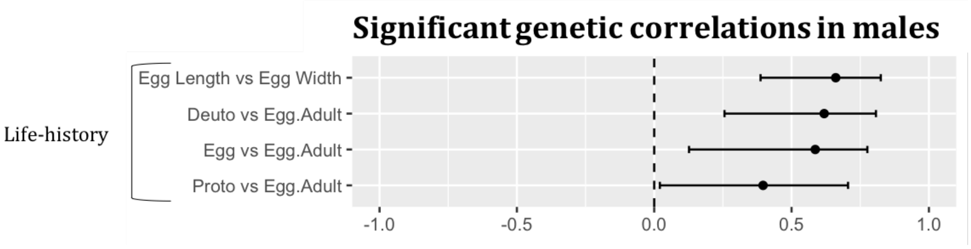

Figure 2.3.2: Significant genetic correlations (r_g +/- credible intervals) between traits measured in the same male individuals.

Life-history traits

Egg length was highly positively correlated with egg width in eggs that produced either females or males (r_g=0.63 and 0.66 for females and males respectively), that is, the longer the eggs are, the wider they are and vice versa, something expected assuming that the shape of the eggs does not change with size.

Most of the traits regarding developmental time were positively genetically correlated to each other, both in the case of females and males. In particular, the time in deutonymph was the most correlated with total developmental time, that is, from egg to adult, with r_gs as high as 0.85 and 0.62 for females and males respectively. In addition, individuals whose genes determined longer developmental times had longer pre-oviposition times. Besides, both developmental and pre-oviposition times were negatively correlated with fecundity in either of its estimates, during the first 10 days and throughout the life of the females. However, being genetically determined to develop later was not correlated to being genetically larger (see Appendix II, p. 215), although a slight positive correlation was apparent when estimates came from isoline means (see Appendix II, p. **Error! Bookmark not defined.** and Figure 2.3.4).

Fecundity and oviposition time were positively correlated, indicating that probably the number of eggs to be laid, fecundity, constraints for how long they are laid.

There was a positive correlation between Post-oviposition time and longevity. Therefore, longevity largely depended on genes that determined the extension of live beyond the reproductive age.

Behavioural traits

Genetic correlations between behavioural traits indicated that individuals genetically determined to walk faster travelled longer distances. In addition, genes that determined the level of activity in individuals seemed to also be involved in making them faster.

Between trait types

In egg-producing females, the length of the egg was correlated positively with the length of the dorsal shield, indicating that bigger eggs produced bigger females. The meaning of this information can be complemented with that of the correlations measured using isoline means (see below).

Genetic correlations by isoline means

Figure 2.3.3 shows all the significant genetic correlations between traits assessed by isoline means weather measured in the same individuals or not. The results (genetic correlation coefficients (r_g), 95% credible intervals, and posterior probabilities) of all possible genetic correlations calculated by isoline means, whether significant or not, can be found in Appendix II, p. **Error! Bookmark not defined.**.

Most of the results matched with the significant genetic correlations between traits measured in the same individuals when calculated through MCMCglmm (see section above and figure 2.3.2). Additionally, calculating the genetic correlations by isoline means, new outcomes are obtained. Among the most remarkable results that can be found, I highlight the following:

Life-history traits

Oviposition time and fecundity were genetically positively correlated to longevity. In addition, the larger the number of eggs they lay at the beginning of their adult live (early fecundity), the longer the post oviposition period at the end of the adult life, although the correlation is rather small (~0.1). Similarly, there is a positive correlation between egg size and egg developmental time indicating that larger eggs take longer to develop.

There is a negative relationship between the number of eggs (early and total fecundity and even oviposition time) and the egg size, a fundamental trade-off, which has been previously reported to have a genetic basis in some organisms (Roff, 1992). However, my estimates were rather small (~-0.1).

Behavioural traits

The three traits are highly positively correlated to each other, strongly suggesting that they can respond synergistically to selection.

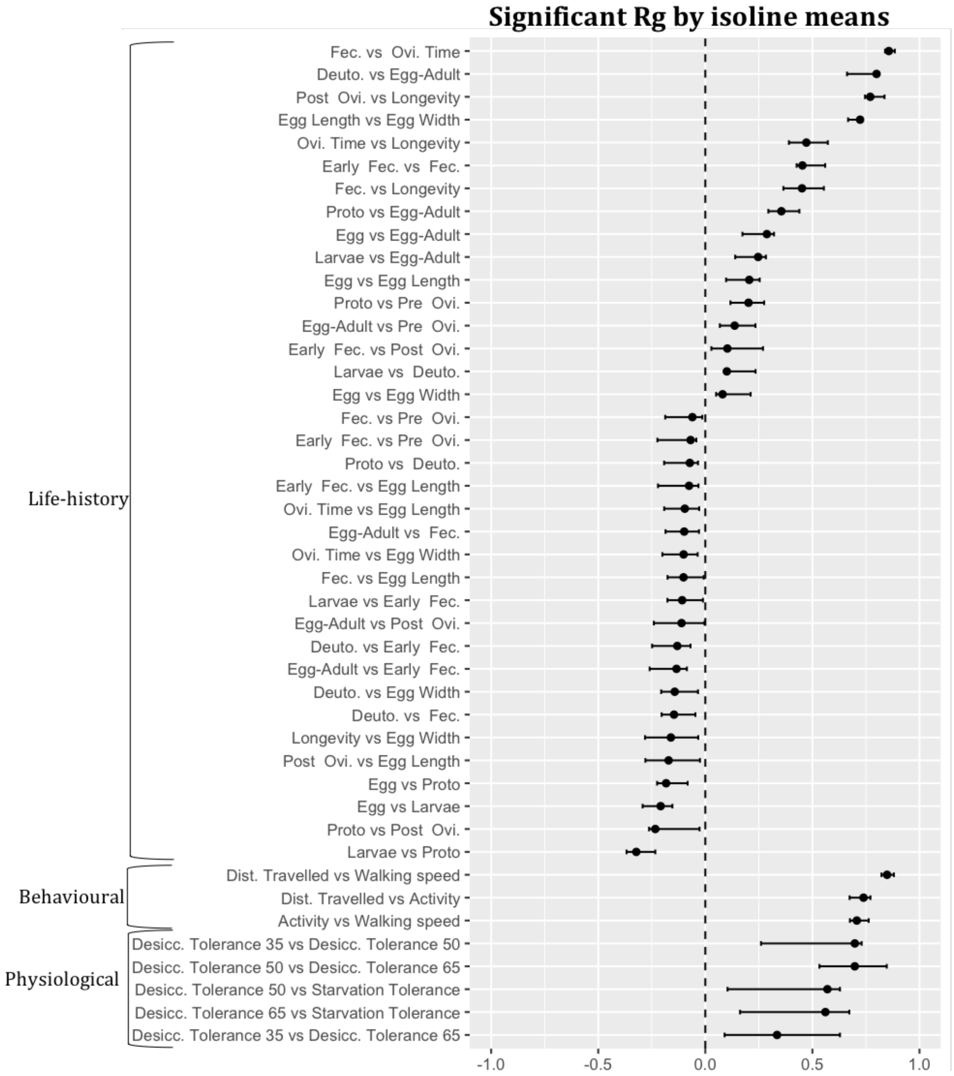

Figure 2.3.3: Significant genetic correlations, calculated from isoline means (r_g +/- credible intervals). Traits are ordered by trait type.

Physiological traits

The traits in which tolerance to desiccation at different relative humidities was evaluated were all positively correlated to each other. Furthermore, both tolerance to desiccation at 50% and 65% RH were positively correlated with starvation tolerance, suggesting that the same mechanisms, which would have a common genetic basis, would be involved in each type of tolerance, therefore, also pointing to a fundamental genetic correlation.

Between trait types

Figure 2.3.4 shows all the significant genetic correlations between traits belonging to different trait types, as calculated by isoline means. Among the new positive genetic correlations appearing between trait types, some of the most remarkable are those between longevity and tolerance to desiccation at 50 and 65% RH. Again, a positive correlation appears between egg and adult sizes. Since this genetic correlation was calculated by isoline means, each egg is not associated with the female in which its size was subsequently measured. Thus, this indicates that in addition to larger eggs giving rise to larger females (positive genetic correlation -see above-) larger females are genetically equipped to lay bigger eggs. Therefore, the heritability of body size may be mediated by its correlation to egg size. Although to a small extent, there are positive correlations between traits related to fecundity (oviposition time, early fecundity, and total fecundity) and behavioural traits (distance travelled, walking speed and activity), indicating that individuals with higher movement abilities are more fertile.

Results revealed that there seems to be a genetic trade-off between traits related to fecundity (mainly fecundity and oviposition time, and Early fecundity in lesser extent) with tolerance to starvation; therefore, the larger the number of eggs that females from an isoline can lay, the lower the chance that those females will survive periods of food deprivation. This is regardless of the number of eggs laid at the point where deprivation was measured. This seems to be a genetic trade-off originated from the cost of investing in reproduction even before reproducing; that is, genetics makes all the machinery to lay a large number of eggs to be on at the time of maturation and this detracts from tolerating starvation, which likely uses some of the same resources. Similarly, there was a negative correlation between early fecundity and tolerance to desiccation at both 50 and 65% RH, although the latter to a much greater degree. Results also indicated that phenotypes with shorter developmental time displayed higher predation rates on *T. urticae* eggs. Also, there was a negative correlation between time spend in egg stage and tolerance to desiccation at 50% RH, that is, the shorter the time in egg, the longer the tolerance to desiccation.

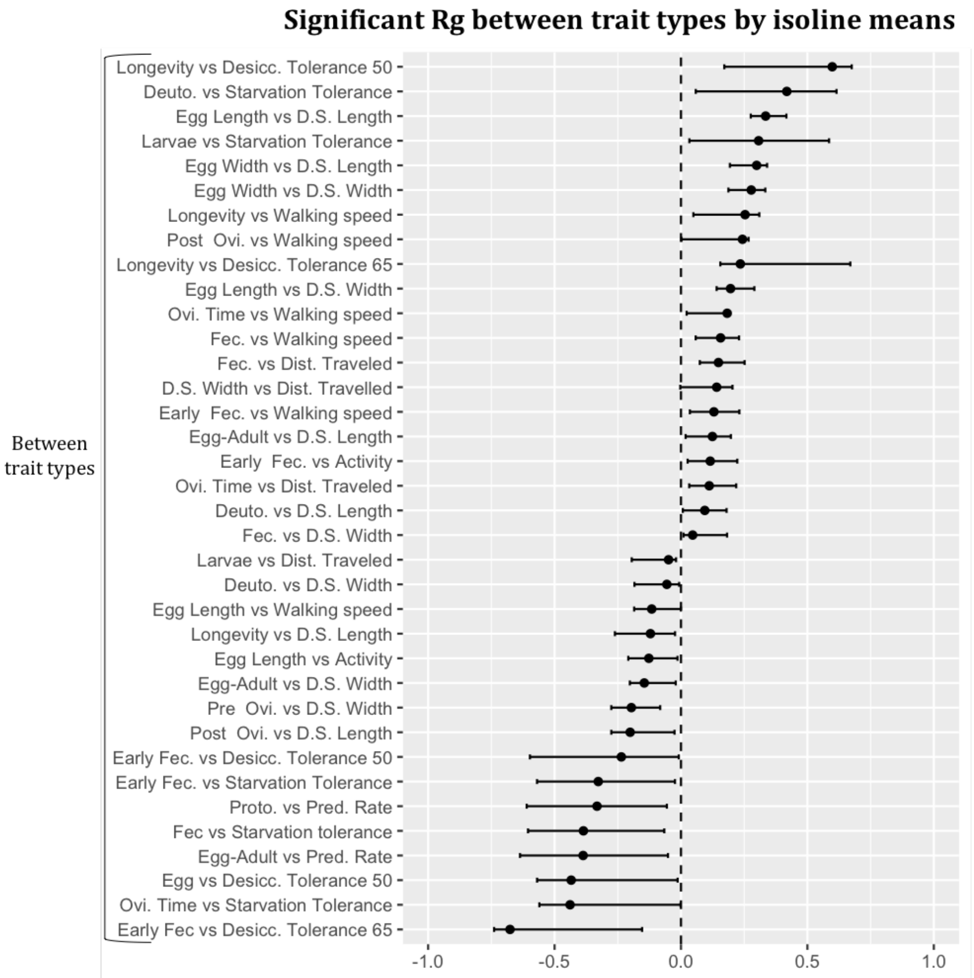

Figure 2.3.4: Significant genetic correlations between trait types as calculated from isoline means (r_g +/- credible intervals).

SECTION 3: Evolutionary responses and costs of adaptation under biotic and abiotic selection forces

Introduction common to Chapters 4 and 5

When biological control agents are released in the field, they may be exposed to environmental stressors that can negatively affect their efficiency at controlling pests (see Chaper1, section "Factors affecting the outcomes of BPC", p. 12). Indeed, biotic interactions such as competition and predation, and abiotic factors such as warming, are strong selection forces that can affect populations via the evolutionary processes they trigger (see Chapter 1, section "Ecology and evolution to improve BPC: a holistic approach", p. 26). Furthermore, these two types of selection forces probably do not act independently but simultaneously, which opens the door for the emergence of potential interactive effects between the two forces on evolutionary processes. Here I intended to disentangle the evolutionary processes driven by either or both biotic and abiotic selective forces using full crossed factorial experimental designs. The goal was to evaluate whether traits holding heritable genetic variability were able to evolve, and towards what direction, when populations were exposed to selection pressures accounting for both the community context (presence of an Intraguild predator) and climate change (high temperatures). This is within the context of integrating this knowledge to improve the methodologies involved in acquiring strains of BCAs directionally selected to improve their performance in the field.

Genetic improvement of BCAs

The genetic improvement of BCAs can be carried out by inducting rapid evolution via evolution experiments (Lirakis & Magalhães, 2019). Yet, other methodologies can also be implemented, such as genomic selection (recombinant DNA techniques) (Leung et al., 2020; Routray et al., 2016). The use of evolution experiments to improve the performance of BCAs is feasible because most of the BCAs are insects or mites (van Lenteren, 2012) that hold characteristics that define them as good candidates for evolution experiments: i) they are easy to maintain in the laboratory; ii) they have short generation times; and iii) they can reach large population sizes (Belliure et al., 2010). Indeed, genetic improvement programs focusing on BCAs have proven to improve their performance by increasing their tolerance to hostile climates, increasing their ability in finding hosts, changing their host preferences, improving the synchronization with their host, increasing their tolerance to insecticides, or selecting for non-diapause strains, among other relevant traits (Belliure et al., 2010; Hoy, 1986; Lirakis & Magalhães, 2019; Routray et al., 2016; Whitten & Hoy, 1999). For example, Arora and Shera (2014) succeeded at selecting two strains of the parasitoid *Trichogramma chilonis* Ishii

(Hymenoptera: Trichogrammetidae), one adapted to high, and another adapted to low, temperatures. This BCA is used to control several moth pests that cause damage to sugarcane, cotton, and vegetable crops. The strain adapted to high temperatures performed better than other strains against the sugarcane stem borer *Chilo auricilius* Dudgeon (Lepidoptera: Crambidae) and the early shoot borer, *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae) during the hot months in summer (Singh et al., 2007, 2008). Similarly, the strain adapted to low temperatures performed better than the non-adapted strain when they were evaluated under laboratory low temperatures, indicating that for its field success in the cooler months in temperate areas, a low temperature adaptation may be required (Jalali et al., 2006).

Methodologies involving evolutionary experiments

The two methodologies for the genetic improvement of BCAs that are based on evolution experiments are artificial selection and experimental evolution (Belliure et al., 2010; Garland & Rose, 2009; Kawecki et al., 2012).

Artificial selection (or selective breeding) consists of directional genetic improvement of populations by scoring (or phenotyping) the trait of interest in individuals within the population. Next, breeders, that is, the parents of the next generation, are chosen based on their score (Garland & Rose, 2009). This methodology is usually applied when the goal is that a specific desired phenotype be over-represented in the population. In artificial directional selection individuals with desired trait values are favoured under indoor conditions. The trait of interest is measured in all individuals within one generation and the individuals holding the highest values for the trait to be improved, are the only ones allowed to mate to each other for the next generation, and so on from generation to generation. The population mean value of this trait will change across generations as long as some additive genetic variance exists and there are no genetic trade-offs in fitness-related traits. There are many examples of successful genetic breeding in BCAS. For example, the predatory bug *O. laevigatus* was artificially selected for better fitness when feeding on a suboptimal diet (Mendoza et al., 2020a), for bigger size (Mendoza et al., 2020b), and resistance to certain pesticides (Balanza et al., 2019). Also, Lommen et al. (2019) applied bidirectional selective breeding from mild wing truncation individuals to get different types of flightless morphs of the two-spotted ladybird beetle *Adalia bipunctata* L. (Coleoptera: Coccinellidae), which is used for the control of aphids in greenhouses. The authors succeeded at selecting downwards a population without any wing tissue, and upwards a population with only very small truncations in wings.

This methodology can have some drawbacks because it requires previous knowledge of the trait, or group of traits, to be selected, which is not an easy task because in some cases the desired trait is not obvious. Indeed, what traits should be selected in BCAs' to improve their performance has been a topic of debate over time (Hopper et al., 1993; Hoy, 1986; Whitten & Hoy, 1999). Leung et al. (2020) suggested that the lack of progress in BCAs' genetic breeding could be due to this reason. Recently, Bielza et al. (2020) highlighted this problem and identified potential candidate traits to be improved. The authors suggested firstly looking at the limiting factors for the BCAs' good performance, and, secondly, focusing on the traits that would allow BCAs to overcome those limitations. Lastly, they provided a list of possible candidate traits for the artificial improvement. Another drawback of artificial selection is that phenotyping of individuals for the trait(s) of interest is required at each generation, which usually is much more costly and time-consuming.

The experimental evolution approach (Garland & Rose, 2009; Kawecki et al., 2012) consists of the study of the populations' evolutionary changes, where individuals are let to evolve in response to selection forces imposed by the experimenter but without any intended directional selection by her/him (Belliure et al., 2010; Garland & Rose, 2009). This way selection can act on any or all traits and genes relevant to fitness under the environmental regimes of interest. This methodology aims at characterizing the evolutionary responses of a population under a given environment. Selection forces act on individuals bearing particular trait values, and if these traits are inheritable, an evolutionary change (evolutionary response) will occur. Any perturbation in an ecosystem can affect <u>response traits</u> affecting differentially the fitness or the mortality of the individuals within a population or community. When these ecological responding traits have a genetic basis, this could result in populations undergoing adaptation processes from natural selection imposing genotype changes. Phenotypic plasticity (i.e., the expression of different phenotypes by the same genotype according to environmental changes) is another way in which traits can respond to ecological perturbations, whether adaptively or not, and which do not involve genetic changes.

Experimental evolution does not have the main drawbacks of artificial selection. With this methodology, the researcher should start with the most possible outbreed population, thus holding the highest possible genetic variability for most of the traits, to increase the population's chances to be able to evolve via "natural selection" acting through the artificially imposed selective forces, generation after generation. This way there is no need to neither know nor choose a priori which trait to select, nor to phenotype individuals at each generation but only at the end of the experiment. However, when feasible, measuring the trait at different points during the experiment could be desirable if one wants to document the ongoing evolutionary dynamics. Therefore, this methodology assumes that the individuals displaying the best phenotypic values under the artificial environment under which they are raised, will be the parents of the next generation, and so on. Furthermore, if selection conditions are very strong, only the survivors will breed; whereas if conditions are not strong enough to cause mortality of individuals, genotypes displaying higher fitness will contribute more progeny to the next generation, compared to less fitted genotypes. Consequently, as generations go by, there will be evolutionary responses causing a change in the frequency of alleles, leading to changes in the trait´s mean within the population. The process will succeed as long as negative genetic correlations with fitness-related traits are not present, or do not involve traits relevant for the particular set of environments under which the experiment is conducted. Therefore, this technique allows the researcher to assess whether traits evolve, and if so, in which direction and to what extent. This results are achieved by comparing trait values of populations exposed to stressful conditions with those not exposed and maintained in neutral environments; i.e., the controls (Kawecki et al., 2012).

Considerations to contemplate when applying experimental evolution

Experimental evolution is a good experimental tool when researchers seek to know what traits evolve, and how, when populations are exposed to given stressful environments. Yet, in experimental evolution several considerations need to be considered: i) The unit of replication is the population, not the individuals. Therefore, it is crucial to run several populations under the same conditions to disentangle adaptation by quasi-natural selection from possible genetic drift, or biased setups (Lirakis & Magalhães, 2019; Sousa et al., 2019); ii) before phenotyping the individuals at the end of the experiment, it is essential to expose populations to the environment without the selection force for one or two generations, to eliminate non-genetic effects derived from phenotypic plasticity or environmental maternal and grand-maternal effects (Kawecki et al., 2012; Lirakis & Magalhães, 2019; Sousa et al., 2019). This is because long-term exposure to stressful environments can change the mean phenotypic value of traits in the populations without a change in the allele frequencies, and iii) populations under experimental evolution should be initiated from a high-enough number of individuals to attain the maximum genetic variance in the initial population. This recommendation is because the underrepresentation of alleles in the initial population could lead to genetic drift or founder effects, which could mask evolutionary responses from the quasi-natural selection (Lirakis & Magalhães, 2019; Sousa et al., 2019).

Objectives of Section 3

This section addresses the Objective 2 of this thesis (p. 41). In short, to use experimental evolution to disentangle evolutionary processes driven by either, or both, biotic and abiotic factors considering realistic scenarios of community contexts and climate change (chapter 4), and evaluate potential adaptations and costs in the exposed populations (chapter 5).

In Chapter 4 I present the results of experimental evolution. Specifically: I exposed populations of the wild strain of *A. swirskii* to two selection forces, one abiotic (high Temperature) and one biotic (presence of the top predator *Orius laevigatus*), using a full-crossed factorial design. At the end of the experiment, I only measured the traits that were susceptible to be response traits, that is, those that held significant H^2 (Chapter 2).

In Chapter 5 I present the results of evolutionary adaptation: Specifically: I measured a) possible adaptation and/or costs to high temperatures: some traits (oviposition rate and size of eggs) were measured in individuals from populations that were previously exposed or not, to high temperatures under experimental evolution when exposed to either mild or hot temperatures, b) possible adaptation and/or costs to high temperatures and/or presence of a predator, measuring survival and number of eggs laid when exposed to a heat shock; and c) adaptation or costs to the presence of higher order predator: survival, number of individuals that ran away from the arena, and number of laid eggs were measured in individuals that came from evolutionarily experimental lines previously exposed, or not, to the presence of the top predator *O. laevigatus* during experimental evolution time, and exposed again (in ecological time) to the presence or absence of the predator.

Material and Methods common to Chapters 4 and 5

Arthropod cultures

Amblyseius swirskii

The experiments presented in this section were conducted using the sub-populations of the wild strain of *A. swirskii* only, because a) the genetic variability of most of the evaluated traits was higher in the wild strain than in the commercial strain and b) the multidimensional space of the genetic variability found in all the traits measured in the commercial population was contained within that found in the wild population (Chapter 2; Figures 2.2.1 and 2.2.2, pp. 78-79)

The wild population of *A. swirskii* was maintained in rearing units at laboratory conditions (25±2°C, 70±10% RH, 16:8 L-D), and it was fed with *C. edulis* pollen supplied *at libitum* three times a week (see "Mite Cultures", p. 55, for more details).

Orius laevigatus

The stock colony of *O. laevigatus* Fieber (Hemiptera: Anthocoridae) was started from ca. 500 individuals provided by Koppert Biological Systems (Thripor-L®). The insect was cultured following the method described in (Bueno et al., 2006). In short, rearing units consisted of Plexiglas bottles (14,5x10cm ∅) with 2 air vents, one at the bottom and the other in the lid, screened with 70µm ∅ fine-mesh nylon gauze. Each bottle contained buckwheat husks as substratum that provided shelter and hiding places to reduce cannibalism. Green bean pods served as oviposition substrates, and 2ml Eppendorf tubes filled with water and cotton wool served as a resource for water. Bugs were fed 3 days per week with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, provided by Biobest (Westerlo, Belgium) and stored at -20°C. The rearings were kept in a room at 25±2°C, 70±10% RH and 16:8h L:D photoperiod. New cultures were made weekly, transferring bean pods with *O. laevigatus* eggs to new bottles.

