# Developmental Plasticity in Sexual Roles of Butterfly Species Drives Mutual Sexual Ornamentation

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Current explanations for why sexual ornaments are found in both sexes include genetic correlation, same sex competition, and mutual mate choice. In this study, we report developmental plasticity in mating behavior as induced by temperature during development in the butterfly *Bicyclus anynana*. Males and females reciprocally change their sexual roles depending on their larval rearing temperatures. This switch is correlated with a change in mating benefits to females and costs to males. The discrete seasonal environments, wet season and dry season, are known to produce the two developmental forms and as a consequence impose alternating, symmetrical patterns of sexual selection, one season on male ornaments, the following season on female ornaments. Thus, reciprocal selection through time may result in mutual sexual ornamentation.

Morphology and behavior can be developmentally plastic and change in response to environmental variation experienced during ontology (*1*). Butterflies often exhibit seasonal polyphenism in wing coloration, a form of developmental plasticity triggered by certain environmental cues experienced during critical ontogenetic periods in larval and pupal development (*2, 3*). To what extent rearing environment also influences adult butterfly behavior is largely unknown. We investigated both courtship and mate preference in the developmentally plastic African butterfly, *Bicyclus anynana*. This species is known to have multiple generations per year and to display two different developmental forms, wet season and dry season (WS and DS) (Fig. 1). In nature, these two forms are separated temporally and are adapted to their respective seasons (*4*). The WS form has large eyespots and a conspicuous transversal band on the ventral wings (Fig. 1A), whereas the DS form has reduced eyespots and cryptic, uniform ground coloration on the ventral wings (*5*) (Fig. 1B). These wing patterns are determined primarily by larval rearing temperature, not genetic differences (*5*). Females are known to mate multiply, and the DS females delay laying eggs until the end of the dry season, whereas the WS form will lay eggs immediately after mating (*6*).

The dorsal forewing eyespots, unlike ventral eyespots, show little size variation with larval rearing temperatures (*7*) (Fig. 1, C to F) and are the WS male sexual ornament (*8*). WS males actively court WS females, and these females prefer males with intact dorsal eyespots, specifically the presence of the white, ultraviolet (UV)-reflective center often called the pupil (*8*). These same ornaments are also present on females. We investigated whether dorsal eyespot pupils are signals in courtship and mate preference used by both sexes and by both developmental forms. We reared the two forms simultaneously in the lab and assessed both male and female courtship behavior among and between forms. We then measured mate response toward individuals with intact versus manipulated dorsal eyespots among and between forms.

We conducted courtship observations using a two-by-two factorial design (sex by developmental form). Courtship in *B. anynana* is highly ritualized (*9, 10*). It involves the courter displaying the dorsal eyespots to the receiver by rapidly opening and closing its wings in front of the eyes of the receiver (*9*). We found DS females courted either male form more often than WS females courted males [analysis of variance (ANOVA) $F_{1,19} = 3.69$, $P = 0.018$; no sex by form interaction] (Fig. 2A). Developmental form also affected male courtship; WS males courted females more often than DS males courted females ($F_{1,19} = 9.04$, $P = 0.0012$; no sex by form interaction) (Fig. 2B). Within developmental forms, we found that WS males courted more than WS females ($F_{1,19} = 9.23$, $P = 0.001$) (Fig. 2), whereas DS females courted more than DS males ($F_{1,19} = 5.10$, $P = 0.004$) (Fig. 2). There is a developmental shift in the intensity of courtship behavior displayed by each sex, irrespective of the developmental form of the receiver.

