Functional Ecology 1990, **4**, 743–751

Adult nutrition affects male virility in *Papilio glaucus* L.

R. C. LEDERHOUSE, M. P. AYRES and J. M. SCRIBER
Department of Entomology, Michigan State University, East Lansing, Michigan 48824, USA

Abstract. As adults many male Lepidoptera are known to supplement their nectar diet by feeding at puddle margins or on damp soil, sometimes in the vicinity of carrion or faeces. Although sodium ions prolong this behaviour, and may directly enhance reproductive success, it is unclear whether sodium ions alone are being sought or whether other nutrients such as amino acids are also important. Newly emerged laboratory-reared males of the tiger swallowtail butterfly, *Papilio glaucus* L. (Lepidoptera: Papilionidae), were allocated to four adult diets: dilute honey-water (20% by weight); honey solution supplemented with electrolytes (lepidopteran Ringer's); honey solution supplemented with amino acids (0·5% casein hydrolysate); or honey solution supplemented with electrolytes and amino acids. We attempted to hand-pair males after 2 days of feeding and then at 2-day intervals to a maximum of four pairings. Males of both electrolyte treatments were more likely to couple than honey-water controls. Males receiving electrolytes plus amino acids produced seven times more hatching larvae than control males. This was chiefly attributable to improved number and success of matings subsequent to the first mating. Spermatophore size was correlated with male pupal mass for the first adult mating; diet affected the size of second and later spermatophores. Male diet had little effect on the longevity of males or their mates.

Key-words: Fertility, longevity, mating success, nutrition, *Papilio glaucus*, reproduction, spermatophore

Introduction

In most lepidopteran species, nutrients allocated to reproduction are derived largely from larval feeding and supplemented to varying degrees through adult feeding (Gilbert, 1972; Boggs, 1981a; Boppre, 1986). Acquisition of energy reserves is relatively easy for larvae on suitable hosts (Scriber & Slansky, 1981). Adult haustellate mouthparts are ideal for harvesting nectar, sap, fruit juices and honeydew. However, these adult food sources are typically low in available nitrogen (Watt, Hoch & Mills, 1974; Baker & Baker, 1975), and nitrogen may limit caterpillar growth on many hosts (Slansky & Feeny, 1977; Mattson, 1980). Because sodium is only required as a micronutrient by most land plants (Brownell & Crossland, 1972), it is not stored in plant tissues and is of limited availability to herbivorous caterpillars (Seastedt & Crossley, 1981; Mattson & Scriber, 1987). Nectar generally contains little sodium (Hiebert & Calder, 1983), especially relative to potassium content (Waller, Carpenter & Ziehl, 1972). Both nitrogen and sodium have been implicated as nutrients limiting reproduction in the Lepidoptera (Gilbert, 1972; Dunlap-Pianka, Boggs & Gilbert, 1977; Boggs, 1981a,b; Pivnick & McNeil, 1987).

Adults of a variety of Lepidoptera apparently supplement their diets by feeding at the edges of puddles, on damp soil or from carrion or faeces (Norris, 1936; Downes, 1973). The presence of sodium ions has been shown to prolong puddling behaviour (Arms, Feeny & Lederhouse, 1974; Adler & Pearson, 1982; Pivnick & McNeil, 1987). Also, amino acids acquired during puddling were incorporated into somatic tissues of *Papilio glaucus* L. (Arms *et al.*, 1974). Most individuals observed at puddles are males (Downes, 1973; Adler, 1982), which often produce spectacular aggregations (Scudder, 1889; Klots, 1958).

