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Seasonal plasticity in morphology and metabolism differs between migratory North American and resident Costa Rican monarch butterflies

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Abstract

Environmental heterogeneity in temperate latitudes is expected to maintain seasonally plastic life-history strategies that include the tuning of morphologies and metabolism that support overwintering. For species that have expanded their ranges into tropical latitudes, it is unclear the extent to which the capacity for plasticity will be maintained or will erode with disuse. The migratory generations of the North American (NA) monarch butterfly *Danaus plexippus* lead distinctly different lives from their summer generation NA parents and their tropical descendants living in Costa Rica (CR). NA migratory monarchs postpone reproduction, travel thousands of kilometers south to overwinter in Mexico, and subsist on little food for months. Whether recently dispersed populations of monarchs such as those in Costa Rica, which are no longer subject to selection imposed by migration, retain ancestral seasonal plasticity is unclear. To investigate differences in seasonal plasticity, we reared NA and CR monarchs in summer and autumn in Illinois, USA, and measured seasonal reaction norms for aspects of morphology and metabolism related to flight. NA monarchs were seasonally plastic in forewing and thorax size, increasing wing area and thorax to body mass ratio in autumn. While CR monarchs increased thorax mass in autumn, they did not increase the area of the forewing. NA monarchs maintained similar resting and maximal flight metabolic rates across seasons. However, CR monarchs had elevated metabolic rates in autumn. Our findings suggest that the recent expansion of monarchs into habitats that support year-round breeding may be accompanied by (1) the loss of some aspects of morphological plasticity as well as (2) the underlying physiological mechanisms that maintain metabolic homeostasis in the face of temperature heterogeneity.

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Keywords

Seasonal plasticity, migration phenotypes, metabolic rate, wing morphology, *Danaus plexippus*, monarch butterfly

Introduction

Fluctuating seasonal environments in temperate habitats are expected to favor the evolution of overwintering strategies that are, by their nature, plastic responses to the environment (Moran 1992; Kingsolver and Huey 1998). For some species, these strategies involve changes in physiology and morphology that accompany overwintering in place, while for other species they involve physiological and morphological changes that support seasonal migration (Arnold et al. 2004; Butler and Woakes 2001). Theory for the evolutionary maintenance and loss of plasticity has been well developed (Via and Lande 1985, 1987; de Jong 1990; Van Tienderen 1991; Gomulkiewicz and Kirkpatrick 1992; Moran 1992; Gavrilets and Scheiner 1993) and there are established empirical frameworks and organismal systems for investigating the evolutionary dynamics of seasonal plasticity (Kingsolver and Huey 1998; Scheiner 1993). Trait plasticity may be lost when species ranges expand out of temperate, seasonal environments and into tropical, constant environments. This loss may occur via costs of plasticity that include a reduced efficacy of selection (Van Tienderen 1991; Kawecki 1994; Whitlock 1996; DeWitt et al. 1998; Van Dyken and Wade 2010) or genetic assimilation whereby adaptation to a new constant environment fixes the trait value (Waddington 1961; Price et al. 2003; Lande 2009; Schleicherová et al. 2013; Wan et al. 2018).

Trait plasticity may be a key factor enabling population persistence as environments across the globe become more variable (Catullo et al. 2019; Matesanz and Ramirez-Valiente 2019; O'Connor et al. 2012; Price et al. 2003; Sgro et al. 2016), motivating a better empirical

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61 understanding of how and when plasticity is lost. The loss of plasticity through assimilation,
62 where some populations evolve a fixed phenotype that maximizes fitness in a new, stable
63 environment (Aubret and Shine 2009; Corl et al. 2018) has been demonstrated in a handful of
64 systems, including at a mechanistic and genetic level for wing coloration in common buckeye
65 butterflies (van der Burg et al. 2020). A series of studies by Cooper et al. (2012, 2014)
66 suggests that cellular membrane plasticity in phospholipid composition can be eroded through
67 disuse or via costs of maintaining plasticity in fruit fly populations evolved in constant
68 thermal environments. Relaxed selection at cold-acclimation genes coincides with range
69 expansion into warmer latitudes in *Arabidopsis* (Zhen and Ungerer 2008; Zhen et al. 2011).
70 However, seasonal plasticity is complex in that it involves suites of traits to support divergent
71 physiologies or life histories across seasons (Williams et al. 2017; Wilsterman 2021).
72 Investigating how this complex multi-trait plasticity is lost (e.g., piecemeal versus wholesale
73 loss) when species ranges expand into less seasonal latitudes may provide insight into both the
74 mechanisms of trait integration and trait loss, as well as identify aspects of seasonal plasticity
75 that may be retained and respond to different environmental cues in the new environment.
76 North American (NA) monarch butterflies (*Danaus plexippus*) are well known for
77 their long-distance seasonal migration plasticity that meets different dispersal, reproductive,
78 and energetic demands across generations (Reppert and de Roode 2018). Summer generations
79 are short-lived and reproduce shortly after adult eclosion. The autumn/winter generation lives
80 for 8-12 months during which they migrate to their overwintering grounds in Mexico where
81 they remain in reproductive diapause until the following spring. They then migrate back into
82 the Southern United States and successive generations recolonize northern latitudes. In N.
83 America, seasonally variable environmental conditions are predicted to maintain plasticity for

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many aspects of morphology and physiology that support the different life-history strategies in summer versus autumn/winter generations. Notably, previous studies comparing summer (non-migratory) and autumn (migratory) generation monarchs have shown that NA monarchs eclose in reproductive diapause, have increased longevity and cold tolerance, greater fat stores, differences in sun compass neuropil volume, and a strong drive to fly south in autumn compared with summer-eclosing monarchs (Barker and Herman 1976; Herman and Tatar 2001; Goehring and Oberhauser 2002; Brower et al. 2006; Zhu et al. 2008, 2009; Heinze et al. 2013; Tenger-Trolander et al. 2019).

NA monarchs have expanded their range through multiple independent dispersal events into tropical latitudes that lack seasonal heterogeneity and support resident, year-round breeding populations (Zhan et al. 2014), making monarchs a good system to investigate the loss of complex multi-trait plasticity. These populations are descendants of the migratory NA population, but have lost long-distance migratory behavior and differ in two migration-relevant phenotypes – wing size and sun compass neuron tuning to sunlight (Altizer and Davis 2010; Freedman et al. 2020; Nguyen et al. 2021). Today, non-migratory populations can be found in Central and South America, the Caribbean, the Iberian Peninsula, Morocco, the Pacific Islands, Australia, and New Zealand (Zhan et al. 2014; Pfeiler et al. 2017). In Australia, there are both migratory and non-migratory populations that exhibit plasticity in reproductive development (James 1984; Dingle et al. 1999; Freedman et al. 2018), suggesting that some aspects of seasonal migration plasticity may be maintained within some dispersed populations.

Here, we quantified plasticity across generations in NA monarchs for wing morphology and metabolic traits that are related to long-distance migration, and then asked whether Costa

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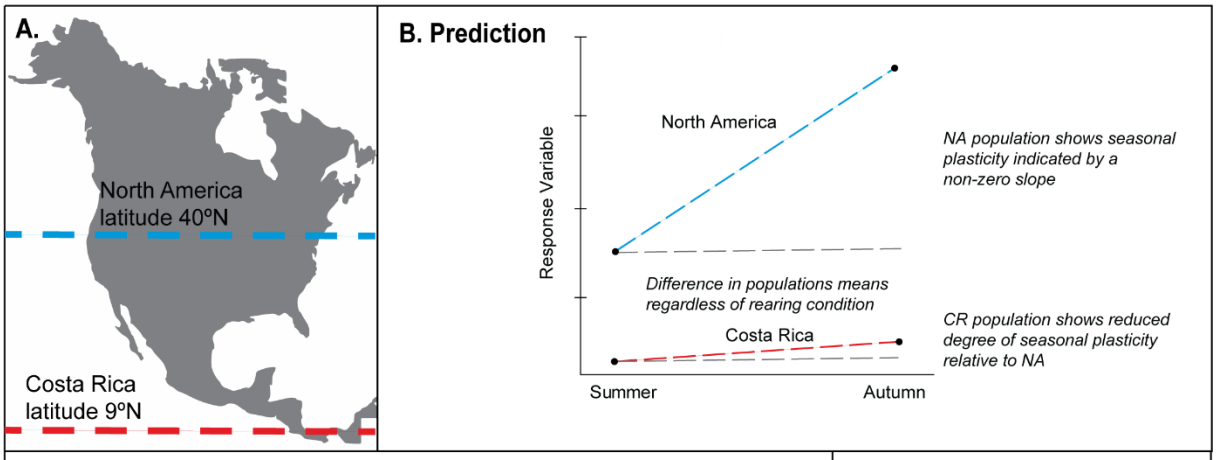
Rican (CR) monarch butterflies have lost or decreased plasticity in these traits (Figure 1). Using a common garden experiment with seasonal rearing of NA and CR monarchs, we tested the prediction that migratory populations have greater plasticity in response to seasonal rearing conditions than do non-migratory populations that no longer experience temperate-latitude seasonality in temperature, day length, and host-plant availability (Figure 1).



C. Results

Response Variables	Predictor	Seasonal plasticity?	
		Costa Rica	North America
Egg count	Season + Population	Yes	Yes
Ratio of thorax:body mass	Season + Population + Sex + Season:Sex + Population:Sex	Yes	Yes
Wing size (area)	Season + Population + Season:Population	No	Yes
Metabolic rates (resting and flight)	Season:Population	Yes	No
Wing shape (circularity, aspect ratio, geometric morphometrics)	None	No	No

Figure 1. Summary of the experiment, predictions, and main findings investigating seasonal plasticity in Costa Rican (CR) and North American (NA) monarchs. A) Map of North and Central America indicating NA (blue) and CR (red) monarch respective latitudes. B) Prediction and potential outcomes of a possible response variable (i.e., a trait). Populations may differ in trait value regardless of seasonal rearing condition. Populations may also differ in seasonal trait plasticity. A non-zero reaction norm between summer and autumn trait values within a population indicates the presence of seasonal plasticity (blue and red vs grey lines), and differences in reaction norm between populations indicate differences in degree of plasticity (different slope of blue and red line). C) List of traits measured in this study, the independent

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variables (population, rearing season, and sex) that explained significant variance in the trait, and whether each population exhibited seasonal trait plasticity.

