

RESEARCH ARTICLE

Population-specific responses of an insect herbivore to variation in host-plant quality

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Abstract

Anthropogenic climate change poses a substantial challenge to many organisms, to which they need to respond to avoid fitness reductions. Investigating responses to environmental change is particularly interesting in herbivores, as they are potentially affected by indirect effects mediated via variation in host-plant quality. We here use the herbivorous insect *Pieris napi* to investigate geographic variation in the response to variation in food quality. We performed a common garden experiment using replicated populations from Germany and Italy, and manipulated host quality by growing host plants at different temperature and water regimes. We found that feeding on plants grown at a higher temperature generally diminished the performance of *P. napi*, evidenced by a prolonged development time and reduced larval growth rate, body mass, fat content, and phenoloxidase activity. Genotype by environment interactions ($G \times E$) were present in several performance traits, indicating that Italian populations (1) respond more strongly to variation in host-plant quality and (2) are more sensitive to poor food quality than German ones. This may reflect a cost of the rapid lifestyle found in Italian populations. Consequently, German populations may be more resilient against environmental perturbations and may perhaps even benefit from warmer temperatures, while Italian populations will likely suffer from the concomitantly reduced host-plant quality. Our study thus exemplifies how investigating $G \times E$ may help to better understand the vulnerability of populations to climate change.

KEYWORDS

anthropogenic climate change, genotype by environment interaction ($G \times E$), indirect effects, pace of life, phenotypic plasticity, *Pieris napi*

1 | INTRODUCTION

Climate change causes rising mean temperatures and increasing frequencies of extreme weather events such as heat waves, severe drought, and heavy rain (Hansen et al., 2012; Rahmstorf &

Coumou, 2011). Organisms are forced to respond to these changes in order to survive, as climate change may otherwise push them beyond their critical limits (Harvey et al., 2020; Soroye et al., 2020). Concomitant responses to novel environmental conditions may include phenotypic plasticity and genetic adaptation (Rodrigues &

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Beldade, 2020; Sgrò et al., 2016). Regarding the latter, populations often exhibit traits that provide an advantage under the specific environmental conditions encountered, termed local adaptation (Kawecki & Ebert, 2004). Consequently, populations often differ in fitness-related traits across their geographical range, which is thought to reflect spatial variation in environmental conditions and corresponding selective pressures (Chown & Gaston, 2010; Posledovich et al., 2014; Sambucetti et al., 2006). A prominent example comprises variation in body size in insects. This may result from Bergmann size clines (larger body size in cooler environments), counter-gradient variation (smaller body size in cooler environments), or differences in season length (with shorter growing seasons resulting in smaller body size; Blanckenhorn & Demont, 2004; Chown & Gaston, 2010; Nygren et al., 2008). Also, plastic responses to environmental variation are common. For instance, heat tolerance is well known to be environment dependent, increasing under warmer conditions and vice versa (Fischer & Karl, 2010; Franke et al., 2019; Günter, Beaulieu, Freiberg, et al., 2020). Finally, such plastic responses may differ among species and populations, reflecting genotype by environment interactions ($G \times E$) (Günter, Beaulieu, Freiberg, et al., 2020; Saltz et al., 2018).

The existence of $G \times E$ is a fundamental concept in evolutionary biology, indicating genetic variation in trait plasticity (Oomen & Hutchings, 2015; Saltz et al., 2018). As a consequence, genotypes may exhibit similar phenotypes in familiar environments, but may respond differently to novel environmental conditions (Murren et al., 2014; Saltz et al., 2018). Therefore, investigating $G \times E$ may help to (1) better understand the vulnerability of populations to climate change, (2) assess the performance of a genotype across environments, and (3) identify traits not having the potential to respond to environmental change (Kelly, 2019; Oostra et al., 2018; Saltz et al., 2018; Sgrò et al., 2016).

To investigate responses to environmental variation, herbivorous insects comprise interesting study systems as they are not only directly but additionally indirectly affected, the latter being mediated by environmentally induced changes in the nutritional quality of their host plants (Harvey et al., 2020). Food quality is an important selective pressure (Chown & Gaston, 2010), and its effects on herbivores have been widely documented (Awmack & Leather, 2002; Scriber & Slansky, 1981). Some recent studies on butterflies, for instance, showed that feeding on plants grown at higher temperatures reduces insect performance, indicated by decreased body size, prolonged development time, and a reduced efficiency of converting food into body matter (Bauerfeind & Fischer, 2013a; Kuczyk, Müller, et al., 2021; Kuczyk, Raharivololoniaina, et al., 2021). With regard to water availability, drought stress may increase plant quality for insects, as evidenced by increased reproductive performance, growth rate, and body size (Franzke & Reinhold, 2011; Kuczyk, Müller, et al., 2021; Larsson, 1989; Salgado & Saastamoinen, 2019). However, to what extent populations from different regions respond variably to changes in plant quality is hitherto largely unexplored.

