國立臺灣大學生命科學院生態學與演化生物學研究所

碩士論文

Institute of Ecology and Evolutionary Biology
College of Life Science
National Taiwan University

Master Thesis

溫度與天敵壓力之互動透過跨世代可塑性對被掠食者之影響

Temperature and predation stressors interactively shape response of prey via transgenerational plasticity

邱楉蒔

JO-SHIH Chiu

指導教授：何傳愷 博士

Advisor: CHUAN-KAI Ho, Ph.D.

中華民國 111 年 8 月

August, 2022

doi:10.6342/NTU202204043

誌謝

首先要感謝我的指導教授何傳愷博士，老師對於治學的嚴謹要求令我獲益匪淺，日後無論從事學術或任何領域，都是大有助益。其次要感謝孫烜駿博士，他在提出研究問題、形成研究架構與檢視研究結果上都給予我莫大幫助，一起做實驗的過程也十分愉快。再來要感謝實驗室的每一位成員，包括正鴻、智升、亞其、明陽、薰逸、暐鈞、季恆以及舒平等，你們在研究過程中給予的支持與協助令我銘感於心。最後，感謝李承叡博士在內共生菌實驗過程的許多協助，張智函博士提供的實驗空間資源，以及行政院農委會高雄農業改良場的周國隆博士所提供的實驗用大豆。

doi:10.6342/NTU202204043

中文摘要

在個體適應環境變遷的過程中，生物性與非生物性壓力扮演了重要的角色；過去壓力所引發的反應，可能透過跨世代的可塑性進而影響個體對新環境的適應性，最後影響所在的生態系統。過去雖有相關研究，但多為針對單一壓力對個體的影響，或僅觀察少數世代的可塑性，缺乏探討在多個壓力交互作用下的個體反應，以及在經歷多個世代後個體的表現。為回答以上問題，本研究利用跨世代實驗，檢視大豆蚜在暖化和／或天敵壓力下的可塑性反應，以及該反應是否能持續作用並影響大豆蚜對新環境的適應。本研究使用一隻雌性蚜蟲進行無性生殖，以產生實驗用的蚜蟲族群，並從中取 30 隻四齡蚜蟲置於大豆植株，使其接受以下其中一種實驗處理：控制組、暖化組（+2 ˚C）、天敵組（加入一隻六條瓢蟲）、暖化+天敵組。七天後（以此為一世代），本研究將 30 隻四齡蚜蟲移至新的大豆上，並使其接受原處理。16 世代後，本研究進行 4x4 交叉試驗，以了解跨世代可塑性是否可維持效果並影響蚜蟲對後續壓力的反應。跨世代實驗結果顯示，暖化會讓蚜蟲體型下降，但大約從第 10 世代起便停止下降。瓢蟲的存在會使蚜蟲族群數量下降，但僅在同時接受暖化處理的情況下於後期停止下降，並有回升趨勢，顯示在多世代的觀察下，暖化與天敵壓力會透過交互作用進而影響蚜蟲。交叉試驗結果顯示，過去曾長期接受天敵處理的蚜蟲具有適應天敵的性狀，但只有在同時接受暖化處理的情況下才有此現象；此結果顯示跨世代可塑性可以延續並影響蚜蟲對後續壓力的反應，但此效果需視壓力種類而定。綜合以上結果，本研究彰顯出生物性與非生物性等多個壓力因子之交互作用會顯著地影響物種的跨世代可塑性，且過去經驗與新環境之間的交互作用亦會影響個體的性狀表現。本研究建議後續關於相關機制的探討，以幫助精確地預測個體對環境變動的適應性。

關鍵字：暖化、天敵、跨世代可塑性、蚜蟲、瓢蟲

doi:10.6342/NTU202204043

ABSTRACT

Stressor plays an important role in driving organisms' adaptation to the changing world. Stress-induced effects may affect organisms' responses to future stressors through transgenerational plasticity and lead to significant impact on ecosystems. However, it remains unclear how multiple stressors may interact and whether the effect of previous stressors will affect that of current stressors. To fill these knowledge gaps, this study investigated evolutionary responses of soybean aphids to warming temperature and predation stressors using an experimental evolution approach. A single founder aphid was used to form the stock through clonal multiplication, from which 30 fourth instar aphids were randomly collected and introduced to a soybean plant, then assigned to one of the four treatments: control, warming (+2 °C), predation (one adult lady beetle), and warming plus predation. After seven days (~ one generation), 30 fourth instar aphids from each plant were collected and transferred to a new plant, and submitted to the same treatment to which they had been exposed. The process was repeated for 16 generations, followed by a reciprocal transplant experiment with 4x4 full factorial design for three generations to test if transgenerational plasticity persists and thus mediates aphids' responses to future stressors. We found that under warming temperature, whether predators were present or not, aphid body size reduced over generations and reached stabilization at around 10^{th} generation onwards. The presence of ladybeetles reduced aphid population size over generations, but at warming conditions, such reduction became less obvious at later stage, suggesting an interaction between abiotic (temperature) and biotic (predation) stressors over generations. Aphids under consistent predation pressure over generations showed a plastic adaption when

doi:10.6342/NTU202204043

they were exposed to predators later in the reciprocal transplant experiment, but only when also exposed to warming at the same time. This suggested that transgenerational plasticity may persist but the effect may depend on future stressor. Overall, the results highlight the important effect of stressor interaction on species' transgenerational plasticity, as well as the interactive effect between previous and current stressors. Further investigations on the underlying mechanisms should help us better forecast organisms' adaptiveness to changing environment.

Keywords: Warming; Predator; Transgenerational plasticity; Aphid; Lady beetle

doi:10.6342/NTU202204043

CONTENTS

doi:10.6342/NTU202204043

LIST OF FIGURES

doi:10.6342/NTU202204043

doi:10.6342/NTU202204043

LIST OF TABLES

doi:10.6342/NTU202204043

Chapter 1 Introduction

Ecosystems regularly experience many stressors, which can mediate the effects of each other through complex interactions and result in combined effects on ecosystems [1]. While many studies have examined either abiotic (e.g., climate warming) or biotic (e.g., predation) stressors, a growing body of research shows that abiotic and biotic stressors can interactively mediate ecological and evolutionary responses. For example, it is found that increased temperature enhanced predator pressure on snails, such that growth efficiency of snails became negative when they were exposed to both predation and warming temperature [2]; warming can enhance predator pressure, leading to reduced coexistence between two Collembola species in warming environment [3]. On the other hand, daphnia reared at warmer temperature evolved higher growth rates when reared with predators at the same time [4]. Therefore, a multistress approach is required to build a more holistic and realistic picture of how ecosystems are impacted by stressors, especially under global change scenarios [5, 6], where natural populations struggle to cope with rapidly changing environments.

In response to environmental changes, species develop phenotypic plasticity, which is the capacity of an individual genotype to generate different phenotypes, and can be expressed as variation in biochemistry, physiology, morphology, behavior, or life history [7]. Empirical studies suggest that rapid adaptation to environmental changes can happen if there is sufficient standing genetic variation, and/or phenotypic plasticity to give fast responses [8]. While phenotypic plasticity is a non-genetic variation that can enhance the chances of species to survive and allow them to adapt rapidly to changing environment, it also serves to enhance adaptive genetic responses by mechanisms such

doi:10.6342/NTU202204043

as genetic assimilation [9]. As a major mechanism of organisms' response to environmental variations [10], there has been growing interest in studying the role of phenotypic plasticity on ecological and evolutionary process [11], which is required to better predict organisms' potential of rapid adaptation to the ongoing climate change.

