SODIUM: A male moth’s gift to its offspring

(Lepidoptera/ Gluphisia/ nuptial gift/ parent investment/ puddling)

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ABSTRACT Males of the moth Gluphisia septentrionis acquire sodium by drinking from mud puddles. Analyses of male and female bodies indicate that such “puddling” behavior enables the male to provide his mate with a nuptial gift of sodium, presumably via the spermatophore. This gift (about 10 µg), amounting to more than half of a puddler male’s total body sodium, is in large measure apportioned by the female to her eggs. Puddler-sired eggs contain 2 to 4 times more sodium than those control-sired; this difference is already apparent in eggs laid the night after mating. Paternal endowment of offspring with sodium had not previously been demonstrated for an insect to our knowledge. The potential adaptive significance of such chemical bestowal is evident, given that the foliar diet of G. septentrionis larvae is extremely low in sodium content.

Naturalists have long been fascinated by the sight of butterflies, aggregated often by the hundreds, drinking at water sources. The phenomenon, which most frequently takes place at the edge of puddles, has appropriately been called “puddling.” Moths also engage in puddling, but they are rarely observed to do so, since they drink at night. Puddling had long ago been suggested to serve for sodium intake (1), and puddling butterflies, in choice experiments, had been shown to favor sodium-containing over sodium-free fluids (2–4). Mystifying was the fact that puddling is heavily sex-biased, being restricted almost exclusively to males. This suggested that puddler males might transfer acquired sodium to females at mating (3), a possibility that recently gained support: in the skipper butterfly Thymelicus lineola, the male loses sodium as a consequence of copulation, while the female is left sodium-enriched (4).

We have investigated puddling in Gluphisia septentrionis, a notodontid moth that manifests the behavior in extreme form (5, 6). A single male Gluphisia, weighing about 80 mg, may puddle uninterruptedly for hours, during which it pumps an astounding 10–50 ml of fluid through its gut. It imbibes the liquid through an especially widened oral cleft, voiding it in the form of anal jets passed at intervals of seconds. The jets may be squirted to distances of 0.4 m or more (Fig. 1). We had earlier shown that as a result of puddling, male Gluphisia gain in the order of 17 µg of sodium, while losing a molar equivalent of potassium. Sodium uptake apparently takes place in the hindgut, which unlike its counterpart in the nonpuddling male, is endowed with a highly expanded absorptive surface (6). We now report that in Gluphisia, as in Thymelicus, the male bestows sodium upon the female as a nuptial gift, but in addition we demonstrate that the female allocates this gift in substantial measure to the eggs.

MATERIALS AND METHODS

To check into nuptial transfer of sodium, we analyzed (i) whole bodies of Gluphisia males that had either puddled (puddlers) or not puddled (controls) and in turn had either mated or not mated; (ii) whole bodies of females that had mated either with puddler or control males or had remained virginal; and (iii) body parts of males and females that had undergone some of these treatments.

To look into transfer of sodium to the eggs, we analyzed eggs sired either by puddler males or by controls.

Experimental Animals. The Gluphisia were field-collected (light traps in Ithaca, NY) or were laboratory-reared progeny of such moths. Reared moths, as larvae, were fed fresh foliage of quaking aspen (Populus tremuloides), the local host plant. Adult moths were maintained as described (6, 7). Experimental moths were all laboratory-reared, except where otherwise specified. Special care was taken throughout all procedures to avoid contamination of moths with unwarranted exogenous sodium (7). Voucher specimens of the moths are on deposit in the Cornell University Insect Collection (lot no. 1214).

Puddler males all drank a standardized solution [aqueous equimolar (1 mM) NaCl/KCl/CaCl2/MgCl2] to which control males had no access, as described (6). Matings were effected by introducing virgin moths into cylindrical cages (fiberglass mesh; 16-cm diameter × 36-cm height) 3–5 hr prior to darkness in a naturally lit room, in which they were confined overnight.

Analyses. Following determination of dry mass (after 48 hr at 55°C), whole moth bodies or dissected body parts were digested as described (6). Egg samples, after determination of dry mass (after 48 hr at 70°C), were digested in the manner of the body parts, except that only 0.20 ml of nitric acid was added. Following digestion, all samples were solubilized (6), stored in sealed polyethylene vials, and refrigerated (4°C) until analyzed.