To address this issue, we here used the temperate zone butterfly *Pieris napi*, which has already served as model organism in various

studies on genetic adaptation and phenotypic plasticity. This species seems to possess pronounced plastic and genetic capacities (Espeland et al., 2007; Günter et al., 2019; Günter, Beaulieu, Franke, et al., 2020; Günter, Beaulieu, Freiberg, et al., 2020; Posledovich et al., 2014). For instance, recent studies documented that Italian as compared with German populations seem to have a selective premium on rapid development, being nevertheless larger in size owing to higher larval growth rates (Günter et al., 2019; Günter, Beaulieu, Franke, et al., 2020; Günter, Beaulieu, Freiberg, et al., 2020). Furthermore, feeding on plants grown at cooler temperatures or under drought stress increased the performance of *P. napi* (Bauerfeind & Fischer, 2013a; Kuczyk, Müller, et al., 2021). However, whether the effects of variation in host-plant quality differ across geographic regions, which is the subject of the present study, has not been investigated thus far. To this end, we here used replicated populations from Germany and Italy in a common garden experiment. We manipulated food quality by exposing host plants to different temperature and water regimes, mimicking effects of climate change. We then analyzed selected morphological and physiological traits, indicative of insect performance, in animals fed on plants of the different treatments. We thus exclusively focus on indirect, plant-mediated effects. We did not additionally investigate direct effects for the following reasons: (1) There are in general many more studies on direct as compared with indirect effects. (2) We have considered both direct and indirect effects in earlier studies on *P. napi* already (Bauerfeind & Fischer, 2013a, 2013b). (3) The focus in this study is on variation in the responses of populations from different origins to indirect effects. Additionally addressing direct effects would have been logistically very difficult based on the concomitantly high number of treatments.

Our earlier work has indicated a much faster lifestyle (i.e., a more rapid development) in Italian than in German individuals, arguably to fit in additional generations per year (Günter et al., 2019; Günter, Beaulieu, Freiberg, et al., 2020). This rapid pace of life also seems to increase oxidative damage (Günter, Beaulieu, Franke, et al., 2020). We, therefore, predict that the more time-stressed Italian populations are more sensitive to poor food quality as compared to their German counterparts, reflecting a cost of rapid development. Thus, we expect $G \times E$ with Italian populations responding more strongly to variation in host-plant quality than German ones.

2 | MATERIALS AND METHODS

2.1 | Study organism

Pieris napi (Linnaeus, 1758) is a temperate zone butterfly that is widely distributed across northern Eurasia (Ebert & Rennwald, 1991). In most parts of its range, the species has two to three more or less overlapping generations. *P. napi* overwinters as pupa (Henriksen & Kreutzer, 1982). The principal larval host plants are several species of the Brassicaceae family, such as *Alliaria petiolata* (Bieb.) and *Cardamine pratensis* (L.). *P. napi* is predicted to suffer from climate change due to its association with moist habitats such as moist meadows and forest ecotones

(Oliver et al., 2012). In contrast to its relatives *P. rapae* and *P. brassicae*, *P. napi* is of limited importance as a pest species (Ebert & Rennwald, 1991). *P. napi* females are polyandrous, that is, they mate repeatedly. Accordingly, males are larger than females due to a positive correlation between body and spermatophore size, with larger spermatophores delaying female remating (Wiklund & Kaitala, 1995).

2.2 | Population sampling

We collected fecund females from three Italian and three German populations (Figure S1: Appendix S1). In Italy, we sampled 22 females near Mantova (45.21°N, 10.75°E), 20 near Pavia (45.12°N, 9.16°E), and 24 near Torino (45.29°N, 7.30°E). In Germany, we collected 22 females near Rostock (54.11°N, 12.12°E), 25 near Wismar (53.54°N, 11.42°E), and 20 near Greifswald (54.04°N, 13.27°E). Caught females were transferred to Greifswald University and kept in a climate chamber (Sanyo MLR-351H) at 26°C, 60% relative humidity, and a L18: D6 photoperiod (with light from 5 a.m. to 23 p.m.). Females were placed individually into small plastic pots (1 L) covered with gauze and were provided with *Alliaria petiolata* as oviposition substrate, and additionally fresh flowers, water, and a highly concentrated sucrose solution (ca. 20 vol%) for adult feeding. Deposited eggs were collected daily and transferred to small plastic boxes.