Methods

Trait Selection

We selected physiological and morphological traits that are likely subject to different selective pressures depending upon seasonal environmental heterogeneity, including egg count, thorax and abdomen mass, wing size, wing shape, and metabolic rate. Plasticity in these traits is thought to impact monarch success in reproduction, migration and overwintering. We counted the number of mature oocytes as a measure of reproductive arrest, a well-known phenomenon in migrating NA monarchs that is correlated with their longevity and overwintering strategy (Barker and Herman 1976; Goehring and Oberhauser 2002). We measured resting and maximal flight metabolic rates to quantify plasticity in energy demand that supports adult maintenance and flight. We massed the thorax and abdomen separately to estimate mass associated with the flight muscles and with the reproductive organs and fat body respectively. We measured forewing size and shape as traits associated with flight efficiency. Larger wings generate more lift due to lower wing loading, and more narrow wings with high aspect ratios decrease drag. (Winkler and Leisler 1992; Senar et al. 1994; Lockwood et al. 1998; Swaddle and Witter 1998; Dudley 2000; Egbert and Belthoff 2003; Wang 2004).

Seasonal rearing

We reared two generations (summer and autumn) of NA and CR monarch butterflies outdoors in Chicago, IL in 2016 and 2017. Rearing was done under permits from USDA-APHIS. Butterflies that emerged in July and August were designated as the summer

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generation and those that emerged in September and October were designated as the autumn generation. In both years, the autumn generation were the offspring of the summer generation. In 2016, we measured metabolic traits in female and male adult monarchs and counted the number of mature oocytes in the females. We repeated these measurements for monarchs reared in 2017, with the addition of morphological measurements, including body mass, forewing size, and forewing shape.

Sample sizes

We reared 576 monarchs (149 individuals in 2016 and 427 individuals in 2017) and measured 573 of these for at least one trait. We measured 179 individual's metabolic rates, dissected 165 females to count the number of mature oocytes present in the abdomen, dried and massed 184 individuals, assayed geometric morphometric shape traits of 254 individuals, and measured the forewing size and shape traits for 237 individuals. Some individuals were used for multiple measurements, but those for which we counted oocytes could not be used for mass measurements and vice versa. In addition, butterflies for which we measured metabolic traits were more likely to be tattered and excluded from wing trait measurements. Further details on the number of individuals measured for each trait by rearing year, season of development, and population can be found in Table 1.

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Table 1. Sample sizes for all traits measured. Top) For samples reared in 2016, we assayed metabolic rate (MR) and number of mature oocytes (MO). MR + MO indicates the subset of individuals from the MR and MO row tallies for which we have measurements of both traits for the same individual. Bottom) For samples reared in 2017, in addition to metabolic rate assays and oocyte counts, we measured morphological traits using wings and bodies. MR + MO indicates the subset of individuals from the MR and MO row tallies for which we have measurements of both traits for the same individual, and Mass + Wings indicates the subset of individuals from the Mass and Wings row tallies for which we have measurements of both traits for the same individual.

	Sample size in 2016 (N = 149)			
	North America (N = 85)		Costa Rica (N = 64)	
	Summer (N = 53)	Autumn (N = 32)	Summer (N = 44)	Autumn (N = 20)
Phenotypes				
Metabolic Rate (MR)	36	32	25	12
Mature Oocyte (MO)	33	10	32	11
MR + MO	16	10	13	3

	Sample size in 2017 (N = 427)			
	North America (N = 233)		Costa Rica (N = 194)	
	Summer (N = 159)	Autumn (N = 74)	Summer (N = 114)	Autumn (N = 80)
Phenotypes				
Metabolic Rate (MR)	20	7	30	17
Mature Oocyte (MO)	33	20	5	16
MR + MO	4	0	0	4
Mass	55	36	60	33
Wings	83	61	48	62
Mass + Wings	7	32	18	32

Genetic composition

We derived CR monarchs from ~20 pupae obtained from a butterfly breeder in Costa Rica in 2016 and in 2017. While Central and South American monarchs remain the most genetically similar to NA monarchs, perhaps as a result of continuing gene flow (Pierce et al. 2014; Freedman et al. 2020), their estimated divergence time of 2,000-3,000 years from the N. American population is the largest among the three dispersals (Zhan et al. 2014).

All NA monarchs reared in 2017 were derived from wild-caught NA monarchs captured in Chicago, IL and morphological traits were only measured in 2017 (Table 1). The

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NA monarchs reared in 2016 were derived from two sources: wild-caught and commercially sourced NA individuals. At the time of the first common garden experiment, we were not aware of the genetic distinctiveness of the commercial NA lineage compared with the wild NA population (Tenger-Trolander et al. 2019). Of the 179 individuals assayed for MR (Supplemental Information, Table S1), only 18 individuals were purely commercial (15 summer-reared and 3 autumn-reared), 21 were NA/Commercial F1 crosses reared in summer, and 29 were backcrosses (NA/Commercial F1 backcrossed to NA) reared in autumn. While a proportion of pure commercial NA monarchs have lost the propensity to orient south in response to autumn rearing conditions (Tenger-Trolander et al. 2019; Tenger-Trolander and Kronforst 2020), the backcrosses (NA/Commercial F1 backcrossed to NA) reared in autumn showed clear southern orientation (Tenger-Trolander et al. 2019). The commercial lineage also enters reproductive diapause when reared outdoors in autumn implying some physiological responses in the wild and commercial NA populations are similar (Tenger-Trolander et al. 2019).

Animal Husbandry

We housed the monarchs from their respective populations in medium size (91.5cm x 30.5cm^2) mesh pop-up cages outdoors with access to the host plant, *Asclepias syriaca*. After females laid eggs, we transferred the eggs to small (30.5cm^3) outdoor mesh pop-up cages and fed larvae on a diet of wild-collected *A. syriaca* cuttings. All pop-up cages were contained inside two large outdoor 1.83m^3 mesh cages separated by population of origin. As individuals eclosed, they were collected as virgin males and females, labeled with a unique ID, and left outdoors for a minimum of three days. Adults were then either shipped to Lincoln, NE for metabolic measurement or measured for morphological traits in Chicago. Adult butterflies

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were shipped overnight in glassine envelopes, spending between 12-24 hours in a dark cardboard box. Upon arrival, butterflies were separated by sex and housed in large collapsible butterfly cages in a laboratory space with natural light. Prior to metabolic measurements, all individuals were given at least 48 hours to acclimate. Individuals kept in Chicago were also separated by sex and housed outdoors in Chicago, IL until frozen for morphological trait measurements. All butterflies had access to a constant supply of artificial nectar (Birds Choice Butterfly Nectar, Chilton, WI).

In 2017, summer-reared monarchs were shipped back to Chicago from Lincoln to found the autumn generation due to a summer die-off caused by the spillover of the pesticide permethrin from a neighboring yard in Chicago. None of the summer-reared monarchs that we measured were present when this exposure occurred, and the subsequent autumn generation of monarchs was founded by individuals not present during the die-off.

Mature oocyte counts

Females were kept separately from males and never mated. We dissected females by making a longitudinal cut down the abdomen to remove eggs. We then counted the number of mature oocytes present. Immature and mature oocytes in monarchs are distinguished by the shape of the chorion. A smooth chorion surface indicates an immature oocyte while a chorion with ridges is considered mature.

Body mass measurements

We removed the wings, antennae, head, and legs from the body. We separated the thorax and abdomen and dried them at 60ºC in an incubator with a silica crystal desiccant for 72 hours. After drying, we weighed the thorax and abdomen both separately and together on an analytical balance.

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Wing size and shape measurements

We placed a single forewing and hindwing on a sheet of gridded paper with 0.635 cm squares or on a white sheet of paper with a metric ruler in view. We photographed the wings using a DSLR Canon EOS 70d camera with an 18-55mm lens. We scaled each photo by number of pixels/cm and converted the color photos to 8-bit black/white images in ImageJ (Schindelin et al. 2012; Rueden et al. 2017). We filled in non-black portions of the forewing with black to measure area (Supplementary Information, Figure S1). Before measuring area or shape attributes, we smoothed the contours of the forewings with the ImageJ plugin 'Shape smoothing' (Erdenetsogt and Wagner 2016). 'Shape smoothing' applies a Fourier transformation to gain Fourier descriptors (FDs). We kept 0.35% of FDs relative to the total number of FDs identified in the image (Supplementary Information, Figure S1). We then measured the area (in cm^2), aspect ratio (length/width), and circularity (4π*area/perimeter^2) of each forewing in ImageJ (Rueden et al. 2017). To measure aspect ratio, ImageJ finds the longest length (major axis) and width (minor axis) of the object while maintaining the perpendicular intersection of both lines and divides the major axis length by the minor. Higher circularity scores indicate a more circular wing shape whereas lower scores more polygonal or angular shapes. Circularity is different than roundness (4*area/(π*major_axis^2)). For example, a hexagon has high circularity and low roundness whereas an oval has low circularity and high roundness.

We also used 2D landmark-based geometric morphometrics to assess shape differences. Using the software tpsDIG2ws, we placed 16 landmarks at homologous points (vein intersections and margins) on each forewing (Rohlf 2006) (Supplementary Information, Figure S2). We analyzed the resulting landmark data in R using the package 'Geomorph'

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(Adams and Collyer 2020). We performed a general Procrustes analysis that removed differences in orientation and size, allowing us to focus exclusively on shape differences (Supplementary Information, Figure S3). We then calculated the mean shape which is the average landmark coordinates for a set of aligned wings. For each of the 16 landmarks, we calculated the distance between the individual's coordinates and the group mean coordinates and summed those distances to find each specimen's total distance from the mean shape.

