

Trade-off mediated by pyrrolizidine alkaloids predicts alternative reproductive tactics in ithomiine butterflies

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Abstract. 1. Trade-offs between male mating success and survival are the basis of alternative male reproductive tactics.

2. Adult male *Greta morgane* butterflies acquire protective pyrrolizidine alkaloids (PAs) from plants and use PA-derived pheromones to attract females. Adult females acquire PAs from male spermatophores, thereby obtaining chemical defence against predation while avoiding costs of PA toxicity and sequestration.

3. We found that males that fed longer on PA-containing plants were more successful at mating, but males fed a diet containing PAs had a shorter lifespan than males fed a diet without PAs. Together, these results demonstrate a trade-off between survival and reproduction in relation to male feeding behaviour.

4. As predicted by the alternative reproductive tactics hypothesis, we found that some males fed preferentially on PA-containing plants while others specialised on PA-free plants.

Key words. chemical ecology, Ithomiini, Lepidoptera, longevity, mate choice, plant use, trade-off.

Introduction

Traits that reduce survival may be favoured by selection if they increase male mating success in the short term (Zahavi, 1975; Kodric-Brown & Brown, 1984; Hunt *et al.*, 2004; Peron *et al.*, 2016). Trade-offs between survival and mating success are the basis of alternative male reproductive tactics, which are often frequency or condition dependent (Gross, 1996; Alonzo & Warner, 2000; Taborsky & Brockmann, 2010). Insects in which males provide females with nuptial gifts, such as captured prey items and spermatophores, provide some of the best documented examples of alternative male reproductive tactics (Gwynne, 2008; Taborsky & Brockmann, 2010).

In Lepidoptera, male spermatophores can include defensive chemicals that protect females and their eggs from predation (Gwynne, 2008; Dussourd *et al.*, 2009). Ithomiine butterflies are generally aposematic in coloration and chemically protected by plant-derived pyrrolizidine alkaloids (PAs) (Pliske *et al.*, 1976; Brown Jr., 1984). In the basal ithomiines, PAs are acquired at the larval stage from host plants and retained into adulthood (Trigo *et al.*, 1994; Schulz *et al.*, 2004). By contrast, in the derived ithomiines, females are not protected chemically until mating

(Masters, 1990). Adult males acquire PAs by feeding on the nectar of PA-containing plants, attract females with PA-derived pheromones, and transfer PAs to their mates through the spermatophore (Pliske, 1975; Schulz *et al.*, 2004). The switch from larval- to adult-acquired PAs was probably a pivotal event in the evolution of ithomiine butterflies that facilitated host plant shifts and increased the rate of diversification (Fordyce, 2010). Trigo and Brown Jr. (1990) compared species that acquire PAs at the larval and adult stages and found that in larval-acquiring species, females consistently have higher levels of PAs than males, while in adult-acquiring species, males have a larger range and higher variance in PA levels than females (Trigo & Brown Jr., 1990). Selection on females to choose males with high PA levels is probably stronger in adult- than larval-acquiring species, because females of adult-acquiring species rely on mating for chemical protection from predation.

Ingested PAs are converted into the *n*-oxide form, which is non-toxic to butterflies, but prior to conversion these chemicals are toxic, and their sequestration has biochemical costs (Trigo *et al.*, 1994; Schulz *et al.*, 2004). Thus, the availability of nectar sources with different PA levels could pose a mating success-survival trade-off for males—precisely the kind of scenario in which alternative male reproductive tactics would be expected to evolve (Gross, 1996; Alonzo & Warner, 2000; Taborsky & Brockmann, 2010).

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Here, we investigate the influence of feeding on the nectar of PA-containing plants on male mating success, and that PA ingestion reduces male survival, in the adult-acquiring species *Greta morgane*. We provide evidence that this trade-off between mating success and survival is associated with alternative reproductive tactics: some males pursue a high mating rate-low survival tactic by feeding preferentially on the nectar of PA-rich plants, while others exhibit a low mating rate-high survival tactic by specialising on the nectar of PA-free plants.

