國立臺灣大學生命科學院生態學與演化生物學研究所

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溫度上升與二氧化碳濃度增加對跨海拔植食性昆蟲的直接與間接影響

Direct and indirect effects of elevated temperature and CO_2 on herbivore performance across altitudes

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口試委員會審定書

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本論文係劉騏銘君（學號R03B44001）在國立臺灣大學生態學與演化生物學研究所完成之碩士學位論文，於民國107年3月8日承下列考試委員審查通過及口試及格，特此證明

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序

起始於 2016 年的旅程，終於漸漸邁向尾聲。回首一路走來，或許不到披荊斬棘的程度，卻也難免遭遇逆境與無法盡如人意的情況。這項兩人合作的實驗，由諸多細小的「零件」所構成。例如:實驗物種的採集、培養，生長箱的設定、校正，實驗結果的統計分析。必須適切的調整各項零件，以得到值得信賴的實驗結果。然而各式各樣的難題，卻會不時造訪，帶來考驗。

隨著難題而來的，是諸多焦慮與自我懷疑的時光，覺得自己的能力似乎不足以應對眼前的實驗規模。對於困境要如何解決，也可能和夥伴意見相左，而需要更多溝通與思考。其中有令人振奮的時刻，例如成功校正生長箱，使其完美呈現溫度曲線。有向現實妥協的時刻，例如栽培葶藶時，因應狀況以根進行無性繁殖或是以種子進行有性繁殖。也有危機處理失敗，導致有效樣本數減少的時刻，例如幼蟲生病死亡與薊馬入侵的事件。在一切實驗操作結束，終於取得資料以後，眼前還有統計的問題等著學習與探討。試著將場景倒轉回到成為研究生的第一天，對照起來，當時的我顯然沒有正確預測出這些考驗的難度，以及考驗現身的形式。另一方面，自己灌注在實驗的信心、鬥志與積極度，似乎沒有達到預期中的水準。結果飼養結束以後，收拾與論文撰寫均花費相當多的時間。

幸好，在眾多夥伴的幫助之下，研究得以在掙扎之中緩慢向前推進。並且幾度在研討會當中與大家分享成果。隨著這一本論文順利誕生，內心裡面的大石頭終於可以放下，那些擔憂的日子終於成為過去。引述陳之藩教授在〈謝天〉當中的句子:「得之於人者太多，出之於己者太少」,「因為需要感謝的人太多了，就感謝天罷」。

從今以後，再次造訪採集地時，能夠盡情享受大自然，放下前途茫茫的緊張感。得以帶著平靜的心情欣賞翩翩飛舞的蝴蝶，而非盤算著如何捕獲足夠數量的雌蝶。人生也要向著下一個階段繼續前進。最後，預祝向期刊投稿的計畫能夠順利，讓這項研究成為後人更向前一步的基石。

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摘要

溫度上升與二氧化碳濃度增加，是近年來全球氣候變遷的兩項重要變化。為了進一步了解它們對生態系統造成的衝擊，本研究探討： (一)溫度上升與二氧化碳濃度增加是否會有交互作用，並且直接地(例如透過生長與發育)、或是間接地(例如:透過影響食草)影響植食性昆蟲的表現。 (二)跨越環境梯度(例如海拔)的生物族群，是否具有種內變異，因而對於氣候變遷產生不同的反應。為了回答前述問題，本研究以緣點白粉蝶(*Pieris canidia*)幼蟲，及其主要食草葶藶(*Rorippa indica*)為材料，進行兩組生長箱實驗: 直接與間接效應實驗。每一實驗共有十二種(3*2*2)處理組合: (現今平均溫度[21.8°C]、+3°C、+6°C)*(環境 CO_2 濃度 500 ppm、預估本世紀末 CO_2 濃度 1000 ppm)*(生物的海拔來源:[低或中海拔])，其中溫度處理具有氣溫日變化。「直接效應」實驗將 *P. canidia* 飼養在各個(溫度*二氧化碳)處理之下，並以統一培養於人工氣候室的食草加以餵食。「間接效應」實驗則是將食草培養在各個(溫度*二氧化碳)處理之下，分別餵食培養於控制組環境的幼蟲。結果顯示，溫度上升和二氧化碳濃度增加，會透過單獨以及彼此交互作用的形式，影響 *P. canidia* 的表現。而溫度上升單獨影響 *P. canidia* 的性狀項目，多於二氧化碳濃度增加所影響的項目。此外，物種的海拔來源會單獨的影響 *P. canidia* 表現，並且與溫度及二氧化碳處理產生交互作用。綜合以上的結果，本研究建議氣候變遷的評估 (一)應該考慮氣候變遷因子之間的交互作用，以及它們如何透過直接與間接效應影響生物表現; (二)應考慮隨環境梯度產生的種內差異，以族群而非整個物種作為評估的反應單位。

關鍵詞: 直接與間接效應、暖化、二氧化碳濃度增加、海拔、緣點白粉蝶、葶藶

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Abstract

Temperature and CO_2 concentration in the atmosphere are predicted to keep rising during this century. Therefore, it becomes increasingly important to understand 1) whether the elevated temperature and CO_2 will interact and influence species performance directly or indirectly (e.g., indirectly via their effects on interacting species, such as herbivores' host plants), and 2) whether these effects will vary spatially (e.g., populations across altitudes might react differently due to their adaption history). To answer the aforementioned questions, we empirically examined the direct and indirect effects of elevated temperature and CO_2 on the performance of *Pieris canidia* (herbivore; *Pieris* hereafter) on *Rorippa indica* (host plant; *Rorippa* hereafter) across altitudinal gradients by conducting two experiments: direct and indirect effect experiments. Each experiment had a 3x2x2 factorial design including temperature treatment (daily fluctuating about a average of 21.8, 24.8, and 27.8°C, representing control, +3°C, and +6°C, respectively), CO_2 treatment (500 and 1000 ppm, representing control and elevation) and altitudinal origin of species (low- and medium-altitude), allowing us to examine the individual and collective effects of temperature and CO_2 on populations across altitude. In the direct effect experiment, *Pieris* larvae were reared under each of six temperature-CO_2 treatments but fed with *Rorippa* plants grown from a common garden. In the indirect effect experiment, *Pieris* larvae were reared under control treatment (21.8°C on average, 500 ppm CO_2) but fed with *Rorippa* plants grown under each of six temperature-CO_2 treatments. *Pieris* and *Rorippa* plants were originally collected from three regions each at low (c.a.100 m a. s. l.) and medium (c.a. 1000 m a. s. l.) altitudes, to avoid potential idiosyncrasies. The results showed that in direct or indirect effect experiments, elevated temperature and CO_2 individually or interactively affected *Pieris* performance, while elevated CO_2 alone affected fewer

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performance traits than elevated temperature did. Furthermore, species' altitudinal origin affected *Pieris* performance and mediated the temperature and CO_2 effects. In conclusion, the results have two implications: 1) Climate change impact assessments may need to consider the interplay between climate change components (e.g., elevated temperature and CO_2), which can directly and indirectly affect species. 2) These assessments should also consider intraspecific variation across spatial gradients, such as treating a population, instead of an entire species, as a responsive unit.

Key term: Direct & Indirect effects, Elevated temperature, Elevated carbon dioxide concentration, Altitude, *Pieris canidia, Rorippa indica*

目錄

List of Tables

doi: 10.6342/NTU201802543

List of Figures

doi: 10.6342/NTU201802543

Introduction

Temperature and CO_2 concentration can critically affect species performance and interactions, such as growth, metabolism, fitness and trophic interactions (Fajer *et al.* 1989, Kukal and Dawson 1989, Bale *et al.* 2002, Bauerfeind and Fischer 2013, Klaiber *et al.* 2013, Murray *et al.* 2013). As temperature and CO_2 concentration in atmosphere are predicted to keep rising during this century (IPCC 2014), it is important to evaluate the impact of these elevations on species. For example, the Intergovernmental Panel on Climate Change (IPCC) predicts that the mean temperature of global surface might elevate between 2°C–4°C (RCP 6.0 and 8.5, respectively), and the CO_2 concentration might double and reach up to 1000 ppm (RCP 8.5) by the year of 2100 (IPCC 2014). Evaluating the effect of this high degree of elevations on species performance and interactions is a critical first step to predict climate change impact on communities and ecosystems.

Elevated temperature and CO_2 may affect species through direct and indirect mechanisms. For example, in plant-herbivore communities, elevated temperature and CO_2 may affect herbivore performance through direct and indirect mechanisms. Directly, temperature could affect herbivore growth, development, fitness, and feeding behavior (Harlow *et al.* 1976, Kukal and Dawson 1989, Bale *et al.* 2002, Karl *et al.* 2011, Lemoine *et al.* 2013). For example, warming often reduced animal body size (Gardner *et al.* 2011, Sheridan and Bickford 2011). This may affect animal fitness because body size and individual fitness often correlates positively (Choudhury *et al.* 1996, Ellers *et al.* 1998, Sokolovska *et al.* 2000). In addition, moderate warming often speeds up animal development (e.g., shorter juvenile or larval period) and increases feeding rate (e.g., herbivory) (Bauerfeind and Fischer 2013, Akbar *et al.* 2015). In contrast to elevated temperature, elevated CO_2 (at the climate change level) may have

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little direct effect on terrestrial animals (herbivores) (Murray *et al.* 2013), although this direct effect was seldom investigated alone (e.g., confounded with indirect effects) (Lincoln *et al.* 1993, Brooks and Whittaker 1998, Hunter 2001, Klaiber *et al.* 2013). While previous studies have investigated how temperature or CO_2 alone directly affects animals (e.g., herbivores) (Harlow *et al.* 1976, Bale *et al.* 2002, Gardner *et al.* 2011, Murray *et al.* 2013, Akbar *et al.* 2015), the direct effect of temperature-CO_2 interaction on herbivores is understudied and remains to be determined (Johns and Hughes 2002, Murray *et al.* 2013) (*knowledge gap 1*).

Elevated temperature and CO_2 concentration may also indirectly affect herbivore performance via changing plant quality. Interestingly, some evidences hint that these indirect effects reduce herbivore performance. For example, host plants raised under elevated temperature could negatively affect herbivores, such as reduced body weight and elongated development in herbivores (Bauerfeind & Fischer 2013). Although the underlying mechanisms are not clear, elevated temperature has been reported to influence plant photosynthesis rate, respiration rate, and plant chemicals (Dury *et al.* 1998, Zvereva and Kozlov 2006, Zhao and Liu 2008, Way and Oren 2010, Liang *et al.* 2013). As for CO_2 effect, elevated CO_2 reportedly changed secondary metabolites related to plant defense and impeded herbivore growth (Srinivasa Rao *et al.* 2012). Elevated CO_2 may also reduce host plant quality for herbivores (e.g., higher leaf C content and C/N ratio) (Lincoln *et al.* 1993, Lawler *et al.* 1996, Cotrufo *et al.* 1998, Williams *et al.* 2000, Johns *et al.* 2003, Gherlenda *et al.* 2015), leading to longer larval period, larger larval weights, and higher food intake in herbivores (Fajer *et al.* 1989, Srinivasa Rao *et al.* 2012, Murray *et al.* 2013). While these aforementioned studies have examined how temperature or CO_2 alone indirectly affects herbivores, the indirect effect of temperature-CO_2 interaction on herbivores is unclear (*knowledge gap 2*).

Although less studied, evaluating the direct and indirect effects of climate change (e.g., elevated temperature and CO_2) on herbivores should consider whether the herbivore responses will vary spatially (e.g., altitude or latitude) (*knowledge gap 3*). An intraspecific variation in herbivore performance across space, if exists, could suggest that different herbivore populations of the same species (e.g., across altitude) may respond differently to the direct or indirect effect of climate change. In other words, climate change impact assessment may need to treat a population, instead of an entire species, as a responsive unit. This speculation is supported by numerous studies where intraspecific variations in performance occurred across spatial gradients (e.g., altitude or latitude) (Kato 2005, Posledovich *et al.* 2015). For example, the body size of a herbivorous species in coastal marshes may increase toward higher latitudes (lower temperature), consistent with the Bergmann's rule (larger body size at colder environment) (Ho *et al.* 2009, Ho and Pennings 2013). Moreover, *Pieris rapae* larvae from higher latitude may be induced to diapause by a longer daytime length, compared to those from lower latitude, likely due to adaption to high latitude climate (e.g., low temperature) (橋本健一 *et al.* 2008).

An integrated study of elevated temperature and CO_2 across spatial gradients is required to help fill the three aforementioned knowledge gaps that will facilitate future climate change impact assessment. How elevated temperature and CO_2 (individually and interactively) may directly (*knowledge gap 1*) and indirectly (*knowledge gap 2*) affect herbivore performance, and whether these effects will vary with spatial gradients (altitude) (*knowledge gap 3*). Specifically, we experimentally examined the direct and indirect effects of temperature and CO_2 on the performance of *Pieris canidia* (herbivore) on *Rorippa indica* (host plant) across altitudinal gradients by conducting two experiments: direct and indirect effect experiments (Figure 1). Each experiment had a 3x2x2 factorial design including daily fluctuating temperature treatment (about an average of 21.8°C [control], 24.8°C [+3°C] and 27.8°C [+6°C] on average, based on real data and the prediction of IPCC), CO_2 treatment (500 ppm [control], 1000 ppm [elevation]) and altitude treatment (low- and medium altitude), allowing us to examine the individual and interactive effects of temperature and CO_2. In the direct effect experiment, *P. canidia* larvae were reared under each of the six temperature-CO_2 treatments but fed with *Rorippa* plants grown from a common garden. In the indirect effect experiment, *P. canidia* larvae were reared under ambient treatment (21.8°C on average, 500 ppm CO_2) but fed with *Rorippa* plants grown under each of the six temperature-CO_2 treatments. In both experiments, *P. canidia* and *Rorippa* plants were originally collected from three sites each at low (c.a. 100 m a. s. l.) and medium (c.a. 1000 m a. s. l.) altitudes, to investigate the effect of altitudinal origin and avoid potential idiosyncrasies. The control temperature (21.8°C on average) reflected the daily temperature fluctuation of *P. canidia* peak season (details in methods), and the control CO_2 concentration (500 ppm) represented the ambient condition of our study location. The elevated temperature (+3 and +6°C) and CO_2 concentration (1000 ppm) were comparable to the Intergovernmental Panel on Climate Change (IPCC) prediction for the year 2100 (IPCC 2007, 2014). We measured *P. canidia* and plant traits to investigate the direct and indirect effects of elevated temperature and CO_2 on *P. canidia* performance across altitude. We expected that elevated temperature would affect herbivore (*P. canidia*) performance both directly and indirectly while elevated CO_2 would affect indirectly. We also expected that herbivore populations from low and medium altitudes would perform differently under climate change (i.e., elevated temperature and CO_2) likely due to their different adaptation history.

Materials & Methods

Study system

We used *P. canidia* (herbivore) and *R. indica* (host plant) in this study. *P. canidia* is a common butterfly species in Taiwan distributed from 0 ~ 2500 m a.s.l., and could be multi-generational (呂至堅 and 陳建仁 2014). In Taipei, their populations are active from December to May and peak between March and April at low altitudes. At medium altitudes, they can be observed from spring to at least summer time (personal observations). *P. canidia* mainly hosts on plants of the *Brassicaceae* family including *R. indica* and several agriculture crops (e.g., *Brassica oleracea*). *R. indica* is widespread in Taiwan across latitude and altitude (鍾明哲 2011) and should be an important food source for *P. canidia* in the wild (personal observations).

Experimental design

To investigate how climate change components (i.e., elevated temperature and CO_2 concentration) may directly and indirectly affect herbivore (*P. canidia*) performance across environmental gradients (e.g., altitude), we raised and monitored *P. canidia* larvae in direct and indirect effect experiments, each of which included temperature (21.8°C [control], 24.8°C [+3°C], and 27.8°C [+6°C] on average), CO_2 (500 ppm [control], 1000ppm [elevation]), and altitude origin (low- and medium-altitude) treatments. Each treatment combination (temperature-CO_2-altitude) generally had 12 replicates (each with one *P. canidia*) and may have up to 15 replicates.

Each direct and indirect effect experiment had a 3x2x2 factorial design (Figure 1): three daily fluctuating temperature setting (Table 2; mean = 21.8°C [control], 24.8°C [+3°C] and 27.8°C [+6°C] respectively) x two constant CO_2 levels (control [500 ppm] and elevation [1000 ppm]) x two altitudinal origin (low and medium altitude). In the direct effect experiment, *P. canidia* larvae were reared in each of the six environmental chambers under different (temperature*CO_2) treatments but fed with plants grown from a common garden in the Phytotron of National Taiwan University (constant temperature: 20°C night/ 25°C day). In the indirect effect experiment, *P. canidia* larvae were reared in environmental chambers under control conditions (mean temperature = 21.8°C, CO_2 = 500 ppm) but fed with plants grown under one of the six (temperature*CO_2) treatments.

The control temperature setting was calculated based on data collected from low altitude of Taipei, Taoyuan and Nantou in April from 2008 to 2015 (Data Bank for Atmospheric and Hydrologic Research, Taiwan Typhoon and Flood Research Institute). To make comparison between populations across altitude, the same control temperature setting was used for populations from medium altitude. Regardless, average temperatures of low- and medium-altitude during our field collection months (low altitude: April and medium altitude: August) were similar (Appendix A). The control CO_2 concentration is based on the measurement in our laboratory. Warming and elevated CO_2 settings were based on IPCC predictions (IPCC 2007, 2014). In both direct and indirect effect experiments, individuals collected from low- and medium-altitude sites and were only fed with *R. indica* from same region and altitude to incorporate their coexisting history at local scales (e.g., local adaptation) and reduce potential experimental biases. Photoperiod (light/ dark = 12 hours/ 12 hours) and relative humidity (70 %) settings were kept constant throughout the experiment.