CHAPTER 4: Evolutionary responses

Material and Methods

Experimental evolution

The wild strain of *A. swirskii* was reared under laboratory conditions for more than two and a half years (approximately 90 generations) before the experiment. Therefore, adaptations to these conditions (mild temperature and absence of predators) could have occurred. Thus, I assumed that the selection pressures used in experimental evolution had not been recently affecting the populations.

Subsets of populations obtained from the wild strain of *A. swirskii* were exposed, in a full factorial crossed design, to two selection forces (Table 3.4.1). One selection force was a *biotic* stressor consisting in exposing the populations to the presence of a top predator, that is, the presence of 2 individuals in pre-adult stages (4-5th nymph instars) of *O. laevigatus*. Exposure to predators consisted of alternating their presence or absence for two consecutive days. This discontinuous exposure was done to control for the high mortality this predator can impose on individuals of *A. swirskii* (Serrano-Carnero personal observation). The second selection force was an *abiotic* stressor consisting in exposing populations to a regime of high temperatures. Six replicates per treatment were carried out.

Table 3.4.1: Design of the evolution experiment in a full factorial crossed design, consisting of two factors with two levels each (abiotic: "Mild" or "Hot" conditions; and biotic factor: "Presence" or "Absence" of a predator). Each treatment had 6 replicates.

Abiotic factor (Temperature)	**Biotic factor (Presence of *O. laevigatus*)**	
	Absence	**Presence**
Mild	x6	x6
Hot	x6	x6

The temperature regime of the exposed populations (treatment "Hot") was 34°C during the day (5h) and 27°C during the night (8h), and a total of 11h of an ascending-descending ramp between both temperatures, with a resultant average/day of 30°C. The control population (treatment "Mild") was exposed to a constant temperature of 25°C. (Figure 3.4.1). All the populations of the experiment were kept at 70±5% RH and 16:8h L:D photoperiod. Mites were fed *ad libitum* three times per week with *C. edulis* pollen.

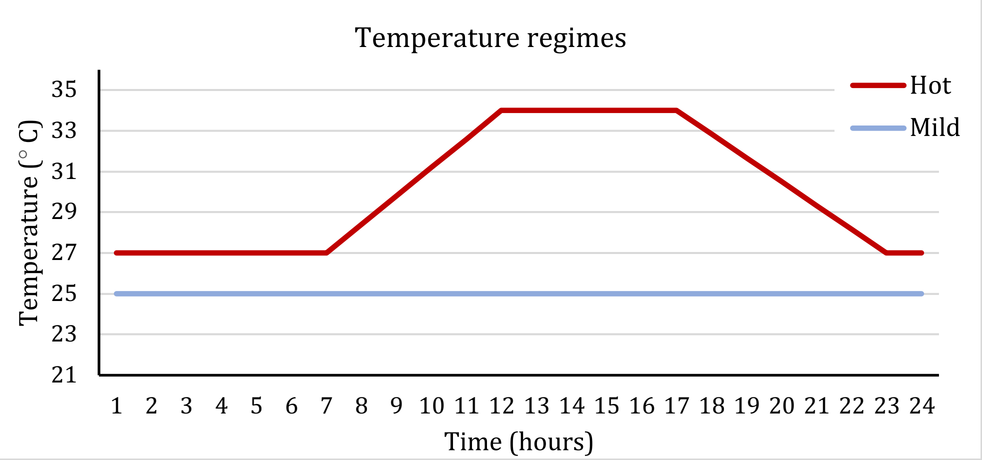

Figure 3.4.1.: Daily temperature regimens from both mild and hot abiotic conditions.

Experimental arenas consisted of 16 x 16cm plastic sheets placed on top of sponges covered by water-soaked cotton in 23,5 x 23,5cm recipients with water. The edges of the plastic sheet were covered with filter paper, serving both as a water source and a barrier to prevent mites from escaping. Four roof-shaped plastic pieces with a cotton thread under them were provided as shelter and/or ovipositing sites.

The methodological procedure consisted of transferring 200 gravid females (founders of each experimental population), taken randomly from the base wild population, to the arenas where they could oviposit. Before their offspring (F_1) became adults, these founder females that remained alive were removed. Then, F_1 individuals were allowed to reach adulthood and to mate randomly. Subsequently, F_1 gravid females were randomly collected (up to 200 when possible, depending on availability) and transferred to new experimental arenas, as founders of the new generation. This procedure was repeated until reaching the 10th generation (F_{10}).

To standardize potential differential effects caused by location, experimental arenas were randomly repositioned within climate chambers at each run.

Once the evolution experiment finished, the resulting populations were kept in a common and non-stressful environment (25±1°C, 70±10% RH, and 16:8h L:D, and absence of top-predators), fed with *C. edulis* pollen, for at least 3 generations, to eliminate potential maternal or grand-maternal effects before the phenotyping of individuals (Figure 3.4.2). Thus, traits were assessed in individuals that had been exposed to control conditions, for at least three generations after the experiment was finished.

Specific methodological details for factor "Abiotic"

The time founder females could oviposit in the arenas depended on the temperature regime to which they were exposed: developmental time of phytoseiid mites is influenced by temperature [i.e., the higher the temperature, the shorter the developmental time, up to a maximum temperature threshold). I used life table results at different temperatures (Lee & Gillespie, 2011; Yousef et al., 1982)], to estimate the times of parents/offspring co-occurrence. Accordingly, founder females at each generation were allowed to oviposit under hot or mild conditions for 5 or 6 days, respectively, before removing them from the arenas, to avoid overlapping of parents and (adult) offspring of consecutive generations, that is, females were allowed to lay eggs for as long as possible before their first eggs reached adulthood. Once founders of each generation were removed, the remaining individuals from populations exposed to either mild or hot conditions were kept in the arenas for 6 or 5 days, respectively, to allow all the mites to reach adulthood and mate randomly.

When the number of founder females in each generation was less than 200 individuals, arenas were resized according to their number, to keep population densities constant at the beginning of each run.

Importantly, when founder females of a generation were less than 50 individuals, the evolution experiment was stopped until the populations recovered. It happened with populations exposed to hot conditions. Because of this high mortality, experimental evolution of these populations reached only up to the F_8. Despite the risk of loss of alleles from bottlenecks, I preferred to take this path over refilling the populations with 150 females from the stock population, because that would have prevented us from seeing any signs of evolution from natural selection.

Specific methodological details for factor "Biotic"

The addition of the top predator caused high mortality in the populations of *A. swirskii*. Consequently, in some rounds it was not possible to obtain the desired initial number of 200 females. Therefore, I used the following criteria as proxy of a constant initial top-predator/predatory mite ratio at the beginning of each round: when the number of *A. swirskii* founder females were <100 I added 1 individual of *O. laevigatus;* when the number of founder females was >100 I added 2 individuals of *O. laevigatus*.

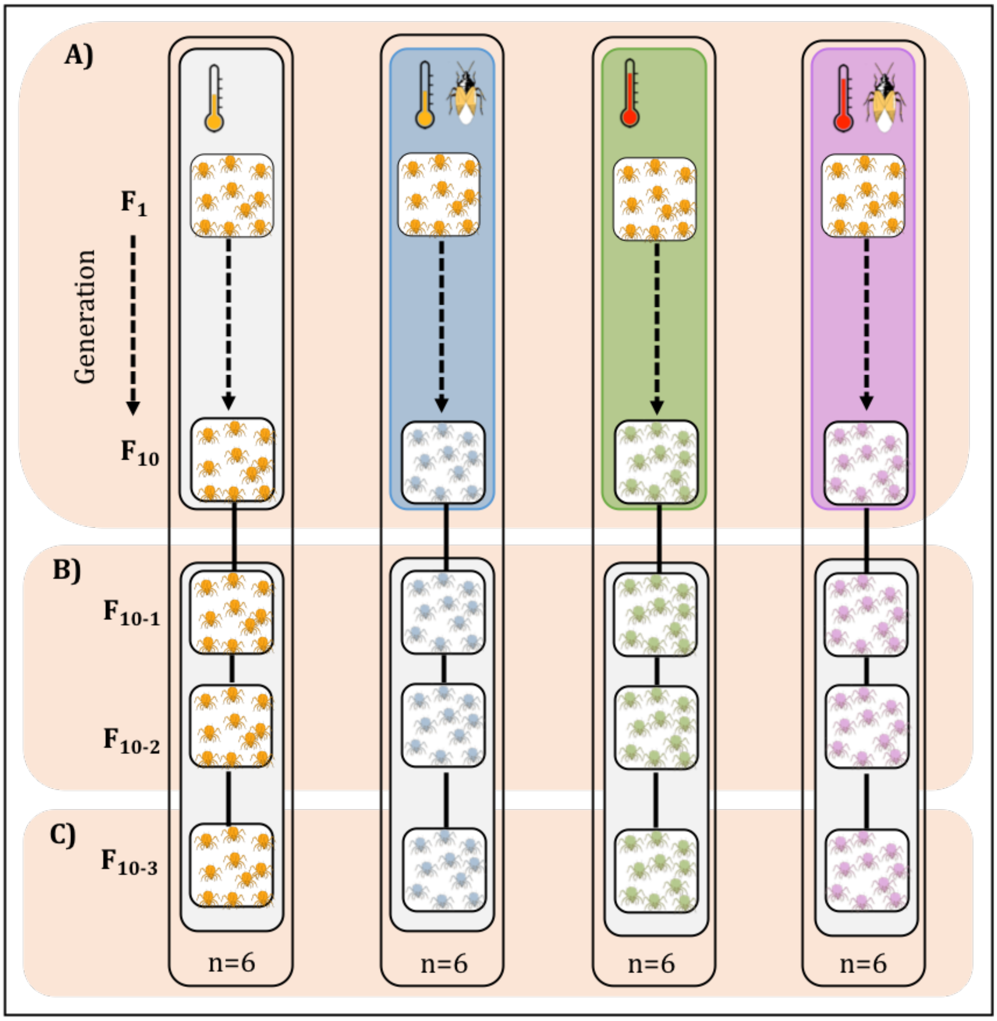

Figure 3.4.2: Scheme of the steps followed for the evolution experiment. From the exposure of the populations to the different treatments to the measurement of traits of the individuals of the resulting populations the steps where: **A)** populations of *A. swirskii* were exposed for 10 generations to different biotic and abiotic regimes (coloured columns). **B)** The resulting populations (F_{10}) were exposed to control conditions (without stressors) for two generations to eliminate maternal and grand-maternal effects (F_{10-1} and F_{10-2}). **C)** Different traits of individuals from the third generation (F_{10-3}) onwards were measured after the experimental evolution ended. These measures were to assess whether the exposure to the different stressors caused evolutionary responses in the target traits. Each treatment was replicated six times.

Measurements of traits

Traits holding significant values of H^2 [i.e., egg and dorsal shield length, and developmental time ("Heritability of representative traits", p. 82)] were evaluated in individuals taken from the populations exposed to experimental evolution. Heritability was not significant for the traits tolerance to desiccation (35, 50 and 65% RH) and tolerance to starvation (100% RH) when H^2 values were estimated in the wild and the commercial populations, separately. However, H^2 of these traits was significant when the two populations were combined, indicating that detection of heritability was dependent on the number of isolines. Because all the variability of the commercial population was contained within the trait space of the wild population (Chapter 2: Figure 2.2.1. and 2.2.2, pp. 78-79) I decided to measure the traits tolerance to desiccation at 35% RH and tolerance to starvation (100% RH).

The methodology to measure traits was the same as that used to assess heritabilities, explained in detail in Section 2 ("Measurement of Traits", p. 58).

Statistical Analysis

The effects of the different selection regimes were analysed for each trait separately. Traits measured in the same individuals were analysed using LMM, function "*lmer*"(package "lme4") (Bates et al., 2015). The traits tolerance to desiccation at 35%RH and tolerance to starvation were analysed using survival regression in Cox Proportional Hazards Regression Mixed Effects Models (Therneau, 2022), with the function "*coxme*" "(package "coxme"). The survival curve graph was done with "ggsurvplot" function ("Survminer" package) (Kassambara et al., 2021). All models included the "Abiotic" condition (mild or hot temperature) and "Biotic" condition (presence or absence of predator) as fixed factors. For traits measured in both males and females (i.e., Egg length and developmental time) the variable "Sex" (male or female) was included as a fixed factor too. An implicit nesting was implemented to account for replicates and blocks within each of the treatments and included as random factors in all the models. Models to analyse Tolerance to desiccation at 35% RH and Tolerance to starvation included "Number of eggs laid" by tested females during the experiment, as a covariate. For all the applied models I followed a backward elimination procedure: if the higher-order interaction among the explanatory variables was not significant and the model without the interaction had a lower Akaike Information Criterion (AIC) (Akaike, 1974) by more than 2 units, this interaction was removed from the model. Subsequently, the same procedure was sequentially followed for lower-order interactions (Crawley, 2013). *Post-*

hoc tests were done using the “*glht*” (package “multcomp”) (Hothorn et al., 2008) with Tukey tests. The significance of random effects was estimated using the “*ranova*” function from the library “lmerTest” (Kuznetsova et al., 2017).

Results

Egg length

The length of the egg was affected by the interaction between Sex and abiotic conditions, and between abiotic and biotic conditions (Table 3.4.2). Indeed, males responded more strongly to selection from abiotic factors than females (Figure 3.4.3, compare upper and lower panels). The interaction Abiotic*Biotic was significant (Table 3.4.2; P<0.05), and the *post-hoc*-test revealed that eggs from the population experiencing both high temperatures and the presence of predators were smaller than those from the other populations (Figure 3.4.3). The random factor Replicate was significant (P<0.001), indicating that there was variability among replicates.

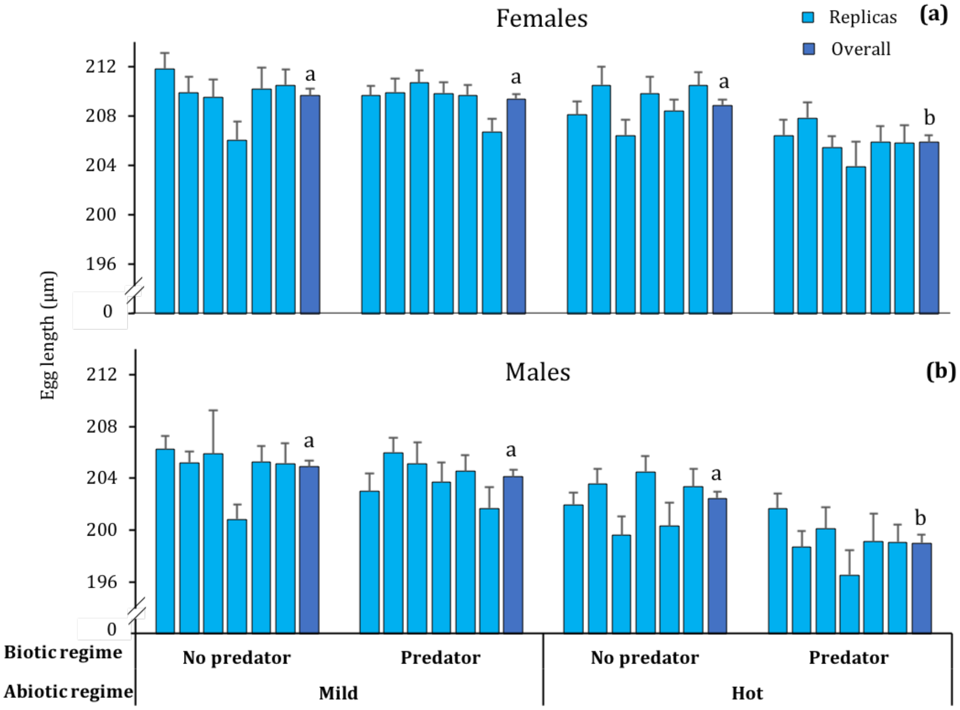

Figure 3.4.3: Averages (± SE) of egg length of a) Females and b) Males from populations exposed to biotic (“No predator” or “Predator”) and abiotic (“Mild” or “Hot”) conditions. Light blue bars represent each of the populations (replicates) under each selection regime whereas the dark blue bars represent the overall for each selection regime. Overall bars capped with different letters are significantly different from others (P<0.05).

Table 3.4.2: Results of the LMM and Survival Mixed Effects Cox Models of the traits assessed from the resultant populations from the evolutionary experiment under a full crossed design of abiotic (Mild or Hot) and biotic (Presence or absence of predator) selection regimes.

Traits	Source of variation	F/χ^2 *	df1;df2	P
Egg length	Intercept	90296.32	1; 28	<0.001
	Abiotic	0.586	1; 28	0.45
	Biotic	12.974	1; 21	<0.001
	Sex	150.289	1; 802	<0.001
	Abiotic*Biotic	4.685	1; 20	0.043
	Abiotic*Sex	4.766	1; 800	0.029
	Biotic*Sex		EXCLUDED	
	Biotic*Abiotic*Sex		EXCLUDED	
Dorsal shield length	Intercept	154160	1; 19	<0.001
	Abiotic	0.277	1; 20	0.604
	Biotic	8.894	1; 18	0.008
	Abiotic*Biotic	5.672	1; 19	0.028
Developmental time	Intercept	45210.476	1; 23	<0.001
	Abiotic	10.435	1; 20	0.004
	Biotic	0.941	1; 21	0.343
	Sex	149.574	1;800	<0.001
	Abiotic*Biotic	0.816	1;20	0.377
	Abiotic*Sex		EXCLUDED	
	Biotic*Sex		EXCLUDED	
	Biotic*Abiotic*Sex		EXCLUDED	
Tolerance to desiccation at 35% RH	Nº eggs laid	8.707	1	0.003
	Abiotic	0.937	1	0.333
	Biotic	3.652	1	0.056
	Abiotic*Biotic	2.367	1	0.124
Tolerance to starvation	Nº eggs laid	2.910	1	0.088
	Abiotic	2.056	1	0.151
	Biotic	0.247	1	0.619
	Abiotic*Biotic	0.245	1	0.620

*F-values are given for linear models, whereas χ^2 are given for survival Cox models.

Dorsal shield length

The length of the females' dorsal shield was strongly affected by the interaction Abiotic*Biotic (Table 3.4.2). Indeed, *post-hoc* tests revealed that females that were exposed to both the abiotic and the biotic selection pressures were smaller in size (Figure 3.4.4). The random factor Replicate was not significant (P=0.175).

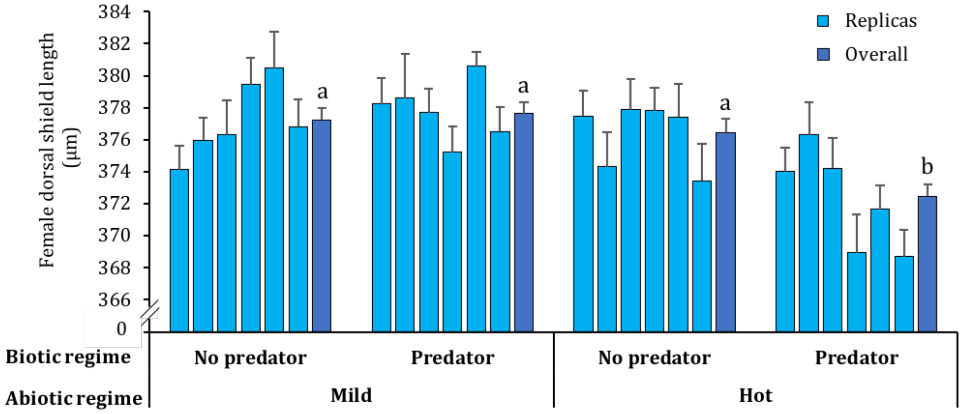

Figure 3.4.4: Averages (± SE) of female dorsal shield length of populations exposed to biotic ("No predator" or "Predator") and abiotic ("Mild" or "Hot") conditions. Light blue bars represent each of the populations (replicates) under each selection regime whereas the dark blue bars represent the overall for each selection regime. Overall bars capped with different letters are significantly different from others (P<0.05).

Developmental time

Results showed that the developmental time was affected by the main factors Sex and Abiotic conditions (Table 3.4.2). Indeed, males developed faster than females, and overall developmental time evolved to be longer under hot conditions than under mild conditions (Figure 3.4.5). The random factor was highly significant (P<0.001), indicating that there was variability among replicates (Figure 3.4.5).

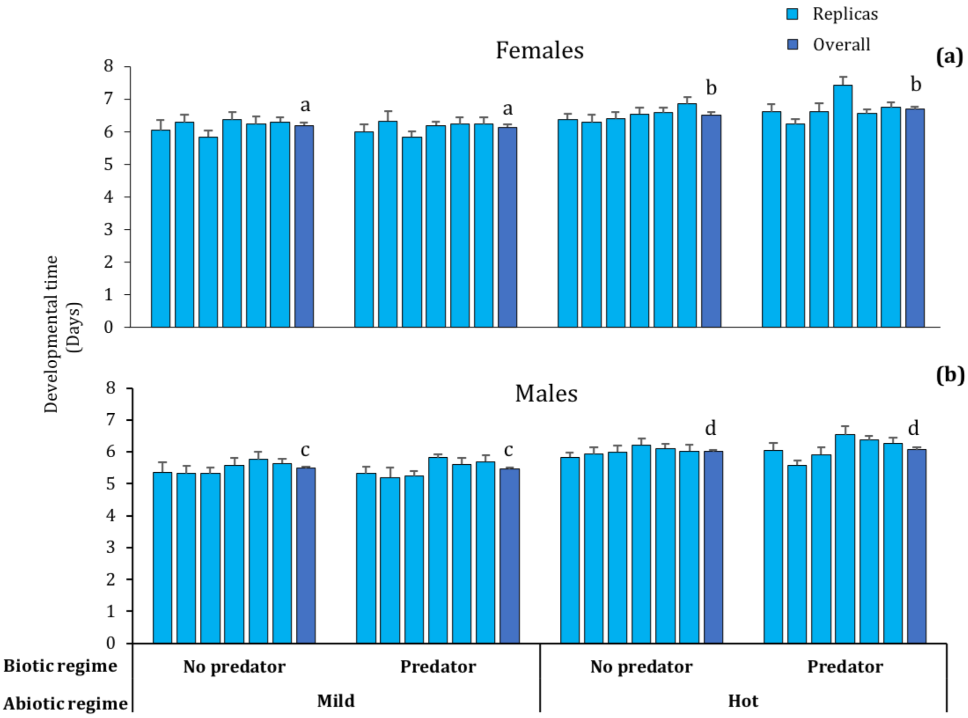

Figure 3.4.5: Averages (± SE) of developmental time (from egg to adult) of a) Females and b) Males of populations exposed to biotic ("No predator" or "Predator") and abiotic ("Mild" or "Hot") conditions. Light blue bars represent each of the populations (replicates) under each selection regime whereas the dark blue bars represent the overall for each selection regime. Overall bars capped with different letters are significantly different from others (P<0.05).

Tolerance to desiccation 35% RH

Neither the main factors nor their interaction were significant (Table 3.4.2, Figure 3.4.6). However, the number of eggs laid during the experiment affected significantly their survival time in all the treatments. Overall, females that laid more eggs had a higher hazard to die, which corresponds to shorter survival times (Coef=0.237, Z=2.95, P=0.003).

Tolerance to starvation

Again, neither the main factors nor their interaction were significant. The number of eggs laid during the experiment had no effect either (Table 3.4.2, Figure 3.4.7).

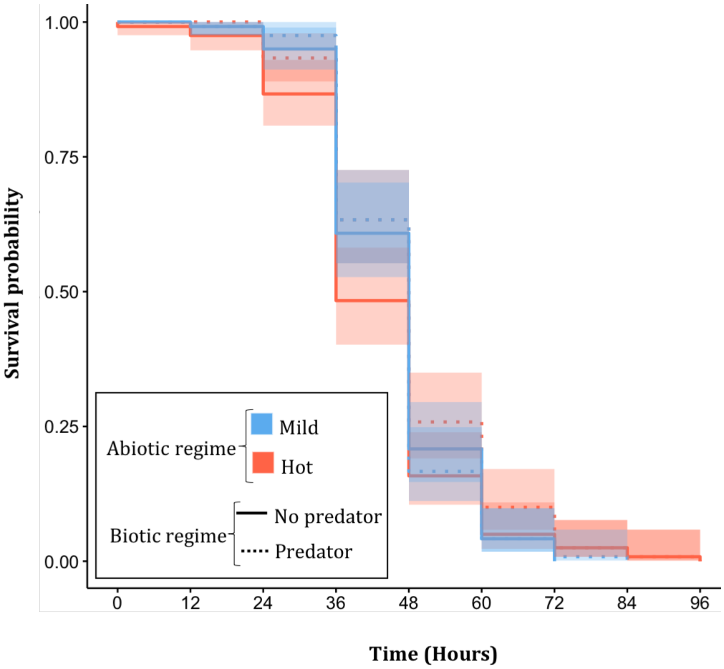

Figure 3.4.6: Survival curves (± confidence intervals) of tolerance to desiccation at 35% RH of populations exposed to biotic ("No predator" or "Predator") and abiotic ("Mild" or "Hot") conditions. No significant differences were found.

