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

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Competing interests

The authors declare no competing financial interests.

Author contributions

Marie-Jeanne Holveck: Conceptualization, Formal analysis, Writing- original draft

Doriane Muller: Formal analysis, Investigation, Writing-original draft

Bertanne Visser: Writing-original draft, Writing-review & editing

Arthur Timmermans: Investigation

Lidwine Colonval: Investigation

Fabrice Jan: Investigation

Michel Crucifix: Investigation

Caroline Nieberding: Conceptualization, Funding acquisition, Project administration, Supervision, Writing-original draft, Writing-review & editing.

Data availability

All data described in this paper is available as online supplementary material and at https://visserlab.be/publications.

Abstract

1. The impact of learning ability and sexual selection on the climate and biodiversity crisis are currently unclear.

2. Using the African butterfly *Bicyclus anynana*, which shows strong phenotypic plasticity (i.e., polyphenism) in response to temperature, we tested whether learning affects mate preferences 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. Experienced females that were exposed to different male seasonal phenotypes during sexual maturation changed sexual preferences.

5. Female fertilization success was reversed for naive compared to experienced females, 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; Climate warming; Seasonal polyphenism; Simulations; Learning

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, which is 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)). Learning is important, because it 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 (Westerman, Hodgins-Davis, Dinwiddie, & Monteiro, 2012). Compared to innate behaviour, learned behaviours may thus produce widely different predictions about species responses to human-induced rapid environmental change. 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). For example, a long-term study with the collard flycatcher *Ficedula albicollis* revealed that rising temperatures altered male ornamentation (i.e., reduced forehead patch size), reducing male mating success (Evans & Gustafsson, 2017). In the fly *Drosophila melanogaster*, lifespan and reproductive success decreased in response to an increase in temperature as a consequence of increased sexual conflict (García-Roa, Chirinos, & Carazo, 2019). 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 a generalist 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 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 and pupal 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 (Muller et al., 2019; San Martin, Bacquet, & Nieberding, 2011). Previous work showed that all seasonal female forms prefer mating with the dry season male phenotype when offered a choice, increasing fitness (i.e., egg number) (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 combined climate modelling to test the effect of realistic climate warming scenarios on butterfly morphology, mating preference 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 an experiment where we let individuals develop under the current and predicted climatic scenarios (experiment 1). Under the most stringent climate

modelling scenario, the dry season temperature in 2100 will be comparable to the wet season temperature in 1988. We thus expected that the predicted increase in temperature under climate warming will lead to the production of wet season individuals at the beginning of the dry season. As a consequence, only maladapted male phenotypes, i.e., wet season, would be available for mating. To test how male seasonal form affects mate preferences, we then exposed wet season females to dry, wet or intermediate male phenotypes and measured female mate preference (experiment 2). We expected that a previous experience with any male phenotype (either wet, dry, or intermediate) would change mate preference towards that male phenotype, meaning that females learn to prefer mates that are actually present in the current environment. To estimate how female experience and male seasonal phenotype affect fitness, we subsequently measured the proportion of successfully hatched eggs, representing fertilization success, and female longevity (experiment 3). Our results revealed that, all else being equal, an increase in temperature will alter the appearance of seasonal forms (more wet season males at the onset of the dry season), reducing mating opportunities with the most adaptive and preferred seasonal form (i.e., dry season). We further showed that a previous experience of females with available males produces learned sexual preferences for intermediate season males that become maladaptive under climate warming by reducing the proportion of successfully hatched eggs and thus fitness.

Methods

Modelling climatic scenarios of the seasonal transition in 1988 and 2100

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.

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 laboratory population. Larvae were fed with maize, *Zea mays*, and adults with moist, organic banana, *Musa acuminata* (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 of 27°C (± 1°C), a 12:12h light:dark cycle, and a high relative humidity of

70% (±5%) representing the wet season in the field (Brakefield et al., 2009). For experiments, eggs were repeatedly collected from the laboratory population. Eggs of all thermal treatments were first reared in the standard rearing environment at 27°C. The developmental temperature experienced from the 4th larval instar onwards determines the expression of adult polyphenism (Bear & Monteiro, 2013; Brakefield & Reitsma, 1991; Kooi & Brakefield, 1999; Oostra et al., 2011a). Larvae were, therefore, subsequently placed in groups for the different thermal treatments from the 4th larval instar until the second half of their pupal stage (Fig. 2). To do so, each group of 4th instar larvae was placed on a single plant inside a sleeve that covers the plant and protects the developing larvae (n = 25-50/sleeve). 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.

Experiment 1: Effects of climatic scenario on survival, time to first emergence, and seasonal phenotype

To test if climate warming would lead to the production of wet season individuals at the beginning of the dry season, we exposed butterflies to the modelled climatic scenarios. 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, Fig. 3). We evaluated the effects of increasing temperature on survival until adult emergence (i.e., the proportion of surviving individuals between the start of the thermal treatment, at the 4th larval stage, and the first day of adult emergence), time to emergence as a proxy for development time (where day 1 is the date at which the first butterfly of a cohort emerged). Emerging adults were subsequently 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).