Species collection

P. canidia and *R. indica* were collected from low- (c.a. 100 m a. s. l.) and medium-altitude (c.a. 1000 m a. s. l.) from three regions in Taiwan (Taipei, Taoyuan, Taichung / Nantou) (Table 1) to avoid potential idiosyncrasies. Field collection of low altitude took

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place in 2016 March to April, we captured adult female butterflies and harvested *R. indica* roots letting the plant reproduce asexually. Field collection of medium altitude took place in 2016 July to August, we captured female butterflies from field and cultivated *Rorippa* from seeds collected previously. We induced female butterflies to lay eggs in the laboratory and used new hatched larvae for experiment. The plants were cultivated in the environment according to their experimental group and would be fertilized twice before fed to larvae (20% N, 20% P, 20% K; HYPONeX No.2; concentration at 1:1000; 100 ml each time).

Trait measurement

The newly hatched 1st instar larvae were kept separately with plastic cups inside environmental chambers. For all treatments, *P. canidia* larvae were checked daily and offered fresh leaves (stale leaves inside the plastic cup would be removed and preserved for estimating leaf consumption). Once *P. canidia* reached adulthood, they were kept in mesh cages and fed honey water (honey: water = 1:6). To quantify and analyze performance of herbivore and plant, several traits were recorded as described below.

Herbivore traits To reflect the performance of *P. canidia* under treatment, we recorded a total of 9 herbivore traits: final larval weight (g), larval period (day), relative growth rate (RGR) (1/day), leaf consumption (g), pupal weight (g), pupal period (day), adult weight (g), forewing length (mm) and adult longevity (day). We measured these traits because arthropod weight, larval period, and wing length might correlate with fitness, predation risk, and migration ability, respectively (Stamp and Bowers 1991, Honěk 1993, Li *et al.* 2016). In this study, final larval weight is the heaviest weight record in 5th instar (we measured the larvae every day since they entered 5th instar). Relative growth rate is calculated as: [ln(final larval weight)- ln(initial weight)]/

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duration, with initial weight estimated from additional larvae that were not used for the direct and indirect effect experiments. Leaf consumption is estimated as the amount of leaf dry weight consumed. Specifically, we measured the fresh weight of leaves before giving them to each larva then applied linear regression (based on additional leaves) converting the fresh weight into dry weight. After feeding to the larva, leaf litter was collected, dried, and weighted to derive total leaf consumption = [initial dry weight of leaves – dry weight of litter]. Adult weight and forewing length were measured at the day of emergence.

Plant traits To explain the indirect effect of climate change components (i.e., temperature and CO_2) on herbivore via host plant quality, 7 traits of plants grown from environmental chambers (temperature*CO_2 treatments) were recorded: plant height (cm), leaf number, leaf area (cm^2), chlorophyll content (SPAD value), carbon content (%), nitrogen content (%) and C/N ratio. Plant height, leaf number, leaf area and chlorophyll content were measured using non- destructive methods ($n = 12$ for each temperature-CO_2-altitude combination) while the plants were about one-month-old. Chlorophyll content was measured with Chlorophyll Meter SPAD-502 Plus and reported as SPAD value. Carbon content, nitrogen content, and C/N ratio were measured with destructive methods with additional plants that were not used for the direct and indirect effect experiments. Leaves were collected and dried under 60°C for three days than ground into powder and sent for further analysis with nitrogen and carbon analyzer (FlashEA 1112, Thermo scientific) in Stable Isotope Laboratory at Institute of Ecology and Evolutionary Biology, National Taiwan University.

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Analysis

We applied generalized linear mixed model (GLMM) to analyze data with temperature, CO_2 and altitudinal origin as fixed factors and the region (i.e., Taipei, Taoyuan, Taichung / Nantou) where the species collected as a random factor. We checked normality and homogeneity of variance with Q-Q plot and residual plot (a plot of residuals versus fitted values). For the post hoc analysis, we used least-squares means and Tukey adjustment. All analyses were done under R version 3.4.1 and R studio version 1.0.143, with the 'lmerTest' and 'car' packages.

Results

In general, the direct or indirect effect experiments showed that elevated temperature and CO_2 individually or interactively affected *Pieris* performance, while elevated CO_2 alone affected fewer performance traits than elevated temperature did. In addition, species' altitudinal origin affected *Pieris* performance and mediated the temperature and CO_2 effects. For clarity, the reported results of the direct and indirect effect experiments are classified according to our focal factors (temperature, CO_2, and altitudinal origin).

Temperature

Direct effect experiment

Temperature alone and Temperature*CO_2*Altitude directly affected *P. canidia* performance (Table 3–5). For temperature effect, elevated temperature alone directly reduced the body size of *P. canidia*, suggested by lower body weight (larval [Figure 2, $P < 0.001$], pupal [Figure 6, $P < 0.001$] and adult stage [Figure 8, $P < 0.001$]) and shorter forewing length (Figure 9, $P < 0.001$). However, elevated temperature alone directly increased the developmental rate of *P. canidia*, revealed by a shorter larval period (Figure 3, $P < 0.001$) and faster RGR (Figure 4, $P < 0.001$). In addition, elevated temperature interacted with other factors. For example, the interaction between elevated temperature, elevated CO_2 and altitudinal origin affected larval period (Table 3).

Indirect effect experiment

Temperature and its interactions with CO_2 and altitude indirectly affected *P. canidia* performance (Table 6–8). Specifically, elevated temperature alone indirectly reduced *P. canidia* performance: host plants cultivated under elevated temperature tended to lead to lower *P. canidia* body weight (larval and pupal weight) (Figure 11, P =

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0.012 and, Figure 15, $P = 0.002$), longer larval period (Figure 12, $P = 0.032$), and shorter adult forewing length (Figure 18, $P = 0.007$). In addition, elevated temperature interacted with elevated CO_2 and altitudinal origin and affected larval period, leaf consumption and RGR (Table 6).

CO_2

Direct effect experiment

CO_2 and its interactions with temperature and altitude affected *P. canidia* performance (Table 3–5). Specifically, elevated CO_2 concentration alone affected forewing length (Figure 9, $P = 0.005$), but the direction of the effect was not straightforward due to a factor interaction. Overall, CO_2 interacted with temperature and/or altitude and affected final larval weight, larval period, adult body weight, adult forewing length and adult longevity (Table 3–5). For example, under control CO_2, there was no difference in the forewing length between low- and medium-altitude *P. canidia*. However, under elevated CO_2, medium-altitude *P. canidia* had larger forewing length than did low-altitude *P. canidia* (Figure 9 and Table 5).

Indirect effect experiment

CO_2 concentration alone did not indirectly affect herbivore performance in our indirect experiments (Table 6–8). However, CO_2 treatment interacted with temperature and altitude and indirectly affected larval period (Figure 12, $P = 0.043$), RGR (Figure 13, $P = 0.031$) and leaf consumption (Figure 14, $P < 0.001$). For example, at control temperature, elevated CO_2 did not affect the pattern in RGR of low- vs. medium-altitude *P. canidia* (higher RGR in medium-altitude *P. canidia*). However, at elevated temperature, elevated CO_2 seemed to reduce the difference in RGR between low- vs. medium-altitude *P. canidia* (Figure 13 and Table 6).

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Altitude

Direct effect experiment

Altitudinal origin and its interactions with temperature and CO_2 directly affected *P. canidia* performance (Table 3–5). Note that *P. canidia* were fed with host plants from the same altitude origin (details in Methods). Specifically, *P. canidia* from medium altitude have larger body size, suggested by higher final larval weight (Figure 2, $P < 0.001$), pupal weight (Figure 6, $P < 0.001$) and adult forewing length (Figure 9, $P = 0.022$). Medium-altitude *P. canidia* also consumed more (Figure 5, $P < 0.001$) and grew faster (Figure 4, $P = 0.022$) than low altitude ones. In addition, altitude effects interacted with other effects. For example, altitude effect was mediated by CO_2. The difference in forewing length between low- and medium-altitude *P. canidia* increased under elevated CO_2 (Figure 9 and Table 5).

Indirect effect experiment

Altitudinal origin and its interactions with temperature and CO_2 indirectly affected *P. canidia* performance (Table 6–8). Note that *P. canidia* were fed with host plants from the same altitude origin (details in Methods). Specifically, medium-altitude *P. canidia* had larger body weight (Figure 11, $P = 0.001$; Figure 15, $P = 0.007$). In addition, altitudinal origin of *P. canidia* interacted with temperature and/or CO_2 and indirectly affected larval period, leaf consumption and RGR (Table 6). For example, at control temperature, low-altitude *P. canidia* had longer larval period than did medium-altitude *P. canidia*. However, at elevated temperature, this difference between altitudes reduced (Figure 12 and Table 6).

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Plant trait (environmental chamber)

Temperature

Temperature alone and its interactions with CO_2 and altitudinal origin affected *R. indica* performance (Table 10-11). Specifically, elevated temperature alone increased plant height (Figure 20, $P = 0.001$) and C/N ratio (Figure 26, $P = 0.001$), decreased carbon (Figure 24, $P = 0.046$) and nitrogen (Figure 25, $P < 0.001$) content, and affected leaf area (Figure 22, $P = 0.016$) and chlorophyll (Figure 23, $P = 0.019$) in a complex fashion. In addition, temperature interacted with CO_2 and/or altitudinal origin and affected leaf number, leaf area, carbon content, nitrogen content and C/N ratio (Table 10-11). For example, elevated temperature seemed to reduce the carbon content difference between low and medium altitude *R. indica* (Figure 24 and Table 11).

CO_2

CO_2 concentration alone did not affect *R. indica* traits (Table 10-11). However, CO_2 interacted with temperature and/or altitude and affected leaf number, leaf area, nitrogen content and C/N ratio in complex ways (Table 10-11).

Altitude

Altitudinal origin alone and its interactions with temperature and CO_2 affected *R. indica* traits (Table 10-11). Specifically, *R. indica* from medium altitude had higher plant height (Figure 20, $P = 0.01$), larger leaf area (Figure 22, $P < 0.001$), higher carbon content (Figure 24, $P < 0.001$), higher nitrogen content (Figure 25, $P < 0.001$), lower leaf number (Figure 21, $P < 0.001$), lower chlorophyll content (Figure 23, $P < 0.001$) and lower C/N ratio (Figure 26, $P < 0.001$) than *R. indica* from low altitude. In addition, altitudinal origin of *R. indica* interacted with temperature and/or CO_2 and affected leaf area, carbon content, nitrogen content and C/N ratio (Table 10-11).

Discussion

To better understand the mechanisms through which climate change components affect plant-herbivore interactions across spatial gradients, we filled three knowledge gaps by empirically examining how elevated temperature and CO_2 would directly (*knowledge gap 1*) and indirectly (*knowledge gap 2*) affect herbivore performance across altitudinal gradients (*knowledge gap 3*). This integrated, pioneering study offers an opportunity to clarify the mechanisms for climate change effects across spatial gradients (altitude). Our important findings include these: 1) Elevated temperature (alone or interacting with CO_2 and altitude) directly and indirectly affected *P. canidia* performance, although some effects were conflicting. 2) Compared to temperature, elevated CO_2 alone directly affected fewer *P. canidia* performance traits (elevated CO_2 alone did not have indirect effects). Importantly, similar to temperature, elevated CO_2 also interacted with other factors and directly and indirectly affected *P. canidia* performance. 3) Altitudinal origin (alone or interacting with temperature and CO_2) affected *P. canidia* performance in direct and indirect experiments (summary in Table 9). The results suggest that future climate change assessments may need to consider the interplay between global change components and the intraspecific variation across spatial gradients. Based on the results, we discuss a) the conflicting effects of elevated temperature on *P. canidia* performance, b) the interactive effects of CO_2 and other factors, c) altitudinal effect, d) plant effect, and e) potential caveats of the experiment.

Conflicting warming effects on species performance

An important contribution of this integrated study to climate change ecology is that it revealed conflicting climate change effects on species. The conflicting effects may need to be included in ecological models in order to precisely predict climate change impact, otherwise, the effects of climate change factors may be overestimated. In addition, our direct and indirect effect experiments showed that these conflicting effects could act through different traits (e.g., warming-induced smaller body size [lower individual fitness] vs. shorter generation time [facilitating population growth]) or the same traits (e.g., warming-induced positive direct vs. negative indirect effects on individual development). For example, while temperature effect (e.g., size reduction under warming) is well known in previous studies (Gardner *et al.* 2011), this study revealed its conflicting effects on herbivore performance via affecting different traits or the same trait. In the case of different traits, warming directly reduced *P. canidia* body size (Figure 2), which might reduce individual fitness and population growth. However, warming also directly increased developmental rate of *P. canidia* (Figure 3), which could lower predation risk, increase number of generations in a year, and benefit population growth. In the case of the same trait, warming directly reduced but indirectly increased larval period (Figure 3 and Figure 12), creating conflicting effects on larval development. While it is difficult to predict the consequences of conflicting effects of climate change components in the field, we suggest that the net effects may be determined by the abiotic (e.g., temperature range) and biotic factors (e.g., trophic interactions) our focal species encounter.

Interactive effects of CO_2 and other factors

While previous studies often examined CO_2 effects alone (Fajer *et al.* 1989, Srinivasa Rao *et al.* 2012, Foss *et al.* 2013), the results of our direct and indirect effect experiments suggest that climate change impact assessment should consider the interaction between CO_2 and other factors because these interactions can play a significant role. For example, in this study, elevated CO_2 interacted with other factors (temperature and altitude) and affected many herbivore traits (e.g., final larval weight,

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larval period, RGR, leaf consumption, adult forewing length, adult weight, adult longevity) (Table 3-6). Therefore, climate change assessment that does not consider the interaction between CO_2 and other factors (e.g., temperature, spatial variation, etc.) will likely underestimate the effect of CO_2 and make a less accurate prediction. Due to the complexity of the direction and strength of these interaction effects, empirical studies like this one can provide valuable parameters for ecological models that help climate change assessment.

This study showed that CO_2 usually interacted with other factors and significantly affected herbivore performance, rather than that CO_2 alone generated these effects (Table 9). This is different from temperature effect that alone or together with other factors significantly affected herbivore performance. Although exploring the underlying mechanisms for the interactions between CO_2 and other factors is outside the scope of this study, possible explanations include these. First, species may have adapted to local CO_2 level at specific altitude (i.e., CO_2-altitude interaction). Second, host plants may respond to the same CO_2 level differently under different temperature and then affect herbivores (i.e., CO_2-temperature interaction). In fact, a CO_2-temperature interaction on host plants was observed in this study (e.g., leaf number, N content, and C/N ratio) (Table 10-11).

Altitudinal effects

The strong altitude effects in our direct and indirect effect experiments suggest significant intraspecific variations in our herbivores across spatial gradients, supporting our speculation that climate change impact assessment may need to consider intraspecific variation by treating a population, instead of an entire species, as a responsive unit. Specifically, this study found significant intraspecific variations in

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herbivore performance across altitude under elevated temperature and CO_2 treatments (e.g., final larval weight, larval period, RGR, leaf consumption, pupal weight, pupal period, adult weight, forewing length and adult longevity) (Table 3-7). In other words, herbivore populations (of the same species) from different altitudes performed differently under climate change scenarios (i.e., elevated temperature and CO_2). Noticeably, herbivore performance was affected by altitude effect alone or its interaction with other factors, similar to the case in warming effect.

It is interesting that our herbivore populations from medium altitude performed better than those from low altitude (e.g., larger body size [larval and pupal weight]) (Figure 2, Figure 6, Figure 11 and Figure 15). Although the underlying mechanisms are unclear and the study design has limitations (see Caveats section below), it is possible that the larger body size at higher altitude (consistent with Bergmann's rule) (Bergmann 1847) may be an adaptation to harsher environment (e.g., starvation resistance hypothesis) (Arnett and Gotelli 2003). The faster growth rate may be an adaption to a shorter growth season at higher altitude, similar to the cases in latitudinal systems (Arnett and Gotelli 1999). Regardless of the underlying mechanisms, the results suggest that future work can investigate whether the better performance of medium altitude herbivores, compared to low altitude conspecifics, is a common phenomenon and will lead to population expansion under climate change.

Plant effects

Our study results in plant traits highlight these potential indirect effects that are often overlooked by current climate change studies: 1) Climate change (e.g., elevated temperature) could affect plant quality and then herbivore performance. 2) Spatial variation (e.g., altitude) in plant quality could affect herbivore performance. The indirect

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experiment of this study revealed some potential correlations between plant and herbivore performance over temperature or altitudinal gradients (Table 12), such as a positive correlation between plant nitrogen content and herbivore performance (Figure 27). Therefore, any effects of climate change or spatial variation on plants may have a consequence on herbivore performance. For example, warming reduced the nitrogen content of host plants in this study (Figure 25), likely contributed to the reduction of herbivore performance (e.g., lower final larval and pupal weight under warming) (Figure 11 and Figure 15). In addition, the nitrogen content of host plants from medium altitude tended to be higher than that from low altitude (Figure 25), partially explaining the better performance in medium-altitude herbivores (e.g., higher final larval weight and pupal weight) (Figure 11 and Figure 15). The correlation between higher plant quality and better herbivore performance at higher altitude in this study is actually consistent with the case in a latitudinal system (Ho *et al.* 2009, Ho and Pennings 2013), suggesting the importance for climate change studies to consider intraspecific variation across spatial gradients.