Metabolic rate measures

Using flow-through respirometry, we estimated resting or routine metabolic rate (MR) and maximal flight MR from the volume of CO_2 (VCO_2) produced by individual adult monarchs ranging from 3-45 days old. While not ideal, this age range was a consequence of shipping logistics between Chicago and Lincoln. Age was not a significant predictor of MR, even when controlling for mass in an analysis of covariance (routine MR, $P = 0.157$; flight MR, $P=0.310$). Older butterflies tended to be smaller (effect of age on mass, $P = 0.009$), and variation in mass was accounted for in our statistical analysis of MR (see below in *Statistical Analyses)*. Butterflies were placed in a 3.3-liter glass cylindrical container covered with a piece of black velvet cloth ensuring complete darkness during resting MR measurements. CO_2-free, dry air was pumped through the container at a rate of 3 liters/minute using a dual pump system (Sable Systems International, Las Vegas, NV, USA) coupled with a mass-flow valve (Sierra Instruments, Monterey, CA, USA). After the air left the measurement container, it was subsampled at 100 ml/min using a SS-4 Sub-Sampler pump (Sable Systems International, Las Vegas, NV, USA), scrubbed of water and then passed into a high-performance CO_2/H_2O differential gas analyzer (LI-7000, Li-Cor, Lincoln, NE, USA) to

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quantify CO_2. All MR data were collected using the Expedata software package (Sable Systems International, Las Vegas, NV, USA).

Individuals rested in the cloth-covered chamber at 21ºC for a minimum of 25 minutes prior to metabolic rate measurement. Before removing the cloth, we recorded resting MR until a stable resting MR was established. We then removed the cloth and exposed the individual to full-spectrum UV light. After 30 seconds of light exposure, we induced flight by gently shaking the container. We recorded 10 minutes of CO_2 production during flight. If butterflies stopped flying during this 10-min period, we gently shook the chamber to induce flight. After flying for 10 minutes, we turned off the light and covered the container to allow the butterfly to return to a stable resting MR. While 21ºC is cooler than others' have used to measure flight metabolic rate (e.g., Zhan et al. 2014; Pocius et al. 2022), we chose this because it was the common garden temperature at which all monarchs were being held in the lab prior to measurements. We experienced no issues inducing flight, which was likely facilitated by warming due to the full-spectrum UV light and monarch thermoregulatory behavior (Masters et al. 1988). The maximal rate of CO_2 production sustained over a 1-min period during the 10-minute flight was used as our estimate of maximal flight MR. Before and after each metabolic measurement, baseline CO_2 values were recorded and drift-corrected using the two-endpoint method in Expedata. To compensate for a response lag in the respirometry system, we utilized the "Z-transformation" function (instantaneous transformation) in Expedata. Raw CO_2 values were converted from parts per million to ml/hr.

Statistical analyses

For analyses of morphological traits, we used the R package 'glmulti' to automatically select the best fit generalized linear model for each trait and determine which of the

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294 independent variables (sex, population, season) were significant predictors of the
295 measurements (Calcagno and de Mazancourt, 2010; R Core Team 2013). We fit thorax mass
296 (grams), thorax:body mass ratio, forewing area (cm^2), and abdomen mass (log-transformed)
297 within the Gaussian family as these traits were normally distributed (Supplementary
298 Information, Figure S4A, Table S2 and S3). For egg counts, we fit the model with a negative
299 binomial distribution (Supplementary Information, Figure S4B and Table S4).
300 To quantify the association between each independent variable and dependent variable
301 for our glms, we calculated effect size with the statistic Eta squared ($\eta 2$) which is the ratio of
302 each group's sum of squares to the total sum of squares. It is interpreted as the percentage of
303 variance accounted for by each variable in the glm. For the negative binomial model, we
304 relied on model coefficients to determine the predominant effect. We further performed the
305 rank-based nonparametric Kruskal-Wallis test to determine whether there were differences
306 between groups and then a post hoc Dunn test (with a Bonferroni correction for multiple
307 testing) to determine which groups were different. Circularity scores, aspect ratios, and mean
308 shape distances were not normally distributed, and various transformations of the
309 measurements did not yield normal distributions (Supplementary Information, Figure S4C). In
310 these cases, we relied on the rank-based nonparametric Kruskal-Wallis test with post hoc
311 Dunn test (with a Bonferroni correction) to identify differences between groups.
312 We used standardized major axis regression (SMA) implemented in the R package
313 "smatr" (Warton et al. 2006; R Core Team 2013) to test for the effects of rearing conditions
314 and population on metabolic rate. SMA controls for the relationship between metabolic rate
315 and mass (in our case, whole-body wet mass) like an analysis of covariance, but it accounts
316 for the fact that both metabolic rate and mass are measured with error. We used SMA to fit the

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metabolic scaling relationship between ln(VCO_2) and ln(mass) for all individuals within particular combinations of the factors sex, population (NA and CR), and rearing season (summer and autumn). We first tested whether the scaling relationship between MR and mass was similar between levels of our independent factors (i.e., testing for difference in slopes). When there was no evidence of a mass x factor interaction, we fit a common slope and then tested whether there was a significant effect of the factor on the elevation of the relationship between MR and mass (i.e., a difference in the mass-specific metabolic rate) or a significant shift along the x-axis between factor levels (i.e., a difference in mass). Because females and males did not differ significantly in the scaling relationship between MR and mass or in mass-specific MR (Supplementary Information, Table S5), the sexes were combined for all subsequent analyses. The scaling exponents relating ln(VCO_2) and ln(mass) were greater than 1. While this deviates from the broad interspecific pattern where metabolic rate scales with mass to the ¾ power, intraspecific scaling exponents frequently deviate from this expectation (Glazier 2005; Greenlee et al. 2014). These scaling exponents may also have differed if we had chosen a different measure for mass, e.g., with wings removed.

We used mass-corrected resting and flight MRs to test for the statistical significance of interactions between population and rearing condition on metabolic rates, which would indicate a difference in plasticity between NA and CR monarchs. Mass-corrected MRs were obtained by taking the residual for each individual from the SMA fits of ln(VCO_2) as a function of ln(mass) and adding back the average ln(VCO_2) to obtain a meaningful scale as in Hoekstra et al. (2013). The mass-corrected MRs were used as the dependent variable in linear models to test for the effects of population, rearing season, and the statistical interaction between population and rearing season.

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Results

Both NA and CR monarchs exhibit seasonal plasticity in female reproduction

The number of mature oocytes present in a female monarch's abdomen was best explained by the additive effects of season and population (Supplementary Information, Table S4). Both CR and NA monarchs had fewer mature oocytes when reared in autumn (Figure 2, Kruskal-Wallis $\chi^2 = 64.32$, df = 3, $P = 7.02e\text{-}14$), consistent with the known seasonal reproductive diapause. Season had a larger effect size estimate (1.31, 95% CI [0.94, 1.66]) than did population (-0.62, 95% CI [-0.97, -0.28]) with little CI overlap. Although our model suggests there was a significant effect of population, differences between CR and NA monarchs within each seasonal rearing environment were not significant after correcting for multiple tests.

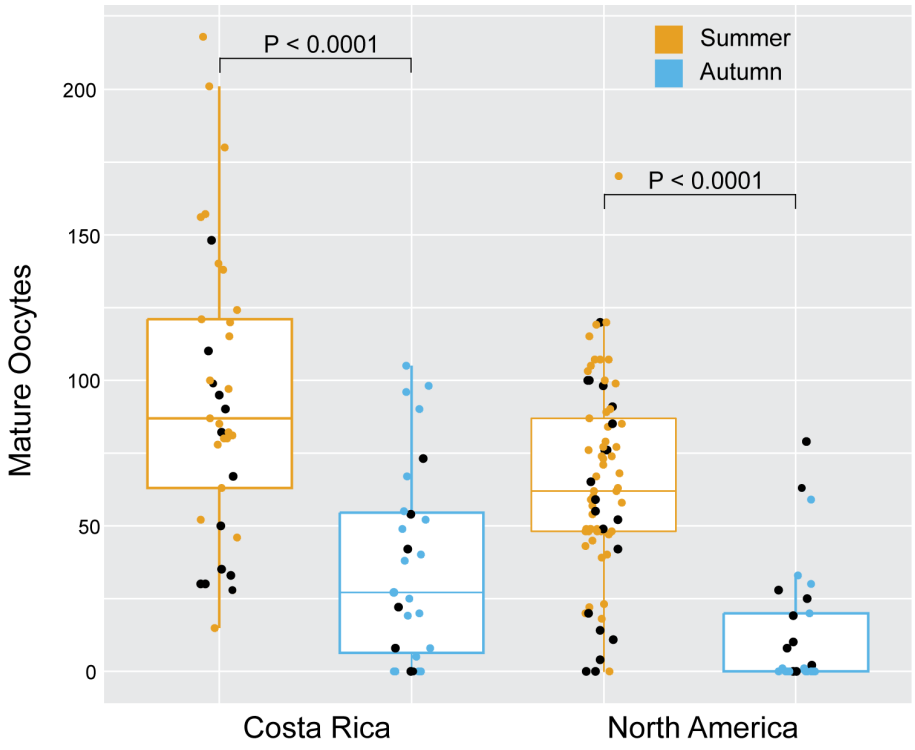

Figure 2. Boxplot of mature oocytes in female monarchs. Both CR and NA monarchs have decreased numbers of mature oocytes in response to autumn rearing, relative to summer rearing.

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Significant differences between rearing seasons are indicated on the plot. Black dots highlight individuals also assayed for metabolic rates.

Components of body mass differ in seasonal plasticity between sexes and populations

Total body mass (the combined mass of the thorax and abdomen) did not differ significantly between populations, rearing seasons, or sexes (mean = 0.0845 grams, Kruskal-Wallis χ^2= 11.4, df = 7, P = 0.12). However, abdomen mass was seasonally plastic in both NA and CR monarchs, and this plasticity differed between the sexes (Table 2). A model including season, sex, and their interaction explained ~10% of variation in abdomen mass (R^2 = 0.096; Table 2). Males reared in summer had lighter abdomens than females reared in summer (female mean = 0.0352g vs male mean = 0.043g, Kruskal-Wallis χ^2= 16.8, df = 7, P = 0.019, Dunn test with Bonferroni correction, P = 0.0035), and males increased abdomen mass in response to autumn (autumn mean = .0454g vs summer mean = 0.0352g, Dunn test with Bonferroni correction, P = 0.0043).

