

Mating systems and sexual division of foraging effort affect puddling behaviour by butterflies

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Abstract. 1. Foraging effort can vary among age classes and between the sexes. In many Lepidoptera, young males feed from mud, dung or carrion in a behaviour known as 'puddling', whereas females rarely puddle. In at least one species, males transfer sodium gained from puddling to females at mating for use in egg production.

2. Here we examine sex- and age-specific puddling patterns in seven montane butterfly species. We also test the hypothesis that among species in which young males predominate at puddles, differences in age- and sex-specific puddling patterns for a given species are related to mean female lifetime mating numbers.

3. For five species, young males fed proportionately more at puddles than other sex and age classes. Two species showed anomalous feeding patterns. In one, young females predominated at puddles; in the other, butterflies were rarely found at flowers.

4. As predicted, among the five species in which young males feed proportionately more at puddles, mean number of lifetime matings by females was negatively correlated with frequency of mud puddling by older females. A second prediction that mean number of lifetime matings by females is positively correlated with frequency of mud puddling by older males was not supported.

5. The results provide support for interspecific variation in division of responsibility between the sexes for resource acquisition for female reproduction, indicating close coordination between the sexes of foraging and life-history tactics.

Key words. Lepidoptera, foraging strategy, mud puddling, sexual dimorphism, nuptial gifts.

Introduction

An integral part of unravelling life history patterns is discerning the pressures affecting acquisition of nutrients needed for survival and reproduction. Organisms' feeding habits need not be constant, but may vary with age and between the sexes. These differences can be qualitative and dramatic, as in insect species with herbivorous larvae and nectivorous adults. Alternatively, the differences may be quantitative; increases or decreases in nutrient intake may occur with age, and the pattern of change may differ between the sexes (Boggs & Ross, 1993). Further, interactions between the sexes may affect age-specific nutrient intake. In some insect species, nutrients are transferred from males to females at

mating through direct feeding or a spermatophore (Thornhill, 1976a; Boggs & Gilbert, 1979; Boggs, 1995). This nutrient transfer can lead to a reduction in female feeding (Downes, 1970; Thornhill, 1976b; Svensson & Petersson, 1987; Boggs, 1990). Such differences among species in the timing and source of nutrients obtained can result in different constraints on possible age-specific survival and reproductive patterns (Pianka, 1976; Boggs, 1981a; Mooney & Chiariello, 1984; Gatto *et al.*, 1989; Boggs, 1992).

Adult Lepidoptera do show age- and sex-specific feeding patterns. For those feeding on nectar, the amount imbibed differs both between the sexes and with age (Boggs & Ross, 1993). Many species also exhibit 'puddling' behaviour, feeding at mud, dung or carrion (reviewed by Adler, 1982). In general, young males are most frequently found puddling (Collenette, 1934; Adler, 1982; Adler & Pearson, 1982). The few females that have been found puddling tend to be old (Adler & Pearson, 1982; Berger & Lederhouse, 1985; Launer *et al.*, 1996). Boggs & Jackson (1991) found that older *Speyeria mormonia* (Nymphalidae) females

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puddle to a greater extent than females of other well-studied species.

Sodium in wet sand stimulates puddling behaviour, at least in *Papilio glaucus* (Papilionidae) (Arms *et al.*, 1974), although butterflies could certainly obtain other nutrients such as amino acids or other salts from puddling (Lederhouse *et al.*, 1990). Alder & Pearson (1982) constructed a sodium budget for *Pieris rapae* (Pieridae), showing that although females spend approximately 75% of their initial adult sodium in eggs, the amount of sodium did not differ between the bodies of newly emerged and older females. Males showed marked decreases in body sodium between newly emerged and older adults. Adler & Pearson suggested that, when combined with the overwhelming predominance of males at puddles, these data are consistent with the hypothesis that males at mating are providing females with sodium needed for egg production. Pivnick & McNeil (1987) further demonstrated that males do transfer significant amounts of sodium to the female at mating in *Thymelicus lineola* (Hesperiidae). They showed that male-derived sodium is used by these females in egg production and is important in reproductive success.