Caveats

This study detected altitude effects on most herbivore performance traits, highlighting the critical role of altitudinal origin in herbivore performance under current or future climate conditions. However, the design of this study does not allow us to dissect the underlying mechanism – whether the result was contributed by plant origin, herbivore origin, or both. This is because we fed herbivore with host plants from the same altitudinal origin (e.g., low-altitude plants for low-altitude herbivores), in order to consider their coexisting history at local scale (e.g., local adaptation) and reduce potential experimental biases. Note that both of the herbivore (*P. canidia*) and host plant (*R. indica*) have reportedly shown the effect of altitudinal origin in common garden:

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medium-altitude *P. canidia* were larger (余淑惠 2016) and medium-altitude *R. indica* were taller and had higher leaf nitrogen content than low-altitude conspecifics (Figure 20 and Figure 25 in this study). To understand the effects of plant origin and herbivore origin on herbivore performance under climate change, we encourage future studies that apply factorial design experiments (e.g., herbivore origin x plant origin) to investigate this topic.

Conclusions

This pioneering, empirical study provides an opportunity to investigate the mechanisms for climate change effects (elevated temperature and CO_2) on herbivores across spatial gradients (altitude). There are two main findings and suggestions: 1) Elevated temperature and CO_2 acted individually or interactively to directly or indirectly affect species performance, suggesting that climate change impact assessments may need to consider the interplay of climate change components. 2) Species' altitudinal origin affected species performance, suggesting that climate change impact assessments should consider intraspecific variation across spatial gradients, such as treating a population, instead of an entire species, as a responsive unit.

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Table 1. Collection sites

P. canidia and *R. indica* were collected from low-altitude (L) (c.a. 100 m a.s.l.) and medium-altitude (M) (c.a. 1000 m a.s.l.) sites from three regions (Taipei, Taoyuan and Taichung-Nantou). (Modified from (顏韶寬 2017)).

Collection site	Altitude (L or M)	Species	Longitude	Latitude
Taipei				
NTU	16 m (L)	*P. canidia* and *R. indica*	121°32'19"E	25°00'56"N
Zhuzihu	701 m (M)	*P. canidia*	121°32'12"E	25°10'31"N
Mt. Datun	1093 m (M)	*R. indica*	121°31'20"E	25°10'37"N
Taoyuan				
NCU	137 m (L)	*P. canidia*	121°11'32"E	24°58'08"N
Cihu	231 m (L)	*R. indica*	121°17'35"E	24°50'24"N
Dongyangshan	925 m (M)	*P. canidia* and R.indica	121°24'35"E	24°49'41"N
Taichung-Nantou				
Central District	88 m (L)	*P. canidia*	120°40'53"E	24°08'11"N
Zhongliao	202 m (L)	*P. canidia* and *R. indica*	120°44'19"E	23°53'21"N
Xitou	1128 m (M)	*P. canidia* and *R. indica*	120°47'00"E	23°40'00"N

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Table 2. Temperature settings for environmental chambers

This study used environmental chambers to investigate three temperature treatment groups (control, +3°C and +6°C). The temperature in each treatment group fluctuated in a 24-hour period to simulate the real temperature dynamics in the field. The control and warming temperatures were determined based on real data.

Time	Control (°C)	+3 (°C)	+6 (°C)
02:00	20.1	23.1	26.1
06:00	19.6	22.6	25.6
07:00	20.2	23.2	26.2
10:00	23.4	26.4	29.4
12:00	24.5	27.5	30.5
13:00	24.5	27.5	30.5
15:00	24.2	27.2	30.2
16:00	23.7	26.7	29.7
17:00	23.1	26.1	29.1
18:00	22.4	25.4	28.4
19:00	21.8	24.8	27.8
20:00	21.4	24.4	27.4
23:00	20.7	23.7	26.7

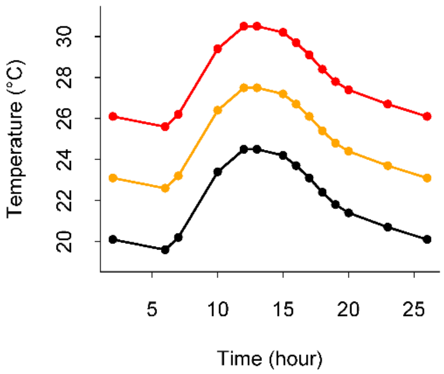

Table 3. Larval performance of *P. canidia* (direct experiment)

Analysis of deviance table of generalized linear mixed model for the larval performance of *P. canidia* (from the direct experiment). Temperature (T) = temperature treatment (control, +3°C and +6°C). CO_2 (C) = CO_2 treatment (control [500 ppm] and elevation [1000 ppm]). Altitude (A) = altitude treatment (low- and medium-altitude origin). Bold numbers indicate statistically significant.

a)

	Final larval weight				**Larval period**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	27.29	2	100.308	**< 0.001**	104.43	2	100.07	**< 0.001**
C	0.19	1	100.899	0.667	0.93	1	100.23	0.338
A	55.30	1	101.697	**< 0.001**	3.39	1	100.51	0.068
T×C	2.70	2	100.326	0.072	0.13	2	100.08	0.876
T×A	2.78	2	100.109	0.067	2.36	2	100.03	0.099
C×A	4.94	1	100.893	**0.028**	3.73	1	100.23	0.056
T×C×A	0.31	2	100.256	0.732	3.44	2	100.06	**0.036**

b)

	Relative growth rate				**Leaf consumption**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	57.75	2	100.127	**< 0.001**	2.54	2	97.291	0.084
C	0.41	1	100.394	0.523	0.01	1	97.794	0.926
A	5.40	1	100.834	**0.022**	279.27	1	98.222	**< 0.001**
T×C	0.75	2	100.139	0.474	1.77	2	97.431	0.176
T×A	1.49	2	100.043	0.230	0.17	2	97.113	0.844
C×A	0.56	1	100.391	0.456	1.43	1	97.787	0.235
T×C×A	1.38	2	100.104	0.255	0.72	2	97.376	0.488

Table 4. Pupal performance of *P. canidia* (direct experiment)

Analysis of deviance table of generalized linear mixed model for the pupal performance of *P. canidia* (from the direct experiment). Temperature (T) = temperature treatment (control, +3°C and +6°C). CO_2 (C) = CO_2 treatment (control [500 ppm] and elevation [1000 ppm]). Altitude (A) = altitude treatment (low- and medium-altitude origin). Bold numbers indicate statistically significant.

	Pupal weight				Pupal period			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	10.41	2	100.845	**< 0.001**	85.95	2	85.042	**< 0.001**
C	1.34	1	101.902	0.250	3.60	1	85.181	0.061
A	42.47	1	99.762	**< 0.001**	2.29	1	85.968	0.134
T×C	0.33	2	100.819	0.722	0.74	2	85.455	0.482
T×A	0.73	2	100.357	0.486	0.15	2	85.051	0.859
C×A	2.81	1	101.902	0.097	0.80	1	85.211	0.374
T×C×A	0.01	2	100.736	0.986	1.57	2	85.438	0.214

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Table 5. Adult performance of *P. canidia* (direct experiment)

Analysis of deviance table of generalized linear mixed model for the adult performance of *P. canidia* (from the direct experiment). Temperature (T) = temperature treatment (control, +3°C and +6°C). CO_2 (C) = CO_2 treatment (control [500 ppm] and elevation [1000 ppm]). Altitude (A) = altitude treatment (low- and medium-altitude origin). Bold numbers indicate statistically significant.

	Adult weight				**Forewing length**				**Adult longevity**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	13.16	2	85.103	< **0.001**	9.53	2	79.157	< **0.001**	2.83	2	85.31	0.065
C	1.49	1	85.438	0.225	8.27	1	79.999	**0.005**	6.18	1	86.23	**0.015**
A	2.72	1	86.848	0.103	5.56	1	53.756	**0.022**	0.24	1	82.67	0.623
T×C	0.10	2	85.994	0.903	1.38	2	79.039	0.257	0.27	2	86.16	0.765
T×A	1.43	2	85.124	0.244	0.01	2	79.12	0.988	1.24	2	85.375	0.293
C×A	5.16	1	85.495	**0.026**	11.12	1	79.781	**0.001**	7.40	1	86.275	**0.008**
T×C×A	2.14	2	85.946	0.124	1.55	2	79.402	0.220	0.98	2	85.807	0.381

Table 6. Larval performance of *P. canidia* (indirect experiment)

Analysis of deviance table of generalized linear mixed model for the larval performance of *P. canidia* (from the indirect experiment). Temperature (T) = temperature treatment (control, +3°C and +6°C). CO_2 (C) = CO_2 treatment (control [500 ppm] and elevation [1000 ppm]). Altitude (A) = altitude treatment (low- and medium-altitude origin). Bold numbers indicate statistically significant.

a)

	Final larval weight				**Larval period**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	4.68	2	76.614	**0.012**	3.59	2	75.646	**0.032**
C	0.03	1	76.999	0.858	1.41	1	75.85	0.239
A	13.01	1	70.544	**0.001**	0.84	1	76.681	0.362
T×C	0.23	2	76.221	0.792	0.54	2	75.399	0.586
T×A	2.04	2	76.275	0.137	6.45	2	75.444	**0.003**
C×A	0.04	1	75.996	0.848	1.33	1	75.249	0.253
T×C×A	0.86	2	76.182	0.425	3.29	2	75.355	**0.043**

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b)

	Relative growth rate				**Leaf consumption**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	4.14	2	75.495	**0.020**	10.83	2	75.156	**< 0.001**
C	1.11	1	75.654	0.295	1.18	1	75.211	0.281
A	2.30	1	76.371	0.134	27.05	1	52.278	**< 0.001**
T×C	0.64	2	75.301	0.531	16.81	2	75.163	**< 0.001**
T×A	9.00	2	75.337	**< 0.001**	0.23	2	75.253	0.795
C×A	1.06	1	75.185	0.307	39.66	1	75.339	**< 0.001**
T×C×A	3.63	2	75.267	**0.031**	16.18	2	75.451	**< 0.001**

Table 7. Pupal performance of *P. canidia* (indirect experiment)

Analysis of deviance table of generalized linear mixed model for the pupal performance of *P. canidia* (from the indirect experiment). Temperature (T) = temperature treatment (control, +3°C and +6°C). CO_2 (C) = CO_2 treatment (control [500 ppm] and elevation [1000 ppm]). Altitude (A) = altitude treatment (low- and medium-altitude origin). Bold numbers indicate statistically significant.

	Pupal weight				**Pupal period**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	7.04	2	76.194	**0.002**	2.23	2	56.225	0.117
C	1.21	1	76.291	0.274	0.08	1	56.975	0.773
A	7.86	1	50.687	**0.007**	3.55	1	36.519	0.068
T×C	0.71	2	76.287	0.495	1.21	2	56.241	0.307
T×A	0.31	2	76.057	0.735	2.95	2	55.717	0.061
C×A	0.06	1	76.559	0.808	0.00	1	56.404	0.981
T×C×A	0.15	2	76.397	0.858	0.15	2	55.823	0.863

Table 8. Adult performance of *P. canidia* (indirect experiment)

Analysis of deviance table of generalized linear mixed model for the adult performance of *P. canidia* (from the indirect experiment). Temperature (T) = temperature treatment (control, +3°C and +6°C). CO_2 (C) = CO_2 treatment (control [500 ppm] and elevation [1000 ppm]). Altitude (A) = altitude treatment (low- and medium-altitude origin). Bold numbers indicate statistically significant.

	Adult weight				**Forewing length**				**Adult longevity**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	1.12	2	56.332	0.335	5.43	2	53.956	**0.007**	0.31	2	55.456	0.735
C	2.24	1	56.923	0.140	0.01	1	54.181	0.927	0.01	1	55.839	0.913
A	4.22	1	21.911	0.052	0.59	1	54.636	0.446	3.05	1	56.996	0.086
T×C	0.84	2	56.455	0.436	1.25	2	53.575	0.296	2.18	2	55.424	0.122
T×A	0.00	2	55.924	0.996	1.07	2	53.556	0.350	1.25	2	55.213	0.295
C×A	1.14	1	56.782	0.290	0.12	1	53.57	0.732	0.00	1	55.392	0.988
T×C×A	0.97	2	56.057	0.386	0.78	2	53.371	0.465	0.07	2	55.245	0.935

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Table 9. Summary of *Pieris* performance

Pieris performance in the direct and indirect effect experiments. Arrows ↑ and ↓ indicate increasing and decreasing patterns, respectively.

a) Direct effect experiment

Stage	Temperature (↑)	CO$_2$ (↑)	Altitude (↑)	Interaction
Larval	Weight (↓) Period (↓) RGR (↑)		Weight (↑) RGR (↑) Consumption (↑)	CO$_2$×Alt: Weight TempxCO$_2$×Alt: Period
Pupal	Weight (↓) Period (↓)		Weight (↑)	
Adult	Weight (↓) Forewing length (↓)	Forewing length Longevity	Forewing length (↑)	CO$_2$×Alt: Weight forewing length longevity

b) Indirect effect experiment

Stage	Temperature (↑)	CO$_2$ (↑)	Altitude (↑)	Interaction
Larval	Weight (↓) Period (↑) RGR Consumption		Weight (↑) Consumption	TempxCO$_2$: Consumption Temp×Alt: Period RGR CO$_2$×Alt: Consumption TempxCO$_2$×Alt: Period RGR Consumption
Pupal	Weight (↓)		Weight (↑)	
Adult	Forewing length(↓)			

Table 10. Performance of *R. indica*

Analysis of deviance table of generalized linear mixed model for the performance of *R. indica*. Temperature (T) = temperature treatment (control, +3°C and +6°C). CO_2 (C) = CO_2 treatment (control [500 ppm] and elevation [1000 ppm]). Altitude (A) = altitude treatment (low- and medium-altitude origin). Bold numbers indicate statistically significant.

a)

	Plant height				Leaf number			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	7.34	2	126.12	**0.001**	2.51	2	126.09	0.085
C	2.10	1	126.13	0.150	0.21	1	126.09	0.645
A	6.79	1	126.13	**0.010**	41.74	1	126.09	**< 0.001**
T×C	2.45	2	126.12	0.090	4.75	2	126.09	**0.010**
T×A	0.76	2	126.12	0.470	0.99	2	126.09	0.376
C×A	0.42	1	126.13	0.518	1.19	1	126.09	0.278
T×C×A	2.08	2	126.12	0.129	2.27	2	126.09	0.107

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b)

	Leaf area				**Chlorophyll**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	4.31	2	126.1	**0.016**	4.07	2	126.02	**0.019**
C	2.90	1	126.11	0.091	0.92	1	126.02	0.340
A	40.80	1	126.11	**< 0.001**	35.85	1	126.02	**< 0.001**
T×C	2.75	2	126.1	0.068	0.12	2	126.02	0.888
T×A	1.00	2	126.1	0.372	2.21	2	126.02	0.114
C×A	0.87	1	126.11	0.354	1.46	1	126.02	0.230
T×C×A	3.27	2	126.1	**0.041**	0.43	2	126.02	0.655

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Table 11. Quality of *R. indica*

Analysis of deviance table of generalized linear mixed model for the quality of *R. indica.* Temperature (T) = temperature treatment (control, +3°C and +6°C). CO_2 (C) = CO_2 treatment (control [500 ppm] and elevation [1000 ppm]). Altitude (A) = altitude treatment (low- and medium-altitude origin). Bold numbers indicate statistically significant.

	C content				**N content**				**C/N ratio**			
	F	Df	Df.res	*P*	*F*	Df	Df.res	*P*	*F*	Df	Df.res	*P*
T	3.17	2	120.064	**0.046**	20.97	2	120.034	**< 0.001**	7.91	2	120.09	**0.001**
C	0.05	1	120.062	0.830	0.61	1	120.033	0.435	0.58	1	120.087	0.447
A	103.23	1	120.038	**< 0.001**	29.59	1	120.02	**< 0.001**	14.24	1	120.054	**< 0.001**
T×C	1.65	2	120.053	0.196	7.81	2	120.028	**0.001**	4.50	2	120.074	**0.013**
T×A	5.24	2	120.064	**0.007**	5.53	2	120.034	**0.005**	4.43	2	120.09	**0.014**
C×A	2.16	1	120.062	0.145	3.91	1	120.033	0.050	0.71	1	120.087	0.402
T×C×A	2.93	2	120.053	0.057	5.47	2	120.028	**0.005**	7.32	2	120.074	**0.001**

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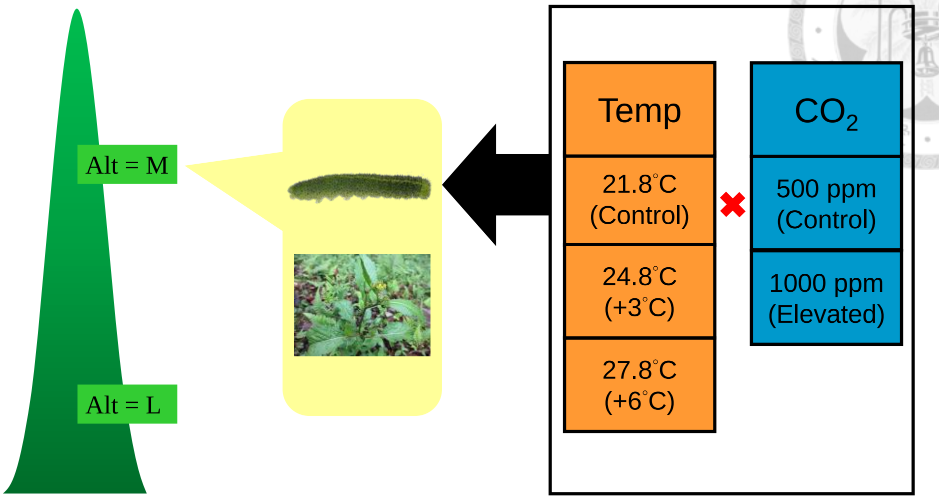
Table 12 Summary of indirect effect experiment

Responses of *P. canidia* and *R. indica* performance to increasing temperature and altitude in the indirect experiment. Arrows ↑ and ↓ indicate increasing and decreasing patterns, respectively.