Transgenerational plasticity—plasticity that persists across generations—reflects parents' environmental effects on body size, population growth, phenology, etc. of offspring [12, 13]. Studies show that transgenerational plasticity is effective in helping organisms cope with rapidly changing environment, and is found in many species that experience various stressors [14, 15]. Understanding species' responses in a longer time scale in terms of transgenerational plasticity is specifically important in climate change contexts, because environmental variability can last for multiple generations in many species [16]. However, the majority of previous studies have focused on species' responses within a generation, or across only a few generations [17-20], which may limit the possibility of detecting adaptive responses due to insufficient time of observation [21]. Although there are studies looking into long-term effects on species with short generation time, such as bacteria [22, 23], long-term studies on multicellular species such as vertebrates or arthropods are limited [24]. Furthermore, how species response to multiple stressors over multiple generations remains to be revealed. Many studies have focused on a single stressor [17], overlooking the fact that most species are faced with multiple stressors simultaneously. Looking into how stressors interactively act on species for multiple generations can help render a more realistic prediction on species' adaptive responses to changing environment and/or future stressors.

Studying transgenerational plasticity under multiple stressors will help clarify not only how species adapt to the past and current environment, but also how species respond to novel or repeated environmental events in the future, however, this type of

doi:10.6342/NTU202204043

studies is scarce. There are many studies, although focusing on single stressors or only a few generations, suggest that previous exposure to stressors may affect species' future responses in new environment. For example, previous exposure of *Escherichia coli* to warmer temperature enhanced its fitness at the same temperature [25], and growing *Arabidopsis thaliana* in warming environment led to great improvement in seed production in offspring submitted to the same warming environment [26]. However, acclimation of sticklebacks to high-CO_2 environment had negative effects on the survivorship of their offspring under the same environment [27]. Although the mixed results suggest that previous exposure to stressors is not always beneficial to organisms through plasticity [28], they raise a need to empirically investigate the effect of focal environmental stressors on species at present and in the future.

To understand the role of transgenerational plasticity in species adaptation to multiple stressors, this study asked the following questions: 1) how species plastically respond to abiotic (warmer temperature) and/or biotic (predation) stressors through multiple generations, and 2) whether these stress-induced effects persist through generations and consequently affect species response to future stressors (a previously exposed stressor, or a novel stressor). To address the first question, we conducted a 16-generation experimental selection with 2x2 factorial design, including temperature as abiotic stressor (control and +2℃ warming temperature), and predation as biotic stressor (presence and absence of predators), using a major pest in the world – soybean aphid (*Aphis glycines*) as the prey. By using a single soybean aphid to form the entire experimental stock, this study avoided initial standing genetic variation [29], allowing us to monitor aphids' phenotypic variations across generations with minimized chance of these variations being contributed to the occurrence of genetic change. To address the second question, we conducted a reciprocal transplant experiment [30] to test for the

doi:10.6342/NTU202204043

persistence of plastic responses after constant exposure to stressors during the experimental selection. Aphid population size and body size were monitored as plastic responses throughout the two experiments, and aphid life history traits were recorded before and after the experimental selection to see if exposure to stressors has changed aphids' life history. We expected to see that temperature and predation act interactively on aphid body size, population size and life history traits, and that the combined effect of temperature and predation is larger than their additive effects when traits respond to the two stressors in the same direction.

doi:10.6342/NTU202204043

Chapter 2 Methods

2.1 Study system

To investigate transgenerational plastic responses to warming and predation stressors, we used a three-level food chain composed of soybean plant *Glycine max* cv. Kaohsiung No. 9, soybean aphid *Aphis glycines and ladybeetle Cheilomenes sexmaculata*. Since *A. glycines* is one of the major pests of soybean plants, this study system also provides insights into the role of species plasticity in pest management, especially in climate warming scenarios. To do so, we cultivated soybean seeds from Kaohsiung District Agricultural Research and Extension Station, and kept the potted soybean plants in growth chambers at control temperature (simulating daily temperature fluctuation ranging from 26.0 to 30.8 °C, average 28.0 °C), 60% relative humidity and under a 12L:12D photoperiod. Soybean plants at vegetative stage V1 – V2 (3 to 4 weeks old) were then used both in experimental selection and reciprocal transplant experiments. The aphid system has been an ideal model to study phenotypic plasticity [31]. Under certain environmental conditions, aphids can reproduce through apomictic parthenogenesis over multiple generations, producing populations that are genetically identical. Using aphid populations produced by a single founder aphid allowed us to investigate organism performance in response to environmental stressors through transgenerational plasticity. To establish the stock, we collected soybean aphids from three farms (soybean farms in Taipei, Tainan, and the National Taiwan University Experimental Farm) and then raised them on soybean plants in mesh cages in growth chambers that simulate field conditions at 17.1 – 22.5 °C, 60% relative humidity and under a 12L:12D photoperiod, with which only viviparous parthenogenic offspring were

doi:10.6342/NTU202204043

produced. The ladybeetle *C. sexmaculata*, which is commonly found in Taiwan, has been used as natural enemy for soybean aphid control. We collected these ladybeetles from the National Taiwan University Experimental Farm, and then maintained them in mesh cages, each with 4 to 5 individuals and one soybean plant infested with soybean aphids ad libitum, and kept in growth chambers at the same conditions as that for soybean plant stocks.

2.2 Experimental design

The experiment consists of three parts (see Fig. 1). We conducted experimental selection for 16 generations to investigate the variation in aphids' plastic responses to warming and predation stressors, followed by a reciprocal transplant experiment to test for the persistence of transgenerational plasticity and its impact on aphids' responses to new environments. We also monitored aphids' life history traits (generation time and fecundity) before and after experimental selection experiment to see how exposure to environmental stressors may affect aphids' life history. More details are listed below.

2.2.1 Experimental selection

A single aphid founder was used to reproduce clonally for 2 weeks at control temperature. The aphid population was then used for the experimental selection study with a 2 x 2 factorial design, including temperature treatment (control, warming) and predation treatment (absence, presence). There were six replicates for each of the four treatment groups: 1) control temperature + predator absence, 2) warming temperature + predator absence, 3) control temperature + predator presence, and 4) warming temperature + predator presence, resulting in 24 experimental populations ('line',

doi:10.6342/NTU202204043

hereafter).

To begin with, 30 fourth instar aphids were randomly picked, transferred to a petri dish, weighed to the nearest 0.1 mg, and then transferred to a soybean plant and assigned to one of the four treatment groups. After seven days (defined as one generation), we counted the total number of aphids on each plant, and then 30 fourth instar aphids from each plant were picked randomly, weighed, transferred to a new soybean plant, and submitted to the same treatment to which they had been exposed. The process was repeated for 16 generations, followed by a reciprocal transplant experiment (details below). The reason why we transferred fourth instar aphids to a new plant was because this method ascertained that each new generation in our experimental selection study would be the immediate offspring of the previous generation. Each of the experimental lines was maintained in an individual mesh cage to prevent aphids and/or ladybeetles from escaping, and kept in growth chambers at control or warming temperature depending on treatments, with 60% relative humidity and a 12L:12D photoperiod.

For the temperature treatment, control temperature was set with within-day temperature fluctuation ranging from 26.0 to 30.8 °C (average 28.0°C) based on the average hourly temperatures in Septembers from 2004 to 2018 (autumn soybean planting season) in Taipei. According to the IPCC's predicted temperature rise in the Fifth Assessment Report [32], we set the warming temperature as 2°C above control temperature, which ranged from 28.0 to 32.8 °C (average 30.0°C). For the predation treatment, on the sixth day from the introduction of aphids to each soybean plant, an adult ladybeetle was randomly picked from the stock, weighed and then introduced to each experimental lines of the predator presence treatment group. Ladybeetles were removed two days after (which is the end of the generation) and weighed before being

doi:10.6342/NTU202204043

returned to the stock. To examine how exposure to stressor(s) affects aphid's fitness over generations, we monitored aphids' population size and body size of each experimental group for each generation.

