Warmer temperatures result in maladaptive learning of sexual preferences

Marie-Jeanne Holveck[1§], Doriane Muller[1§], Bertanne Visser[2], Arthur Timmermans[1], Lidwine Colonval[1], Fabrice Jan[1], Michel Crucifix[3] & Caroline M. Nieberding[1*]

[§]Co-first authors

[1] Evolutionary Ecology and Genetics group, Earth and Life Institute, UCLouvain, Louvain-la-Neuve, Belgium.

[2] Evolution and Ecophysiology group, Department of Functional and Evolutionary Entomology, University of Liège - Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium

[3] Earth and Climate, Earth and Life Institute, UCLouvain, Louvain-la-Neuve, Belgium.

*Corresponding author: Caroline Nieberding, +32(0)10473488, caroline.nieberding@uclouvain.be; Evolutionary Ecology and Genetics group, Earth and Life Institute, UCLouvain, Croix du Sud 4-5, 1348 Louvain-la-Neuve, Belgium

Acknowledgements

We would like to thank Philippe Vernon, Franjo Weissing, Magdalena Kozielska, and Georges Lognay for careful and insightful comments on previous versions of this manuscript. This research was supported by a "FRIA" PhD grant to DM and a "Chargée de Recherches" postdoctoral grant to MJH provided by the Fonds National de Recherche Scientifique (FNRS) of Belgium. This work was further funded through UCLouvain and the Fédération Wallonie-Bruxelles (Grant ARC 17/22-086) to CN. BV was supported by the Fonds National de Recherche Scientifique.

Competing interests

The authors declare no competing financial interests.

Data availability

All data described in this paper is available at https://visserlab.be/publications.

Abstract

1. Empirical studies predicting the effects of the climate crisis on biodiversity loss that take into account learning ability and sexual selection are currently lacking.

2. Using the African butterfly *Bicyclus anynana*, which shows strong phenotypic plasticity (i.e., polyphenism) in response to temperature, we find that learning produces a maladaptive mate preference under climate warming.

3. We first modelled climate warming scenarios and then showed experimentally that as temperature becomes an unreliable cue to the onset of the dry season, adult butterflies displayed the wet season rather than the dry season form.

4. Female learning further suppressed their innate, adaptive sexual preference for dry season males. Instead, females learned to prefer a phenotype transiently present during the seasonal transition.

5. Female fertility and longevity were also indirectly affected by learning, likely reducing female fitness following climate warming.

6. Our results emphasize the importance of sexual selection, learning, and their fitness consequences for understanding (mal)adaptation of natural populations to climate warming.

Keywords

Reproductive fitness; Global warming; Seasonal polyphenism; Simulations; Social exposure

Introduction

Climate warming is expected to lead to a global extinction rate of 15% by 2100 (Cahill et al., 2013; Urban, 2015). This predicted extinction rate should be taken with caution, however, because estimation models do not incorporate key biological responses of species (Urban, 2015), such as learning ability (Candolin & Wong, 2012a). Learning, i.e., a modification of behaviour resulting from experience with the environment, has emerged as a major response of species to human-induced rapid environmental change (see Table 1 in (Barrett, Zepeda, Pollack, Munson, & Sih, 2019) for empirical examples; (Nieberding, Marcantonio, Voda, Enriquez, & Visser, 2021)). Learned behaviours for species responses to climate change are important, because learning can significantly change behaviours, for example aversive or reversal learning in response to danger or fear (Ozawa & Johansen, 2018; Rodrigues, Goodner, & Weiss, 2010; Tedjakumala & Giurfa, 2013) or biased learning toward the easiest cues (Westerman, Hodgins-Davis, Dinwiddie, & Monteiro, 2012). Learned behavioural responses of animals may thus produce widely different predictions about species responses to human-induced rapid environmental change compared to innate behaviours. Moreover, the most recent documented cases of learning in response to human-induced rapid environmental change have shown that learned behaviours are actually maladaptive (Barrett et al., 2019; Greggor, Trimmer, Barrett, & Sih, 2019). Maladaptation occurs whenever a “strategy” in a population becomes prevalent while it decreases the fitness of individuals (Crespi, 2000; Nesse, 2005). Learning may, therefore, accentuate the extinction risk of species, rather than alleviate it (as is usually assumed (Thorpe, 1963)), leading to so-called evolutionary traps (i.e., adaptive traits become maladaptive (Robertson, Rehage, & Sih, 2013; Schlaepfer, Runge, & Sherman, 2002)).

Most work on climate warming documents (mal)adaptation under natural selection, but another key biological mechanism to consider for improving the forecasting of species extinction rates is sexual selection. Over 50% of recorded climate change impacts are not due

to direct effects of increased temperature, but to biotic interactions (Cahill et al., 2013). Sexual selection is an important biotic force of evolution (West-Eberhard, 2014) that is also affected by climate warming (Candolin & Heuschele, 2008; Candolin & Wong, 2012b; Grazer & Martin, 2012b). Sexual selection is further important to consider, because it can reduce local adaptation due to the cost of sexual ornaments (handicap hypothesis) and sexual conflict (Servedio & Boughman, 2017). Sexual selection may thus act against natural selection, increasing the risk of population extinction ((Kokko & Brooks, 2003; Martínez-Ruiz & Knell, 2017; Tanaka, 1996), but see (Svensson & Connallon, 2019)).

Sexual interactions are strongly affected by learning (in vertebrates: (Verzijden et al., 2012), and insects: (Dion, Monteiro, & Nieberding, 2019)), but whether and how animal responses to the climate crisis are mitigated by learned sexual behaviours remains virtually unexplored (Barrett et al., 2019; Greggor et al., 2019). We used an ectothermic insect as a model, the African butterfly *Bicyclus anynana* (Butler 1879; Nymphalidae), which has become one of the best-known examples of developmental plasticity in response to temperature. During the wet season, which lasts about ~5 months, temperature is high (Fig. 1) and there is an abundance of resources (Brakefield & Mazzotta, 1995; Brakefield, Pijpe, & Zwaan, 2007; Brakefield & Reitsma, 1991; Windig, Brakefield, Reitsma, & Wilson, 1994). High temperatures (> 23ºC; (Brakefield & Mazzotta, 1995)) lead to the development of a typical wet season male butterfly morph that is characterized by conspicuous eyespots to avoid predation (Chan, Rafi, & Monteiro, 2019; Kooi & Brakefield, 1999; Lyytinen, Brakefield, Lindstrfom, & Mappes, 2004). Several successive generations of wet season butterflies appear during the wet season. During the seasonal transition, from wet to dry, temperatures are lower (between ~19ºC and ~23ºC; (Brakefield & Mazzotta, 1995)), leading to a higher frequency and development of an intermediate male phenotype (Fig. 1). During the dry season, there is reduced vegetation cover and an overall harsher, more stressful environment. A low temperature (<19ºC; (Brakefield &

Mazzotta, 1995)) leads to development of the typical dry season male form, which is dull with reduced eyespots (Fig. 1). Only one generation spans the entire dry season, which lasts ~7 months. During the seasonal transition in nature, the three male phenotypes co-occur (Brakefield & Reitsma, 1991). Wet, intermediate, and dry season wing patterns are determined primarily by larval rearing temperature (Brakefield & Mazzotta, 1995). Both wet and dry season forms are adapted to the conditions of their respective seasons: wet season individuals favour reproduction, while dry season individuals have adapted to cope with limited food availability, while increasing longevity (Brakefield et al., 2007). Mating and mate preference have been studied predominantly in the wet season phenotype, where males show extensive courtship activity and females choose a mate based on visual and chemical cues (San Martin, Bacquet, & Nieberding, 2011). Previous work showed that females prefer mating with the dry season male phenotype when offered a choice, increasing fitness (i.e., egg numbers) (Prudic, Jeon, Cao, & Monteiro, 2011). Another study revealed that a previous exposure to a male with more eyespots increases female preference for these artificially enhanced ornaments (Westerman, Chirathivat, Schyling, & Monteiro, 2014). *B. anynana* thus provides morphological evidence of adaptation to changing, seasonal climates that is easy to track (Muller et al., 2019), while mate preferences are under strong selection, particularly in changing environments.