Laboratory-reared *P. glaucus* males that did not have the opportunity to puddle but were fed a maintenance diet of honey-water had high rates of reproductive failure when hand-paired to virgin females in our laboratory (Lederhouse & Scriber, 1987). Hand-pairings using field-collected males were considerably more successful. Ejaculate components in addition to sperm may be important in maximizing reproduction (Boggs & Gilbert, 1979), which could explain why the egg viability of *P. glaucus* females declined significantly when the interval between mating and oviposition was over 1 week (Lederhouse, 1981; Lederhouse & Scriber, 1987). This study was designed to test whether supplementing the male's diet with

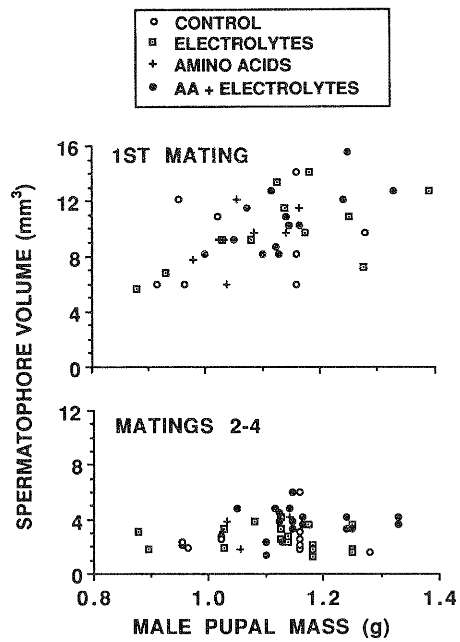

Fig. 3. Spermatophore volume as a function of pupal mass in *Papilio glaucus* males mating for the first time (top) and in subsequent matings (bottom). The four different diets are indicated.

0·02), but was affected by adult diet. Males receiving amino acids plus electrolytes produced significantly larger second and subsequent spermatophores than control males (mean ± SE = 3·80 ± 0·36 *vs* 2·46 ± 0·40 mm^3, P = 0·012) or electrolyte males (2·58 ± 0·31, P = 0·010). Males receiving amino acids alone also tended to produce larger second spermatophores (3·25 ± 0·68). However, so few amino acid males remated (5) that this is difficult to interpret; they did not differ significantly from the control males (P = 0·29). First spermatophores of all treatments combined averaged three times larger than subsequent spermatophores (least-square means ± SE = 9·41 ± 0·40 *vs* 3·00 ± 0·23 mm^3, $P < 0·0001$).

Male diet did not significantly affect the number of eggs laid by their mates (P = 0·50, Table 1). Nor did male mating history significantly affect egg production (P = 0·16). Furthermore, total oviposition was not correlated with spermatophore volume either among first matings (r = 0·17, n = 41) or later matings (r = 0·09, n = 55), as might be expected from Rutowski, Gilchrist & Terkanian (1987). Unfortunately, we are unable to reject confidently the existence of biologically meaningful effects on egg production. Given the high variance in egg production (which has been typical for *P. glaucus* in our laboratory; Lederhouse & Scriber, 1987) sample sizes were too small to provide a robust test (grand mean = 14·5 eggs, s = 29·7, range = 0–153, n = 96 females).

The hatching success of eggs produced from matings to control and electrolyte treatment males were generally very low (Fig. 4). No third or fourth mates of these males produced any eggs that hatched. Although most mates of amino acid treatment males had low hatching success, one especially fecund female had high hatching success (132 of 153 eggs hatched). The majority (13 of 23) of matings with males fed amino acids plus electrolytes had intermediate to high hatching success (>20%); this was true even for females that were second or third mates of these males. The overall contrast between control and amino acid plus electrolyte diets fell just short of significance (least-square means ± SE = 13 ± 8 *vs* 34 ± 7 per cent hatch, respectively, P = 0·06). However, in matings beyond the first, eggs sired by amino acid plus electrolyte males were significantly more likely to hatch (per cent hatch = 39 ± 10 *vs* 11 ± 8, P = 0·048). The mean values for per cent hatch seem surprisingly low. However, if the zero values are eliminated, the mean per cent hatch (± SE) for electrolyte/amino acid males becomes 57 ± 7, which is consistent with values calculated in the same way for large samples of field-collected, wild-mated *P. glaucus* females brought into our

Table 1. ANOVA results testing the effect of adult diet and mating history (first matings *vs* later matings) on the reproductive performance of male *Papilio glaucus*. All results are based on the same fixed effects model using transformed data as indicated.

