GENERAL INFORMATION

Title

Seasonal plasticity for anti-predatory strategies: matching colour and colour preference for effective crypsis

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ABSTRACT

Effective anti-predatory strategies typically require matching morphology and behaviour in prey and there are many compelling examples of behavioural strategies that enhance the effect of morphological defences. When protective adult morphologies are induced by a developmental environment predictive of future predation risk, adult behaviour should be adjusted accordingly to maximize predator avoidance. Behaviour is typically strongly affected by the adult environment but an effective match with a developmentally plastic morphology might be ensured when behaviour is affected the same pre-adult environmental cues. We study early and late life environmental effects on a suite of traits that are expected to work together for effective crypsis. We focused on body colour and colour preference in *Bicyclus anynana*, a model of developmental plasticity that relies on crypsis as a seasonal strategy for predator avoidance. Using a full-factorial design, we disentangle effects of developmental and adult thermal environments on both appearance and behaviour. We show that developmental conditions affect both adult colour and colour preference, with temperatures that simulate dry season conditions leading to browner butterflies with a perching preference for brown backgrounds. Our results reveal that coordinated environmentally-induced responses can increase effective seasonal anti-predatory strategies in species coping with environmental heterogeneity.

KEYWORDS

animal colouration – background matching – seasonal environments – phenotypic plasticity

1. INTRODUCTION

Predation is an important selective pressure and organisms have evolved a wide range of adaptations to improve chances of survival in the presence of predators. Such adaptations typically include a suite of traits working together to maximize predator avoidance. For example, spider-mimicking moths both look and act like their predators to improve their chances of survival [1], and butterflies bearing large vertebrate-eye-like eyespots generally only expose these when threatened to increase their chances of startling predators [2,3]. While defensive mechanisms are often constitutive properties of prey species (e.g. the spines of the hedgehog), they can also be induced by specific environmental conditions. For example, the protective morphology of *Daphnia* is induced by chemical cues that signal predator presence [4,5]. Plasticity in anti-predator strategies also occurs in response to abiotic factors such as those that affect the visual background under which species need to escape predators. For example, in many polyphenic butterflies, seasonal strategies to avoid predation are associated with seasonal fluctuations in vegetation cover [6]. While such environmental regulation of development, or developmental plasticity, often leads to changes in adult morphology that are irreversible, adult behaviour is generally more responsive to environmental conditions experienced during adulthood. The question of whether plastic responses of morphological and behavioural anti-predator traits are coordinated, to ensure that animals use a behavioural repertoire that matches their appearance, remains under explored.

The tropical butterfly *Bicyclus anynana* is well suited to test hypotheses about the integration of multiple environmentally-induced anti-predator traits. This species exhibits seasonal polyphenism as an adaptive response to dry-wet seasonal environments, with crypsis and deflection as seasonally alternating anti-predator strategies [6]. Dry season individuals with small inconspicuous wing pattern elements are camouflaged against the brown background of dry leaves, while wet season individuals have large conspicuous marginal eyespots that deflect predators' attention away from the more vulnerable body. This seasonal variation in wing patterns is induced by the temperature during development, with cooler temperatures leading to small pattern elements [7]. Mark-release-

recapture experiments in the field [8] and experimental work with different putative predators have provided support for the adaptive advantage of seasonal variation in eyespot size [9,10].

Here we use thermal plasticity in *B. anynana* and a full factorial design to study the combined effects of developmental and adult environments on anti-predator pigmentation and behaviour. While thermal plasticity for *B. anynana* eyespot size is well characterized, other traits that could enhance the crypsis of dry season individuals, such as actual wing colour and colour preference, have largely been ignored (but see [6,9]). We investigated thermal plasticity in the colours of wing patterns as well as in behaviours that are predicted to make crypsis more effective. These behaviours are background matching, by which animals choose to rest on backgrounds that resemble their appearance [11,12], and motionlessness, since camouflage is known to be less effective when animals are in motion [13]. We test the hypotheses that the cryptic dry season forms, that are produced when development occurs in cooler temperatures, will have not only smaller pattern elements, but also colours that are less conspicuous. Moreover, we hypothesise that these cryptic individuals will be less active and rest preferentially on brown backgrounds reminiscent of the natural dry foliage. Globally, this work explores the extent to which seasonal strategies for avoiding predation involve synchronized responses to environmental information, to prevent a potentially fatal mismatch between anti-predatory morphology and behaviour.

2. METHODS

(a) Butterflies and rearing conditions

Eggs from a laboratory population of *B. anynana* were allocated to one of two climate-controlled rooms (65% RH, 12L:12D) differing in ambient temperature to simulate the conditions of the dry (Td=19°C) and wet seasons (Td=27°C). Groups of 250 larvae were reared in large population cages and fed with young maize plants. Upon eclosion, males and females were separated and allocated to one of two adult temperature regimes (Ta; 19°C and 27°C); yielding four treatments with different

88 combinations of Td and Ta (electronic supplementary material, figure S1). Adults were kept at
89 densities of 25 same-sex individuals per cage, and fed with moist banana.

90 *(b) Wing pigmentation analysis*

91 Using a colour-calibrated digital scanner (Epson V600), we scanned the ventral surfaces of
92 hindwings of 16 female and 16 male butterflies per treatment (N=128). The resulting images were
93 analysed with custom-made interactive Mathematica notebooks [14]. We first drew two contiguous
94 transects defined by five landmarks on the wing compartment bearing the eyespot that is often used
95 to characterize seasonal polyphenism in *Bicyclus* and related genera [15]. We then specified the
96 limits of the central band and each of the eyespot colour rings along the transect. The colours of the
97 wing background and different pattern elements were quantified by extracting the mean RGB value
98 of 3×3 pixel squares centred on the transect (figure 1*a*). For each individual, we then calculated the
99 Euclidean distance in RGB colour space between the colour of the different pattern elements and
100 that of the background of the wing. These distances were used as a proxy for colour
101 conspicuousness. We also calculated the distance from each pixel along the transect to the brown
102 patches used in our behavioural assay and used the mean of these as a proxy for crypsis in our
103 experimental setup (electronic supplementary material, figure S2).

104 *(c) Behavioural assays*

105 The behavioural assays were performed in large flight cages (150×50×54 cm) that allow expression
106 of a more natural behaviour [16]. The bottoms, backs, and sides of the cages were covered with
107 canvas depicting a mosaic of green (Hue=130°, Saturation=60%, Brightness=50%) and brown
108 (HSB 25°, 60%, 50%) squares (16.7 cm^2). The fronts were covered with mesh netting and the tops
109 had three fluorescent lamps (Osram L58W/840). Digital cameras (Canon EOD 1000D), connected
110 to a Wireless Remote Control (Hähnel - Giga T Pro), were positioned in front of the cage. Cohorts
111 of 20 same-sex, 6-8 day old individuals were released into the flight cage and left to habituate for
112 two hours. Subsequently, about 8 hours after the lights were switched on, 15 time-lapse photos were

taken at four-minute intervals for a period of 60 minutes. For each photo, we registered the number of butterflies at rest on each of the two background colours, as well as the number of individuals resting at exactly the same position as in the previous time point (i.e. no re-location). For perching preference we used the number of new arrivals to brown patches and the number of new arrivals to green patches, for each time point, as a 2-vector dependent variable. Likewise, for activity levels, we used the number of inactive individuals and the number relocated individuals. Sixteen cohorts were tested for each experimental treatment, eight for each of the sexes ($N_{cohort} = 64$).