To test the effect of developmental plasticity on mate preference, we manipulated the sexual ornaments of each sex in turn and evaluated mate preference in each form. We eliminated the dorsal eyespot pupils, the white, UV-reflective centers, by concealing them with paint (*8, 10*). Consistent with previous work (*8*), we found WS females preferred to mate with males with intact dorsal eyespot pupils ($F_{1,19} = 5.27$, $P = 0.003$) (Fig. 3A). However, DS females showed no preference
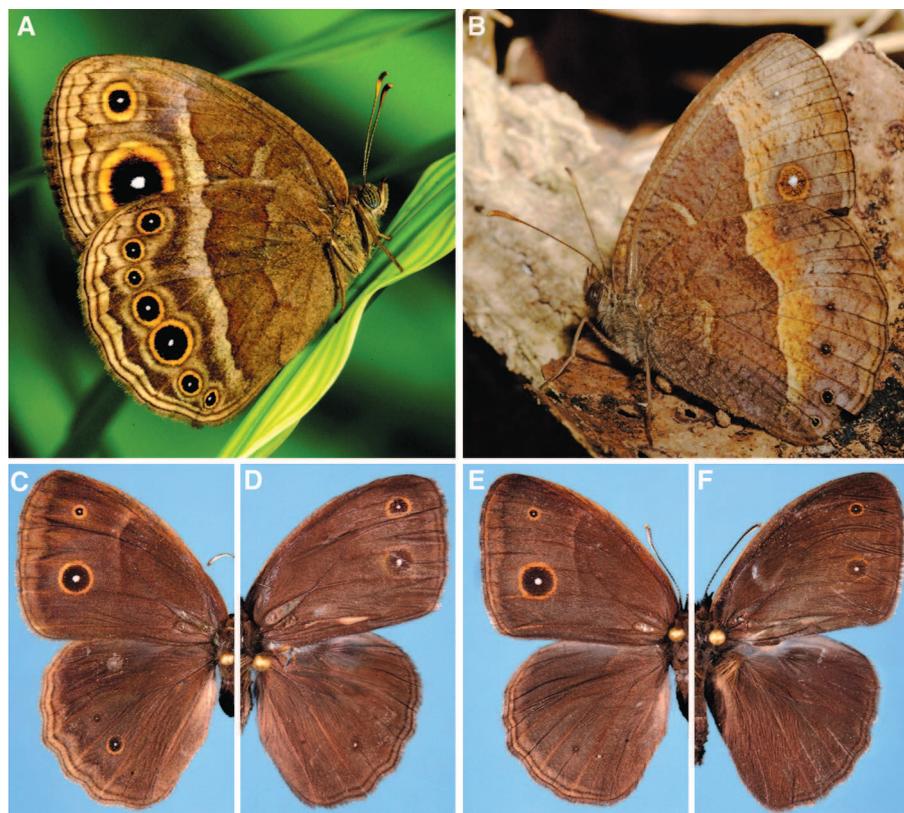


**Fig. 1.** Wet and dry seasonal forms of *B. anynana*. (**A**) WS ventral female, (**B**) DS ventral female, (**C**) WS dorsal female, (**D**) WS dorsal male, (**E**) DS dorsal female, (**F**) DS dorsal male. Note the difference in degree of plasticity between ventral and dorsal surfaces. Dorsal forewing eyespots (the sexual ornament) are not plastic compared with ventral eyespots.

