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# Male disguised females: costs and benefits of female-limited dimorphism in a butterfly

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**Abstract.** 1. In polymorphic species, two or more discrete phenotypes co-occur simultaneously. Sex-limited polymorphism is a particular case of polymorphism, in which several discrete morphs coexist within one of the two sexes only. Several hypotheses were proposed to explain the existence and the maintenance of sex-limited polymorphism in insects: (i) the morphs have similar fitness, such as similar survival and expected fecundity, and their frequencies vary randomly (i.e. the null hypothesis); (ii) harassment by males is reduced towards the less common female morph, in this case andromorph females (i.e. the male mimicry and learned mate recognition hypotheses); (iii) morphs differ in predation risk (i.e. the predation hypothesis); or (iv) morphs differ in thermoregulation ability (i.e. the thermoregulation hypothesis).

2. Field observations and experiments were employed to compare the relative support of these hypotheses using dimorphic females of the bog fritillary butterfly. Differences were detected between morphs in survival, fecundity, harassment by males, predation pressure and thermal properties, thereby rejecting the null hypothesis.

3. The lifestyle of both morphs is associated with different costs and benefits, with advantages in daily survival and precocious emergence for the gynomorph females, and advantages in fecundity, predation and male harassment for the andromorph females. Besides, as the bog fritillary butterfly is protandrous (i.e. males emerge before females), the longer development of andromorph females puts them at risk of emerging when all the males are dead. The results raise the question as to which mechanisms control the ontogenetic pathways driving the production of the two morphs (i.e. genetic polymorphism or phenotypic plasticity).

**Key words.** *Boloria eunomia*, colour polymorphism, density-dependent predation, learned mate recognition hypothesis, male mimicry hypothesis, sex-limited polymorphism.

## Introduction

Polymorphism is defined as 'the presence of two or more distinct, genetically determined morphs within a single interbreeding population, the rarest of which is too frequent to be solely the result of recurrent mutation' (Ford, 1945; Gray & McKinnon, 2007). Morphs coexisting in a population often differ in their suite of traits (e.g. morphology, physiology or behaviour), each suite of traits conferring optimal adaptation to particular environmental conditions. The origin of polymorphism may thus rely on disruptive selection in spatially and/or temporally variable environments (Gray & McKinnon, 2007). The maintenance of polymorphism often relies on frequency-dependent selection: the fitness of individuals pertaining to a given morph is conversely dependent on the frequency of the morph in the population. It can also rely on habitat heterogeneity. Accordingly, a snapshot measure of morph frequency in the population (e.g. during a particular year or generation) should indicate significant fitness differences between individuals of

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each morph, either in space or in time. Differential survival of morphs according to local conditions will lead to balanced polymorphism in space. As an example, predation rates on the different colour morphs of the snail *Cepaea nemoralis* by the song thrush (*Turdus philomelos*) depend on the background on which the snails are living (Cain & Sheppard, 1954). Sexual selection with advantage to the rarer morph will lead to cycles of morph replacement within the population (Sinervo & Lively, 1996) and balanced polymorphism in time.

In many species, colour polymorphism, a conspicuous easily observable polymorphism, is expressed in one sex only. One female morph is generally coloured like the conspecific male (i.e. andromorph or androchrome female) while the other is dissimilar (i.e. gynomorph or gynochrome female). Such female-limited colour polymorphism has been extensively documented in damselflies and dragonflies [e.g., among others, *Ischnura graellsii* in Cordero (1992), *Enallagma hageni* and *Enallagma boreale* in Fincke (1994), *Ischnura elegans* in Cordero & Sanchez-Guillen (2007), and *Ischnura senegalensis* in Takahashi & Watanabe (2010)]. In comparison, we found few documented examples of female-limited colour polymorphism in butterflies, namely in some species of the *Colias* group [where it is present in 35 out of the 57 known species according to Gilchrist & Rutowski (1986), e.g. *Colias philodice* in Gerould (1911) and *Colias eurytheme* in Watt (1973)] and in three *Papilio* species [*Papilio memnon* in Gerould (1911), *Papilio glaucus* in Aardema & Scriber (2013) and *Papilio dardanus* in Cook *et al.* (1994)]. Mechanisms driving this colour polymorphism in the *Colias* genus have long been investigated and have revealed a genetic origin, with a dominant allele coding for the white coloration of females, never expressed in males and transmitted in a Mendelian way (Remington, 1954).