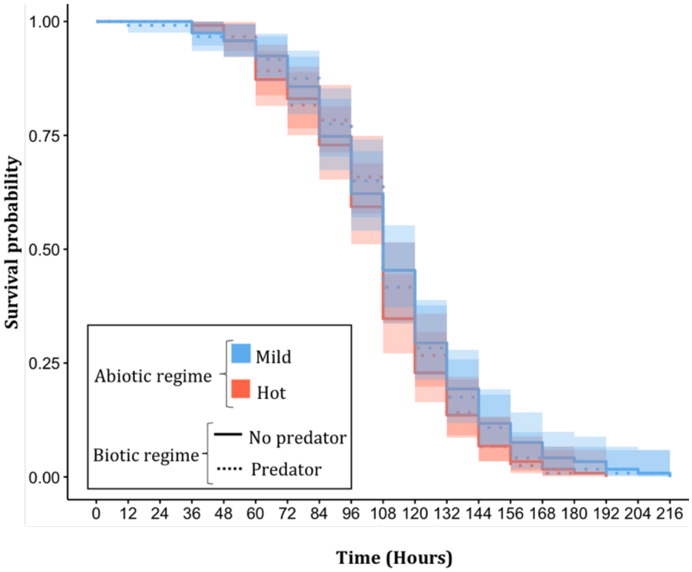

Figure 3.4.7: Survival curves (± confidence intervals) of tolerance to starvation of populations exposed to biotic ("No predator" or "Predator") and abiotic ("Mild" or "Hot") conditions. No significant differences were found (P<0.05).

CHAPTER 5: Adaptation and evolutionary costs

Material and methods

Measurements of traits

The experimental design consisted of exposing individuals from populations exposed or not to high temperatures or to the presence or not of an IG predator (*O. laevigatus*) during experimental evolution, to two environments, one stressful and the other not, in full crossed designs. These new environments are described below. The initial hypotheses were that a) if adaptation (or simply responding to natural selection) had occurred, individuals from populations that had experienced experimental evolution in stressful environments should perform better in the current stressing environment than individuals from populations exposed to benign environments; and b) if adaptation entailed a cost, individuals from populations that had experienced experimental evolution in stressful environments should perform worse in benign environments than individuals from populations that did not experience any stress.

Because the populations that were exposed to both selection factors were in low numbers, I decided to assess possible adaptations or costs only in populations previously exposed to the main selection factors, that is, only Biotic or only Abiotic, not in the populations exposed to both. For the sake of readability, hereafter I will refer to populations that experienced experimental evolution at high temperatures ("Hot" condition) as $\text{Populations}_{evolved\_abio}$; to populations that experienced experimental evolution in the presence of the IG predator *O. laevigatus* as $\text{Populations}_{evolved\_bio}$; and to populations that did not experience any stressful condition under experimental evolution (that is, when populations experienced "Mild" temperatures and "Absence" of *O. laevigatus*) as $\text{Populations}_{not\_evolved}$.

Experimental arenas

Experimental arenas were a downsized version of those from the evolution experiments (see "Experimental evolution", p. 107), to mimic similar environmental conditions and avoid extra sources of variation. To maintain population densities like those in the evolution experiment, I adjusted the initial number of females to the size of the arenas (1 individual/cm2 approx.). Arenas consisted of a 3x3 cm plastic sheet surrounded by tissue paper, which served as a source of water and prevented mites from escaping, all of it resting on top of a water-soaked cotton layer into 5x5cm square Petri dishes. In each arena (replicate) I added 10 gravid females, 12-14 days old. In addition, a roof-shaped plastic with cotton threads was provided as a shelter and oviposition substrate. Mites were fed with *C. edulis* pollen.

Adaptation to abiotic stress (high temperatures)

Females from Populations$_{evolved\_abio}$ and Populations$_{not\_evolved}$ were used to measure adaptations or costs resulting from prolonged exposure to high temperatures. The experiments consisted of exposing females from these populations to stressful and not stressful temperature environments in a crossed design. There were two different stressful environments, high non-lethal temperature, or high lethal temperature (heat shock), each of them imposed in different experiments, whereas the non-stressful environment was common to both experiments. All of them are defined below.

The temperature is an environmental factor affecting life history traits in ectotherms. When temperature is stressful, ectotherms usually respond reallocating their metabolic energy to components of life history related to the individual's survival or growth. Thus, under high temperatures it is expected a reduction in the per capita oviposition rate (Burger et al., 2019).

Another trait in ectotherms that is affected by high temperatures is size. Indeed, high temperatures usually increases the frequency of smaller individuals within populations (e.g., smaller eggs) (Blanckenhorn, 2000; Liefting et al., 2010; Sun & Niu, 2012). However, the extent to which this is an evolutionarily or just a plastic response is still debated. Actually, a recent review and meta-analysis shows that there is no evidence that smaller sizes are an evolutionary response to an increase in temperature (Siepielski et al., 2019).

The starting hypothesis was that populations that were exposed to high temperatures for several generations (i.e., Populations$_{evolved\_abio}$) should perform better under high temperatures than populations that were maintained at mild temperatures (i.e., Populations$_{not\_evolved}$). This response is to be expected if the former underwent adaptation processes during the long-term exposure to the hot environment. Thus, my expectations were that in hot environments a) the oviposition rate of individuals from Populations$_{evolved\_abio}$ would be higher than those from Populations$_{not\_evolved}$; b) size of the eggs laid by females from Populations$_{evolved\_abio}$ would be larger than those laid by females from Populations$_{not\_evolved}$, because this would prevent overheating from higher Surface/Volume when exposing females to high temperatures. In addition, egg size is an important life-history trait that can affect the offspring's fitness, so that the larger the egg, the greater the size at hatching, survival, growth rates, and stress tolerance (Xu et al., 2019) ; c) the survival probability of individuals from Populations$_{evolved\_abio}$ would be higher than that of individuals from Populations$_{not\_evolved}$.

Thus, the traits I measured to assess whether there was any adaptation and/or cost associated to prolonged exposure to high temperatures in the evolution experiment were oviposition rate, size of the eggs laid and survival probability and number of laid eggs by females exposed to heat shock. The methodological details are explained below:

High-temperature stress

-<u>Oviposition rate</u>: Between 29 and 36 groups of 10 gravid females, 12-14 days old, taken from Populations$_{evolved\_abio}$ and Populations$_{not\_evolved}$ were isolated in experimental arenas for 3 days, exposing them to either hot or mild treatment conditions in a full-crossed design. Females were allowed to lay eggs for 3 days. Eggs from the first day were discarded to exclude eggs produced when females were not under the experimental conditions. The number of eggs laid by 10 females per arena during the 2nd and 3rd days was recorded. Next, I calculated the average number of eggs produced per arena during the last two days.

-<u>Egg length:</u> Eggs laid during the experiment above were isolated individually in small arenas consisting of 1.8cm ∅ leaf discs of *P. vulgaris* placed upside down on cotton wool water-soaked in 5.5cm ∅ plastic glasses. Two photos of each egg were taken and egg lengths were measured. For the statistical analysis, only the longest measure of the 2 photos was used (see Section 2: "Measurements of traits" for more details, p. 58). Subsequently, eggs were kept under rearing conditions (25±2°C, 70±5% RH and fed with *C. edulis* pollen) until becoming adults for sex determination.

Heat shock stress

-*<u>Survival probability under heat shock</u>*: Survival was measured in Populations$_{evolved\_abio}$ and Populations$_{not\_evolved}$. In each of the experimental arenas (See above for more details), I introduced 10 gravid females, 12-14 days old, obtained from populations exposed or not to "Hot" conditions in the evolution experiment. Half of the arenas per treatment were exposed to the heat-shock treatment and the other half to the control treatment. The heat shock treatment consisted of exposing the arenas to 40°C, 70±5% RH and 16:8 L:D, and in the control treatment to 25°C, 70±5% RH and 16:8 L:D, abiotic conditions. Every 8 hours, I recorded the number of individuals alive/dead until all individuals died. There were 8 replicates per population. Because the previous experimental evolution had 6 replicates (subpopulations), a total of 48 replicates per treatment were established.

-*<u>Eggs laid under heat shock</u>:* In addition, the number of eggs laid per arena was recorded.

Adaptation to biotic stress (Presence of O. laevigatus)

The general experimental design consisted of exposing individuals obtained from Populations$_{evolved\_bio}$ and Populations$_{not\_evolved}$ to two new environments, one stressful and another biotically mild, in a crossed design. These new environments are defined below. The initial hypotheses were that a) if adaptation had occurred, females from Populations$_{evolved\_bio}$ would perform better in the stress environment than females from Populations$_{not\_evolved}$; and b) if adaptation entailed a cost, females from Populations$_{evolved\_bio}$ would perform worse in the benign environment than females from Populations$_{not\_evolved}$.

I measured possible adaptations related to anti-predator behaviour, such as the ability to survive, to run away, or to retain eggs, in the presence of a top predator. If adaptation had occurred, my expectations were that, in the presence of a predator, a) survival of individuals from Populations$_{evolved\_bio}$ would be higher than those from Populations$_{not\_evolved}$; b) adapted populations would avoid patches with the presence of predators in higher rates than not-evolved populations, as shown in whiteflies (Nomikou et al., 2003a). Therefore, I expected an increase in the tendency of the mites to run away from the patch; c) lastly, given that phytoseiid mites have the ability to retain their eggs to protect them from the attack of predators (Montserrat et al., 2007), I expected individuals from Populations$_{evolved\_bio}$ to avoid egg-laying in patches with the presence of a predator, and perhaps retaining the eggs inside their bodies, in higher frequency than individuals from Populations$_{not\_evolved}$. The methodological details are explained below:

Sixteen groups of 10 gravid females, 12-14 days old, taken at random from Populations$_{evolved\_bio}$ and Populations$_{not\_evolved}$, were isolated in experimental arenas for 24 hours, half of each in the presence or absence of *O. laevigatus*, and the other half in its absence. Arenas were kept in a climate chamber at 25°C, 70±5% RH and 16:8 L:D. Arenas contained *C. edulis* pollen as a food resource.

The traits measured after 24 h of exposure to the predator or no predator conditions were:

-N° of surviving females*:* the number of surviving mites.

-N° of escapees*:* the number of mites that ran away from the experimental arenas.

-N° of eggs laid*:* the number of eggs laid by all individuals in the arena.

Statistical Analysis

All the statistical analyses were ran using the computer environment R (v. 4.0.2) (R Core Team, 2022).

All the models, independently of the trait assessed, included two main factors and their interaction. One main factor referred to the population from which tested individuals came from ("Population" factor, with two levels ("evolved" or "not evolved" to the stress conditions imposed in the evolution experiment). The other main factor was "Treatment", and referred to the new environment individuals were tested in. The levels of the factor "Treatment" depended on the experiment (see below). In addition, because there were 6 subpopulations (replicates) per treatment in the previous evolution experiment, "replicate" was included in the model as a random factor.

Adaptation to abiotic stress (high temperatures)

-High-temperature stress
Models for oviposition rate and egg length were adjusted with Linear Mix Models (LMM), using the function "*lmer*" from the package "lme4" (Bates et al., 2015), including "Population", and "Treatment" ("Mild" or "Hot" conditions) as main factors, and their interaction.

-Heat shock stress
Survival probability was adjusted with a survival regression in Cox Proportional Hazards Regression Mixed Effects Models, using the function "coxme" (Package "coxme") (Therneau, 2022). The number of eggs laid was fitted with GLMM using maximum likelihood estimation with the function "*glmmTMB*" (package "glmmTMB") (Brooks et al., 2017) with family="*nbinom2*" distribution of errors to prevent overdispersion. In both cases, models included "Population" factor, "Treatment" (heat shock "Yes" or "No"), and its interactions. In addition, the mean number of survivors in the arenas throughout the experiment "Surv_mean" was added as a covariate. When the interaction was significant, *post-hoc* multiple comparisons were performed and corrected using Holm`s Sequential Bonferroni Method (Holm, 1979). The survival curve graph was done with "ggsurvplot" function ("Survminer" package) (Kassambara et al., 2021).

Adaptation to biotic stress (Presence of O. laevigatus)

Models applied were GLMM, using the function "*glmmTMB*" (package "glmmTMB") (Brooks et al., 2017). Models included "Population" and "Treatment" ("Presence" or "Absence" of the predator). For the variable

"Number of surviving females", I assumed a "*Poisson*" distribution of errors. Because of the overdispersion of data in the variables "Number of escapees" and "Number of eggs laid", models assumed a negative binomial ("*nbinom2*") distribution of errors. Tests to detect overdispersion in data were performed with "*testDispersion*" (Package "DHARMa") (Hartig & Lohse, 2022).

Results

Adaptation to abiotic stress (high temperatures)

High temperatures stress

-Oviposition rate*:* Results from the LMM revealed that neither the main factor "Population" nor the interaction "Population * Treatment" affected the oviposition rate of individuals (Table 3.5.1). Oviposition rates were strongly affected by the main factor "Treatment" (Table 3.5.1), pointing to a plastic response, in which oviposition rates were higher in the groups of females that were exposed to mild temperatures. Therefore, oviposition rates were only determined by temperature, not by the previous history experienced by populations (Figure 3.5.1). Thus, I did not detect any adaptation process, nor any cost, in the trait oviposition rate in the populations that experienced experimental evolution under high temperatures (Figure 3.5.1). The random effect was not significant either (P=0.688).

Table 3.5.1: Results of the LMM applied to the traits "oviposition rate" and "egg size" assessed in individuals obtained from Populations$_{evolved\_abio}$ and Populations$_{not\_evolved}$, exposed to two temperatures ("Mild" or "Hot") in a full crossed design.

Traits	Source of variation	*F*	df1;df	*P*
Oviposition rate	Intercept	309.532	1; 20	<0.001
	Population	0.009	1; 17	0.924
	Treatment	70.289	1; 20	<0.001
	Population*Treatment	0.063	1; 19	0.801
Egg Length	Intercept	57602.29	1; 21	<0.001
	Population	0.518	1; 20	0.48
	Treatment	0.154	1; 31	0.698
	Sex	113.682	1;1551	<0.001
	Population*Treatment	0.055	1; 29	0.814
	Population*Sex	0.466	1;1550	0.495
	Treatment*Sex	1.738	1;1564	0.188
	Population*Treatment*Sex	0.968	1;1561	0.325

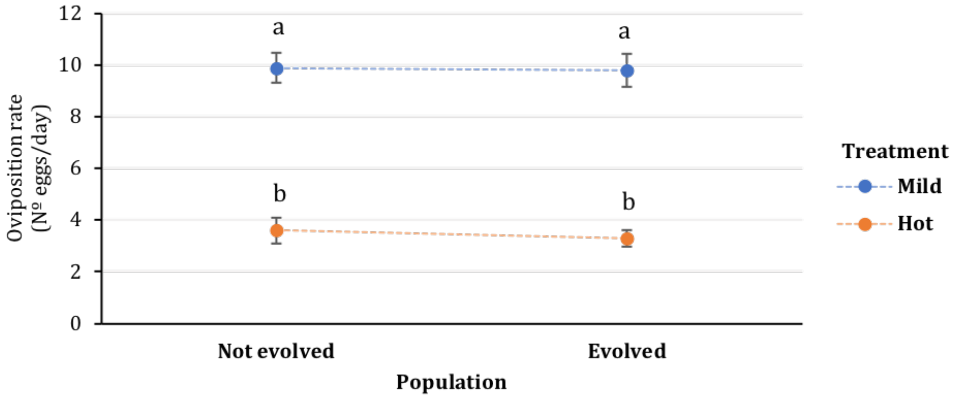

Figure 3.5.1: Oviposition rate (average ± SE) of individuals obtained from Populations$_{evolved\_abio}$, and Populations$_{not\_evolved}$, exposed to two temperatures (“Mild” or “Hot”) in a full crossed design. Different letters indicate significant differences (P<0.05).

-<u>Egg length</u>: None of the main factors, but “Sex”, nor their interactions, were significant (Table 3.5.1). Again, eggs producing males were overall much smaller than those producing females (Figure 3.5.2). The random effect was significant (P<0.001). Therefore, I did not detect any adaptation or cost derived from long-term exposure to high temperatures, for the trait egg length.

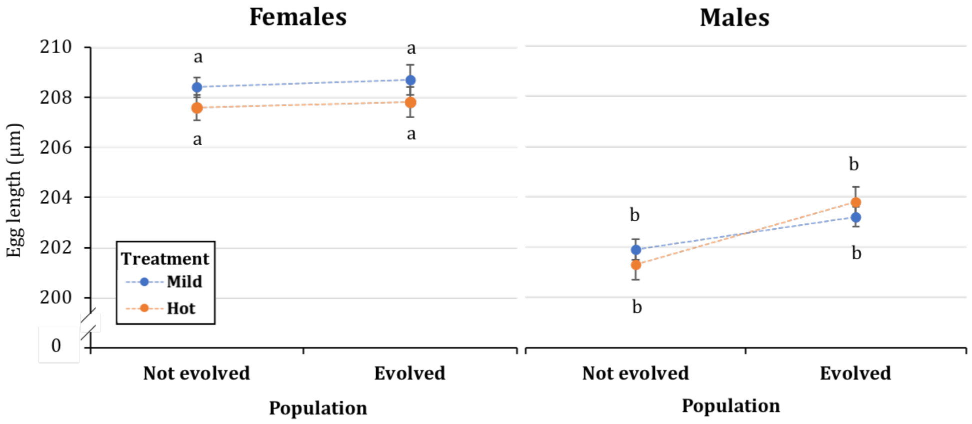

Figure 3.5.2: Female and male egg length (average ± SE) of individuals obtained from Populations$_{evolved\_abio}$, and Populations$_{not\_evolved}$, exposed to two temperatures (“Mild” or “Hot”) in a full crossed design. Different letters indicate significant differences (P<0.05).

Heat shock stress

-Survival probability under heat shock: Results revealed that the treatment “heat shock” was highly significant (Table 3.5.2). Indeed, exposure to very high temperatures reduced significantly the overall probability of survival (Figure 3.5.3). However, the survival analysis also revealed a significant interaction between the two main factors, “Heat shock * Population” (Table 3.5.2). Pair-wise comparisons corrected by the Sequential Holm-Bonferroni method revealed that under control conditions populations that prior experienced long-term exposure to high temperatures had less chance to survive than those that did not (P=0.018, $\alpha_{Bonf.}$=0.025), suggesting that prolonged exposure to hot environments entailed a cost from natural selection (Figure 3.5.3).

Table 3.5.2: Results of the Survival Mixed Effects Cox Models and GLMM analysis applied to the traits “time to death” and “Nº of eggs laid” assessed in individuals obtained from Populations$_{evolved\_abio}$ and Populations$_{not\_evolved}$, subsequently exposed to the Treatment conditions (Heat shock “Yes” or “No”) in a full crossed design.

Traits	Source of variation	χ^2	Estimate	df	P
Survival hazard rate	Heat shock	356.64		1	<0.001
	Population	3.043		1	0.081
	Heat_shock*Population	4.789		1	0.029
Eggs laid	Intercept	10.754		1	0.001
	Surv_mean	17.835	0.222	1	<0.001
	Heat shock	68.758		1	<0.001
	Population	1.184		1	0.413
	Heat shock*Population	0.391		1	0.982

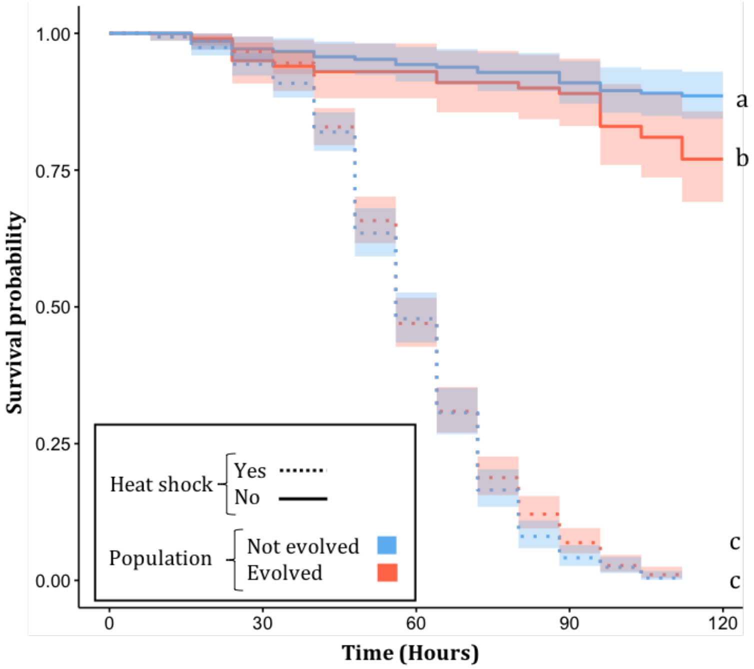

Figure 3.5.3: Survival probability curves (± confidence interval) of individuals obtained from Populations$_{evolved\_abio}$ and Populations$_{not\_evolved}$, subsequently exposed to the Treatment conditions (Heat shock "Yes" or "No"). Different letters indicate significant differences (P<0.05).

-<u>Eggs laid under heat shock</u>*:* Heat shock affected oviposition rates in all tested individuals, independently of their origin (Table 3.5.2). Indeed, individuals exposed to heat shock produced hardly any egg (Figure 3.5.4). The covariate "survival mean" significantly and positively influenced the number of eggs laid. Thus, individuals from populations$_{evolved\_abio}$ did not perform better than individuals from populations$_{not\_evolved}$ under heat-shock conditions.

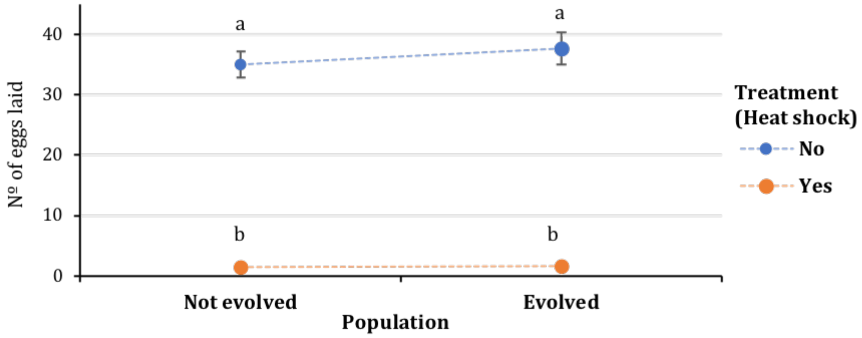

Figure 3.5.4: Number of laid egg (average ± SE) of individuals obtained from Populations$_{evolved\_abio}$ and Populations$_{not\_evolved}$, subsequently exposed to the Treatment conditions (Heat shock "Yes" or "No"). Different letters indicate significant differences (P<0.05).

Adaptation to biotic stress (presence of top predator)

-<u>N° of surviving females</u>*:* Exposure to *O. laevigatus* affected the number of surviving females, but neither treatment nor the interaction between the two main factors had any effect on survival (Table 3.5.3). Indeed, the presence of the predator increased mortality independently of the origin of the population. Thus, in my experimental set-up, I did not detect any adaptation in populations$_{evolved\_bio}$ to the presence of the predator (Figure 3.5.5).

Table 3.5.3: Results of the GLMM analysis applied to the traits "Nº of surviving females", "Nº of escapees" and "Nº of eggs laid" assessed in individuals obtained from Populations$_{evolved\_bio}$ and Populations$_{not\_evolved}$, exposed to two treatment conditions ("Absence" or "Presence" of *O. laevigatus* in a full crossed design.