		Pairing duration $\sqrt{\text{min}}$			Spermatophore volume $\sqrt{\text{mm}^3}$			Eggs/mating $\text{Log}_{10}(\text{eggs} + 1)$		
Source	d.f.	MS	F	P	MS	F	P	MS	F	P
Diet	3	2·15	2·01	0·12	0·272	1·70	0·17	0·369	0·80	0·50
Mating history	1	5·41	5·07	0·027	35·527	221·65	0·0001	0·944	2·05	0·16
Diet × mating history	3	1·07	1·00	0·40	0·195	1·22	0·31	0·135	0·29	0·83
Error	88	1·07			0·160			0·461		

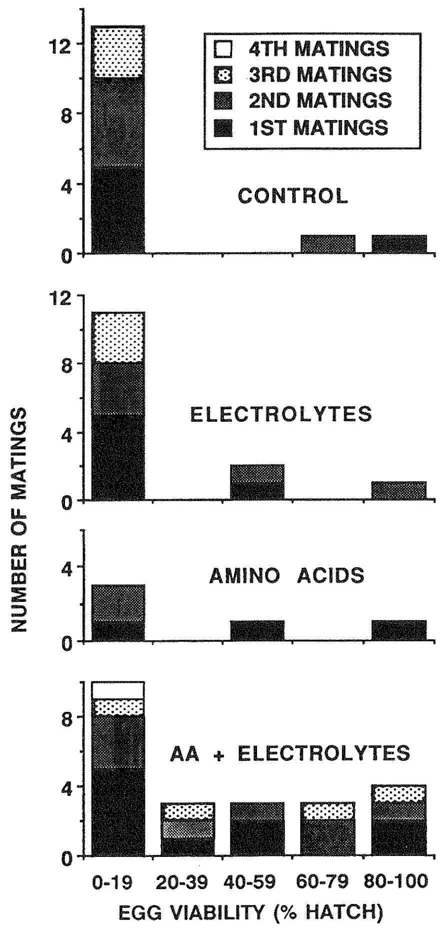

Fig. 4. The hatching success of eggs resulting from matings with *Papilio glaucus* males fed different diets as adults.

laboratory (Lederhouse & Scriber, 1987). These low values are not likely to be the result of laboratory conditions. We monitored six samples of eggs found on hosts in the field. These samples totalling 159 eggs had a mean hatching rate of 59 ± 5%.

The overall test for an effect of diet on the production of first instar progeny per mating opportunity was marginally insignificant at P = 0·08 (Table 2). However, the contrast with control males showed significantly elevated reproductive success in electrolyte/amino acid males (P = 0·049). Electrolyte/amino acid males also produced more progeny than males fed either electrolytes or amino acids separately (P = 0·048 and P = 0·024, respectively). None of the other three pairwise contrasts among diets were significant. Significant differences among males (P = 0·0034) reflect a failure of initially unsuccessful males to compensate in later mating opportunities. Following their initial opportunity, males were generally less likely to convert subsequent mating opportunities into progeny (Time effect, Table 2).

Lifetime reproductive performance of males receiving the four diet treatments is partitioned and summarized in Table 3. The greatest effects of adult diet were manifest after the first mating (rows e–h, Table 3). Males fed electrolytes, with or without amino acids, were more likely to remate (row e). The female mates of males fed electrolyte plus amino acid tended to lay more eggs (row f, differences not significant), which were more likely to hatch (row g, significantly greater than control). The cumulative effect of more matings, more eggs and higher hatching success was a seven-fold increase in hatching larvae per electrolyte/amino acid male compared with control honey-water males (16·9 *vs* 2·0 larvae per male; row i, Table 3).