Several non-exclusive hypotheses have been invoked to explain the existence and maintenance of female-limited polymorphism in insects. First, a null hypothesis has been proposed by Fincke (1994) according to which the frequency of the morphs varies among populations as a result of founder effects and random genetic drift. Under this hypothesis, genetic drift may lead to the fixation of some variants in populations, or else to the maintenance of a certain level of polymorphism under a mutation or drift equilibrium. This means that variability exists across time; in other words, there is a phenotype turnover due to the appearance of variability through mutations and the loss of variability through genetic drift. However, in a given time window, the null hypothesis can result in the coexistence of different unselected phenotypes. Second, the learned mate recognition (LMR) hypothesis suggests that, assuming males have a biased recognition towards the most frequent female morph, this morph should receive a higher number of mating attempts (Fincke, 2004). This was observed in *Colias* species, where the less frequent morph is less often courted by males (5 : 1 ratio), leading to lower (re-)mating in those females (Ley & Watt, 1989). Although differentially courted by males, both female morphs of the *C. eurytheme* received spermatophores (see Kemp & Macedonia, 2007). The male mimicry (MM) hypothesis considers that gynomorph females will be more sexually harassed than andromorph females, whatever their frequency. Excessively recognised and harassed females can have a reduced fitness due to the loss of time for other activities [feeding, search for suitable egg-laying sites and oviposition (Gilchrist & Rutowski, 1986; Gosden & Svensson, 2007)]. Consequently, andromorph females may be advantaged at high population density (through male harassment avoidance) but disadvantaged at low population density (through a risk of dying unmated) (Cordero, 1992). The LMR and MM hypotheses have been validated in many damselfly species (e.g. Fincke, 1994; Sherratt, 2001; Gosden & Svensson, 2009; but see also Andrés *et al.,* 2002). Thirdly, density-dependent predation might be another mechanism at work (Van Gossum *et al.,* 2007). Female morphs may differ in selection by predators, while the search image for the most common prey could be density-dependent [i.e. 'apostatic selection' (Allen & Greenwood, 1988; Gray & McKinnon, 2007)]. This has been studied in detail with a virtual ecology approach (Bond & Kamil, 2002), demonstrating perceptual switching in predators and morph cycles in the prey. However, empirical evidence supporting the idea that apostatic selection can maintain polymorphism, especially in non-cryptic organisms, remains scarce (Ajuria Ibarra & Reader, 2013). Finally, polymorphism may have a frequency-independent adaptive function. Different wing colours have different absorption properties: darker individuals will increase their body temperature faster than lighter ones (i.e. thermal property hypothesis). As ectotherm behaviour is dependent on optimal body temperature, faster heating under cooler environmental conditions or slower cooling under warmer environmental conditions may confer fitness benefits to one or other morph by extending their daily activity period (Watt, 1968).

In the bog fritillary butterfly *Boloria eunomia* (ESPER 1799), we found that female-limited polymorphism exists in several populations that are part of a long-term metapopulation study in Belgium (e.g. Schtickzelle & Baguette, 2004; Baguette *et al.*, 2011). The underside of the wings of males is yellow, whereas the underside of the wings of females can be either white or yellow, as in males (Fig. 1), in a strictly bimodal distribution. Hereafter, white and yellow females will therefore be called gynomorph and andromorph females, respectively. To investigate the relative advantage/disadvantage of each female colour morph, we performed a series of field observations and experiments to test the four hypotheses listed earlier. First, to obtain insights into the morph distribution and dynamics, we assessed the spatial and temporal variation in polymorphism frequency. Second, we tested the first null hypothesis (i.e. equality of fitness traits) by comparing the expected fitness (survival and fecundity) of females of each morph. Under this null hypothesis, we should observe no difference in these traits between the female morphs. Third, as the gynomorph females occur more frequently than the andromorph females within *B. eunomia* populations, we cannot discriminate between the LMR and MM hypotheses with field experiments (Sherratt, 2001). However, both hypotheses should lead to the same prediction: we should detect that *B. eunomia* male harassment is more often targeted towards gynomorph females. In this case, we can conclude that the polymorphism in *B. eunomia* is linked to sexual conflict and potentially density-dependent selection. Accordingly, we assessed in the field whether andromorph and gynomorph females suffered from the same male harassment intensity. Fourth, the white gynomorph females of *B. eunomia* may be more conspicuous to predators, especially birds. Under this hypothesis we expect a lower predation rate on the less frequent, andromorph females, which means that here again the polymorphism in *B. eunomia* would be linked to density-dependent selection. Consequently, we investigated whether resting females of both morphs experienced the same predation rate. Finally, as andromorph females are darker than gynomorph females, we should observe a faster heating rate in the former. We thus measured the heating rate of females from both morphs under controlled conditions.