2.2.2 Life history traits of individual aphids before and after experimental selection

To test if exposure to different stressors affects aphids' life history traits, before the experimental selection, we used first instar aphids from the stock and monitored their generation time (the time required to grow from first instar to giving birth to the first offspring), development time (the time required to grow from first instar to adulthood), and fecundity (offspring number) in control and warming conditions. After the experimental selection, using the first instar aphids collected from each experimental lines at the end of generation 15, we monitored the same traits in control and warming conditions (the same temperature treatment to which each experimental population had been exposed). The lines under predation selection were monitored in control and warming conditions only but not in predation conditions (absence and presence). This was because the predation stress was too strong for us to observe any trait variations. In both life history trials, first instar aphids were randomly picked, and each was introduced to a soybean plant. There were three replicates for each line. We observed the aphid nymphs once every 12 hours to record their developmental stage. Upon reaching adulthood, we monitored the number of offspring produced by each adult aphid on a daily basis until its death, and removed the offspring after every observation to ensure accurate counting.

doi:10.6342/NTU202204043

2.2.3 Reciprocal transplant experiment

To test if transgenerational plasticity persists and affects organisms' response to future stressors, which could be novel or repeated, we performed a reciprocal transplant experiment. After 16 generations of experimental selection, 30 fourth instar aphids from each of the experimental lines were randomly picked, weighed, and transferred to a new plant, and then introduced to a common garden environment for one week, which is the same as "control temperature + predator absence" environment. This is to minimize any maternal effects, such that variations observed during reciprocal transplant experiment could be attributed to transgenerational plasticity. We then performed the reciprocal transplant experiment in a 4 x 4 full factorial design, such that 120 offspring (4 subpopulations of 30 fourth instar aphids) from each of the 24 experimental lines were submitted to all four treatments: 1) control temperature + predator absence, 2) warming temperature + predator absence, 3) control temperature + predator presence, and 4) warming temperature + predator presence. This resulted in 96 experimental lines in total (24 lines x 4 treatments). Similar to experimental selection, we counted the total number of aphids on a plant, after which randomly picked 30 fourth instar aphids, weighed them, and transferred them to a new plant. The process was repeated for three generations. To see how aphids' fitness has changed over the three generations, we monitored population size and body size of each of 96 experimental groups for each generation.

2.3 Data analysis

For experimental selection, we used generalized linear mixed models (GLMMs) to test for the effects of temperature, predation, generation and their interactions on aphids' population size (log-transformed) and body size (mean aphid body weight).

doi:10.6342/NTU202204043

Temperature (control, warming) and predation (absence, presence) were included as categorical explanatory variables, generation was included as a continuous explanatory variable, and generation^2 was also included to test for quadratic effects. To investigate the relationship between body size and population size, we tested the effects of body size and treatments on aphid population size by including temperature, predation, generation, and body size as explanatory variables. We compared pairwise the slopes of fitted lines of body size-population size relationship to further examine how such relationship varies across two stages – early stage (generation 1-10) and later stage (generation 11-16).

To test how temperature affects aphid consumption by ladybeetles, we first defined the strength of predator stressor as changes in aphid population biomass before and after ladybeetle introduction per body weight of ladybeetles [33], and then used GLMM to test if the strength of predator stressor was affected by temperature and generation. For aphid changes in life history traits before and after experimental selection, we used three separate GLMMs to test how temperature and predation affect generation time, development time and fecundity, with temperature and predation included as explanatory variables.

For the reciprocal transplant experiment, we used GLMMs to a) analyze the effects of temperature and predation stressors during experimental selection and during reciprocal transplant on aphids' population size and body size during reciprocal transplant, and b) examine if aphid performance during reciprocal transplant was affected by previous experience during the experimental selection. 'Selection temperature' and 'selection predation' (temperature and predation treatments during experimental selection), 'assay temperature' and 'assay predation' (temperature and predation treatments during reciprocal transplant experiment), and two generations of

doi:10.6342/NTU202204043

experimental selection and reciprocal transplant experiment respectively, were included as explanatory variables. For all models, population sizes were all log-transformed, and experimental population ID was included as random factor.

We used Akaike Information Criterion (AIC) [34] to select well-fit models, and post-hoc Tukey tests to examine significant differences among means. All analyses were performed in R 4.0.4 [35], with GLMMs fitted using *lme4* package [36] and post-hoc Tukey tests performed using *lsmeans* package [37].

doi:10.6342/NTU202204043

Chapter 3 Results

3.1 Experimental selection: variation in aphid body size and population size

Overall, temperature, predator, and generation interactively affected aphid body size (p = 0.017, Fig. 2 and Table 1). Predator presence reduced aphid body size, when exposed to control temperature, the difference between control group and predator group became larger across generations. However, when exposed to predator and warming temperature at the same time, the difference in body size between warming group and warming + predator group remained similar across generations (Fig. 2). The interaction between temperature and generation^2 influenced aphid body size (p = 0.007). Specifically, warming reduced aphid body size initially, but the reduction seemed to be less obvious over the last few generations, especially (Fig. 2).

Temperature, predator, and generation interactively affected aphid population size (p < 0.001, Fig. 3). At control temperature, predator presence reduced aphid population size, and the extent of reduction became greater in later generations. However, at warming temperature, predator presence reduced aphid population size largely in early but not late generations. Furthermore, the interaction between temperature and generation^2 influenced aphid population size (p < 0.002). It seemed that warming reduced aphid population size initially, but the reduction became less evident in later generations, especially compared with the predator treatment group (with control temperature) (Fig. 3).

To understand the role of ladybeetle predation in regulating aphid population size, this study also estimated the temperature effects on aphid consumption by ladybeetles

doi:10.6342/NTU202204043

(details in Methods). The result showed that ladybeetles consumed more aphids when introduced to warming environment (p = 0.028), suggesting that the larger reduction in aphid population size in response to predator at warming condition might be related to the greater predation pressure under warming. Since this greater predation pressure under warming did not change with generation (p = 0.232), the recovery of aphid populations under warming + predator treatment at later generations (Fig. 3) may not be due to a change in predation pressure.

Since arthropod body size often predicts population size [38], this study examined whether the aphid body size – population size relationship shifted over generations. The results showed that aphid population size was affected by the interaction among temperature, predator, generation, and aphid body size (p = 0.01). At early stage (generation 1 to 10, Fig. 4A), both warming (p < 0.001) and predation (p < 0.001) affected body size-population size relationship, the slope of the body size – population size relationship was steeper in warming (p < 0.001) and warming + predator treatment groups (p = 0.048, Table 4) than in the control group. At later stage (generation 11 to 16, Fig. 4B), warming had marginal effect on (p = 0.055) on body size-population size relationship, predator did not affect the relationship (p = 0.350). While the slope of the body size – population size relationship at later stage was steeper in the warming treatment group than in the control group (p = 0.034). At either early or later stage, the steeper slopes in the warming or warming+predator treatment group suggest that population size change in response to stressors (i.e., warming and/or predation) would be more drastic than body size change (Fig. 4). For example, size reduction in aphids likely led to a reduction in aphid population size for the control group, but this population size reduction would be more prominent for the warming or warming + predator treatment group. This may suggest that smaller aphids bear more fitness cost in

doi:10.6342/NTU202204043

face of stressors (e.g., warming or predation). To disentangle how body size-population size relationship changed through generation within each treatment group, we tested the effect of stage (i.e., early vs. later stage) and found that body size-population size relationship changed with stages (p = 0.0046) in the warming + predator treatment group but not the control + predator group (p = 0.1463), suggesting that warming temperature may have mediated aphid's response to predators. No stage effect was found in other treatment groups.

3.2 Changes in life history traits after experimental selection

For the generation time of aphids (the time needed for aphids to grow from first instar to giving birth to first offspring; Table 2), it was prolonged under warming (p = 0.007; Table 2) but not affected by predation (p = 0.156). This longer generation time under warming was likely due to the delay of aphid reproduction (i.e., giving birth to the first offspring), because aphid developmental time (the time needed for aphids to grow from first instar to adulthood; Table 2) was not affected by warming (p = 0.114) or predation (p = 0.402). For offspring number (Table 2), aphids exposed to warming temperature produced less offspring (p = 0.034), different from the result before our experimental selection (marginal effect of warming, p = 0.057), suggesting that warming effect on aphid fecundity may have accumulated over the experimental selection process. In addition, aphids exposed to predator produced more offspring (p = 0.01).

doi:10.6342/NTU202204043

3.3 Reciprocal transplant experiment: persistence and consequences of transgenerational plasticity

Overall, aphid body size at reciprocal transplant stage reduced, but this was only mediated by temperature and predator effects during reciprocal transplant stage. While aphid population size was affected by warming temperature at experimental selection and reciprocal transplant interactively Notably, selection predation led to larger population size during reciprocal transplant experiment, when compared with aphid groups that had never been selected under predation, but this effect was only evident when assayed at warming temperature. The results may suggest that organisms' response to previous stressors may mediate the response to new environment through transgenerational plasticity, but the effect is dependent on the type of the stressors.