Traits	Factor	χ^2	df	*P*
N° of surviving females	Intercept	2682.275	1	<0.001
	Population	0.079	1	0.779
	Treatment	144.651	1	<0.001
	Population*Treatment	1.015	1	0.314
N° of escapees	Intercept	23.415	1	<0.001
	Population	2.024	1	0.155
	Treatment	43.902	1	<0.001
	Population*Treatment	1.394	1	0.238
N° of eggs laid	Intercept	2276.949	1	<0.001
	Population	0.0513	1	0.821
	Treatment	122.211	1	<0.001
	Population*Treatment	0.758	1	0.384

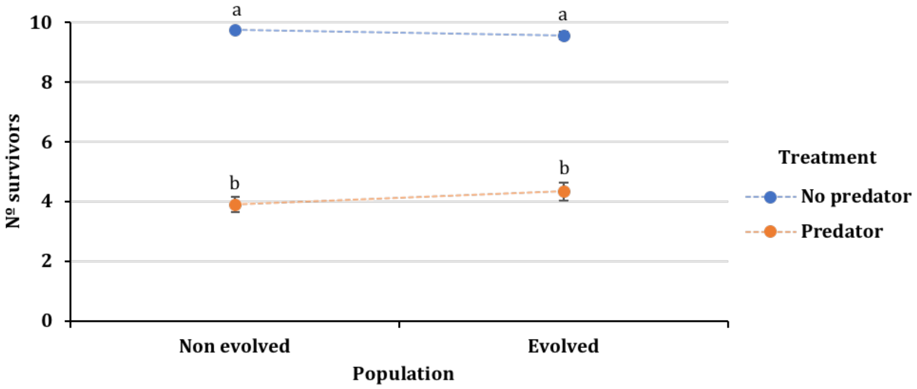

Figure 3.5.5: Number of survival females (average ± SE) obtained from Populations$_{evolved\_bio}$ and Populations$_{not\_evolved}$, subsequently exposed to the Treatment conditions ("Absence" or "Presence" of *O. laevigatus*). Different letters indicate significant differences (P<0.05).

-<u>N° of escapees</u>*:* As above, results showed the number of females escaping from the arenas only depended on the presence of *O. laevigatus* (Table 3.5.3; Figure 3.5.6). Again, no adaptation to the presence of a top predator was detected in Populations$_{evolved\_bio}$.

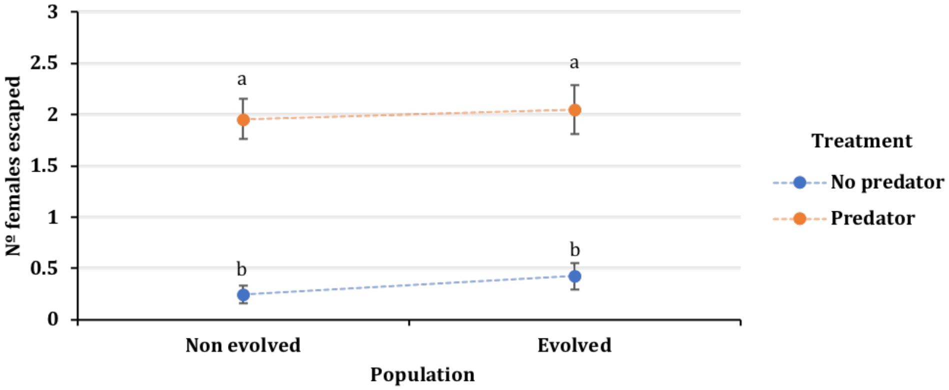

Figure 3.5.6: Number of female escapees (average ± SE) from Populations$_{evolved\_bio}$ and Populations$_{not\_evolved}$, subsequently exposed to the Treatment condition ("Absence" or "Presence" of *O. laevigatus*). Different letters indicate significant differences (P<0.05).

-<u>N° of eggs laid</u>*:* Once more, I did not detect any differential effect between populations (Table 3.5.3, Figure 3.5.7). However, females responded plastically decreasing oviposition when exposed to predators. These results suggest an anti-predator behaviour in the form of egg retention (Montserrat et al., 2007) displayed by the *A. swirskii* as a consequence of the *O. laevigatus* presence.

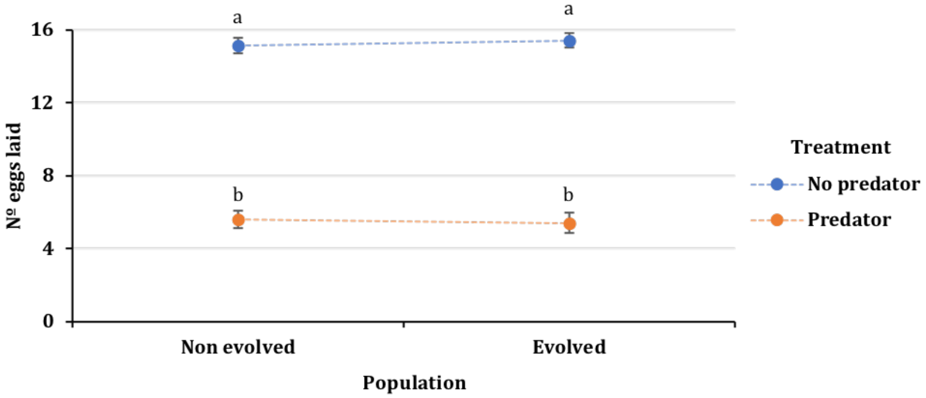

Figure 3.5.7: Number of laid eggs (average ± SE) of individuals obtained from Populations$_{evolved\_bio}$ and Populations$_{not\_evolved}$, subsequently exposed to the Treatment conditions ("Absence" or "Presence" of *O. laevigatus*). Different letters indicate significant differences (P<0.05).

CHAPTER 6: General discussion

This thesis is embedded within the framework of FWE, an innovative tool designed to improve the performance of BCAs exposed to hostile environments. Basically, FWE seeks to exploit the natural intraspecific genetic variability of beneficial organisms to prepare them, beforehand, to the conditions of the environments they will be released in. This would be done via improving traits that may be relevant for their establishment in these environments, and for their efficiency at controlling pests, especially if these environments are hostile. To that aim, this thesis explores the genetic architecture of relevant traits in a BCA, tests it in subpopulations exposed to biotic and abiotic selection pressures to identify potential response functional traits, and it evaluates whether these traits respond to the selection pressures of interest, and in which direction.

Genetic variability in wild and commercial populations

Differences between populations

Most of the traits in which I found differences between the wild and the commercial populations were related to development and reproduction. Indeed, both males and females from the commercial population developed significantly faster than those from the wild population (Table 2.2.1.b, p. 71), and early fecundity was significantly higher in females from the former than in those from the later populations (Table 2.2.2.b, p. 73), although females from both the commercial and the wild populations laid similar number of eggs through their life-time (Table 2.2.2.b). These results indicate that the commercial population likely underwent evolutionary processes during the progression of mass-rearing, being these intended or not.

Captive conditions almost inevitably select for faster development and fecundity (Nunney, 2003). Indeed, the literature provides several examples of rapid adaptation in mass-reared populations to the rearing conditions, resulting in faster development [e.g., in medfies -*Ceratitis capitata* Wiedemann (Diptera: Tephritidae)- (Rössler, 1975; Vargas & Carey, 1989; Wong & Nakahara, 1978), oriental fruit fly *Dacus dorsalis* Hendel (Diptera: Tephritidae) (Foote & Carey, 1987), in the Caribbean fruit fly *Anastrepha suspensa* Loew (Diptera: Tephritidae) (Leppla et al., 1976), and melon fly *Bractocera cucurbitae* Coquillett (Diptera: Tephritidae) (Miyatake, 1993; Miyatake & Yamagishi, 1999)] and earlier mating and oviposition [e.g., in the melon fly: (Miyatake, 1998); in medflies: (Rössler, 1975; Vargas & Carey, 1989; Wong & Nakahara, 1978), oriental fruit fly: (Foote & Carey, 1987) or in tobacco budworm *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) (Raulston, 1975)]. Such adaptation to mass-rearing conditions can occur relatively fast. For example, Raulston

(1975) reported that a wild population of the tobacco budworm oviposited significantly latter than the adapted laboratory stain, but these differences vanished after only 3 generations of the wild strain being exposed to the laboratory conditions.

Behavioural traits were not different between populations; yet, the values of some traits were similar to those recorded by other authors. For example, Buitenhuis et al. (2014) measured activity time and walking speed in *A. swirskii* on plants with varying densities of trichomes and on plastic arenas, to address how trichomes affected movement. The values the authors obtained in plastic arenas were similar to those I obtained in my artificial arenas: the walking speed reported by Buitenhuis et al. (2014) was 0.18 ± 0.05 cm/s, similar to the values I obtained [(0.18 ± 0.03 (wild) and 0.17 ± 0.03 cm/s (commercial)]; although the percentage of time individuals were active was shorter than in my experiments (76 ± 9.5%, vs. 86 ± 7 (wild) and 84 ± 8% (commercial), respectively). This difference could be caused by differences in the size of the arenas as the total recording time of each individual was not so different, 10 min in their experiments compared to 12 min in mine. However, my experimental arenas were two times larger, and mites could have invested longer in exploring the arena, thus increasing the time being active. Yet, I could not find in the literature any work supporting this hypothesis. Still, similar results obtained from different methodologies provides robustness to the data obtained from the recordings made with the robot.

The commercial population was significantly less tolerant to starvation than the wild population. This result indicates that individuals from the commercial population might have become “adapted” to benign and constant environments with abundant food, typical from rearing conditions, which may have limited their ability to withstand food deprivation. Indeed, commercial mass rearing is conducted under controlled conditions that do not reflect the environmental variations that wild populations face in the field. For example, Hoffmann et al. (2001) observed a rapid loss of tolerance to starvation in the species *Drosophila melanogaster* Fallen (Diptera: Drosophilidae) caused by adaptation to the benign laboratory conditions. In this study, I found that tolerance to starvation is positively correlated with developmental time and could trade-off with fecundity (Figure 2.3.4, p. 95), two traits that are potential targets by breeding companies. Indeed, in my experiments the commercial population, in addition to have lower tolerance to starvation, developed faster, and its early oviposition was higher than that of the wild population. Similar findings were reported in studies with *D. melanogaster*, where selection for desiccation and starvation tolerance affected early fecundity negatively

(Hoffmann & Parsons, 1989a). Other works with this fruit fly species also detected the existence of this trade off (Service et al., 1985; Service & Rose, 1985) or showed that strains selected for higher tolerance to starvation had slower developmental times (Chippindale et al., 1996; Harshman et al., 1999). If these trade-offs are typical in ectotherm species, they could explain why the wild population had higher tolerance to starvation and longer developmental times. In other words, selection for shorter developmental times in laboratory conditions could indirectly select for lower starvation resistance.

A proximate explanation arises from strong evidence supporting that an increase in the body lipid content is related to tolerance to starvation, and it accounts for almost all the variation in starvation tolerance (Chippindale et al., 1996). Furthermore, increased tolerance to environmental stresses is associated with a reduction in metabolic rates (Hoffmann & Parsons, 1989). Assuming that wild populations are more closely adapted to environmental stresses than captive populations, the former should have lower metabolic rates, explaining, therefore, why the wild population has higher tolerance to starvation and longer developmental times than the commercial population. Unfortunately, I did not measure the lipid content of individuals, to confirm this hypothesis.

Heritability of traits

H^2 was estimated for 12 traits measured in females and 2 in males, in each population (wild and commercial). In the wild population, relevant H^2 was detected in life history (egg length and developmental time) and morphological traits (dorsal shield length). These traits are often heritable (Roff, 1997) and could be essential for the persistence of populations dwelling in variable environments (Barrett & Schluter, 2008). Life-history traits are closely related to reproductive fitness, and thus, their genetic variance should be reduced to a minimum because they are subjected to stronger selection (Fisher, 1930). However, additive variance is sometimes found to be similar (Coltman et al., 2005; Kruuk et al., 2000; McCleery et al., 2004) or even at higher levels (Houle, 1992; Merilä & Sheldon, 2000) in life-history traits than in traits less closely related to fitness, such as morphological and physiological (Teplitsky et al., 2009). I found higher H^2 value for morphological traits (e.g., dorsal shield length) than for life-history traits (e.g., developmental time). However, H^2 is estimated from V_G, and thus, it does not provide what is the contribution of the V_A to the total V_G. Yet, heritability of life-history traits, such as egg length, developmental time and longevity, which can be very closely related to fitness (Falconer & Mackay, 1996), can potentially be maintained via genetic correlations with the highly heritable morphological traits (Figure 2.3.4, p. 95).

Heritability in other traits, such as early fecundity, desiccation tolerance, and starvation tolerance, was only detected when analyses included all the 42 isolines. These results could indicate that part of the variation of these traits in both populations was additive, that is, it did not completely overlap, and thus increasing the number of isolines increased the total variability. However, my results showed that the variance of the commercial population was contained within that of the wild population (Figures 2.2.1 and 2.2.2, pp. 78-79). Therefore, it is more probable that the statistical power in the detection of heritability increased with the number of isofemale lines. Indeed, when the isofemale lines of the two populations were included in the analyses, I found significant H^2 in six traits that yielded no heritability when each population was analysed separately (Tables 2.2.7.a and b, pp. 84-85), five in females and one in males.

Tolerance to desiccation and starvation yielded significant heritability. These are stress-related traits directly associated to the ability of *A. swirskii* to counter the effects of drought and food scarcity. High levels of tolerance to desiccation are associated to adaptation to arid environments in *Drosophila* sp. (Kimura & Beppu, 1993), which suggests that my target species could still evolve further resistance to environmental drought from natural selection.

None of the behavioural traits held significant H^2. This result could be due to the very nature of this type of traits, which are complex and regulated by genes involving multiple functions; e.g., metabolic pathways, nervous system development, biomechanics, and environmental cues; e.g., sensory ecology, context dependency. Indeed, behavioural traits are assumed to be highly plastic (West-Eberhard, 2003), suggesting that the magnitude of the genetic variation relative to the effects of the environment on behaviour, should be low. However, the three behavioural traits I measured were highly positively correlated among them (Figure 2.3.3, p. 93), suggesting that they may be part of the same behavioural syndrome (Réale et al., 2007) and respond cohesively to selection (Dingemanse & Réale, 2010). Additionally, they were genetically correlated with other traits that showed relevant heritability (e.g., egg length and early fecundity) (Figure 2.3.4, p. 95). Therefore, there is some heritability left in these traits, even though it was not detected in my analyses, probably because a lack of power to detect very small effect sizes. Indeed, behavioural traits show generally low heritability (Roff, 1997). Recently, in the parasitoid species *Trychogramma evanenscens* Westwood (Hymenoptera: Trichogrammatidae), Lartigue et al. (2021) detected very low H^2 for similar behavioural variables like the ones I measured.

Consequences for mass rearing of biological control agents

Interestingly, none of the traits estimated in the commercial population were heritable (Table 2.2.7.a and b, pp. 84-85). This result indicates that the commercial population may have undergone processes of selection, whether intentional or not, that led to a decrease in its genetic variability. Indeed, all the genetic variance of the commercial population was contained within that of the wild population, and no heritability was found for any of the measured traits in the commercial population. Wild populations are commonly exposed to fluctuating environments which contribute to the maintenance of higher genetic variation than that in captive-bred organisms, usually kept in constant environments (Barrett & Schluter, 2008). For example, the genetic variability of a long-term kept in captivity population of *Drosophila subobscura* Collin (Diptera: Drosophilidae) was reported to decrease drastically because of adaptation to lab conditions (Matos et al., 2000; Santos et al., 2012).

The environmental conditions typical of mass-rearing facilities, or labs, can trigger unintentional selection favoring certain genotypes over others at a very high rates (Hoffmann & Ross, 2018). For example, mass-rearing methodologies usually select for faster developmental times (Nunney, 2003). Alternatively, if during the process of mass-rearing populations undergo bottlenecks some genotypes may have been unintendingly favoured, because of founder effects (Santos et al., 2012; Szűcs et al., 2019). In both cases, however, the genetic variability of the captive population would be reduced. There are multiple examples in the literature showing that populations reared in captivity have less genetic variability than natural populations (Nunney, 2003). For example in the generalist aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) (Postic et al., 2021), the ectoparasitic wasp *Mastrus ridens* Horstmann (Hymenoptera: Ichneumonidae) (Retamal et al., 2016), the Arlequin ladybird *Harmonia axyridis* (Tayeh et al., 2012), and in the predatory bug *Orius majusculus* Reuter (Hemiptera: Anthocoridae) (Rasmussen et al., 2018); also in other species of human interest such as parasitic worms (Bian et al., 2015; Stohler et al., 2004) and mosquitoes (Norris et al., 2001).

Unintentional adaptations to long-term captive conditions can have drastic effects on the performance of BCAs once they are released in the field (Sørensen et al., 2012). Upon release, individuals may experience biotic and abiotic conditions to which they have never been exposed before, which may undermine their efficiency as BCAs (Hoffmann & Ross, 2018). For example, the parasitoid *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae) maintained during multiple generations on a factitious host (*Ephestia kuehniella*) had lower fecundity, lower rates of emergence, and lower fitness, when tested on its natural host, the sugarcane borer, *Diatraea saccharalis* Fabr. (Lepidoptera: Crambidae) than that of populations that were cultured using the borer as food. Thus, the former would likely perform worst as BCA in the field than the later (Bertin et al., 2017).

The low genetic variability of commercial populations calls for attention. Indeed, heritability is a crucial component of evolutionary adaptation, and low genetic variability in commercial populations may limit their resilience and potential for adaptation in response to environmental changes, ultimately jeopardizing pest control. The lower genetic variability found in the commercial population compared to the wild is probably caused by processes intimately related to the mass-rearing conditions and methodologies. This pattern of lower variability in commercial populations has been also observed by other authors. For example, in a recent study using DNA microsatellite markers, Paspati et al*.* (2021) reported that the *A. swirskii* Koppert commercial population was 2.5 times less heterozygous than another wild population from Israel, indicating that the former had less genetic variability than the later.

Genetic correlations

Genetic architecture obtained from correlations using individuals vs isofemale line means

Significant genetic correlations within trait types when they were calculated from traits assessed in the same individuals were half the number of those when they were calculated using isofemale means. Because the former method includes within line in addition to between line variation in the estimates, these are more accurate because environmental variation is considered. The second method only uses the between line variation component, taking the mean across individuals within each line for calculations, ignoring environmental variability. This involves less accuracy in estimates, which could lead to overestimation of the parameters. That is, the inclusion of part of the variability among individuals (environmental component) in the variability between isolines could result on a higher number of significant genetic correlations if this leads to an inflation or deflation of the trait values in a given direction from environmental effects alone. However, the fact that more correlations were detected using directly the isoline means poses the question of whether power (i.e., more significant inaccurate estimates) over accuracy (i.e., less significant but more accurate estimates) would be desirable. Simulations with constructed data could help solving this issue.

In general, the nature of the genetic correlations I found was very diverse. The maintenance of the genetic variability in some traits may involve correlational selection (Sinervo & Svensson, 2002), that is, when selection acts on a combination of trait values (statistical interaction) rather than additively on the traits. Note that correlational selection differs from the correlated response to selection, which is when one trait changes through generations as a result of selection acting on another, genetically linked, trait (Falconer & Mackay, 1996). Indeed, a hypothesis supports that genetic correlations are generated by correlational selection (Roff & Fairbairn, 2012). One example could be the negative correlation between developmental time and fecundity (Hiraizumi, 1961), found in *D. melanogaster* [see also Roff (2000)] or the positive genetic correlations between highly related traits, such as those belonging to fecundity [oviposition time, early fecundity and total fecundity (Roff, 1992)] or behaviour [distance travelled, walking speed and activity (Lartigue et al., 2021)]. For correlational selection to be behind the evolution and maintenance of genetic correlations, different trait combinations should translate into equal relative fitness to the holding individuals. Thus, correlational selection is a necessary but not sufficient condition for genetic correlations to evolve. Different trait combinations hold by individuals that translate into identical fitness among individuals is also necessary. This is usually shown as a ridge in the adaptive landscape [e.g., (Arnold et al., 2008)]. One of the best tests making the hypothesis of correlational selection being behind the evolution of genetic correlations is the finding of a strong positive correlation between correlational selection gradients and genetic correlations between pairs of traits (Roff & Fairbairn, 2012). Additionally, physiological constraints may be behind some other genetic correlations, such as the positive correlations between egg size and egg developmental time (Maino et al., 2017; Marshall & Bolton, 2007) and between fecundity and oviposition time (this study). However, physiological constraints *per se* do not explain the genetic basis of the correlation. For the correlation to be genetic in addition to be subject to physiological constraints, other mechanisms, such as correlational selection should be at play.

Genetic correlations between traits measured in the same individuals

Regarding the genetic correlations between traits belonging to the same categories (Figure 2.3.1, p. 90), I found that most of the traits related to development were positively correlated to each other, indicating that the same genes are probably involved in the developmental processes, for example as a consequence of pleiotropy. Developmental time, however, was negatively correlated with traits related to fecundity. In addition, individuals whose genes determined longer developmental times had longer pre-oviposition times, and the two traits were in turn negatively correlated with both early and total fecundity. Overall, the negative relationship between developmental time and fecundity is counterintuitive, although this correlation was also reported in guppies (Reznick, 1983), albeit with no genetic basis documented. One would rather expect a genetic trade-off which would arise from a positive, not a negative correlation (i.e., developing faster, and probably at a smaller size, trades-off with the number of eggs laid). Additionally, the hypothesis of weak past selection as a cause to maintain positive genetic correlations does not seem relevant, as fecundity shows very low heritability, indicating that selection has been strong in the past which everything else equal would lead to negative, not positive genetic correlations (Roff, 1996). However, one possible explanation for females developing faster, laying more eggs, despite probably at the expense of being smaller (Roff, 2000), could be past correlational selection. In changing and competitive environments, natural selection favours individuals with faster and more efficient reproduction (Stearns, 1992). Therefore, those organisms with shorter developmental times have the advantage of reaching sexual maturity faster and reproducing earlier than those with longer developmental times, which additionally allows the former to escape mortality more likely via viability selection; that is, selection acting on the probability of reaching maturity. Furthermore, since there is a negative genetic relationship with fecundity, that is, individuals developing faster lay more eggs, this gives them an even greater evolutionary advantage. However, for correlational selection to be at the origin of this genetic correlation other trait value combinations should provide with relatively higher fitness. For instance, longer developmental times laying fewer but large eggs could give some advantage if egg quality is important. All cases showed in Roff (2000) presented this negative correlation, although the values presented were all phenotypic. To my knowledge, this is the first time that such negative genetic correlation has been reported. This negative relationship deserves further attention as this means that selection for fast development would involve higher fecundities both of which are desirable properties in breeding programs. Notwithstanding, the commercial population has both higher fecundities and shorted developmental times than the wild population, suggesting that artificial selection has been applied to captive populations whether intendedly or not. At the same time, fecundity was positively correlated to oviposition time, suggesting that the physiological limit imposed in the time for building up each egg determines total oviposition time.

Behavioral traits were positively correlated to each other (Figure 2.3.1, p. 90). This suggests that they can respond cohesively to selection acting as a behavioural syndrome, indicating common genetic and physiological pathways in the three behavioural traits (Réale et al., 2007). However, distance travelled could be just a consequence of both activity and walking speed. Furthermore, the existence of genetic correlations among behavioural traits may indicate that there is still some heritability left in these traits, albeit probably very low and a lack of power impeded detection in my analyses. Again, correlational selection for individuals that are both fast and move frequently (e.g., reach food patches earlier) may explain the raise of this positive genetic correlation, but this is so if also other trait combinations provide with high fitness (e.g., moving slowly and unfrequently may contribute to encounter fewer predators, and/or save more energy).

Regarding the genetic correlations between traits belonging to different categories (Figure 2.3.1), I only found a positive correlation between the size of the eggs and that of females. Since eggs were measured at the beginning and the resulting female size was measured at the end, this means that females genetically programmed to invest in production of bigger eggs will produce bigger females. Additionally, the opposite pattern, that larger females lay larger eggs could be granted because the same genes that affect female size affect egg size. See however a more detailed explanation in the next section. This result makes sense since it is often assumed that larger eggs are related to egg quality, and represent higher energy investment for developing offspring (Bernardo, 1996), resulting in larger individuals and therefore higher survival probability (Krist, 2011), even though these eggs take longer to develop (Maino et al., 2017; Marshall & Bolton, 2007).