Methods

Study species

Ithomiini is a tribe of 350 species of Neotropical butterflies that includes members of several wing-coloration mimicry rings (Beccaloni, 1997; Jiggins *et al.*, 2006). *Greta morgane* (Lepidoptera: Nymphalidae; Geyer [1833]) is a medium-sized (forewing length, 33–38 mm), clear-winged butterfly that ranges from Mexico to Panama and uses the host plants *Cestrum lanthanum* and *C. standleyi* (DeVries, 1987).

Rearing

Greta morgane is often raised commercially in Costa Rica for butterfly houses. The butterflies used in this study were raised in the El Castillo Butterfly Conservatory at ambient temperatures in net cages within a rearing lab room. Ambient temperatures during the time of this study were between 18 and 26 °C. As pupae emerged, they were transferred to large net houses for testing. The plant preference and mating portions of this study were conducted daily, over several weeks, in one large enclosure at the Butterfly Conservatory in El Castillo, Costa Rica in December and January 2011. The enclosures were approximately 10 m × 10 m and 5 m in height, and made of black mesh.

Plant use experiment

Upon emergence, each butterfly received an individual number on its wing, and sex and date of emergence were recorded. Only butterflies that emerged within the same 3-day period were used. To test male use of alkaloid plants, four vases of flowers were set up in the enclosure at a distance of at least two metres from one another. Two vases contained PA-containing *Ageratum conyzoides* and the other two contained PA-free *Tithonia diversifolia*. Both *Ageratum conyzoides* and *Tithonia diversifolia* are found growing naturally in abundance in the area and ithomiines feed on both plants in the wild (A.G.-K. Pers. Obs). *Ageratum conyzoides* contains the pyrrolizidine alkaloids lycopsamine and echinatine (Wiedenfeld & Röder, 1991). Each vase was watched for 10-min periods and the duration of nectar feeding by each butterfly was recorded to the nearest second. Vases were rotated on a daily basis to eliminate preference due to light or other differences between vase locations. On an hourly basis, the enclosure was searched for mating pairs. To evaluate

whether any matings are likely to have been missed, a sample of matings was timed ($n = 22$). To test for size differences between mating and non-mating individuals, the body length of butterflies of both sexes was measured to the nearest 0.1 mm using callipers.

Preliminary data visualisation of plant use by males indicated that individuals specialised on either *Ageratum conyzoides* (PA-containing) or *Tithonia diversifolia*, based on both time and frequency of plant use. To determine whether specialisation occurred more often than expected by chance, data on male plant use were randomised and redistributed across plant types and a chi-squared test was applied to the redistributed data 10 000 times. Observed chi-square values were then compared with the distribution of chi-square values generated by randomisation. Comparisons between plant use of males that mated and males that did not mate were performed using a Wilcoxon rank sum test to account for unequal variances. Statistical analyses were done in STATA.

PA feeding experiment

To test the impact of PAs on longevity, freshly eclosed male *Greta morgane* were individually syringe-fed artificial nectar containing either 0 or 3 µg/ml of the PA heliotrine (Laotaxan, France). Heliotrine was chosen both on the basis of the use of ithomiine butterflies of *Heliotropium* in nature and its use in previous studies (Pliske *et al.*, 1976; Masters, 1990). The level of 3 µg/ml was chosen because it is within the range naturally found in nectar of PA-containing plants (Smith & Culvenor, 1981). Both PA and PA-free artificial nectar contained 30% sucrose as well as five drops of Kikkoman soy sauce per litre for salt and amino acid content, and all butterflies were syringe-fed 10 ml per day. A previous study found 20% storage over the long term, and 80% in the short term, of PAs in males, which equates to an average of 70 µg body load in the experimental group, which is within the range found in nature (Trigo *et al.*, 1996b; Brückmann *et al.*, 2000). Butterflies were kept in small mesh enclosures (15 × 15 × 30 cm). Butterflies were kept in groups of three males per cage and individuals were labelled with individual numeric labels and monitored daily. Non-parametric Cox proportional hazards survival analysis was used to test for effects of the PA diet treatment on survival.