	R. indica	*P. canidia*
Temperature (↑)	Nitrogen content (↓) Carbon content (↓) Plant height (↑) C/N ratio (↑)	Final larval weight (↓) Pupal weight (↓) Forewing length (↓) Larval period (↑)
Altitude (↑)	Leaf number (↓) Chlorophyll (↓) C/N ratio (↓) Carbon content (↑) Nitrogen content (↑) Leaf area (↑)	Final larval weight (↑) Pupal weight (↑)

a) Direct experiment



b) Indirect experiment

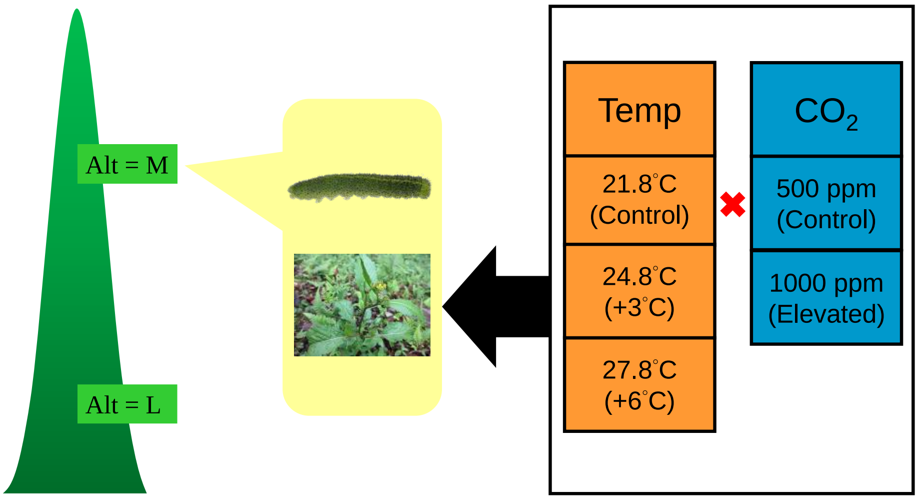


Figure 1. Concept map

This laboratory study empirically examined 1) how climate change components (i.e., warming and elevated CO$_2$ concentration) may affect herbivore (*P. canidia*) performance directly (a) and indirectly (b; via changing the host plant *R. indica*), and 2) whether these effects will vary with spatial gradients (altitude). To investigate the effect of warming and elevated CO$_2$ on herbivore performance, this study crossed temperature treatment (mean = 21.8°C [control], 24.8°C [+3°C] and 27.8°C [+6°C] respectively) with CO$_2$ treatment (control [500 ppm] and elevation [1000 ppm]). Herbivores and plants were originally collected from low (L; c.a. 100 m a. s. l.) and medium (M; c.a. 1000 m a. s. l.) altitude (Alt). The altitudinal origin of herbivores and plants were paired in the direct and indirect experiments. In the direct experiment, temperature and CO$_2$ treatment applied to herbivores. In the indirect experiment, temperature and CO$_2$ treatment applied to host plants.

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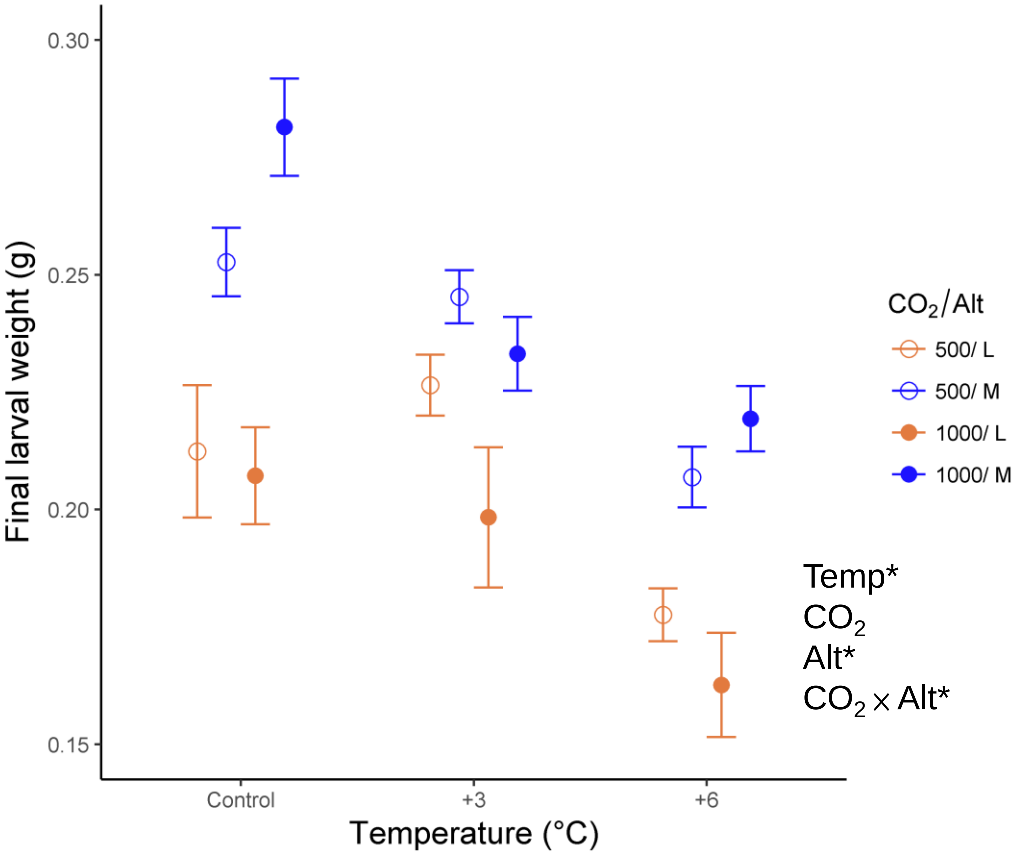

Figure 2. Final larval weight of *P. canidia* (direct experiment)

Final larval weight of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 114). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	6	7	8
500	M	14	13	12
1000	L	6	4	5
1000	M	13	13	13

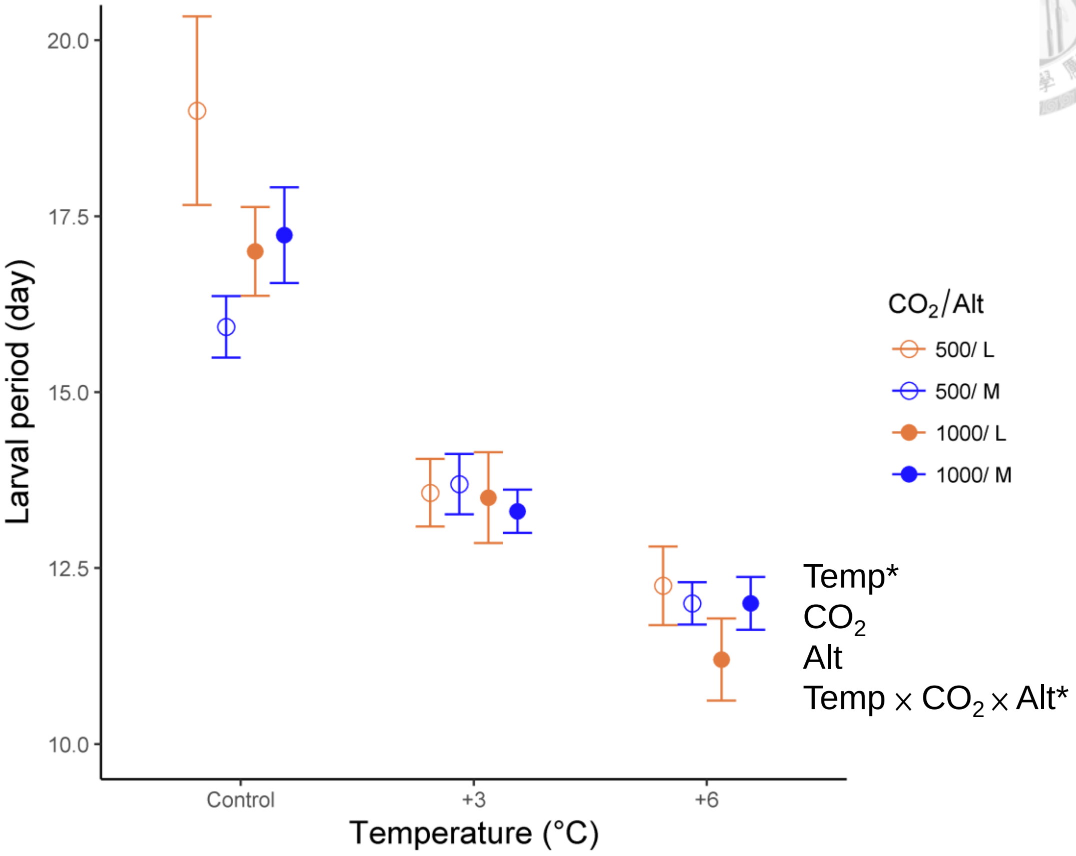

Figure 3. Larval period of *P. canidia* (direct experiment)

Larval period of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 114). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	6	7	8
500	M	14	13	12
1000	L	6	4	5
1000	M	13	13	13

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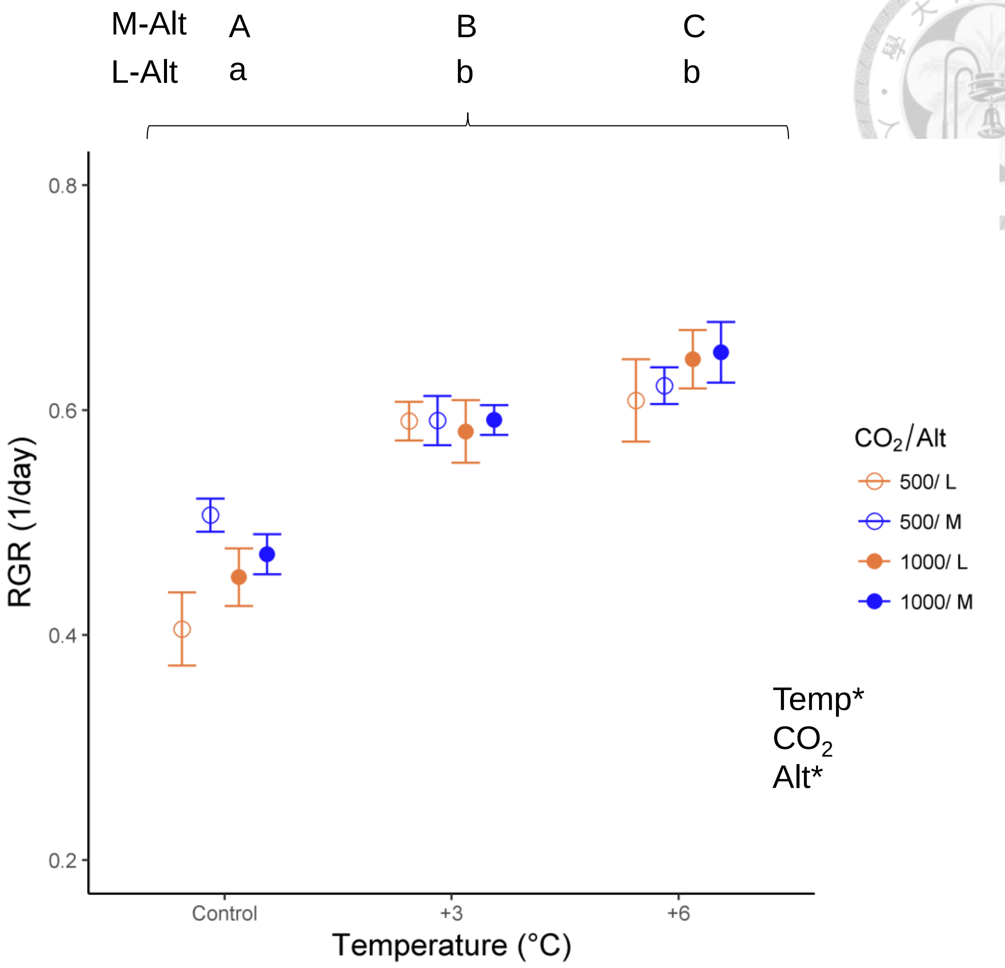

Figure 4. Relative growth rate (RGR) of *P. canidia* (direct experiment)

Relative growth rate (RGR) of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 114). Asterisk indicates significant difference based on GLMM ($P < 0.05$). Post hoc pairwise comparisons across temperature were conducted using Tukey test separately for low and medium altitude populations. Within each altitude, the same letter indicates no significant difference across temperature.

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	6	7	8
500	M	14	13	12
1000	L	6	4	5
1000	M	13	13	13

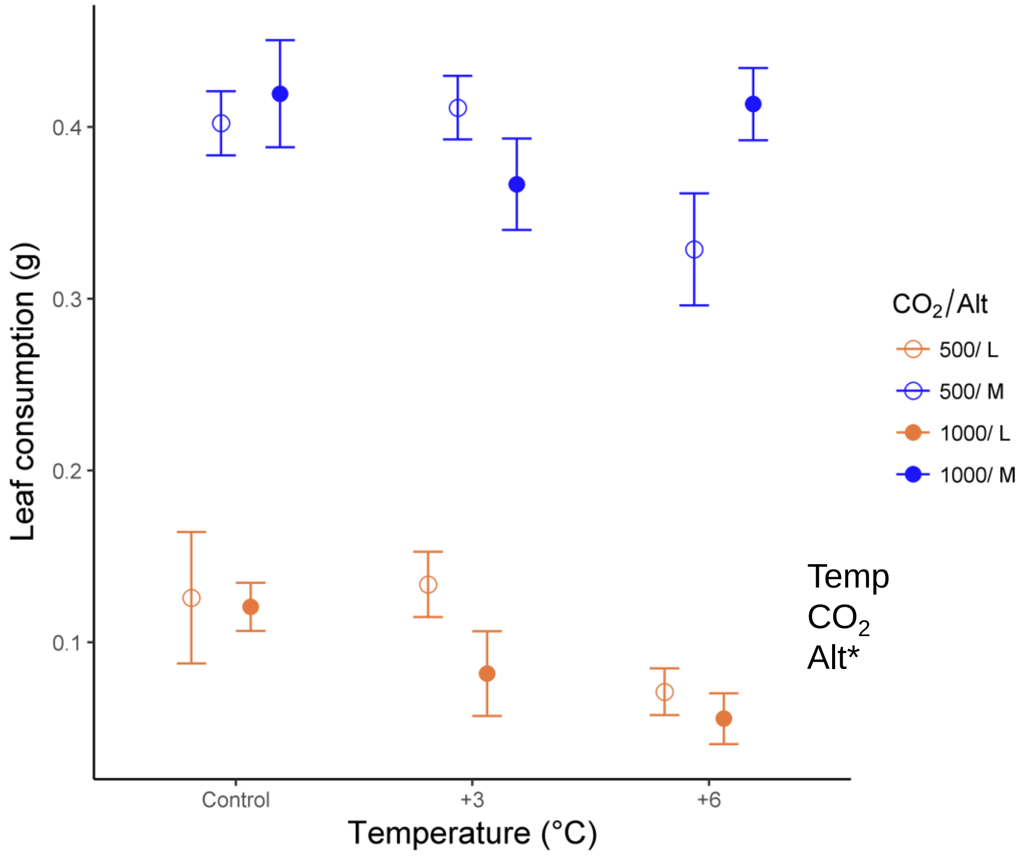

Figure 5. Leaf consumption of *P. canidia* (direct experiment)

Leaf consumption of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 111). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	6	6	8
500	M	14	13	12
1000	L	5	4	4
1000	M	13	13	13

doi: 10.6342/NTU201802543

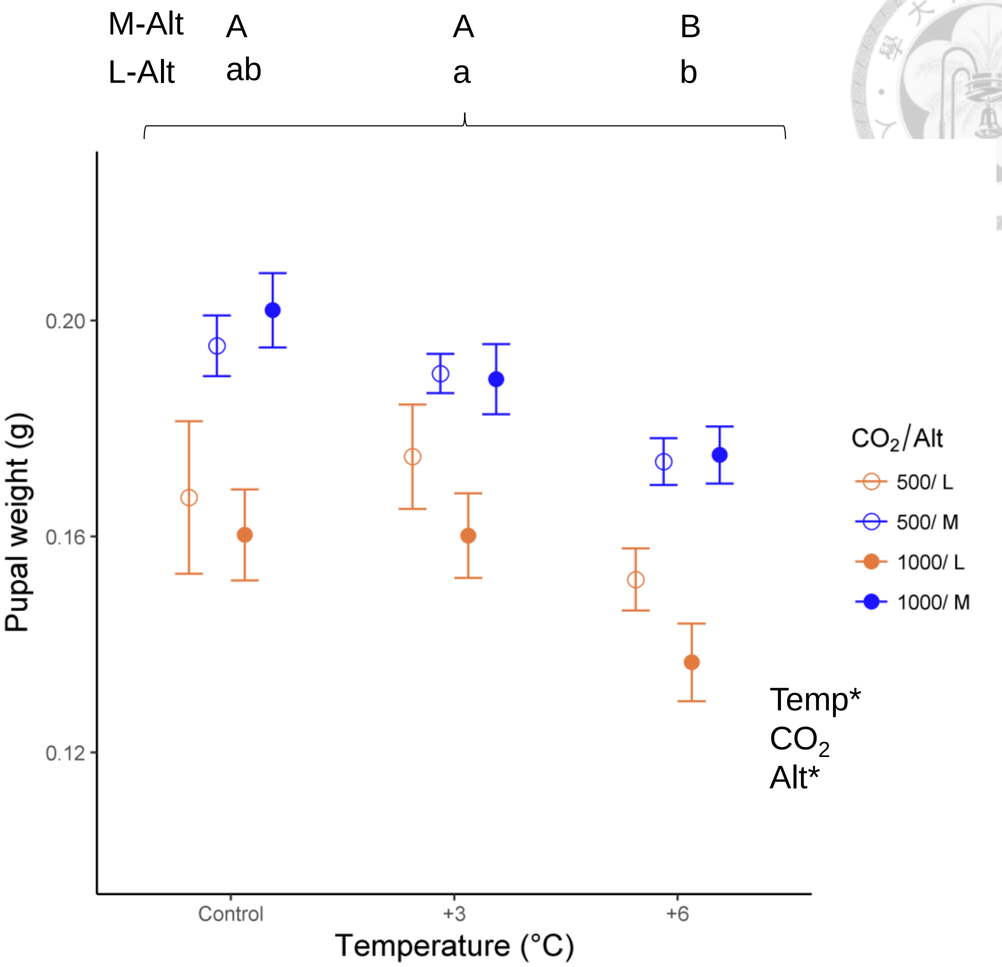

Figure 6. Pupal weight of *P. canidia* (direct experiment)

Pupal weight of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 114). Asterisk indicates significant difference based on GLMM ($P < 0.05$). Post hoc pairwise comparisons across temperature were conducted using Tukey test separately for low and medium altitude populations. Within each altitude, the same letter indicates no significant difference across temperature.