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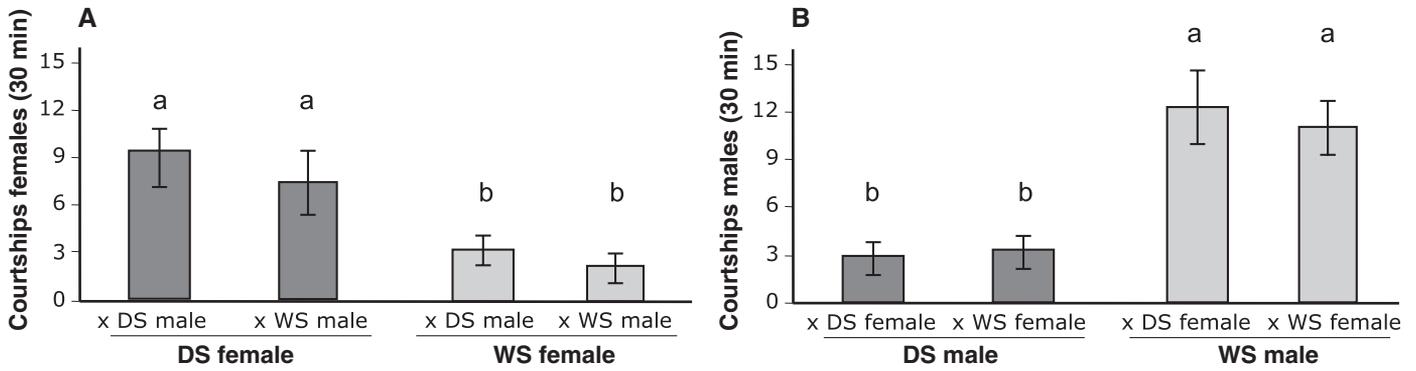
**Fig. 2.** Courtship behavior of DS and WS *B. anynana*. (**A**) Female behavior and (**B**) male behavior. Shade denotes the developmental form of the courter, not whom they courted: medium gray for dry season, light gray for wet season. Different letters above graphs represent significant differences. Error bars represent 95% CI of means.
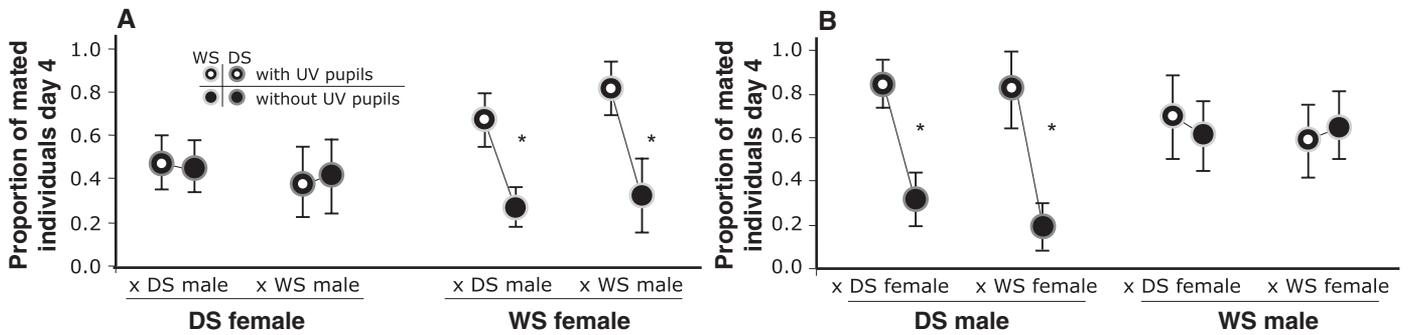


**Fig. 3.** Mate choice relative to presence or absence of intact dorsal eyespots in both developmental forms. (**A**) Female preference and (**B**) male preference. Shade denotes the developmental form of the discriminating sex: medium gray for dry season and light gray for wet season. Eyespot shape denotes UV pupil intact on the manipulated sex, and solid shape denotes UV pupil blocked with paint on the manipulated sex. Asterisks (*) indicate significant differences. Error bars represent 95% CI of means.

between males with or without pupils ($F_{1,19}$ = 0.41, $P$ = 0.690) (Fig. 3A). In the male choice experiments, we found DS males preferred to mate with females with intact pupils ($F_{1,19}$ = 4.93, $P$ = 0.006) (Fig. 3B), but WS males showed no preference ($F_{1,19}$ = 0.41, $P$ = 0.170) (Fig. 3B). Each developmental form was of similar genetic stock, and all individuals experienced the same environmental cues during the behavior experiments. Thus, early temperature differences during larvae and/or pupal development cause the switch in courtship and mate preference behavior.

Dorsal eyespot pupils are thought to be consistent in UV reflectance and size between sexes and seasonal forms, representing monomorphic ornaments (7). Our detailed measurements of these small wing pattern elements indicated that there is both a UV reflectance and size difference between DS and WS forms. WS males have greater UV reflectance than DS males [DS males = 47% with 4% confidence interval (CI), WS males = 58% with 6% CI, $F_{1,19}$ = 9.63, $P$ = 0.006], whereas DS females have greater UV reflectance than WS females (DS females = 56% with 4% CI, WS females = 50% with 3% CI, $F_{1,19}$ = 4.67, $P$ = 0.044). Pupil size is also larger in WS males relative to DS males, both in absolute size and relative to wing size (DS males = 0.056 mm² with 0.02 mm² CI, WS males = 0.111 mm² with 0.05 mm² CI, $F_{1,19}$ = 4.93, $P$ = 0.006), but pupil

size differences were not observed in females (DS females = 0.310 mm² with 0.05 mm² CI, WS females = 0.310 mm² with 0.04 mm² CI, $F_{1,19}$ = 0.35, $P$ = 0.563). Thus, the dorsal eyespots pupils in *B. anynana* are cryptic, seasonally dimorphic sexual ornaments.