Results

The timed matings took 3–4 h (mean = 3.44, SD = 1.13, $n = 22$), and thus it is reasonable to assume that all matings that occurred during the experiment were recorded in the hourly checks. Males that mated during the experiment spent more time feeding on the PA-containing plant *Ageratum conyzoides*, compared with males that did not mate during the experiment (Wilcoxon, mated = 8, unmated = 34, $z = -3.08$, $P = 0.002$, Fig. 1). Mated and unmated males did not differ in time spent feeding on the non-PA-containing plant *Tithonia diversifolia* (Wilcoxon, mated = 8, unmated = 34, $z = -1.19$, $P = 0.23$).

Overall, males fed longer on *Ageratum conyzoides* than *Tithonia diversifolia* (Wilcoxon test, $n = 57$, $z = -4.23$, $P = 0.04$),

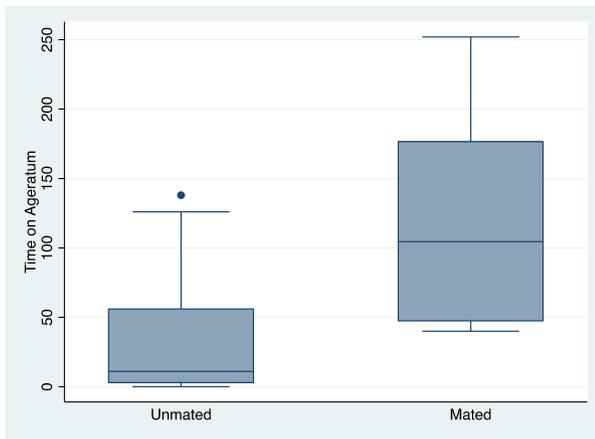


Fig. 1. Compared with unmated males, mated males spent more time feeding on the PA-containing plant *Ageratum conyzoides*. [Colour figure can be viewed at wileyonlinelibrary.com].

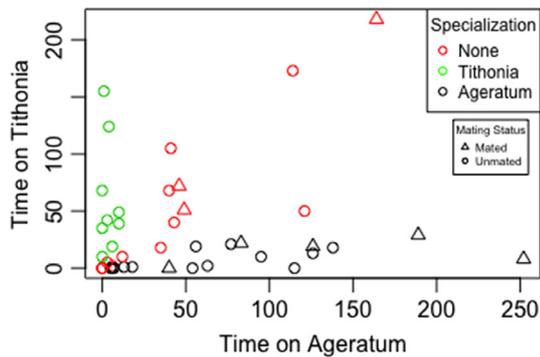


Fig. 2. Males varied in the degree to which they used one nectar source or the other. Specialisation on the PA-containing plant *Ageratum conyzoides* was not a requirement for mating, but no males specialising on the non-PA containing plant *Tithonia diversifolia* mated during the study. [Colour figure can be viewed at wileyonlinelibrary.com].

whereas females fed longer on *Tithonia diversifolia* than *Ageratum conyzoides* ($n = 42$, $z = 2.01$, $P < 0.00001$). Males that mated successfully did not differ in body length from males that failed to mate (Wilcoxon, mated = 8, unmated = 34, $z = 0.40$, $P = 0.69$).

Males used one plant source more than the other significantly more often than would be predicted by a chance distribution of plant use (Fig. 2, Chi-squared = 10.89, $P < 0.001$). We defined males as specialised if they used one plant species at least three times more than the other. An equal number of males specialised on *Ageratum conyzoides* and *Tithonia diversifolia* (Fig. 2; Chi-squared = 0.2727, $P = 0.602$). Males that mated did not always use one plant species significantly more than the other but generally spent more time on *Ageratum conyzoides* than did non-mating males (Fig. 2). When males that mated specialised on one species of plant, it was *Ageratum conyzoides*, the PA-containing plant. None of the males specialising on *Tithonia diversifolia* mated during the experiment. However, some males did not specialise (Fig. 2). Males that specialised

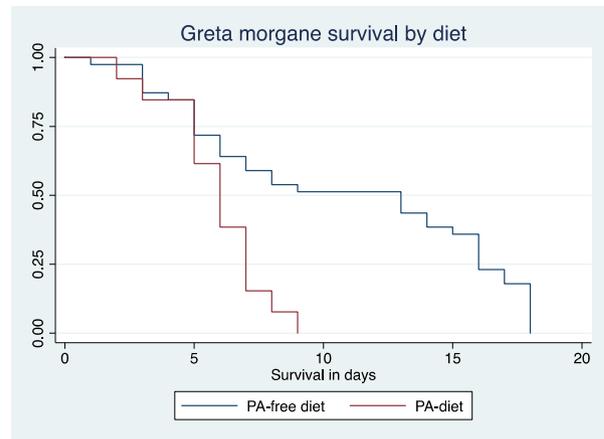


Fig. 3. Male *Greta morgane* survived longer on a PA-free diet. [Colour figure can be viewed at wileyonlinelibrary.com].

on *Tithonia diversifolia* were less likely to mate than males that did not specialise (Fig. 2, Chi-squared = 6.0676, $P = 0.048$).