Here, we used a two-pronged approach to test the effect of climate warming on butterfly morphology, mating success, and fitness. We first modelled the seasonal transition as it will occur in 2100 in the native population of our African butterfly, located in Malawi. We then performed experiments, where we let individuals develop under the current and predicted climatic scenarios. We predicted the following: *1)* Climate warming will lead to the production of wet season individuals at the beginning of the dry season due to an overall increase in temperature. *2)* Naive females prefer dry season males, as mating with dry season males increases fitness. *3)* Social exposure to a male seasonal phenotype changes mate preference of

experienced females towards the available seasonal form. *4)* Female experience and male seasonal phenotypes affect fertilization success. Our results revealed that, all else being equal, an increase in temperature will alter the appearance of seasonal forms, reducing mating opportunities of females with the most adaptive and preferred seasonal form (dry season). We further showed that social learning of females about the availability of males produces learned sexual preferences (for intermediate season males) that become maladaptive under climate warming (i.e., reducing fitness).

Methods

Insect rearing

Experiments were performed with a laboratory population of *Bicyclus anynana* that was originally established from 80 gravid females collected in Malawi in 1988 (Brakefield, Beldade, & Zwaan, 2009). Between 400 and 600 butterflies per generation were used to maintain high levels of heterozygosity and polymorphism (Van't Hof et al., 2005) to avoid loss of genetic diversity in the lab population. Larvae were fed with maize, *Zea mays*, and adults with moist, organic banana, *Musa acuminate* (Brakefield et al., 2009). Insects were reared, kept, and tested in a large climate-controlled room (5.8 x 5.7 m and 2.27 m high) under a standard temperature regime (27 ± 1°C), a 12:12h light:dark and a high relative humidity (70 ± 5%) representing the wet season in the field (Brakefield et al., 2009), except when explicitly mentioned otherwise. An overview of the experimental design can be found in Fig. S1. For experiments, eggs were repeatedly collected from the stock population as described below. Eggs of all thermal treatments were first reared in the standard rearing environment at 27°C. Larvae were then placed in groups for the different thermal treatments from the 4th larval instar until the second half of their pupal stage (in sleeves; n = 25-50/sleeve), because the developmental temperature experienced during this time window determines the expression of adult polyphenism (Bear &

Monteiro, 2013; Brakefield & Reitsma, 1991; Kooi & Brakefield, 1999; Oostra et al., 2011a). Newly emerged adults were sexed and individually marked on the ventral forewing with an indelible felt-tip pen. Groups of the same sex, seasonal form, and age of at most 10 males or 20 females were placed in cylindrical netted cages (diameter: 30 cm; height: 38 cm), except when stated otherwise. All experiments with adult butterflies were performed at a temperature of 27°C, unless mentioned otherwise.

Climate warming and proportions of seasonal forms

To test if climate warming would lead to the production of wet season individuals at the beginning of the dry season, we modelled past and future natural, hourly, circadian temperature cycles for 104 consecutive days representing the transition from the wet to the dry season in Malawi (from March 30th to July 12th). We modelled three climatic scenarios: the past climate in 1988, and two future scenarios, i.e., RCP4.5 and RCP8.5 (with a 3°C and 6°C increase in the region of interest, respectively). The photoperiod followed the light decrease throughout the seasonal transition with a 15-min monthly step. We encoded the three modelled climatic scenarios in three separate incubators. Given the difficulty of obtaining reliable, in-situ meteorological data, we constructed an idealized, smooth seasonal cycle of temperature by averaging 10 years of meteorological data during 1979-1989 [data from the Modern-Era Retrospective analysis for Research and Applications (MERRA), at latitude 12S and longitude 34E (Global-Modeling-and-Assimilation-Office-(GMAO)]. We then considered climate modulations available from the CMIP5 database at the same location (Taylor, Stouffer, & Meehl, 2012). Specifically, we computed the seasonal cycle of temperature anomalies (period 2080-2099 *versus* 1970-1990) of three models (CCSM4, HadGEM2-ES, and IPSL-CM5A-MR; at the time these data were downloaded, the HadGEM2-ES historical data were available until 1984 only), and added them to the reference scenario. We retained the HadGEM2-ES model,

because its climate sensitivity is intermediate and considers two future climatic scenarios, i.e., RCP4.5 (about 3°C increase in the region of interest) and RCP8.5 (6°C increase)(IPCC, Pachauri, & Meyer, 2014), in addition to reference climatology for 1979-1989.

To cover the entire seasonal transition, we successively reared three cohorts of butterflies: the first 24 days for cohort 1, the following 28 days for cohort 2, and the final 35 to 50 days for cohort 3 (Fig. 1). We evaluated the effects of increasing temperature on survival until adult emergence (proportion of surviving individuals between the start of the thermal treatment and the first day of emergence), developmental time (day 1 is the date of emergence of the first butterfly per cohort). In addition, adults were frozen at emergence at -80°C to measure adult polyphenism using the relative area of the fifth eyespot on the right ventral hindwing (i.e., hv5; Fig. 2). These traits were chosen, because they show the largest differences between seasonal forms (Muller et al., 2019).

Mating success of different male seasonal forms with naive and experienced females

To test how the seasonal phenotype of males during the seasonal transition affects sexual selection, we produced the wet (temperature of 27°C), intermediate (23°C), and dry (17°C) seasonal forms (Brakefield et al., 2007; Brakefield & Reitsma, 1991; Muller et al., 2019; Oostra et al., 2011a; Prudic et al., 2011). We obtained naive wet season females by placing all wet season pupae individually in closed plastic cylindrical containers (diameter 3.5 cm, height 7 cm, 60 ml) and adding a piece of white paper sheet to prevent visual, olfactory, gustatory and acoustic contact with other individuals. We obtained experienced wet season females by exposing freshly emerged females to 8-16-day-old males of one seasonal form in a flight cage (10 to 15 males per cage of 60 x 60 x 60 cm) for 3 hours. Males within the same age range were used for all exposures during experiments to more accurately mimic natural conditions. This experimental setup prevents male courtship activity (Supplementary Fig. 1). Experienced

females were kept separately in their container with a custom-modified, wire mesh cap (7 x 7 mm), allowing them to see, smell and touch the flying males above them, but preventing any mating or visual and gustative contacts with the other females exposed at the same time (with a maximum of 30 females spaced 5 cm apart). The males used for exposure were different than those used for subsequent mating experiments. All females were fed with a slice of banana in their individual container.

We used male courtship activity as a proxy for mating proportions because *B. anynana* females, as in many insects, do not engage in mating activities unless a male initiates courtship. In addition, male courtship activity is known to *(i)* reduce butterfly survival (Wedell, 2010), *(ii)* differ among seasonal forms (Bear & Monteiro, 2013), and *(iii)* predict male mating success (Holveck, Gauthier, & Nieberding, 2015; Nieberding & Holveck, 2017, 2018). We ran four mating experiments: one with naive wet season females (20 replicates) and three with experienced wet season females, exposed the day of their emergence to wet (20 replicates), intermediate (17 replicates), or dry season males (15 replicates). For each replicate 15 virgin males (i.e., 5 selected randomly from each of the three thermal treatments), 8 to 11 days of age, were left to compete in the presence of 5 virgin, wet season females, 3 to 6 days of age, under semi-natural conditions (see below)(Holveck et al., 2015; Nieberding & Holveck, 2017, 2018). Eight to 11-day-old virgin males were used, because all three components of the male sex pheromone are present at significant levels at this age (Heuskin et al., 2014; Nieberding et al., 2008, 2012). We used 3-6-day-old virgin females as females readily mate at this age in the laboratory (Brakefield et al., 2009; Nieberding et al., 2008, 2012). These ages also match the general age of females in behavioural experiments with this species (Holveck et al., 2015; Nieberding & Holveck, 2017). We first introduced the males into the experimental area and left them to adapt and interact with one another for 1 hour, after which females were added (Supplementary Fig. 1). Experimental cages (120 x 59 x 60 cm) containing two maize plants to

increase perching and hiding opportunities were used, and moist cotton with banana slices provided (Supplementary Fig. 1). This cage size, a density below the recommended threshold of 0.1 individual/dm^3 for *B. anynana*, and a sex ratio of 3 males for 1 female within the species-specific range creates semi-natural conditions and allows the full expression of courtship display by males (including flight), and of female mate choice that allows rejection of a male by flying away from an unwanted male (Holveck et al., 2015; Nieberding & Holveck, 2017, 2018). Each replicate was observed for 10 min every 30 min, with total observation duration of 30 min per replicate. Direct behavioural observations for these 10 min were encoded using the software The Observer 5.0 (Noldus Information Technology, Wageningen, the Netherlands) and when a male started courting a female, we followed this courtship until it ended before focusing on another male. Throughout each experiment, we noted the identity of mates of all formed couples.