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	6	7	8
500	M	14	13	12
1000	L	6	4	5
1000	M	13	13	13

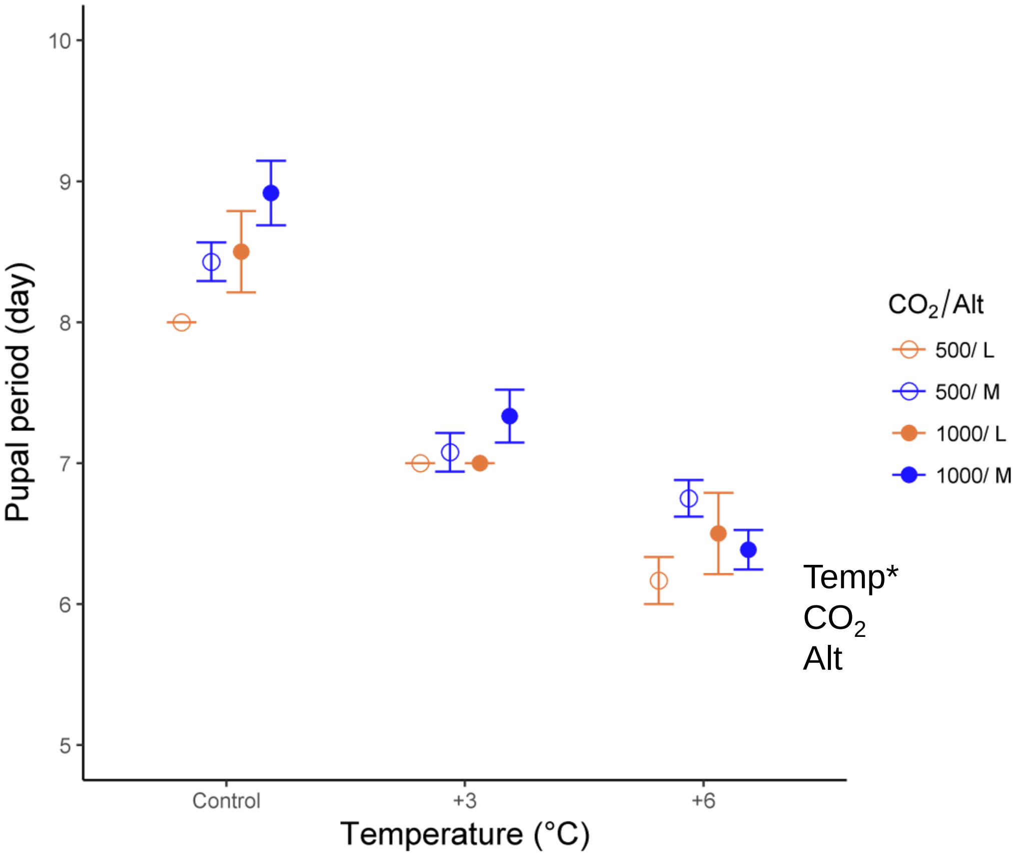

Figure 7. Pupal period of *P. canidia* (direct experiment)

Pupal period of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 99). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	2	4	6
500	M	14	13	12
1000	L	4	3	4
1000	M	12	12	13

doi: 10.6342/NTU201802543

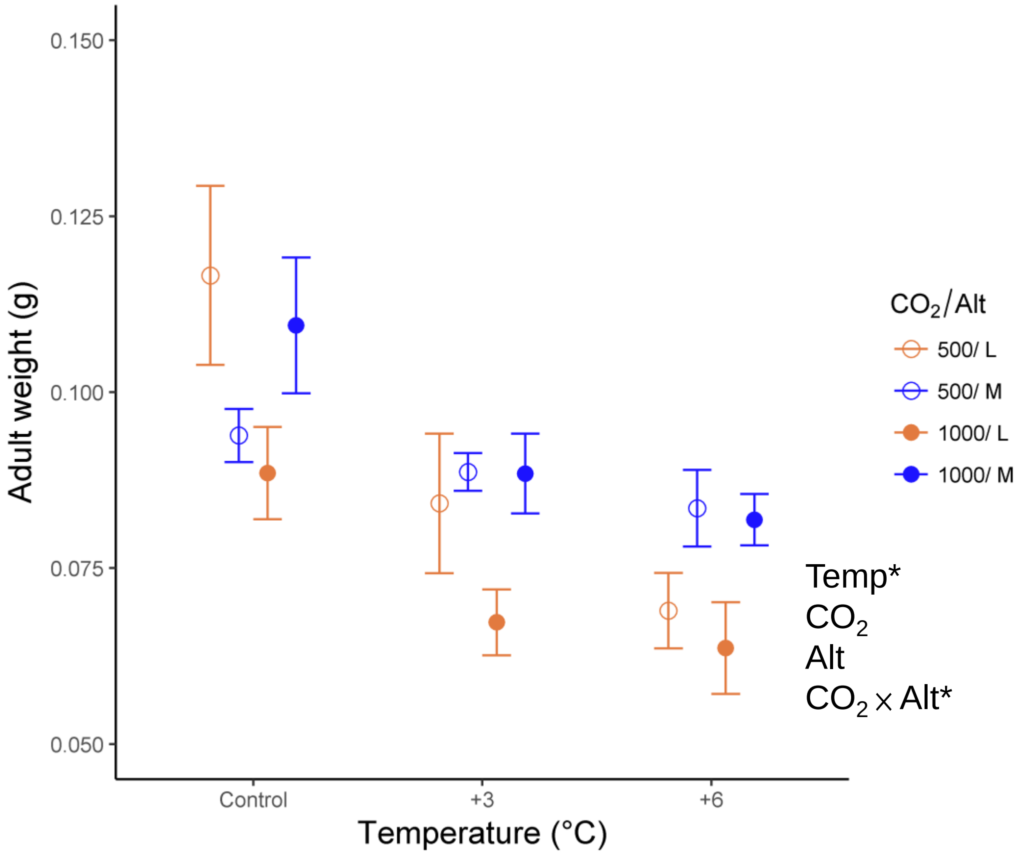

Figure 8. Adult weight of *P. canidia* (direct experiment)

Adult weight of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 99). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	2	4	6
500	M	14	13	12
1000	L	4	3	4
1000	M	12	12	13

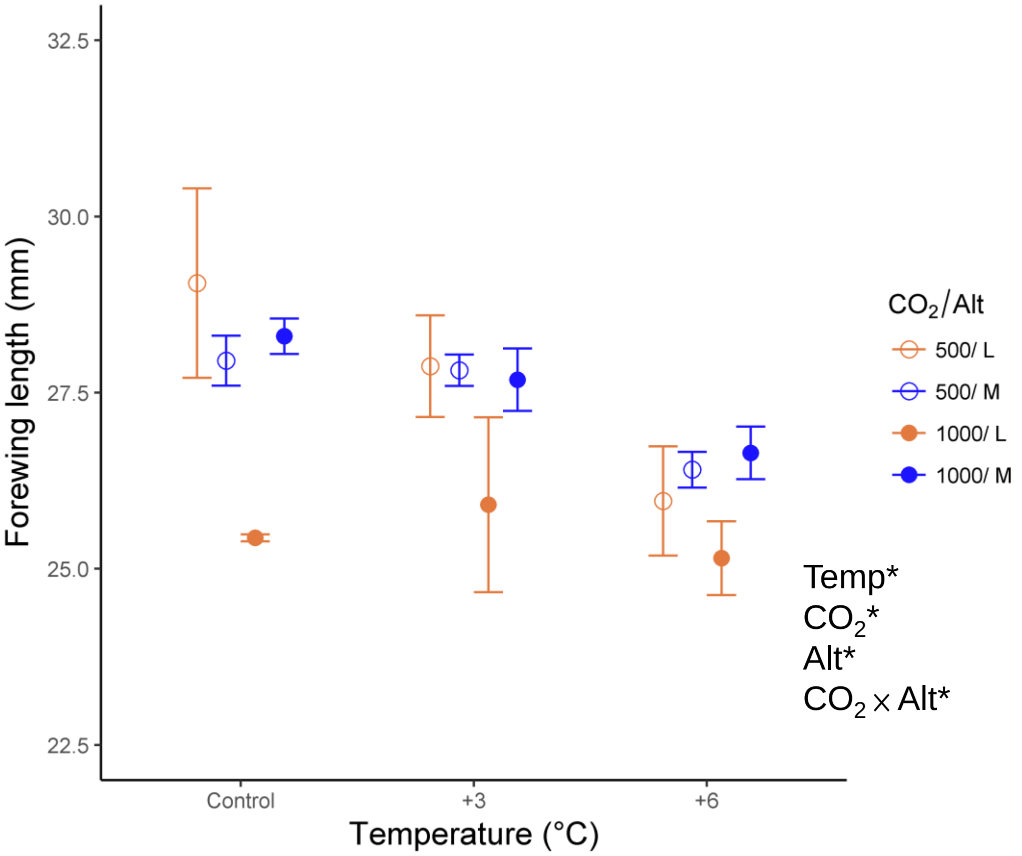

Figure 9. Forewing length of *P. canidia* (direct experiment)

Forewing length of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 92). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	2	4	4
500	M	14	13	12
1000	L	2	2	3
1000	M	11	12	13

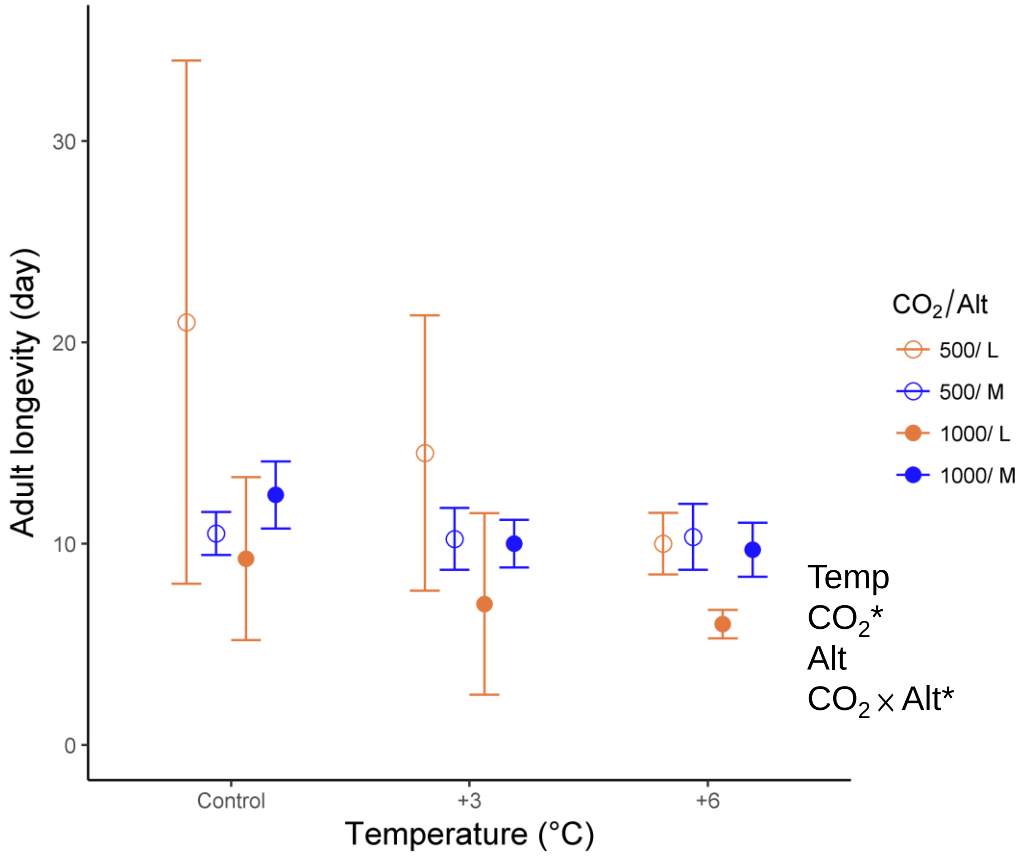

Figure 10. Adult longevity of *P. canidia* (direct experiment)

Adult longevity of low (L; orange color) and medium (M; blue color) altitude *P. canidia* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the direct experiment (mean ± SE, N = 99). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	2	4	6
500	M	14	13	12
1000	L	4	3	4
1000	M	12	12	13

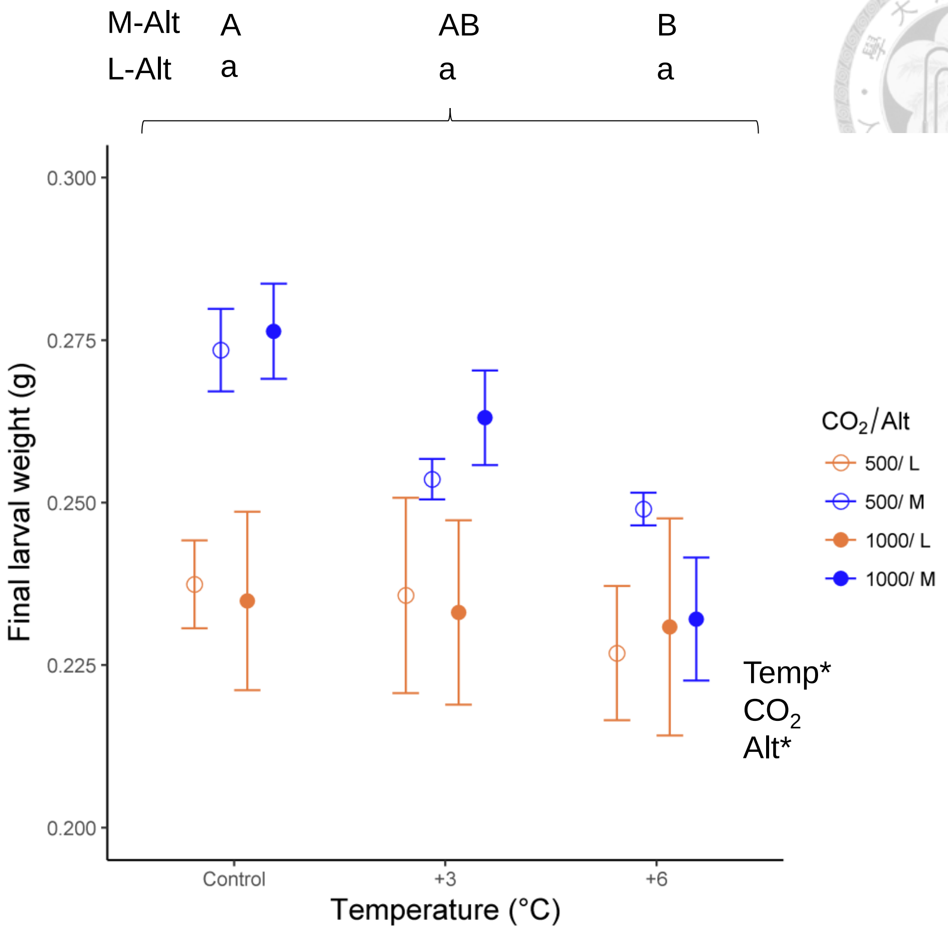

Figure 11. Final larval weight of *P. canidia* (indirect experiment)

Final larval weight of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 89). Asterisk indicates significant difference based on GLMM ($P < 0.05$). Post hoc pairwise comparisons across temperature were conducted using Tukey test separately for low and medium altitude populations. Within each altitude, the same letter indicates no significant difference across temperature.