Male *Greta morgane* fed a diet containing PAs from the time of eclosion had shorter lifespans than males on the PA-free diet (Fig. 3, Cox proportional hazard, Chi = 7.22, d.f. = 1, $P = 0.007$).

Discussion

Male ithomiine butterflies likely trade longevity for sex appeal. Male *Greta morgane* that spend more time feeding on pyrrolizidine alkaloid-containing plants are more likely to mate. However, the alkaloids, which are used as precursors for pheromones and as part of the nuptial gift, also reduce male lifespans. These experiments indicate a potential trade-off and multiple male strategies for resource acquisition and prioritisation. As perspectives on costs to males of mating and reproduction are changing, physiological and other costs of nuptial gifts is a category of costs of mating that is largely born by males (Wedell & Karlsson, 2003; Torres-Vila & Jennions, 2004; Ferkau & Fischer, 2006). In the moth *U. ornatix*, a PA-using species of arctiid moth, higher levels of alkaloids carry a physiological cost for both sexes (Conner *et al.*, 2000). In other species of butterflies, nuptial gifts have been shown to be costly for the males that produce them (Karlsson, 1998; Ferkau & Fischer, 2006; Duploux *et al.*, 2017). In ithomiine butterflies that acquire PAs as adults, males alone suffer the cost of acquisition and conversion of PAs for courtship and nuptial gift of chemical defence to females.

Although we did not find evidence of condition dependence of investment in sequestration, male behaviour may ultimately be condition dependent in a way not measured by this study and may relate to larval growth rate or parasite load. Although we did not find any difference between mating and non-mating males in other qualities, it is possible that PA quantity represents some other measure of quality not measured here and that alkaloid sequestration opposed to patterns are condition dependent. Strategies for prioritising PA acquisition over nectar-only

resources may be more viable at different times, for example, seasonally, in relation to climate factors or predation risk. In fact, the levels of alkaloids in ithomiines fluctuate seasonally, although this is likely related to seasonality in availability of PA-containing plants locally (Trigo *et al.*, 1996a).

Many elements of the costs and risks of mating, such as predation, were not addressed here as these experiments were by necessity conducted in enclosures. Previous experiments indicate that butterflies released into open environments without chemical defences do not survive for long (Schneider *et al.*, 1975). Feeding studies with ithomiines indicate that mated females are unpalatable while unmated females are palatable, and freshly emerged males are palatable until they have fed on a PA source (Masters, 1990). Because male lifespans are shown to be reduced by PAs, it seems likely that the resource acquisition by males and nuptial gift in ithomiines increases female lifespans (Pliske *et al.*, 1976; Conner *et al.*, 2000). Additionally, in related species some defensive compounds are excreted during oviposition and so eggs and freshly emerged larvae may also be protected (Dussourd *et al.*, 2009). Both sexes would benefit from the increased longevity of the females and the survival of offspring.

It is possible that while this experiment demonstrates the physiological costs of alkaloid sequestration, lifespan in nature would be curtailed by predation if alkaloid levels were too low. However, part of the defensive function of ithomiine aggregations may be to protect recently emerged individuals, or individuals with low levels of PAs and these aggregations may enable multiple male strategies. The possibility for strategies including lower levels of defensive compounds is especially interesting in the context of ithomiine natural history. Ithomiines are unusual among butterflies in having multi-species aggregations. These aggregations likely serve as a protected space (Haber, 1978). These aggregation spaces likely buffer both palatable females and low-alkaloid males against predation. Further research is warranted into the ecology and behaviour of ithomiines to understand the implications in nature of this trade-off between mating success and lifespan.

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The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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