Fitness-related trait measurements of wet season females mated with different male seasonal forms

To test the fitness consequences of social learning in females, we measured fertilization success of eggs laid by either naive or experienced wet season females mated with males of different seasonal forms, as well as female longevity. After experiments were finished, both naive and experienced females were placed singly in a 1-dm^3 plastic box with wet cotton as water supply and a maize leaf for oviposition (Brakefield et al., 2009). Feeding status differed between females, because part of the females obtained from the mating success experiments (i.e., all 57 naive females, and 9 females exposed to wet season males) also received banana slices *ad libitum*. Every three days, we replaced leaves and recorded egg fertilization success, as well as female longevity (i.e., the number of days from butterfly emergence to death).

Statistical analyses

We performed all statistics with RStudio 1.0.143 (RStudio-Team, 2016). We analysed the effect of the climatic scenarios in interaction with cohort on the *survival until adult emergence* using generalized linear mixed models GLMM (lme4 package in R (Bates, Maechler, Bolker, & Walker, 2015) with a binomial distribution (logit link function) and bobyqa optimizer. Random factors were sleeve identity nested in cohort identity nested in scenario identity. The model formula was Survival ~ Scenario * Cohort + (random effects: Scenario/Cohort/SleeveID) (Script S1 and Dataset S1). We added the interaction with sex (categorical variable) to analyse fixed effects on *developmental time* and on *polyphenism index* (with a Box-Cox transformation to achieve normality) using GLMM with a Poisson distribution, a bobyqa optimizer and an observation-level random effect to capture overdispersion (Elston, Moss, Boulinier, Arrowsmith, & Lambin, 2001; Holveck et al., 2015), and with a normal distribution, respectively. Random and nested factors were scenario(cohort(sleeve)). The model formulae were Developmental time ~ Scenario*Cohort*Sex + (random effects: Scenario/Cohort/SleeveID) (see Script S2 and Dataset S2) and Polyphenism index ~ Scenario*Cohort*Sex + (random effects: Scenario/Cohort/SleeveID) (Script S3 and Dataset S3), respectively.

We analysed the effect of male seasonal phenotype (dry when reared at 17°C, intermediate when reared at 23°C, or wet at 27°C) and female experience (naive or exposed to wet season males) on *female fitness and longevity* as follows: *(i)* the effects of male seasonal phenotype in interaction with female experience on *fertilization success* as a binary variable. We added the age at mating of both sexes and the thermal treatment duration as fixed factors and experimenter(experiment(replicate)) as random factors. We used a GLMM with a binomial distribution, a bobyqa optimizer and an observation-level random effect to capture overdispersion. Feeding status after mating was starved for all females. The model formula was

Egg fertilization success ~ Male seasonal phenotype * Female experience + Female age at mating + Male age at mating + Treatment duration + (random effects: Experimenter/Experiment/Replicate). *(ii)* We ran a similar GLMM on *female longevity* with a Poisson distribution, but added female feeding status after mating (all were starved) in interaction with male seasonal phenotype, and whether or not females could choose among males of different seasonal forms as fixed factors. The model formula was Longevity ~ Male seasonal phenotype * Female experience + Male seasonal phenotype ***** Feeding status + Mate choice for seasonal forms + Female age at mating + Male age at mating + Treatment duration + (random effects: Experimenter/Experiment/Replicate). In all these analyses (Script S4 and Dataset S3), we rescaled and centred the continuous explanatory variables on the mean, thus generating standardized z-scores, to evaluate the relative effects of variables measured on different scales and to lessen the correlation between the interactions and their component variables.

In the mating success experiments, we tested for homogeneity among replicates per experiment using a Pearson's Chi-squared test (with simulated *P*-value based on 1^{e+06} replicates to obtain a correct approximation; all $P > 0.05$) before performing analyses on the pooled data set. We then tested differences in *mating probabilities* among males of different seasonal phenotypes by comparing the observed counts to the theoretical counts of mating of the three male thermal treatments per experiment using Pearson's Chi-squared tests of goodness-of-fit. We subsequently searched for the male phenotype(s) responsible for these differences by comparing the observed counts to the theoretical counts of mating for each of the three male thermal treatments per experiment and in paired comparisons between male phenotypes per experiment, applying Pearson's Chi-squared tests and reporting *P*-values with and without Holm-Bonferroni correction for multiple comparisons (Millot, 2009). We also performed

Fisher's Exact tests when the expected frequencies were below 5 (Script S5, which contains the raw data).

Results

Higher temperatures lead to a maladaptive increase in wet season forms at the onset of the dry season

We mimicked the entire wet to dry seasonal transition (120 days) as it occurs in Malawi (Fig. 1; (Brakefield et al., 2009)) by implementing hour-to-hour modelled changes in temperature and monthly changes in photoperiod. Overall, climatic modelling showed that the alternation of wet and dry seasons was maintained in 2100, with a wet season of 5 to 6 months. Under the warmest scenario, however, the average temperature of both seasons increased by a quantity comparable to the amplitude of the seasonal cycle (6°C). Consequently, the higher temperatures typical of the wet season in 1988 will be common during the dry season in 2100. We then reared three successive cohorts of *B. anynana* larvae under these climatic conditions until adult emergence. Comparing the 1988 data to the +6°C climatic scenario, climate warming increased survival by 21% (Fig. 2a; Table S1), and reduced development time by 50% at the beginning of the seasonal transition (from 15.4 to 7.8 days), and by 74% (from 27.4 to 7.2 days) at the end (Fig. 2b; Table S1). An increase in survival and reduced development time are indeed generally expected for ectothermic species (Colinet, Sinclair, Vernon, & Renault, 2015). Regarding seasonal phenotypes, we found that climate warming increased the expression of the wet season phenotype throughout the seasonal transition (Fig. 2c). Although butterflies displayed an increasingly dry season phenotype throughout the seasonal transition under the current scenario (which can be expected as temperature decreases during the seasonal transition; Fig. 2c), development under the +3°C and +6°C climatic scenarios produced a wet season phenotype at the end of the seasonal transition. Under the +6°C climatic scenario, the phenotype during the

dry season is indistinguishable from the phenotype in the 1988 wet season, supporting our first prediction (Fig. 2c).

Exposure to male seasonal forms modifies female mate preference towards intermediate seasonal males

Naive females preferred to mate with dry season males over intermediate or wet season males (Fig. 3a; Table S2): the mating proportions of dry season males were significantly higher, while the mating proportions of intermediate and wet season males were lower than expected based on phenotype-specific male courtship rates, in line with our second prediction. Exposure of virgin wet season females to males of different seasonal forms induced a long-term (3 to 6 days) suppression of innate female preference for dry season males (Fig. 3; Table S2), meaning that long-term memory formation was involved. In addition, exposure to different male seasonal forms increased female mate preference towards intermediate season males, compared to naive females for dry seasonal males (Fig. 3; Table S2). Indeed, mating proportions of intermediate season males either matched (Fig. 3b, d) or exceeded (Fig. 3c) the expected mating rate based on courtship activity, as compared to their lower than expected mating numbers with naive females (Fig. 3a). The preference of wet season females for intermediate season males was highest when wet season females were exposed to intermediate season males (Fig. 3c; Table S2), in line with our third prediction. The sexual preference of experienced females was, however, not higher for dry season males or wet season males after exposure to such males.

Sexual preference of experienced females for wet season males reduces fertilization success

Experienced females displayed reversed fertilization rates with different male seasonal phenotypes compared to naive females (Fig. 4a and 4.b). The proportion of successfully fertilized eggs of naive females was lower when females mated with dry rather than with

intermediate or wet season males (Fig. 4a). Experienced females, however, had highest fertilization rates when mating with dry season males (Fig. 4b). In line with our fourth prediction, female experience and male seasonal phenotypes affect fertilization success, and mating with wet season males is maladaptive for females following exposure to these males during sexual maturation. We further found that experienced females had reduced longevity (Fig. 4c), suggesting that social learning generates some costs (Barrett et al., 2019).