## Material and methods

### *Biology of the species*

The bog fritillary *B. eunomia* is a specialist species inhabiting wet meadows and peat bogs. Caterpillars and adults use a single food source: the bistort *Persicaria bistorta*. Females are capital breeders (i.e. they emerge from the chrysalid with almost fully developed eggs) and lay clusters of 1–30 eggs (mean = 7, $n = 19$). In the study area (‘Plateau des Tailles’, southern Belgium), *B. eunomia* flies in May and June and hibernates as a caterpillar. *B. eunomia* is a protandrous species in this area: males emerge before the females. At the end of the season, only females can be found and, in our intensive mark–release–recapture (MRR) sessions performed every year since 1992, we observed lags of female peaks (compared with male peaks) of up to 21 days. All the populations in this area are connected by regular gene flow and can thus be considered a metapopulation (Vandewoestijne & Baguette, 2004).

### *Spatial and temporal variations in polymorphism frequency*

In May to June 2011, the spatial and temporal variations in female dimorphism frequency were first investigated in detail in the ‘Prés de la Lienne’ (PL) patchy population (50°18′N 5°47′E) with an MRR study. In this population, the two morphs have co-occurred for at least 14 years, as revealed by specimens in collections. During the flight period of the species in summer 2011, the study site was visited 14 times under suitable weather conditions (no wind, no cloud, air temperature >15 °C). Each individual encountered was caught, marked and immediately released. For each (re-)capture, we recorded the date, location (i.e. one of the several habitat patches), mark, sex and female morph. We extracted the temporal pattern of each female morph appearance and its variation among habitat patches. Moreover, we tested if the proportion of the two female morphs recorded in each patch was similar using a one-way $\chi^2$ test. Finally, we investigated whether there was a temporal relationship between the proportion of andromorph females and the proportion of males across all the patches using a linear regression model.

Furthermore, we compared the relative female morph frequency over a larger spatial area, by recording a snapshot measure at the end of the flying period in four additional populations: the ‘Grande Fange’ (50°14′N 5°46′E), the ‘Bièvres’ (50°14′N 5°50′E), the ‘Bihain’ upstream (50°14′N 5°48′E) and downstream (50°14′N 5°48′E).

### *Survival and catchability of each morph*

Survival of the two female morphs was inferred from the MRR data described previously in the PL population. From these data, we compared the catchability and survival of female morphs using Cormack–Jolly–Seber models implemented in the MARK software (White & Burnham, 1999). The procedure for estimating these demographic parameters in the same way in the same species is described in detail in Schtickzelle *et al.* (2002), including the review of the assumptions of this type of analysis.

### *Morphology and potential fecundity*

First, during the flight period of *B. eunomia*, we recorded the morph and the wing size (with calipers) on 108 live females in the PL population (26 andromorph and 82 gynomorph females). The difference in wing size according to female morph was tested using a two-sample *t*-test.

Second, abdomen and thorax weights have been shown to be good proxies for potential fecundity and mobility in butterflies, respectively (Honek, 1993; Berger *et al.,* 2008; Turlure *et al.,* 2010). Five females of each morph were collected alive in the PL population, just after emergence (i.e. with bright and undamaged wings) on the same day, to prevent potential differences due to female age. Their thoraxes and abdomens were dried and weighed after assessing their thermal properties (see later). The area of one forewing per female was estimated from pictures analysed using the IMAGEJ software (Schneider *et al.,* 2012). We compared the thorax and abdomen dry weights and the area of the forewing between the two morphs using two-sample *t*-tests. We also tested for abdomen weight – forewing area and thorax weight – forewing area allometric relationships using linear models, with extra morph effect and morph × weight interaction.

### *Male harassment avoidance*

In June 2011, we followed 15 males for around 10 min each in three different sites (i.e. 5 males per site) hosting one large *B. eunomia* population each: the ‘Pisserotte’ (50°13′N 5°47′E), the ‘Grande Fange’ and the ‘Bièvres’ nature reserves. For each male followed, we recorded how many females of each morph were visited. We also recorded the number of females of each morph encountered within 2 m of the males’ path as a measure of relative morph availability at that time in that population. All observations were performed under weather conditions that were suitable for male activity. A generalised mixed model (with a binomial distribution, logit link function and Laplace likelihood approximation) was used to assess the effect of female morph, population and their interaction on the probability of a female being visited by a male. Male identity was added as a random variable.