Experiment 2: Mate preference of naive and experienced females for different male seasonal forms

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 experienced wet season females by exposing freshly emerged females to 8-16-day-old males of one seasonal form inside a flight cage (10 to 15 males per cage of 60 x 60 x 60 cm) for 3 hours (Fig. 2b). During exposure, females were kept separately in a small plastic cylindrical container (diameter 3.5 cm, height 7 cm, 60mL) with a custom-modified, wire mesh cap (7 x 7 mm), allowing the females to see, smell and touch the flying males above them, but preventing mating. This setup further prevented any visual and gustative contacts with females that were exposed in the flight case at the same time (with a maximum of 30 females spaced 5 cm apart). All females were fed with a slice of banana in their individual container. Males used for exposure were different than those used for subsequent mating experiments.

Mate preference experiments were performed with naive wet season females (20 replicates; control), as well as experienced wet season females exposed 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. 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, which plays a key role in mate choice decisions (Heuskin et al., 2014; Nieberding et al., 2008, 2012). We used 3-6-day-old virgin females, because 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 typically used in behavioural experiments with this species (Holveck, Gauthier, & Nieberding, 2015; Nieberding & Holveck, 2017). Before experiments started, we first introduced the males into the experimental area and left them to adapt and interact for 1 hour, after which females were added (Fig. 2b). The experimental area consisted of experimental cages (120 x 59 x 60 cm) containing two maize plants to increase perching and hiding opportunities and food. Using this larger cage size results in butterfly densities that are below the recommended threshold of 0.1 individual/dm^3 for *B. anynana.* Together with a sex ratio of 3:1 males:females, this set-up creates a semi-natural condition that allows on the one hand the full expression of male courtship displays (including flight) and on the other hand female mate choice, where the female can reject a male by flying away (Holveck et al., 2015; Nieberding & Holveck, 2017, 2018). Each replicate was observed for 10 min every 30 min, with a total observation duration of 30 minutes per replicate. Direct behavioural observations for these 10 min were encoded using the software The Observer 5.0 (Noldus Information Technology, Wageningen, the Netherlands). When a male started courting a female, we followed the stereotyped courtship sequence until it ended before focusing on another male. Throughout each experiment, we noted the identity of all formed couples. As in many insects, *B. anynana* females do not engage in mating unless a male initiates courtship. We used mating proportions for analyses, representing the number of mated females out of the total number of tested females, i.e., observed mating proportions. These observed mating proportions were then corrected by male courtship number, i.e., theoretical mating proportions, because courtship activity differs among seasonal forms (Bear

& Monteiro, 2013) and predicts mating success (Holveck et al., 2015; Nieberding & Holveck, 2017, 2018).

Experiment 3: Fitness-related trait measurements of wet season females mated with different male seasonal forms

To test the fitness consequences of a previous experience in females, we measured fertilization success as the proportion of successfully hatched eggs laid by either naive or experienced wet season females mated with males of different seasonal forms. We further measured female longevity. To do so, both naive and experienced females were placed singly in a 1-dm^3 plastic box with wet cotton wool and a maize leaf for oviposition (Brakefield et al., 2009) after experiment 2 was finished. Feeding status differed between females, because part of the females obtained from the mate preference experiment (i.e., all 57 naive females, and 9 females exposed to wet season males) also received banana slices *ad libitum*. Leaves were replaced and egg fertilization success was recorded every three days. Longevity was recorded daily and calculated as the number of days from butterfly emergence to death.

Statistical analyses

Experiment 1 Climate warming. We performed all statistics with Rstudio 1.0.143 (RStudio-Team, 2016). For all statistical models, we used model simplification based on the AIC values. We analysed the effect of the climatic scenarios in interaction with cohort on the survival 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 (see (Barr, Levy, Scheepers, & Tily, 2013) for a discussion on using factorial variables as fixed and random effect). 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 development time (time to emergence) 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 Development 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.

Experiment 2 Mate preference and learning. 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 between observed and theoretical mating proportions 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 observed to theoretical mating proportions 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 (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 S4, which contains the raw data).

Experiment 3 Fitness. We analysed how female fertilization success (as a binary variable) varied in response to mating with each seasonal phenotype and female experience (naive or exposed to wet season males). 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. 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). Next, we ran a similar GLMM on female longevity with a Poisson distribution, but added female feeding status after mating 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 S5 and Dataset S3), we rescaled and centred the continuous explanatory variables on the mean.

Results

Modelling predicts that 1988 wet season temperatures become common during the dry season in 2100

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.

Experiment 1: Higher temperatures lead to a maladaptive increase in wet season forms at the onset of the dry season

Comparing the 1988 data to the +6°C climatic scenario, climate warming increased survival by 21% (Fig. 3a; Table 1). Climate warming further reduced the time to emergence (as a proxy for 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. 3b; Table 1). Regarding seasonal phenotypes, we found that climate warming increased the degree of polyphenism, with more individuals showing the wet season phenotype throughout the seasonal transition (Fig. 3c): 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. 3c), but 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, in line with our expectation (Fig. 2c).