This developmental polyphenism in mating behavior and sexual ornaments should be caused by a corresponding shift in the relative costs and benefits of mating. Because mating in insects is often associated with female fitness benefits via the transfer of a spermatophore or some other nuptial gift (11, 12), we investigated changes in the direct benefit to females between mating with WS and DS males. The relative effects of mating on male and female longevity were measured as the number of days until death in the absence of all adult resources except water (10). Both WS and DS female longevity (DS female $F_{1,35}$ = 24.54, $P$ = 0.0001; WS female $F_{1,35}$ = 22.71, $P$ = 0.0001) and number of eggs laid (DS female $F_{1,23}$ = 18.98, $P$ = 0.0001; WS female $F_{1,23}$ = 3.18, $P$ = 0.052) increased when females mated with a DS male (Fig. 4A) (Fig. 4C). However, female longevity did not differ between unmated females and those that mated with a WS male (DS female $F_{1,35}$ = 1.39, $P$ = 0.157; WS female $F_{1,35}$ = 0.19, $P$ = 0.762) (Fig. 4A). Conversely, DS male longevity was negatively affected by mating, whereas WS male longevity was not (DS

male $F_{1,35}$ = 18.22, $P$ = 0.001; WS male $F_{1,35}$ = 2.11, $P$ = 0.132) (Fig. 4B). These results suggest that females can increase their longevity by soliciting copulations and receiving a more beneficial nuptial gift from DS males. DS females may be actively seeking mating opportunities and the resultant male nuptial gifts, as seen in other butterfly species (13, 14). The number of copulations may limit female survival and subsequent probability of reproduction, and females may compete for mates in the dry season. We found no obvious preliminary difference in spermatophore size between WS and DS males, so additional research is necessary to elucidate the underlying mechanism promoting female longevity. Although we did not measure costs to males of multiple matings, it is possible that these costs could accumulate and affect subsequent mating opportunities. This hypothesis is bolstered by two findings in WS males: As the number of matings increase, (i) smaller spermataphores are produced (15), and (ii) less sperm is transferred (16).

We have shown that courtship, mate preference, and ornament UV reflectance in *B. anynana* changes reciprocally with developmental temperature, suggesting that seasonal temperature variation in the field drives a complex polyphenism in mating behavior and morphology. Compared with congeners, *B. anynana* is not obviously sexually dimorphic (17). We found that the seem-
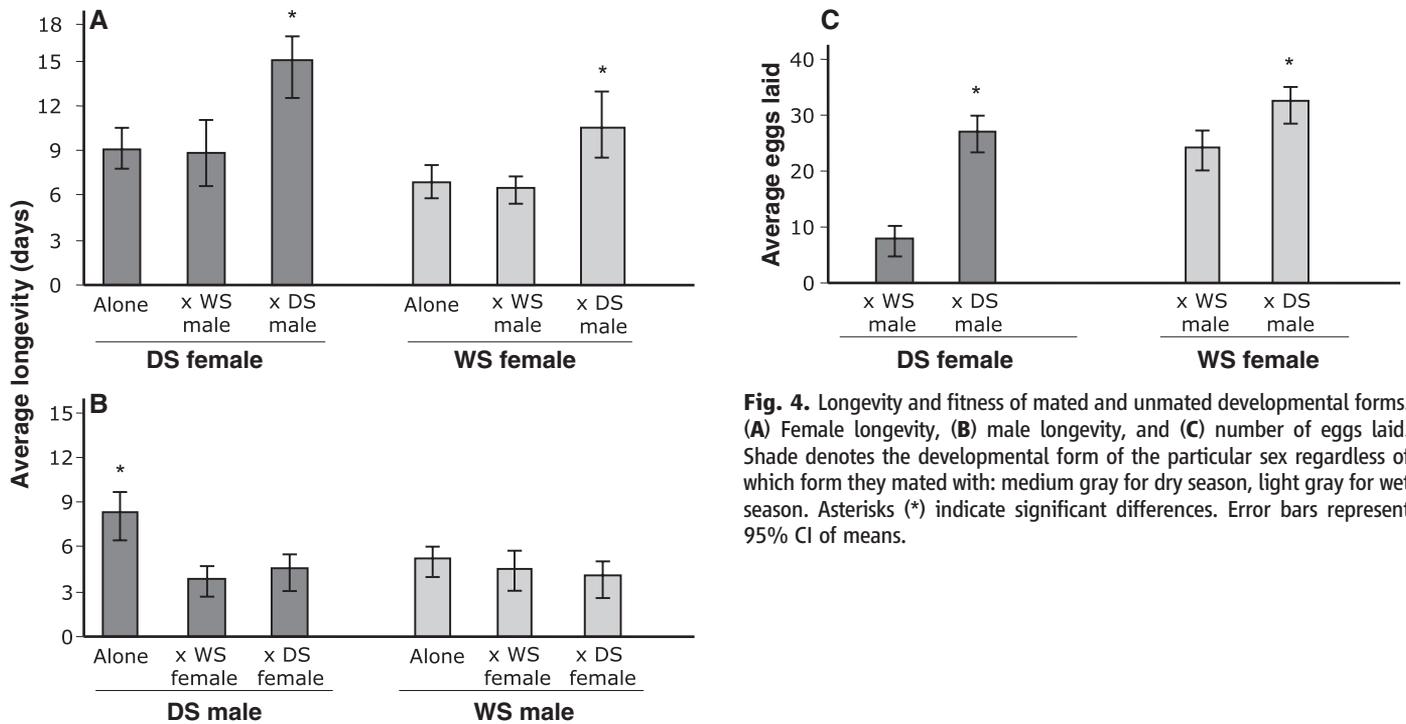
**Fig. 4.** Longevity and fitness of mated and unmated developmental forms. (**A**) Female longevity, (**B**) male longevity, and (**C**) number of eggs laid. Shade denotes the developmental form of the particular sex regardless of which form they mated with: medium gray for dry season, light gray for wet season. Asterisks (*) indicate significant differences. Error bars represent 95% CI of means.