After the experimental selection, all of the lines were introduced to a common garden environment for one week to remove potential transient effects, such that no difference in aphid body size was found for all selection treatments ($p = 0.988$). Body size reduced during reciprocal transplant stage, but this was affected only by exposure to warming temperature ($p < 0.001$, Table 3) and predation ($p = 0.001$) during reciprocal transplant stage. While previous exposure to warming and predator during experiment selection stage had no effect. There was no interaction between selection treatments and reciprocal transplant treatments ($p > 0.05$).

For aphid population size, temperature treatment during selection stage and reciprocal transplant stage tended to interactively affect population size ($p = 0.053$; Fig. 5 and Table 3). Specifically, aphids exposed to warming environment at selection stage had marginally smaller population size when assayed in control temperature ($p = 0.054$), but this effect was not found at warming assay temperatures ($p = 0.742$) at the reciprocal

doi:10.6342/NTU202204043

stage.

However, previous exposure to predator during selection stage affected aphids' response to predator during reciprocal transplant stage. This predator effect was independent of generation ($p > 0.05$), but dependent on assay temperature (selection predation * assay temperature * assay predation, $p = 0.035$; Fig. 6 and Table 3). When assayed in control temperature at the reciprocal transplant stage, aphid population size was not affected by whether aphids were previously exposed to predators during the selection stage ($p = 0.357$; Fig. 6A). On the contrary, when assayed in warming temperature at the reciprocal transplant stage, aphid population size was affected by previous exposure to predator during the selection stage (selection predation * assay predation, $p = 0.058$; Fig. 6B). Specifically, predation-selected lines had marginally larger population size under warming temperature ($p = 0.083$).

doi:10.6342/NTU202204043

Chapter 4 Discussion

To investigate the transgenerational plastic effects in response to multiple stressors, we conducted an experimental selection where soybean aphids were exposed to warming temperature and/or predators. The main findings include: 1) Predator and temperature treatments interactively affected aphid body size, population size, and the body size-population size relationship over generations, suggesting that multiple stressors (e.g., abiotic and biotic) can interactively affect species' adaptation via transgenerational plasticity; 2) While the treatments in the experiment selection and reciprocal transplant experiment did not interact and affect aphid body size, they interactively affect aphid population size (selection temperature * assay temperature. $p = 0.053$; selection predation * assay temperature * assay predation, $p = 0.035$; Fig. 6). This suggests that species adaptation to environmental changes via plasticity may depend on both the previous stressors (e.g., experiment selection in this study) and current stressors (e.g., reciprocal transplant experiment in this study). We further discuss the aforementioned results below.

While previous adaptation studies focused on single stressors over a short time period [18, 19], this study highlights the important interactive effect of multiple stressors on species adaptation over multiple generations. For example, this study showed the interaction among temperature, predation, and generation. Specifically, predators reduced aphid body size, but the reduction in body size became less obvious near the end of experimental selection in warming + predator treatment group (Fig. 2). Regarding population size, at control temperature, predators reduced aphid population size, especially in later generations. However, at warming temperature, predators

doi:10.6342/NTU202204043

reduced aphid population size in early but not late generations (Fig 3). The explanation for such interactions may involve complex mechanisms and require further studies. However, our study revealed a potential mechanism for the temperature – predation interaction: either warming or predation can individually lead to smaller body size in species [39, 40]; since predation pressure was greater in warming environment in this study (see Results for temperature effect on ladybeetle consumption), predation and warming together may have a synergistic effect on aphid body size. Further study in attempt to clarify how such interactions between stressors may change over time will help predict the impacts of multiple stressors in climate change scenarios [41].

Regarding an individual abiotic stressor, this study found that warming reduced aphid body size (Fig. 2), which is consistent with the temperature-body size rule shown by many previous studies [42] that warmer environments tend to reduce body size due to shorter development time. However, although aphid body size reduced in warming environment, aphid development time found in our study was not affected by warming temperature. This could be because our temperature regime, which was on average 28°C for control and 30°C for warming treatments, fell within optimal and suboptimal temperature for aphids [43]. Notably, we found body size of aphids exposed to warming temperature reducing in the early stage of the experimental selection, likely due to accumulating warming effects, but the effect became less evident at around 10th generation onwards, suggesting that aphids may have adapted to the warmer temperature, or aphids may have reached the physiological constraint beyond which aphids cannot successfully grow. Another possible explanation on warming effect on aphid performance may be due to a change in endosymbiont composition, as previous work on pea aphids shows that certain facultative symbionts helped recovering aphids' fitness after being exposed to heat shock through physiological variations [44].

doi:10.6342/NTU202204043

Regarding an individual biotic stressor, this study showed that predation reduced aphid body size (Fig. 2), which is consistent to previous studies on the same predator-prey pair [45]. While this size reduction was suggested to be driven by size-selective predation, i.e., predators preferred larger aphid, this suggestion is not supported by our study, where aphid body size variance was independent of selection treatments (p = 0.911). Nevertheless, the result of our study seems to be in accordance with the theory of nonselective predation, where models predict that size of prey at maturity decreases in response to increased predation risk [40].

We found similar patterns in the response of aphid body size and population size to warming and predation stressors (Fig. 3). This is possibly due to the positive relationship between body size and fecundity, such that larger aphids reproduce more offspring [18, 38]. While warming led to smallest body size and population size, when aphids were exposed to predators at the same time, an increase in population size happened at around generation 10 onwards, and went on to the extent of recovering aphid population size to approaching the level comparable with that of the beginning of selection, implicating that aphids selected under interaction of multiple stressors became adaptive to the environment by increasing fecundity. The result also suggests that there was an interactive effect of multiple stressors that would not have been observed with single stressor settings. Furthermore, the effect would not have been observed within only a few generations. While the positive relationship of body size and population size is supported by this study (Fig. 4), our further analysis suggests that the slope of this relationship may shift over generations. When controlling for body size in predicting population size, population size was found to be affected interactively by treatments, generations, and body size (p = 0.01). Specifically, the body size-population size relationship of aphids exposed to warming + predator environment was different

doi:10.6342/NTU202204043

between early and later stages, suggesting that aphids' response to predator presence in terms of body size-population size relationship may have been mediated by warming temperature. Our study highlighted the importance of taking into account the interactive effect of multiple stressors to better predict impacts of environmental changes on ecosystems.

By examining how life history traits have changed after experimental selection, we found that warming temperature prolonged generation time, but did not affect developmental time, implying that the prolonged generation time may be due to the delay of reproduction. However, although previous work shows that predator presence may shorten development time due to a reduction in body size [46, 47], this is not found in this study. As for fecundity, aphids exposed to warming temperature reproduced less offspring; on the contrary, predator presence enhanced aphid fecundity (see Results), suggesting that life history traits were selected in different direction under warming and predation stressors.

Our reciprocal transplant experiment highlights that species adaptation to environmental changes via plasticity may depend on both the previous and current stressors. In other words, previous exposure to stressors can prepare aphids to better deal with new environments, but such effect is dependent on type of stressors. For example, previous exposure to warming environment mediated aphid population size in response to future environment, regardless of whether aphids experienced predation (Fig. 5). While previous exposure to predator presence helped aphids to cope with predators in new environment, this occurred only when new environment contains warming temperature stressor (Fig. 6). The contrasting results may be due to different mechanisms of selection in response to warming temperature and predator presence. For instance, previous exposure to warming temperature reduced aphid's fecundity; in

doi:10.6342/NTU202204043

addition, the body size-population size relationship of warming groups shows steeper slope when compared with control groups. Therefore, these aphids with smaller body size and lower fecundity would show smaller population size when assayed at control temperature, compared with aphids that were exposed to the control environment at selection stage. Whereas previous exposure to predators enhanced aphids' fecundity, as a result, these aphids had better performance when assayed with predator presence comparing to naïve aphids that have never experienced predators, but the effect is only observed when aphids were assayed in warmer environment, possibly because smaller body size was favored at warming temperature.