Experiment 2: Experience with different male seasonal forms modifies female mate preference

Naive females preferred to mate with dry season males over intermediate or wet season males (Fig. 4a; Table 2): the mating proportions with dry season males were significantly higher, while the mating proportions with intermediate and wet season males were lower than expected. 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. 4; Table 2), meaning that long-term memory formation was involved. Furthermore, exposure to different male seasonal forms increased female mate preference towards intermediate season males compared to naive females (Fig. 4; Table 2). Indeed, observed mating proportions with

intermediate season males either matched (Fig. 4b, d) or exceeded (Fig. 4c) the theoretical mating proportions (corrected for courtship activity)(Fig. 4a). The preference of wet season females for intermediate season males was highest when wet season females were exposed to intermediate season males, as expected (Fig. 4c; Table 2). This means that wet season females learn to prefer mating with intermediate season males after a previous exposure to intermediate males. More complex learned mate preferences were observed when females were exposed to dry or wet season males (Fig. 4b, 4d).

Experiment 3: Learning affects fertilization success and reduces longevity

Experienced females displayed reversed fertilization success with different male seasonal phenotypes compared to naive females (Fig. 5a and 5b). 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. 5a). Experienced females, however, had highest fertilization success when mating with dry season males (Fig. 5b). As expected, female experience and male seasonal phenotypes affect fertilization success. Furthermore, 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. 5c), suggesting that 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 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 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 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 naive, females when mating with different male phenotypes better reflects what happens in the field. 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 reproductive fitness. Our results thus reveal that using behavioural data from naive individuals to predict sexual responses of animals may lead to erroneous conclusions. We further found that learning reduced longevity. 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 also 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 larger 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; Nieberding et al., 2021; 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., (Evans & Gustafsson, 2017; García-Roa et al., 2019)). 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 et al., 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. Indeed, 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.

Barr, D. J., Levy, R., Scheepers, C., & Tily, H. J. (2013). Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language*, *68*, 255–278. doi:10.1016/j.jml.2012.11.001

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.

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*(2). doi:10.1038/s41559-016-0039

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.

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.

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*, 415–424.

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.

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.