Thorax mass was seasonally plastic in both populations, with no evidence for a statistical interaction between season and population (Table 2). A model including rearing season, sex, and population explained 19% (R^2 = 0.19) of the variation in thorax mass (Table 2). An individual's thorax was likely to be heavier if population was NA, sex was male, and season of development was autumn (Supplementary Information, Figure S5). A post-hoc test found no significant differences between NA males and females or CR males and females reared in either season, though the difference between CR males and females was nearly significant in autumn (Kruskal-Wallis χ^2= 38.67, df = 7, P < 0.0001, Dunn test with Bonferroni correction, NA: P = 0.88, P = 1.0, CR: P = 0.48, P = 0.08, summer and autumn respectively).

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Table 2. Summary of the best fit general linear model (glm) for abdomen mas, thorax mass, the ratio of thorax:body mass, and forewing area. Each model's R^2 is reported along with the significance and effect size of each independent variable in the model. η2 (Eta squared) is a measure of effect size that can be interpreted as the amount of variance accounted for by each variable in the best fit glm.

Abdomen mass, R^2 = 0.096

Independent variable	df	Sum Sq	Mean Sq	F value	P value		η2
Sex	1	0.31	0.3105	3.382	0.06755	.	0.017
Season	1	0.284	0.2843	3.097	0.08015	.	0.016
Sex:Season	1	1.168	1.1684	12.728	0.00046	***	0.064

Thorax mass, R^2 = 0.189

Independent variable	df	Sum Sq	Mean Sq	F value	P value		η2
Season	1	0.00078	0.000775	10.39	0.0015	**	0.047
Sex	1	0.00129	0.001294	17.36	4.81E-05	***	0.078
Population	1	0.00106	0.001058	14.19	0.00022	***	0.064

Thorax:body ratio, R^2 = 0.274

Independent variable	df	Sum Sq	Mean Sq	F value	P value		η2
Season	1	0.001	0.00095	0.272	0.60253		0.001
Sex	1	0.1072	0.10724	30.591	1.12E-07	***	0.125
Population	1	0.0417	0.04172	11.9	0.0007	***	0.049
Season:Sex	1	0.0614	0.06138	17.51	4.48E-05	***	0.071
Sex:Population	1	0.024	0.02399	6.844	0.00966	**	0.028

Forewing area, R^2 = 0.37

Independent variable	df	Sum Sq	Mean Sq	F value	P value		η2
Season	1	7.62	7.62	19.564	1.49E-05	***	0.053
Population	1	43.29	43.29	111.09	< 2e-16	***	0.3
Season:Population	1	2.41	2.41	6.173	0.0137	*	0.017

The ratio of thorax mass to total body mass was seasonally plastic in females, particularly in NA monarchs (Table 2). A model including population and sex, as well as interactions between population and sex and between sex and season explained 27% of the variation in the thorax:body mass ratio (R^2 = 0.27). Sex had the largest effect with males having higher thorax:body mass ratios than females. NA monarchs had higher thorax:body mass ratios than did CR monarchs. Females increased the thorax:body mass ratio when reared in autumn relative to summer, and this effect of season was significant in NA but not in CR females (Figure 3, Kruskal-Wallis χ^2= 52.97, df = 7, $P < 0.0001$, Dunn test with Bonferroni

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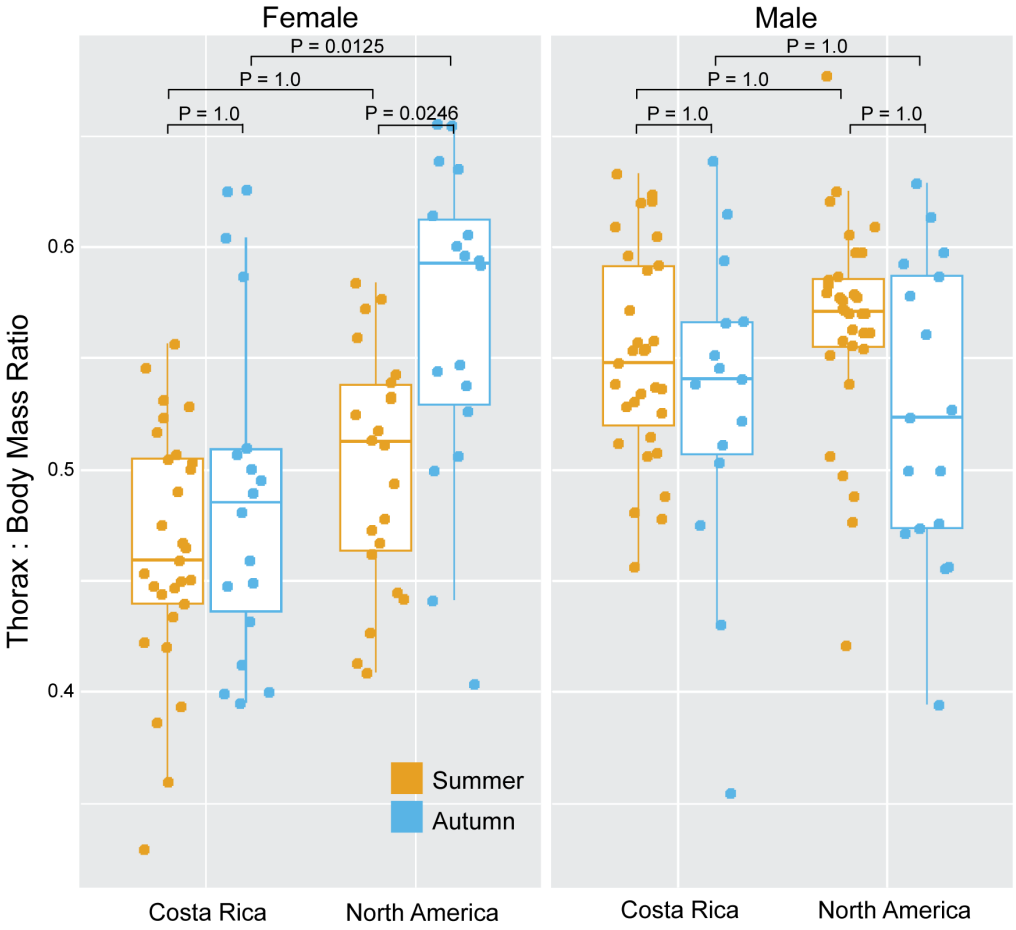
391 correction, CR: $P = 1$ and NA: $P = 0.0246$). Autumn-reared NA female thorax:body mass
392 ratios were significantly greater than those of autumn-reared CR females (Figure 3, Dunn test
393 with Bonferroni correction $P = 0.0125$). In summary, investment in thorax mass as a fraction
394 of total body mass exhibits a sex-specific plasticity that was significant in NA monarchs, with
395 NA females developing a more male-like pattern of investment in autumn versus summer.



396

397 **Figure 3.** Boxplot of the ratio of thorax mass to total body (thorax + abdomen) mass. Scores
398 above 0.5 indicate an individual has invested more of their total mass in the thorax than in
399 abdomen. NA female monarchs increase investment in thorax tissue in autumn. P-values for
400 differences between season, sex, and population are indicated on the plot.

401

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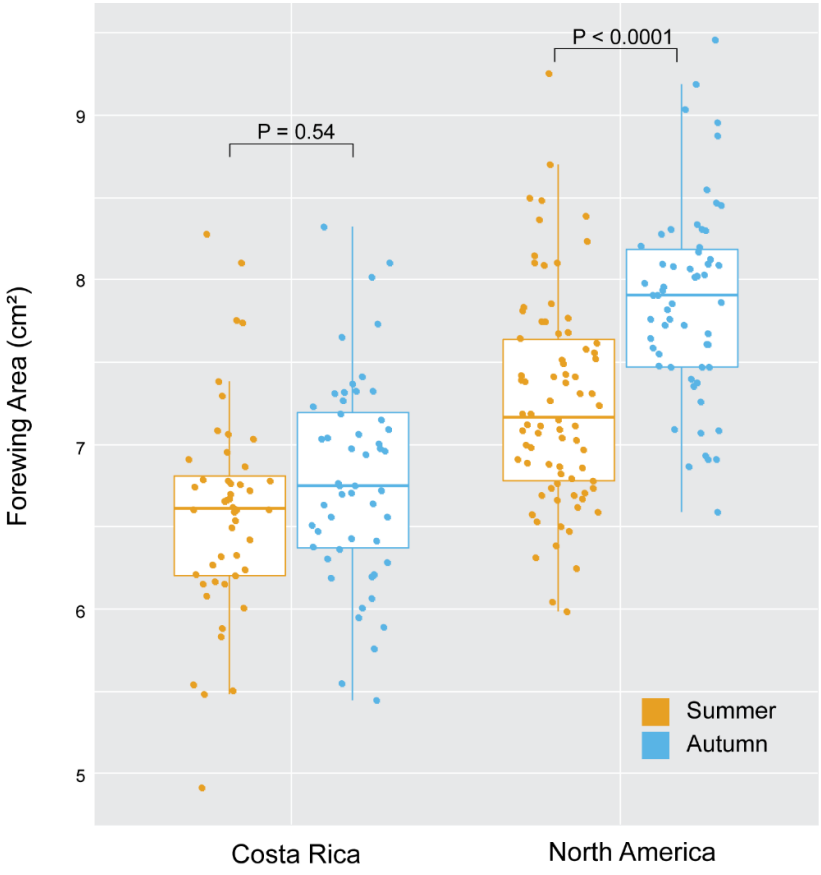
402 *Only NA monarchs exhibit seasonal plasticity in wing size*

403 Wing area was seasonally plastic in NA monarchs. Variation in wing area was best
404 explained by a model that included the effects of rearing season and population, as well as
405 their interaction ($R^2 = 0.37$; Table 2). NA monarchs reared in autumn had on average 8%
406 larger forewings than the NA summer-reared monarchs (Figure 4, summer mean =7.26 cm^2 vs
407 autumn mean = 7.87 cm^2, Kruskal-Wallis $\chi^2 = 90.68$, df = 3, $P < 0.0001$, Dunn test with
408 Bonferroni correction, $P < 0.0001$) and 16% larger forewings than the CR autumn-reared
409 monarchs. CR monarch forewing area was not seasonally plastic (Figure 4, summer mean =
410 6.56 cm^2 vs autumn mean = 6.79 cm^2, Dunn test with Bonferroni correction, $P = 0.54$).