### *Experimental assessment of predation*

Predation pressure on the two female morphs was investigated using fake butterflies (printed female pictures)

**Fig. 1.** Pictures of *Boloria eunomia* females. (a) Gynomorph female; (b) andromorph female; and (c) a pair of fake females stapled on to *Deschampsia cespitosa* stems, close to the host plant (white gynomorph female on the left, yellow andromorph female on the right).

in a semi-controlled experiment. Sixty fake butterflies were stapled directly on to host plant leaves or stems in pairs (1 gynomorph and 1 andromorph; Fig. 1) in two different habitat configurations (15 locations close to bushes and 15 in the centre of habitat patches) in the PL site. After 1 day, we recorded the presence of bird predation marks on each fake female. This experiment was repeated six times on different days. First, a logistic regression model was used to analyse the probability of being preyed upon relative to female morph, habitat configuration and their interaction. Second, we compared the proportions of attacked fake butterflies between the two morphs using Fisher's exact tests, for each habitat configuration separately.

### *Thermal properties of each morph*

To estimate the thermal properties of each female morph, we collected five live females of each morph after emergence (i.e. with bright and undamaged wings) in the PL population. In a first step, we placed females in a fridge for 15 min so that they reached an identical low body temperature. Then, they were removed from the fridge and their body temperature recorded every minute for 5 min using an infrared thermometer (resolution = 0.1 °C, precision = 0.2 °C). To control for the potential confounding effect of individual size on heating speed, we paired each gynomorph female with the andromorph female having the most similar wing size. We then calculated the difference in body temperature between the two females at each time step, and checked for a linear and quadratic change with time of this difference, which would indicate a difference in the heating speed between morphs.

## Results

### *Spatial and temporal variations in polymorphism frequency*

In the PL population, andromorph females were less numerous than gynomorph females (30 vs. 102). Their relative frequencies were not significantly different among habitat patches (two-way $\chi^2$ test, $\chi^2_6 = 7.39$, $P = 0.29$). Andromorph females appeared 11 days after gynomorph ones and their maximum relative frequency was observed on day 24 (30% of females were andromorphs; Fig. 2). This temporal change was significantly and negatively related to the proportion of males (linear regression model, slope = −0.372, $t_9 = -2.68$, $P = 0.027$). Overall, the same temporal pattern was observed in all the patches.

The frequency of andromorph females recorded in four other populations in the same landscape varied between 18% and 57%. Andromorph females appeared between day 29 and 34 and their maximum relative frequency was observed on day 31 and 32.

### *Catchability, survival and fecundity of each morph*

Andromorph females had a significantly lower catchability and a lower daily survival than gynomorph females over

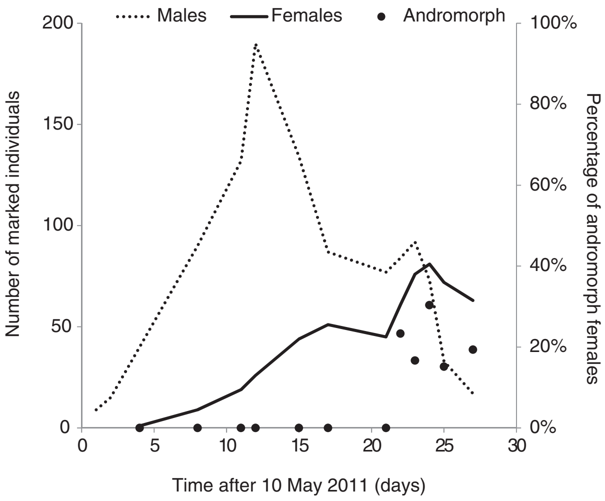

**Fig. 2.** Temporal change in the number of marked individuals and proportion of andromorph females for the 'Prés de la Lienne' population. *Y*-axes: (left) total number of marked males (dotted line) and females (black line) in the population; (right) proportion of andromorph females (dots). On the *x*-axis: zero corresponds to the day at first emergence (i.e. 10 May 2011).