(d) Statistical analyses

All statistical tests were done using R [17]. We examined the effects of developmental temperature (Td), adult temperature (Ta), and sex on wing pigmentation using linear mixed models (electronic supplementary material, table S1). The effects of Td, Ta, sex on perching preference and on levels of activity were assessed using generalized linear mixed models with a binomial distribution and a logit link. The cohort, and the time point (i.e. consecutive photos) for the behavioural data, were included as random effects in the models. For both behavioural variables, minimum adequate models were obtained by backward elimination, starting from the full model and using the Bayesian Information Criterion (BIC). P-values were calculated via likelihood ratio tests and by parametric bootstrapping (nsim=999), using the package *afex* [18]. Post-hoc pairwise comparisons (alpha=0.05) were conducted using the package *lsmeans* (electronic supplementary material, tables S2 and S3).

3. RESULTS

We used a full factorial design, with two developmental temperatures (Td; 19°C and 27°C) and two adult temperatures (Ta; 19°C and 27°C) that simulate the natural dry and wet seasons, respectively, to study thermal plasticity in pigmentation and behaviour and partition environmentally-induced variation into early and late life effects.

138 *(a) Thermal plasticity in wing colours*

139 Using a custom image analysis tool, we quantified the colour of different wing pattern components
140 (central band and eyespot rings; figure 1*a*) and of the wing background of adults from each of the
141 four combinations of Td and Ta. We found that developmental temperature affected many of these
142 aspects of wing pigmentation in both males and females. Pattern elements of individuals reared at
143 cooler temperatures were not only smaller, as had been shown previously [15,19], but they were
144 also of less conspicuous colours. Specifically, the colours of the eyespot's external ring (Td;
145 $F=278.38$, $P<0.001$) and central focus (Td; $F=757.57$, $P<0.001$) and, in females, the central band
146 (Td; $F=47.65$, $P<0.001$) were closer to the background colour of their respective wings (figure
147 1*b*,*c*). Moreover, overall colouration of individuals reared at 19°C was also closer to the brown
148 background patches used in our behavioural assays (figure 1*d*), with the colours of males being
149 darker and closer to the brown patches (Sex; $F=171.40$, $P<0.001$). Adult temperature had, as
150 expected, no effect on wing pigmentation.

151 *(b) Thermal plasticity in colour preference*

152 The behaviour of males and females from the four temperature treatments was monitored in large
153 flying cages containing a mosaic of green and brown patches. Using time-lapse photographs, we
154 monitored preference to rest on brown versus green, as well as overall levels of activity (electronic
155 supplementary material, figure S3). We found that developmental temperature affected adult colour
156 preference (figure 2*a*). Compared to their more conspicuous wet-season counterparts (Td=27°C),
157 individuals with more cryptic colouration (Td=19°C) were more likely to alight in brown patches
158 (Td; $\chi^2=20.66$, $P<0.001$). This was true for both sexes and in both adult temperatures. The
159 preference for brown backgrounds was stronger in females (Sex; $\chi^2=62.53$, $P<0.001$) and these sex-
160 specific colour preferences were stronger when animals were tested at low adult temperatures
161 (Ta:Sex; $\chi^2=14.26$, $P<0.001$). Activity levels of females were not affected by either Td or Ta while,
162 in contrast to our prediction, males were more active when reared (Td:Sex; $\chi^2=7.79$, $P=0.005$) or
163 kept (Ta:Sex; $\chi^2=22.91$, $P<0.001$) at cooler temperatures (figure 2*b*).

4. DISCUSSION

Using a full factorial design that allows separating the effects of developmental and adult environments [20], we showed that both appearance and behaviour are affected by thermal conditions during development in *B. anynana*. We demonstrated for the first time that developmental temperatures affect wing pigmentation beyond the size of the ornamental pattern elements. For better camouflage against the dry brown foliage, dry-season individuals not only develop smaller pattern elements but the colours of these contrast less with their wings' brown background colour. Moreover, we also showed that individuals reared at cooler temperatures (i.e. the browner ones) also had stronger preferences for brown backgrounds. Such a concerted response in adult colour and colour preference can help ensure that the development of a cryptic adult appearance is matched by a behavioural repertoire that enhances crypsis.

The integrated background colour preferences that we demonstrate here may reduce the risk of detection by predators when cryptic individuals are confronted with visually heterogeneous environments [21]. For B. a*nynana* this is especially relevant during seasonal transitions when dead brown leaves and green vegetation co-occur and butterflies can choose among a variety of backgrounds to rest on. We found that the preference for brown backgrounds was stronger in females. In fact, overall, males favoured green backgrounds which may be related to sex-specific trade-offs between survival and reproduction in the wild. Males of *B. anynana* pursue a perch-and-chase strategy to procure mates and are typically found in patches of green sunlit vegetation that improve the probability of detecting potential mates [16,22]. In contrast, females tend to fly more closely to the ground searching for suitable host plants for oviposition. While our data supports the hypothesis that cryptic individuals show stronger preference for the background colour that presumably maximizes camouflage, we found no evidence for responses in another type of behaviour that could enhance crypsis; motionlessness. Field observations had suggested that cryptic

189 adults spend the dry season being relatively inactive, likely to conserve energy and avoid detection
190 [6], but we found no evidence for reduced activity in dry-season-like individuals in this study.

191 We demonstrated that environmental conditions during early life can affect wing colouration and
192 lead to "fixed" changes in adult behaviour, as had been shown in insects and vertebrates (e.g.
193 [23,24]). The mechanisms underlying the environmentally-induced differences in colour and colour
194 preference remain unclear, though traits responding in an integrated manner to environmental cues
195 typically share underlying regulators. Traits making up the *B. anynana* seasonal syndrome,
196 including eyespot size [19], life-history traits [25] and courtship behaviour [23], are known to be
197 mediated by ecdysteroid hormone dynamics during the late-larval and early pupal stages. Moreover,
198 sex differences in ecdysteroid titres during development have been shown to influence patterns of
199 sex-specific pigmentation in dorsal eyespots [26], which are not analysed here nor involved in
200 predator avoidance [27,28]. Whether and how ecdysone dynamics can have a direct effect on the
201 biosynthetic pathways for pigment production [29–31] is unclear, but effects of developmental
202 temperatures on these pathways have been studied in detail in *Drosophila melanogaster* [32], a
203 well-described model for thermal plasticity in body pigmentation.

204 Temperature-induced changes in ecdysteroid signalling may also alter neuronal activity and
205 connectivity [33,34] and/or mediate visual sensitivity of adults through the regulation of eye
206 development [35]. In fact, the size of the *B. anynana* compound eye is known to be
207 developmentally plastic and sexually dimorphic [36]. Wet-season individuals and males have larger
208 eyes than dry-season individuals and females, respectively, which could affect colour vision and
209 preferences. These published data on thermal plasticity in eye size [36] correlate strongly with our
210 behavioural data; individuals reared at lower temperatures have smaller eyes and stronger
211 preferences for brown patches (electronic supplementary material, figure S4).