In conclusion, this study demonstrates the importance of considering 1) multiple stressors – including abiotic and biotic stressors – and their interaction, and 2) the interactive effect of previous and current stressors on species' transgenerational plasticity in order to understand species adaptation. Understanding these interactions will have important implications on ecological and evolutionary consequence in the context of accelerating environmental changes foreseen for the near future, and help better evaluate and predict the impact of environmental changes on ecosystems.

doi:10.6342/NTU202204043

REFERENCE

1. Thompson, P.L., M.M. MacLennan, and R.D. Vinebrooke, *An improved null model for assessing the net effects of multiple stressors on communities.* Global Change Biology, 2018. **24**(1): p. 517-525.
2. Matassa, C.M. and G.C. Trussell, *Effects of predation risk across a latitudinal temperature gradient.* Oecologia, 2015. **177**(3): p. 775-784.
3. Thakur, M.P., et al., *Warming magnifies predation and reduces prey coexistence in a model litter arthropod system.* Proceedings of the Royal Society B: Biological Sciences, 2017. **284**(1851): p. 20162570.
4. Tseng, M. and M.I. O'Connor, *Predators modify the evolutionary response of prey to temperature change.* Biology Letters, 2015. **11**(12): p. 20150798.
5. Cuenca-Cambronero, M., et al., *Evolutionary mechanisms underpinning fitness response to multiple stressors in Daphnia.* Evolutionary Applications, 2021. **14**(10): p. 2457-2469.
6. Segner, H., M. Schmitt-Jansen, and S. Sabater, *Assessing the Impact of Multiple Stressors on Aquatic Biota: The Receptor's Side Matters.* Environmental Science & Technology, 2014. **48**(14): p. 7690-7696.
7. Whitman, D. and A. Agrawal, *What is Phenotypic Plasticity and Why is it Important?* Phenotypic plasticity of insects, 2009.
8. Shama, L.N.S., *The mean and variance of climate change in the oceans: hidden evolutionary potential under stochastic environmental variability in marine sticklebacks.* Sci Rep, 2017. **7**(1): p. 8889.
9. Ghalambor, C.K., et al., *Adaptive versus non-adaptive phenotypic plasticity and*

doi:10.6342/NTU202204043

the potential for contemporary adaptation in new environments. Functional Ecology, 2007. **21**(3): p. 394-407.

10. Bonamour, S., et al., *Phenotypic plasticity in response to climate change: the importance of cue variation.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2019. **374**(1768): p. 20180178.
11. Mooney, K. and A. Agrawal, *Phenotypic Plasticity*. 2008. p. 43-57.
12. Wadgymar, S.M., R.M. Mactavish, and J.T. Anderson, *Transgenerational and Within-Generation Plasticity in Response to Climate Change: Insights from a Manipulative Field Experiment across an Elevational Gradient.* The American Naturalist, 2018. **192**(6): p. 698-714.
13. Herman, J.J., et al., *How Stable 'Should' Epigenetic Modifications Be? Insights From Adaptive Plasticity and Bet Hedging.* Evolution, 2014. **68**(3): p. 632-643.
14. Shama, L.N.S., et al., *Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean.* Functional Ecology, 2014. **28**(6): p. 1482-1493.
15. Herrel, A., D. Joly, and E. Danchin, *Epigenetics in ecology and evolution.* Functional Ecology, 2020. **34**(2): p. 381-384.
16. Wootton, R.J., *The evolution of life histories: Theory and analysis.* Reviews in Fish Biology and Fisheries, 1993. **3**(4): p. 384-385.
17. Sha, Y., S.V.M. Tesson, and L.-A. Hansson, *Diverging responses to threats across generations in zooplankton.* Ecology, 2020. **101**(11): p. e03145.
18. Hayden, M.T., K.D. Holmes, and L.M. Arcila Hernández, *Multigenerational consequences of aphid size on offspring phenotype and reproduction.* Entomologia Experimentalis et Applicata, 2021. **169**(10): p. 947-958.
19. Crain, C.M., K. Kroeker, and B.S. Halpern, *Interactive and cumulative effects of*

doi:10.6342/NTU202204043

multiple human stressors in marine systems. Ecol Lett, 2008. **11**(12): p. 1304-15.

20. Shama, L.N.S. and K.M. Wegner, *Grandparental effects in marine sticklebacks: transgenerational plasticity across multiple generations.* Journal of Evolutionary Biology, 2014. **27**(11): p. 2297-2307.
21. Salinas, S. and S.B. Munch, *Thermal legacies: transgenerational effects of temperature on growth in a vertebrate.* Ecol Lett, 2012. **15**(2): p. 159-63.
22. Lenski, R.E. and M. Travisano, *Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations.* Proceedings of the National Academy of Sciences, 1994. **91**(15): p. 6808-6814.
23. Johnson, M.S., et al., *Phenotypic and molecular evolution across 10,000 generations in laboratory budding yeast populations.* eLife, 2021. **10**: p. e63910.
24. Donelson, J.M., et al., *Transgenerational plasticity and climate change experiments: Where do we go from here?* Global Change Biology, 2018. **24**(1): p. 13-34.
25. Leroi, A.M., A.F. Bennett, and R.E. Lenski, *Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption.* Proceedings of the National Academy of Sciences, 1994. **91**(5): p. 1917-1921.
26. Whittle, C.A., et al., *Adaptive epigenetic memory of ancestral temperature regime in Arabidopsis thalianaThis paper is one of a selection of papers published in a Special Issue from the National Research Council of Canada – Plant Biotechnology Institute.* Botany, 2009. **87**(6): p. 650-657.
27. Schade, F.M., C. Clemmesen, and K. Mathias Wegner, *Within- and transgenerational effects of ocean acidification on life history of marine three-spined stickleback (Gasterosteus aculeatus).* Marine Biology, 2014. **161**(7):

doi:10.6342/NTU202204043

p. 1667-1676.

28. Brady, S.P., et al., *Causes of maladaptation.* Evolutionary Applications, 2019. **12**(7): p. 1229-1242.

29. Sentis, A., et al., *Evolution without standing genetic variation: change in transgenerational plastic response under persistent predation pressure.* Heredity, 2018. **121**(3): p. 266-281.

30. Catullo, R.A., et al., *The Potential for Rapid Evolution under Anthropogenic Climate Change.* Current Biology, 2019. **29**(19): p. R996-R1007.

31. Srinivasan, D.G. and J.A. Brisson, *Aphids: a model for polyphenism and epigenetics.* Genetics research international, 2012. **2012**: p. 431531-431531.

32. IPCC, *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* 2014: p. 151 pp.

33. Schwarz, T. and T. Frank, *Aphid feeding by lady beetles: higher consumption at higher temperature.* BioControl, 2019. **64**(3): p. 323-332.

34. Akaike, H., *A new look at the statistical model identification.* IEEE Transactions on Automatic Control, 1974. **19**(6): p. 716-723.

35. R Core Team, *R: A language and environment for statistical computing.* Vienna, Austria. Available at: https://www.R-project.org/. 2018.

36. Bates, D., et al., *Fitting Linear Mixed-Effects Models Using lme4.* Journal of Statistical Software, 2015. **67**(1): p. 1 - 48.