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

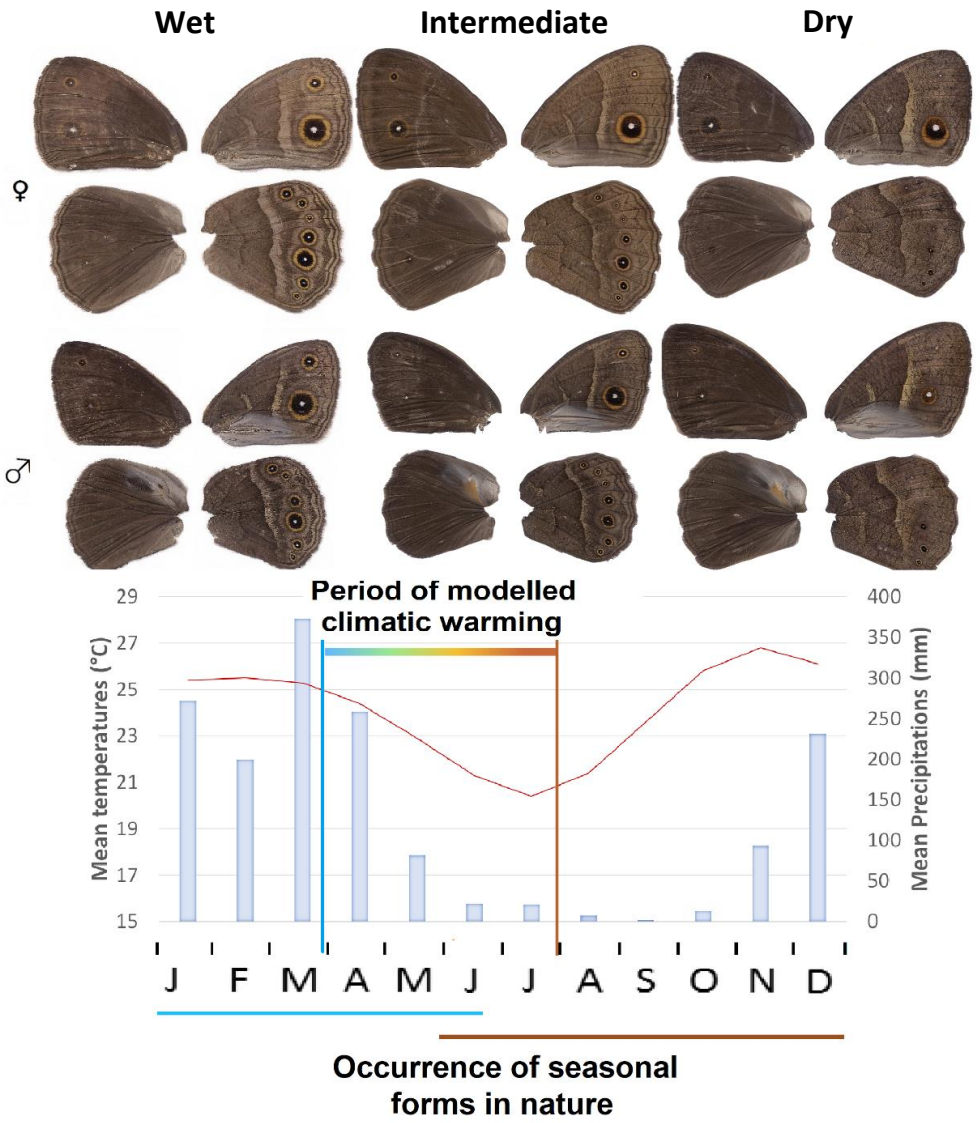

Fig. 1. Morphological differences of females (top 2 wing pairs) and males (bottom 2 wing pairs) between dry, intermediate, and wet season phenotypes (Brakefield & Reitsma, 1991), as well as monthly average values of precipitation (blue bars) and temperature (red curve) in the field in Malawi, Africa (data available at (World-Bank-Group, 2019). The duration of the wet season is indicated by the blue line underneath the graph, while the duration of the dry season is represented by the orange line. The 120-day period of the climatic warming we modelled (Julian day 88 to 192, as in Fig. 3) is also depicted.

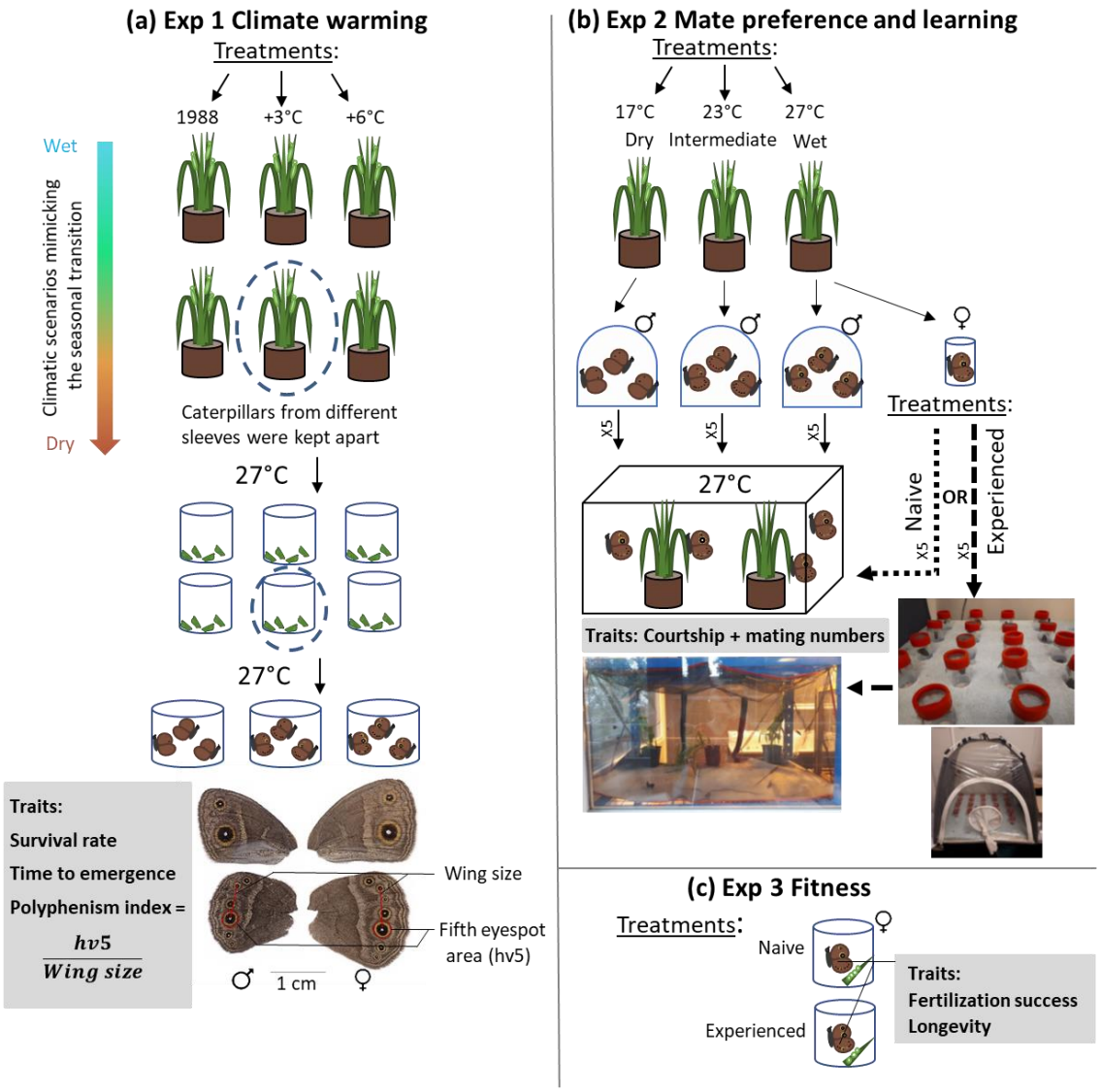

Fig. 2: Experimental design for the climate warming experiment (a), the mate preference and learning experiment (b), and the fitness experiment (c), including the traits that were measured for each experiment. Prior to experiments, eggs were collected and kept at 27°C for 15 days; hence 15 day old larvae were used for subsequent experiments.