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	4	6	7
500	M	12	9	10
1000	L	9	4	6
1000	M	10	6	6

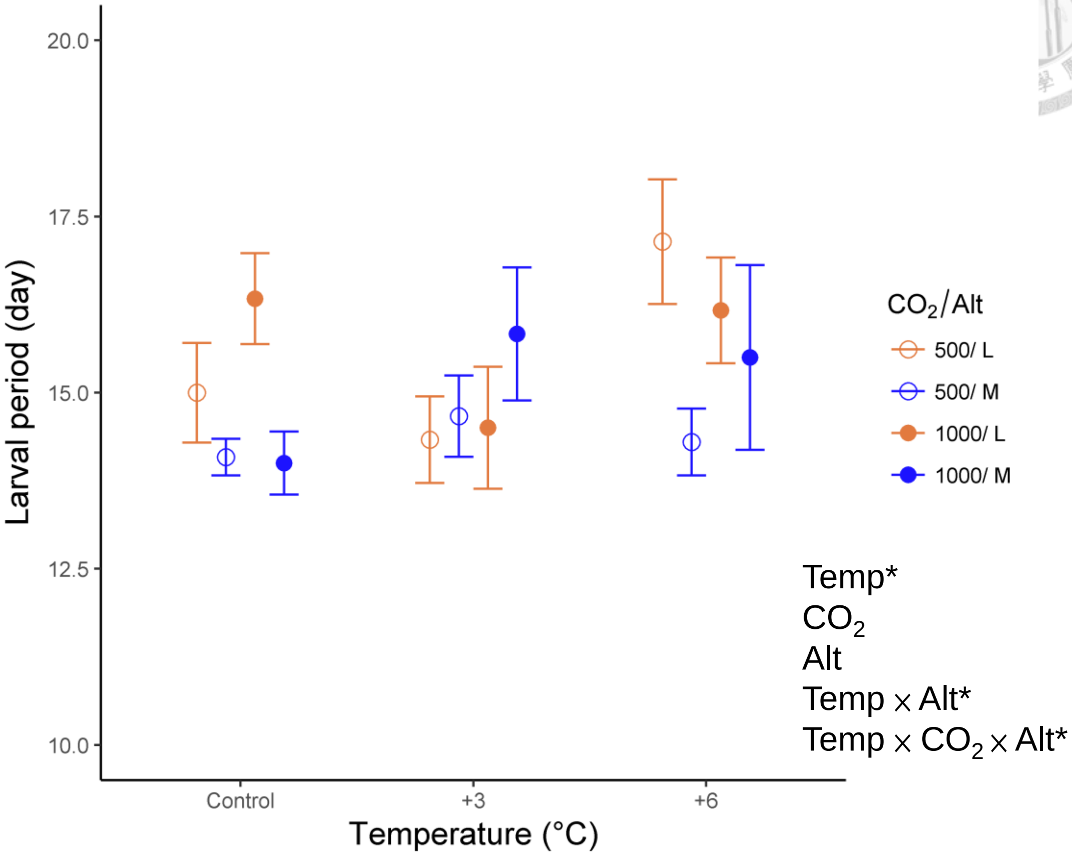

Figure 12. Larval period of *P. canidia* (indirect experiment)

Larval period of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 89). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	4	6	7
500	M	12	9	10
1000	L	9	4	6
1000	M	10	6	6

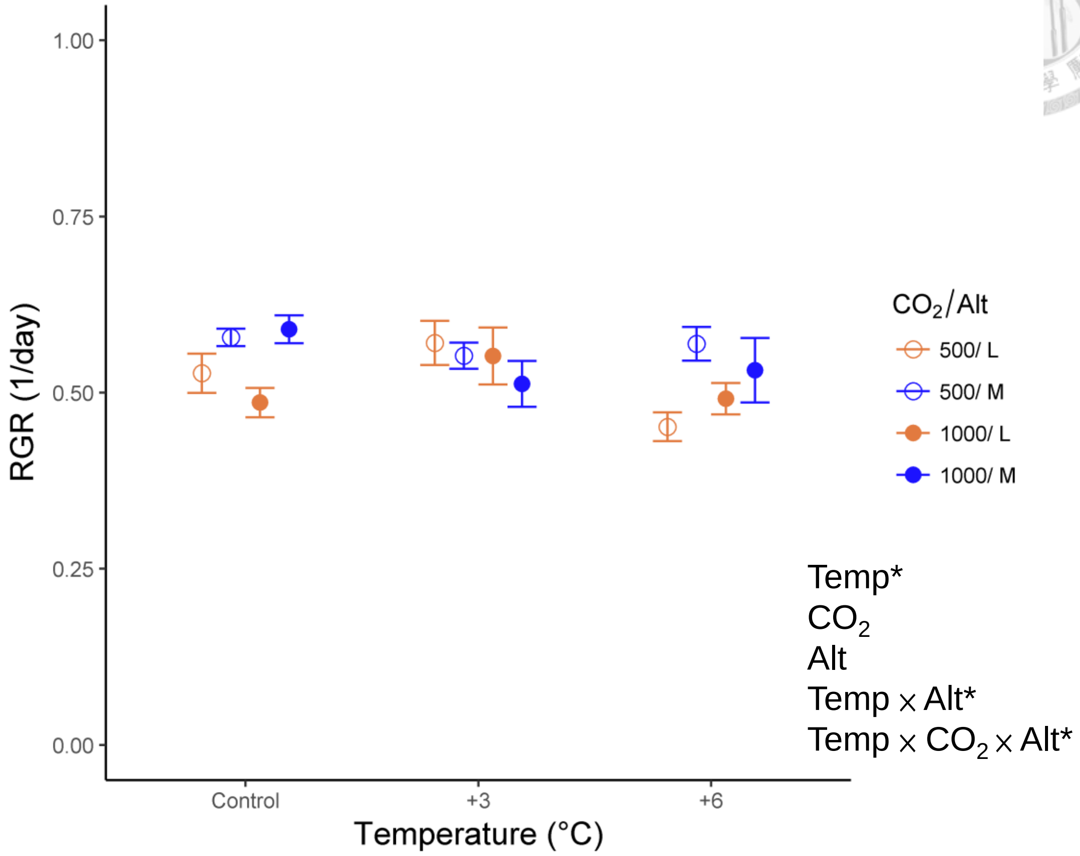

Figure 13. Relative growth rate (RGR) of *P. canidia* (indirect experiment)

Relative growth rate (RGR) of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 89). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	4	6	7
500	M	12	9	10
1000	L	9	4	6
1000	M	10	6	6

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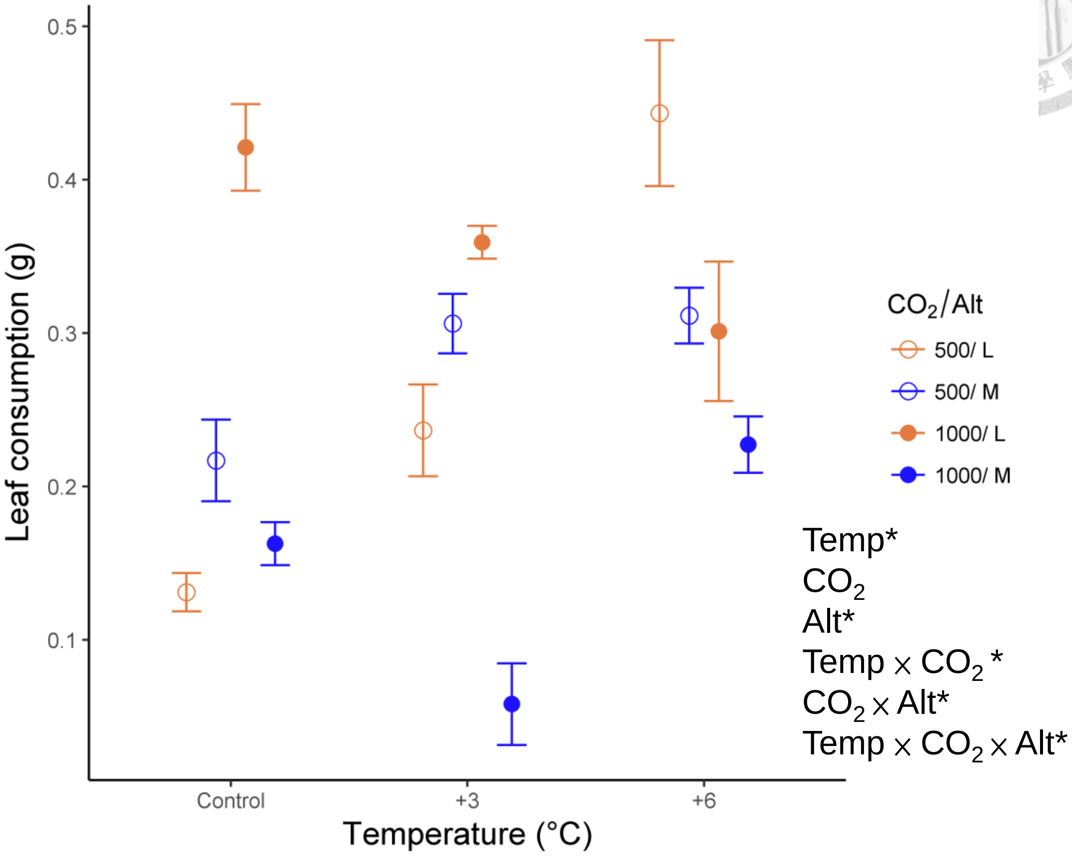

Figure 14. Leaf consumption of *P. canidia* (indirect experiment)

Leaf consumption of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 88). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	4	6	7
500	M	12	9	10
1000	L	9	4	6
1000	M	10	5	6

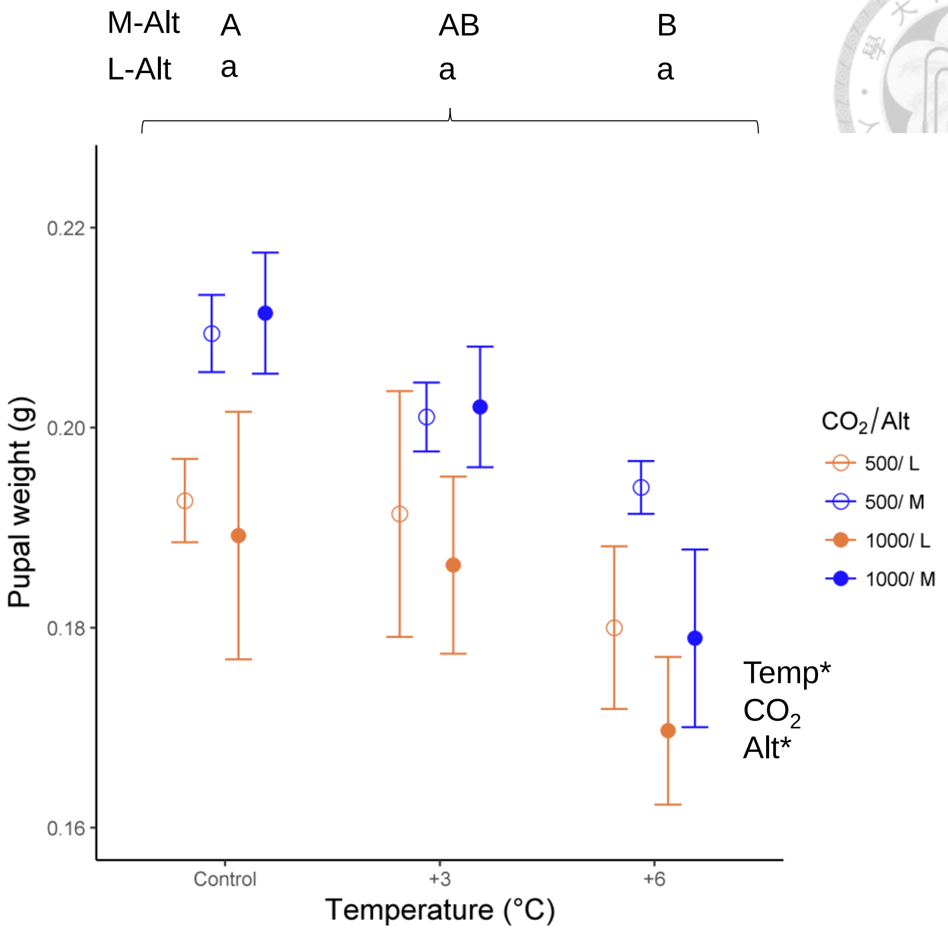

Figure 15. Pupal weight of *P. canidia* (indirect experiment)

Pupal weight of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 89). Asterisk indicates significant difference based on GLMM ($P < 0.05$). Post hoc pairwise comparisons across temperature were conducted using Tukey test separately for low and medium altitude populations. Within each altitude, the same letter indicates no significant difference across temperature.

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	4	6	7
500	M	12	9	10
1000	L	9	4	6
1000	M	10	6	6

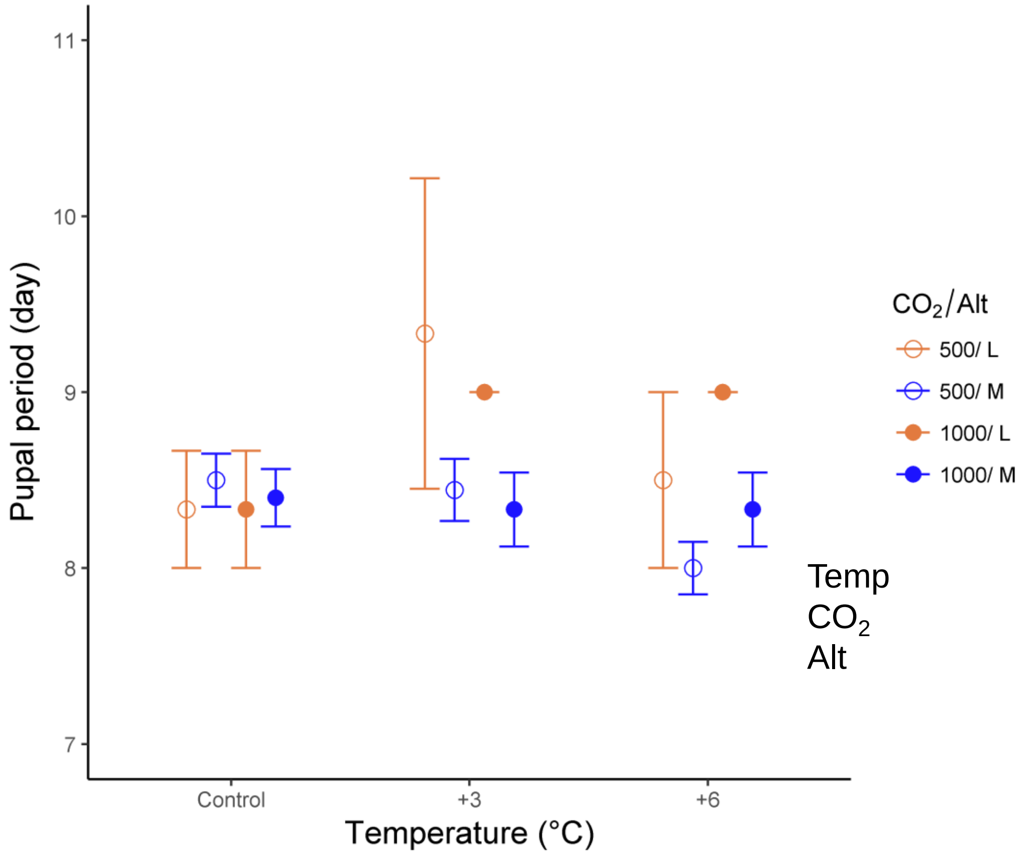

Figure 16. Pupal period of *P. canidia* (indirect experiment)

Pupal period of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 69). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	3	3	2
500	M	12	9	10
1000	L	3	3	2
1000	M	10	6	6

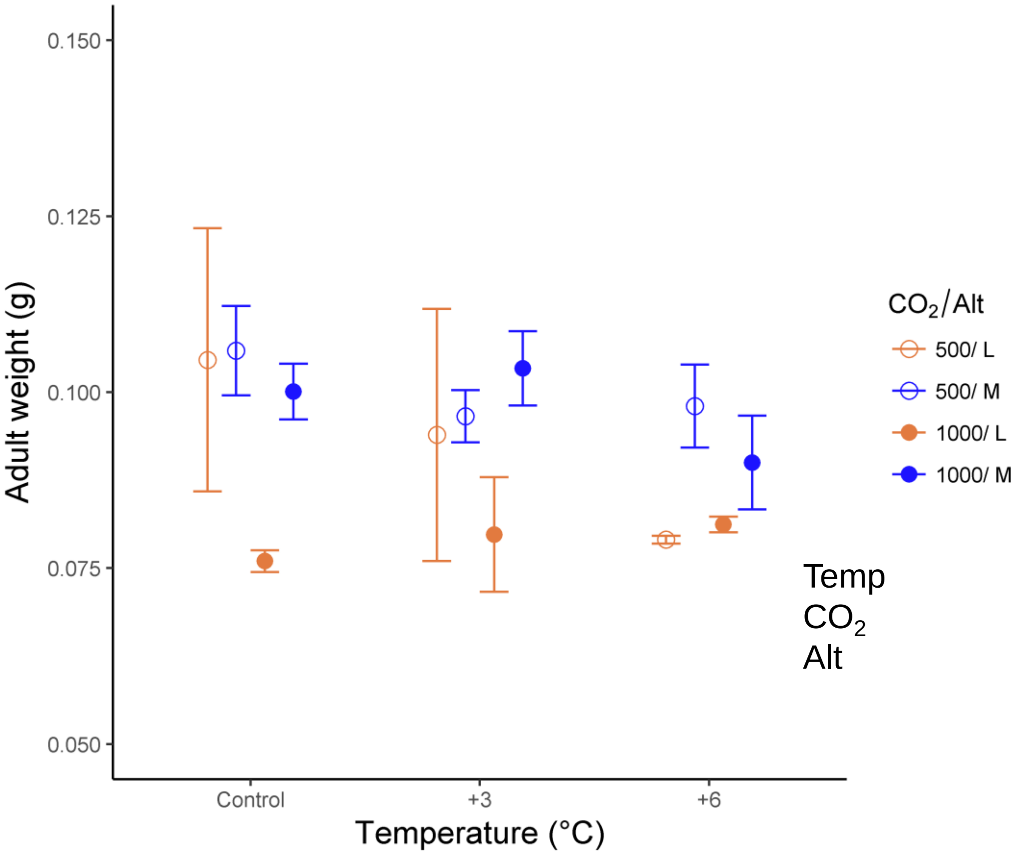

Figure 17. Adult weight of *P. canidia* (indirect experiment)