Discussion

This study provides, to the best of our knowledge, a first example of a learned sexual interaction that produces maladaptation under climate warming (Barrett et al., 2019; Greggor et al., 2019). As environmental cues become unreliable, in the case of *B. anynana* through temperature values that no longer predict the onset of the dry season, several maladaptive responses became apparent: *i)* production of the wet season form; *ii)* suppression of naive mate preference for dry season males after exposure to wet or intermediate season males; and *iii)* reduced longevity and fertilization success by experienced females mating with wet season males.

Our experimental results are relevant and important to consider for predicting the fitness consequences of sexual selection after the climate has warmed in nature. In the field, most virgin females are expected to get socially exposed to potential mates during the few days of sexual maturation following adult emergence, because this species is protandrous (i.e., males emerge before females), which we show induces a form of sexual imprinting. Females remaining naive from any type of social exposure to available males until mating are likely rare in the field, also because this species is abundant. We thus expect that the reproductive fitness effect of experienced, rather than naïve, females when mating with different male phenotypes better reflects what happens in the field. The overall effect on fitness of naive females mating with dry or wet seasonal males may, however, need further analysis, because our results on

fertilization rates (i.e., that are lower when mating with dry season males) contradicts earlier results where only egg numbers were measured (i.e., that were higher when mating occurred with dry season males)(Prudic et al., 2011). Overall, male availability, the thermal history of females, but also other factors, such as rainfall, can have major effects on fitness and survival of the population in nature.

The broader relevance of our results rests on two pillars. First, while learning is usually assumed to improve immediate adaptation to environmental variation (within the lifetime of an organism), the fitness effects of learning remain rarely assessed (Candolin & Wong, 2012b; Dion et al., 2019; Morand-Ferron, Cole, & Quinn, 2016; Nieberding, Van Dyck, & Chittka, 2018). Here, we discovered that learning reverses both mate preference and its effects on longevity and reproductive fitness. Our results thus reveal that using behavioural data from naive individuals to predict sexual responses of animals may lead to erroneous conclusions. Whether learning increases or limits adaptation to sexual selection under climate warming remains largely undocumented so far (but see (Botero, Boogert, Vehrencamp, & Lovette, 2009) who found that more complex, learned, male bird songs were present in more unpredictable and unfavourable climatic environments). Maladaptive or suboptimal learning was, however, previously found for foraging (Avarguès-Weber, Lachlan, & Chittka, 2018) and two recent reviews document that learning in response to human-induced rapid environmental change is maladaptive for a large number of environmental threats and behaviours (Barrett et al., 2019; Greggor et al., 2019). We suggest that maladaptive learning in sexual interactions under human-induced rapid environmental change may occur in many species, as most animals studied to date modify their sexual behaviours through learning (Dion et al., 2019; Verzijden et al., 2012), which could lead to evolutionary traps (Robertson et al., 2013).

Second, our results highlight the importance of sexual selection as a strong evolutionary force to include for quantifying species responses to human-induced rapid environmental

change, such as climate warming (Candolin, 2019; Candolin & Heuschele, 2008). The effect of natural selection on species responses to the climate crisis has received considerable interest. Studies have shown that at the intraspecific level, climate warming leads to adaptive plastic and genetic changes in key phenotypic traits, including dispersal capacity and other morphological, physiological, behavioural, and life-history traits associated to phenology (Musolin & Saulich, 2012; Parmesan, 2006; Thackeray et al., 2016). However, the effects of anthropogenic environmental changes on sexual selection matter as well, because sexual selection may increase the extinction risk of populations (Holland, 2002; Martínez-Ruiz & Knell, 2017; Parrett & Knell, 2018; Plesnar-Bielak, Skrzynecka, Prokop, & Radwan, 2012). It is indeed increasingly acknowledged that human activities disturb important aspects of sexual selection (e.g., eutrophication and turbidity including algal blooms (Seehausen, van Alphen, & Witte, 1997), urban noise (Montague, Danek-Gontard, & Kunc, 2013), selective harvest in hunting (Knell & Martínez-Ruiz, 2017)). This can be expected, as sexual selection has a strong environmental component (Ingleby, Hunt, & Hosken, 2010; Parrett & Knell, 2018). Regarding the climate crisis, sexual selection is altered through the availability of sexual partners (Twiss, Thomas, Poland, Graves, & Pomeroy, 2007) with potential effects on the proportion of extra-pair mating opportunities (Bichet, Allainé, Sauzet, & Cohas, 2016), relative fitness of mono- versus polyandrous females (Grazer & Martin, 2012a), assortative mating (Santos, Vieira, & Monteiro, 2018), sexual conflict (García-Roa, Chirinos, & Carazo, 2019), secondary sexual traits (Evans & Gustafsson, 2017), and mate preferences (Candolin, 2019; Fuxjäger et al., 2019).

Predicting the effects of maladaptive learned mating preference on the long-term maintenance of the butterfly population in the wild requires consideration of the effects of climate warming on the reduction in generation time and increased survival of the butterflies. Moreover, as our climate modelling predicted that a true “dry season” will be maintained in

2100, the lack of dry season males could have consequences beyond reproductive outcomes, assuming that wet season males are incapable of surviving the dry season. Physiologically, dry season males are more resistant to starvation, have a lower resting metabolic rate and accumulate more fat compared to wet season males (Brakefield et al., 2007; Brakefield & Reitsma, 1991; Oostra et al., 2011b). Dry season males are predicted to become rare after climate warming, meaning that females will have to mate with wet season males, which reduces reproductive fitness. Mating with wet instead of dry season males may thus drastically reduce the proportion of females and developing eggs surviving the harsh, dry season until the next wet season by cascading effects of learned sexual interactions and fitness of reproducing females at the onset of the dry, harsh, tropical season. It remains difficult, however, to predict the fate of *B. anynana* populations in Malawi based on available information.

Our case study using the tropical butterfly *B. anynana* may be representative of a more general pattern, because butterflies are arthropods that represent most of the animal biomass and species diversity on Earth (Bar-On, Phillips, & Milo, 2018), and generally show strong phenotypic plasticity to cope with alternating seasons (Simpson, Sword, & Lo, 2011). Furthermore, tropical biomes both contain most biodiversity and are highly threatened by climate warming (Deutsch et al., 2008; Hoffmann, Chown, & Clusella-Trullas, 2013; Lister & Garcia, 2018; Sunday, Bates, & Dulvy, 2011), but see (Vasseur et al., 2014; Williams, Henry, & Sinclair, 2014). Understanding how maladaptations arise is increasingly important as humans rapidly transform the Earth into an environment where maladaptation is expected to become prevalent for most species (Crespi, 2000; Robertson et al., 2013). Our results suggest that global warming can have additive effects reducing, more than has been considered so far, the long-term survival probabilities of various taxa once learning and sexual selection are included as important biological mechanisms of species responses (Bell, 2013; Bradshaw & McNeilly,

1991). Our results thus advocate for integrating sexual selection, learning, and their fitness consequences to better understand (mal)adaptation to climate warming.

References

Avarguès-Weber, A., Lachlan, R., & Chittka, L. (2018). Bumblebee social learning can lead to suboptimal foraging choices. *Animal Behaviour*, *135*, 209–214.

Bar-On, Y. M., Phillips, R., & Milo, R. (2018). The biomass distribution on Earth. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 6506.

Barrett, B., Zepeda, E., Pollack, L., Munson, A., & Sih, A. (2019). Counter-Culture: Does social learning help or hinder adaptive response to human-induced rapid environmental change? *Frontiers in Ecology and Evolution*, *7*, 1–18.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Bear, A., & Monteiro, A. (2013). Male courtship rate plasticity in the butterfly *Bicyclus anynana* is controlled by temperature experienced during the pupal and adult stages. *PLoS ONE*, *8*, 5–10.

Bell, G. (2013). Evolutionary rescue and the limits of adaptation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *368*, 20120080.

Bichet, C., Allainé, D., Sauzet, S., & Cohas, A. (2016). Faithful or not: direct and indirect effects of climate on extra-pair paternities in a population of Alpine marmots. *Proceedings of the Royal Society B: Biological Sciences*, *283*, 20162240.

Botero, C. A., Boogert, N. J., Vehrencamp, S. L., & Lovette, I. J. (2009). Climatic patterns predict the elaboration of song displays in mockingbirds. *Current Biology*, *19*, 1151–1155.

Bradshaw, A. D., & McNeilly, T. (1991). Evolutionary response to global climatic change. *Annals of Botany*, *67*, 5–14. Retrieved from http://www.jstor.org/stable/42758386

Brakefield, P. M., Beldade, P., & Zwaan, B. J. (2009). The African butterfly Bicyclus anynana: A model for evolutionary genetics and evolutionary developmental biology. *Cold Spring Harbor Protocols*, *4*, 1–10.