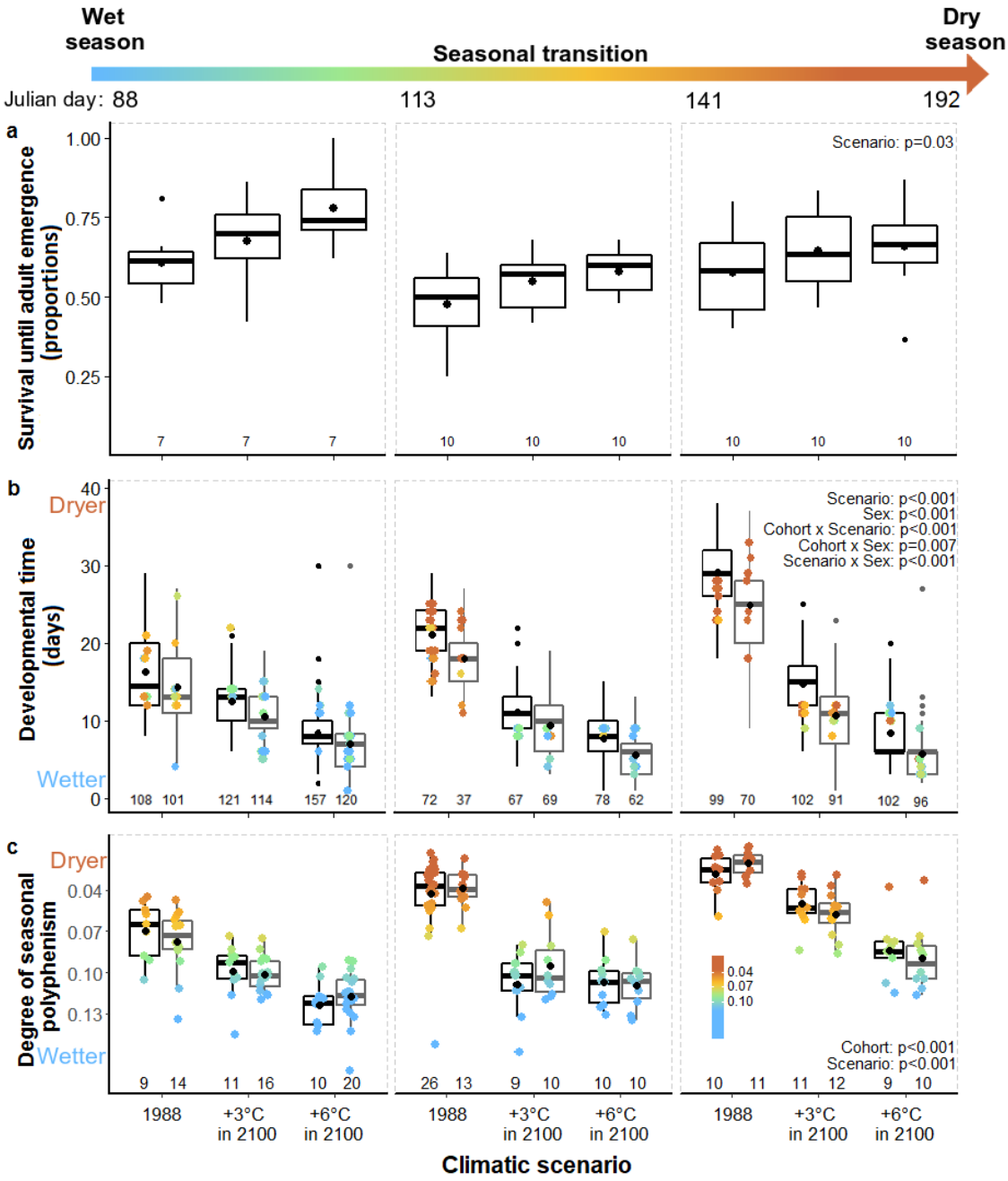

Fig. 3. Climate warming during the wet to dry seasonal transition increases the proportion of surviving larvae until adult emergence (a), reduces the time to first emergence (i.e., development time); (b), and produces wet season butterflies at the onset of the dry season (based on the polyphenism index, as described in Fig. 1)(c). Statistics are presented in Table 1. The colour scale (in the bottom right graph) 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 the number of sleeves (n = 25-50 larvae/sleeve) in (a) and individuals in (b-c).