The sodium and potassium content of samples was determined by inductively coupled plasma emission spectrometry (Thermo Jarrell–Ash Model 975 [ICAP 61 upgrade]; forward power = 1.25 kW at 27.1 MHz; coolant, plasma, and sample gas [argon] flow rates = 18.0, 0.8, and 0.8 liter/min, respectively; sample uptake rate = 2.2 ml/min). Cation values were read on a supplementary 0.75-m polychromator (1800 lines per mm grating). To maximize resolution of weak sodium signals resulting from the inherently small masses of egg samples, the forward power was reduced to 1.00 kW, and an auxiliary argon supply line was added, bypassing the nebulizer and entering directly at the mixing chamber.

We express quantitative ionic data in terms of concentration (ppm of dry mass). Since masses were invariant with treatment [P > 0.18 in all cases; ANOVAs (whole bodies), t tests (body parts)], concentration differences, where found, are accurately reflective of net content differences. The single exception is specified in the text.

Nuptial Transfer of Sodium. Female and male whole bodies. A group of virgin males was subdivided into pairs, ensuring that members of each pair were closely matched in emergence mass and postemergence age. One male of each pair was randomly assigned to puddle, while the other was designated as control. The next day each male of a pair was given a small

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white identifying marker (Liquid Paper), and the two were released, together with a randomly selected virgin female, into a mating chamber. The individual male that mated was identified either by being seen in copula or by its postcopulatory mass loss [a reliable indicator of mating (7)], or most often by both criteria. Females mated only once per night. The next day all moths were frozen (−14°C) and thus kept until analyzed (white marker was removed before analysis).

**Female body parts.** Individual virgin females were randomly paired in mating chambers with individual virgin males that had either puddled or not puddled the night before (puddling status was randomly assigned). The following day, less than 14 hr after the end of copulation, the females were killed by chilling (−14°C for 20 min), autopsied, and, if found to contain a spermatophore, dissected into four parts: (i) bursa plus spermatheca and accessory glands, (ii) ovaries, (iii) alimentary canal plus malpighian tubules, and (iv) remains (aggregate of all leftover parts). Dissections were performed dry to minimize leaching of tissue solutes. Samples were then analyzed.

**Male body parts.** Field-collected males that had puddled in the laboratory the previous night were randomly assigned either to mating with a virgin female or to solitary confinement. Copulation was confirmed by visual check of the mating cage or was assumed to have occurred if the eggs subsequently laid by the female hatched.

On the following day, less than 12 hr after copulation ended, the males were killed by chilling and were dissected into four component parts: (i) testes plus simplex and accessory glands, (ii) alimentary canal plus malpighian tubules, (iii) coremata (the pheromonal scent brushes) plus genitalia, and (iv) remains (aggregate of leftover parts). Samples were then analyzed.

**Transfer of Sodium to Eggs.** Individual virgin females were randomly designated to mate either with a virgin pudler male or a virgin control male (puddling status itself was randomly assigned). Puddling took place the night before pairing. Occurrence of copulation was verified visually or by subsequent confirmation of egg viability.

The day following pairing, the females were transferred individually to cylindrical, wax-paper-lined oviposition chambers (8-cm diameter and height; deionized water provided on cotton wad) under a 16-hr light/8-hr dark photocycle. Females were transferred daily to new chambers, thereby permitting separate collection of eggs laid each night.

Four egg samples were collected per female. The first three were from batches laid respectively on nights 1 to 3 following the night of mating. The fourth sample was a collective batch of any eggs laid from day 4 until the end of oviposition (in a few instances females died before day 4). The eggs were kept live for 4 days (by which time developing embryos were detectable) and then were stored frozen (−14°C) for later analyses. Eggs were removed with a steel microspatula from their wax paper backing, counted, collectively transferred to a Teflon boat, and then analyzed. Whenever possible, 60 eggs were collected per sample (preliminary analyses proved this number to be ample for appropriate measurement of sodium levels). Only eggs that were free of adhering scales or other visible contaminant were chosen.

Eggs from light-trapped females were collected in similar fashion (all proved viable, indicating natural insemination). Since the date of mating for such females was unknown, their first day in captivity was assigned day 2 status, given that at least one day had in all likelihood transpired since their insemination. Thus, only three egg samples (designated batches 2, 3, and 4 for purposes of comparison with the eggs from reared females) were collected from these females.