2.3 | Host-plant treatments

To investigate the effects of host-plant manipulation on *P. napi* performance, we used *Sinapis alba* L. (Brassicaceae), a widespread annual plant of the temperate zone with a short life cycle (Dong et al., 2012). It is a common crop plant but also grows on ruderal places. Plants were grown from seeds (Rühlemann's, Horstedt, Germany) in commercially available potting soil in plastic pots (11 × 11 × 12 cm; 5–6 plants per pot) kept in climate cabinets (Sanyo MLR-351H) at 70% relative humidity and a L18: D6 photoperiod. Seedlings were divided among four treatments once the first leaves following the cotyledons had appeared. We used 2 temperature and 2 water regimes with 10 plant pots per treatment: (1) 17°C, water control (150 ml of water per day and pot); (2) 17°C, drought (50 ml water per day); (3) 24°C, water control; (4) 24°C, drought. The water control treatment was chosen to keep plants fully turgid until the next watering bout, based on pre-trials. Leaves were harvested for larval feeding after plants had reached the eight-leaf stage. The above plant treatments have been shown to affect *S. alba* chemistry by increasing leaf glucosinolate and decreasing C:N ratio at higher temperatures (Kuczyk, Müller, et al., 2021).

2.4 | Insect rearing and analyses

After hatching, F1 larvae were randomly divided among the four host-plant treatments, using 100 individuals per population and

treatment (i.e., 2400 in total, 6 populations × 4 treatments × 100). Five larvae each were reared within small plastic boxes (125 ml) in a climate chamber (Sanyo MLR-351H) under the same conditions as used for oviposition (26°C, 60% relative humidity, L18:D6 photoperiod). Larvae were supplied daily with fresh leaves from the respective host-plant treatment ad libitum. Boxes were checked daily and larvae were supplied with fresh leaves as necessary. Resulting pupae were weighted one day after pupation (Sartorius CPA225D) and afterwards kept individually until eclosion. Development time was scored from hatching until adult eclosion, and larval growth rate was calculated as mass gain per day (pupal mass divided by larval development time). After eclosion, adult butterflies were frozen at –80°C until further analyses.

We measured, in addition, the following morphological and physiological parameters as indicators of butterfly performance: total body mass, thorax mass, abdomen mass, thorax-abdomen ratio (as proxies of body size and the relative investment into flight versus reproduction), forewing length, wing loading and wing aspect ratio (as measures of flight performance), abdomen fat content, and phenoloxidase (PO) activity (reflecting general condition in insects; Freitak et al., 2003; González-Santoyo & Córdoba-Aguilar, 2011). Thus, we considered an array of traits as fitness proxies, including development time and growth rate, body size, flight performance, and condition, which may all be well relevant for survivorship and reproduction. First, total body mass was determined to the nearest 0.01 mg. Then, wings, head, and legs were removed. Thorax and abdomen were separated and afterward weighed. Forewing length and area were measured using digital images of left forewings. Images were captured ventrally with a digital camera mounted on a stereo microscope. Wing loading was calculated as total body mass divided by forewing area and wing aspect ratio as $4 \times \text{forewing length}^2$ divided by forewing area (Berwaerts et al., 2002).

Abdomen fat content was measured according to Fischer et al. (2003) but using acetone instead of dichloromethane. In short, abdomens were dried to constant weight for 2 days at 70°C (UN110, Memmert) and initial dry mass was taken. Thereafter, fat was extracted twice (2 × 2 days) using 1.5 ml of acetone for each butterfly. Then, abdomens were dried again for 2 days and the fat-free dry mass was measured. Relative fat content was determined as the mass difference between initial and final abdomen dry mass and is given in percent. For PO measurements, thoraces were homogenized (Tissuelyser II, Qiagen) with 200 µl of a phosphate-buffered saline (PBS) and centrifuged for 10 min at 14,000 rpm. Afterward, 60 µl of the supernatant or PBS buffer (for blank measurements) was transferred to a microtiter plate (96 wells) and 140 µl L-Dopa (dihydrophenyl-L-alanine; 10 mM in PBS buffer) was added. Readings were taken every 30 s on a spectrophotometric plate reader (BioTek EL 808) at 30°C and 490 nm for 45 min. Enzyme activity was measured as the slope during the linear phase of the reaction, during which the enzyme catalyzes the transition from L-Dopa to dopachrome. PO activity was assayed twice per individual. The mean of both readings, corrected for blank values, was used for statistical analyses. Total protein content was quantified using the Roti

Nanoquant protein assay based on the Bradford method (Bradford, 1976), following the manufacturer's instructions (Roth).

2.5 | Statistical analyses

All traits were analyzed with general linear mixed models (GLMMs) using population origin, host-plant temperature, host-plant water treatment, butterfly sex, and all interactions as fixed factors. Population (nested within origin) was included as a random effect. Protein was added as a covariate in the analysis of PO activity. Minimum adequate models were built by sequentially removing non-significant interaction terms. Pupal mass was ln-transformed prior to analyses to meet GLMM requirements. Mean values depicted in the tables and figures refer to untransformed data. All means are given \pm 1 SE. All statistical tests were performed using Statistica 8.0 (StatSoft, 2007).