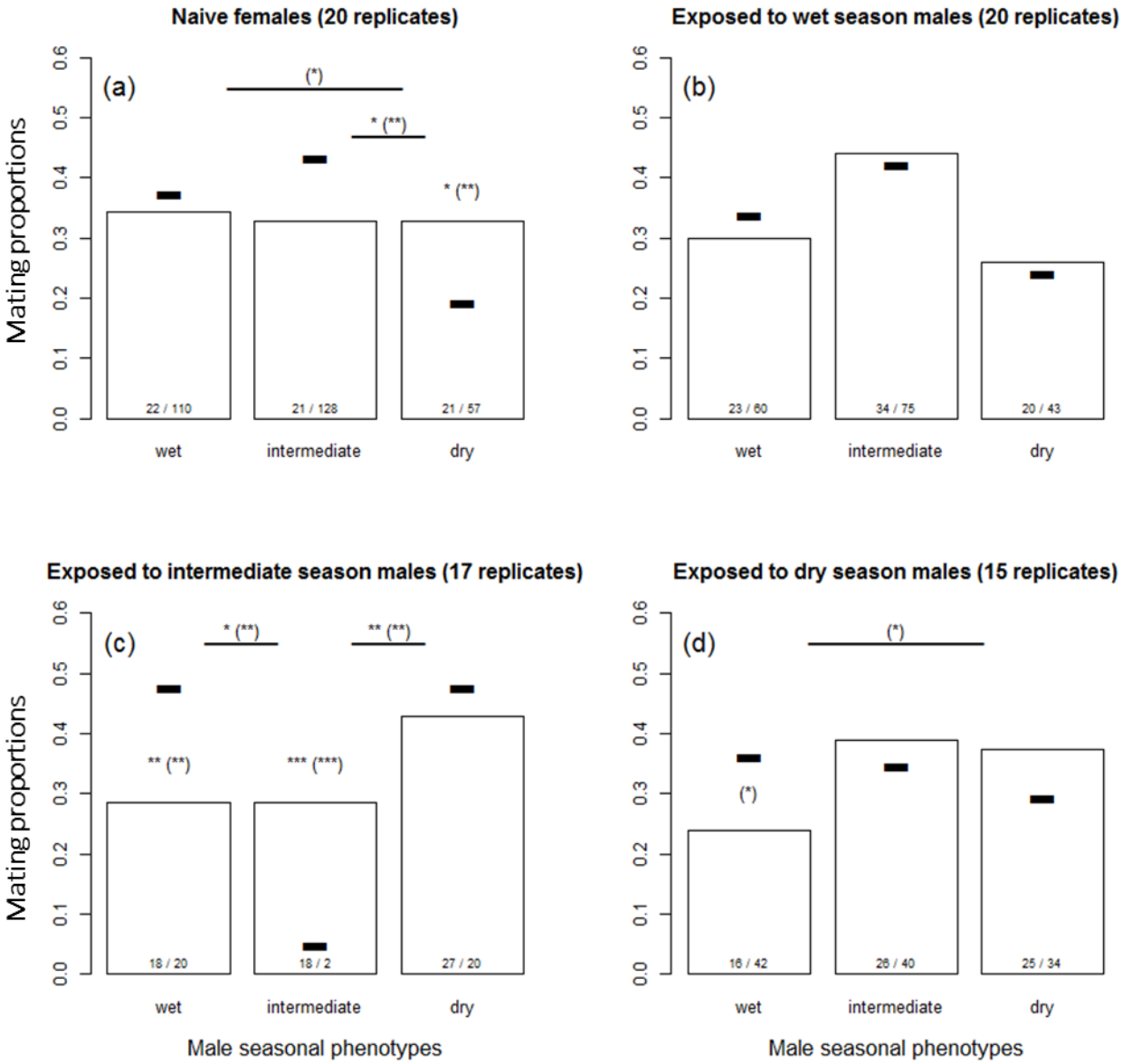

Fig. 4. Female mate preference represented by observed (bars) and theoretical (black dashes) mating proportions. Significant differences between observed and theoretical mating proportions are indicated: *<0.5, **<0.01, ***<0.001 (using adjusted Holm-Bonferroni *P*-values and uncorrected *P*-values in brackets). Comparisons are made for each male phenotype and in paired comparisons between phenotypes. Samples sizes inside each bar are numbers of male matings/courtships. Statistics are presented in Table 2.