The observation that males transfer sodium to females at mating, combined with interspecific variance in sex ratios at puddles, suggests that mating and puddling patterns may covary among species. That is, for species whose females mate only once, with one infusion of sodium (and/or other resources obtained from puddles), females may deplete their sodium stores as they age and turn to mud-puddling to replenish sodium (Boggs & Jackson, 1991). However, if females mate multiple times throughout their lives, they may receive multiple inputs of puddle nutrients, and never need to puddle. In these cases, males are foraging for nutrients for females.

The present study tested the following two predictions: (i) Species from a high elevation montane habitat exhibit the same qualitative patterns of age- and sex-specific puddling behaviour described in the literature for other species, as outlined above. Using seven butterfly species from three families, we predicted that young males should predominate at puddles. (ii) Among species in which young males predominate at puddles, such males are gathering sodium and other resources to give to females at mating. For such species, quantitative differences among species in the age- and sex-specific puddling patterns reflect differences in division between the sexes of resource acquisition by adults for female reproduction. If this hypothesis holds, we expect an inverse relationship between number of female matings for a species and the proportion of older females of that species mud puddling. A complementary prediction from the same hypothesis is that mean female mating number and the proportion of older males puddling are positively correlated among species. Both predictions are for species in which young males predominate at puddles, as these are most likely to fit the assumption that males gather extra sodium to pass to females at mating.

An important assumption here is that spermatophores or other male-derived compounds serve a nutritive function rather than a mate-guarding function (cf. Boggs, 1981b). Should male-derived compounds solely have a mate-guarding function, the qualitative pattern predicted above should not be seen. With no puddle nutrients transferred to and used by females, female puddling should be independent of mating habits. Male puddling may still be correlated with mating habits, if puddle nutrients are used in

spermatophore construction or other reproductive products involved in mate guarding.

Materials and Methods

The study was conducted near Rocky Mountain Biological Laboratory, Gothic, Gunnison County, Colorado, from 27 June 1991 to 16 August 1991 and from 29 June 1993 to 16 August 1993. Two study sites were used. Each contained a puddling site and area with abundant flowers visited by butterflies. The first site was located approximately 1 km south of Gothic on the Kettle Ponds Road, and was the same site used by Boggs & Jackson (1991). This site contained a 6.25×1.5 m mud puddle created by a small stream crossing a road (after flowing through an old horse corral which was used infrequently during summers only) and a 60×120 m open fescue grassland (Langenheim, 1962). The second site was located 0.5 km northeast of Gothic on the Copper Creek Trail. A stream flowing out of an abandoned beaver pond and crossing the trail created one 4.2×1.1 m mud puddle, while another 5×3.2 m seep provide a mud puddling site where the stream met Copper Creek. Adjacent to the trail puddle was a 85×30 m rocky meadow with abundant flowers.

For each site, equal time was spent searching for and capturing butterflies feeding at mud and at flowers in the surrounding meadow area. Searching occurred between 10.00 and 16.00 hours. Captured individuals were numbered on the hindwing with a permanent marker. The species, sex, activity (feeding at mud or flowers) and wing wear were recorded. Wing wear was used to approximate the age of the adult butterfly, as wings are not repaired with age. Wing wear was rated using the 1.5–5.0 scale used by Boggs (1987), where 1.5 = wings not fully hardened and 2.0 = dry, but undamaged wings. Wear rating increases in increments of 0.5 as scale erosion and wing tearing increases.

Resulting data were converted to captures at mud divided by total captures. These proportions were subject to arc-sin square root transformation before analysis. Wing wear classes were grouped into young (wear rating 1.5–2.0) and old (wear rating 2.5–5.0) for purposes of data analysis.