3 | RESULTS

Origin significantly affected fat content only, while replicate populations differed significantly in all traits (Table 1). Fat content was higher in German than in Italian individuals ($G: 11.9 \pm 0.2\% > I: 4.9 \pm 0.2\%$). However, origin was involved in interactions with three other factors, indicating that animals from different origins showed differential responses to environmental variation or among sexes (see further below).

Host-plant water treatment did not significantly affect any butterfly trait directly. However, host-plant temperature caused significant variation in development time, larval growth rate, pupal mass, thorax mass, abdomen mass, fat content, and PO activity (Table 1). Feeding on plants grown at the higher compared with the lower temperature decreased growth rates ($17^\circ\text{C}: 0.389 \pm 0.002 \text{ mg/day} > 24^\circ\text{C}: 0.380 \pm 0.002 \text{ mg/day}$), pupal mass ($17^\circ\text{C}: 107.7 \pm 0.7 \text{ mg} > 24^\circ\text{C}: 105.2 \pm 0.7 \text{ mg}$), thorax mass ($17^\circ\text{C}: 14.3 \pm 0.1 \text{ mg} > 24^\circ\text{C}: 13.7 \pm 0.1 \text{ mg}$), abdomen mass ($17^\circ\text{C}: 16.3 \pm 0.2 \text{ mg} > 24^\circ\text{C}: 15.6 \pm 0.2 \text{ mg}$), fat content ($17^\circ\text{C}: 8.9 \pm 0.2\% > 24^\circ\text{C}: 7.9 \pm 0.2\%$), and PO activity ($17^\circ\text{C}: 5.0 \pm 0.2 \text{ mOD/ml} > 24^\circ\text{C}: 4.1 \pm 0.2 \text{ mOD/ml}$) but increased development time ($17^\circ\text{C}: 20.03 \pm 0.06 \text{ days} < 24^\circ\text{C}: 20.30 \pm 0.06 \text{ days}$).

Butterfly sex significantly influenced development time, larval growth rate, pupal mass, thorax mass, abdomen mass, thorax-abdomen ratio, and wing aspect ratio (Table 1). Males compared to females had higher larval growth rates ($\delta: 0.393 \pm 0.002 \text{ mg/day} > \text{f}: 0.376 \pm 0.002 \text{ mg/day}$), pupal masses ($\delta: 112.5 \pm 0.7 \text{ mg} > \text{f}: 100.4 \pm 0.7 \text{ mg}$), thorax masses ($\delta: 15.0 \pm 0.1 \text{ mg} > \text{f}: 12.9 \pm 0.1 \text{ mg}$), abdomen masses ($\delta: 16.4 \pm 0.2 \text{ mg} > \text{f}: 15.5 \pm 0.2 \text{ mg}$), thorax-abdomen ratios ($\delta: 0.97 \pm 0.01 > \text{f}: 0.88 \pm 0.01$), wing aspect ratios ($\delta: 10.44 \pm 0.02 > \text{f}: 10.33 \pm 0.02$), and a shorter larval development time ($\delta: 19.99 \pm 0.06 \text{ days} < \text{f}: 20.33 \pm 0.06 \text{ days}$).

Significant interactions between butterfly origin and host-plant temperature were found for development time, larval growth rate,

TABLE 1 Results of general linear mixed models for the effects of population origin, host-plant temperature, host-plant water regime, and sex (all fixed) on various traits in *Pieris napi*

	MS	df	F	p
Development time				
Origin	4080.8	1	3.13	.1518
Population [Origin]	1310.9	4	675.31	<.0001
Temperature	18.3	1	9.43	.0022
Water	2.3	1	1.18	.2782
Sex	29.3	1	15.10	.0001
Origin \times Temp.	8.7	1	4.50	.0342
Origin \times Water	13.8	1	7.11	.0078
Error	1.9	1003		
Larval growth rate				
Origin	2.62	1	2.58	.1833
Population [Origin]	1.02	4	473.94	<.0001
Temperature	0.02	1	7.96	.0049
Water	<0.01	1	0.03	.8710
Sex	0.07	1	33.24	<.0001
Origin \times Temp.	0.02	1	9.34	.0023
Origin \times Water	0.02	1	10.16	.0015
Error	<0.01	1002		
Pupal mass				
Origin	4.52	1	3.63	.1296
Population [Origin]	1.25	4	54.27	<.0001
Temperature	0.17	1	7.50	.0063
Water	0.06	1	2.79	.0949
Sex	3.30	1	143.02	<.0001
Origin \times Temp.	0.29	1	12.47	.0004
Error	0.02	1003		
Thorax mass				
Origin	2703.5	1	5.11	.0865
Population [Origin]	530.8	4	58.01	<.0001
Temperature	108.5	1	11.85	.0006
Water	8.1	1	0.88	.3477
Sex	1107.5	1	121.03	<.0001
Origin \times Temp.	72.2	1	7.89	.0051
Error	9.2	1004		
Abdomen mass				
Origin	5701.8	1	3.93	.1186
Population [Origin]	1458.4	4	52.63	<.0001
Temperature	115.9	1	4.18	.0411
Water	2.9	1	0.11	.7449
Sex	178.5	1	6.44	.0113