the flight season (catchability, 0.11 ± 0.032 vs. 0.29 ± 0.023; survival, 0.72 ± 0.070 vs. 0.98 ± 0.007); this was also the case when averaged over the period where both were present together to control for the impact of environmental conditions on survival (catchability, 0.13 ± 0.042 vs. 0.32 ± 0.025; survival, 0.62 ± 0.084 vs. 0.95 ± 0.014). The abdomen weight of 10 females used for the thermoregulation experiment (five andromorph and five gynomorph females) was significantly heavier for the andromorph females than for the gynomorph females (two-sample *t*-test, $t_{4.32} = -3.73$; $P = 0.0175$; 16.79 ± 1.78 mg for andromorph females vs. 10.02 ± 0.36 mg for gynomorph females). The thorax weight was not significantly different between the two morphs (two-sample *t*-tests: thorax weight, $t_8 = -1.45$; $P = 0.19$). However, we detected a strong difference between morphs in the allometric relations between forewing area and both the thorax weight and the abdomen weight (Table 1). In comparison to gynomorph females, abdomen and thorax weights were much higher and increased faster with forewing area in andromorph females.

### *Male harassment avoidance*

Of the 162 females encountered on the path of these 15 males (79 andromorph and 83 gynomorph females), only 55 were visited by a male (17 andromorph vs. 38 gynomorph females). The proportion of andromorph and gynomorph females encountered by males did not change significantly between populations (48% of andromorph females in the 'Pisserotte', 44% in the 'Grande Fange' and 53% in the 'Bièvres'; two-way $X^2 = 0.732$, dF = 2, $P = 0.69$). However, the probability of being visited by a male was higher for gynomorph females but did not differ between populations (Table 1c).

### *Experimental assessment of predation*

We recorded the presence of predation marks on only 33 out of 288 fake females (21 gynomorph vs. 12 andromorph females). Thirty-six pairs of fake butterflies were previously discarded from the analysis because at least one butterfly in the pair was destroyed by slugs. The probability of being predated was marginally related to the female morph and not related to the habitat configuration or their interaction (Table 1). Close to the bushes, the proportion of fake butterflies that were attacked was significantly higher for gynomorph females than for andromorph females (Fisher's exact one-tailed test, $P = 0.031$), which was not the case in open areas ($P = 0.50$). In addition, andromorph females had a lower catchability (0.13 vs. 0.32) than gynomorph females when averaged over the period where both were present together.

### *Thermal properties of each morph*

Andromorph females heated up faster than gynomorph females (Fig. 3a); the andromorph–gynomorph difference in body temperature was only positive and increased with time (linear time effect, $t_1 = 2.12$, $P = 0.046$; Fig. 3b). As the body temperature seemed to reach a threshold between 4 and 5 min, at least for andromorph females, the andromorph–gynomorph difference in body temperature decreased at the end of the experiment (quadratic time effect, $t_1 = -2.52$, $P = 0.019$; Fig. 3b).

## Discussion

In *B. eunomia* populations, andromorph females occur less frequently than gynomorph ones, and appear around 10 days later in the flight season. Given that the two morphs coexist (no spatial segregation of the morphs and no balanced polymorphism in space) with a higher potential fecundity for andromorph females and a higher daily survival for gynomorph females, we hypothesise that this non-negligible delay (the flight season lasts, on average, 28 days) has a crucial role in the maintenance of the observed female-limited dimorphism in *B. eunomia*. As we investigated for and detected differences in survival, fecundity, male harassment avoidance, predation pressure and thermal properties between the two morphs, we thus clearly reject the null hypothesis that both morphs had a similar fitness. Interestingly, our results match the predictions of all other hypotheses: female-limited dimorphism in *B. eunomia* was related to sexual conflict, density-dependent processes and fitness trade-offs. Hereafter, we discuss the general framework possibly explaining the existence and maintenance of female-limited polymorphism in this species, and conclude that there are unexplored but insightful issues.

In *B. eunomia*, andromorph and gynomorph females had similar wing and thorax morphology. However, andromorph females had much heavier abdomens than gynomorph females (+6.77 mg, i.e. 67% of the abdomen dry weight). If the whole difference translates into egg production, andromorph females will have a much higher potential fecundity than gynomorph

**Table 1.** Results of the models are presented for: thorax weight as a function of female morph, forewing area and their interaction (generalized regression model, normal distribution, identity link); abdomen weight as a function of female morph, forewing area and their interaction (generalized regression model, normal distribution, identity link); the probability of being visited by a male as a function of female morph, population and their interaction (generalized mixed model, binomial distribution, logit link); and the probability of being predated as a function of female morph, habitat configuration and their interaction (generalized regression model, binomial distribution, logit link).