Genetic correlations by isoline means

Using the Pearson correlations between the mean isoline values, the analysis yielded significant correlations that, although they were detected when traits were linked to each individual, provide additional information, as in the case of the positive correlation between egg and adult sizes (see above) (Figure 2.3.4, p. 95). Since this genetic correlation was calculated by isoline means, each egg is not associated with the female in which its size was subsequently measured. Thus, this indicates more directly that larger females are genetically equipped to lay bigger eggs. This is consistent with the fact that egg size might be a function of body size and that such relationship has a genetic basis (Fox & Czesak, 2000). Additionally, given the compelling evidence indicating the presence of significant factors that limit the development of larger body size

(Blanckenhorn, 2000), if a robust genetic correlation between the latter and egg size is present, the evolution of egg size would be limited by the same forces affecting body size evolution (Pick et al., 2016). Altogether, taking into account this positive correlation estimated by both methods, it can be concluded that larger females are genetically equipped to lay bigger eggs and that this produces larger females, although at the expense of increasing their developmental time (see below).

I also found a positive correlation between egg size and egg developmental time (Figure 2.3.3, p. 93). The physiological constraints imposed by lower surface-volume in larger eggs to diffusing heat and enzymatic processes (Glaser, 2012), and that larger eggs give rise to larger young specimens (Segers & Taborsky, 2011) makes these eggs require more time for the embryo to grow, or the inverse correlation between size and mass-specific metabolic rate (Steele, 1977) could drive this correlation (Gillooly et al., 2002). Actually, the correlation between embryonic developmental time and egg size is considered to be fundamental, and it has to be necessarily corrected to estimate the effect of temperature on egg developmental time (Gillooly et al., 2002). Indeed, after reviewing 68 different studies across 98 species a positive correlation has been reported as a general trend across insect species, (Maino et al., 2017) [see also Steele (1977)]. However, to my knowledge this is the first time that a correlation between egg size and developmental time at the intraspecific level has been reported to have a genetic basis. Again, if past correlational selection were responsible to the arising of such genetic correlation benefits between both small eggs and fast development, as well as large eggs and slow development, it should be at play. However, additionally, egg size depends on another, fundamental trade-off, that with egg number.

The classical negative correlation between egg size and fecundity (C. C. Smith & Fretwell, 1974; Winkler & Wallin, 1987) emerged only in the correlations estimated from isofemale line means, and the value of the correlation was quite low (Figure 2.3.3, p. 93). It makes sense that it only appears when genetic correlations are made from the means of the isolines because the original egg is that of the individual whose fecundity was subsequently measured. In other words, egg size of female offspring and female fecundity have not been measured in the same individual females. Albeit the value is small, few studies have shown the genetic basis of this trade-off in arthropods (Fox & Czesak, 2000) as it is the case for *Daphnia magna* Straus and *Daphnia pulex* L. (Cladocera: Daphniidae) (Ebert, 1993; Lynch, 1946) or for the seed beetle *Stator limbatus* Horn (Coleoptera: Bruchidae) (Czesak & Fox, 2003).

Additionally, this genetic trade-off has been also reported in other type of animals as in poultry and fish (Francesch et al., 1997; Snyder, 1991).

Tolerance to desiccation was positively correlated to tolerance to starvation (Figure 2.3.3). These results suggest mechanisms with a common genetic basis underlying some of the variations in these traits. Indeed, Hoffmann and Parson (1999) stated that genetic correlations between tolerance to different environmental stresses tend to be positive. For example, positive correlations between tolerance to desiccation and starvation have been reported in *Drosophila* spp. by several authors (Hoffmann & Harshman, 1999; Hoffmann & Parsons, 1989a; Service et al., 1985). Other studies reported a positive relationship between tolerance to starvation and lipid content (Chippindale et al., 1996; Djawdan et al., 1998; Harshman et al., 1999). Also, tolerance to food deprivation is often associated with increased glycogen levels, which are an energy source as well (Djawdan et al., 1998). This suggests that accumulating metabolic energy in the form of lipid or glycogen reservoirs may contribute to overcome physiological stresses, and provide a potential physiological basis for tolerance to both traits (Bradley et al., 1999; Chippindale et al., 1998; Graves et al., 1992), as I argue below.

The fundamental elements underlying desiccation tolerance are thought to involve reducing the rates of water loss and increasing the capacity for water storage (Folk et al., 2001; Gibbs & Matzkin, 2001; Hoffmann & Harshman, 1999). Indeed, glycogen might function as a sponge, and once it is metabolized, more water would be available (Gibbs et al., 1997). Thus, the importance of glycogen in the evolution of desiccation tolerance appears to be crucial (Chippindale et al., 1998). Furthermore, a reduced metabolic rate is also associated with tolerance to stresses such as desiccation and starvation (Hoffmann & Parsons, 1989a, 1989b), and populations presenting tolerances to these stresses had also lower metabolic rates and increased lipid and glycogen body contents (Graves et al., 1992; Service, 1987).

Another remarkable result is that there is a moderate positive genetic correlation between longevity and tolerance to desiccation (Figure 2.3.4, p. 95). This result, can be explained for instance if genotypes that are more tolerant to starvation and desiccation have also lower metabolic rates (see above), because, according to the rate of living theory (Rubner, 1908), lower rates of metabolism increase longevity. This is the basis for the caloric restriction hypothesis of aging (Shanley & Kirkwood, 2000). Indeed, in stressful environments, such as during periods of drought and food scarcity, reduced energetic requirements for maintenance should be advantageous and, therefore, individuals with lower metabolic rates would be favoured from natural selection (Harshman et al., 1999).

That tolerance to starvation could be explained as a consequence of lower metabolic rates could also explain the positive genetic correlation between starvation resistance and the time spent as larva and deutonymph stages (Figure 2.3.4), as individuals with low metabolic rates are known to have slower developmental times (Pettersen et al., 2016).

The negative correlation between early fecundity and tolerance to starvation and desiccation could be explained from physiological constraints underlaying the cost of reproduction. The two possible scenarios that could explain this trade-off would be: i) reproduction results in direct somatic damage, causing increased vulnerability to environmental stress, and ii) reproduction drains somatic energy reserves (e.g. carbohydrate and lipid), thus limiting the energy available for biochemical mechanisms that protect the body from damage caused by those stressors (Harshman & Zera, 2007). These negative correlations have also been reported from artificial selection experiments in *D. melanogaster*, in which lines selected for tolerance to desiccation have lower early fecundity (Hoffmann & Parsons, 1989a; Service et al., 1985; Service & Rose, 1985).

Regarding correlations obtained between traits related to fecundity (oviposition time, early fecundity, and total fecundity) and behavioural traits (distance travelled, walking speed and activity), it is unlikely that weak selection favouring both types of traits could explain the occurrence of the positive correlations (Figure 2.3.4, p. 95) because both early fecundity and behavioural traits show low heritability (Table 2.2.7.a, p. 84), and thus, there is some evidence that strong selection has operated on them. Since this strong selection has not led to negative genetic correlations (Roff, 1996), correlational selection is again a potential explanation for the maintenance of this genetic correlation with different combinations of behaviour and fecundity trait values equally leading to high fitness. These correlation values, however, are not very high (Figure 2.3.4.), and since they have been detected using different individuals should be considered with caution.

There is a negative genetic correlation between developmental time and predation rate on eggs of *T. urticae* (Figure 2.3.4, p. 95). Since body size was not affected by this shortening of developmental time (i.e., shorter developmental time did not lead to smaller adults), this shows that the more voracious individuals develop at a higher rate and the less voracious individuals develop at a lower rate but at the same time the later expose themselves less to predation. This is consistent with the hypothesis of the pace-of-life syndrome

(POLS) (Réale et al., 2010), as extended to behaviour at the intrapopulation level. Therefore, this can maintain the genetic correlation of a continuum of strategies in POLS, with fast living individuals that are highly voracious and slow living individuals that are less voracious.

Perhaps, the positive genetic correlation between post-oviposition time and Longevity (Figure 2.3.3, p. 93) could be a residual correlation and be due to an artefact as a consequence of having food *ad libitum*. In nature, due to the great fluctuation of conditions and low availability of food, they should not lay eggs at as high rates as in laboratory populations, that is, in nature, they need to use all their longevity and therefore the post-oviposition time would be much shorter than in laboratory populations.

Experimental evolution:

Aiming at finding out which response traits evolve and towards what direction, I subjected subpopulations of the wild strain of *A. swirskii* to two selection forces, one biotic (presence of the anthocorid *O. laevigatus*) and the other abiotic (exposure to high temperatures), in a full crossed design. After exposure to selective forces during 8 generations, traits related to development and size evolved, not always intuitively, as follows:

High temperatures do not always lead to the evolution of accelerated development

Exposure to high temperatures resulted in the evolution of juveniles requiring more time to fulfil development (Figure 3.4.5, p. 115). This is, again, another counterintuitive result. Indeed, metabolic rates in ectotherms are fueled by temperature (J. H. Brown et al., 2004; Gillooly et al., 2001) and thus, higher temperatures should speed up developmental rates up to a limit (Gillooly et al., 2002). However, phenotypical adjustments in response to changing temperatures may not be possible under all conditions because of energetic constraints (Walczyńska et al., 2016) if, for example, at very high temperatures performance is compromised (Dell et al., 2011). This way, if developing faster under high temperatures requires an energy demand that is not fulfilled by individuals when they forage to acquire energy, this could decrease developmental rate (and conversely increase developmental time), especially if at temperatures beyond optimal anabolism entail high costs. Indeed, it is well known that beyond optimal temperatures enzymatic reactions and all the temperature-dependent traits associated with performance, are compromised [e.g., Dell at al. (2011)]. For example, individuals genetically codified to develop faster that were not capable of accruing all the elements (nutrients, enzymatic reactions) for growth and development due to the high imposed temperature in the "Hot" treatment could have been eliminated selectively, resulting in populations with longer developmental time.

According to Lee and Gillespie (2011), the shortest developmental time of *A. swirskii* occurs at 32°C. During experimental evolution, the daily average temperature under the "Hot" treatment was 30°C. However, there were 5 hours per day in which the temperature was 34°C (Figure 3.4.1, p. 114). This temperature regime, with periods of temperature way beyond the optimal, triggered an evolutionary response in the populations. Other works that did not include evolution reported that the shortest developmental time of *A. swirskii* occurs at 35°C (Farazmand et al., 2020). In my evolution experiment this temperature was never reached, but, contrary to Farazmand´s work, my experiments were the result of a long-term exposure, for several generations, which trigger occurrence of evolutionary processes. Therefore, this mismatch between theirs and my results reflects the relevance of performing experiments across several generations as unexpected results may emerge.

The temperature-size rule does not always rule

The temperature-size rule in ectotherms (TSR) (Atkinson, 1994) states that "individuals reared at colder temperatures reach maturity at a larger size than when reared at warmer temperatures". That is, individuals inhabiting warm environments will develop faster, but at the cost of becoming smaller (Angilletta Jr. et al., 2004). This rule has indeed been observed in many ectotherm species in the literature (Atkinson, 1994; Daufresne et al., 2009; Gardner et al., 2011; Sheridan & Bickford, 2011). However, there have been as well many examples in which the TSR did not hold (Atkinson, 1995; Walters & Hassall, 2006). Recently, other authors claimed that organisms only follow this rule when temperatures are between a minimum and optimal temperatures, a range that is species-specific, while the opposite pattern is observed at temperatures beyond the optimal (Walczyńska et al., 2016); or that a key factor for organisms to follow the temperature-size rule is the timing of exposure to high temperatures, with longer exposures having more of an effect (Peralta-Maraver & Rezende, 2021). Even more, a recent review and meta-analysis revealed no evidence for higher temperatures selecting for smaller body sizes in the wild, indicating that responses of size to temperature are mostly due to phenotypic plasticity (Siepielski et al., 2019). Indeed, there is a lack of studies demonstrating that the reduction in body size with increasing temperatures is genetically based

(Gienapp et al., 2008). I did find that temperature yielded smaller individuals, but not when this source of stress was acting alone.

Both egg size and adult body size evolved to be smaller only when two stressors were acting together, one abiotic, temperature, and another biotic, predator presence (Figures 3.4.3 and 3.4.4, pp. 112 and 114). This is a novel finding, and, to my knowledge, it is the first time that body size is documented to respond evolutionarily to both temperature and predation pressure. I hypothesize that the mechanisms leading to a selective evolutionary response yielding smaller body sizes were several folded: Firstly, high temperatures increase the activity levels, and thus the energy demand of ectotherms, thereby increasing the encounter and attack rates between predator and prey (J. H. Brown et al., 2004; Moya-Larano, 2010). Therefore, predation pressure exerted by *O. laevigatus* on *A. swirskii* was likely higher at high temperatures. Secondly, *O. laevigatus* is a visual predator, and if it forages optimally it would preferentially select for bigger prey items that maximize its reproductive success (Stephens & Krebs, 1986), as long as prey size is within catchable range (Brose et al., 2006). Therefore, a combination of predator preferences for larger individuals, an increase in encounter and predation rates, would favor smaller individuals.

Contrary to the effects observed when the two selection pressures acted independently, their combination acted synergistically, indicating that the interaction between biotic and abiotic factors can yield predictable results that would be very difficult to predict otherwise.

Lack of evidence for adaptation to temperature or predation when tested separately but evolutionary costs associated to the former

After exposing subpopulations of *A. swirskii* to two selection forces, I wanted to assess whether prolonged exposure to each selection force had entailed any adaptive process and/or evolutionary cost to the individuals.
In my experiments, I found no evidence of adaptation to high temperatures (see figures 3.5.1, 3.5.2, 3.5.3 and 3.5.4, pp. 125-127).

However, I was able to detect an evolutionary cost in the populations that evolved under hot temperatures. When these populations were exposed to mild conditions, individuals showed a lower survival probability than those from populations that were not exposed to such conditions (Figure 3.5.3, p. 127). These results indicate a loss of plasticity as a consequence of is long-time exposure to hot temperatures.

Somehow, these results highlight the severity of the problems associated with warming and the increase in frequency of heat waves in the context of climate change for the health of agro-ecosystems under BPC management.

Yet, because of logistical problems, I could only evaluate few traits and under each selection force separately. Thus, the interaction of the two factors, which is when I observed evolution towards smaller sizes, was not tested. Furthermore, developmental time (the only trait that responded to temperature only) and adult body size were not tested. This was due to the lack of enough time to analyse the results of possible evolutionary responses once the evolution experiment was over. For this reason, and because of the large number of experiments to be carried out in the shortest possible time, to avoid possible dilution of the evolutionary responses in the absence of such selection pressures (in the case these would entail a cost), the evaluation of the developmental time was not considered because is high time demanding and due to constraints imposed by the completion of the project.

Furthermore, I have not been able to report any adaptive response (or evolutionary cost) regarding long exposure to the presence of a predator (Figures 3.5.5, 3.5.6 and 3.5.7, pp. 128-129). Perhaps, one of the reasons could be the lack of heterogeneity in the habitat structure in the experimental arenas, both during the evolution experiment and during the evaluation of possible adaptation or evolutionary costs. Indeed, one of the main factors for prey to be able to successfully exert anti-predator behaviour is the structure or heterogeneity of the space (Janssen et al., 2007). This could have prevented mites from displaying anti-predator behaviour and thus, having selected those individuals who were able to avoid an encounter with the predator. Therefore, if the habitat structure had been complex, perhaps *A. swirskii* females could have used it to take advantage of it, displaying anti-predator behaviour and actively using it as a refuge, which would have allowed them to increase their survival (Persson & Eklov, 1995).

Additionally, the interaction between the two stress factors, temperature and presence of predators, which is the most relevant, was not possible to be tested due to their low population numbers at the time when they were to be measured. However, it is important to realize that a test for adaptation of smaller body sizes under high temperatures and predation pressure would have involved mixing individuals of the evolved population with larger individuals of the source population, thereby providing the predator with a choice to catch larger prey. If testing only the individuals of the small population, probably predation would be higher, not lower upon them, just because the predator would need to kill more individuals in order to meet its energy demands under higher temperatures, thus providing with the inaccurate conclusion of no adaptation.

CONCLUSIONS

In this thesis dissertation I performed extensive quantitative genetic analyses for a total of 23 life-history, behavioral, morphological and physiological traits in a predator widely used in biological pest control (*A. swirskii*). To that end, I collected individuals from the wild population in Israel and from a comercial population, and brought them to the lab to create 20 and 22 isofemale lines respectively from each population.

Using PCA on the traits measured on these isofemale lines, I reported genetic associations among many of the traits spread across two PCA orthogonal axes. This served to pick the most relevant of them to assess broad sense heritability, showing that:

1) Developmental time showed the highest heritability, followed by fecundity and body size (with similar estimates) and desiccation and starvation resistance
2) I failed to find heritability for the three behavioral traits, namely walking speed, activity and distance travelled. However, the fact that they were positively genetically correlated to each other suggests that some, albeit low genetic variation is left in the behavioural traits.

Also, the PCA figures showed that:

3) The genetic variation across two PC axes is much smaller in the comercial population than in the wild population, and that in fact the multidimensional variation of the former is fully included within the second.

This, along with differences in mean values between the two populations, suggest that:

4) The comercial population has been subjected to strong selection occurring in mass-rearing procedures which could compromise their future success as biological control agent due to strong selection

Regarding genetic correlations, they were widespread both among traits of the same type (life-history, morphological, physiological) and between traits of different type.

5) Negative genetic correlations can constraint evolution but may arise merely by strong past selection acting on each of the traits inolved. Among them, interesting traits truly involving potential evolutionary constraints were the negative correlations between developmental time and predation rate, and the tolerance to desiccation and starvation with traits related to fecundity (early or total fecundity and oviposition time).

6) Positive genetic correlations, on the other hand, may be maintained by correlational selection, particularly if selection has been strong in the past, as suggested by the low heritabilites of many of the traits I found to be involved in these positive correlations. Among them, the correlations between the behavioural traits, between egg size and egg developmental time, and between the total developmental time and pre-oviposition time.

Experimental evolution manipulating a central abiotic factor in climate change (temperature) and a relevant biotic factor (predation pressure) ended up in:

7) *A. swirskii* evolving to smaller body sizes under high temperatures, but only when predators were present.
8) Populations that evolved under high temperatures experienced a lengthening of their developmental time. This result does not align with the published developmental times of *A. swirskii* at different temperatures. However, the assessment in the later studies was only conducted in one generation, likely involving mere plasticity.

Consequently,

9) This highlights the importance of considering the potential evolutionary responses over many generations, as unexpected, previously unanticipated results may emerge.

Therefore, results from the evolution experiment show

10) The importance of approaching BPC strategies in a more realistic and comprehensive way, considering existing abiotic and biotic factors and their interaction, as well as the evolutionary responses derived from them.

Finally, these results were possible likely because I used the wild population for experimental evolution, which holds much higher genetic variation than the comercial one. Therefore,

11) If BCA are to be improved in the face of global change, much attention must be paid to the actual amount of genetic variation present in wild populations.

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GLOSSARY

Glossary

Words included in this glossary are underlined in the main text of this thesis.

Apparent competition: Indirect interaction that may occur between two species (A and B) that share a predator enemy. The mechanism underlying apparent competition is that the presence of a second prey species (let's say B) increases the abundance of available food to the predator, which, consequently, increases in numbers. The higher numerical response of the predator due to the presence of species B may result in higher overall predation of the predator on species A (despite the per capita predation rate might be lower) and thus, in lower densities of species A at equilibrium than those in the absence of species B. This term was coined by (Holt, 1977), to distinguish two similar community outputs resulting from negative indirect interactions between two prey species, one mediated by exploitative competition and the other mediated by predation.

Apparent mutualism: In a two-prey one-predator system, apparent mutualism is an indirect interaction between pest species in which the presence of a second prey (Prey B) affects prey A densities positively as a result of the satiation of their shared predator. Predators may switch to the most abundant prey species, thus releasing the other species from predation. This should occur in the short term, before the predator´s numerical response increases. (Abrams & Matsuda, 1996; Holt & Lawton, 1994).

Artificial selection: A method used in the production of plant cultivars and animal breeds to achieved desired phenotypes. It consists of the selective breeding of individuals in a population with more extreme target characteristics, promoting a change in the mean of the selected trait across generations. In addition, this methodology is used to examine the genetics of quantitative traits, describing the extent to which traits that show continuous phenotypic variation will be able to adapt to selective forces (Brakefield, 2003; Conner, 2003).

Community modules: Small subsets of species integrating food webs that are characterized by strong interactions. Usually consist of 2-4 species interacting through predation, competition, mutualism, or apparent competition (Holt, 1997).

Correlational selection: Process that occurs when the relationship between an individual's trait value and expected fitness for one trait depends on that individual's trait values for other traits. Natural selection does not act independently on each trait, but selection acting on one trait depends on the values taken by another trait. Correlational selection can operate in both discrete trait combinations and quantitative characters (Sinervo & Svensson, 2002; Svensson et al., 2021).

Effect trait: Those traits which have an impact on ecosystem properties, ecosystem functions and ecosystem services and do not represent an adaptative advantage to the individual itself. For example, the trophic cascade produced by biological characteristics of natural enemies/predators in the biological pest control (Díaz et al., 2013; Violle et al., 2007).

Experimental evolution: It is a generic term that involves the use of experiments to investigate the process of evolution under controlled conditions where typically the researcher manipulates one or more environmental or genetic factors and observes how the population of organisms responds to the selection pressures imposed by these changes across generations, allowing to follow the evolution of populations in real-time. It can be used to test the hypotheses about the evolutionary outcomes of different scenarios such as the adaptation of organisms to changing environmental conditions.

Founder effect: The founder effect occurs when a subgroup of a population leaves the main population and establishes a new population. Although a population can increase, the genes carried by all its members are derived from the few genes originally present in the founders, so there is a much lower genetic diversity as compared from the source population.

Functional traits: Morpho-physio-behavioural traits that impact the fitness of individual species via their effects on growth, reproduction and survival, the three components of the individual performance (Violle et al., 2007). As such, a functional trait determines the organism's response to pressures (Response trait), and/or its effects on ecosystem processes or services (Effect trait) (Harrington et al., 2010).

Genetic drift: It is a random change in allele frequency from one generation to another. Normally, from probability alone, there is a loss of the less frequent alleles and a fixation of the most frequent ones, resulting in a decrease in the genetic diversity of the population. This effect is stronger under low population numbers such as it is the case during bottlenecks or founder effects.

Genetic trade-off: The concept is an integral part of life-history theory and it usually involves a negative additive genetic correlation between two traits that are positively associated to fitness. When a genetic trade-off between two traits (A and B) exists among individuals in a population, a selective pressure favouring trait A decreases at the same time individual fitness from trait B, thereby constraining evolutionary change (Fabian & Flatt, 2012; Roff, 1992).

Heritability: It summarizes how much of the variation in a trait is determined by the alleles that are transmitted from the parents to the offspring in a population level. It depends on the frequencies of alleles in the population and in the environment under which it is measured. It measures the resemblance between the parents and their offspring beyond the potential common environment in which they live and its values are ranged between 0 and 1. There are two main types of heritability. In broad-sense heritability determines the extent to which individuals' phenotypes are determined by their genotypes (both additive and non-additive genetic variance; e.g., from dominance or epistasis) ($H^2=V_G/V_P$) whereas narrow-sense heritability captures only the variation of individuals' phenotypes that is due to additive genetic variance ($h^2=V_{AD}/V_P$). (Falconer & Mackay, 1996; Roff, 1997).

Inbreeding: Inbreeding occurs when two individuals that are related to each other by common ancestry produce offspring by mating with each other. Inbreeding results in higher consanguinity and homozygosity, and therefore a loss of genetic variability. In addition, high inbreeding could increase the chances of offspring being affected by deleterious alleles and a decreased biological fitness in the population (inbreeding depression).

Intraspecific genetic variability: It is the part of the phenotypic variability within or across populations that is explained by the total amount of alleles present in the species. It is responsible for species to adapt to changes that occur in the environment, influencing not only the evolution of populations, but also their ecological effects. Its extent within populations gives the adaptive potential to the species and largely determines the ability to its populations to persist over time from adaptive changes in genetic frequencies.