(Continues)

TABLE 1 (Continued)

	MS	df	F	p
Error	27.7	1005		
Thorax-abdomen ratio				
Origin	1.03	1	3.69	.1270
Population [Origin]	0.28	4	7.92	<.0001
Temperature	0.02	1	0.45	.5004
Water	0.04	1	1.10	.2948
Sex	2.28	1	64.65	<.0001
Temp. × Water	0.31	1	8.67	.0033
Error	0.04	1004		
Forewing length				
Origin	109.6	1	1.86	.2442
Population [Origin]	64.5	1	26.64	<.0001
Temperature	3.7	4	1.54	.2141
Water	8.5	1	3.50	.0615
Sex	2.4	1	0.97	.3246
Origin × Temp.	16.4	1	6.79	.0093
Origin × Sex	16.8	1	6.96	.0085
Error	2.4	1320		
Wing loading				
Origin	0.017	1	0.07	.8028
Population [Origin]	0.268	4	110.26	<.0001
Temperature	0.004	1	1.67	.1962
Water	0.002	1	0.70	.4027
Sex	0.004	1	1.80	.1796
Error	0.002	1320		
Wing aspect ratio				
Origin	3.49	1	3.44	.1348
Population [Origin]	1.09	4	5.26	.0003
Temperature	0.04	1	0.21	.6448
Water	0.44	1	2.13	.1450
Sex	3.27	1	15.80	<.0001
Origin × Sex	2.34	1	11.34	.0008
Error	0.21	1319		
Fat content %				
Origin	12293.5	1	18.77	.0123
Population [Origin]	657.7	4	24.70	<.0001
Temperature	251.2	1	9.43	.0022
Water	6.7	1	0.25	.6173
Sex	50.2	1	1.89	.1700
Error	26.6	1003		

TABLE 1 (Continued)

	MS	df	F	p
Phenoloxidase activity				
Origin	188.7	1	0.58	.4869
Population [Origin]	402.0	4	16.37	<.0001
Temperature	182.6	1	7.44	.0065
Water	10.3	1	0.42	.5183
Sex	69.6	1	2.83	.0926
Protein	785.6	1	32.00	<.0001
Error	24.6	1004		

Note: Replicate population was added as a random factor and nested within origin. Protein content was added as a covariate in the analysis of phenoloxidase activity. Models were constructed by sequentially removing nonsignificant interaction terms. Significant *p*-values are given in bold. Development time = larval time + pupal time.

pupal mass, thorax mass, and forewing length (Table 1). These indicate that the effects of host-plant temperature were restricted to Italian individuals while German ones remained unaffected. Feeding on plants grown at the higher temperature decreased larval growth rate, pupal mass, and thorax mass but increased development time and wing length in the Italian individuals (Figure 1). The interaction between butterfly origin and plant water treatment was significant for development time and larval growth rate (Table 1). Similar to above, effects of the host-plant water treatment were found in Italian individuals only. They showed longer development times and lower larval growth rates when feeding on drought-stressed plants compared with control plants (Figure 2). The interaction between origin and sex was significant for forewing length and wing aspect ratio (Table 1). For both traits, there was no significant difference between the sexes in Italian individuals, while German males had longer wings and higher wing aspect ratios than German females (Figure 3). Additionally, the interaction between host-plant temperature and water treatment significantly affected the thorax-abdomen ratio (Table 1). Feeding on plants from the drought treatment increased the thorax-abdomen ratio when in combination with the low temperature treatment (17°C drought: 0.95 ± 0.01 ; 17°C control: 0.91 ± 0.01), but tended to decrease it when in combination with the high temperature treatment (24°C drought: 0.91 ± 0.01 ; 24°C control: 0.93 ± 0.01).

4 | DISCUSSION

Organisms are increasingly confronted with environmental variability and extremes owing to anthropogenic climate change (Harvey et al., 2020; Soroye et al., 2020). Phenotypic plasticity is a potentially powerful mechanism to cope with such variability. Here, we discuss patterns of geographic variation in phenotypic plasticity in an insect herbivore in response to changes in host-plant quality, and its potential significance for dealing with climate change.