411 **Figure 4.** Boxplot of the forewing area measured in cm^2. NA monarchs increase the size of
412 their forewing in response to autumn while CR monarchs do not. P-values for differences
413 between seasons in each population are indicated on the plot.
414

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Neither NA nor CR monarchs exhibit seasonal plasticity in wing shape

In contrast, measures of forewing shape did not differ between NA and CR monarchs and showed little to no within population plasticity. Variation in forewing aspect ratio was not explained by sex, season, population, or any of their interactions (Kruskal-Wallis χ^2= 7.37, df = 7, P = 0.39). Circularity of the forewing, where a value of 1 is a perfect circle and decreasing scores indicate more polygonal (angular) forewings, was not seasonally plastic in either population, although NA monarchs trended towards more angular wings in autumn (Supplementary Information, Figure S8A, Kruskal-Wallis χ^2= 8.72, df = 3, P = 0.03, Dunn test with Bonferroni correction, NA: P = 0.085 and CR: P = 0.85). Geometric morphometric analysis did not reveal any differences in mean shape between NA and CR forewings in either season, and neither population exhibited any seasonal plasticity in this measure of forewing shape (Supplementary Information, Figure S6 and S7). To quantify variability in forewing shape, we measured the distance of each individual forewing's landmark to the respective consensus mean landmark and summed those distances. Total distance from the mean shape did not vary by population or season (Supplementary Information, Figure S8B, Kruskal-Wallis χ^2= 3.58, df = 3, P = 0.31).

Metabolic rates were seasonally plastic in CR but not NA monarchs

Resting MR of NA monarchs was not seasonally plastic (mass x rearing, P = 0.55; rearing, P = 0.37) (Figure 5B and Supplementary Information, Table S6). However, autumn-reared CR monarchs had significantly greater resting MR relative to summer-reared CR monarchs (mass x rearing, P = 0.86; rearing, P = 2.63E-08) (Figure 5A and Supplementary Information, Table S6). Variation in mass-corrected MR was explained by a significant interaction between rearing season and population (P = 0.004; Table 3), with only CR

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438 monarchs exhibiting seasonal plasticity (Figure 5C). Resting MR of summer-reared NA and
439 CR monarchs were not significantly different, but resting MR of CR monarchs was
440 significantly greater than that of NA monarchs reared in autumn (Supplementary Information,
441 Table S7).

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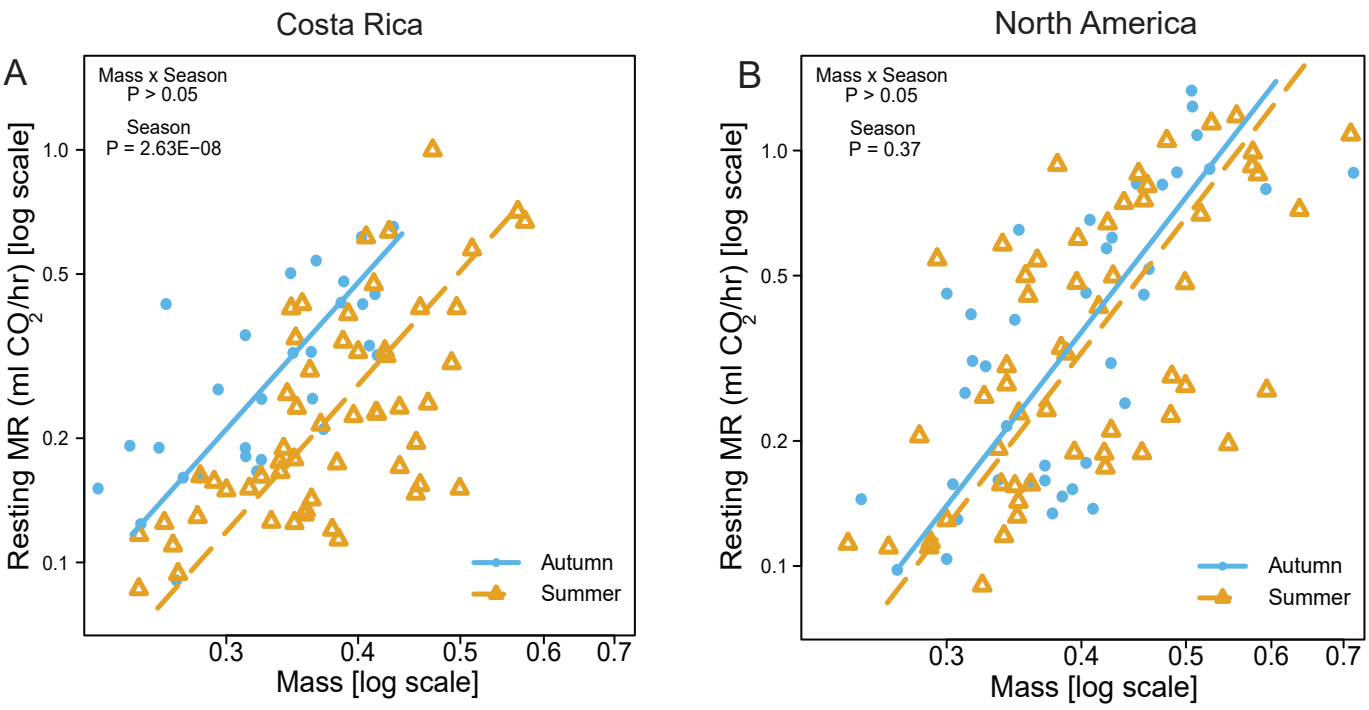

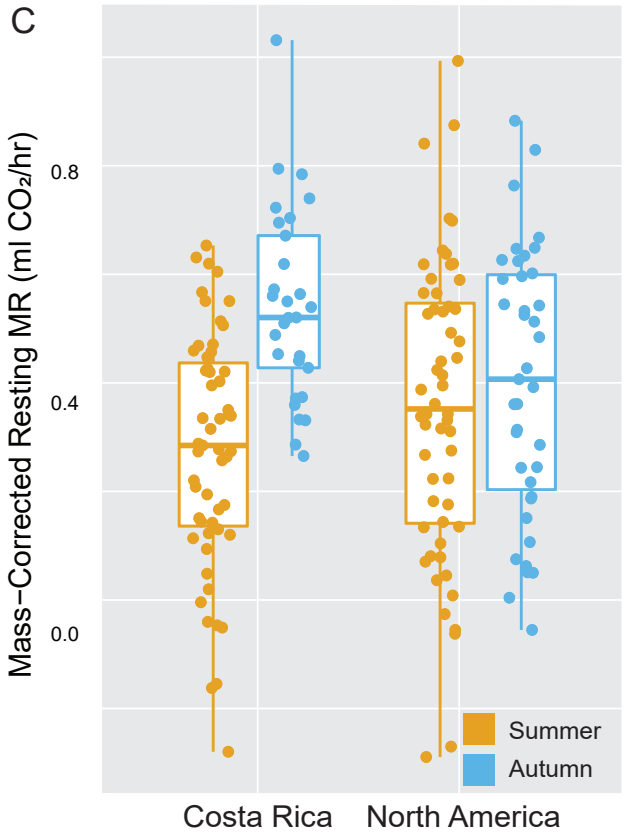

Figure 5. Effects of population and seasonal rearing conditions on resting metabolic rates (MR). A) Resting MR were significantly increased in CR monarchs reared in autumn relative to summer, B) while rearing season did not affect the resting MR of NA monarchs. C) There was a significant effect of the interaction between population and rearing season on mass-corrected resting MR (population x rearing, P = 0.004), with the maintenance of similar resting metabolic rates across seasonal rearing environments in NA but not in CR monarchs. Male and female data are plotted together, as the sexes did not differ in patterns of MR (Supplementary Information, Table S5).

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Similar to patterns for resting MR, CR monarchs had elevated flight MR when reared in autumn relative to summer (mass x rearing, $P = 0.19$, rearing $P = 0.04$) (Figure 6A and Supplementary Information, Table S6), but maximal flight MR in NA monarchs was not seasonally plastic (mass x rearing, $P = 0.97$; rearing $P = 0.19$) (Figure 6B and Supplementary Information, Table S6). When we corrected flight MR for mass, there was a significant effect of season ($P = 0.0283$; Table 3) but no significant interaction between rearing season and population (Table 3). However, the magnitude of seasonal plasticity in mass-corrected flight MR appeared larger in CR relative to NA monarchs (Figure 6C). Flight MR of summer-reared NA and CR monarchs were not significantly different, but autumn-reared CR and NA monarchs differed in the scaling relationship with mass, with larger NA monarchs maintaining lower flight MR than CR monarchs (Supplementary Information, Table S7).

Table 3. Summary of general linear model used to test for effects of population and rearing season on mass-corrected MR.

Trait	Independent Variable	df	Sum Sq	Mean Sq	F-value	P-value
Mass-corrected resting MR	Population	1	0.001	0.0014	0.03	0.8746
	Season	1	0.879	0.8789	15.64	0.0001 ***
	Population:Season	1	0.490	0.4900	8.72	0.0036 **
Mass-corrected flight MR	Population	1	0.036	0.0360	0.70	0.404
	Season	1	0.252	0.2515	4.89	0.0283 *
	Population:Season	1	0.023	0.0232	0.45	0.5026