37. Lenth, R., *Least-Squares Means: The R Package lsmeans.* Journal of Statistical Software, 2016. **69**.

38. Honěk, A., *Intraspecific Variation in Body Size and Fecundity in Insects: A General Relationship.* Oikos, 1993. **66**(3): p. 483-492.

doi:10.6342/NTU202204043

39. Gardner, J.L., et al., *Declining body size: a third universal response to warming?* Trends in Ecology & Evolution, 2011. **26**(6): p. 285-291.

40. Abrams, P.A. and L. Rowe, *The effects of predation on the age and size of maturity of prey.* Evolution, 1996. **50**(3): p. 1052-1061.

41. Orr, J.A., et al., *Rapid evolution generates synergism between multiple stressors: Linking theory and an evolution experiment.* Global Change Biology, 2022. **28**(5): p. 1740-1752.

42. Atkinson, D., *Temperature and organism size-A biological law for ectotherms? Advances in Ecological Research 25: 1.* Res., 1994. **25**.

43. Wang, Y.-J., T. Nakazawa, and C.-K. Ho, *Warming impact on herbivore population composition affects top-down control by predators.* Scientific Reports, 2017. **7**(1): p. 941.

44. Heyworth, E.R., M.R. Smee, and J. Ferrari, *Aphid Facultative Symbionts Aid Recovery of Their Obligate Symbiont and Their Host After Heat Stress.* Frontiers in Ecology and Evolution, 2020. **8**.

45. Sentis, A., J.L. Hemptinne, and J. Brodeur, *Non-additive effects of simulated heat waves and predators on prey phenotype and transgenerational phenotypic plasticity.* Glob Chang Biol, 2017. **23**(11): p. 4598-4608.

46. Roff, D., *Optimizing development time in a seasonal environment: The 'ups and downs' of clinal variation.* Oecologia, 1980. **45**(2): p. 202-208.

47. Beckerman, A.P., G.M. Rodgers, and S.R. Dennis, *The reaction norm of size and age at maturity under multiple predator risk.* J Anim Ecol, 2010. **79**(5): p. 1069-76.

doi:10.6342/NTU202204043

Appendix

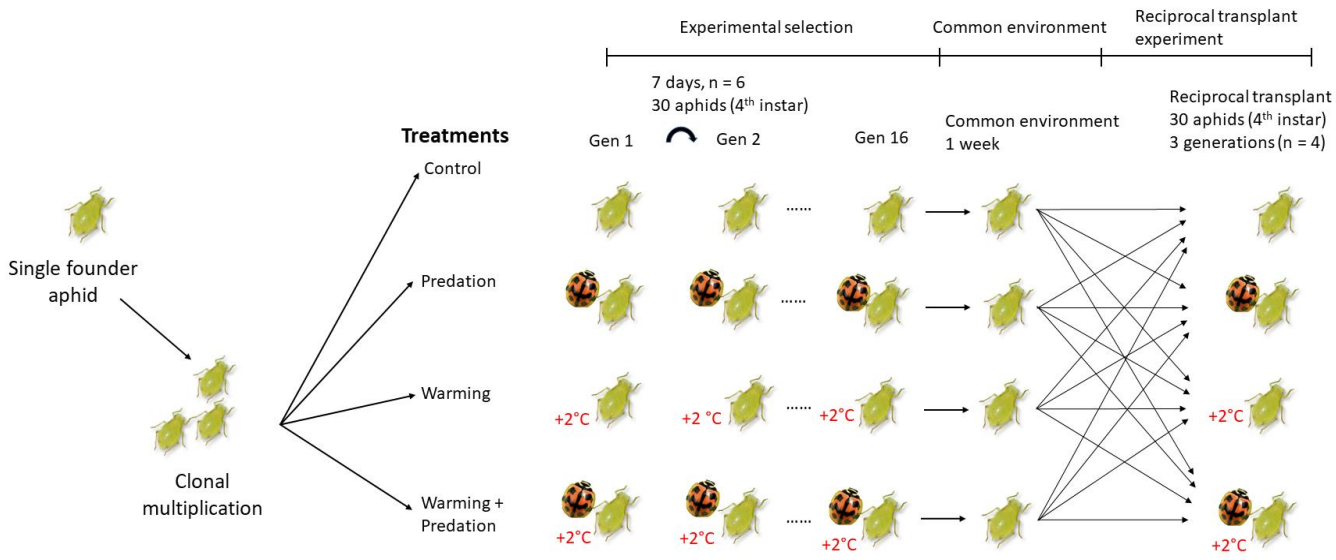

Figure 1. Experimental design. The study consists of an experimental selection (16 generations) to examine transgenerational plasticity in response to warming temperature and predation stressors, and a reciprocal transplant experiment (3 generations) to test for persistence of transgenerational plasticity and its effect on aphid performance in new environment.

doi:10.6342/NTU202204043

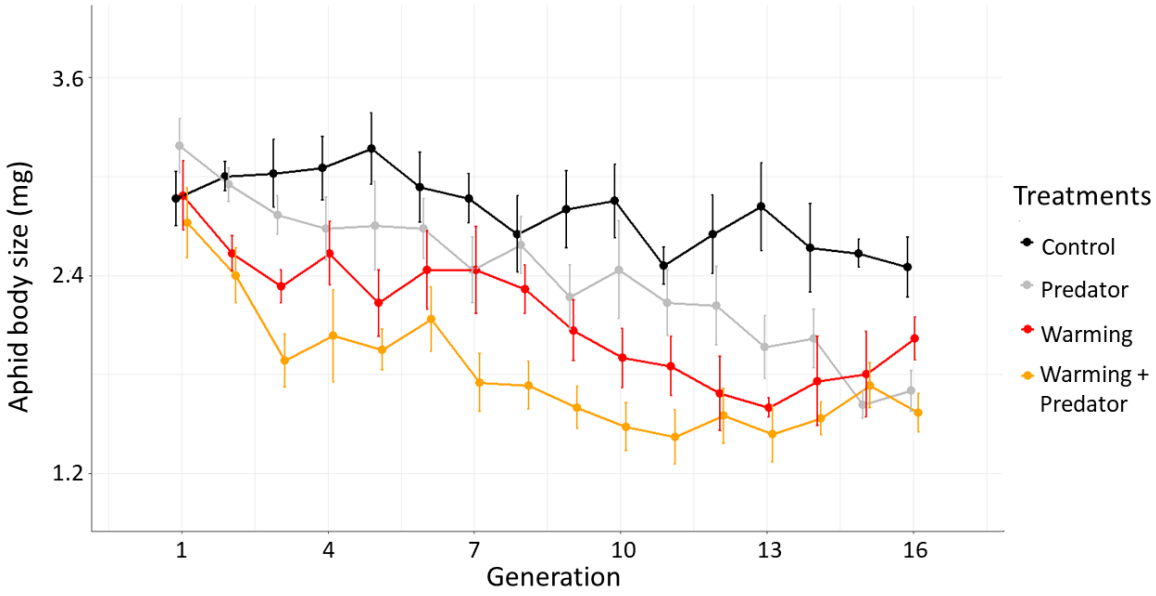

Figure 2. Aphid body size across 16 generations (mean ± SE) in experimental selection, with control (in black), predator (in grey), warming (in red) and warming plus predator (in orange) treatments.