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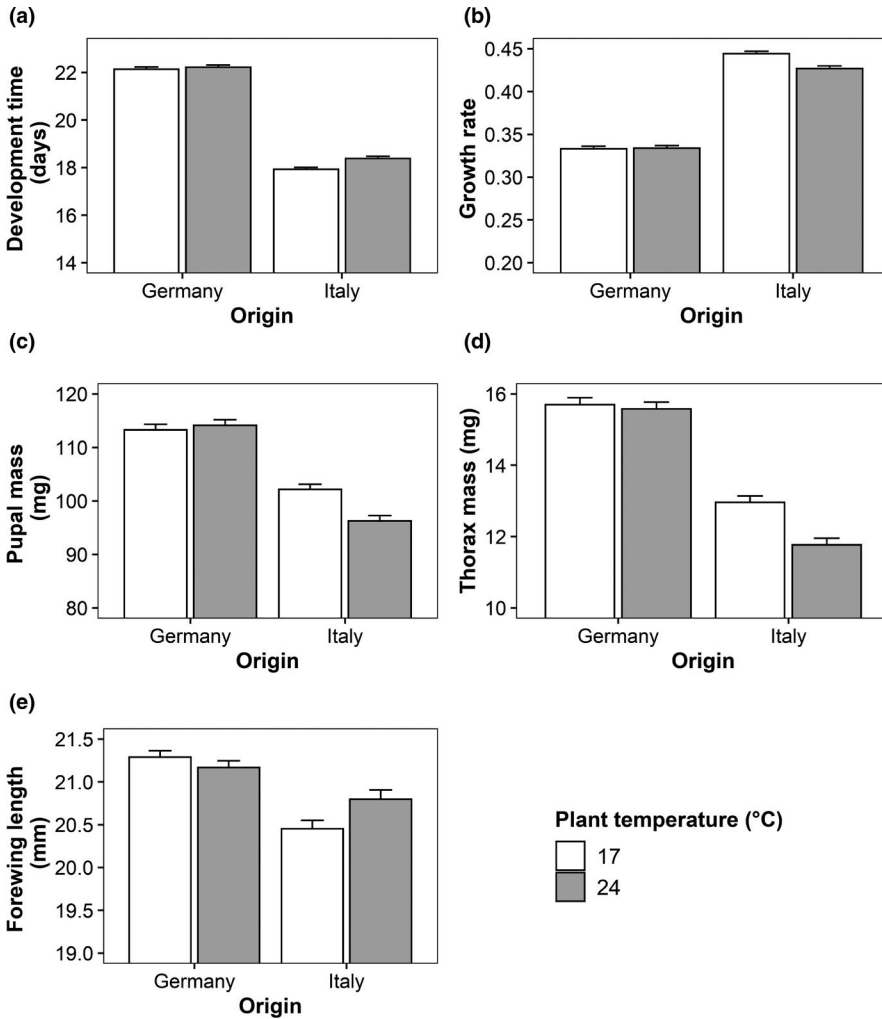


FIGURE 1 Effects of origin (Germany vs. Italy) and host-plant temperature (17 vs. 24°C) on development time (a), larval growth rate (b), pupal mass (c), thorax mass (d), and forewing length (e) in *Pieris napi*. Given are means + SE