**Statistics.** Statistical analyses were performed with [SYSTAT](8). In instances where within-group variances were heteroscedastic (P < 0.05, Bartlett’s test) and rectification by logarithmic transformation was unsuccessful, a nonparametric ANOVA (Kruskal–Wallis) was used. Following ANOVAs that indicated significant differences (P < 0.05), multiple comparison tests were performed (Tukey–Kramer, experiment-wide α = 0.05). All t tests compared means of independent samples. Values are presented throughout as means ± 1 SD.

**RESULTS**

**Female and Male Whole Bodies.** Sodium concentration for females (Fig. 2A) varied between samples (P < 0.001, Kruskal–Wallis test). Puddler-mated females had significantly higher sodium levels than did control-mated females, whose levels did not differ from those of virgins. Potassium levels (Fig. 2B) did not vary among the three female categories (P = 0.996, ANOVA).

For males, sodium concentration (Fig. 3A) differed between samples as a function both of puddling history and reproductive status (P < 0.001, Kruskal–Wallis test). Sodium levels were highest in virginal puddlers. Puddlers that mated contained distinctly lower quantities of sodium but not as little as either virgin or mated nonpuddlers. Levels in the latter two types did not differ from one another. Potassium levels (Fig. 3B) did not differ among the four male categories (P = 0.24, ANOVA).

**Female Body Parts.** Sodium concentration was higher in body parts of puddler-mated than control-mated females (Fig. 4A). The value was highest for the Repro category, which included the structures that can be expected to have received the male’s seminal infusion. For potassium there was no comparable indication of nuptial bestowal upon the female (Fig. 4B).
Male Body Parts. Without exception, these contained lower sodium levels in puddlers that mated than in those that remained virginal (Fig. 5A). For the reproductive parts (Repro), which could be expected to have undergone seminal depletion as a consequence of mating, the mass was lower in mated individuals ($P = 0.03$, $t$ test); the difference in net sodium content between such males and virgins was therefore actually greater than suggested by the given concentration.

The net sodium loss undergone by the reproductive organs as a consequence of mating was actually considerable. Prior to mating, the reproductive parts contained 38 ± 6% ($n = 9$) of the sum total of sodium in the body parts. After mating the amount was decreased to 22 ± 4% ($n = 6$) ($P < 0.001$, $t$ test on arcsine-square root transformed percentages).
The data for potassium (Fig. 5B) indicated no pattern of concentration change paralleling that for sodium. The reproductive organs, certainly, underwent no potassium loss as a concomitant of mating.

**Estimated Magnitude of Sodium Transfer.** The preceding data provide for separate bases for calculating the quantity of sodium delivered by the male at mating. These calculations, for which figures for net sodium content rather than sodium concentrations were used, yielded comparable values (Table 1). We conclude that the nuptial gift bestowed by puddler males is in the order of 10 μg of sodium.

**Transfer of Sodium to Eggs.** Egg sodium content was clearly a function of whether the sire had puddled or not (Fig. 6A, statistical results in legend). Puddler-sired eggs contained higher sodium levels irrespective of when after mating they were laid. On the first day after mating, such eggs were somewhat underendowed relative to those laid on the next 2 days. Control-sired eggs consistently contained low sodium levels, invariant with time after mating. Potassium concentration remained constant over time and did not differ in puddler-sired and control-sired eggs (Fig. 6B).

The eggs of field-collected females (n = 9 females) contained sodium levels (batches 2, 3, and ≥4 contained [Na], respectively, of 4.6 ± 1.2, 4.4 ± 1.5, and 2.5 ± 1.6 ppm × 10^2) equivalent to those sired by puddlers in the laboratory (Fig. 6A) (P = 0.14, two-way ANOVA; see Materials and Methods for the procedure of comparison).

The net sodium content of control-sired eggs was consistently 3 ng of sodium per egg. In contrast, puddler-sired eggs contained 7 ng per egg on the first day of laying, and an approximately constant 13 ng per egg on days thereafter. Fecundity did not differ between puddler-mated females (521 ± 157 eggs per female; n = 11) and control-mated females (426 ± 151; n = 11) (P = 0.16, t test). Puddler-mated females therefore passed substantially more sodium to their eggs (5.1 ± 1.8 μg of sodium per female) than those control-mated (1.3 ± 0.7 μg of sodium per female) (P < 0.001, t test).