Our data clearly show that morphological and behavioural anti-predator traits are affected in concert by the conditions experienced during development and underscore the tight integration of form and function necessary for survival under heterogeneous environments.

Data accessibility

Raw data will be archived in the Dryad Digital Repository upon acceptance of the manuscript.

Authors' contributions

EvB and PB conceived and designed the experiments. EvB performed the experiments and analysed the data. EvB and PB wrote the manuscript.

Competing interests

The authors declare no competing financial interest.

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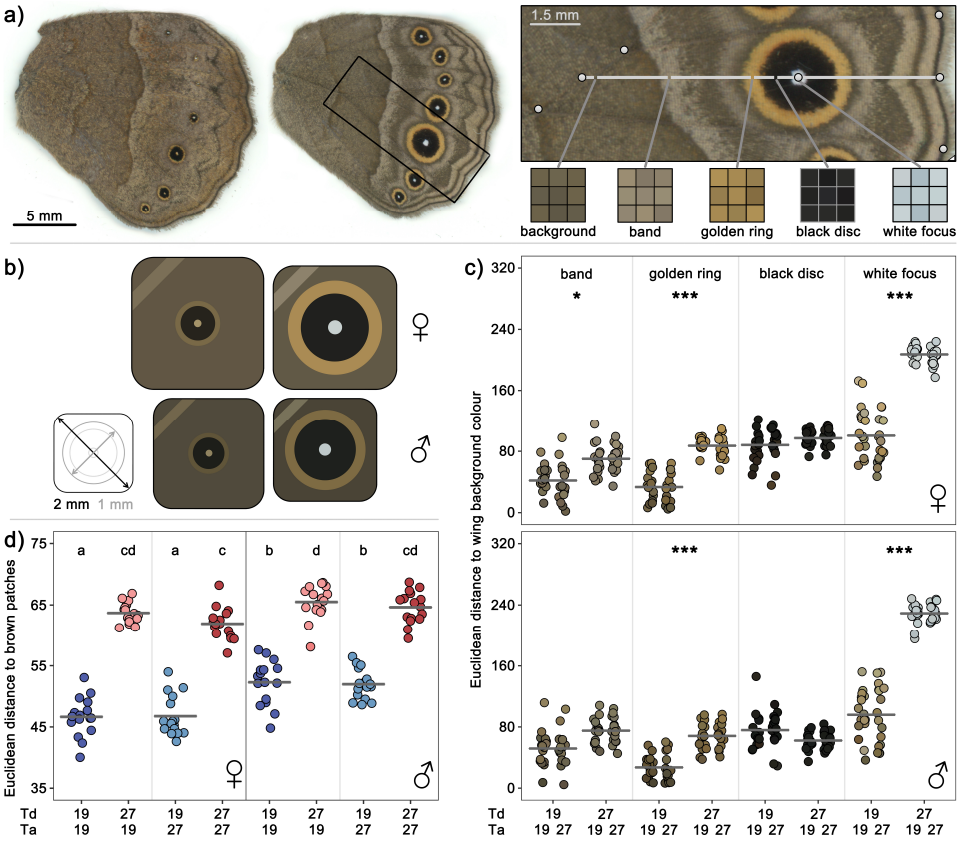
238 **FIGURE LEGENDS**

239 **Figure 1** Environmentally-induced wing pigmentation. (*a*) Examples of ventral hindwing
240 surfaces from females reared at Td=19°C (left) and Td=27°C (right; with section highlighted).
241 Section of a hindwing showing landmarks (white circles) that defined two contiguous transects
242 (white line) passing through the centre of the eyespot. The colours of the background, central band,
243 and eyespot rings were quantified by extracting the mean RGB values of 3×3 pixel squares. (*b*)
244 Schematic representation of the colour of the background (square fill), central band (stripe on top
245 left corner) and eyespot rings (circles), as well as the size of the wings and eyespot rings (cf. scale
246 on left-most drawing), for individuals reared at Td=19°C (left) and Td=27°C (right). (*c*) Euclidian
247 distance in RGB colour space between the colour of the background and the colour of different
248 pattern elements for females are males from four thermal treatments. Colour conspicuousness was
249 not affected by adult temperature (Ta), statistical significance for effect of developmental
250 temperature (Td) is indicated as: * for P<0.05 and *** for P<0.001. (*d*) Mean Euclidian distance in
251 RGB colour space, using all pixels along the transect, to the colour of the brown patches used in our
252 behavioural assay. Significant differences between groups (Tukey's HSD, P<0.05) are indicated by
253 different letters. Details of statistical tests in electronic supplementary material, table S1.