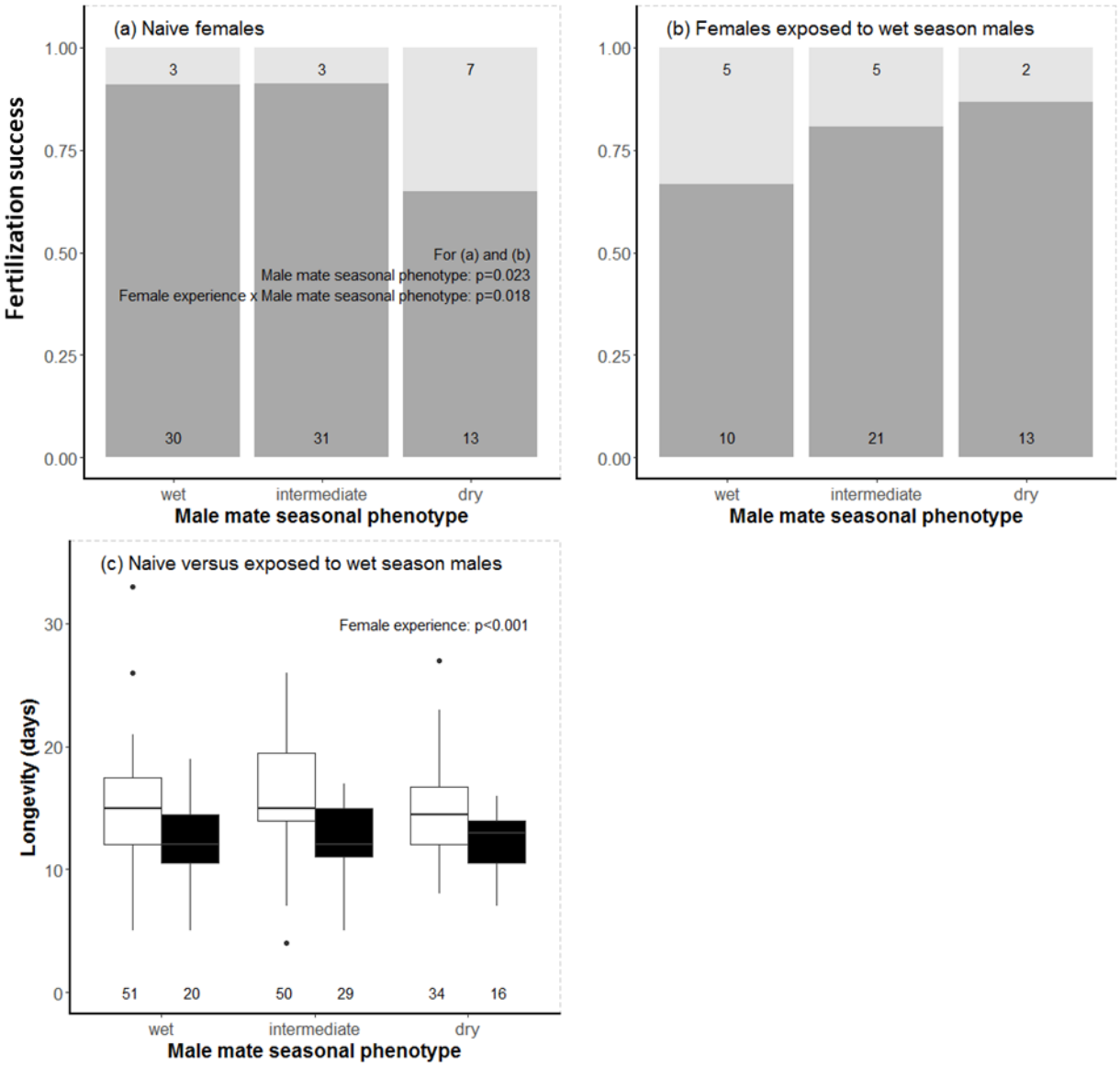

Fig. 5. The proportion of wet season females whose eggs were fertilized (in dark grey) differ depending on whether the females are naive (a) or have been exposed previously to wet season males (b). The same was found for longevity, where naive and experienced females are depicted by white or black boxplots, 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.

Table 1. A) Statistical output for experiment 1 presented in Figure 3. B) Statistical output for experiment 2 presented in Figure 5. For both experiments, we used model-averaged standardized coefficients following conditional average of the set of supported candidate models for each response variable. For each conditional average of supported candidate models ($\Delta_i < 2$), we provide the relative importance of explanatory variables ($P \leq 0.05$ in bold). Of note, sexes differ in their developmental response to climate warming (Fig. 3b): males emerged earlier than females; female time to emergence (i.e., development time) decreased faster with warming (scenario-by-sex interaction) and increased faster throughout the seasonal transition (cohort-by-sex interaction) compared to males. These sex differences become smallest under the warmer scenario and at the beginning of the seasonal transition. Δ_i = difference in AIC[b] (or AICc[a]) of model *i* with the best candidate model (i.e., the model with the smallest AIC[b] or AICc[a]). SE = Conditional standard errors; Adjusted SE = Adjusted standard error estimator with improved coverage. w_+ = sum of the normalized Akaike weights across all candidate models (Δ_i <2) in which the variable occurred. NA = not applicable.

A.