| Parameter | d.f. | $\chi^2$ | $P$ | Estimate | Standard error |
|---|---|---|---|---|---|
| *Thorax weight* | | | | | |
| Intercept | | | | −6.69 | 1.79 |
| Female morph | | | | | |
| Gynomorph | 1 | 6.05 | 0.014 | 7.19 | 2.49 |
| Andromorph | | | | 0 | – |
| Forewing area | 1 | 21.81 | <0.0001 | 9.10 | 1.09 |
| Female morph × forewing area | | | | | |
| Gynomorph | 1 | 7.95 | 0.005 | −5.13 | 1.47 |
| Andromorph | | | | 0 | – |
| *Abdomen weight* | | | | | |
| Intercept | | | | −30.71 | 11.38 |
| Female morph | | | | | |
| Gynomorph | 1 | 3.52 | 0.061 | 32.529 | 15.82 |
| Andromorph | | | | 0 | – |
| Forewing area | 1 | 8.33 | 0.004 | 29.01 | 6.94 |
| Female morph × forewing area | | | | | |
| Gynomorph | 1 | 5.17 | 0.023 | −24.33 | 9.35 |
| Andromorph | | | | 0 | – |
| *Probability of being visited by a male* | | | | | |
| Intercept | | | | −1.22 | 0.30 |
| Female morph | | | | | |
| Gynomorph | 1 | 5.30 | 0.023 | 0.86 | 0.36 |
| Andromorph | | | | 0 | – |
| Population | 2 | 0.71 | 0.493 | – | – |
| Female morph × population | 2 | 0.12 | 0.887 | – | – |
| *Probability of being predated* | | | | | |
| Intercept | | | | −2.40 | 0.30 |
| Female morph | | | | | |
| Gynomorph | 1 | 3.04 | 0.081 | 0.63 | 0.38 |
| Andromorph | | | | 0 | – |
| Habitat configuration | 1 | 0.59 | 0.442 | – | – |
| Female morph × habitat configuration | 1 | 1.93 | 0.165 | – | – |

Allometric relationships between forewing area and both thorax and abdomen weight differed between female morphs; in comparison to gynomorph females, abdomen and thorax weights were much higher and increased faster with forewing area in andromorph females. The probability of being visited by a male differed according to female morph only, gynomorph females being more likely to be visited than andromorph females. The probability of being predated differed slightly according to female morph, gynomorph females being more predated than andromorph females. Statistical analyses were performed with SAS software.

ones (e.g a fresh egg in the related butterfly *Euphydryas editha* weighs *c.* 0.25 mg; Murphy *et al.,* 1983). In insects, female fecundity indeed scales positively with abdomen mass (e.g. Honek, 1993; Berger *et al.,* 2008). As there is a time lag of several days after emergence before the first eggs are laid and as the collected females had bright and undamaged wings (wings fade after a few hours of activity), we can reasonably assume that they were less than 24 h old (Kemp, 2006) and that none had laid its first egg mass.

In addition, female-realised fecundity is usually reduced compared with potential fecundity due to restricted laying opportunities. In the damselfly *Ischnura senegalensis*, Takahashi and Watanabe (2010) found that gynomorph females laid 35% fewer eggs than andromorph females. They proposed that harassment may interrupt, and hence limit, feeding activities and food intake needed for egg production in gynomorph females, consequently affecting their reproductive success. Similarly, in *B. eunomia*, we observed that gynomorph females were more courted and harassed by males than were andromorph females, probably reducing their energy budget and in turn the amount of eggs they can lay. Also, several additional factors may affect female fitness, such as offspring competition and survival, and temporal changes in host plant quality, which were not investigated in this study. Therefore, the observed difference in potential fecundity between female morphs might not directly translate into differences in realised fecundity.