254 **Figure 2** Environmentally-induced behaviours. (*a*) Probability of relocating to a brown patch
255 for females and males from different combinations of Td and Ta. Within the same testing
256 temperature and sex, cryptic individuals (Td=19°C; blue dots) are more likely to alight in brown
257 patches than individuals with a more conspicuous colouration (Td=27°C; red dots). Preference for
258 brown patches is stronger in females and sex-specific colour preferences are stronger when tested at
259 cooler temperatures (Ta=19°C). Asterisks indicate groups having probabilities of relocating to
260 brown that are significantly different from a 1:1 ratio. (*b*) Probability of relocating for females and
261 males from different treatments. Males were more active when reared or kept at cooler temperatures
262 while activity levels of females were not affected by either Td or Ta. Dots in both panels represent
263 the probability of relocating (to brown) for each cohort based on the total number of relocations (to
264 brown) observed throughout the assay. Significant differences between groups (Tukey's HSD,
265 $P<0.05$) are indicated by different letters. Details of statistical tests in electronic supplementary
266 material, table S2 and S3.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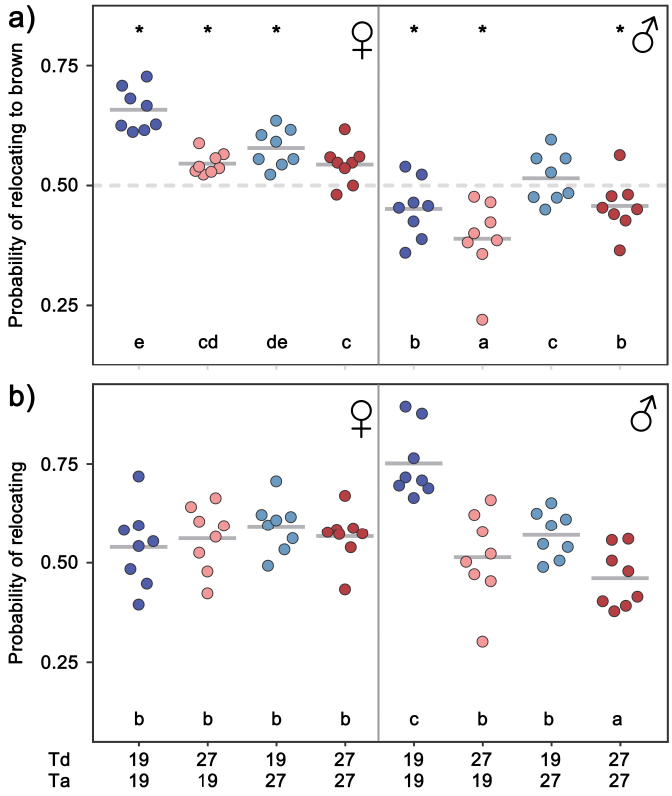
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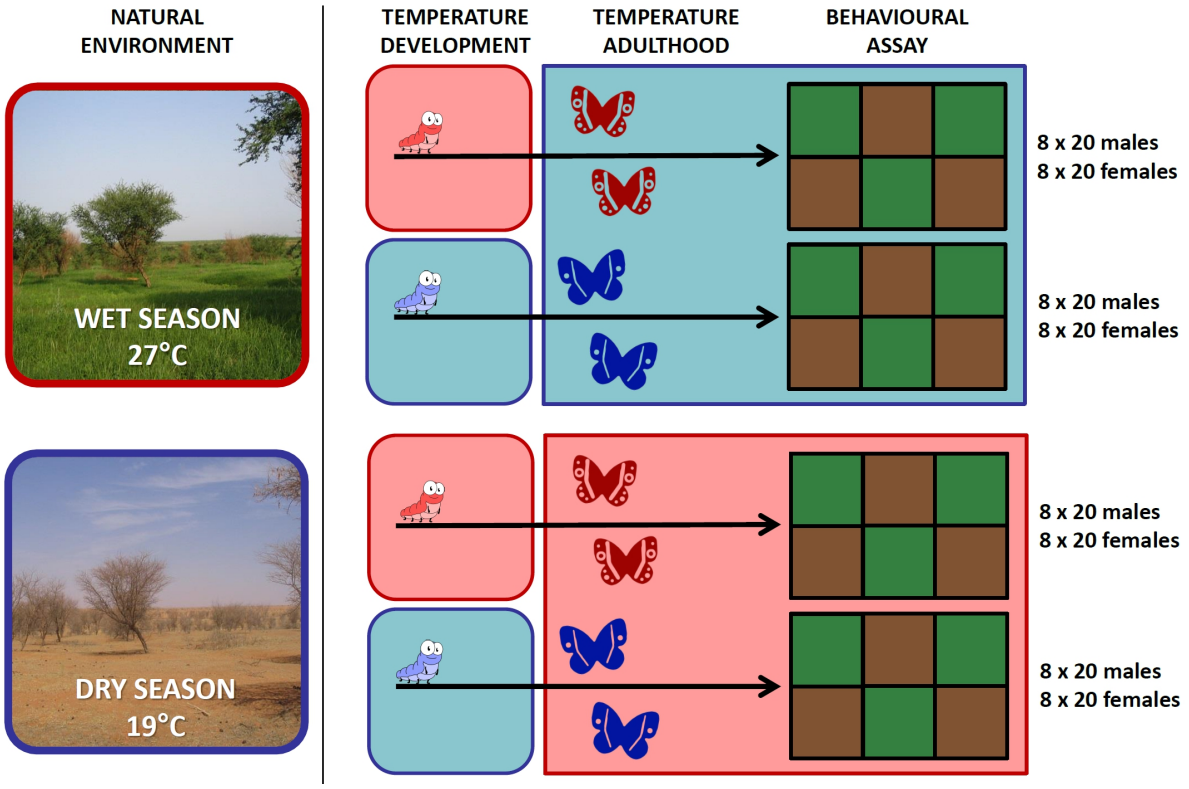
SUPPLEMENTARY MATERIALS

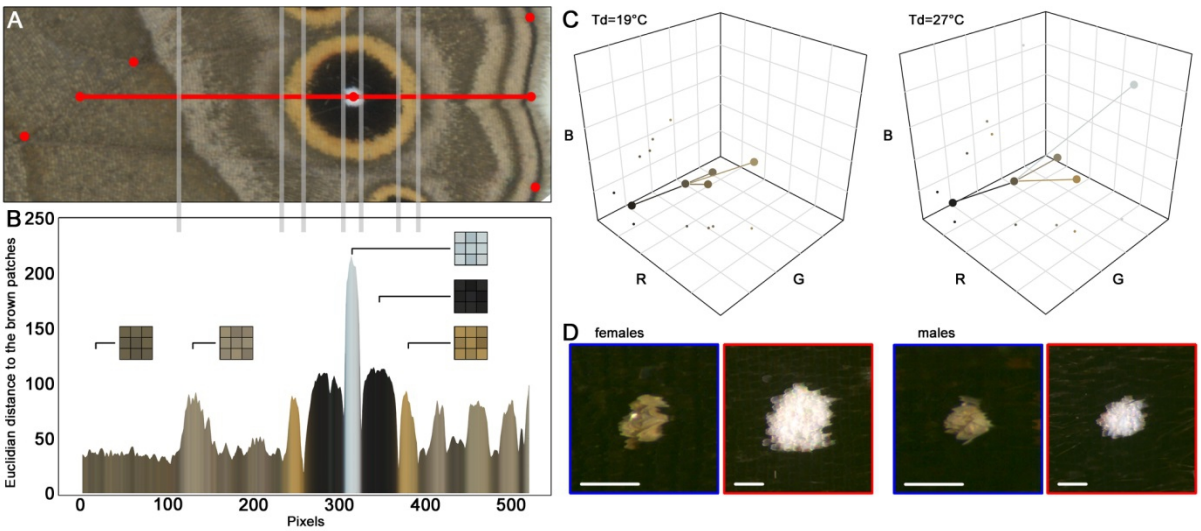
Supplementary Fig. 1	Schematic representation of the experimental design.
Supplementary Fig. 2	Quantification of crypsis-enhancing pigmentation traits.
Supplementary Fig. 3	Behavioural data per cohort.
Supplementary Fig. 4	Correlations between eye size and perching preference.

Supplementary Table 1	Colours and sizes of the wing pattern elements across treatments.
Supplementary Table 2	Generalized linear mixed models for perching preference.
Supplementary Table 3	Generalized linear mixed models for activity levels.
Supplementary Table 4*	Behavioural dataset used in this study.
Supplementary Table 5*	Morphological dataset used in this study.