**DISCUSSION**

The fate of sodium acquired by male *G. septentrionalis* through puddling appears established: the male transmits the ion to the female at mating, and the female apportions the gift to the eggs. Puddler males, after mating, contained diminished sodium levels, while females, after mating with puddlers, contained elevated levels. Furthermore, eggs sired by puddlers had higher sodium content than those sired by nonpuddlers. We do not know for certain that the male bestows his sodium by way of the spermatophore, but we suspect that he does.

<table>
<thead>
<tr>
<th>Data source</th>
<th>Sodium content in each of the two categories,* μg (mean ± 1 SD)</th>
<th>Sodium transferred, μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females [(puddler-mated) – (control-mated)]</td>
<td>(12.2 ± 2.0) – (1.6 ± 0.4) = 10.6</td>
<td></td>
</tr>
<tr>
<td>Whole bodies</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Body parts</td>
<td>(10.9 ± 1.5) – (1.9 ± 0.3) = 9.0</td>
<td>10</td>
</tr>
<tr>
<td>Males [(puddler/virgin) – (puddler/mated)]</td>
<td>(19.0 ± 5.8) – (11.3 ± 8.0) = 7.7</td>
<td>28</td>
</tr>
<tr>
<td>Whole bodies</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Body parts</td>
<td>(20.9 ± 5.5) – (10.6 ± 2.8) = 10.3</td>
<td>9</td>
</tr>
</tbody>
</table>

*Comparison (t tests) (i) of the total sodium content of whole bodies and (ii) of the sum totals of sodium contents of parts in the two categories of females and of males; , P < 0.01; , 0.01 < P ≤ 0.05.

Given that the ion is present in substantial quantity in the male’s reproductive system prior to mating, and in the female’s reproductive system (specifically in the ejaculate-receiving structures) after the event.

Quantitatively, our earlier demonstration that the male sequesters in the order of 17 μg of sodium by puddling (6) is now supplemented by the finding that he relinquishes at mating about 10 μg of this supply, of which the female then passes ~5 μg to the eggs. Thus, about a third of the sodium initially procured by a male makes its way to the offspring of his first mate. Male *G. histiasia*, in the laboratory, mate at least three times during their brief (6 day) lifespan (7). Whether in nature they also mate more than once and puddle between matings to replenish their sodium remains questionable. Puddling and copulation both take considerable time, and each may preclude the other from occurring on the same night.

A number of other conclusions can be drawn from the data. If the spermatophore is indeed the vehicle of sodium transmission, then it must be viewed as a “well-salted” package: it amounts to only 9% of male body mass, yet conveys over half of a puddler male’s body sodium. By the same token, the female, upon receipt of sodium, appears to translocate the ion quickly: within hours after mating, acquired sodium is already detectable in structures other than the ejaculate-receiving organs (e.g., the ovaries). Apportionment of received sodium appears to occur promptly: within the day after mating eggs already contain a significant allocation of sodium. Even females that mate with nonpuddlers give priority to sodium provisioning of their offspring. Although they deliver a total of only 1.3 μg of sodium to the eggs, this represents fully 80% of their systemic content: puddler-mated females, in delivering 5.1 μg, part with only about half of their supply.
From an adaptive point of view, sodium sequestration by male Gluphisia and the subsequent transmission of the ion to female and eggs makes sense. Herbivores on land often face sodium shortages (9, 10), given that terrestrial plants typically contain low titers of the ion. P. tremuloides, the Gluphisia food plant, is particularly deficient in sodium (6, 11–13). Puddling may therefore be the specific means by which Gluphisia remedies this ionic insufficiency. Potassium acquisition and transfer do not appear to be concomitants of puddling. The ion is in plentiful supply in land plants, and insect herbivores generally show no lack of it. Indeed, Gluphisia actually voids potassium when puddling (6), and no significant quantities of the ion are passed to the female at mating or subsequently to her eggs.

While nuptial transfer of sodium had been shown previously to occur in a lepidopteran (4), there is no precedent for the demonstration that such ion is invested in the eggs. But the finding is new only insofar as it applies to sodium. Phosphorus (14) and zinc (15), both of male origin, are apparently transmitted to eggs in certain moths. Best documented for insects are cases involving male donation of nutrients, which the female invests in egg production (16–20), and of defensive substances, such as pyrrolizidine alkaloids (21, 22) or cantharidin (23, 24), which protect eggs against predation. The major conclusion to be derived from this growing body of evidence is that paternal contribution of chemicals to eggs may be more widespread than suspected, certainly in insects, but perhaps in other animals as well.

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