Despite the lower frequency of andromorph females, we observed the same number of andromorph and gynomorph females on the paths of tracked males. This suggests that either gynomorph females are less active than andromorph ones, or

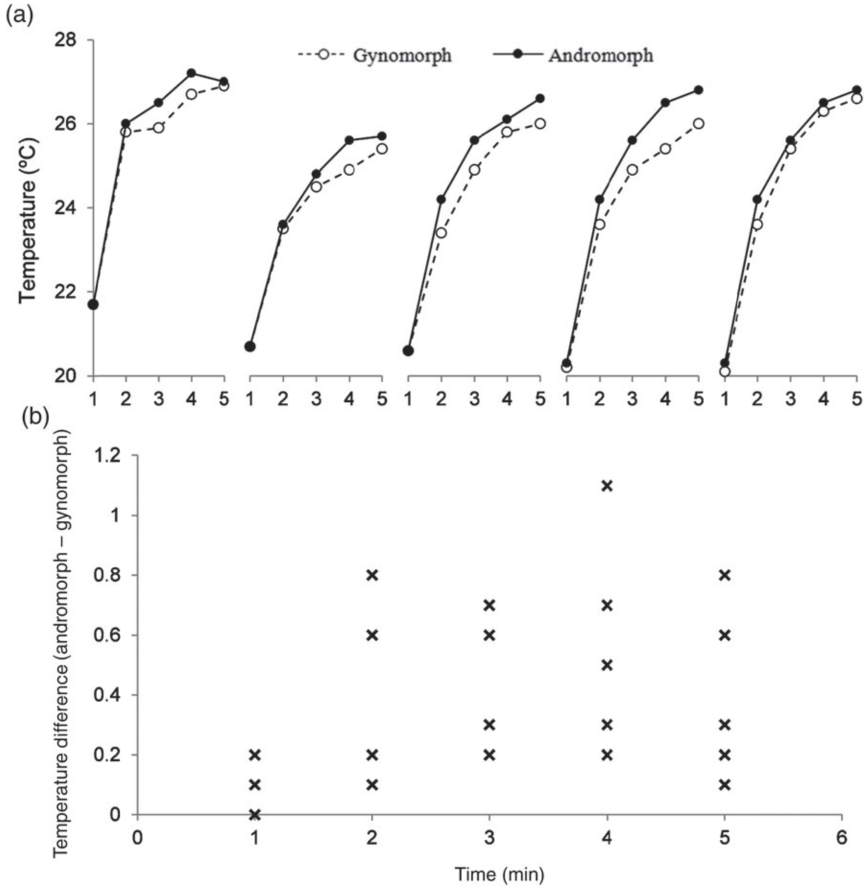

**Fig. 3.** (a) Female body temperature as a function of reheating time after females have been cooled down to a standardized temperature. Each panel is a size-matched pair of females (one gynomorph and one andromorph). (b) Difference in body temperature (andromorph − gynomorph) at each time step. This difference is only positive and changed in a quadratic way with time, indicating that andromorph females heated up more quickly than gynomorph females before 5 min.

that harassment by males leads to a higher emigration of the gynomorph females, as observed for the harassed females in *C. eurytheme* (Gilchrist & Rutowski, 1986). Both could be considered as strategies to avoid being chased by males. As males appear before females in *B. eunomia* (i.e. protandry), and as gynomorph females emerge before andromorph females (Fig. 2), males may encounter more gynomorph females in their lifetime, leading to a bias towards more frequent harassment of gynomorph females (i.e. the LMR hypothesis). Consequently, frequency-dependent male harassment could have been a key factor in producing and maintaining the female dimorphism in *B. eunomia*, by directly limiting the fecundity of gynomorphs, as exemplified in other insect species (Cordero, 1992; Svensson *et al.,* 2005; but see contradictory examples in Cordero & Sanchez-Guillen, 2007; Hammers & Van Gossum, 2008). If andromorph females are suspected to have a higher potential and realised fecundity, their daily survival was, by contrast, lower (0.62 vs 0.95, i.e. −26%). Two non-exclusive mechanisms might be at work to explain this difference.

First, andromorph females might be more fragile due to the costs associated with the production of a more coloured phenotype (Talloen *et al.,* 2004). In the ‘alba’ female morph of *C. eurytheme*, the overall pteridine pigment production is lower than in the yellow female morph of the same species, except for the leucopterin ones (Table 5 in Watt, 1973). This suggests that the supply of nitrogenous pigment precursors has been reduced in white forms (or, conversely, increased in coloured forms) (e.g. Watt, 1973) or diverted to some other metabolic end. Watt suggested that the pupal nitrogen reserves of the white morphs may be reallocated and confer higher survival, reproduction or shorter pupal development time. This is in line with the differences we observed in emergence time and survival. In addition, the allometric relationships indicate that, for a given wing size, andromorph females invest more in both the abdomen and the thorax than do gynomorph females. This may lead to lower somatic maintenance and/or higher flight costs, and hence contribute to a lower survival.