Isofemale line (Isoline): An isoline is a highly inbred family founded from a single male and female from the base population. Generating isolines is a technique used for investigating the genetic architecture of a population and it was coined by Parsons & Hosgood, 1967. Once originated, an isoline is then propagated by mass mating in a laboratory culture (David et al., 2005). Several isolines are used to characterize the genetic architecture of a population.

Maternal effect: It is an indirect effect by which the genotype and/or the environment of a mother, or even grandmother, influences its offspring's phenotype of many metric characteristics even when measured as adult. Therefore, the presence of maternal effects leads to the transgenerational transmission of environmental sources of variation which can indirectly influence evolutionary responses to selection (Mousseau & Fox, 1998; Wolf et al., 1998). To eliminate possible maternal or grand-maternal effects on offspring from experimental regimes (different environmental, demographical or social conditions) that parents could have experienced, resulting populations should be reared in a common environment for one or two generations respectively (Kawecki et al., 2012).

The metabolic theory of ecology: The metabolic theory of ecology (MTE) is an extension of the Metabolic Scaling Theory (West et al., 1997) and Kleiber´s law. It holds that the metabolic rate of organisms is the most fundamental biological rate that controls most of the ecological processes at all levels of organization from individuals to the biosphere. MTE provide a quantitative and unified theory for the importance of metabolism in driving pattern and biological processes based on the relationship among metabolic rate, body size and temperature (J. H. Brown et al., 2004).

Next Generational Individual-Based Model: Individual-Based Models (IBMs), also called Agent-Based Models (AGMs), are a type of model that simulates populations or communities composed of discrete individual organisms where the behaviour of populations emerges from interactions between individuals and these individuals with the abiotic factors. Each individual has a set of state variables or attributes (i.e.: spatial location, physiological and behavioural traits) that vary among the individuals and can change through time, and behaviours (DeAngelis & Grimm, 2014). The IBMs allow for investigating questions about complex ecological systems that have been difficult or impossible to study using classical approaches. Today, ecological modelling has a permit to develop Next-Generation Individuals-Based Models (NG-IBMs) characterized by three essential elements that IBMs do not have in their entirety: 1) indicators of realism, 2) emergence of ecological phenomena at a population, community, or ecosystem level and patterns from individuals, and 3) the outputs of the model should be considered as solid predictions (Grimm & Berger, 2016).

Phenotypic variance: In is the set of different values of each of the observable characteristics that the individuals of a population present. This is the total observed variation in a particular trait within a population. It is the sum of the genetic and environmental variances that contribute to the variation in the trait. It is continuously distributed among all individuals that compose a natural population, usually following a normal probability distribution (Mackay, 2009).

Quantitative traits: Any morphometrical, physiological or behavioural attribute (trait) that varies continuously in a population and depends on the cumulative actions of many genes (polygenic characters) and the environment. This contrasts with the more classic Mendelian traits (e.g., eye colour) which are affected by genes of large effects at a single locus. However, the genes involved in polygenic traits are also assumed to have Mendelian inheritance.

Response trait: A Functional trait of a species that influences or changes the ability of populations to colonize a habitat and persist under environmental changes. For example, in biological pest control a response trait in a natural enemy/predator may change either plastically or evolutionarily (e.g.: acari) under different community contexts (trophic chain, intraguild predation or apparent competition) (Díaz et al., 2013; Violle et al., 2007).

Trophic cascade: Ecological concept that refers to when, in a food web, organisms of higher trophic levels (e.g., secondary consumers) indirectly influence the abundances of organisms of two trophic levels below (e.g., primary producers) via the direct trophic pressure they exert on their adjacent trophic level (e.g., primary consumers). The term was first coined by (Paine, 1980).

Top-down control: In food webs, top-down control occurs when populations of organisms of higher trophic levels control/regulate the populations of organisms of lower trophic levels. For example, populations of herbivores are controlled by their natural enemies. It is, in fact, the mechanism that explains trophic cascades. This mechanism was first described by (Hairston et al., 1960) in their “Green World Hypothesis”, and it was later extended to include ecosystem productivity gradients by (Oksanen et al., 1981) in their “Exploitation Ecosystem Hypothesis”.

LIST OF ABBREVIATIONS

BC: Biological Control
BCA: Biological Control Agent
BPC: Biological Pest Control
CAP: Common Agricultural Policy
CBD: Convention on Biological Diversity
EPPO: European and Mediterranean Plant Protection Organization
ERA: Environmental Risk Assessments
EU: European Union
FAO: Food and Agriculture Organization
FWE: Food Web Engineering
GLM: Generalized Linear Model
GLMM: Generalized Linear Mix Model
IBM: Individual Base Model
IGP: Intra-Guild Predation
ILT: Isofemale line technique
IPCC: Intergovernmental Panel on Climate Change
IPPC: International Plant Protection Convention
L:D: Light:Dark (Photoperiod)
LMM: Linear Mix Model
MCMC: Markov Chain Monte Carlo
NAPPO: North American Plant Protection Organization
NG-IBM: Next-Generation Individual Base Model
PCA: Principal Component Analysis
R_g: Genetic regression
RH: Relative Humidity
Temp.: Temperature
TSR: Temperature-size rule
UV-B: Ultraviolet-B radiation
V_{ad}: Additive genetic variance
V_d: Dominance variance
V_E: Environmental variance
V_G: Genetic variance
V_i: Interaction (epistatic) dominance
V_p: Phenotypic variance

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APPENDICES

Appendix I (Supplementary material Chapter 2)

Results of PCA of the means by isoline of all the traits assessed on females in both populations for the PC1 and PC2. "Cor" is the correlation between the variable and the dimension, "ctr" is the contribution of each variable and "cos2" is the Cos2 for the variable.

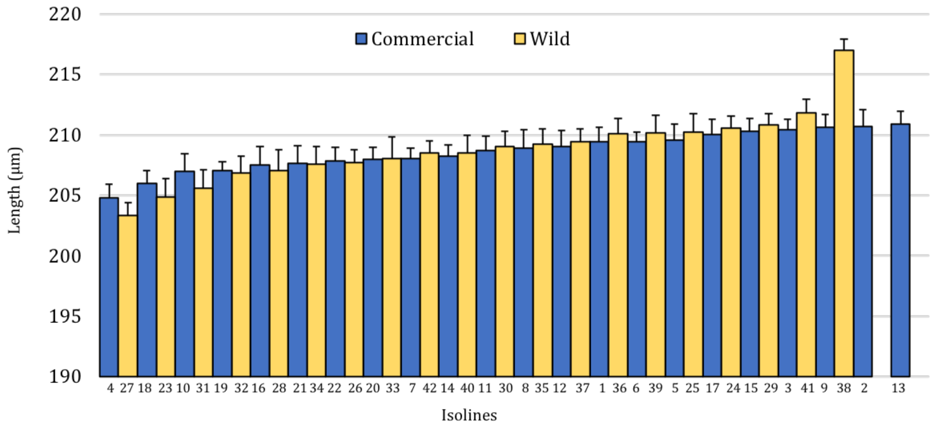
Trait		**PC1**			**PC2**		
		Cor	**ctr**	**cos2**	**Cor**	**ctr**	**cos2**
Egg length		0.348	2.103	0.121	-0.199	1.010	0.040
Egg Width		0.323	1.809	0.104	-0.427	4.626	0.182
Dorsal shield length		0.465	3.759	0.216	-0.473	5.688	0.224
Dorsal shield width		-0.145	0.366	0.021	-0.311	2.461	0.097
Egg		-0.037	0.023	0.001	-0.269	1.841	0.025
Larva		0.557	5.396	0.311	0.157	0.630	0.025
Protonymph		0.705	8.626	0.496	0.219	1.223	0.048
Deutonymph		0.842	12.309	0.708	0.218	1.210	0.048
Egg-Adult		0.859	12.814	0.737	0.193	0.943	0.037
Longevity		-0.134	0.311	0.018	0.808	16.564	0.652
Pre-oviposition time		0.584	5.929	0.341	-0.117	0.346	0.014
Oviposition time		0.371	2.391	0.138	0.584	8.655	0.341
Post-oviposition time		-0.586	5.970	0.343	0.548	7.625	0.300
Early fecundity		-0.756	9.944	0.572	-0.306	2.384	0.094
Fecundity		-0.281	1.372	0.079	0.413	4.337	0.171
Distance travelled		0.671	7.825	0.450	-0.282	2.020	0.080
Walking speed		0.604	4.636	0.365	-0.284	2.263	0.089
Activity time		0.516	6.350	0.267	-0.298	2.046	0.081
Tolerance to desiccation	35% RH	-0.042	0.030	0.002	0.272	1.878	0.074
	50% RH	0.074	0.096	0.006	0.764	14.842	0.584
	65% RH	0.157	0.427	0.025	0.682	11.822	0.465
Tolerance to starvation		0.502	4.388	0.252	0.361	3.308	0.130
Predation rate		-0.424	3.127	0.180	-0.299	2.278	0.090

Results of the PCA of the means by isoline of all the traits assessed on females and males in both populations. “Cor” is the correlation between the variable and the dimension, “ctr” is the contribution of each variable and “cos2” is the Cos2 for the variable.

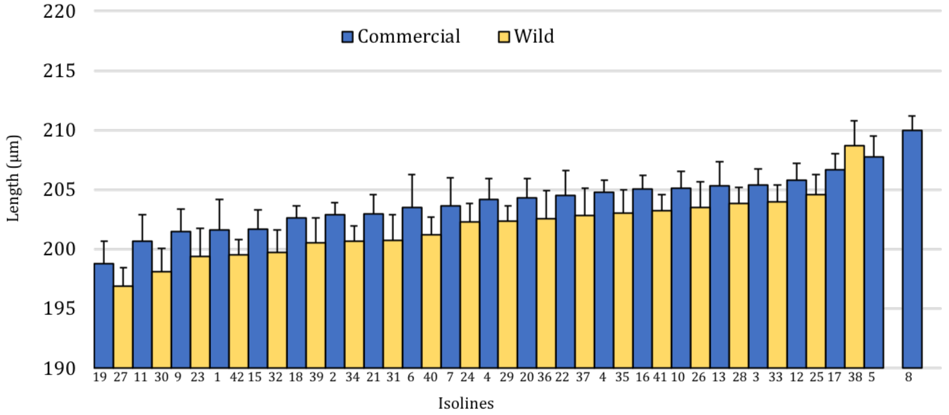
Trait	**PC1**			**PC2**		
	Cor	**ctr**	**cos2**	**Cor**	**ctr**	**cos2**
Egg length	0.802	1.350	0.047	0.426	13.960	0.182
Egg Width	0.800	0.002	0.000	0.687	36.316	0.472
Egg	-0.218	19.035	0.668	0.104	0.838	0.011
Larva	-0.007	21.508	0.755	0.298	6.824	0.089
Protonymph	0.817	21.538	0.756	0.465	16.657	0.217
Deutonymph	0.869	18.330	0.643	-0.415	13.243	0.172
Egg-Adult	0.869	18.238	0.640	-0.398	12.162	0.158

Graphs of the mean values (Mean ± SE) of each of the isolines of both populations for each evaluated trait.

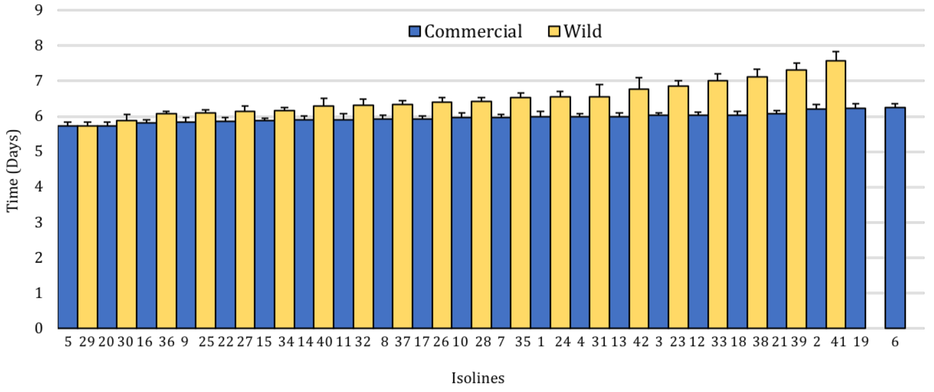
Female egg length

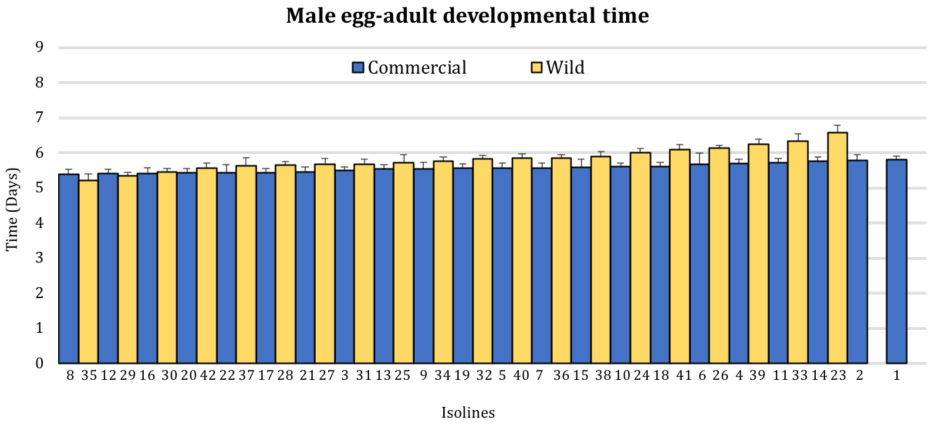


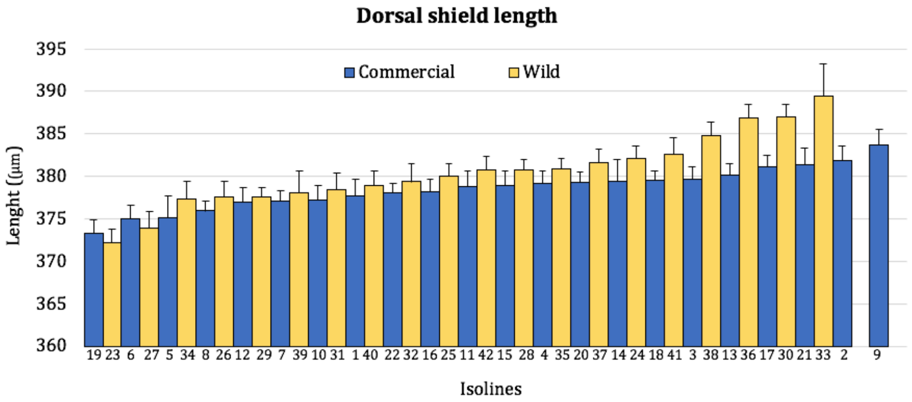
Male egg length

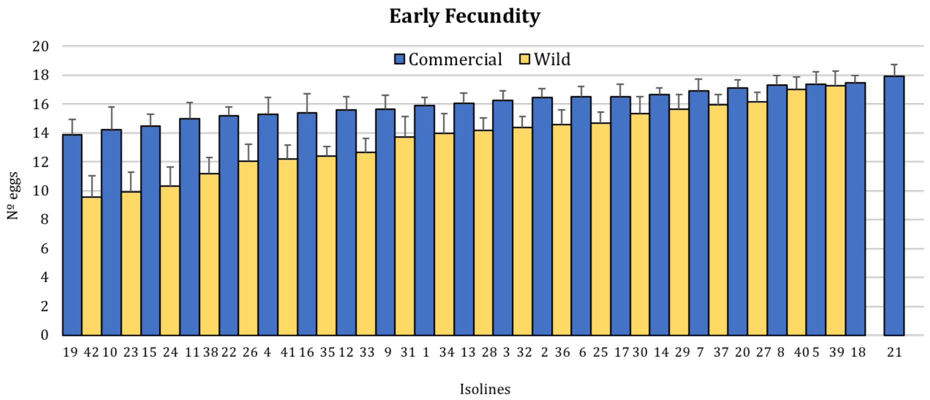


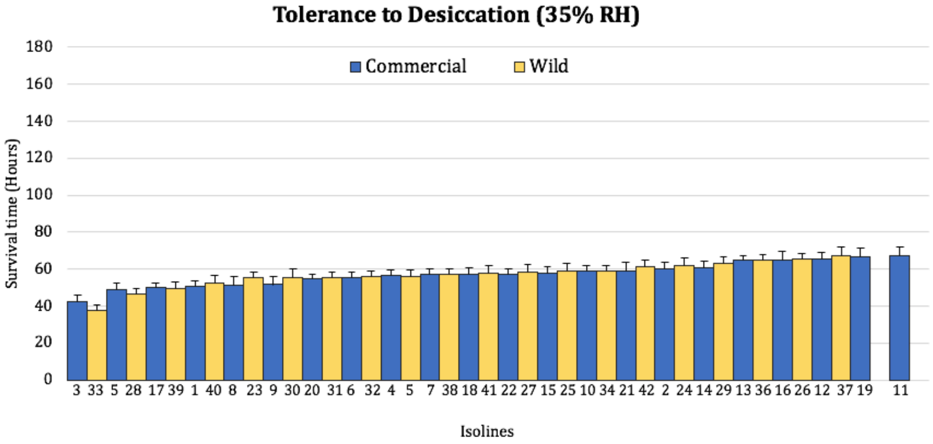
Female egg-adult developmental time

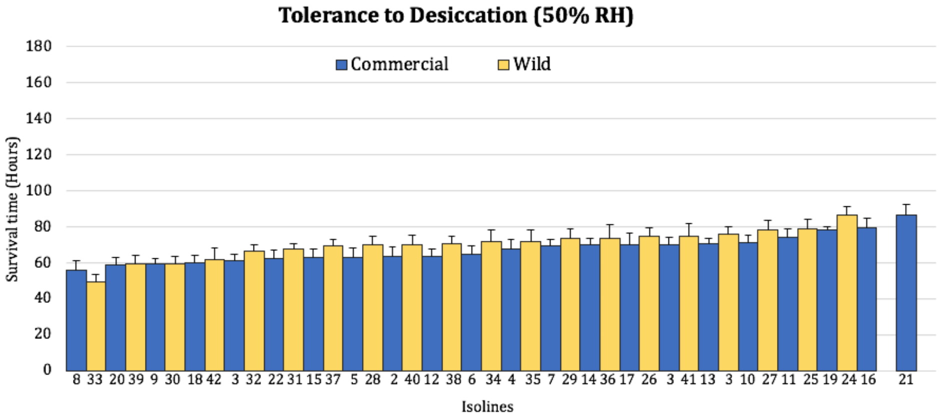


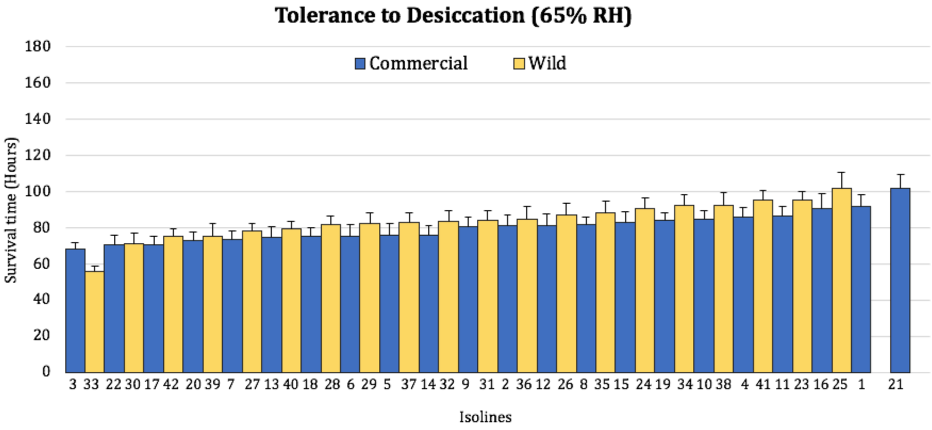

Male egg-adult developmental time
Commercial
Wild
Time (Days)
9
8
7
6
5
4
3
2
1
0
8 35 12 29 16 30 20 42 22 37 17 28 21 27 3 31 13 25 9 34 19 32 5 40 7 36 15 38 10 24 18 41 6 26 4 39 11 33 14 23 2 1
Isolines

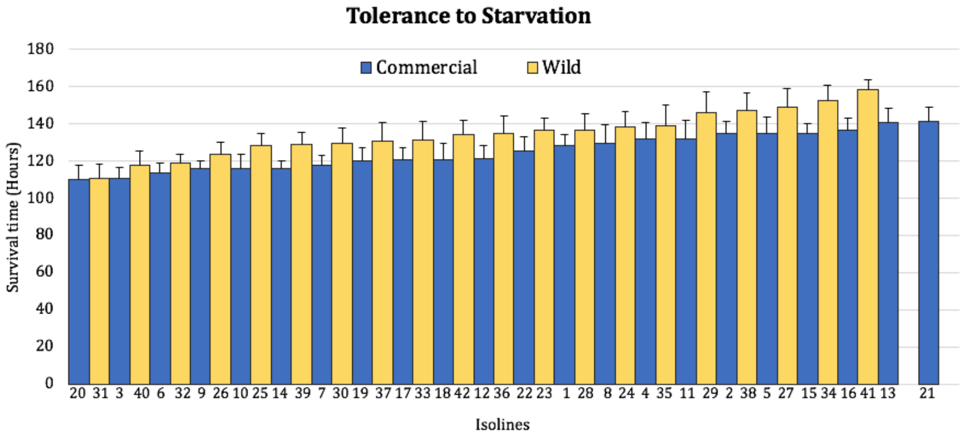

Dorsal shield length
Commercial
Wild
Lenght (μm)
395
390
385
380
375
370
365
360
19 23 6 27 5 34 8 26 12 29 7 39 10 31 1 40 22 32 16 25 11 42 15 28 4 35 20 37 14 24 18 41 3 38 13 36 17 30 21 33 2 9
Isolines

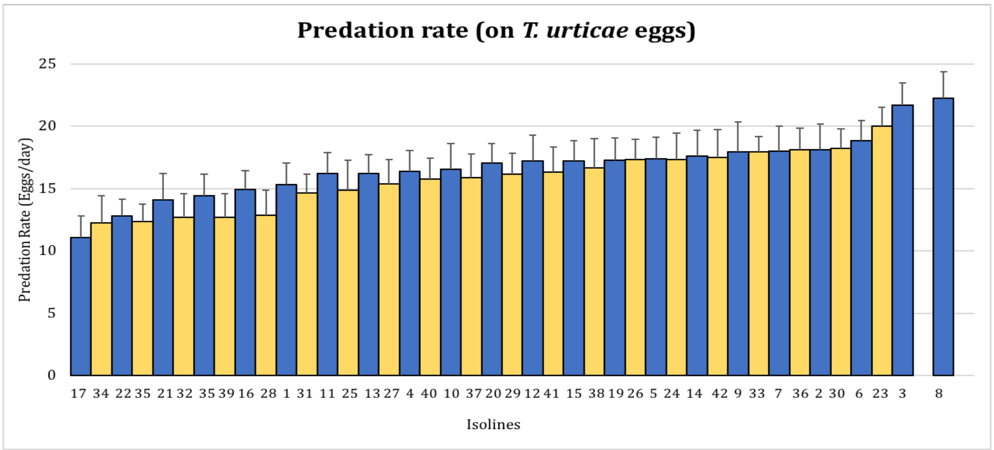

Early Fecundity
Commercial
Wild
Nº eggs
20
18
16
14
12
10
8
6
4
2
0
19 42 10 23 15 24 11 38 22 26 4 41 16 35 12 33 9 31 1 34 13 28 3 32 2 36 6 25 17 30 14 29 7 37 20 27 8 40 5 39 18 21
Isolines