Adult weight of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 69). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	3	3	2
500	M	12	9	10
1000	L	3	3	2
1000	M	10	6	6

doi: 10.6342/NTU201802543

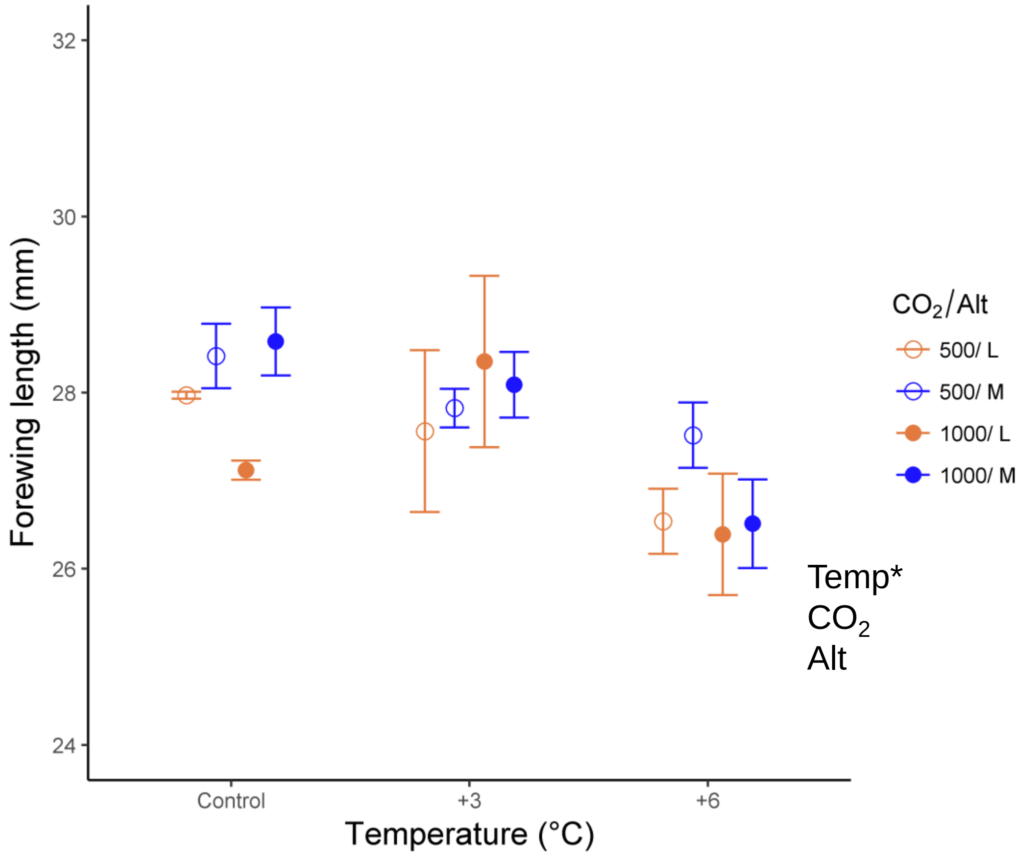

Figure 18. Forewing length of *P. canidia* (indirect experiment)

Forewing length of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 67). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	2	3	2
500	M	12	9	10
1000	L	2	3	2
1000	M	10	6	6

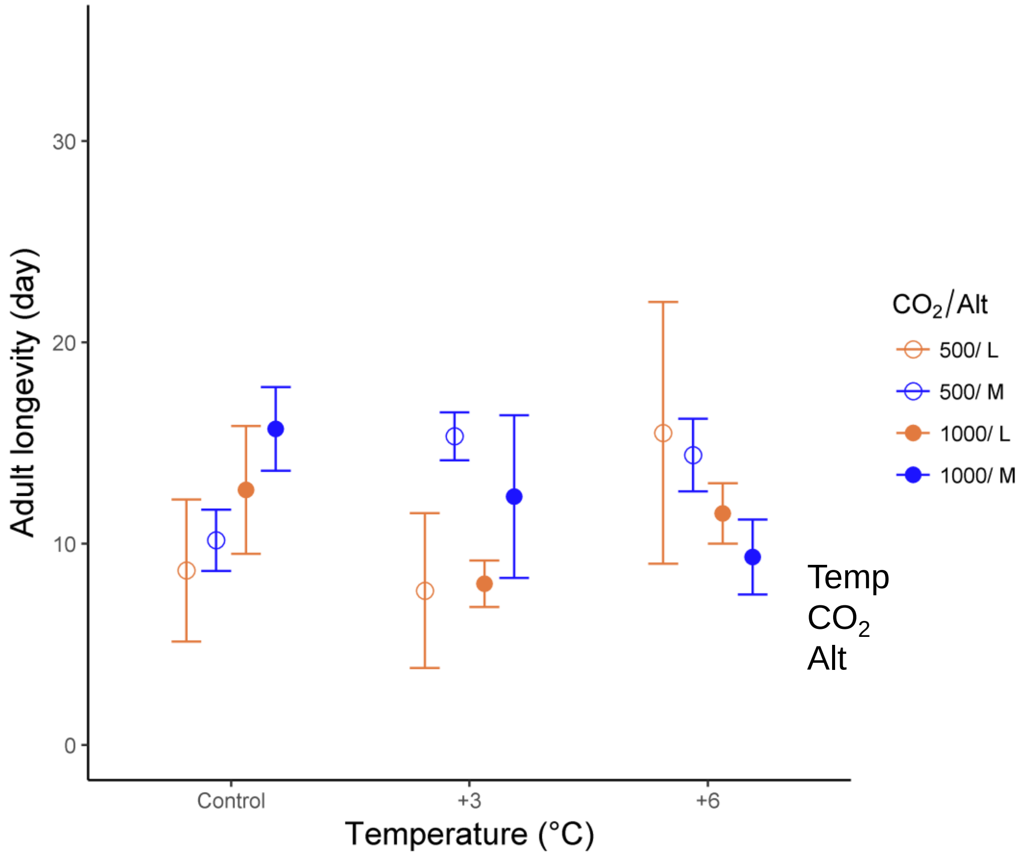

Figure 19. Adult longevity of *P. canidia* (indirect experiment)

Adult longevity of low (L; orange color) and medium (M; blue color) altitude *P. canidia* feeding on *Rorippa* plants that were under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) from the indirect experiment (mean ± SE, N = 69). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	3	3	2
500	M	12	9	10
1000	L	3	3	2
1000	M	10	6	6

doi: 10.6342/NTU201802543

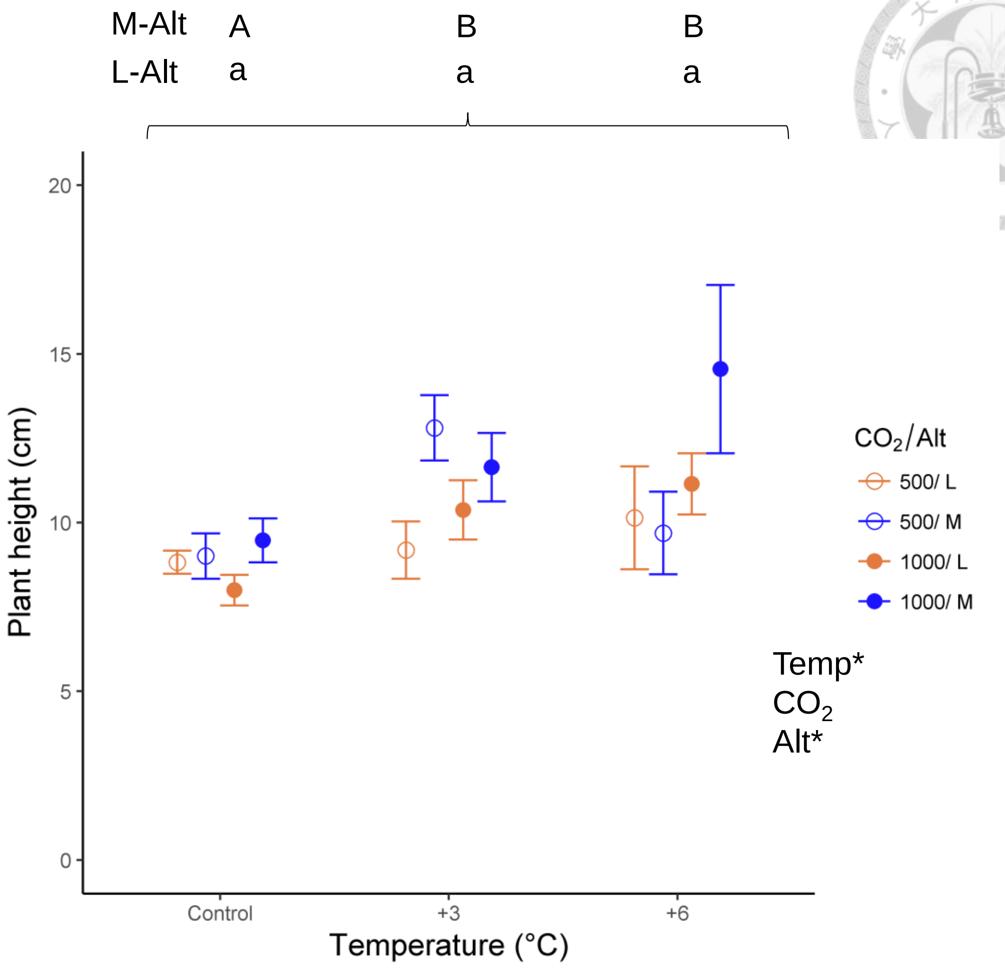

Figure 20. Plant height of *R. indica*

Plant height of low (L; orange color) and medium (M; blue color) altitude *R. indica* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) (mean ± SE, N = 140). Asterisk indicates significant difference based on GLMM ($P < 0.05$). Post hoc pairwise comparisons across temperature were conducted using Tukey test separately for low and medium altitude populations. Within each altitude, the same letter indicates no significant difference across temperature.

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	12	12	12
500	M	12	12	12
1000	L	12	12	12
1000	M	12	12	8

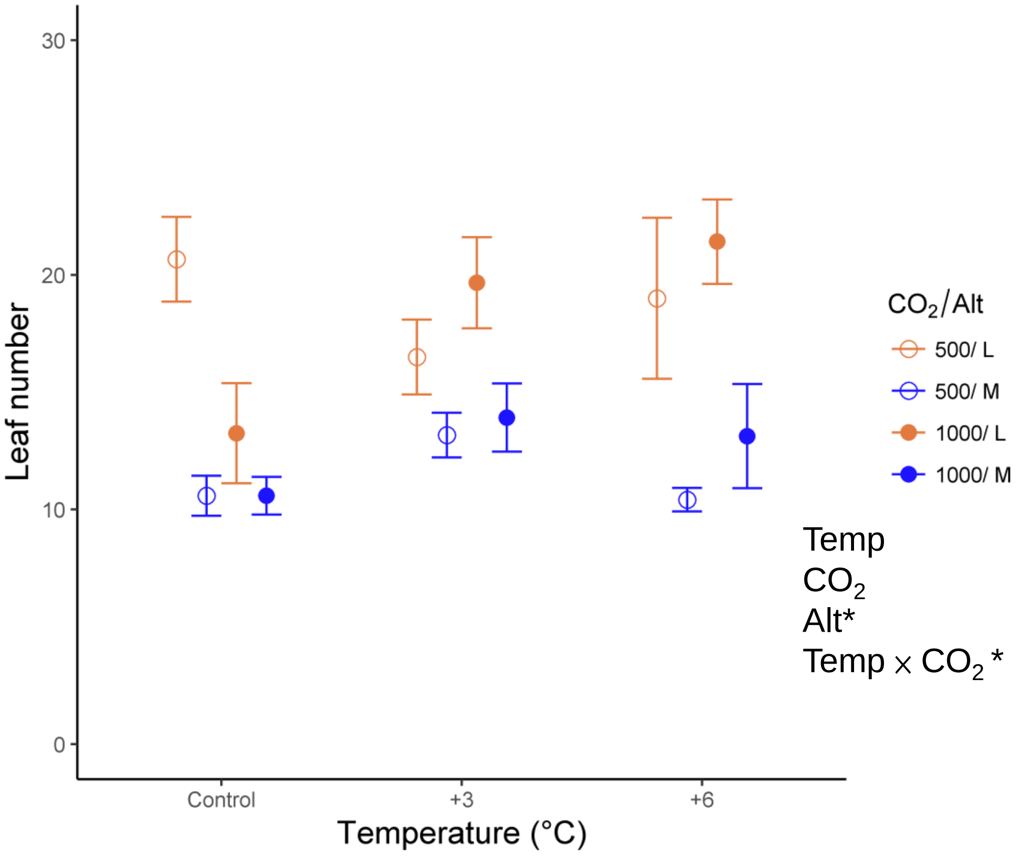

Figure 21. Leaf number of *R. indica*

Leaf number of low (L; orange color) and medium (M; blue color) altitude *R. indica* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) (mean ± SE, N = 140). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	12	12	12
500	M	12	12	12
1000	L	12	12	12
1000	M	12	12	8

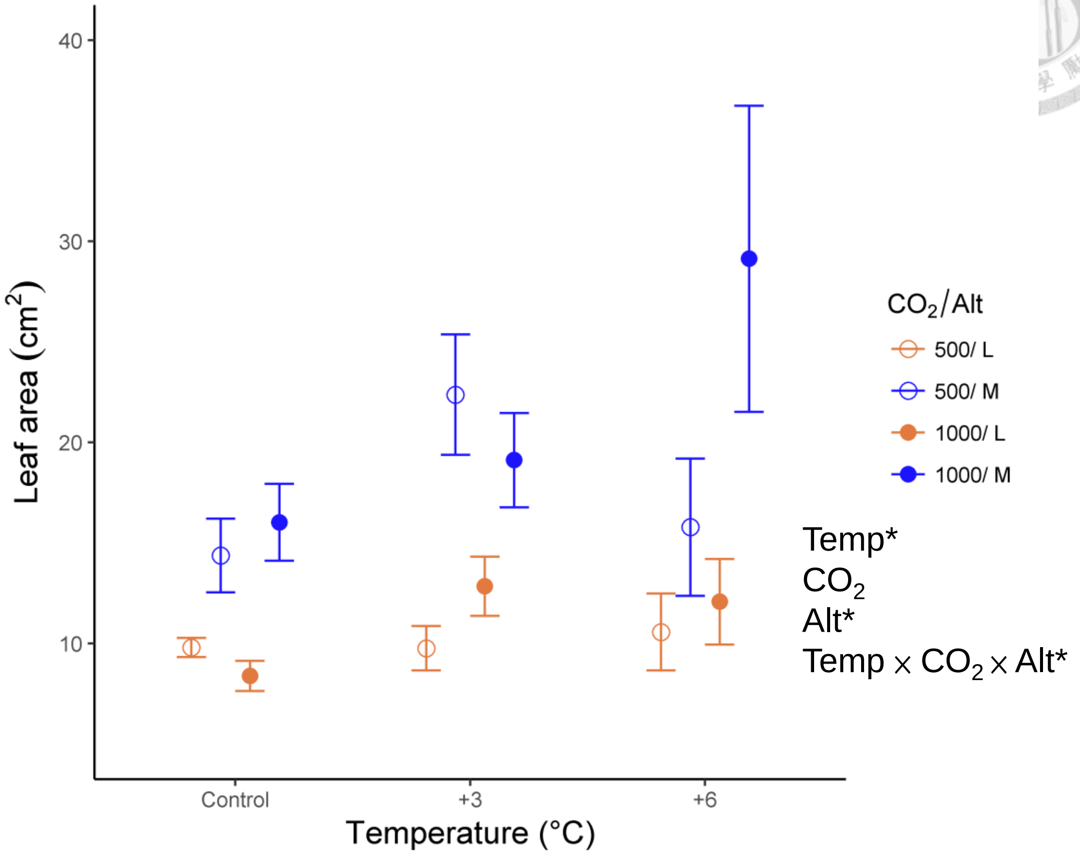

Figure 22. Leaf area of *R. indica*

Leaf area of low (L; orange color) and medium (M; blue color) altitude *R. indica* under temperature treatment (control, +3°C and +6°C) and CO$_2$ treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) (mean ± SE, N = 140). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO$_2$	Alt	Temperature		
		Control	+3	+6
500	L	12	12	12
500	M	12	12	12
1000	L	12	12	12
1000	M	12	12	8