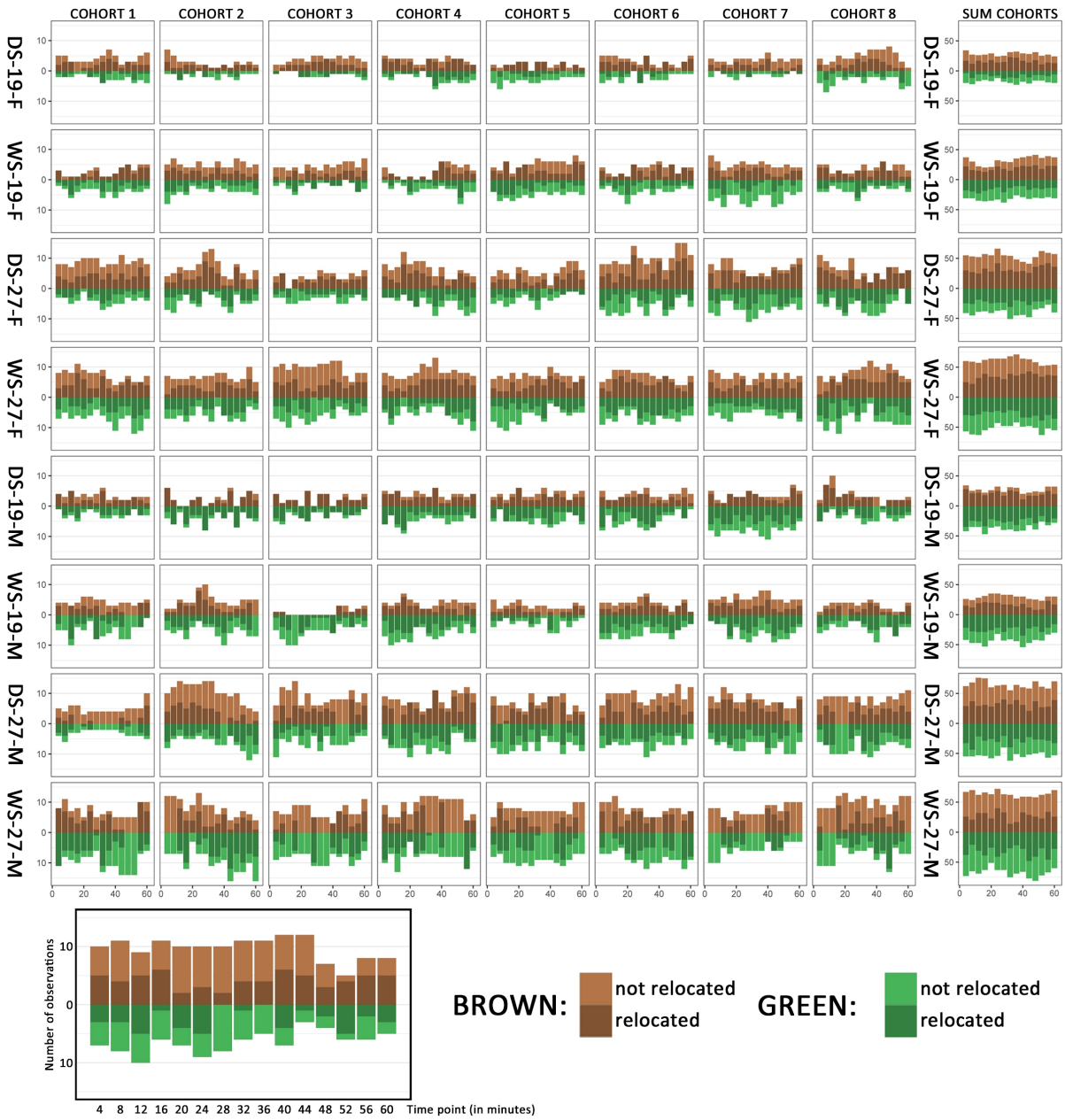
* Supplementary Table 4 and 5 will be uploaded to Dryad upon acceptance for publication.



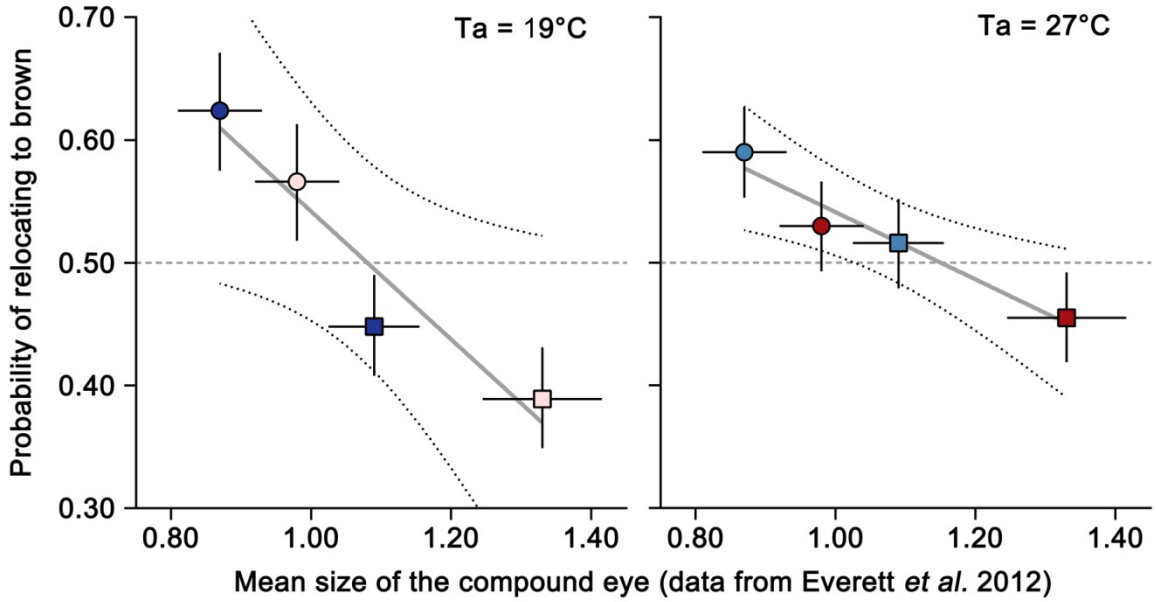
Supplementary Figure 1. Schematic representation of the experimental design. Panels on the left-hand side depict the natural environment of *B. anynana* during the wet season (upper) and dry season (lower) while panels on the right-hand side provide a schematic representation of our experimental design. Individuals were reared, from egg to adult eclosion, at one of two developmental temperatures (Td; 19°C and 27°C) that simulate the ambient temperatures of the dry and wet season, respectively. Variation in rearing temperature is sufficient to induce seasonal polymorphism in this species, with cool and warm temperatures inducing dry season form (DS) and wet season form (WS) individuals, respectively. On the day of eclosion, the butterflies were allocated to one of two adult temperatures (Ta; 19°C and 27°C), yielding four experimental treatments with different combinations of Td and Ta. Behavioural assays were performed at the temperature of the adult regime using cohorts of 20 six to eight day old males or females (N_{cohort} = 64). The red colours in this scheme represent warm temperatures or individuals reared at warm temperatures. Cool temperatures, or individuals reared at cool temperatures, are represented by blue colours.



Supplementary Figure 2. Quantification of crypsis-enhancing pigmentation traits. A. We used a set of custom-made interactive Mathematica notebooks to analyse the ventral hindwing surfaces of 16 female and 16 male butterflies per experimental treatment (i.e. two individuals per cohort, N=128). Two contiguous transects were defined by five landmarks (red dots) in the wing compartment bearing the large fifth eyespot (Cu1). Secondary landmarks, represented by grey lines, were used to specify the edge of the central band and the limits of each of the colour rings along the transect line. **B.** The colours of the eyespot rings were quantified by extracting the mean RGB value of 3×3 pixel squares centred on the intersection between the midpoints of the secondary landmarks and the transect line. The mean RGB values of the background and central band were extracted from a 3×3 cluster located 10 pixels after the associated secondary landmarks on the marginal side. Moreover, for each pixels we calculated the Euclidean distance in RGB colour space to the colour of the brown patches used in our behavioural assays. These distances are presented on the y-axis of the graph. **C.** We determined the Euclidean distance in RGB colour space between the colour of the each pattern element and the colour of the wing's background. The 3D-plots represent the mean RGB values for the each pattern element for females reared at cool (Td=19°C; left) and warm (Td=27°C; right) temperatures and lines represent the Euclidean distances for the band, golden ring, black disc and the white focus. **D.** Detailed images of the wing scales that make up the white focus for females (left) and males (right). The blue coloured outline represents individuals reared at cool temperatures (Td=19°C) while red outlines represents individuals reared at warm temperatures (Td=27°C). Scale bars represent 200 µm.