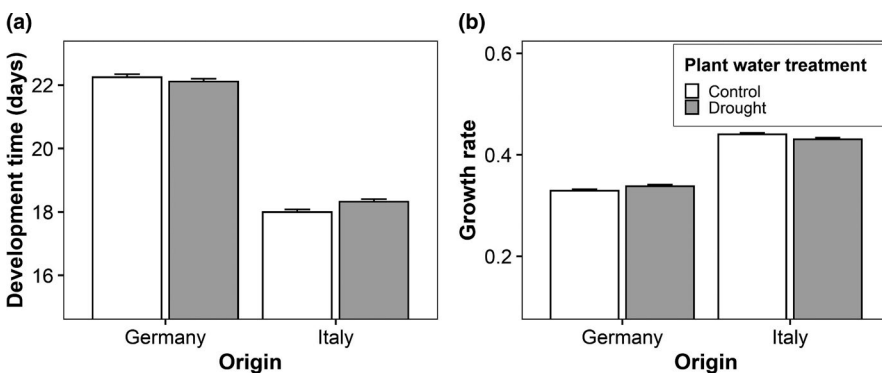


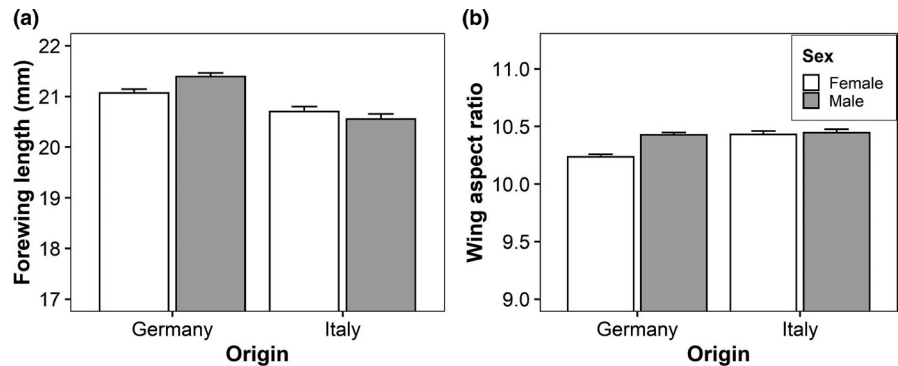
FIGURE 2 Effects of origin (Germany vs. Italy) and host-plant water treatment (control vs. drought) on development time (a) and larval growth rate (b) in *Pieris napi*. Given are means + SE

4.1 | Effects of geographic origin

With regard to the main effects of geographic origin, a significant difference was found only for fat content. The higher fat content found in German butterflies fits the previously observed differences in lifestyles (Günter, Beaulieu, Franke, et al., 2020; Günter, Beaulieu, Freiberg, et al., 2020). It may thus indicate that storage reserves are more strongly reduced in the rapidly developing Italian individuals compared with German individuals. However, we could not confirm the earlier results on differences

in development time, growth rates, and body size among Italian and German populations (Günter, Beaulieu, Franke, et al., 2020; Günter, Beaulieu, Freiberg, et al., 2020). This is, at least in part, likely due to the pronounced variation among populations within countries (see Figure S2: Appendix S1 for further illustration), in combination with the low statistical power of nested GLMs when the number of replicates is low. Note the substantial phenotypic difference in development time and growth rate between German and Italian populations, in accordance with previous results, also in the present study (Figure 1).

FIGURE 3 Effects of origin (Germany vs. Italy) and butterfly sex on forewing length (a) and wing aspect ratio (b) in *Pieris napi*. Given are means + SE



Indeed, populations from Germany and Italy differed in their responses to host-plant quality, as indicated by significant $G \times E$ suggesting genetic variation in phenotypic plasticity (Via & Lande, 1985). Such interactions occurred most frequently between origin and host-plant temperature (five significant interactions). Specifically, feeding on plants grown at the higher temperature diminished performance of Italian individuals by decreasing larval growth rate, pupal mass, and thorax mass but increasing development time, while German individuals remained unaffected (Figure 1). Furthermore, two significant interactions between origin and plant water treatment were found, again showing that the plant treatment only affected Italian individuals. These showed longer development times and lower larval growth rates when feeding on drought-stressed compared with control plants (Figure 2). The above findings indicate, as predicted, that Italian populations (1) respond more strongly to variation in host-plant quality and (2) are more sensitive to poor food quality than German ones. This may, consequently, reflect a cost of the rapid lifestyle of Italian individuals (Günter, Beaulieu, Franke, et al., 2020; Günter, Beaulieu, Freiberg, et al., 2020). The fact that wing length actually increased when feeding on plants grown at the higher temperature, despite reduced pupal and thorax mass, may seem contradictory upon first sight. This is because wing length is typically positively related to body mass. However, we suggest that it may reflect increased investment into dispersal ability to escape areas with adverse, that is, very warm, environmental conditions (Matthysen, 2012), in which plants are of reduced quality.

4.2 | Effects of host-plant treatment

Overall, our results clearly indicate detrimental effects of high plant-growing temperature on the performance of *P. napi*. This is evidenced by a prolonged development time and reduced larval growth rate, body mass, fat content, and PO activity when having fed on plants grown at the higher rather than lower temperature. These findings are in line with other studies on insects (Bauerfeind & Fischer, 2013a; Kuczyk, Müller, et al., 2021; Kuczyk, Raharivololoniaina, et al., 2021; but Raharivololoniaina et al., 2021). The specific mechanisms underlying the reduced performance are

unclear, but temperature-mediated changes in leaf glucosinolate, carbon, and carbon:nitrogen ratio, as have been documented in *S. alba*, may play a significant role (Kuczyk, Müller, et al., 2021). However, plant water regime did not affect any trait directly, and was involved in two interactions only. Thus, we could not confirm that drought stress increases food quality for herbivores in line with the plant-stress hypothesis (Franzke & Reinhold, 2011; Kuczyk, Müller, et al., 2021; Larsson, 1989; Salgado & Saastamoinen, 2019). Note that we have even found some weak evidence for negative effects of drought stress, as development times were longer and larval growth rates reduced when feeding on drought-stressed compared with control plants (Figure 2). We have no explanation for this deviation, but the results may depend on the specific treatments applied. Thus, we speculate that the severity of drought stress and interactions with temperature, with high temperatures increasing drought stress, may play a role (Kuczyk, Müller, et al., 2021). Assay conditions are well known to affect the outcome of biological experiments (e.g., Fischer et al., 2011; Petavy et al., 2001).