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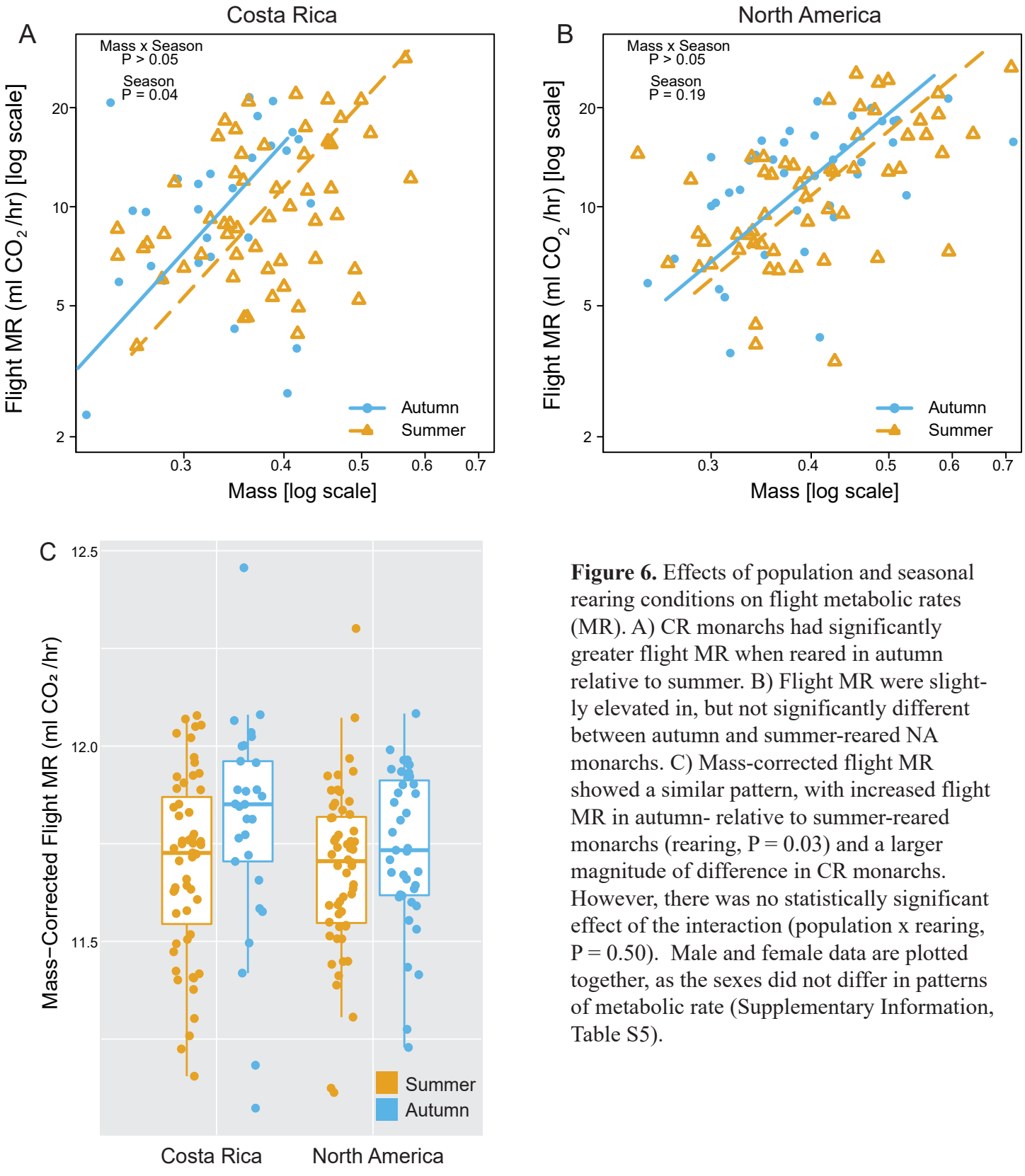

Figure 6. Effects of population and seasonal rearing conditions on flight metabolic rates (MR). A) CR monarchs had significantly greater flight MR when reared in autumn relative to summer. B) Flight MR were slightly elevated in, but not significantly different between autumn and summer-reared NA monarchs. C) Mass-corrected flight MR showed a similar pattern, with increased flight MR in autumn- relative to summer-reared monarchs (rearing, P = 0.03) and a larger magnitude of difference in CR monarchs. However, there was no statistically significant effect of the interaction (population x rearing, P = 0.50). Male and female data are plotted together, as the sexes did not differ in patterns of metabolic rate (Supplementary Information, Table S5).

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Discussion

We compared ancestral temperate (NA) and derived tropical (CR) monarch populations for the extent of seasonal plasticity in physiological and morphological traits suspected to be adaptive for monarch migration and overwintering. We predicted that plasticity would be lost in monarch populations that have dispersed into more stable, tropical habitats, such as Costa Rica. We found that the non-migratory CR descendants of the migratory NA population retain some, but not all ancestral seasonal trait plasticity. This suggests that seasonal plasticity in monarchs can be lost in a piecemeal fashion in the absence of selective pressures for its maintenance. The maintenance of metabolic rates in autumn compared to summer, plus the increase in wing size and thorax mass relative to total body mass in NA monarchs, suggest that these traits may be important for migration success and that the regulation of these traits may be critical to maintaining alternative summer and autumn phenotypes.

Mass differences in the abdomen and thorax are consistent with different selective pressures facing females versus males as well as NA versus CR populations. Autumn rearing induces an apparent shift in resources in females presumably from egg mass to flight muscle, consistent with the idea that successful autumn migration is critical for both sexes. While NA male and female monarchs were not significantly different in thorax mass in either season in our comparisons, a previous experiment that compared thorax mass in NA monarchs found significant differences between thorax mass in males and females (Davis and Holden 2015). Particularly in summer, we saw a similar trend towards larger male thoraxes, and sex was a significant predictor of thorax mass in our glm. Though not to the same degree as NA females, CR females also responded to autumn by increasing the thorax to body mass ratio

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480 though the difference comes from a decrease in abdomen mass rather than an increase in
481 thorax mass in autumn. In summary, CR females retained seasonally plastic reproduction, but
482 the seasonal shift in allocation to thorax mass may be eroding. Further investigation of
483 plasticity in resource allocation into reproductive and flight muscle tissues are warranted, as
484 well as investigation of whether other abiotic factors (e.g., drought or host-plant quality) may
485 induce reproductive diapause and maintain plasticity for this trait in tropical monarch
486 populations.

487 Forewing size was the most divergent morphological trait between NA and CR
488 monarchs. Consistent with other work comparing migratory and resident monarch
489 populations, we found CR monarchs had smaller wings than NA monarchs (Beall and
490 Williams 1945; Dockx 2007; Altizer and Davis 2010; Li et al. 2016; Freedman et al. 2020).
491 However, unlike previous work, our study explicitly compared monarchs reared in the NA
492 monarch's migratory range in summer and autumn. We found that forewing size was
493 seasonally plastic in NA but not in CR monarchs. Previous measurements from a study of
494 museum specimens collected in North America between 1878-2017 noted that autumn-
495 collected individuals had larger wings than summer (Freedman and Dingle 2018). Our data
496 suggest that this difference is at least partly explained by seasonal plasticity in wing size
497 rather than differential mortality during migration (Flockhart et al. 2017; Davis et al. 2020).
498 The smaller forewing size of CR and other resident monarch populations plus the CR
499 monarchs' lack of plasticity suggests that adaptation to the local environment post-dispersal
500 may have selected for smaller wing size. Meanwhile, large wing size is likely under constant
501 selection in migratory NA monarch populations during autumn, as large wing size is
502 associated with longer flight in butterflies (Altizer and Davis 2010, Li et al. 2016, Flockhart et

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503 al. 2017). Thus, this might be an example where seasonal heterogeneity maintains plasticity in
504 wing size in NA monarchs, with the summer-like small wing trait fixed in resident monarch
505 populations that experience more summer-like conditions throughout the year. Investigating
506 the flight and fitness consequences of these changes in wing morphology would be
507 particularly useful for assessing whether this is an example of the loss of plasticity through
508 adaptive assimilation.

509 The importance of wing shape to migration is less clear. Previous work found
510 differences in shape between some resident and migratory monarchs (Dockx 2007; Altizer
511 and Davis 2010; Satterfield and Davis 2014; Freedman et al. 2020), while other population
512 comparisons did not find differences (Li et al. 2016; Freedman et al. 2020). Between our three
513 measures of wing shape (geometric morphometrics, aspect ratio, and circularity), the only
514 significant shape difference was in forewing circularity between autumn-reared NA monarchs
515 and summer-reared CR monarchs, but the difference was small and the distributions were
516 largely overlapping. We suggest that the difference seen in circularity when comparing wild-
517 caught CR monarchs to NA monarchs (Altizer and Davis 2010) could be driven by
518 developmental environment rather than population. However, we found no evidence of
519 seasonal plasticity in wing shape in either population, consistent with findings from Flockhart
520 et al. (2017) which found no relationship between wing roundness or aspect ratio and distance
521 flown in NA migrators. However, others have noted differences in aspect ratio when
522 comparing wild-caught to indoor-reared NA monarchs (Davis et al. 2020) and when
523 comparing NA individuals caught earlier in the migration season to individuals caught later
524 (Satterfield and Davis 2014).

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525 In contrast to the prediction that NA monarchs relative to CR monarchs might exhibit
526 greater plasticity in metabolic rates to support flight during migration, we observed that
527 metabolic rates were affected by seasonal rearing only in CR monarchs. Autumn-reared CR
528 monarchs had elevated resting and flight metabolic rates relative to summer-reared monarchs,
529 while NA monarchs maintained similar and lower resting and flight metabolic rates across
530 seasons. There are two, non-mutually exclusive, ways to interpret this pattern. First, the NA
531 population may have seasonal plasticity in underlying physiology that maintains similar
532 metabolic rates across seasonal environments, with the plastic mechanisms that maintain
533 metabolic rate across the seasons lost in the CR population. Second, if the CR population has
534 lost either the maternal provisioning or developmental mechanisms appropriate for the shorter
535 photoperiod days of autumn, then the elevated metabolic rates in autumn-reared CR monarchs
536 may be the consequence of coping with environmental stress during development. That stress,
537 however, cannot be attributed to differences between reproductive output or host plant
538 between the populations, as monarchs from both populations significantly decreased egg
539 counts in response to autumn and consumed common milkweed in both summer and autumn
540 in our common garden experiment. We note that while common milkweed differs from CR's
541 native tropical milkweed host (*Asclepias curassavica*), this did not result in differences in
542 metabolic rate between the populations in the summer, suggesting that any effect of host plant
543 on population differences in metabolic rate in our study must interact with the effect of
544 seasonal rearing.