Species used in the study included *Pieris napi* (Pieridae), *Coenonympha tullia ochracea* and *Erebia epipsodea* (Nymphalidae: Satyrinae), *Speyeria mormonia* (Nymphalidae: Nymphalinae), and *Agriades rustica*, *Everes amyntula* and *Plebejus acmon* (Lycaenidae). Data collected in 1993 for *S. mormonia* were combined with that for 1987 or 1989 (Boggs & Jackson, 1991) when there was no significant difference between years in the proportion of butterflies found puddling.

Ten older females of each of *C.t. ochracea* and *A. rustica* were dissected, and the number of spermatophores was counted to determine the average number of matings for females of these species. Mean number of female matings for other species used in this study were obtained from Ehrlich & Ehrlich (1978) or, for *S. mormonia*, from Boggs (1986).

Results

Qualitative patterns of age- and sex-specific puddling

C.t. ochracea and *E. amyntula* stand out as having different

Table 1. Number of individuals caught feeding at mud/total number of individuals caught. Proportions are given in parentheses, and years during which the data were collected are given in brackets.

Species	Young males	Old males	Young females	Old females
Pieridae				
<i>P.napi</i>	80/88 (0.91) [1993]	47/69 (0.68) [1993]	2/6 (0.33) [1993]	1/9 (0.11) [1993]
Nymphalidae				
Satyrinae				
<i>C.t.ochracea</i>	3/34 (0.08) [1991, 93]	7/52 (0.13) [1991, 93]	5/15 (0.33) [1991, 93]	3/14 (0.21) [1993]
<i>E.episodea</i>	10/20 (0.50) [1991, 93]	11/55 (0.20) [1991, 93]	0/2 (0.00) [1993]	2/11 (0.18) [1993]
Nymphalinae				
<i>S.mormonia</i>	56/76 (0.74) [1993]	34/90 (0.38) [1987, 93]	3/77 (0.04) [1989, 93]	7/21 (0.33) [1987, 93]
Lycaenidae				
<i>A.rustica</i>	32/37 (0.86) [1993]	14/48 (0.29) [1991, 93]	2/6 (0.33) [1993]	8/12 (0.67) [1993]
<i>E.amyntula</i>	42/44 (0.95) [1993]	40/42 (0.95) [1993]	0/0 [1993]	4/4 (1.00) [1993]
<i>Pacmon</i>	10/13 (0.79) [1993]	4/8 (0.50) [1993]	0/3 (0.00) [1993]	0/6 (0.00) [1993]

puddling patterns than the other five species studied (Table 1). Young males of *C.t.ochracea* were found at mud puddles significantly less often than young males of the other six species (for all species, $G = 26.60$, 6 d.f., $P < 0.005$; without *C.t.ochracea*, $G = 3.08$, 5 d.f., ns). Across all ages, female *C.t.ochracea* were found at puddles proportionately more often than were males (Goldstein's $x^* = 2.04$, $0.05 > P > 0.01$).

No young female *E.amyntula* were caught, either at flowers or puddling. There were no significant differences in proportion puddling among young males and older individuals of either sex ($G = 0.004$, 2 d.f., ns). Of ninety animals caught, only four of any sex or age were found on flowers.

Among the remaining five species, young males generally puddled to a greater extent than older males or females of any age. The proportion of young males found at puddles was

significantly greater than that for old males, with the exception of *Pacmon*, which had the smallest sample size (Table 1; Goldstein's x^* : *P.napi*, 3.60, $P < 0.001$; *E.episodea*, 2.56, $0.05 < P < 0.01$; *S.mormonia*, 4.63, $P < 0.001$; *A.rustica*, 5.26, $P < 0.001$; *Pacmon*, 1.27, ns). Further, the proportion of young males at puddles was also significantly greater than that for all females (Table 1; Goldstein's x^* : *P.napi*, 6.42, $P < 0.001$; *E.episodea*, 2.02, $0.05 > P > 0.01$; *S.mormonia*, 8.56, $P < 0.001$; *A.rustica*, 2.53, $0.05 < P < 0.01$; *Pacmon*, 3.56, $P < 0.001$).