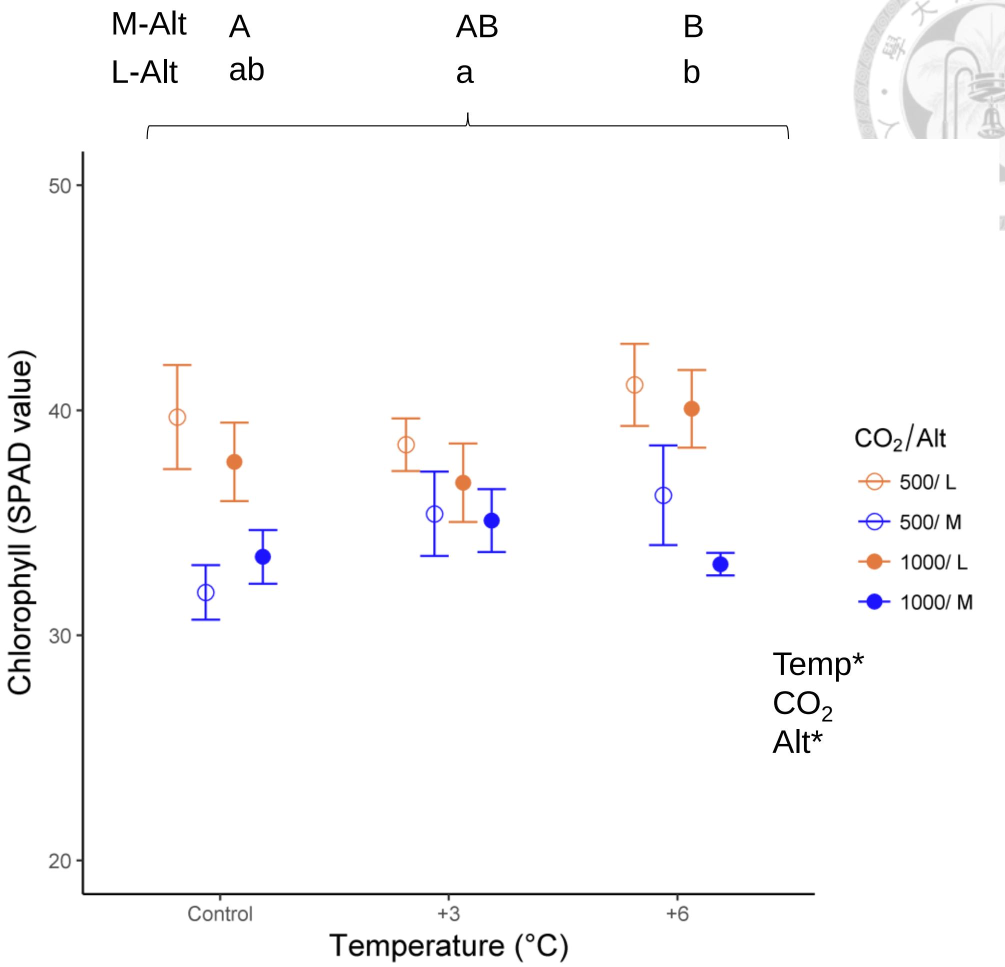

Figure 23. Chlorophyll content of *R. indica*

Chlorophyll content of low (L; orange color) and medium (M; blue color) altitude *R. indica* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) (mean ± SE, N = 140). Asterisk indicates significant difference based on GLMM ($P < 0.05$). Post hoc pairwise comparisons across temperature were conducted using Tukey test separately for low and medium altitude populations. Within each altitude, the same letter indicates no significant difference across temperature.

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	12	12	12
500	M	12	12	12
1000	L	12	12	12
1000	M	12	12	8

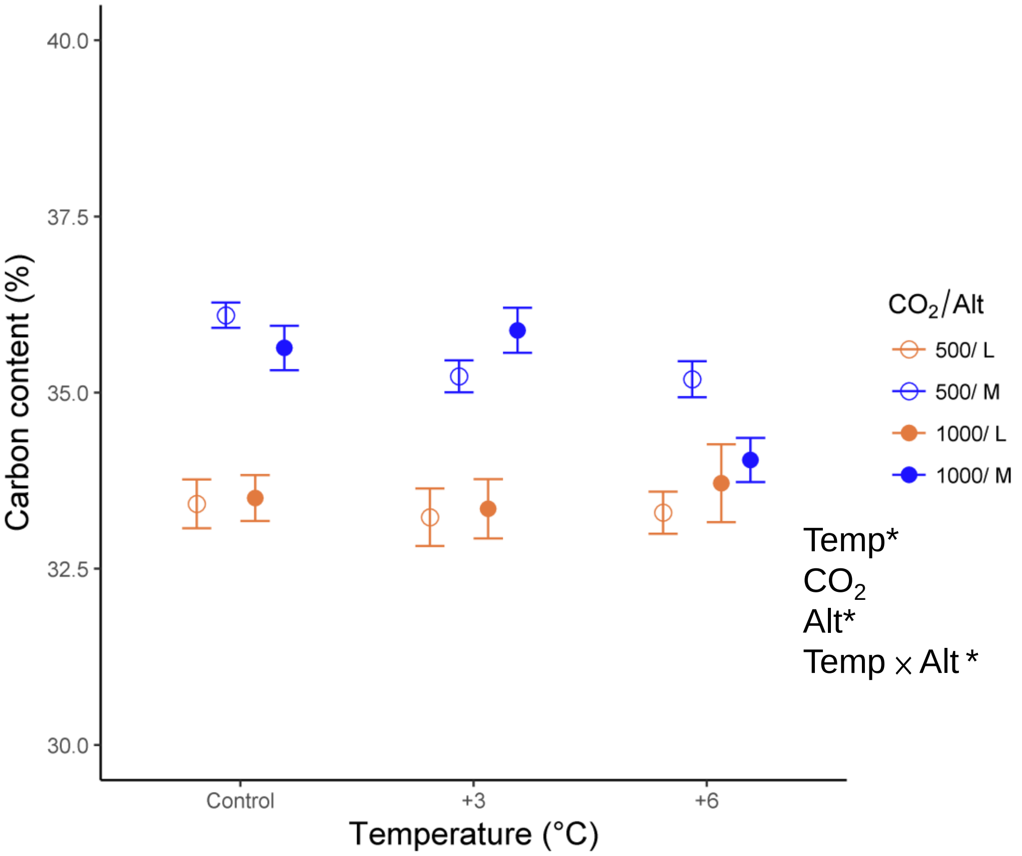

Figure 24. Carbon content of *R. indica*

Carbon content of low (L; orange color) and medium (M; blue color) altitude *R. indica* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) (mean ± SE, N = 134). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	4	11	12
500	M	12	12	12
1000	L	12	12	11
1000	M	12	12	12

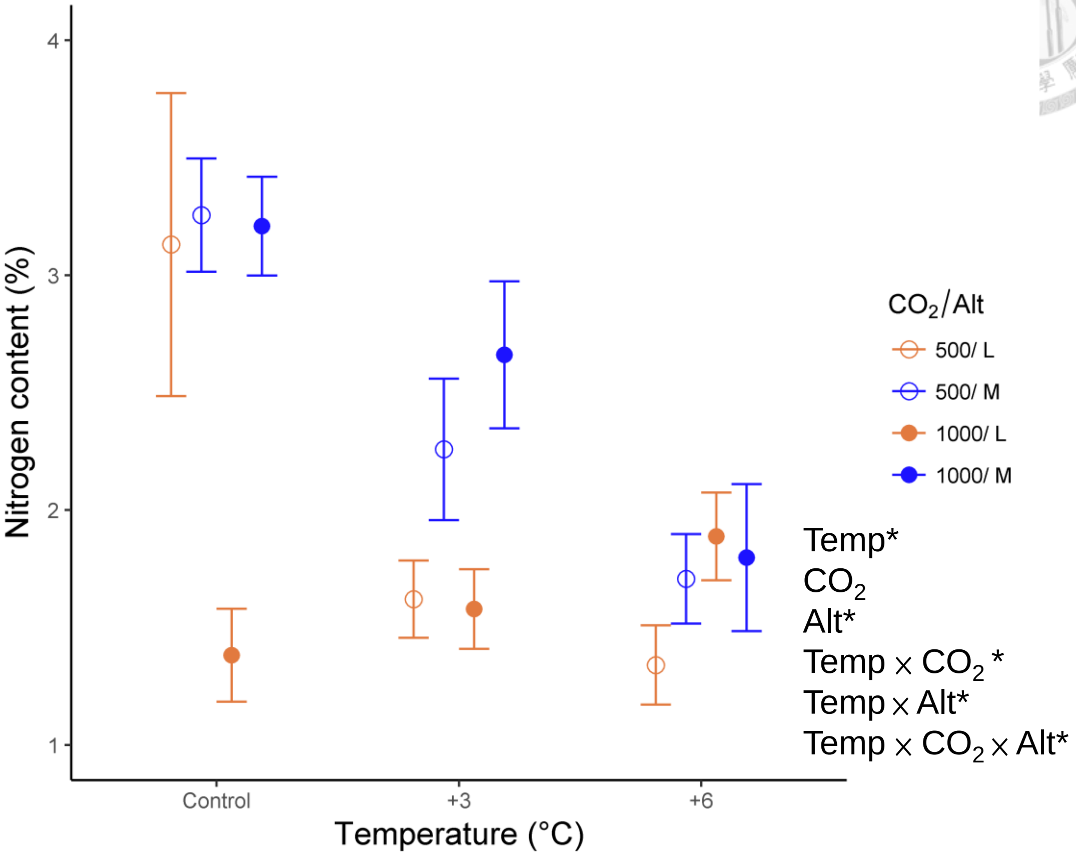

Figure 25. Nitrogen content of *R. indica*

Nitrogen content of low (L; orange color) and medium (M; blue color) altitude *R. indica* under temperature treatment (control, +3°C and +6°C) and CO₂ treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) (mean ± SE, N = 134). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	4	11	12
500	M	12	12	12
1000	L	12	12	11
1000	M	12	12	12

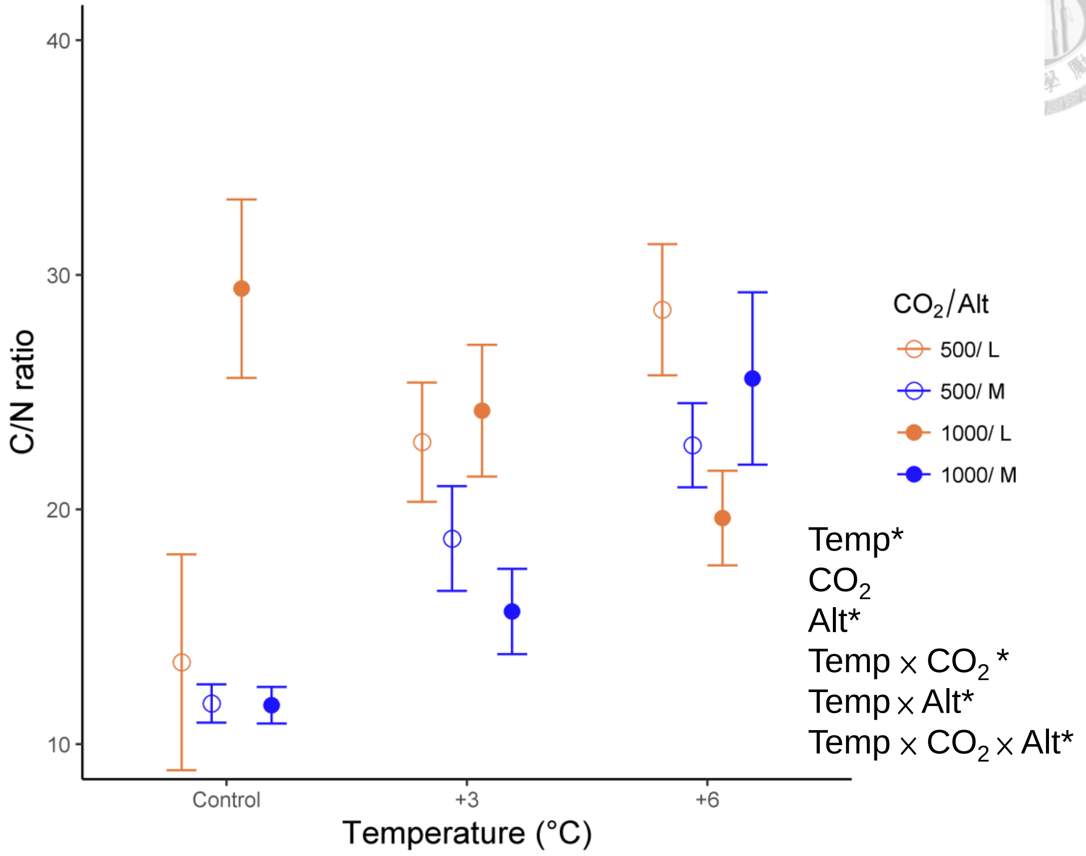

Figure 26. C/N ratio of *R. indica*

C/N ratio of low (L; orange color) and medium (M; blue color) altitude *R. indica* under temperature treatment (control, +3°C and +6°C) and CO_2 treatment (control [500 ppm; open circle] and elevation [1000 ppm; solid circle]) (mean ± SE, N = 134). Asterisk indicates significant difference based on GLMM ($P < 0.05$).

Final sample size:

CO_2	Alt	Temperature		
		Control	+3	+6
500	L	4	11	12
500	M	12	12	12
1000	L	12	12	11
1000	M	12	12	12

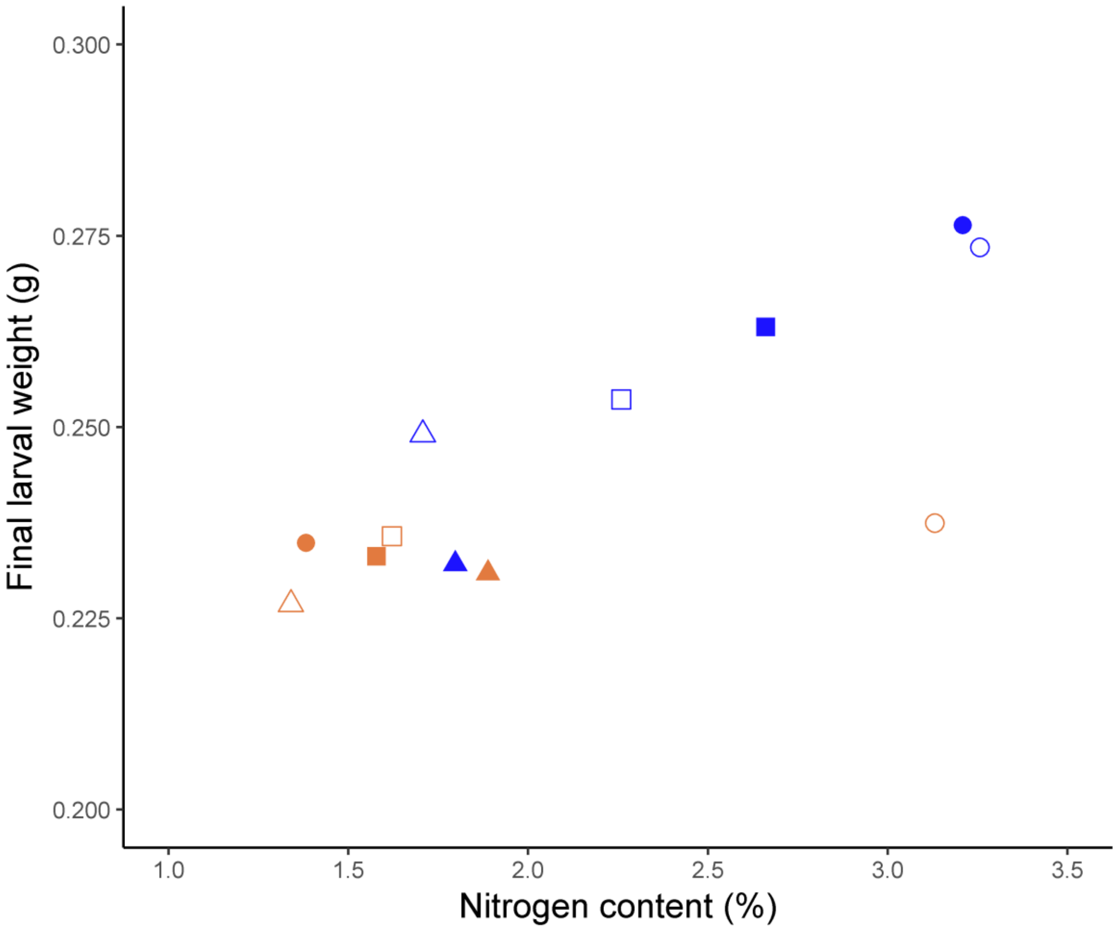

Figure 27. Correlation between leaf nitrogen content and final larval weight

Each data point corresponds to mean final larval weight (g) and mean leaf nitrogen content (%) of the respective treatment combination. Shapes indicate temperature treatment (circle: control, square: +3°C and triangle: +6°C). Colors indicate altitude (orange: low altitude and blue: medium altitude). Solid or open shapes indicate CO_2 treatment (open: 500 ppm and solid 1000 ppm). Adjusted R-squared = 0.517.

Appendix A. Mean temperatures of our collection sites

The mean temperatures of our low and medium altitude sites. The April temperatures from the low altitude sites were calculated from Data Bank for Atmospheric and Hydrologic Research. The August temperatures from the medium altitude sites were calculated from our own data loggers in the field. (Modified from (顏韶寬 2017))

Low altitude (April, 2008 ~ 2015)			
Shihlin (Taipei)	Zhongli (Taoyuan)	Zhushan (Nantou)	Average
22.04 ± 0.27°C	20.61 ± 0.28°C	22.83 ± 0.51°C	21.83 ± 0.36°C
Medium altitude (August 2015)			
Mt. Datun (Taipei)	Dongyangshan (Taoyuan)	Xitou (Nantou)	Average
20.56 ± 0.23°C	21.30 ± 0.26°C	20.86 ± 0.43°C	20.90 ± 0.30°C

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