Brakefield, P. M., & Mazzotta, V. (1995). Matching field and laboratory environments: effects of neglecting daily temperature variation on insect reaction norms. *Journal of Evolutionary Biology*, *8*, 559–573.

Brakefield, P. M., Pijpe, J., & Zwaan, B. J. (2007). Developmental plasticity and acclimation both contribute to adaptive responses to alternating seasons of plenty and of stress in Bicyclus butterflies. *Journal of Biosciences*, *32*, 465–475.

Brakefield, P. M., & Reitsma, N. (1991). Phenotypic plasticity, seasonal climate and the population biology of Bicyclus butterflies (Satyridae) in Malawi. *Ecological Entomology*, *16*, 291–303.

Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., Karanewsky, C. J., Ryu, H. Y., … Wiens, J. J. (2013). How does climate change cause extinction? *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20121890.

Candolin, U. (2019). Mate choice in a changing world. *Biological Reviews*, *94*, 1246–1260.

Candolin, U., & Heuschele, J. (2008). Is sexual selection beneficial during adaptation to environmental change? *Trends in Ecology and Evolution*, *23*, 446–452.

Candolin, U., & Wong, B. B. M. (2012a). *Behavioural Responses to a Changing World. Mechanisms and Consequences*. Oxford University Press.

Candolin, U., & Wong, B. B. M. (2012b). Sexual selection in changing environments: consequences for individuals and populations. In U. Candolin & B. B. M. Wong (Eds.), *Behavioural Responses to a Changing World. Mechanisms and Consequences* (First Edit, pp. 201–215). UK: Oxford University Press.

Chan, I. Z. W., Rafi, F. Z., & Monteiro, A. (2019). Interacting effects of eyespot number and ultraviolet reflectivity on predation risk in *Bicyclus anynana* (Lepidoptera: Nymphalidae). *Journal of Insect Science*, *19*, 1–7.

Colinet, H., Sinclair, B. J., Vernon, P., & Renault, D. (2015). Insects in fluctuating thermal

environments. *Annual Review of Entomology*, *60*, 123–140.

Crespi, B. J. (2000). The evolution of maladaptation. *Heredity*, *84*, 623–629.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 6668.

Dion, E., Monteiro, A., & Nieberding, C. M. (2019). The role of learning on insect and spider sexual behaviors, sexual trait evolution, and speciation. *Frontiers in Ecology and Evolution*, *6*.

Elston, D. A., Moss, R., Boulinier, T., Arrowsmith, C., & Lambin, X. (2001). Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology*, *122*, 563–569.

Evans, S. R., & Gustafsson, L. (2017). Climate change upends selection on ornamentation in a wild bird. *Nature Ecology and Evolution*, *1*, 0039.

Fuxjäger, L., Wanzenböck, S., Ringler, E., Wegner, K. M., Ahnelt, H., & Shama Lisa, N. S. (2019). Within-generation and transgenerational plasticity of mate choice in oceanic stickleback under climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*, 20180183.

García-Roa, R., Chirinos, V., & Carazo, P. (2019). The ecology of sexual conflict: temperature variation in the social environment can drastically modulate male harm to females. *Functional Ecology*, *33*, 681–692.

Global-Modeling-and-Assimilation-Office-(GMAO). (n.d.). MAT1NXSLV (Goddard Space Flight Center Distributed Active Archive Center (GSFC DAAC), Greenbelt, MD, USA, 2008).

Grazer, V. M., & Martin, O. Y. (2012a). Elevated temperature changes female costs and benefits of reproduction. *Evolutionary Ecology*, *26*, 625–637.

Grazer, V. M., & Martin, O. Y. (2012b). Investigating climate change and reproduction: experimental tools from evolutionary biology. *Biology*, *1*, 411–438.

Greggor, A. L., Trimmer, P. C., Barrett, B. J., & Sih, A. (2019). Challenges of learning to escape evolutionary traps. *Frontiers in Ecology and Evolution*, *7*(408).

Heuskin, S., Vanderplanck, M., Bacquet, P., Holveck, M. J., Kaltenpoth, M., Engl, T., … Nieberding, C. M. (2014). The composition of cuticular compounds indicates body parts, sex and age in the model butterfly *Bicyclus anynana*. *Frontiers in Ecology and Evolution*, *2*, 37. doi:10.3389/fevo.2014.00037

Hoffmann, A. A., Chown, S. L., & Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology*, *27*, 934–949. doi:10.1111/j.1365-2435.2012.02036.x

Holland, B. (2002). Sexual selection fails to promote adaptation to a new environment. *Evolution*, *56*, 721–730.

Holveck, M.-J., Gauthier, A.-L., & Nieberding, C. M. (2015). Dense, small and male-biased cages exacerbate male-male competition and reduce female choosiness in *Bicyclus anynana*. *Animal Behaviour*, *104*, 229–245.

Ingleby, F. C., Hunt, J., & Hosken, D. J. (2010). The role of genotype-by-environment interactions in sexual selection. *Journal of Evolutionary Biology*, *23*, 2031–2045.

IPCC, Pachauri, R., & Meyer, L. (2014). *Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Geneva, Zwitzerland.

Knell, R. J., & Martínez-Ruiz, C. (2017). Selective harvest focused on sexual signal traits can lead to extinction under directional environmental change. *Proceedings of the Royal Society B: Biological Sciences*, *284*.

Kokko, H., & Brooks, R. (2003). Sexy to die for? Sexual selection and the risk of extinction. *Annales Zoologici Fennici*, *40*, 207–219.

Kooi, R. E., & Brakefield, P. M. (1999). The critical period for wing pattern induction in the polyphenic tropical butterfly Bicyclus anynana (Satyrinae). *Journal of Insect Physiology*, *45*, 201–212.

Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, E10397.

Lyytinen, A., Brakefield, P. M., Lindstrom, L., & Mappes, J. (2004). Does predation maintain eyespot plasticity in Bicyclus anynana? *Proceedings of the Royal Society B: Biological Sciences*, *271*, 279–283.

Martínez-Ruiz, C., & Knell, R. J. (2017). Sexual selection can both increase and decrease extinction probability: reconciling demographic and evolutionary factors. *Journal of Animal Ecology*, *86*, 117–127.

Millot, G. (2009). *Comprendre et réaliser les tests statistiques à l'aide de R* (Vol. 2ème éditi). De Boeck.

Montague, M. J., Danek-Gontard, M., & Kunc, H. P. (2013). Phenotypic plasticity affects the response of a sexually selected trait to anthropogenic noise. *Behavioral Ecology*, *24*, 342–348.

Morand-Ferron, J., Cole, E. F., & Quinn, J. L. (2016). Studying the evolutionary ecology of cognition in the wild: a review of practical and conceptual challenges. *Biological Reviews*, *91*, 367–389. doi:10.1111/brv.12174

Muller, D., Elias, B., Collard, L., Pels, C., Holveck, M.-J., & Nieberding, C. M. (2019). Polyphenism of visual and chemical secondary sexually-selected wing traits in the butterfly Bicyclus anynana: How different is the intermediate phenotype? *PLOS ONE*, *14*, e0225003.

Musolin, D. L., & Saulich, A. K. (2012). Responses of insects to the current climate changes: from physiology and behavior to range shifts. *Entomological Review*, *92*, 715–740.

Nesse, R. M. (2005). Maladaptation and natural selection. *The Quarterly Review of Biology*, *80*, 62–70.

Nieberding, C. M., de Vos, H., Schneider, M. V, Lassance, J. M., Estramil, N., Andersson, J., … Brakefield, P. M. (2008). The male sex pheromone of the butterfly Bicyclus anynana: Towards an evolutionary analysis. *PLoS ONE*, *3*, 1–12.

Nieberding, C. M., Fischer, K., Saastamoinen, M., Allen, C. E., Wallin, E. A., Hedenström, E., & Brakefield, P. M. (2012). Cracking the olfactory code of a butterfly: the scent of ageing. *Ecology Letters*, *15*(5), 415–424. doi:10.1111/j.1461-0248.2012.01748.x

Nieberding, C. M., & Holveck, M.-J. (2017). Laboratory social environment biases mating outcome: a first quantitative synthesis in a butterfly. *Behavioral Ecology and Sociobiology*, *71*, 117.