Sexual division of resource acquisition via puddling behaviour

C.ochracea and *E.amyntula* did not meet our baseline assumption that young males puddle disproportionately more than

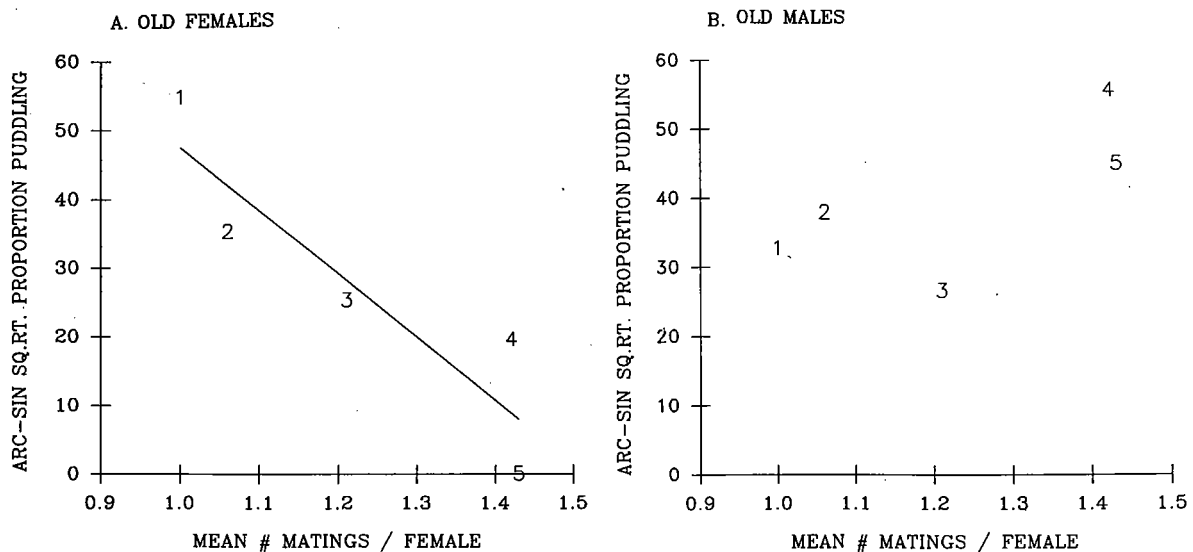


Fig. 1. 1 = *A.rustica*, 2 = *S.mormonia*, 3 = *E.episodea*, 4 = *P.napi*, 5 = *Pacmon*. (a) Arc-sin square root of older females caught at mud/total number of older females caught, as a function of the average number of matings per female. $y = 139.8 - 92.2x$, $r^2 = 0.83$, $F_{1,3} = 14.3$, $P = 0.03$. (b) Arc-sin square root of older males caught at mud/total number of older males caught, as a function of the average number of matings per female. $y = -8.9 + 39.6x$, $r^2 = 0.49$, $F_{1,3} = 2.8$, $P = 0.19$.

other age or sex groups. Therefore we excluded these two species from tests of the predictions that female mating numbers are correlated with tendency of older females or males to mud puddle. Among the remaining five species there was a significant negative linear regression among species for the arc-sin square root transform of proportion of older females puddling against average number of matings per female (Fig. 1a). The regression of arc-sin square root transform of proportion of older males puddling against average number of female matings had a positive slope, but was not significant (Fig. 1b).

Discussion

Two of the seven species examined, *C.t.ochracea* and *E.amyntula*, showed unusual sex- and age-specific patterns of mud puddle use. The data for *C.t.ochracea*, with a greater proportion of females than males puddling, suggest that males do not contribute significant quantities of puddle-derived nutrients to females at mating. Females of this species mate only once, suggesting that spermatophores may function primarily in mate guarding rather than as a nutrient source.

