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Effects of sodium puddling on male mating success, courtship and flight in a swallowtail butterfly

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In many Lepidoptera species usually only males puddle for sodium. Two explanations have been offered for this: (1) neuromuscular activity: males need increased sodium for flight because they are more active flyers than females; and (2) direct benefits: sodium is a type of direct benefit provided by males to females via ejaculate during mating. Surprisingly, there is little direct experimental evidence for either of these. In this study, we examined both explanations using the pipevine swallowtail butterfly, *Battus philenor* L. If sodium increases neuromuscular activity, males consuming sodium should be better fliers than males without sodium. If males collect sodium for nuptial gifts that benefit their mates, males consuming sodium may have greater mating success than males without sodium. In that case, females then need an honest cue/signal of the quality of male-provided direct benefits that they can assess before mating. If sodium affects male courtship flight by increasing neuromuscular activity, how a male courts could serve as such a premating cue/signal of male benefit quality. Therefore, sodium may benefit males in terms of obtaining mates by increasing their neuromuscular activity. In this study we found that males that consumed sodium courted more vigorously and had greater mating success than males that consumed water. In addition, the courtship displays of males consuming sodium were significantly different from those of males consuming water, providing a possible honest cue/signal of male benefit quality that females can assess. Interestingly, we did not find evidence that sodium consumption affects male flight outside of courtship. That only aspects of male flight related to mating were affected by sodium, while aspects of general flight were not, is consistent with the idea that sodium may benefit males in terms of obtaining mates via effects on neuromuscular activity.

Mud-puddling behaviour, seen in many species of Lepidoptera as well as in some other insects (reviewed in Molleman, 2010), refers to individuals feeding on soil, excrement, carrion, etc. to obtain micronutrients such as sodium and nitrogen. In many Lepidoptera species, usually only males puddle (Boggs & Jackson, 1991). Previous studies have found that sodium is most commonly sought during puddling (e.g. Arms, Feeny, & Lederhouse, 1974; Boggs & Dau, 2004; Smedley & Eisner, 1995), although some species seek nitrogenous compounds (e.g. Beck, Muhlenberg, & Fiedler, 1999; Boggs & Dau, 2004). To date, there have been two main explanations for why, in many species of Lepidoptera, usually only males puddle for sodium (reviewed in Molleman, 2010). First, it has been suggested that sodium may be a type of male-provided direct benefit to females, provided to them via male ejaculate during mating. Second, it has been suggested that sodium may be needed more by males because they are the more active flyers, and sodium may promote neuromuscular function (Arms et al., 1974). Both explanations are only weakly supported.

In choosing mates, females should be selected to mate with males that provide them with high fitness benefits (Andersson, 1994). These benefits can take different forms, and can be direct, such as material resources or parental care that increase the fitness of the female or her offspring (e.g. Price, Schluter, & Heckman, 1993; South & Lewis, 2011; Vahed, 1998), or indirect, where males provide their offspring with alleles that increase viability of offspring or increase the attractiveness of male offspring (e.g. Fisher, 1930; Lande, 1981; Zahavi, 1975). For the evolution of adaptive female choice under either scenario, males must vary in benefit quality and females must reliably assess the benefit quality that males can provide prior to mating. For direct benefits to favour

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adaptive female choice, there needs to be a mechanism that keeps males from cheating (i.e. not providing the benefit to a female mate; Wagner, 2011).

Some evidence consistent with the first explanation, hereafter referred to as the direct-benefits explanation, for why males puddle more often than females, has been reported among the Lepidoptera. Nutrients provided in ejaculates by males during mating have been found in eggs (Boggs & Gilbert, 1979): males transfer sodium to females during mating, and eggs of females mated to males that have puddled on sodium solution have higher sodium levels (Smedley & Eisner, 1996). In addition, in the few species where both sexes puddle for sodium, male spermatophores have been shown to contain little sodium (Molleman, Grunsven, Liefting, Zwaan, & Brakefield, 2005). Sodium may be a desired benefit by females for several reasons. First, because sodium is important to the function of the digestive, excretory and neuromuscular systems of insects (reviewed in Molleman, 2010), females may benefit by receiving it during mating. Second, adult butterflies are sodium limited (Arms et al., 1974; Smedley & Eisner, 1996). Third, a previous study found that, when fed sodium, previously mated males showed increased spermatophore size, mass of accessory gland substances and number of sperm relative to virgin males (Niihara & Watanabe, 2009).