Discussion

The reproductive potential of *P. glaucus* males is severely limited unless males supplement larval nutrient accumulations through adult feeding. Although nectars of butterfly flowers frequently contain lipids, amino acids and sodium ions, the concentrations of these nutrients are generally low (Baker & Baker, 1975; Hiebert & Calder, 1983). Tiger swallowtail males are avid visitors of mud puddles, manure and carrion, which provide concentrations of both amino acids and sodium (Arms *et al.*, 1974). Typically, puddling *P. glaucus* males are newly emerged, puddle during their first adult day or two, form large aggregations and then disperse considerable distances from puddling sites (Lederhouse, 1982). Although females do puddle in certain instances (Berger & Lederhouse, 1985; Scriber, 1987), they are often older individuals carrying a depleted spermatophore and do not

Table 2. Repeated measures ANOVA testing the effect of adult diet on the reproductive success (number of hatching larvae per mating opportunity) of *Papilio glaucus* males. All males were given the opportunity to mate 2, 4, 6 and 8 days after the establishment of feeding regimes.

	First instar progeny $\log_{10}(x+1)$				
Source	d.f.	MS	Denominator	F	P
Diet	3	0·492	MS_M	2·35	0·08
Males (Diet)	48	0·210	MS_E	1·83	0·0034
Time	3	0·461	MS_E	4·02	0·0088
Diet × time	9	0·158	MS_E	1·38	0·20
Error	144	0·115			

Table 3. The reproductive performance of *Papilio glaucus* males fed different diets as adults. Lifetime reproduction is partitioned between that attributable to first matings *vs* later matings.

	Control	Electrolytes	Amino acids	Amino acids + electrolytes
First Mating				
a Proportion of males mating at least once	0·70 (10)	0·85 (13)	0·62 (13)	1·00 (14)
b Eggs/mating	10 ± 5(7)	20 ± 7 (11)	24 ± 19 (8)	18 ± 7 (14)
c Hatchlings/egg	0·16 ± 0·13 (6)	0·11 ± 0·07 (7)	0·48 ± 0·25 (3)	0·29 ± 0·11 (10)
d Larvae/male (a×b×c)	1·1	1·9	7·1	5·2
Later Matings				
e Matings/male beyond 1st mating	1·20	1·38	0·38	1·43
f Eggs/mating	8 ± 3(12)	7 ± 3 (18)	1 ± 1 (5)	21 ± 10 (20)
g Hatchlings/egg	0·11 ± 0·08 (9)	0·22 ± 0·13 (8)	0 ± 0 (2)	0·39 ± 0·10 (13)
h Larvae/male (e×f×g)	1·1	2·1	0·0	11·7
Lifetime				
i Larvae/male (d + h)	2·2	4·0	7·1	16·9

Mean ± SE (n).

aggregate. Males are attracted to conspecific aggregations and are therefore able to locate resource concentrations more easily than females (Arms *et al.,* 1974; Pivnick & McNeil, 1987), which are likely to be harrassed in puddling aggregations (Berger & Lederhouse, 1985). An important cost to puddling is that adults may experience greater risk of predation (Morris, 1953; Rawson & Bellinger, 1953). Male *P. glaucus* generally emerge before females (Berger, 1986), providing time to supplement larval reserves before they are likely to mate. Thus, the nutritional requirements of adult males may select for protandry in *P. glaucus,* although protandry also occurs in some taxa where adults do not feed (Waldbauer, 1978).

Electrolyte supplementation alone led to increased mating frequency in male *P. glaucus,* but did not improve the effectiveness of those matings. Similar effects of sodium supplementation occurred in the European skipper, *Thymelicus lineola* (Pivnick & McNeil, 1987). European skipper males that received sodium mated more often over their lifetimes and remated at shorter intervals. Although Pivnick & McNeil (1987) reported that supplemented males had a significantly higher proportion of successful matings, they used a χ^2-test with inappropriately low expected frequencies (Zar, 1984). Reanalysis of their data using a Fisher Exact Probability Test (Siegel, 1956) indicates that the difference between sodium-supplemented males and control males only approached significance ($P = 0{\cdot}07$). In contrast to *P. glaucus* males, many *T. lineola* males stored enough sodium from larval feeding to mate successfully even without adult supplementation (Pivnick & McNeil, 1987).