E.amyntula males and older females were overwhelmingly found at puddles rather than flowers, and no young females were ever caught, even at flowers. Scott (1986) reports that *Oeneis* spp. (Nymphalidae: Satyrinae) rarely feed at flowers, but are found primarily at mud. Our finding of a similar pattern in a lycaenid suggests that variation in adult dependence on nectar may be widespread across phylogenetic groups, raising the possibility of comparative analyses of factors determining species-specific adult feeding sources.

For the five remaining butterfly species, the proportion of older females using puddles decreased among species with increasing mean lifetime number of matings by females. This finding for females supports our hypothesis that, by increasing the lifetime number of matings, females obtain a higher proportion of nutrients from males, reducing the need for female foraging at puddles. If the time females spend in second and subsequent matings is less than the time they would spend puddling to gain equivalent nutrients, then females may gain oviposition or flower feeding time through obtaining puddle nutrients from males. Females might also benefit from a reduction in time spent puddling if predation rates at puddles were higher than for other activities.

Among five species, the proportion of older males puddling increased with increasing number of female matings, but not significantly. There are at least two possible explanations of this result. First, experimental error in measuring the proportion of individuals puddling may be greater for older males than for older females, requiring a larger species sample size from a more species-rich location. This possibility results from a likely difference between the sexes in variance in mating numbers, and hence sample variance in proportion puddling. Mating is driven by opportunity in male Lepidoptera, with little or no refractory time for males after a mating (e.g. Rutowski & Gilchrist, 1986; Oberhauser, 1988; Gwynne, 1990). Given the resulting male-biased sex ratios for individuals available to mate, variance in male mating numbers within a butterfly species is generally greater than variance in female mating numbers, with 50% or more of males failing to mate at all (Boggs, 1979; Elgar & Pierce,

1988). If our hypothesis is true, that older males that have mated frequently are more likely to puddle and older females that have mated frequently are less likely to puddle, sample variance within a species for proportion puddling should be greater for males than for females, given the greater variance in mating success.

A second possibility is that species differ independently of mean mating number in (a) the relative amounts of puddle nutrients transferred at each mating, (b) the relative need for puddle nutrients for female reproduction, and/or (c) the amount of nutrients such as sodium obtained from larval host plants. Such differences should result in strong relationships between mean number of matings and male puddling behaviour, but only within groups of species that were similar in accessory gland content, female reproductive investment and/or the nutritional quality of larval host plants.

Each species in the correlation analysis was treated as an independent data point. There was no *a priori* reason to do otherwise. First, the taxonomic level at which phylogenetic constraints might operate (e.g. genus, family) is unclear. Second, the two lycaenid species in our sample spanned the range of mean number of matings for females, indicating relatively large variance within families.

In summary, our data indicate a complex pattern of feeding at mud, relative to nectar, by seven butterfly species. We observed species-specific differences between the sexes and among age classes in division of foraging effort for female reproductive nutrients. Foraging by males to obtain specific nutrients to transfer to females has been noted for secondary compounds such as pyrrolizidine alkaloids in butterflies (e.g. Brown, 1984; Dussourd *et al.*, 1988), and *Heliconius cydno* (Nymphalidae) females show an inverse relationship between lifetime number of matings and amount of pollen eaten (Boggs, 1990). However, this is the first demonstration of a broader correlation among species in sex-specific feeding habits and mating numbers. Finally, the two species with anomalous sex- and age-specific puddling patterns deserve closer scrutiny. They may represent interesting variants on the spectrum of quality of the adult diet, allowing more detailed examination of the constraints that foraging habits impose on resource allocation, life-history traits and, ultimately, population persistence in variable environments.

Acknowledgments

We thank V. Iyengar and M. Marvier for assistance, and A. Launer, V. Rashbrook, and W. Watt for comments on the manuscript. C.E.S. was funded by a NSF Research Experience for Undergraduates site grant (BIR 93-46367) to Rocky Mountain Biological Laboratory.

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Accepted 17 October 1995