doi:10.6342/NTU202204043

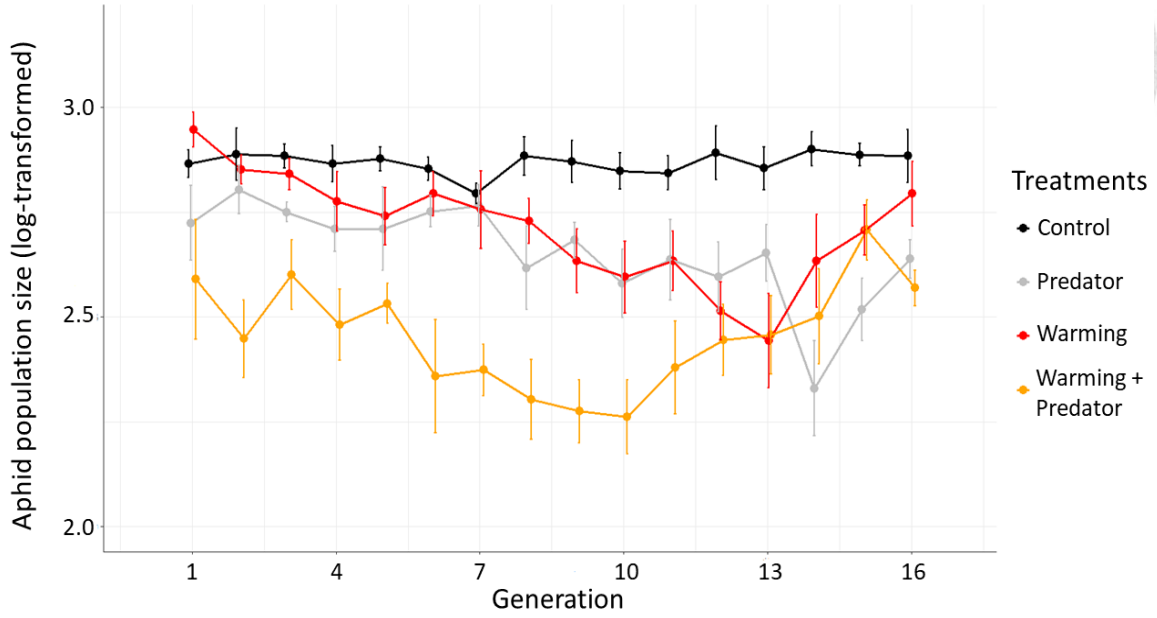

Figure 3. Aphid population size across 16 generations (mean ± SE) in experimental selection, with control (in black), predator (in grey), warming (in red) and warming plus predator (in orange) treatments

doi:10.6342/NTU202204043

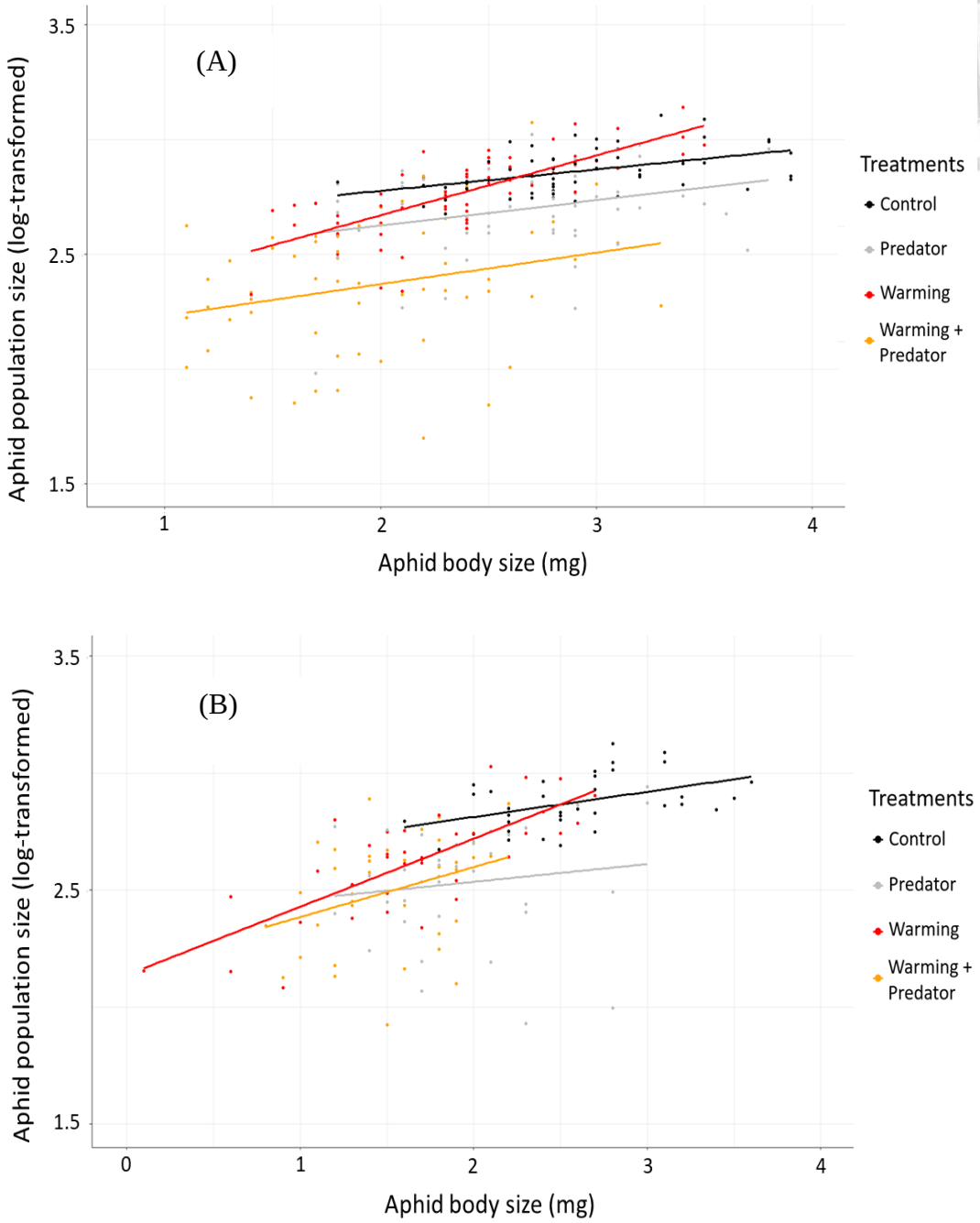

Figure 4. Relationship between aphid body size and population size at (A) early stage of experimental selection (generation 1 – 10) and (B) later stage of experimental selection (generation 11 – 16), with control (in black), predator (in grey), warming (in red) and warming plus predator (in orange) treatments. Each datapoint represents an experimental line.

doi:10.6342/NTU202204043

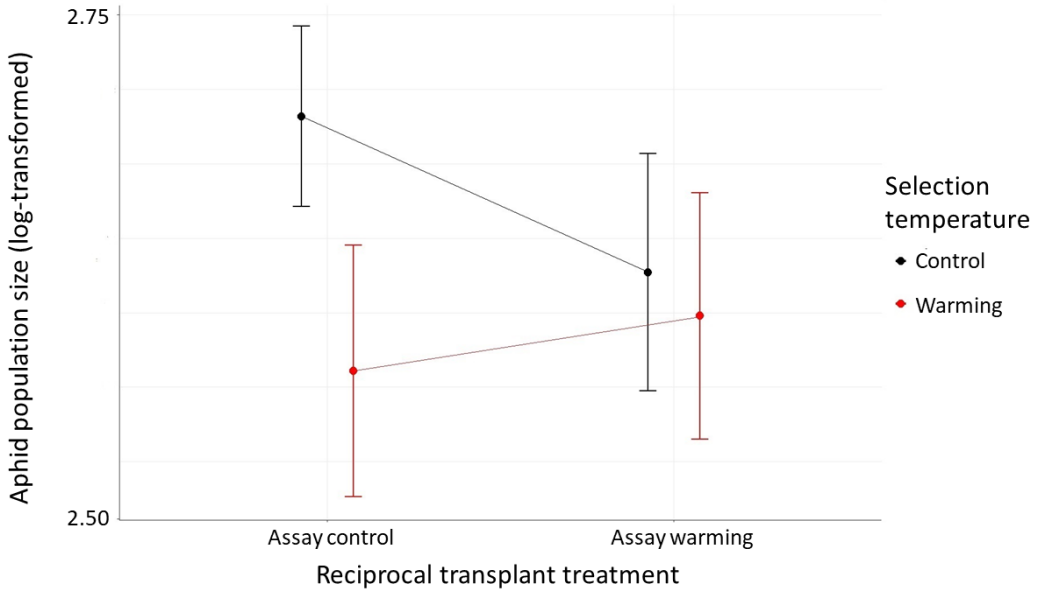

Figure 5. Interactive effect of reciprocal transplant temperature (assay control and assay warming) and selection temperature (control and warming) on population size (mean ± SE), aphids were exposed to control (in black) or warming (in red) treatment during experimental selection.