Nieberding, C. M., Marcantonio, M., Voda, R., Enriquez, T., & Visser, B. (2021). The evolutionary relevance of social learning and transmission in non-social arthropods with a focus on oviposition-related behaviors. *Genes*, *12*, 1–14.

Nieberding, & Holveck, M.-J. (2018). Commentary on Kehl et al. "Young male mating success is associated with sperm number but not with male sex pheromone titres." *Frontiers in Zoology*, *15*, 18. doi:10.1186/s12983-018-0256-y

Nieberding, Van Dyck, H., & Chittka, L. (2018). Adaptive learning in non-social insects: from theory to field work, and back. *Current Opinion in Insect Science*, *27*, 75–81.

Oostra, V., de Jong, M. A., Invergo, B. M., Kesbeke, F., Wende, F., Brakefield, P. M., & Zwaan, B. J. (2011a). Translating environmental gradients into discontinuous reaction norms via hormone signalling in a polyphenic butterfly. *Proceedings of the Royal Society B: Biological Sciences*, *278*, 789–797.

Oostra, V., de Jong, M. A., Invergo, B. M., Kesbeke, F., Wende, F., Brakefield, P. M., & Zwaan, B. J. (2011b). Translating environmental gradients into discontinuous reaction norms via hormone signalling in a polyphenic butterfly. *Proceedings of the Royal Society B: Biological Sciences*,

278, 789–797. doi:10.1098/rspb.2010.1560

Ozawa, T., & Johansen, J. P. (2018). Learning rules for aversive associative memory formation. *Current Opinion in Neurobiology*, *49*, 148–157.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change.

Parrett, J. M., & Knell, R. J. (2018). The effect of sexual selection on adaptation and extinction under increasing temperatures. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1877).

Plesnar-Bielak, A., Skrzynecka, A. M., Prokop, Z. M., & Radwan, J. (2012). Mating system affects population performance and extinction risk under environmental challenge. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 4661–4667.

Prudic, K. L., Jeon, C., Cao, H., & Monteiro, A. (2011). Developmental plasticity in sexual roles of butterfly species drives mutual sexual ornamentation. *Science*, *331*, 73–5.

Robertson, B. A., Rehage, J. S., & Sih, A. (2013). Ecological novelty and the emergence of evolutionary traps. *Trends in Ecology & Evolution*, *28*, 552–560.

Rodrigues, D., Goodner, B. W., & Weiss, M. R. (2010). Reversal learning and risk-averse foraging behavior in the monarch butterfly, Danaus plexippus (Lepidoptera: Nymphalidae). *Ethology*, *116*(3), 270–280. doi:10.1111/j.1439-0310.2009.01737.x

RStudio-Team. (2016). RStudio: integrated development for R. Boston, USA: RStudio, Inc.

San Martin, G., Bacquet, P., & Nieberding, C. M. (2011). Mate choice and sexual selection in a model butterfly species, Bicyclus anynana: state of the art. *Proceedings of the Netherlands Entomological Society Meeting*, *22*, 9–22.

Santos, M. R., Vieira, N., & Monteiro, N. M. (2018). High temperatures disrupt Artemia franciscana mating patterns and impact sexual selection intensity. *Estuarine, Coastal and Shelf Science*, *207*, 209–214.

Schlaepfer, M. A., Runge, M. C., & Sherman, P. W. (2002). Ecological and evolutionary traps. *Trends in Ecology & Evolution*, *17*, 474–480.

Seehausen, O., van Alphen, J. J. M., & Witte, F. (1997). Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, *277*, 1808–1811.

Servedio, M. R., & Boughman, J. W. (2017). The Role of Sexual Selection in Local Adaptation and Speciation. *Annual Review of Ecology, Evolution, and Systematics*.

Simpson, S. J., Sword, G. A., & Lo, N. (2011). Polyphenism in insects. *Current Biology*, *21*, R738--R749.

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences*, *278*, 1823–1830.

Svensson, E. I., & Connallon, T. (2019). How frequency-dependent selection affects population fitness, maladaptation and evolutionary rescue. *Evolutionary Applications*, *12*, 1243–1258.

Tanaka, Y. (1996). Sexual selection enhances population extinction in a changing environment. *Journal of Theoretical Biology*, *180*, 197–206.

Taylor, K. E., Stouffer, R. J., & Meehl, G. A. (2012). An overview of CMIP5 and the experiment design. *Bulletin of the American Meteorological Society*, *93*, 485–498.

Tedjakumala, S. R., & Giurfa, M. (2013). Rules and mechanisms of punishment learning in honey bees: the aversive conditioning of the sting extension response. *The Journal of Experimental Biology*, *216*, 2985.

Thackeray, S. J., Henrys, P. A., Hemming, D., Bell, J. R., Botham, M. S., Burthe, S., … Wanless, S. (2016). Phenological sensitivity to climate across taxa and trophic levels. *Nature*, *535*, 241.

Thorpe, W. H. (1963). *Learning and Instinct in Animals*. London: Methuen and Co. Ltd.

Twiss, S. D., Thomas, C., Poland, V., Graves, J. A., & Pomeroy, P. (2007). The impact of climatic

variation on the opportunity for sexual selection. *Biology Letters*, *3*(1), 12–15.

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*, 571.

Van't Hof, A. E., Zwaan, B. J., Saccheri, I. J., Daly, D., Bot, A. N. M., & Brakefield, P. M. (2005). Characterization of 28 microsatellite loci for the butterfly Bicyclus anynana. *Molecular Ecology Notes*, *5*, 169–172.

Vasseur, D. A., DeLong, J. P., Gilbert, B., Greig, H. S., Harley, C. D. G., McCann, K. S., … O'Connor, M. I. (2014). Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society B: Biological Sciences*, (1779).

Verzijden, M. N., ten Cate, C., Servedio, M. R., Kozak, G. M., Boughman, J. W., & Svensson, E. (2012). The impact of learning on sexual selection and speciation. *Trends in Ecology & Evolution*, *27*, 511–519.

Wedell, N. (2010). Variation in male courtship costs in butterflies. *Behavioral Ecology and Sociobiology*, *64*, 1385–1391.

West-Eberhard, M. J. (2014). Darwin's forgotten idea: The social essence of sexual selection. *Neuroscience and Biobehavioral Reviews*, *46*(P4), 501–508. doi:10.1016/j.neubiorev.2014.06.015

Westerman, E. L., Chirathivat, N., Schyling, E., & Monteiro, A. (2014). Mate preference for a phenotypically plastic trait is learned, and may facilitate preference-phenotype matching. *Evolution*, *68*, 1661–1670.

Westerman, E. L., Hodgins-Davis, A., Dinwiddie, A., & Monteiro, A. (2012). Biased learning affects mate choice in a butterfly. *Proceedings of the National Academy of Sciences*, *109*, 10948–10953.

Williams, C. M., Henry, H. A. L., & Sinclair, B. J. (2014). Cold truths: How winter drives responses of terrestrial organisms to climate change. *Biological Reviews*, *90*, 214–235.

Windig, J. J., Brakefield, P. M., Reitsma, N., & Wilson, J. G. M. (1994). Seasonal polyphenism in the

wild: survey of wing patterns in five species of *Bicyclus* butterflies in Malawi. *Ecological Entomology*, *19*, 285–298.

World-Bank-Group. (2019). Climate Change Knowledge Portal.