Second, higher predation rates of the andromorph females might reduce their daily survival. However, we observed a slightly higher predation probability on the most frequent female morph (i.e. gynomorph), especially in bushy areas. This may be because females of the brighter white morph are more conspicuous to visual predators. Adding camera traps in further experiments would help to identify predators. MRR data indicated that the catchability of gynomorph females was higher, which means that andromorph females were less conspicuous to human observers, and potentially to other organisms such as predators. This second mechanism thus seems less plausible in explaining the lower survival of andromorph females. However, density-dependent predation may still be at play to explain the maintenance of female polymorphism.

Heating up was faster in andromorph females compared with gynomorph females. Because of the limited number of individuals we were able to test in this experiment, we do not want to go beyond the support given by these data, but note that this result is consistent and in line with results from other researches. First, it has been shown that body colours can result in different body temperatures, darker wings in butterflies absorbing more heat than lighter ones (Watt, 1968; Schmitz, 1994). Differences in thermal properties as a function of colour, size or number of spots were shown in several insect species (Cooper, 2010; Umbers *et al.*, 2013, Table 1); e.g. body temperature of the chameleon grasshopper *Kosciuscola tristis* reached a threshold after 5 min, similar to what we observed. Also, Watt (1968, 1969) exemplified how the change of colour in *Colias* butterflies is linked to thermoregulation, darker individuals having more efficient energy absorption and hence being more adapted to colder climatic conditions. As butterfly activities are governed by body temperature, faster heating under the same environmental conditions may confer fitness benefits to the darker morphs. Thus, *B. eunomia* andromorph females, heating faster, might be active in cooler environment and/or under cooler weather conditions, which may compensate for their lower daily survival.

To sum up, the lifestyle of both morphs is associated with different costs and benefits. Advantages in daily survival and emergence time for the gynomorphs are traded off against a lower fecundity, higher predation risk and higher rate of male harassment (and conversely for the andromorphs). Indeed, andromorph females have heavier abdomens than gynomorph females for a given size, which suggests that they have a higher potential fecundity. They are also less predated and harassed by males than are gynomorph females, which should translate into a higher realised fecundity per day. Nonetheless, gynomorph females have a higher survival and a shorter development time. Our >20-year-long series of yearly population dynamics indicates unambiguously that *B. eunomia* is a protandrous species, with the peak of male abundance in the population occurring 2–14 days before the peak of females (Schtickzelle *et al.*, 2002 and N. Schtickzelle, unpublished). Male competition for access to virgin females (females mate only once in *B. eunomia*; Baguette & Nève, 1994) is usually considered the proximal factor driving the evolution of protandry (e.g. Wiklund & Fagerström, 1977). Given their longer development (around 10 days, for a flight season lasting 28 days on average), andromorph females are at risk of emerging when all males are dead and hence being ‘lost in time, lonely and single’ (Calabrese & Fagan, 2004). Careful observations and modelling indicate that monomorphic females in protandrous butterfly species have a high probability (0.06–0.19) of dying unmated (Calabrese *et al.,* 2008). The comparison of this proportion between the two morphs of *B. eunomia* will provide insights into how the risk of dying unmated influences the frequency of andromorph females in the population.

Moreover, the presence of two colour morphs in *B. eunomia* opens up new perspectives in the study of interactions between species in our study system, as it may reduce interference competition (Watt *et al.,* 1989; Nielsen & Watt, 2000). Also, sampling more sites and pursuing long-term population monitoring would allow one to test whether some habitat properties (e.g. thermal properties) or population characteristics (e.g. phenology) are likely to create balanced polymorphism in space or time. Finally, we think that the mechanisms driving the production of the two morphs (i.e. genetic polymorphism or phenotypic plasticity) should be thoroughly investigated for a better understanding of the interactions between polymorphism and adaptive value of each form. It is known in *Colias* butterflies or in damselfly species where a single allelic autosomal locus is involved, with a sex-linked expression (Gerould, 1911; Johnson, 1964; Watt, 1973; Cordero, 1990). This is probably the case in *B. eunomia*, too, although we cannot currently exclude the role of phenotypic plasticity. At this stage, we can only affirm that there is no ontogenetic pathway through adult life in the wing coloration in *B. eunomia*, as indicated by the total absence of change in wing colour of females recaptured several times in our MRR samples.

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CT, DL and MB designed the study. CT, DL and MB performed data collection. CT and NS performed data analyses. CT and MB wrote the manuscript, and all authors contributed to revisions. All authors read and approved the final version of the paper.

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