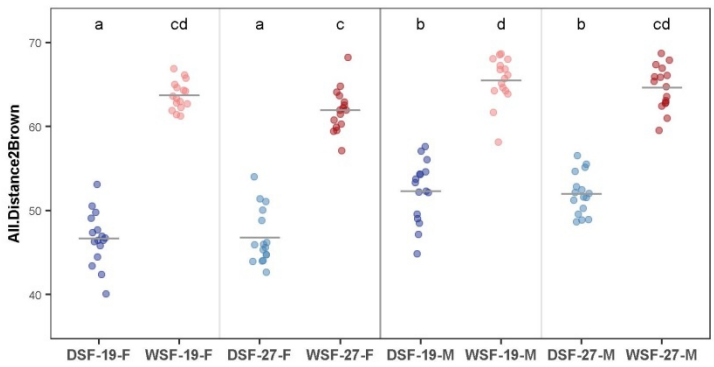
Supplementary Figure 3. Behavioural data per cohort. Panels represent all behavioural data per cohort. The number of butterflies at rest on each of the two background colours is represented by the brown and green bars for each time point. The number of individuals resting at exactly the same position as in the previous time point (i.e. no re-location) is depicted by lighter colours inside each bar. Note that individuals that were resting on the mesh netting that covered the front of the cage or on the upper surface of the cage, or were airborne when the time-lapse photos were taken were denoted as 'unaccounted for' in the data. The number of individuals that was unaccounted for was higher at cooler temperatures (Ta=19°C) for both males and females.



Supplementary Figure 4. Correlations between eye size and perching preference. The size of the *B. anynana* compound eye is known to be developmentally plastic and sexually dimorphic. Individuals reared at warm temperatures (wet-season; red dots) have larger eyes than individuals reared at cold temperatures (dry-season; blue dots) and females (circles) have smaller eyes than males (squares); data from Everett *et al.* 2012. Our behavioural data on perching preferences (y-axis) correlates strongly with the published data on thermal plasticity in eye size (x-axis) such that individuals with smaller eyes have stronger preferences for brown patches. Left-hand panel gives the correlation between eye size and perching preferences measured at cool temperatures (Ta=19°C) while the right-hand panel shows the correlation with behavioural responses assessed at warm temperatures (Ta=27°C).

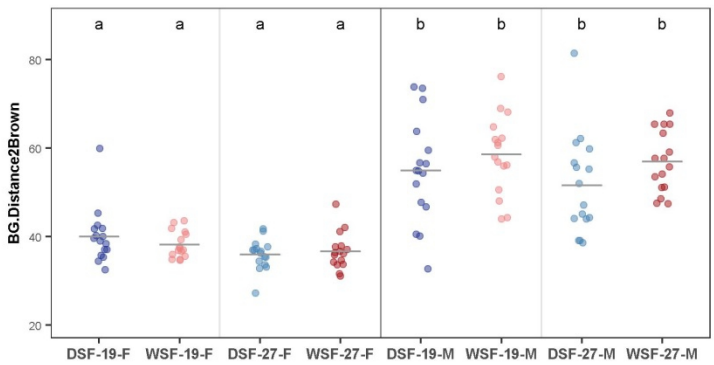
Supplementary Table 1. Colours and sizes of the wing pattern elements across treatments. Left-hand panels represent the Euclidian distance in RGB colour space from the colour of the brown patches used in the experiment to **A)** all pixels along the transect and **B)** the colour of background. Panels C to F represent the Euclidian distance from the background colour of each individual to **C)** the central band, **D)** the golden ring, **E)** the black disc and **F)** the white focus. Panels G to K are related to (relative) sizes and panels represent **G)** the length of the transect (i.e. proxy for wing size), **H)** the relative distance from the start of the transect to the proximal edge of the central band (i.e. inverse proxy for the width of the band, which is difficult to measure directly because of its indistinct distal edge), **I)** the relative width of the golden ring, **J)** the relative width of the black disc and **K)** the relative width of the white focus. Relative widths were calculated by dividing the absolute size by the length of the transect. Tables on the right-hand side of each panel give the effects of developmental temperature (Td), adult temperature (Ta), sex and their interactions on the phenotypic trait measured. Effects with P<0.01 are shown in bold The experimental cohort was included as a random factor and denominator degrees of freedom were determined with the Kenward-Roger method, using the package *afex*. Post-hoc pairwise comparisons (alpha=0.05) were conducted using the package *lsmeans*. Differences between experimental treatments and sexes are given in the graphs, with groups that are not significantly different from each other sharing a common letter.

A)



	F-value	df_{KR}	P_{KR}
T_D	**706.86**	**1,56**	**<0.001**
T_A	1.67	1,56	0.201
Sex	**49.42**	**1,56**	**<0.001**
T_D:T_A	1.26	1,56	0.267
T_D:Sex	**8.48**	**1,56**	**0.005**
T_A:Sex	0.05	1,56	0.828
T_D:T_A:Sex	0.37	1,56	0.546

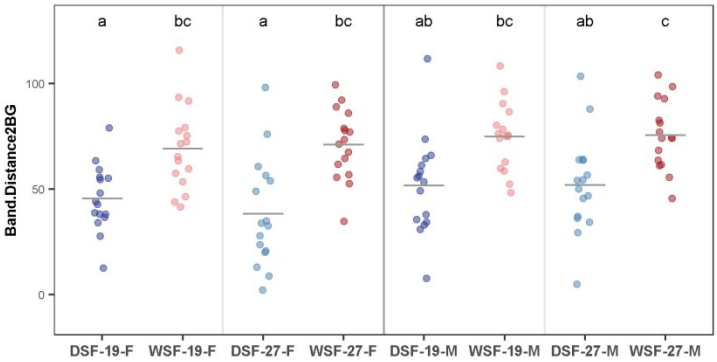
B)



	F-value	df_{KR}	P_{KR}
T_D	2.13	1,56	0.150
T_A	3.79	1,56	0.056
Sex	**171.40**	**1,56**	**<0.001**
T_D:T_A	0.60	1,56	0.441
T_D:Sex	3.49	1,56	0.067
T_A:Sex	0.01	1,56	0.914
T_D:T_A:Sex	0.03	1,56	0.861

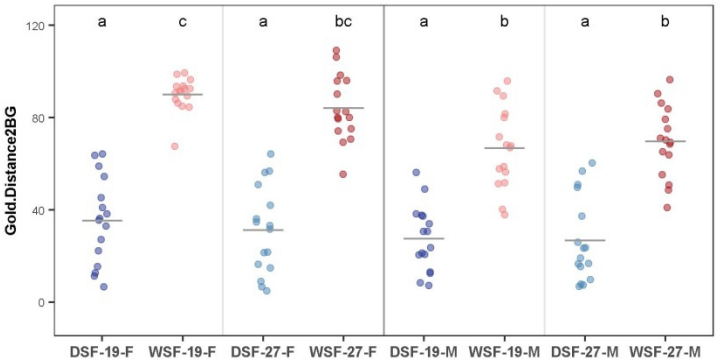
Continuation of Supplementary Table 1.