545 Our results were similar to previous studies of metabolic rates in NA and CR
546 monarchs that were reared in summer (Zhan et al. 2014), although that study used somewhat
547 different measures of metabolic rates and did detect differences in flight metabolic rate

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548 between migratory NA and resident Florida monarchs. Zhan et al. (2014) also found evidence
549 for positive selection and divergent expression of collagen IV alpha-1 and alpha-2 in adult
550 thoracic muscle tissue between migratory and non-migratory populations of monarchs. These
551 proteins are essential for muscle morphogenesis and function (Schnorrer et al. 2010), and have
552 been interpreted as evidence for the evolution of flight efficiency in migrating monarchs
553 (Zhan et al. 2014). Flight is energetically demanding, and selection for long-distance
554 migratory flight may favor more efficient flight relative to shorter duration flight (Rankin and
555 Burchsted 1992). Our results lend support to this hypothesis, as we found that NA monarchs
556 maintained similar resting and flight metabolic rates across seasons. We suggest that
557 migration is supported not by increased metabolic output but likely through other seasonally
558 plastic changes (e.g., in wing area, as we observed, and/or muscle structures) that enable more
559 efficient flight. These results contrast with some other migratory and dispersing insects that
560 have higher metabolic rates compared to their non-migratory and non-dispersing counterparts
561 (Tanaka and Okuda 1996; Zera et al. 1997; Crnokrak and Roff 2002; Niitepõld et al. 2009).
562 Of these examples, NA monarchs migrate the farthest and live the longest. Thus, the
563 maintenance of low metabolic rates may enable monarchs to better survive the months-long
564 overwintering period in Mexico where they consume very little food. Our observation that NA
565 butterflies are able to maintain low flight MR unlike CR butterflies reared in autumn may also
566 indicate that NA monarch physiology enables more efficient flight in the presence of
567 accumulated lipid reserves during migration (Gibo and McCurdy 1993; Brower et al. 2006;
568 Schroeder et al. 2020).

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Conflict of Interest Statement

The authors declare no conflicts of interest.

Author Contributions

All authors participated in conceiving the ideas and designing methodology; AT-T, WL and CRJ collected the data; AT-T, CRJ and KLM analysed the data; AT-T and CRJ led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data Availability

All data are included in supplemental file Data.xlsx.

References

Adams, D.C., and M. Collyer. 2020. Geomorph: Software for geometric morphometric analyses, R package version 3.2.1.

Altizer, S., and A.K. Davis. 2010. Populations of monarch butterflies with different migratory behaviors show divergence in wing morphology: wing morphology and monarch migration. Evolution 64:1018–1028.

Arnold, W., T. Ruf, S. Reimoser, F. Tataruch, K. Onderscheka, and F. Schober. 2004. Nocturnal hypometabolism as an overwintering strategy of red deer (*Cervus elaphus*). American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 286:R174–R181. American Physiological Society.

Aubret, A., and R. Shine. 2009. Genetic assimilation and the postcolonization erosion of phenotypic plasticity in Island Tiger Snakes. Current Biology 19:1932-1936

Barker, J.F., and W.S. Herman. 1976. Effect of photoperiod and temperature on reproduction of the monarch butterfly. Journal of Insect Physiology 22:1565–1568.

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.17.495480; this version posted June 18, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

606 Beall, G., and C.B. Williams. 1945. Geographical variation in the wing length of *Danaus*
607 *plexippus* (lep. Rhopalocera). Proceedings of the Royal Entomological Society of
608 London. Series A, General Entomology 20:65–76.
609 Brower, L.P., L.S. Fink, and P. Walford. 2006. Fueling the fall migration of the monarch
610 butterfly. Integrative and Comparative Biology 46:1123–1142.
611 Butler, P.J., and A.J. Woakes. 2001. Seasonal hypothermia in a large migrating bird: saving
612 energy for fat deposition? Journal of Experimental Biology 204:1361–1367.
613 Calcagno, V., and C. de Mazancourt. 2010. glmulti: An R package for easy automated model
614 selection with (generalized) linear models. Journal of Statistical Software 34.
615 Catullo, R.A., J. Llewelyn, B.L. Phillips, and C.C. Moritz. 2019. The potential for rapid
616 evolution under anthropogenic climate change. Current Biology 29:R996–R1007.
617 Cooper, B.S., L.A. Hammad, N.P. Fisher, J.A. Karty, and K.L. Montooth. 2012. In a variable
618 thermal environment selection favors greater plasticity of cell membranes in *Drosophila*
619 *melanogaster*. Evolution 66:1976–1984.
620 Cooper, B.S., L. A. Hammad, and K.L. Montooth. 2014. Thermal adaptation of cellular
621 membranes in natural populations of *Drosophila melanogaster*. Functional Ecology
622 28:886–894.
623 Corl, A., K. Bi, C. Luke, A.S. Challa, A.J. Stern, B. Sinervo, and R. Nielsen. 2018. The
624 genetic basis of adaptation following plastic changes in coloration in a novel
625 environment. Curr. Biol. 28, 2970– 2977.
626 Crnokrak, P., and D.A. Roff. 2002. Trade-offs to flight capability in *Gryllus firmus*: the
627 influence of whole-organism respiration rate on fitness. Journal of Evolutionary Biology
628 15:388–398.
629 Davis, A.K., and M.T. Holden. 2015. Measuring intraspecific variation in flight-related
630 morphology of monarch butterflies (*Danaus plexippus*): Which sex has the best flying
631 gear? Journal of Insects 591705.
632 Davis, A.K., F.M. Smith, and A.M. Ballew. 2020. A poor substitute for the real thing: captive-
633 reared monarch butterflies are weaker, paler and have less elongated wings than wild
634 migrants. Biology Letters 16:20190922.
635 de Jong, G. 1990. Quantitative genetics of reaction norms. Journal of Evolutionary Biology
636 3:447–468.

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.17.495480; this version posted June 18, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

637 DeWitt, T.J., A. Sih, and D.S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends
638 in Ecology & Evolution 13:77–81.
639 Dingle, H., M.P. Zalucki, and W.A. Rochester. 1999. Season-specific directional movement in
640 migratory Australian butterflies. Australian Journal of Entomology 38:323–329.
641 Dockx, C. 2007. Directional and stabilizing selection on wing size and shape in migrant and
642 resident monarch butterflies, *Danaus plexippus* (L.), in Cuba. Biological Journal of the
643 Linnean Society 92:605–616.
644 Dudley, R. 2000. Kinematics and aerodynamics of flight. Pp. 75–158 *in* The Biomechanics of
645 Insect Flight. Princeton University Press.
646 Egbert, J.R., and J.R. Belthoff. 2003. Wing shape in house finches differs relative to
647 migratory habit in eastern and western north america. The Condor 105:825–829.
648 Erdenetsogt, U., and T. Wagner. 2016. Shape Smoothing.
649 Flockhart, D.T.T., B. Fitz-gerald, L.P. Brower, R. Derbyshire, S. Altizer, K.A. Hobson, L.I.
650 Wassenaar, and D.R. Norris. 2017. Migration distance as a selective episode for wing
651 morphology in a migratory insect. Movement Ecology 5(1):1-9.
652 Freedman, M.G., and H. Dingle. 2018. Wing morphology in migratory North American
653 monarchs: characterizing sources of variation and understanding changes through time.
654 Animal Migration 5:61–73.
655 Freedman, M.G., H. Dingle, S.Y. Strauss, and S.R. Ramírez. 2020. Two centuries of monarch
656 butterfly collections reveal contrasting effects of range expansion and migration loss on
657 wing traits. Proceedings of the National Academy of Sciences of the United States of
658 America 117:28887–28893.
659 Freedman, M.G., H. Dingle, C.A. Tabuloc, J.C. Chiu, L.H. Yang, and M.P. Zalucki. 2018.
660 Non-migratory monarch butterflies, *Danaus plexippus* (L.), retain developmental
661 plasticity and a navigational mechanism associated with migration. Biological Journal
662 of the Linnean Society 123:265–278.
663 Gavrilets, S., and S.M. Scheiner. 1993. The genetics of phenotypic plasticity. V. Evolution of
664 reaction norm shape. Journal of Evolutionary Biology 6:31–48.
665 Gibo, D.L., and J.A. McCurdy. 1993. Lipid accumulation by migrating monarch butterflies
666 (*Danaus plexippus* L.). Canadian Journal of Zoology 71:76-82.

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.17.495480; this version posted June 18, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

667 Glazier, D.S. 2005. Beyond the '3/4-power law': variation in the intra-and interspecific
668 scaling of metabolic rate in animals. Biological Reviews 80:611–662.
669 Goehring, L., and K.S. Oberhauser. 2002. Effects of photoperiod, temperature, and host plant
670 age on induction of reproductive diapause and development time in *Danaus plexippus*.
671 Ecological Entomology 27:674–685.
672 Gomulkiewicz, R., and M. Kirkpatrick. 1992. Quantitative genetics and the evolution of
673 reaction norms. Evolution 46:390–411.
674 Greenlee, K.J., K.L. Montooth, and B.R. Helm. 2014. Predicting Performance and Plasticity
675 in the Development of Respiratory Structures and Metabolic Systems. Integrative and
676 Comparative Biology 54:307–322.
677 Heinze, S., J. Florman, S. Asokaraj, B. el Jundi, and S.M. Reppert. 2013. Anatomical basis of
678 sun compass navigation II: The neuronal composition of the central complex of the
679 monarch butterfly. Journal of Comparative Neurology 521:267–298.
680 Herman, W.S., and M. Tatar. 2001. Juvenile hormone regulation of longevity in the migratory
681 monarch butterfly. Proceedings of the Royal Society B: Biological Sciences 268:2509–
682 2514.
683 Hoekstra, L.A., M.A. Siddiq, and K. L. Montooth. 2013. Pleiotropic effects of a
684 mitochondrial–nuclear incompatibility depend upon the accelerating effect of
685 temperature in *Drosophila*. Genetics 195:1129–1139.
686 James, D.G. 1984. Migration and clustering phenology of *Danaus plexippus* (l.) (lepidoptera:
687 Nymphalidae) in Australia. Australian Journal of Entomology 23:199–204.
688 Kawecki, T.J. 1994. Accumulation of deleterious mutations and the evolutionary cost of being
689 a generalist. The American Naturalist 144:833–838.
690 Kingsolver, J.G., and R.B. Huey. 1998. Evolutionary analyses of morphological and
691 physiological plasticity in thermally variable environments. American Zoologist 383:
692 545–560.
693 Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic
694 plasticity and genetic assimilation. Journal of Evolutionary Biology 22:1435–1446.
695 Li, Y., A.A. Pierce, and J.C. de Roode. 2016. Variation in forewing size linked to migratory
696 status in monarch butterflies. Animal Migration 3:27-34.