ingly monomorphic wing pattern is actually seasonally dimorphic and is likely being maintained by the alternating sexual roles of males and females across seasons. The morphological differences between the sexual ornaments are cryptic to humans because the signal is in the UV range. It is possible that genetic correlations across sexes prevent the complete removal of eyespot pupils in the season where they are not being used in sexual display. Alternatively, dorsal eyespot pupils may play an additional role in predator-prey interactions beneficial to both sexes.

Previous work on vertebrates demonstrates that both males and females may simultaneously use their ornaments in making mating decisions (*18*) and that mate preference can change through adult life (*19, 20*). Similarly, other insect studies have shown seasonal changes in mating behavior within adult individuals based on changes in environmental conditions (*21, 22*). When adult resources are limiting in tettigonid bushcrickets, males become the choosier sex, whereas females initiate courtship more and compete more with each other for mating opportunities (*20–23*). These behaviors are not fixed during development as seen in *B. anynana*; instead, they can change in adults with the introduction of more food resources (*21*). Furthermore, the sexual ornament in bushcricket mate signaling is currently not known or not important in this mating system, although female size is a factor in mate preference (*22, 23*). In other butterfly species, there are examples of seasonal changes in mate preference in one sex (*24, 25*), but our study demonstrates a complete switch in mating behavior between the sexes.

Our study reveals another mechanism by which sexual ornaments may be maintained in both sexes beyond genetic correlation (*26*), same

sex competition (*27*), or mutual mate choice (*28*). Like certain morphological traits, courtship behavior and mate preference is seasonally plastic in *B. anynana*. This developmental control of mating behavior in different environments may be a generalized phenomenon, especially in insects. Many insect species have multiple generations per year and consistent seasonal differences in form between generations (*29*). These seasonal forms can have profound differences in wing pattern, mating behavior, and nuptial gifts (*29–31*). In these situations, developmental plasticity in sexual roles may help explain the evolution of both male and female ornaments. Adult mating behavior encoded during early ontology may be common in insects that have short reproductive periods relative to the length of the season and predictable environmental fluctuations. Seasonal developmental polyphenism in mating behavior would allow adults to immediately perform the correct behavior for a particular season with minimal learning costs. Thus, the complete reversal observed in *B. anynana* adds to our current understanding of environmentally induced sexual role reversal and suggests a dynamic mechanism promoting the evolution and maintenance of sexual ornaments in both males and females.

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