There are two conspicuous gaps in our knowledge with regard to the direct-benefits explanation for male puddling behaviour. First, only two studies to our knowledge have examined the effects of male-provided sodium on females or their offspring, and these studies found weak and/or no effects: one study found that, under drought conditions, egg mortality was marginally lower for females mated to males that puddled on sodium solution (Pivnick & McNeil, 1987), and a second study found no effect of sodium on female egg production or fertility (Molleman, Zwaan, & Brakefield, 2004). If sodium puddling is a male-provided direct benefit, then females mated to males that have puddled on sodium solution should be expected to gain fitness benefits. Second, while there is evidence that sodium gained by males during puddling is transferred to females during mating and may end up in eggs (Smedley & Eisner, 1996), and some evidence that sodium uptake improves mating success (Pivnick & McNeil, 1987), there is no evidence to explain how sodium uptake might make males more attractive. In this study, we set about addressing this second gap.

There is also some evidence for the second explanation, hereafter referred to as the neuromuscular activity explanation, for why usually only males puddle for sodium. Comparing 124 species in 41 genera in the family Riodinidae, Hall and Willmott (2000) found that species with relatively higher wing loading (indicative of the amount of weight that an individual can carry per unit wing area) puddled more than species with lower wing loading. However, Molleman et al. (2005) found no correlation between puddling and wing loading in a group of 98 species of African fruit-feeding butterflies from three subfamilies, Charaxinae, Nymphalianae and Satyrinae, from the family Nymphalidae. Lastly, sodium deprivation as larvae decreased adult flight speed in *Helicoverpa armigera* (Xiao, Shen, Zhong, & Li, 2010), and increasing the concentration of sodium in larval diet resulted in increased flight muscle mass of male cabbage white butterflies, *Pieris rapae* (Snell-Rood, Espeset, Boser, White, & Smykalski, 2014). However, there have been no direct tests of the effects of adult sodium consumption on flight.

It is worth noting that the direct-benefits and neuromuscular activity explanations are not mutually exclusive. Males of many Lepidoptera species perform intricate aerial courtship displays (Rutowski, Nahm, & Macedonia, 2010). It is conceivable that flight performance during courtship is improved with sodium consumption by males, and may serve as an honest signal to females of the quality of the direct benefit provided by the male.

In this study, we examined both explanations for why males puddle. Specifically, we addressed the following questions in the pipevine swallowtail, *Battus philenor* L. (1) Do males with access to sodium have greater mating success than males that are not provided sodium? (2) Does sodium affect their courtship flight behaviour? (3) Does sodium affect male flight outside courtship? We predicted that, (a) if males with access to more sodium are providing females with better-quality direct benefits and if females prefer these males, they should have higher mating success than males without access to sodium; (b) if males advertise the quality of the direct benefits they can provide via improved courtship, then sodium-treated and water-treated males should differ in aspects of their courtship flight; and (c) if males puddle for sodium because it improves their flight, as implied by Arms et al. (1974), then sodium-treated males should fly faster, fly longer, and/or be able to generate more lift than water-treated males.

METHODS

Study Species

Our study species was the pipevine swallowtail butterfly, *B. philenor*, a large black papilionid occurring widely through North and Central America. We conducted our experiments in southern Arizona, where this butterfly is common in most years between March and October. In this region the larval host plant is Watson's Dutchman's pipe, *Aristolochia watsonii*.

Individuals of this species have wing spans of 7–13 cm, and the undersides of hindwings are brightly coloured, with orange submarginal spots on iridescent blue. In addition, males have bright iridescent blue or blue-green coloration on the upper surface of the hindwings. Previous research has shown that ablation of this iridescence on males decreases their mating success (Rutowski & Rajyaguru, 2013). Males court females by flying in loops around them; they approach from behind, fly beneath the female, then fly up in front of her, and drop back behind her to restart the aerial manoeuvre (Rutowski, Alcock, & Carey, 1989; C. Mitra, personal observation). This pattern of flight probably acts to advertise the male's iridescent blue dorsal hindwing coloration to females.

Mud puddling for sodium by males is an excellent behaviour to study, especially in *B. philenor*, for four main reasons. First, quantity of sodium is a simple benefit to control, as we can manipulate male access to sodium while controlling for other environmental variables. Therefore, any differences between sodium-treated males and water-treated (control) males must be due to differences in sodium availability. Second, in *B. philenor*, as in many puddling species, sodium is transferred to females via a spermatophore during mating (Mitra, Papaj, & Davidowitz, n.d.); therefore, if females prefer males that have consumed sodium, there must be a cue/signal that females assess before mating that is correlated with sodium consumption status. Third, previous work in this species has shown that there is substantial variation in the size of the spermatophores that male *B. philenor* transfer to females (mean ± SE = 6.5 ± 2.02 mg, $N = 75$, as reported in Rajyaguru, Pegram, Kingston, & Rutowski, 2013), suggesting that direct benefit quality may vary considerably among males. Lastly, most *B. philenor* females only mate once or twice in their lifetime (Burns, 1968; Rutowski et al., 1989), suggesting that if male sodium increases the fitness of his offspring, natural selection on males may decrease the likelihood that deception may evolve in males of this species.

General Methods

Individuals used in experiments were wild *B. philenor* collected in our *Aristolochia fimbriata* garden plots in Tucson, Arizona. We