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.17.495480; this version posted June 18, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Lockwood, R., J.P. Swaddle, and J.M.V. Rayner. 1998. Avian wingtip shape reconsidered: wingtip shape indices and morphological adaptations to migration. Journal of Avian Biology 29:273–292. [Nordic Society Oikos, Wiley].

Masters, A.R., S.B. Malcolm, and L.P. Brower. 1988. Monarch butterfly (*Danaus plexippus*) thermoregulatory behavior and adaptations for overwintering in Mexico. Ecology 69: 458–467.

Matesanz, S., and J.A. Ramírez-Valiente. 2019. A review and meta-analysis of intraspecific differences in phenotypic plasticity: Implications to forecast plant responses to climate change. Global Ecology and Biogeography 28:1682–1694.

Moran, N.A. 1992. The evolutionary maintenance of alternative phenotypes. The American Naturalist 139:971–989.

Nguyen, T.A.T., M.J. Beetz, C. Merlin, and B. el Jundi. 2021. Sun compass neurons are tuned to migratory orientation in monarch butterflies. Proceedings of the Royal Society B; Biological Sciences. 288:20202988.

Niitepõld, K., A.D. Smith, J.L. Osborne, D.R. Reynolds, N.L. Carreck, A.P. Martin, J.H. Marden, O. Ovaskainen, and I. Hanski. 2009. Flight metabolic rate and *Pgi* genotype influence butterfly dispersal rate in the field. Ecology 90:2223–2232.

O'Connor, M.I., E.R. Selig, M.L. Pinsky, and F. Altermatt. 2012. Toward a conceptual synthesis for climate change responses. Global Ecology and Biogeography 21:693–703.

Pfeiler, E., N.O. Nazario-Yepiz, F. Pérez-Gálvez, C.A. Chávez-Mora, M.R.L. Laclette, E. Rendón-Salinas, and T.A. Markow. 2017. Population genetics of overwintering monarch butterflies, *Danaus plexippus* (Linnaeus), from Central Mexico Inferred from mitochondrial DNA and microsatellite markers. Journal of Heredity 108:163–175.

Pierce, A.A., M.P. Zalucki, M. Bangura, M. Udawatta, M.R. Kronforst, S. Altizer, J.F. Haeger, and J. C. de Roode. 2014. Serial founder effects and genetic differentiation during worldwide range expansion of monarch butterflies. Proceedings of the Royal Society B: Biological Sciences 281:20142230–20142230.

Pocius, V.M., S. Cibotti, S. Ray, O. Ankoma-Darko, N.B. McCartney, R.J. Schilder, and J.G. Ali. 2022. Impacts of larval host plant species on dispersal traits and free-flight energetics of adult butterflies. Communications Biology 5:1–9.

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.17.495480; this version posted June 18, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

727 Price, T.D., A. Qvarnström, and D.E. Irwin. 2003. The role of phenotypic plasticity in driving
728 genetic evolution. Proceedings of the Royal Society of London. Series B: Biological
729 Sciences 270:1433–1440. Royal Society.
730 R Core Team. 2013. R: A language and environment for statistical computing. Vienna,
731 Austria.
732 Rankin, M.A., and J.C.A. Burchsted. 1992. The cost of migration in insects. Annual Review
733 of Entomology 37:533–559.
734 Reppert, S.M., and J.C. de Roode. 2018. Demystifying monarch butterfly migration. Current
735 Biology 28:R1009–R1022.
736 Rohlf, F.J. 2006. tpsDig. Department of Ecology and Evolution, State University of New
737 York at Stony Brook.
738 Rueden, C.T., J. Schindelin, M.C. Hiner, B.E. DeZonia, A.E. Walter, E.T. Arena, and K. W.
739 Eliceiri. 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC
740 Bioinformatics 18:529.
741 Satterfield, D.A., and A.K. Davis. 2014. Variation in wing characteristics of monarch
742 butterflies during migration: Earlier migrants have redder and more elongated wings.
743 Animal Migration 2:1-7.
744 Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. Annual Review of
745 Ecology and Systematics 24:35–68.
746 Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch,
747 C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D.J. White, V. Hartenstein, K.
748 Eliceiri, P. Tomancak, and A. Cardona. 2012. Fiji: an open-source platform for
749 biological-image analysis. Nature Methods 9:676–682.
750 Schleicherová, D., G. Sella, and M.C. Lorenzi. 2013. Do stable environments select against
751 phenotypic plasticity in hermaphroditic sex allocation? Italian Journal of Zoology
752 80:358–363. Taylor & Francis.
753 Schnorrer, F., C. Schönbauer, C.C.H. Langer, G. Dietzl, M. Novatchkova, K. Schernhuber, M.
754 Fellner, A. Azaryan, M. Radolf, A. Stark, K. Keleman, and B.J. Dickson. 2010.
755 Systematic genetic analysis of muscle morphogenesis and function in Drosophila.
756 Nature 464:287–291.

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Schroeder H., A. Majewska, and S. Alitzer. 2020. Monarch butterflies reared under autumn-like conditions have more efficient flight and lower post-flight metabolism. Ecological Entomology. 2020; 45:562–572.

Senar, J. C., J. Lleonart, and N.B. Metcalfe. 1994. Wing-shape variation between resident and transient wintering siskins *carduelis spinus*. Journal of Avian Biology 25:50–54.

Sgrò, C. M., J.S. Terblanche, and A.A. Hoffmann. 2016. What Can Plasticity Contribute to Insect Responses to Climate Change? Annual Review of Entomology 61:433–451.

Swaddle, J.P., and M.S. Witter. 1998. Cluttered habitats reduce wing asymmetry and increase flight performance in European starlings. Behavioral Ecology and Sociobiology 42:281–287. Springer.

Tanaka, S., and T. Okuda. 1996. Life cycles, diapause and developmental characteristics in subtropical locusts, *Nomadacris succincta* and *N. japonica* (Orthoptera: Acrididae). Japanese Journal of Entomology 64:189–201.

Tenger-Trolander, A., and M.R. Kronforst. 2020. Migration behaviour of commercial monarchs reared outdoors and wild-derived monarchs reared indoors. Proceedings of the Royal Society B; Biological Sciences 287:20201326.

Tenger-Trolander, A., W. Lu, M. Noyes, and M.R. Kronforst. 2019. Contemporary loss of migration in monarch butterflies. Proceedings of the National Academy of Sciences of the United States of America 116:14671–14676.

Van Tienderen, P.H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. Evolution 45:1317–1331.

Van der Burg, K.R.L, J.J. Lewis, B.J. Brack, R.A. Fandino, A. Mazo-Vargas, and R.D. Reed. 2020. Genomic architecture of a genetically assimilated seasonal color pattern Science 370:721–725.

Van Dyken, D., and M.J. Wade. 2010. The genetic signature of conditional expression. Genetics 184:557–570.

Via, S., and R. Lande. 1985. Genotype-Environment interaction and the evolution of phenotypic plasticity. Evolution 39: 505–522.

Via, S., and R. Lande. 1987. Evolution of genetic variability in a spatially heterogeneous environment: effects of genotype–environment interaction. Genetics Research 49:147–156. Cambridge University Press.

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.17.495480; this version posted June 18, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

788 Waddington, C.H. 1961. Genetic assimilation. Pp. 257–293 *in* E. W. Caspari and J. M.
789 Thoday, eds. Advances in Genetics. Academic Press.
790 Wan, J.S.H., F. Fazlioglu, and S.P. Bonser. 2018. Loss of plasticity in life-history strategy
791 associated with secondary invasion into stressful environments in invasive narrowleaf
792 plantain (*Plantago lanceolata*). Austral Ecology 43:752–762.
793 Wang, Z.J. 2004. The role of drag in insect hovering. J Exp Biol 207:4147–4155.
794 Warton, D.I., I.J. Wright, D.S. Falster, and M. Westoby. 2006. Bivariate line-fitting methods
795 for allometry. Biol. Rev. Camb. Philos. Soc. 81:259–291.
796 Williams, C.M., G.J. Ragland, G. Betini, L.B. Buckley, Z.A. Cheviron, K. Donohue, J.
797 Hereford, M.M. Humphries, S. Lisovski, K.E. Marshall, P.S. Schmidt, K.S. Sheldon, Ø.
798 Varpe, and M.E. Visser. 2017. Understanding evolutionary impacts of seasonality: an
799 introduction to the symposium. Integrative and Comparative Biology 57:921–
800 933.Winkler, H., and B. Leisler. 1992. On the ecomorphology of migrants. Ibis 134:21–
801 28.
802 Wilsterman, K., M.A. Ballinger, and C.M. Williams. 2021. A unifying, eco-physiological
803 framework for animal dormancy. Functional Ecology 35:11–31.
804 Whitlock, M.C. 1996. The red queen beats the jack-of-all-trades: the limitations on the
805 evolution of phenotypic plasticity and niche breadth. The American Naturalist 148:S65–
806 S77.
807 Zhen, Y., and M.C. Ungerer. 2008. Clinal variation in freezing tolerance among natural
808 accessions of Arabidopsis thaliana. New Phytologist 177:419–427.
809 Zhen, Y., P. Dhakal, and M.C. Ungerer. 2011. Fitness Benefits and Costs of Cold Acclimation
810 in Arabidopsis thaliana. The American Naturalist 178:44–52. The University of Chicago
811 Press.
812 Zera, A.J., J. Sall, and K. Grudzinski. 1997. Flight-muscle polymorphism in the cricket
813 *Gryllus firmus:* Muscle characteristics and their influence on the evolution of
814 flightlessness. Physiological Zoology 70:519–529.
815 Zhan, S., W. Zhang, K. Niitepõld, J. Hsu, J.F. Haeger, M.P. Zalucki, S. Altizer, J.C. de
816 Roode, S.M. Reppert, and M.R. Kronforst. 2014. The genetics of monarch butterfly
817 migration and warning colouration. Nature 514:317–321.

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818 Zhu, H., A. Casselman, and S.M. Reppert. 2008. Chasing migration genes: A brain expressed
819 sequence tag resource for summer and migratory monarch butterflies (*Danaus*
820 *plexippus*). PLoS ONE 3: e1345.
821 Zhu, H., R.J. Gegear, A. Casselman, S. Kanginakudru, and S.M. Reppert. 2009. Defining
822 behavioral and molecular differences between summer and migratory monarch
823 butterflies. BMC Biology 7:14.