Figures

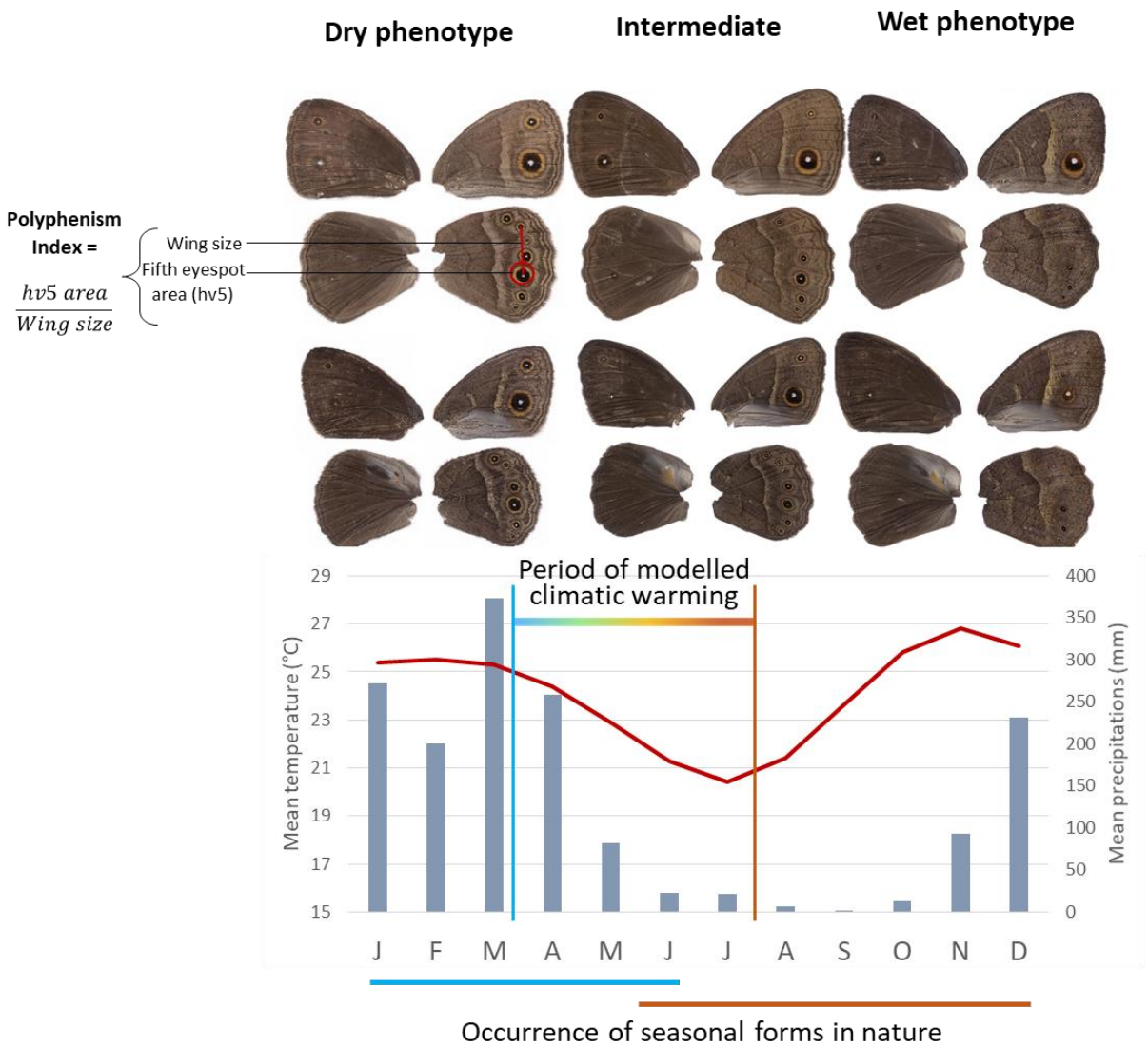

Fig. 1. Morphological differences of males and females between wet and dry season phenotypes and their temporal distribution across seasons in Malawi (Brakefield & Reitsma, 1991), in relation with monthly average values of precipitation (blue bars) and temperature (red curve) in the field (data available at (World-Bank-Group, 2019). The 120-day period of the climatic warming we modelled (Julian day 88 to 192, as in Fig. 2) is depicted.

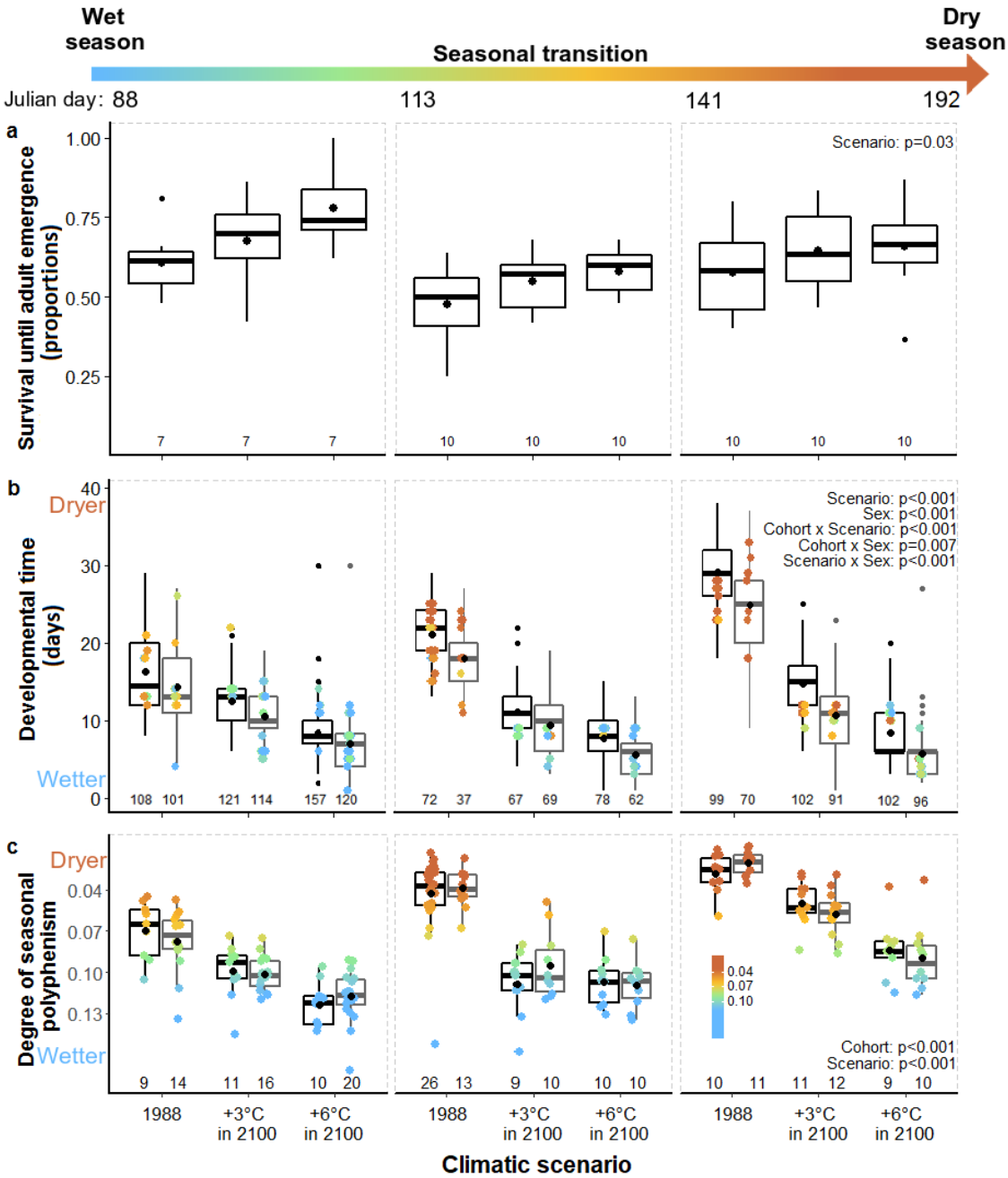

Fig. 2. Climate warming during the wet to dry seasonal transition increases the proportion of surviving larvae until adult emergence (a), reduces developmental time (day 1 = first emerged butterfly per cohort; b), and produces wet season butterflies at the onset of the dry season (based on the polyphenism index, as described in Fig. 1)(c) (Table S1). The color scale (bottom right) represents the change in polyphenism from extremely dry (red) to extremely wet (blue). Data are presented from left to right using three cohorts of larvae whose development cover the entire seasonal transition (cohort 1: from the 88th day of the year to the 112th, cohort 2 from the 113th to the 140th day and cohort 3 from the 141st to the 192th day). For (b-c), colours indicate whether the individuals display the dry (brown), intermediate (yellow and green), or wet (blue) season forms, based on the relative eyespot area of females (black) and males (grey) (as in (Muller et

al., 2019)). Mean values are indicated (black dot inside each boxplot) and samples sizes (below each boxplot) are numbers of sleeves (n = 25-50 larvae/sleeve) in (a) and individuals in (b-c).