C)



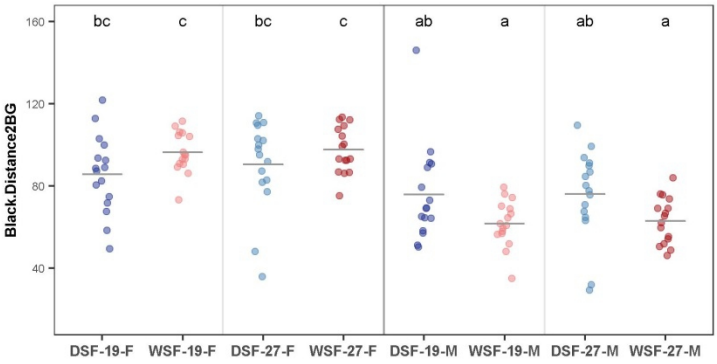
	F-value	df_{KR}	P_{KR}
T_D	**47.65**	**1,56**	**<0.001**
T_A	0.09	1,56	0.765
Sex	4.062	1,56	0.049
T_D:T_A	0.417	1,56	0.521
T_D:Sex	0.453	1,56	0.503
T_A:Sex	0.173	1,56	0.678
T_D:T_A:Sex	0.330	1,56	0.568

D)



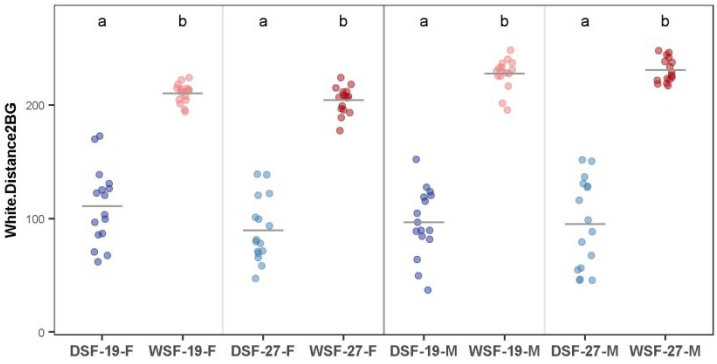
	F-value	df_{KR}	P_{KR}
T_D	**278.38**	**1,56**	**<0.001**
T_A	0.47	1,56	0.494
Sex	**19.45**	**1,56**	**<0.001**
T_D:T_A	0.03	1,56	0.861
T_D:Sex	5.01	1,56	0.029
T_A:Sex	1.16	1,56	0.287
T_D:T_A:Sex	0.25	1,56	0.619

E)



	F-value	df_{KR}	P_{KR}
T_D	0.57	1,56	0.452
T_A	0.37	1,56	0.548
Sex	**59.72**	**1,56**	**<0.001**
T_D:T_A	0.03	1,56	0.860
T_D:Sex	**13.80**	**1,56**	**<0.001**
T_A:Sex	0.15	1,56	0.695
T_D:T_A:Sex	0.14	1,56	0.705

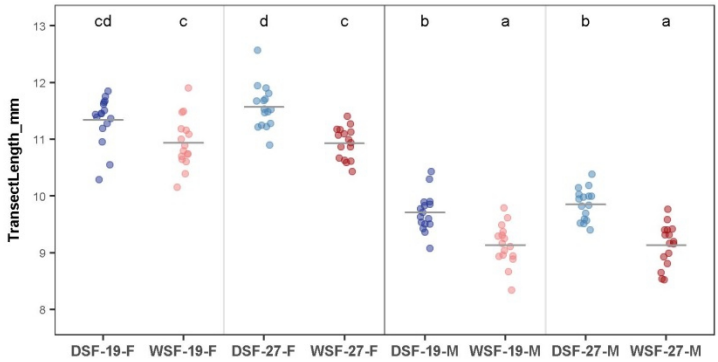
F)



	F-value	df_{KR}	P_{KR}
T_D	**757.57**	**1,56**	**<0.001**
T_A	2.11	1,56	0.151
Sex	3.94	1,56	0.051
T_D:T_A	1.29	1,56	0.261
T_D:Sex	**9.13**	**1,56**	**0.004**
T_A:Sex	2.82	1,56	0.099
T_D:T_A:Sex	0.39	1,56	0.536

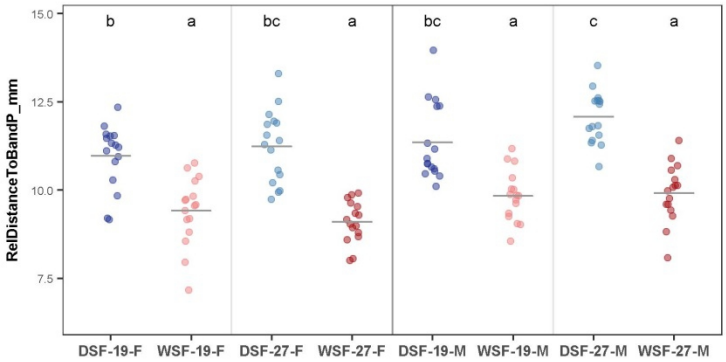
Continuation of Supplementary Table 1.

G)



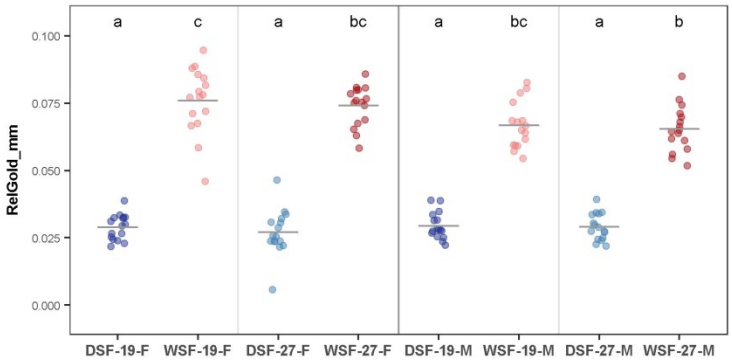
	F-value	df_{KR}	P_{KR}
T_D	**74.60**	**1,56**	**<0.001**
T_A	1.89	1,56	0.174
Sex	**662.76**	**1,56**	**<0.001**
T_D:T_A	1.97	1,56	0.166
T_D:Sex	0.83	1,56	0.366
T_A:Sex	0.11	1,56	0.740
T_D:T_A:Sex	0.12	1,56	0.731

H)



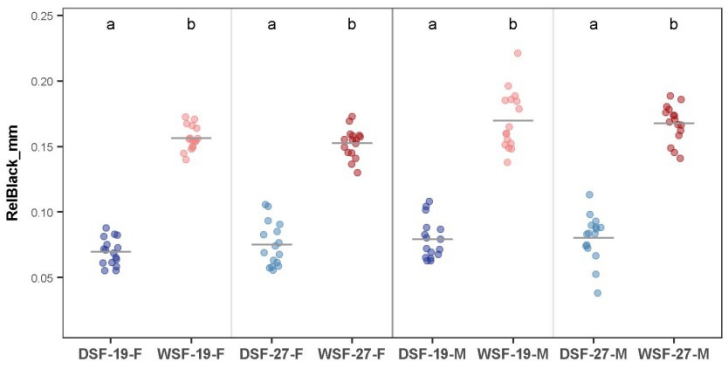
	F-value	df_{KR}	P_{KR}
T_D	**142.75**	**1,56**	**<0.001**
T_A	1.58	1,56	0.214
Sex	**15.87**	**1,56**	**<0.001**
T_D:T_A	4.09	1,56	0.048
T_D:Sex	<0.01	1,56	0.975
T_A:Sex	1.92	1,56	0.171
T_D:T_A:Sex	0.01	1,56	0.934