4.3 | Sexual differences

We found several sex differences that are typical for *P. napi* (Günter, Beaulieu, Freiberg, et al., 2020; Kuczyk, Müller, et al., 2021). In particular, males showed protandry, that is, they eclosed before females, to maximize mating opportunity (Fagerström & Wiklund, 1982; Wiklund & Fagerstrom, 1977), which was facilitated by higher larval growth rates. Males were larger than females, which is quite exceptional for an insect, but in the case of *P. napi*, this is driven by a covariance between body and spermatophore size. As males of this species are polyandrous, transferring a larger spermatophore ensures paternity by delaying remating (Wiklund & Kaitala, 1995). The lower thorax-abdomen ratio found in females likely reflects increased investment into the abdomen and thus fecundity selection, while males invest relatively more into flight muscles (Berwaerts et al., 2002; Honěk, 1993). A higher wing aspect ratio, finally, has been repeatedly found in male butterflies. It may increase flight ability, in particular maneuverability, which might be beneficial during mate location and courtship (Berwaerts et al., 2002, 2006).

5 | CONCLUSIONS

In general, *P. napi* exhibits pronounced genetic and plastic variation, which may indicate high adaptive capacities (Günter, Beaulieu, Freiberg, et al., 2020). While previous studies on this species have assessed exclusively direct (e.g., Bauerfeind & Fischer, 2013c; Bauerfeind & Fischer, 2014) or both direct and indirect effects (Bauerfeind & Fischer, 2013a, 2013b), we here explicitly focused on variation in response to indirect effects across populations. Our recent findings suggest that Italian populations respond more strongly to variation in host-plant quality and are more sensitive to poor food quality than German ones. In terms of vulnerability to climate change, two conclusions seem important. First, German individuals appear to respond less plastically to variation in food quality. This, however, does not seem to be disadvantageous facing climate change. Rather, it seems that these slow developing populations are more resilient against environmental perturbations (at least with regard to food quality), and may therefore potentially even benefit from warmer temperatures, perhaps allowing additional generations per year. Second, Italian individuals with their rapid lifestyle are probably not able to benefit from warmer conditions which reduce development time, as they will likely suffer from the concomitantly reduced host-plant quality. These “warm-adapted” populations appear, therefore, to be more at risk from climate change than their northern counterparts, potentially reflecting a cost of their rapid lifestyle (Günter, Beaulieu, Freiberg, et al., 2020; Günter, Beaulieu, Freiberg, et al., 2020). We believe that our results have wide-ranging implications also for other herbivorous insects, as it seems likely that climate change will have pronounced effects on plant chemistry (DeLucia et al., 2012; Kuczyk, Müller, et al., 2021; Kuczyk, Raharivololoniaina, et al., 2021; Suseela & Tharayil, 2018). It seems also conceivable that time-stressed species or populations respond generally more strongly to poor food quality, based on a high demand for nutrients to fuel rapid development. However, since we did not consider the combination of both direct and indirect effects, which may both be of relevance in nature, our results should be interpreted with caution. Nevertheless, our study exemplifies how investigating $G \times E$ may help to better understand the vulnerability of populations to climate change.

ACKNOWLEDGMENTS

We thank Elisabeth Reim and Massimo Brunetti for help with catching butterflies, Christin Park and Bianca Sorge for support during experiments, and two anonymous reviewers for valuable input. This work was supported by the Universities of Greifswald and Koblenz-Landau.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Josephine Kuczyk: Data curation (equal); Formal analysis (equal); Investigation (lead); Validation (equal); Visualization (equal);

Writing-original draft (equal); Writing-review & editing (equal). **Ange Raharivololoniaina:** Formal analysis (equal); Validation (equal); Visualization (equal); Writing-original draft (equal); Writing-review & editing (equal). **Klaus Fischer:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (lead); Investigation (supporting); Methodology (equal); Project administration (equal); Resources (lead); Supervision (lead); Validation (equal); Writing-original draft (supporting); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

Data from this study are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.g1jwstqs1>.

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How to cite this article: Kuczyk, J., Raharivoloniaina, A., & Fischer, K. (2021). Population-specific responses of an insect herbivore to variation in host-plant quality. *Ecology and Evolution*, 11, 17963–17972. <https://doi.org/10.1002/ece3.8392>