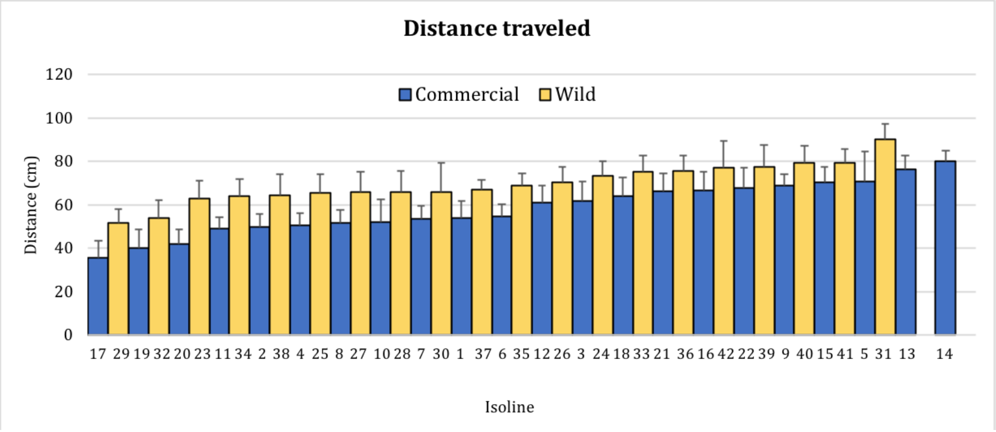

Tolerance to Desiccation (35% RH)
Commercial
Wild
180
160
140
120
100
80
60
40
20
0
Survival time (Hours)
3 33 5 28 17 39 1 40 8 23 9 30 20 31 6 32 4 5 7 38 18 41 22 27 15 25 10 34 21 42 2 24 14 29 13 36 16 26 12 37 19 11
Isolines

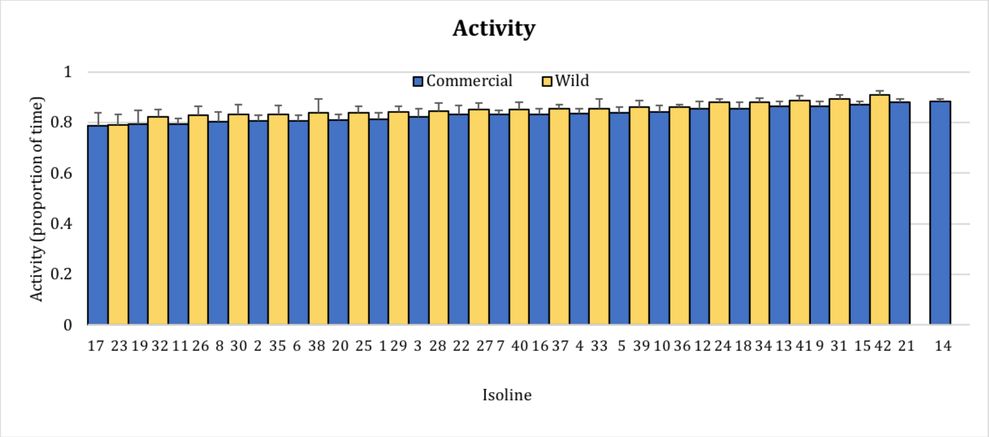

Tolerance to Desiccation (50% RH)
Commercial
Wild
180
160
140
120
100
80
60
40
20
0
Survival time (Hours)
8 33 20 39 9 30 18 42 3 32 22 31 15 37 5 28 2 40 12 38 6 34 4 35 7 29 14 36 17 26 3 41 13 3 10 27 11 25 19 24 16 21
Isolines

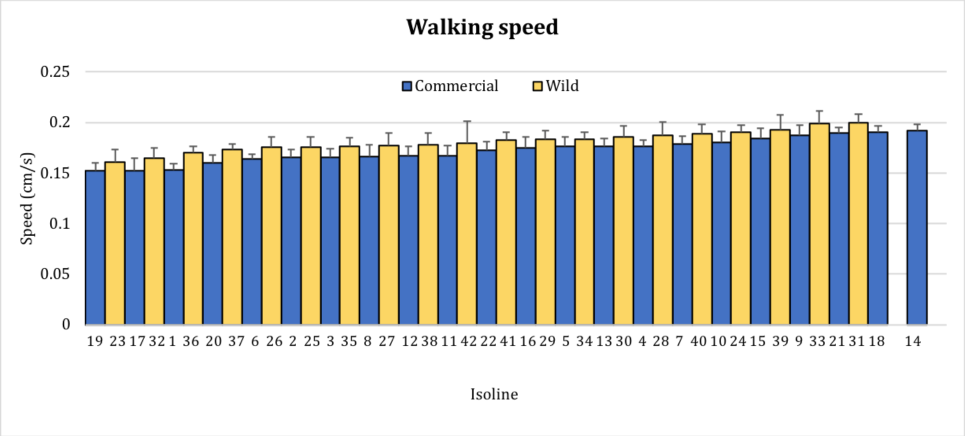

Tolerance to Desiccation (65% RH)
Commercial
Wild
180
160
140
120
100
80
60
40
20
0
Survival time (Hours)
3 33 22 30 17 42 20 39 7 27 13 40 18 28 6 29 5 37 14 32 9 31 2 36 12 26 8 35 15 24 19 34 10 38 4 41 11 23 16 25 1 21
Isolines


Tolerance to Starvation
Commercial
Wild
Survival time (Hours)
0
20
40
60
80
100
120
140
160
180
20 31 3 40 6 32 9 26 10 25 14 39 7 30 19 37 17 33 18 42 12 36 22 23 1 28 8 24 4 35 11 29 2 38 5 27 15 34 16 41 13 21
Isolines


Predation rate (on *T. urticae* eggs)
Predation Rate (Eggs/day)
0
5
10
15
20
25
17 34 22 35 21 32 35 39 16 28 1 31 11 25 13 27 4 40 10 37 20 29 12 41 15 38 19 26 5 24 14 42 9 33 7 36 2 30 6 23 3 8
Isolines


Distance traveled
Commercial
Wild
Distance (cm)
0
20
40
60
80
100
120
17 29 19 32 20 23 11 34 2 38 4 25 8 27 10 28 7 30 1 37 6 35 12 26 3 24 18 33 21 36 16 42 22 39 9 40 15 41 5 31 13 14
Isoline


Activity
Commercial
Wild
Activity (proportion of time)
1
0.8
0.6
0.4
0.2
0
17 23 19 32 11 26 8 30 2 35 6 38 20 25 1 29 3 28 22 27 7 40 16 37 4 33 5 39 10 36 12 24 18 34 13 41 9 31 15 42 21 14
Isoline


Walking speed
Commercial
Wild
Speed (cm/s)
0.25
0.2
0.15
0.1
0.05
0
19 23 17 32 1 36 20 37 6 26 2 25 3 35 8 27 12 38 11 42 22 41 16 29 5 34 13 30 4 28 7 40 10 24 15 39 9 33 21 31 18 14
Isoline

Results of the statistical analysis of the calculation of H^2

Egg length						
	Female			**Male**		
	Comm	Wild	Total	Comm	Wild	Total
Gelman criterion:	1	1	1	1	1	1
Chisq isoline	3.427	43.715	44.748	7.249	4.659	19.203
P-value isoline	0.032	<0.001	<0.001	0.004	0.015	<0.001
DIC with isoline	1539.03	1152.81	2685.95	716.73	735.61	0.004
DIC null	1543.98	1211.93	2752.9	726.66	740.85	1464.51
deltaDIC	-4.943	-59.112	-66.946	-9.931	-5.236	-30.944

Egg to adult developmental time						
	Female			**Male**		
	Comm	Wild	Total	Comm	Wild	Total
Gelman criterion:	1	1	1	1	1	1
Chisq isoline	0.471	69.842	186.522	-5.172	20.437	30.295
P-value isoline	0.246	<0.001	<0.001	1	<0.001	<0.001
DIC with isoline	1133.77	873.18	1858.83	622.657	467.62	1067.87
DIC null	1140.99	970.721	2108.71	621.644	505.30	1123.96
deltaDIC	-7.217	-97.543	-249.875	1.012	-37.68	-56.094

Dorsal shield length			
	Commercial	Wild	Total
Gelman criterion	1	1	1
Chi-square isoline	8.120	43.537	61.108
P-value isoline	0.002	<0.001	<0.001
DIC with isoline	1183.804	925.093	2088.099
DIC null	1194.900	984.901	2176.811
deltaDIC	-11.096	-59.808	-88.711

Early Fecundity			
	Commercial	Wild	Total
Gelman criterion	1	1	1
Chi-square isoline	-0.779	17.464	39.567
P-value isoline	1	<0.001	<0.001
DIC with isoline	922.488	646.965	1530.298
DIC null	922.469	672.740	1592.208
deltaDIC	0.0184	-25.775	-61.91

Tolerance to desiccation (35% RH)			
	Commercial	Wild	Total
Gelman criterion:	1	1	1
Chi-square isoline	11.021	14.96	28.822
P-value isoline	<0.001	<0.001	<0.001
DIC with isoline	1089.444	984.297	2070.777
DIC null	1112.602	1013.271	2122.881
deltaDIC	-23.158	-28.974	-52.104

Tolerance to desiccation (50% RH)

	Commercial	Wild	Total
Gelman criterion:	1	1	1
Chi-square isoline	10.152	5.836	20.324
P-value isoline	<0.001	0.008	<0.001
DIC with isoline	1091.522	985.3126	2070.326
DIC null	1112.602	999.075	2108.709
deltaDIC	-21.08	-13.762	-38.383

Tolerance to desiccation (65% RH)

	Commercial	Wild	Total
Gelman criterion:	1	1	1
Chi-square isoline	2.764	11.724	18.453
P-value isoline	0.048	<0.001	<0.001
DIC with isoline	1089.726	965.226	2047.911
DIC null	1098.426	987.739	2083.155
deltaDIC	-8.7	-22.513	-35.243

Tolerance to starvation

	Commercial	Wild	Total
Gelman criterion:	1	1	1
Chi-square isoline	1.159	3.302	14.986
P-value isoline	0.141	0.035	<0.001
DIC with isoline	1098.620	974.032	2054.334
DIC null	1104.1	982.062	2083.155
deltaDIC	-5.48	-8.030	-28.820

Predation rate (on *T. urticae* eggs)

	Commercial	Wild	Total
Gelman criterion:	1	1	1
Chi-square isoline	-5.714	-6.598	-5.366
P-value isoline	1	1	1
DIC with isoline	963.417	873.748	1833.371
DIC null	965.033	874.209	1836.254
deltaDIC	-1.616	-0.4614	-2.883

Distance travelled

	Commercial	Wild	Total
Gelman criterion:	1	1	1
Chi-square isoline			
P-value isoline			
DIC with isoline	389.377	437.974	813.280
DIC block (recording)	389.112	440.900	816.597
deltaDIC	0.265	-2.926	-3.317

Activity time			
	Commercial	Wild	Total
Gelman criterion:	1	1	1
Chi-square isoline			
P-value isoline			
DIC with isoline	791.166	690.918	1468.214
DIC block (recording)	791.907	691.51	1469.781
deltaDIC	-0.741	-0.591	-1.567

Walking speed			
	Commercial	Wild	Total
Gelman criterion:	1	1	1
Chi-square isoline			
P-value isoline			
DIC with isoline	807.578	695.430	1493.008
DIC block (recording)	805.888	694.159	1491.214
deltaDIC	1.690	1.271	1.794

Appendix II (Supplementary materials Chapter 3)

Genetic correlations between traits measured in the same female individuals as estimated from variance components in a MCMCglmm model of both commercial and wild populations. R_g = Genetic correlation, LCI =lower credible interval, UCI = Upper credible interval, PP = Posterior probabilities (the probability that the hypothesis of a non-zero correlation is supported; only values close to one support the hypothesis). Further support is given when credible intervals do no overlap zero.

Female genetic correlations					
Type	**Traits**	**R_g**	**LCI**	**UCI**	**PP**
Life history	Deuto. *vs* Early Fecundity	-0.61	-0.81	-0.29	1
	Deuto. *vs* Egg Length	0.1	-0.24	0.49	0.78
	Deuto. *vs* Egg Width	0.09	-0.15	0.52	0.85
	Deuto. *vs* Egg-Adult	0.85	0.72	0.93	1
	Deuto. *vs* Fecundity	-0.48	-0.76	-0.14	0.99
	Deuto. *vs* Longevity	0.02	-0.44	0.47	0.55
	Deuto. *vs* Ovi. Time	-0.24	-0.51	0.28	0.72
	Deuto. *vs* Post Ovi.	-0.46	-0.74	0.02	0.95
	Deuto. *vs* Pre Ovi.	0.7	0.48	0.87	1
	Early Fec. *vs* Egg Length	-0.09	-0.5	0.27	0.7
	Early Fec. *vs* Egg Width	-0.03	-0.38	0.4	0.45
	Early Fec. vs Fecundity	0.26	-0.04	0.67	0.95
	Early Fec. *vs* Longevity	-0.3	-0.6	0.28	0.77
	Early Fec. *vs* Ovi. Time	-0.32	-0.63	0.11	0.89
	Early Fec. *vs* Post Ovi. Time	0.26	-0.27	0.61	0.76
	Early Fec. *vs* Pre Ovi.	-0.65	-0.8	-0.27	1
	Egg Length *vs* Egg Width	0.63	0.38	0.81	1
	Egg *vs* Deuto.	-0.1	-0.44	0.32	0.67
	Egg *vs* Early Fec.	0.4	-0.08	0.64	0.93
	Egg *vs* Egg Length	0.05	-0.36	0.42	0.59
	Egg *vs* Egg Width	0.03	-0.33	0.43	0.63
	Egg *vs* Egg-Adult	0.08	-0.35	0.4	0.59
	Egg *vs* Fecundity	-0.01	-0.37	0.4	0.5
	Egg *vs* Larva	-0.03	-0.36	0.4	0.5
	Egg *vs* Longevity	-0.06	-0.47	0.42	0.54
	Egg *vs* Ovi. Time	0.01	-0.43	0.34	0.43
	Egg *vs* Post Ovi.	0.01	-0.45	0.44	0.51
	Egg *vs* Pre Ovi.	-0.05	-0.55	0.25	0.78
	Egg *vs* Proto	0.02	-0.42	0.31	0.41
	Egg-Adult *vs* Early Fecundity	-0.6	-0.79	-0.26	1
	Egg-Adult *vs* Egg Length	0.18	-0.17	0.53	0.83

	Egg-Adult *vs* Egg Width	0.22	-0.12	0.55	0.9
	Egg-Adult *vs* Fecundity	-0.36	-0.72	-0.05	0.98
	Egg-Adult *vs* Longevity	-0.04	-0.53	0.38	0.63
	Egg-Adult *vs* Ovi. Time	-0.05	-0.47	0.34	0.63
	Egg-Adult *vs* Post Ovi.	-0.45	-0.75	-0.01	0.96
	Egg-Adult *vs* Pre Ovi.	0.68	0.43	0.85	1
	Fec. *vs* Egg Length	-0.18	-0.52	0.28	0.76
	Fec. *vs* Longevity	0.36	-0.18	0.65	0.86
	Fec. *vs* Ovi. Time	0.34	0	0.67	0.97
	Fecundity *vs* Egg Width	-0.15	-0.57	0.18	0.85
	Fecundity *vs* Post Ovi.	0.04	-0.37	0.53	0.64
	Fecundity *vs* Pre Ovi.	-0.54	-0.78	-0.18	1
	Larva *vs* Deuto.	0.53	0.11	0.72	0.99
	Larva *vs* Early Fec.	-0.37	-0.67	0.03	0.96
	Larva *vs* Egg Length	0.2	-0.27	0.48	0.73
	Larva *vs* Egg Width	0.1	-0.28	0.48	0.68
	Larva *vs* Egg-Adult	0.42	0.12	0.72	0.99
	Larva *vs* Fec.	-0.17	-0.5	0.25	0.74
	Larva *vs* Longevity	-0.08	-0.43	0.45	0.49
	Larva *vs* Ovi. Time	0.04	-0.35	0.42	0.55
	Larva *vs* Post Ovi.	-0.2	-0.53	0.35	0.71
	Larva *vs* Pre Ovi.	0.51	0.03	0.71	0.97
	Larva *vs* Proto	0.32	-0.21	0.53	0.83
	Longevity *vs* Egg Length	0.07	-0.42	0.49	0.55
	Longevity *vs* Egg Width	-0.28	-0.64	0.25	0.82
	Ovi. Time *vs* Post Ovi.	0	-0.46	0.42	0.56
	Ovi. Time *vs* Egg Length	-0.03	-0.49	0.29	0.7
	Ovi. Time *vs* Egg Width	-0.4	-0.66	0.09	0.92
	Ovi. Time *vs* Longevity	0.28	-0.14	0.68	0.89
	Post. Ovi. *vs* Egg Length	-0.14	-0.59	0.29	0.74
	Post. Ovi. *vs* Egg Width	-0.25	-0.65	0.22	0.83
	Post. Ovi. *vs* Longevity	0.46	0.08	0.77	0.98
	Pre Ovi. *vs* Ovi. Time	-0.3	-0.61	0.17	0.87
	Pre Ovi. *vs* Post Ovi.	-0.26	-0.59	0.28	0.75
	Pre. Ovi. *vs* Egg Length	0.13	-0.3	0.48	0.63
	Pre. Ovi. *vs* Egg Width	0.21	-0.21	0.54	0.8
	Pre. Ovi. *vs* Longevity	-0.04	-0.46	0.43	0.53
	Proto. *vs* Deuto.	0.62	0.38	0.83	1
	Proto. *vs* Early Fecundity	-0.51	-0.71	-0.05	0.99

	Proto. *vs* Egg Length	0.17	-0.23	0.49	0.78
	Proto. *vs* Egg Width	0.24	-0.18	0.53	0.86
	Proto. *vs* Egg-Adult	0.75	0.57	0.89	1
	Proto. *vs* Fecundity	-0.08	-0.54	0.21	0.8
	Proto. *vs* Longevity	-0.09	-0.55	0.33	0.66
	Proto. *vs* Ovi. Time	0.09	-0.35	0.45	0.61
	Proto. *vs* Post Ovi.	-0.15	-0.58	0.3	0.75
	Proto. *vs* Pre Ovi.	0.45	0.12	0.74	0.99
Morphological	D.S. Length *vs* D.S. Width	0.26	-0.17	0.53	0.85
Behavioural*	Activity *vs* Walking speed	0.44	0.08	0.7	0.99
	Dist. Travelled *vs* Activity	0.43	-0.04	0.67	0.94
	Dist. Travelled *vs* Walking speed	0.57	0.15	0.75	0.99
Between trait types	Deuto. *vs* Activity	0.04	-0.31	0.49	0.66
	Deuto. *vs* Dist. Travelled	0.25	-0.19	0.56	0.86
	Deuto. *vs* D.S. Length	0.33	-0.05	0.63	0.95
	Deuto. *vs* D.S. Width	-0.2	-0.55	0.18	0.84
	Deuto. *vs* Walking speed	0.14	-0.24	0.52	0.77
	D.S. Length *vs* Activity	0.22	-0.23	0.56	0.8
	D.S. Length *vs* Dist. Travelled	0.2	-0.16	0.63	0.87
	D.S. Length *vs* Walking speed	0.3	-0.23	0.57	0.82
	D.S. Width *vs* Activity	0.28	-0.22	0.6	0.83
	D.S. Width *vs* Dist. Travelled	0.15	-0.27	0.54	0.75
	D.S. Width *vs* Walking speed	0.23	-0.28	0.55	0.76
	Early Fec. *vs* Activity	0.04	-0.39	0.43	0.54
	Early Fec. *vs* Dist. Travelled	-0.07	-0.45	0.36	0.61
	Early Fec. *vs* D.S. Length	-0.39	-0.61	0.12	0.91
	Early Fec. *vs* D.S. Width	0.16	-0.31	0.5	0.71
	Early Fec. *vs* Walking speed	0.15	-0.38	0.45	0.56
	Egg Length *vs* Activity	0.06	-0.32	0.49	0.67
	Egg Length vs Dist. Travelled	-0.05	-0.41	0.4	0.53
	Egg Length *vs* D.S. Length	0.39	0.07	0.68	0.99
	Egg Length *vs* D.S. Width	0.05	-0.3	0.44	0.66
	Egg Length *vs* Walking speed	0.07	-0.39	0.42	0.53
	Egg *vs* Activity	-0.09	-0.5	0.27	0.67
	Egg *vs* Dist. Travelled	0.02	-0.42	0.38	0.47
	Egg *vs* D.S. Length	-0.05	-0.42	0.38	0.51
	Egg *vs* D.S. Width	-0.09	-0.53	0.26	0.74
	Egg *vs* Walking speed	-0.06	-0.49	0.31	0.67

	Egg Width *vs* Activity	0.15	-0.28	0.52	0.73
	Egg Width *vs* Dist. Travelled	0.19	-0.26	0.52	0.74
	Egg Width *vs* D.S. Length	0.48	0.13	0.72	0.99
	Egg Width *vs* D.S. Width	0.1	-0.28	0.47	0.64
	Egg Width *vs* Walking speed	0.07	-0.35	0.47	0.62
	Egg-Adult *vs* Activity	-0.04	-0.35	0.49	0.38
	Egg-Adult *vs* Dist. Travelled	0.38	-0.06	0.67	0.93
	Egg-Adult *vs* D.S. Length	0.31	-0.09	0.58	0.91
	Egg-Adult *vs* D.S. Width	-0.28	-0.58	0.12	0.91
	Egg-Adult *vs* Walking speed	0.22	-0.2	0.56	0.82
	Fec. *vs* Dist. Travelled	0.07	-0.39	0.42	0.55
	Fec. *vs* D.S. Length	-0.4	-0.65	0.12	0.9
	Fec. *vs* D.S. Width	0.15	-0.32	0.48	0.65
	Fec. *vs* Walking speed	0.14	-0.32	0.49	0.69
	Fecundity *vs* Activity	0.06	-0.38	0.4	0.54
	Larva *vs* Activity	0.04	-0.37	0.43	0.57
	Larva *vs* Dist. Travelled	0.2	-0.27	0.51	0.75
	Larva *vs* D.S. Length	-0.12	-0.5	0.3	0.64
	Larva *vs* D.S. Width	-0.04	-0.47	0.33	0.61
	Larva *vs* Walking speed	0.01	-0.27	0.49	0.69
	Longevity *vs* Activity	-0.07	-0.44	0.48	0.49
	Longevity *vs* Dist. Travelled	-0.05	-0.5	0.4	0.58
	Longevity *vs* D.S. Length	-0.06	-0.53	0.38	0.61
	Longevity *vs* D.S. Width	-0.12	-0.54	0.36	0.63
	Longevity *vs* Walking speed	-0.18	-0.51	0.4	0.6
	Ovi. Time *vs* Dist. Travelled	-0.12	-0.48	0.31	0.65
	Ovi. Time *vs* Walking speed	-0.01	-0.41	0.4	0.5
	Ovi. Time *vs* Activity	-0.11	-0.43	0.35	0.55
	Ovi. Time *vs* D.S. Length	-0.45	-0.71	0	0.96
	Ovi. Time *vs* D.S. Width	-0.11	-0.51	0.32	0.68
	Post Ovi. *vs* Activity	-0.08	-0.48	0.43	0.55
	Post Ovi. *vs* Dist. Travelled	-0.32	-0.65	0.23	0.8
	Post. Ovi. *vs* D.S. Length	-0.24	-0.62	0.27	0.82
	Post. Ovi. *vs* D.S. Width	0.19	-0.44	0.47	0.56
	Post. Ovi. *vs* Walking speed	-0.19	-0.57	0.32	0.71
	Pre. Ovi. *vs* Activity	0.14	-0.42	0.42	0.57
	Pre. Ovi. *vs* Dist. Travelled	0.17	-0.28	0.52	0.75
	Pre. Ovi. *vs* D.S. Length	0.18	-0.2	0.56	0.82
	Pre. Ovi. *vs* D.S. Width	-0.13	-0.52	0.24	0.76

	Pre. Ovi. *vs* Walking speed	-0.01	-0.41	0.43	0.49
	Proto *vs* Activity	-0.02	-0.39	0.41	0.47
	Proto *vs* Dist. Travelled	0.22	-0.17	0.59	0.86
	Proto *vs* D.S. Length	0.28	-0.15	0.55	0.88
	Proto *vs* D.S. Width	-0.21	-0.52	0.23	0.78
	Proto *vs* Walking speed	0.16	-0.29	0.51	0.74

* Behavioural traits were corrected for the number of recordings by including this variable as a covariate in the models.

Genetic correlations between traits measured in the same female individuals as estimated from variance components in a MCMCglmm model of both commercial and wild populations. R_g = Genetic correlation, LCI =lower credible interval, UCI = Upper credible interval, PP = Posterior probabilities (the probability that the hypothesis of a non-zero correlation is supported; only values close to one support the hypothesis). Further support is given when credible intervals do no overlap zero.