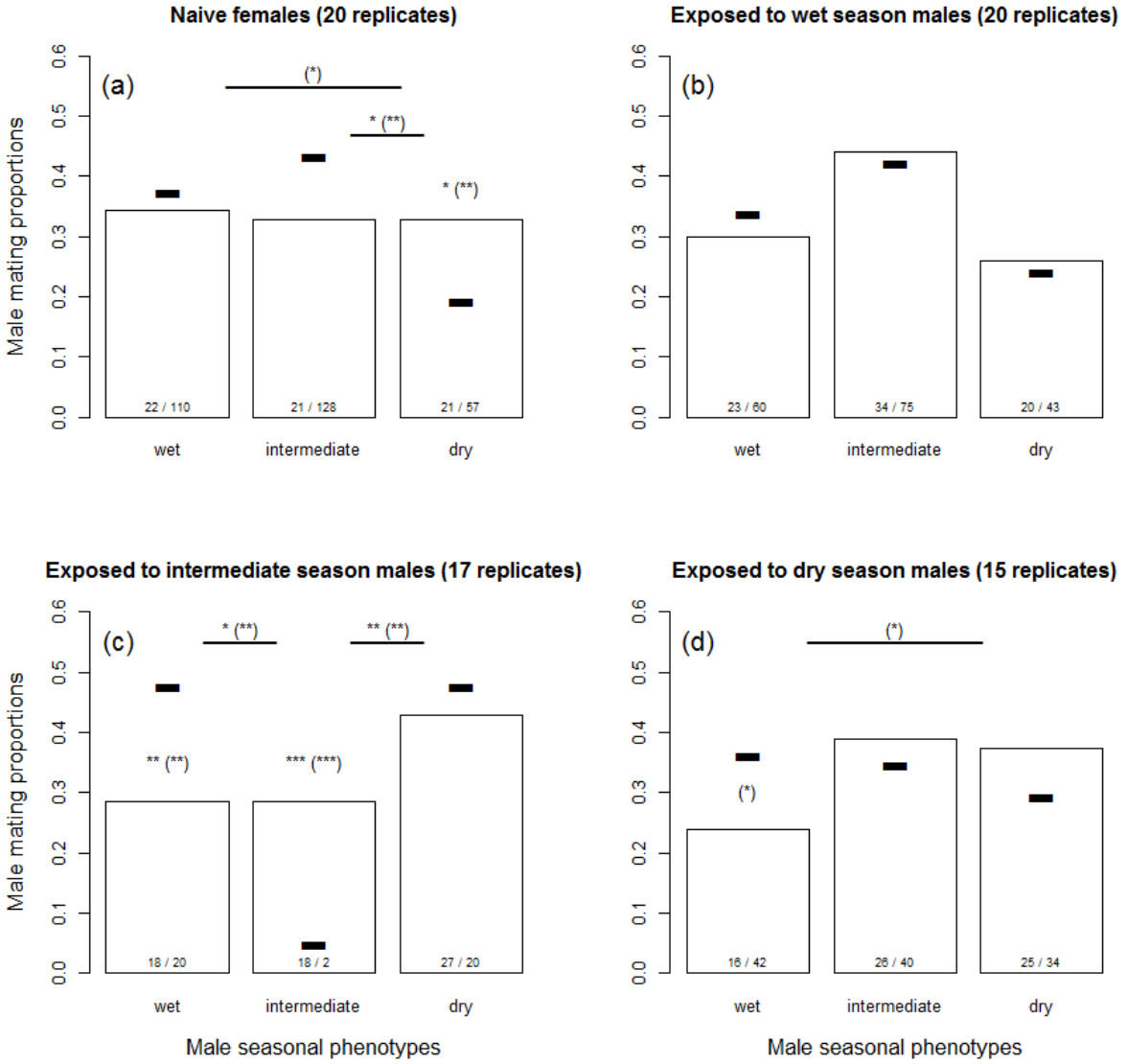

Fig. 3. Male mating success depends on the seasonal form (wet, intermediate or dry) and whether females are naive (a) or have been previously exposed to wet (b), intermediate (c) or dry season males (d). Adjusted Holm-Bonferroni *P*-values (and uncorrected *P*-values in brackets) indicate significant differences (*<0.5, **<0.01, ***<0.001) between observed (bars) and theoretical (dashes) mating proportions, after adjustment for courtship activity, for each male phenotype and in paired comparisons between phenotypes (reported for each bar and comparison; Table S2). Samples sizes inside each bar are numbers of male matings/courtships.

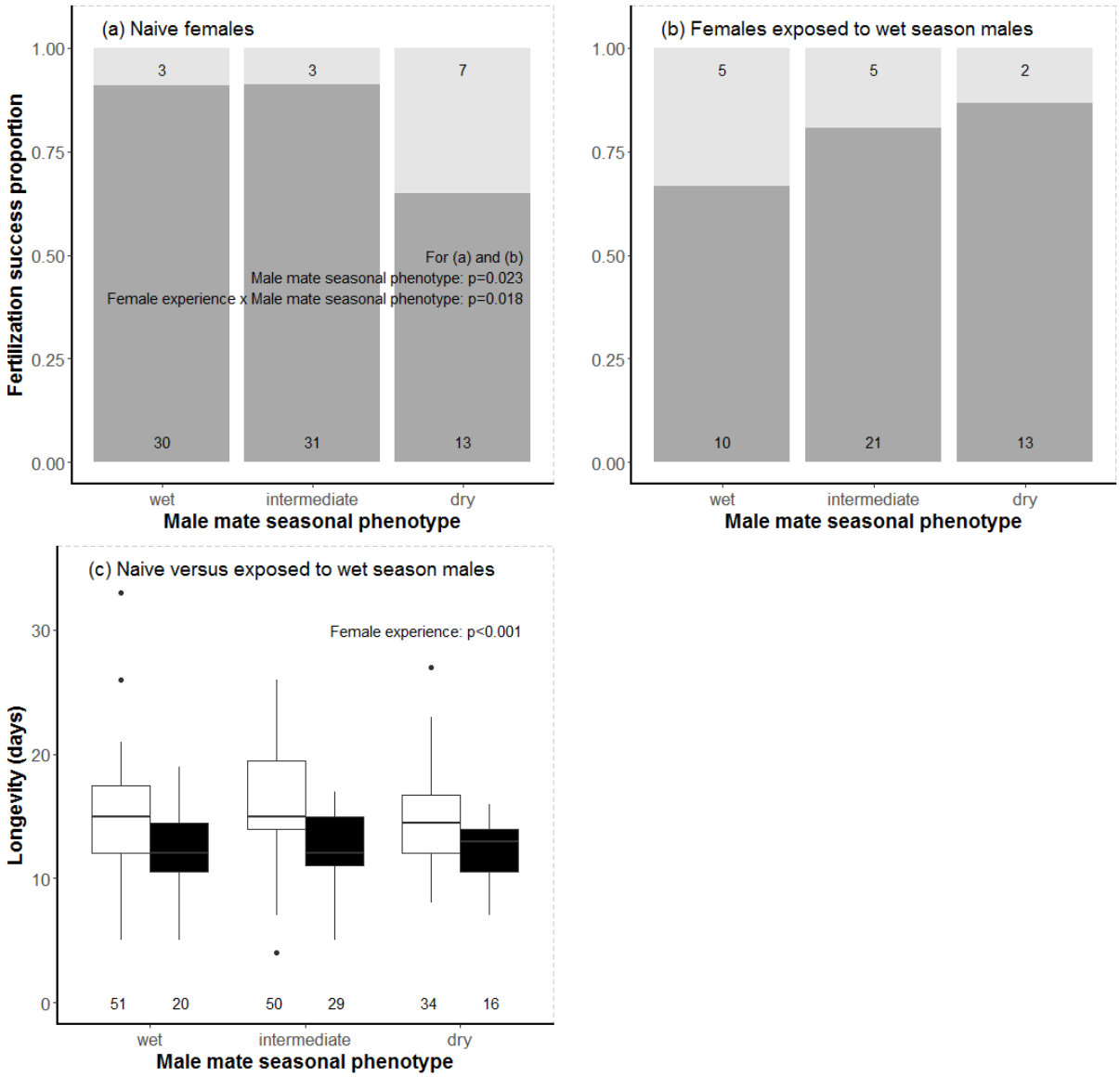

Fig. 4. The proportion of wet season females whose eggs were fertilized (in dark grey) differ depending on whether the females are naive (a) or have been exposed previously to wet season males (b). The same was found for longevity, where naive and experienced females are depicted by white or black boxplots, respectively (c). Fertilization success, but not longevity, also depends on the seasonal form (wet, intermediate, dry) of males. Sample sizes are given inside each bar or below each boxplot.

715