doi:10.6342/NTU202204043

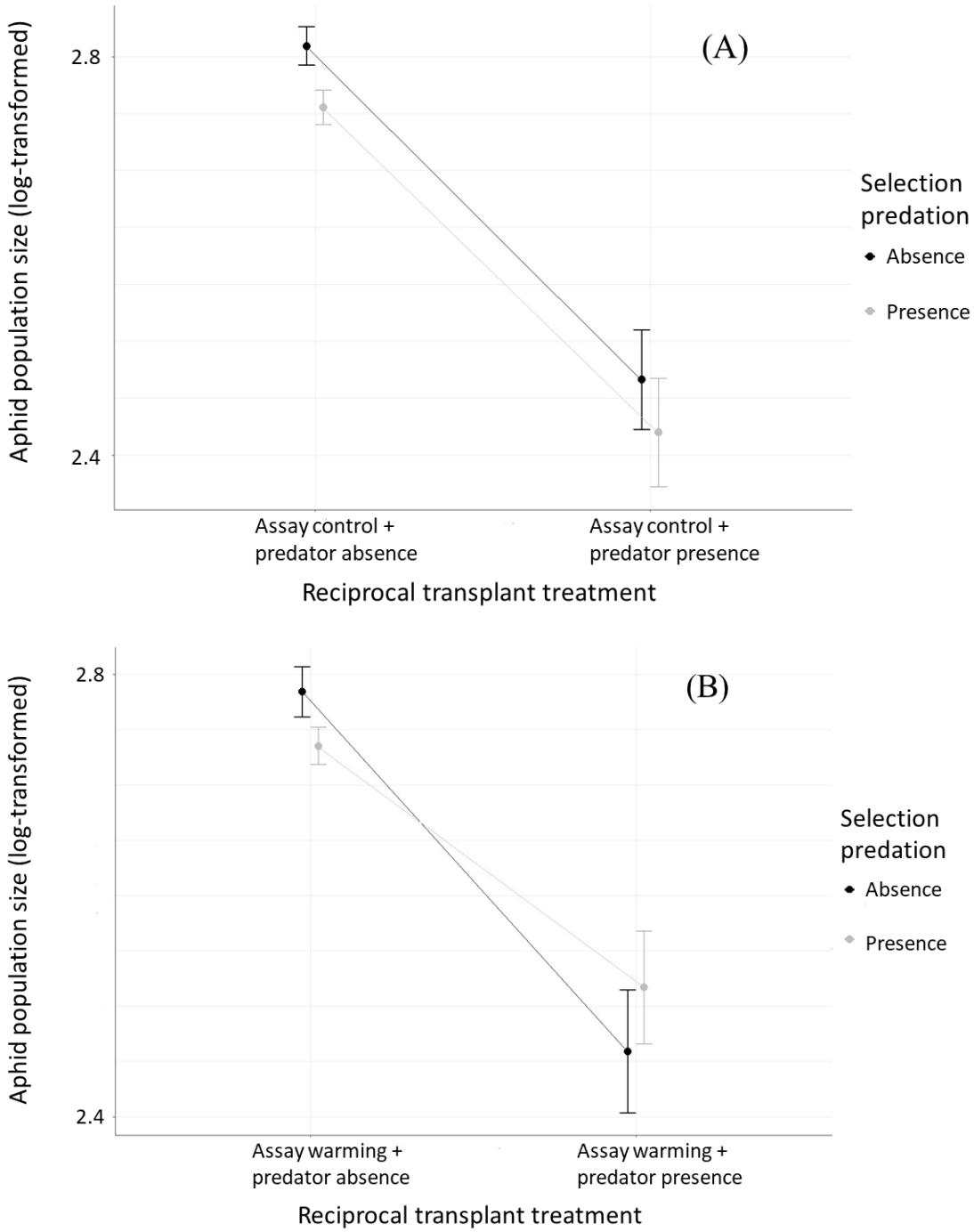

Figure 6. Interactive effect of reciprocal transplant temperature (A: assay control; B: assay warming), reciprocal transplant predation (predator absence and presence), and selection predation (predator absence and presence) on population size (mean ± SE), aphids were selected without predator (in black) or with predator (in grey) during experimental selection.

doi:10.6342/NTU202204043

Table 1. Results of ANOVA of aphid body size and population size in response to warming temperature and predation stressors during experimental selection.

Dependent variable	**Explanatory variables**	χ^2	**d.f.**	***p* value**
Body size	Temperature	27.24	1	**<0.001**
	Predator	10.36	1	**0.001**
	Generation	16.63	1	**<0.001**
	Generation²	0.19	1	0.660
	Temperature x Predator	0.33	1	0.565
	Temperature x Generation	1.63	1	0.201
	Temperature x Generation²	7.17	1	**0.007**
	Predator x Generation	6.50	1	**0.011**
	Temperature x Predator x Generation	5.69	1	**0.017**
Population size	Temperature	12.33	1	**<0.001**
	Predator	26.90	1	**<0.001**
	Generation	0.04	1	0.842
	Generation²	0.36	1	0.548
	Temperature x Predator	1.11	1	0.291
	Temperature x Generation	10.69	1	**0.001**
	Temperature x Generation²	15.52	1	**<0.001**
	Predator x Generation	12.55	1	**<0.001**
	Temperature x Predator x Generation	27.89	1	**<0.001**

Bold: *p* values <0.05

doi:10.6342/NTU202204043

Table 2. Results of ANOVA of aphid life history traits after generation 15.

Dependent variable	**Explanatory variables**	χ^2	**d.f.**	***p* value**
Generation time	Temperature	7.32	1	**<0.007**
	Predator	2.01	1	0.156
Development time	Temperature	2.49	1	0.114
	Predator	0.70	1	0.402
Offspring number	Temperature	4.51	1	**0.034**
	Predator	6.61	1	**0.010**

Bold: *p* values <0.05

doi:10.6342/NTU202204043

Table 3. Results of ANOVA of aphid body size and population size in response to warming temperature and predation stressors during reciprocal transplant experiment.

Dependent variable	**Explanatory variables**	χ^2	**d.f.**	***p* value**
Body size	Temperature selection	0.00	1	0.954
	Predator selection	10.36	1	0.370
	Generation	0.08	1	0.773
	Temperature assay	11.16	1	**<0.001**
	Predator assay	10.73	1	**0.001**
Population size	Temperature selection	3.91	1	**0.048**
	Predator selection	1.32	1	0.250
	Generation	1.60	1	0.206
	Temperature assay	0.86	1	0.354
	Predator assay	18.27	1	**<0.001**
	Temperature selection x Temperature assay	3.74	1	0.053
	Predator selection x Temperature assay	0.00	1	0.998
	Predator selection x Predator assay	0.55	1	0.460
	Temperature assay x Predator assay	2.20	1	0.138
	Predator selection x Temperature assay x Predator assay	4.45	1	**0.035**

Bold: *p* values <0.05

doi:10.6342/NTU202204043

Table 4. Pairwise comparison of slopes of fitted lines of body size- population size relationship of early stage (generation 1-10) and later stage (generation 11-16).

Stage	**Contrast**	***z* ratio**	***p* value**
Early stage	Control – Warming	-4.389	**0.0001**
	Control – Warming + Predator	-2.582	**0.0483**
	Control – Predator	-1.736	0.3049
	Predator – Warming	-2.132	0.1430
	Predator – Warming + Predator	-0.755	0.8745
	Warming – Warming + Predator	1.313	0.5543
Later stage	Control – Warming	-2.712	**0.0338**
	Control – Warming + Predator	-1.501	0.4371
	Control – Predator	-0.051	1.0000
	Predator – Warming	-2.287	0.1009
	Predator – Warming + Predator	-1.342	0.5358
	Warming – Warming + Predator	0.452	0.9692

Bold: *p* values <0.05

doi:10.6342/NTU202204043