Male genetic correlations

Type	Traits	R_g	LCI	UCI	PP
Life history	Deuto. *vs* Egg Length	0.04	-0.42	0.44	0.5
	Deuto. *vs* Egg Width	0.05	-0.41	0.43	0.52
	Deuto. *vs* Egg-Adult	0.62	0.26	0.81	1
	Egg *vs* Deuto.	0.36	-0.1	0.64	0.93
	Egg *vs* Egg Length	0.22	-0.2	0.66	0.79
	Egg *vs* Egg Width	0.4	-0.04	0.71	0.95
	Egg *vs* Egg-Adult	0.59	0.13	0.78	0.99
	Egg *vs* Larva	0.06	-0.43	0.4	0.47
	Egg *vs* Proto.	-0.02	-0.47	0.35	0.63
	Egg-Adult *vs* Egg Length	0.01	-0.39	0.44	0.54
	Egg-Adult *vs* Egg Width	0.09	-0.33	0.46	0.65
	Egg Length *vs* Egg Width	0.66	0.39	0.82	1
	Larva *vs* Deuto.	-0.15	-0.54	0.3	0.67
	Larva *vs* Egg Length	-0.16	-0.57	0.29	0.74
	Larva *vs* Egg Width	-0.2	-0.61	0.2	0.84
	Larva *vs* Egg-Adult	0.07	-0.36	0.48	0.58
	Larva *vs* Proto.	0.03	-0.52	0.32	0.33
	Proto *vs* Deuto.	0.05	-0.32	0.51	0.67
	Proto. *vs* Egg Length	-0.03	-0.48	0.4	0.51
	Proto. *vs* Egg Width	-0.02	-0.47	0.42	0.56
	Proto. *vs* Egg-Adult	0.4	0.02	0.71	0.97

Genetic correlations as estimated from isoline means of both commercial and wild populations, calculated by isoline means. R_g= correlation coefficient, LCI=lower credible interval, UCI=Upper credible interval, PP= Posterior probabilities (the probability that the hypothesis of a non-zero correlation is supported; only values close to 1 support the hypothesis). Further support is given when credible intervals do not overlap zero.

Type	Traits	Rg	LCI	UCI	PP
Life history	Egg Length *vs* Egg Width	0.72	0.67	0.73	1
	Egg *vs* Egg-Adult	0.29	0.17	0.32	1
	Egg *vs* Larva	-0.21	-0.29	-0.15	1
	Egg *vs* Proto.	-0.18	-0.23	-0.08	1
	Larva *vs* Deuto.	0.10	0.09	0.23	1
	Larva *vs* Egg-Adult	0.25	0.14	0.28	1
	Larva *vs* Proto.	-0.32	-0.37	-0.23	1
	Proto. *vs* Egg-Adult	0.36	0.29	0.44	1
	Proto. *vs* Deuto.	-0.07	-0.19	-0.03	1
	Deuto. *vs* Egg-Adult	0.80	0.66	0.80	1
	Early Fec. *vs* Fec.	0.45	0.43	0.56	1
	Fec. *vs* Ovi. Time	0.86	0.84	0.88	1
	Early Fec. *vs* Pre-Ovi.	-0.07	-0.22	-0.04	1
	Early Fec. *vs* Post-Ovi.	0.10	0.03	0.27	0.99
	Fec. *vs* Pre-Ovi.	-0.06	-0.19	-0.01	0.99
	Egg *vs* Egg Length	0.21	0.10	0.25	1
	Egg-Adult *vs* Early Fec.	-0.13	-0.26	-0.09	1
	Fec. *vs* Longevity	0.45	0.36	0.55	1
	Ovi. Time *vs* Longevity	0.47	0.39	0.57	1
	Post-Ovi. *vs* Longevity	0.77	0.75	0.84	1
	Proto. *vs* Pre-Ovi.	0.20	0.12	0.27	1
	Deuto. *vs* Early Fec.	-0.13	-0.25	-0.07	1
	Egg-Adult *vs* Pre-Ovi.	0.14	0.07	0.23	1
	Egg *vs* Egg Width	0.08	0.05	0.21	1
	Deuto. *vs* Fec.	-0.15	-0.20	-0.05	1
	Deuto. *vs* Egg Width	-0.14	-0.21	-0.03	1
	Egg-Adult *vs* Fec.	-0.10	-0.19	-0.03	1
	Ovi. Time *vs* Egg Width	-0.10	-0.20	-0.04	1
	Early Fec. *vs* Egg Length	-0.08	-0.22	-0.03	1
	Ovi. Time *vs* Egg Length	-0.10	-0.19	-0.03	1
	Longevity *vs* Egg Width	-0.16	-0.28	-0.03	0.99
	Larva *vs* Early Fec.	-0.11	-0.18	-0.01	0.99
	Proto. *vs* Post-Ovi.	-0.23	-0.26	-0.03	0.99
	Post-Ovi. *vs* Egg Length	-0.17	-0.28	-0.02	0.99

	Fec. *vs* Egg Length	-0.10	-0.18	-0.01	0.99
	Egg-Adult *vs* Post-Ovi.	-0.11	-0.24	0.00	0.98
	Longevity *vs* Egg Length	-0.05	-0.25	0.00	0.97
	Proto. *vs* Early Fec.	-0.01	-0.17	0.01	0.96
	Fec. *vs* Post-Ovi.	0.03	-0.02	0.22	0.96
	Post-Ovi. *vs* Egg Width	-0.13	-0.23	0.03	0.94
	Deuto. *vs* Egg Length	-0.09	-0.15	0.02	0.94
	Larva *vs* Longevity	0.07	-0.03	0.20	0.92
	Egg-Adult *vs* Egg Width	-0.07	-0.15	0.03	0.92
	Proto. *vs* Longevity	-0.11	-0.19	0.04	0.92
	Fec. *vs* Egg Width	-0.09	-0.14	0.03	0.91
	Deuto. *vs* Ovi. Time	-0.01	-0.13	0.03	0.91
	Egg *vs* Fec.	-0.04	-0.12	0.02	0.90
	Larva *vs* Egg Length	0.05	-0.03	0.13	0.87
	Proto. *vs* Ovi. Time	0.08	-0.04	0.12	0.84
	Early Fec. *vs* Longevity	-0.12	-0.18	0.07	0.84
	Pre-Ovi. *vs* Post-Ovi.	-0.10	-0.19	0.07	0.83
	Deuto. *vs* Longevity	0.08	-0.06	0.17	0.82
	Deuto. *vs* Pre-Ovi.	0.05	-0.04	0.13	0.80
	Early Fec. *vs* Ovi. Time	-0.06	-0.13	0.05	0.80
	Larva *vs* Ovi. Time	0.04	-0.04	0.11	0.78
	Larva *vs* Pre-Ovi.	0.04	-0.05	0.12	0.76
	Deuto. *vs* Post-Ovi.	-0.06	-0.16	0.08	0.75
	Egg *vs* Pre-Ovi.	0.02	-0.05	0.12	0.75
	Proto. *vs* Egg Length	-0.09	-0.12	0.05	0.75
	Proto. *vs* Egg Width	-0.05	-0.12	0.06	0.75
	Ovi. Time *vs* Post-Ovi.	0.01	-0.16	0.08	0.74
	Pre-Ovi. *vs* Ovi. Time	-0.01	-0.11	0.06	0.74
	Pre-Ovi. *vs* Egg Width	0.00	-0.06	0.13	0.73
	Egg *vs* Ovi. Time	-0.05	-0.10	0.05	0.73
	Proto. *vs* Fec.	-0.03	-0.09	0.06	0.69
	Egg *vs* Deuto.	0.05	-0.05	0.10	0.69
	Egg *vs* Early Fec.	-0.01	-0.10	0.06	0.66
	Larva *vs* Post-Ovi.	0.02	-0.13	0.10	0.65
	Egg *vs* Post-Ovi.	-0.06	-0.13	0.09	0.64
	Egg *vs* Longevity	-0.01	-0.13	0.10	0.58
	Early Fec. *vs* Egg Width	-0.04	-0.09	0.11	0.58
	Pre-Ovi. *vs* Egg Length	0.04	-0.09	0.10	0.55
	Larva *vs* Egg Width	-0.02	-0.09	0.08	0.55

	Egg-Adult *vs* Egg Length	0.07	-0.09	0.09	0.54
	Pre-Ovi. *vs* Longevity	0.01	-0.12	0.15	0.54
	Egg-Adult *vs* Longevity	0.00	-0.12	0.12	0.53
	Larva *vs* Fec.	0.02	-0.08	0.08	0.52
	Egg-Adult *vs* Ovi. Time	0.01	-0.08	0.08	0.51
Morphological	D.S. Length *vs* D.S. Width	0.00	-0.06	0.09	0.66
Behavioural	Activity *vs* Walking speed	0.71	0.68	0.76	1
	Dist. Travelled *vs* Activity	0.74	0.67	0.77	1
	Dist. Travelled *vs* Walking speed	0.85	0.82	0.88	1
Physiological	Desicc. Tolerance 35% *vs* Desicc. Tolerance 50%	0.70	0.26	0.73	1
	Desicc. Tolerance 35% *vs* Desicc. Tolerance 65	0.34	0.09	0.63	0.99
	Desicc. Tolerance 35% *vs* Pred. Rate	-0.08	-0.41	0.22	0.72
	Desicc. Tolerance 35% *vs* Starvation Tolerance	0.3	-0.09	0.49	0.91
	Desicc. Tolerance 50% *vs* Starvation Tolerance	0.57	0.10	0.63	0.99
	Desicc. Tolerance 65% *vs* Pred. Rate	-0.25	-0.47	0.22	0.78
	Desicc. Tolerance 65% *vs* Starvation Tolerance	0.56	0.16	0.67	1
	Desicc.50 *vs* Desicc. Tolerance 65%	0.70	0.53	0.85	1
	Desicc.50 *vs* Pred. Rate	-0.27	-0.45	0.19	0.79
Between trait type	Activity *vs* Desicc. Tolerance 35%	0.33	-0.18	0.42	0.78
	Activity *vs* Desicc. Tolerance 50%	-0.08	-0.25	0.37	0.62
	Activity *vs* Desicc. Tolerance 65%	-0.02	-0.25	0.36	0.66
	Activity *vs* Pred. Rate	-0.13	-0.43	0.18	0.77
	Activity *vs* Starvation tolerance	0.32	-0.09	0.50	0.91
	Deuto. *vs* Activity	0.02	-0.09	0.09	0.56
	Deuto. *vs* Desicc. Tolerance 35%	0.16	-0.40	0.22	0.70
	Deuto. *vs* Desicc. Tolerance 50%	0.44	-0.17	0.45	0.81
	Deuto. *vs* Desicc. Tolerance 65%	0.54	-0.04	0.57	0.95
	Deuto. *vs* Dist. Travelled	-0.02	-0.06	0.12	0.74
	Deuto. *vs* D.S. Length	0.09	0.01	0.18	0.98
	Deuto. *vs* D.S. Width	-0.06	-0.18	-0.01	0.98
	Deuto. *vs* Pred. Rate	-0.25	-0.57	0.01	0.96
	Deuto. *vs* Starvation tolerance	0.42	0.06	0.62	0.98
	Deuto. *vs* Walking speed	0.00	-0.09	0.09	0.51

	Dist. Travelled *vs* Desicc. Tolerance 35%	0.06	-0.35	0.30	0.55
	Dist. Travelled *vs* Desicc. Tolerance 50%	-0.18	-0.37	0.24	0.67
	Dist. Travelled *vs* Desicc. Tolerance 65%	-0.09	-0.35	0.28	0.58
	Dist. Travelled *vs* Pred. Rate	-0.15	-0.48	0.12	0.88
	Dist. Travelled *vs* Starvation tolerance	0.33	-0.12	0.48	0.88
	D.S. Length *vs* Activity	-0.05	-0.10	0.10	0.52
	D.S. Length *vs* Desicc. Tolerance 50%	-0.21	-0.45	0.17	0.83
	D.S. Length *vs* Desicc. Tolerance 65%	-0.09	-0.41	0.24	0.69
	D.S. Length *vs* Desicc. Tolerance 35%	-0.10	-0.42	0.23	0.72
	D.S. Length *vs* Dist. Travelled	0.00	-0.06	0.14	0.76
	D.S. Length *vs* Pred. Rate	-0.26	-0.43	0.22	0.78
	D.S. Length *vs* Starvation tolerance	0.06	-0.15	0.46	0.83
	D.S. Length *vs* Walking speed	0.08	-0.08	0.12	0.63
	D.S. Width *vs* Activity	0.11	-0.02	0.18	0.94
	D.S. Width *vs* Desicc. Tolerance 35%	0.16	-0.29	0.33	0.58
	D.S. Width *vs* Desicc. Tolerance 50%	-0.27	-0.45	0.16	0.82
	D.S. Width *vs* Desicc. Tolerance 65%	0.05	-0.37	0.22	0.70
	D.S. Width *vs* Dist. Travelled	0.14	0.00	0.20	0.98
	D.S. Width *vs* Pred. Rate	0.10	-0.20	0.41	0.76
	D.S. Width *vs* Starvation tolerance	-0.05	-0.24	0.38	0.70
	D.S. Width *vs* Walking speed	0.04	-0.02	0.18	0.94
	Early Fec. *vs* Activity	0.12	0.03	0.22	0.99
	Early Fec. *vs* Desicc. Tolerance 35%	-0.17	-0.48	0.13	0.85
	Early Fec. *vs* Desicc. Tolerance 65%	-0.68	-0.74	-0.15	1
	Early Fec. *vs* Dist. Travelled	0.02	-0.03	0.17	0.92
	Early Fec. *vs* D.S. Length	-0.07	-0.14	0.05	0.84
	Early Fec. *vs* D.S. Width	-0.03	-0.09	0.10	0.58
	Early Fec. *vs* Pred. Rate	0.25	-0.08	0.52	0.92
	Early Fec. *vs* Starvation tolerance	-0.33	-0.57	-0.02	0.98
	Early Fec. *vs* Walking speed	0.13	0.04	0.23	1
	Early Fec. *vs* Desicc. Tolerance 50%	-0.24	-0.60	-0.01	0.97
	Egg Length *vs* Activity	-0.13	-0.21	-0.01	0.99
	Egg Length *vs* Desicc. Tolerance 35%	-0.01	-0.32	0.30	0.50
	Egg Length *vs* Desicc. Tolerance 50%	-0.14	-0.39	0.23	0.69
	Egg Length *vs* Desicc. Tolerance 65%	-0.17	-0.31	0.34	0.53
	Egg Length *vs* Dist. Travelled	-0.15	-0.16	0.04	0.88
	Egg Length *vs* D.S. Length	0.33	0.28	0.42	1
	Egg Length *vs* D.S. Width	0.20	0.14	0.29	1
	Egg Length *vs* Pred. Rate	-0.03	-0.26	0.34	0.61
	Egg Length *vs* Starvation tolerance	0.24	-0.10	0.51	0.91

	Egg Length *vs* Walking speed	-0.12	-0.19	0.00	0.98
	Egg *vs* Activity	-0.03	-0.12	0.05	0.81
	Egg *vs* Desicc. Tolerance 50%	-0.43	-0.57	-0.01	0.98
	Egg *vs* Desicc. Tolerance 35%	-0.15	-0.47	0.13	0.86
	Egg *vs* Desicc. Tolerance 65%	-0.33	-0.57	0.00	0.96
	Egg *vs* Dist. Travelled	-0.03	-0.07	0.10	0.60
	Egg *vs* D.S. Length	0.03	-0.05	0.12	0.80
	Egg *vs* D.S. Width	-0.06	-0.14	0.03	0.90
	Egg *vs* Pred. Rate	-0.08	-0.29	0.34	0.58
	Egg *vs* Starvation tolerance	-0.26	-0.49	0.09	0.91
	Egg *vs* Walking speed	0.04	-0.11	0.06	0.74
	Egg Width *vs* Activity	-0.01	-0.15	0.05	0.85
	Egg Width *vs* Desicc. Tolerance 35%	0.09	-0.40	0.22	0.71
	Egg Width *vs* Desicc. Tolerance 50%	-0.42	-0.56	0.00	0.97
	Egg Width *vs* Desicc. Tolerance 65%	-0.34	-0.51	0.11	0.87
	Egg Width *vs* Dist. Travelled	0.05	-0.11	0.09	0.59
	Egg Width *vs* D.S. Length	0.30	0.19	0.34	1
	Egg Width *vs* D.S. Width	0.28	0.19	0.33	1
	Egg Width *vs* Pred. Rate	-0.22	-0.36	0.24	0.67
	Egg Width *vs* Starvation tolerance	-0.07	-0.17	0.48	0.81
	Egg Width *vs* Walking speed	-0.11	-0.14	0.05	0.82
	Egg-Adult *vs* Activity	-0.04	-0.11	0.08	0.60
	Egg-Adult *vs* Dist. Travelled	0.00	-0.07	0.11	0.66
	Egg-Adult *vs* D.S. Length	0.12	0.02	0.20	0.99
	Egg-Adult *vs* D.S. Width	-0.15	-0.20	-0.02	0.99
	Egg-Adult *vs* Walking speed	0.00	-0.11	0.08	0.58
	Egg.Adult *vs* Desicc. Tolerance 35%	-0.27	-0.43	0.20	0.76
	Egg.Adult *vs* Desicc. Tolerance 50%	0.01	-0.24	0.40	0.70
	Egg.Adult *vs* Desicc. Tolerance 65%	0.34	-0.09	0.55	0.91
	Egg.Adult *vs* Pred. Rate	-0.39	-0.64	-0.05	0.98
	Egg.Adult *vs* Starvation tolerance	0.46	-0.02	0.58	0.96
	Fec. *vs* Activity	0.09	-0.01	0.17	0.96
	Fec. *vs* Desicc. Tolerance 35%	-0.25	-0.39	0.21	0.74
	Fec. *vs* Desicc. Tolerance 50%	-0.19	-0.31	0.33	0.50
	Fec. *vs* Desicc. Tolerance 65%	-0.11	-0.46	0.21	0.78
	Fec. *vs* Dist. Travelled	0.15	0.07	0.25	1
	Fec. *vs* D.S. Length	-0.09	-0.15	0.02	0.92
	Fec. *vs* D.S. Width	0.05	0.01	0.18	0.98
	Fec. *vs* Pred. Rate	0.25	-0.14	0.50	0.86
	Fec. *vs* Starvation tolerance	-0.39	-0.60	-0.07	0.99
	Fec. *vs* Walking speed	0.16	0.06	0.23	1
	Larva *vs* Activity	-0.06	-0.16	0.01	0.96
	Larva *vs* Desicc. Tolerance 35%	0.10	-0.43	0.18	0.77

	Larva *vs* Desicc. Tolerance 50%	0.04	-0.30	0.32	0.52
	Larva *vs* Desicc. Tolerance 65%	-0.02	-0.26	0.35	0.64
	Larva *vs* Dist. Travelled	-0.05	-0.19	-0.02	0.99
	Larva *vs* D.S. Length	0.06	-0.01	0.16	0.96
	Larva *vs* D.S. Width	0.00	-0.09	0.08	0.54
	Larva *vs* Pred. Rate	-0.06	-0.56	0.04	0.94
	Larva *vs* Starvation tolerance	0.31	0.03	0.59	0.98
	Larva *vs* Walking speed	-0.11	-0.15	0.02	0.94
	Longevity *vs* Activity	0.07	-0.05	0.22	0.90
	Longevity *vs* Desicc. Tolerance 35%	-0.02	-0.36	0.27	0.66
	Longevity *vs* Desicc. Tolerance 50%	0.60	0.17	0.68	1
	Longevity *vs* Desicc. Tolerance 65%	0.23	0.16	0.67	1
	Longevity *vs* Dist. Travelled	0.12	-0.02	0.24	0.95
	Longevity *vs* D.S. Length	-0.12	-0.26	-0.02	0.99
	Longevity *vs* D.S. Width	-0.08	-0.18	0.06	0.85
	Longevity *vs* Pred. Rate	0.03	-0.27	0.35	0.59
	Longevity *vs* Starvation tolerance	-0.06	-0.37	0.26	0.63
	Longevity *vs* Walking speed	0.25	0.05	0.31	1
	Ovi. Time *vs* Activity	0.05	-0.03	0.15	0.91
	Ovi. Time *vs* Desicc. Tolerance 35%	0.00	-0.25	0.37	0.65
	Ovi. Time *vs* Desicc. Tolerance 50%	-0.15	-0.19	0.41	0.75
	Ovi. Time *vs* Desicc. Tolerance 65%	0.09	-0.26	0.37	0.64
	Ovi. Time *vs* Dist. Travelled	0.11	0.03	0.22	1
	Ovi. Time *vs* D.S. Length	-0.07	-0.14	0.03	0.89
	Ovi. Time *vs* D.S. Width	-0.05	-0.10	0.08	0.54
	Ovi. Time *vs* Pred. Rate	-0.03	-0.27	0.36	0.58
	Ovi. Time *vs* Starvation tolerance	-0.44	-0.56	0.00	0.98
	Ovi. Time *vs* Walking speed	0.18	0.02	0.19	1
	Post-Ovi. *vs* Activity	0.01	-0.12	0.17	0.67
	Post-Ovi. *vs* Desicc. Tolerance 35%	-0.22	-0.42	0.22	0.71
	Post-Ovi. *vs* Desicc. Tolerance 50%	0.20	-0.02	0.55	0.96
	Post-Ovi. *vs* Desicc. Tolerance 65%	0.27	-0.08	0.52	0.92
	Post-Ovi. *vs* Dist. Travelled	0.08	-0.08	0.20	0.81
	Post-Ovi. *vs* D.S. Length	-0.20	-0.28	-0.03	0.99
	Post-Ovi. *vs* D.S. Width	0.01	-0.11	0.14	0.61
	Post-Ovi. *vs* Pred. Rate	0.28	-0.22	0.39	0.68
	Post-Ovi. *vs* Starvation tolerance	-0.07	-0.40	0.22	0.77
	Post-Ovi. *vs* Walking speed	0.24	0.00	0.27	0.98
	Pre-Ovi. *vs* Activity	-0.06	-0.15	0.05	0.84
	Pre-Ovi. *vs* Desicc. Tolerance 35%	-0.10	-0.25	0.41	0.69
	Pre-Ovi. *vs* Desicc. Tolerance 50%	0.35	-0.13	0.53	0.91
	Pre-Ovi. *vs* Desicc. Tolerance 65%	0.25	-0.11	0.53	0.91
	Pre-Ovi. *vs* Dist. Travelled	-0.01	-0.11	0.08	0.62

	Pre-Ovi. *vs* D.S. Length	0.03	-0.11	0.09	0.58
	Pre-Ovi. *vs* D.S. Width	-0.20	-0.28	-0.08	1
	Pre-Ovi. *vs* Pred. Rate	-0.37	-0.58	0.04	0.95
	Pre-Ovi. *vs* Starvation tolerance	0.32	-0.14	0.50	0.87
	Pre-Ovi. *vs* Walking speed	-0.04	-0.10	0.09	0.55
	Proto. *vs* Activity	0.05	-0.04	0.14	0.84
	Proto. *vs* Desicc. Tolerance 35%	0.21	-0.38	0.23	0.69
	Proto. *vs* Desicc. Tolerance 50%	0.30	-0.22	0.39	0.69
	Proto. *vs* Desicc. Tolerance 65%	0.28	-0.05	0.54	0.94
	Proto. *vs* Dist. Travelled	0.11	0.00	0.17	0.97
	Proto. *vs* D.S. Length	0.00	-0.10	0.08	0.59
	Proto. *vs* D.S. Width	-0.03	-0.16	0.02	0.93
	Proto. *vs* Pred. Rate	-0.33	-0.61	-0.06	0.99
	Proto. *vs* Starvation tolerance	0.22	-0.08	0.52	0.92
	Proto. *vs* Walking speed	0.03	-0.03	0.14	0.90
	Starvation Tolerance *vs* Pred. Rate	-0.32	-0.5	0.08	0.92
	Walking speed *vs* Desicc. Tolerance 35%	-0.23	-0.44	0.17	0.82
	Walking speed *vs* Desicc. Tolerance 50%	-0.17	-0.37	0.25	0.67
	Walking speed *vs* Desicc. Tolerance 65%	-0.12	-0.36	0.26	0.66
	Walking speed *vs* Pred. Rate	-0.01	-0.39	0.23	0.66
	Walking speed *vs* Starvation tolerance	0.15	-0.16	0.44	0.82