I)



	F-value	df_{KR}	P_{KR}
T_D	**741.30**	**1,56**	**<0.001**
T_A	0.85	1,56	0.361
Sex	6.22	1,56	0.016
T_D:T_A	0.02	1,56	0.883
T_D:Sex	**10.88**	**1,56**	**0.002**
T_A:Sex	0.12	1,56	0.727
T_D:T_A:Sex	0.02	1,56	0.878

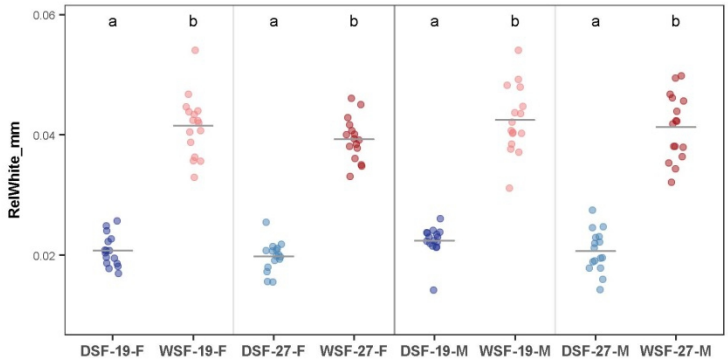
J)



	F-value	df_{KR}	P_{KR}
T_D	**784.99**	**1,56**	**<0.001**
T_A	0.01	1,56	0.941
Sex	**12.55**	**1,56**	**<0.001**
T_D:T_A	1.10	1,56	0.299
T_D:Sex	1.30	1,56	0.258
T_A:Sex	0.04	1,56	0.837
T_D:T_A:Sex	0.25	1,56	0.618

Continuation of Supplementary Table 1.

K)



	F-value	df_{KR}	P_{KR}
T_D	**633.18**	**1,56**	**<0.001**
T_A	3.54	1,56	0.065
Sex	2.95	1,56	0.091
T_D:T_A	0.06	1,56	0.807
T_D:Sex	0.02	1,56	0.894
T_A:Sex	0.01	1,56	0.932
T_D:T_A:Sex	0.30	1,56	0.584

Supplementary Table 2: GLMM for perching preference (related to Fig 2a in the main text). **A.** Minimum adequate models were obtained by backward elimination, starting from the full model and using Bayesian Information Criterion (BIC). **B.** P-values for the minimum adequate model were calculated via likelihood ratio tests and parametric bootstrap (nsim=999), using the package *afex*. **C.** Mean probabilities, as well as upper and lower confidence limits, are given for each sex and experimental treatment. Post-hoc pairwise comparisons (alpha=0.05), obtained using the package *lsmeans*, specify differences between experimental treatments and sexes.

A. Perching preference: cbind(newBrown,newGreen)			BIC
M_{full} :	T_D * T_A * Sex	+ (1|Cohort) + (1|Timepoint)	2846.96
M_2 :	T_D + T_A + Sex + T_D:T_A + T_D:Sex + T_A:Sex	+ (1|Cohort) + (1|Timepoint)	2842.95
M_3 :	T_D + T_A + Sex + T_D:Sex + T_A:Sex	+ (1|Cohort) + (1|Timepoint)	2836.78
M_{mam} :	**T_D + T_A + Sex + T_A:Sex**	**+ (1|Cohort) + (1|Timepoint)**	**2829.95**
M_5 :	T_D + T_A + Sex	+ (1|Cohort) + (1|Timepoint)	2837.34

B.	Chisq	P_{LRT}	$P_{PB\ (n=999)}$
T_D	**20.66**	**<0.001**	**0.001**
T_A	1.32	0.251	0.245
Sex	**62.53**	**<0.001**	**0.001**
T_A:Sex	**14.26**	**<0.001**	**0.001**

C.	Sex	T_A	T_D	mean	LCL	UCL	pairs
	F	19	DS	0.624	0.575	0.671	E
	F	19	WS	0.566	0.518	0.613	CD
	F	27	DS	0.590	0.553	0.627	DE
	F	27	WS	0.530	0.493	0.566	C
	M	19	DS	0.448	0.408	0.490	B
	M	19	WS	0.389	0.349	0.431	A
	M	27	DS	0.516	0.479	0.552	C
	M	27	WS	0.455	0.419	0.492	B

Supplementary Table 3: GLMM for activity levels (related to Fig 2b in the main text). **A.** Minimum adequate models were obtained by backward elimination, starting from the full model and using Bayesian Information Criterion (BIC). **B.** P-values for the minimum adequate model were calculated via likelihood ratio tests and parametric bootstrap (nsim=999), using the package *afex*. **C.** Mean probabilities, as well as upper and lower confidence limits, are given for each sex and experimental treatment. Post-hoc pairwise comparisons (alpha=0.05), obtained using the package *lsmeans*, specify differences between experimental treatments and sexes.

A. Activity level: cbind(leaving,staying)			BIC
M_{full}:	$T_D * T_A *$ Sex	+ (1|Cohort) + (1|Timepoint)	4362.39
M_2:	$T_D + T_A +$ Sex $+ T_D{:}T_A + T_D{:}$Sex $+ T_A{:}$Sex	+ (1|Cohort) + (1|Timepoint)	4360.09
M_{mam}:	**$T_D + T_A +$ Sex $+ T_D{:}$Sex $+ T_A{:}$Sex**	**+ (1|Cohort) + (1|Timepoint)**	**4354.53**
M_4:	$T_D + T_A +$ Sex $+ T_A{:}$Sex	+ (1|Cohort) + (1|Timepoint)	4355.46
M_5:	$T_D + T_A +$ Sex $+ T_D{:}$Sex	+ (1|Cohort) + (1|Timepoint)	4370.57

B.	Chisq	P_{LRT}	$P_{PB\ (n=999)}$
T_D	**8.54**	**<0.001**	**0.006**
T_A	**13.14**	**<0.001**	**0.001**
Sex	0.25	0.620	0.661
T_D:Sex	**7.79**	**0.005**	**0.013**
T_A:Sex	**22.91**	**<0.001**	**0.001**

C.	Sex	T_A	T_D	mean	LCL	UCL	pairs
	F	19	DS	0.554	0.472	0.634	B
	F	19	WS	0.551	0.470	0.629	B
	F	27	DS	0.588	0.511	0.661	B
	F	27	WS	0.585	0.508	0.657	B
	M	19	DS	0.738	0.669	0.797	C
	M	19	WS	0.617	0.540	0.689	B
	M	27	DS	0.544	0.467	0.619	B
	M	27	WS	0.406	0.333	0.482	A