Response	Explanatory	Estimate	SE	Adjusted SE	*z* value	*P* value	w_+
Survival until adult emergence[a]	Intercept	0	0	0	NA	NA	0.70
	Scenario	0.026	0.012	0.012	2.2	**0.03**	0.49
Development time[b]	Intercept	0	0	0	NA	NA	0.94
	Cohort	-0.008	0.006	0.006	1.5	0.15	0.94
	Scenario	-0.046	0.005	0.005	8.4	**< 0.001**	0.94
	Sex	-0.015	0.001	0.001	13.8	**< 0.001**	0.94
	Cohort x Scenario	0.019	0.006	0.006	3.4	**< 0.001**	0.94
	Cohort x Sex	0.004	0.002	0.002	2.7	**0.007**	0.94
	Scenario x Sex	-0.006	0.001	0.001	3.9	**< 0.001**	0.94
	Cohort x Scenario x Sex	0.002	0.001	0.001	1.6	0.11	0.54
Relative area of adult polyphenic hv5 eyespot pupils[a]	Intercept	0	0	0	NA	NA	0.61
	Cohort	0.470	0.075	0.075	6.2	**< 0.001**	0.61
	Scenario	0.662	0.078	0.079	8.4	**< 0.001**	0.61
	Cohort x Scenario	-0.095	0.071	0.071	1.3	0.18	0.27

B.

Fertilization success[a]	Intercept	0	0	0	NA	NA	0.35
	Female experience	-0.139	0.913	0.921	0.2	0.9	0.35
	Female age at mating	1.861	0.977	0.986	1.9	0.059	0.35
	Male mate thermal treatment	2.111	0.918	0.925	2.3	**0.023**	0.35
	Female experience x Male mate thermal treatment	-2.151	0.900	0.907	2.4	**0.018**	0.35
	Thermal treatment duration	-0.616	0.985	0.993	0.6	0.5	0.10
Female longevity[a]	Intercept	0	0	0	NA	NA	0.27
	Female experience	-0.025	0.006	0.006	3.9	**<0.001**	0.27
	Mate choice allowed	-0.006	0.006	0.006	1.0	0.3	0.14
	Female feeding status after mating	-0.005	0.005	0.005	0.9	0.3	0.14
	Male mate age at mating	-0.005	0.006	0.006	0.8	0.4	0.14
	Male mate thermal treatment	0.002	0.005	0.005	0.5	0.6	0.13

Table 2. Statistical output of experiment 2 presented in Figure 4. Using Chi-squared tests for given probabilities, we first tested for differences in mating proportions with the different seasonal phenotypes; second, we searched for differences between observed and theoretical mating proportions for each of the three seasonal phenotypes, and in paired comparisons between seasonal phenotypes, by computing *P*-values with and without (in brackets) Holm-Bonferroni correction for multiple comparisons. $P \leq 0.05$ are in bold.

			Naive females (20 replicates; Fig. 3a)			Females exposed to wet males (20 replicates; Fig. 3b)			Females exposed to intermediate males (17 replicates; Fig. 3c)			Females exposed to dry males (15 replicates; Fig. 3d)		
Tests		Comparisons	Chi²	df	*P*	Chi²	df	*P*	Chi²	df	*P*	Chi²	df	*P*
Comparison of mating proportions for seasonal phenotypes		wet *vs.* intermediate *vs.* dry forms	7.8	2	**0.02**	0.5	2	0.8	80	2	**<0.001**[a]	4.6	2	0.098
Searching for differences in mating proportions	Per phenotype	wet form (*vs.*intermediate and dry)	0.2	1	0.6 (0.6)	0.5	1	1.0 (0.5)	9.2	1	**0.005 (0.002)**	4.4	1	0.11 **(0.036)**
		intermediate form (*vs.*wet and dry)	2.9	1	0.2 (0.088)	0.1	1	1.0 (0.7)	78.8	1	**<0.001 (<0.001)**[b]	0.6	1	0.5 (0.5)
		dry form (*vs.*wet and intermediate)	7.5	1	**0.019 (0.006)**	0.1	1	1.0 (0.7)	0.6	1	0.4 (0.4)	2.1	1	0.3 (0.15)
	In paired comparisons	wet *vs.* intermediate forms	0.4	1	0.5 (0.5)	0.4	1	1.0 (0.5)	73	1	**<0.001 (<0.001)**[c]	2.9	1	0.2 (0.09)
		wet *vs.* dry forms	4.1	1	0.084 **(0.042)**	0.4	1	1.0 (0.5)	1.8	1	0.2 (0.2)	4.4	1	0.11 **(0.037)**
		intermediate *vs.* dry forms	7.3	1	**0.021 (0.007)**	0.01	1	0.9 (0.9)	52	1	**<0.001 (<0.001)**[d]	0.2	1	0.7 (0.7)

Wet = wet season form (27°C). Intermediate = intermediate season form (23°C). Dry = dry season form (17°C). When expected frequencies were below 5, we also report the *P*-value of Fisher's exact test: [a]$P = 0.042$, [b]$P < 0.001$ (<0.001), [c]$P = 0.014$ (0.007), [d]$P = 0.008$ (0.003).