Amino acid supplementation alone led to greater male longevity, but significantly reduced the likelihood that a *P. glaucus* male would mate. We can offer no explanation for this reduced ability or willingness to couple. When males received either electrolytes or amino acids alone their reproductive success increased somewhat over that of control males (2·0–3·2-fold). However, the effect of electrolytes and amino acids in combination was greater than a simple additive effect (7·7-fold increase over control males). The presence of sodium is the proximate stimulus that releases puddling behaviour (Arms *et al.,* 1974), but males will ingest and incorporate amino acids when present with sodium. Male *P. glaucus* did not actively feed on soil containing amino acids but very low levels of sodium (Arms *et al.,* 1974). Where sodium is the result of animal decomposition (carrion), sodium and amino acid concentrations will both tend to be high. Carnivore manure is reportedly more attractive to Lepidoptera than is herbivore manure (Sevastopulo, 1974).

Supplementation of diets of adult Lepidoptera with electrolytes and amino acids has considerable practical application whenever it is desirable to maintain laboratory cultures over several generations with addition of field-captured individuals. In particular, attempts to study quantitative genetics in Lepidoptera have suffered from low success of matings with laboratory-reared males. In addition, there have been considerable problems in assessing fertility of F_1s or back-crosses of interspecific crosses (Ae, 1979; Oliver, 1979; Lorkovic, 1986; Blanchard & Descimon, 1988). In our laboratory, we feed a modification of the dietary supplements used in this study to males every other day, alternating with the straight honey-water solution. Males appear to feed more readily on this modified diet (1·0 g NaCl, 5·2 g KCl, 0·24 g $CaCl_2 \times H_2O$, and 5·0 g salt-free casein acid hydrolysate per litre H_2O). We typically maintain this feeding protocol for about 5 days prior to mating the males. In 1986, prior to establishing this regimen, pairings using laboratory-reared males resulted in 19% of 75 matings producing some larvae (14 fertile females averaging 27 larvae from 73 eggs). In 1987, pairing using males fed our modified diet resulted in 90% of 20 matings with some larvae (18 fertile females averaging 44 larvae from 105 eggs). This translates into an eight-fold increase in larvae per mated female. However, this falls well short of the reproductive performance of wild males captured from the field as adults. During 1987, 84% of 31 hand-pairings using wild males produced some larvae (26 fertile females averaging 87 larvae from 115 eggs), which represents a 14-fold increase in larvae per mated female above that obtained from laboratory-reared males fed only honey-water. Hence, our modified diet allowed laboratory males to achieve about half the reproductive success of wild males. Yet, our diet is apparently not providing all the resources routinely acquired by wild males through puddling or other feeding activities. Nonetheless many presently unanswered questions requiring mating by laboratory-reared males have become experimentally tractable with the incorporation of this diet.

The size of the initial spermatophore of *P. glaucus* males reflects differences in larval nutrient procurement. A similar positive relationship between male size and initial spermatophore size exists for various butterflies (Boggs, 1981b; Svard & Wiklund, 1986; Oberhauser, 1988; but see Svard, 1985). Subsequent *P. glaucus* spermatophores are independent of male mass and only one-third the volume of first spermatophores (Fig. 3). A similar decrease in spermatophore size with increasing number of matings has been observed in a variety of Lepidoptera (Sims, 1979; Boggs & Watt, 1981; Svard & Wiklund, 1986; Pivnick & McNeil, 1987). However, the spermatophore size of male *P. glaucus* receiving both electrolytes and amino acids decreased significantly less with additional matings than that of males on other diets (Fig. 3). Wild *P. glaucus* females that received small spermatophores during their first matings were more likely to remate than those receiving large spermatophores (Lederhouse, Ayres & Scriber, 1989).

In this study, males that received supplemental sodium and nitrogen sired seven times more offspring than control males. In various other Lepidoptera, males contribute nutrients for the production of offspring through the spermatophore and accessory fluids (Boggs & Gilbert, 1979, Boggs, 1981b; Boggs & Watt, 1981; Greenfield, 1982; Drummond, 1984; Pivnick & McNeil, 1987; but see Jones, Odendall & Ehrlich, 1986). *Colias* females mating with males that produced small spermatophores laid fewer eggs per day and had shorter life-spans (Rutowski *et al.*, 1987). Similarly, multiply mated *P. xuthus* females, compared to singly mated control females, increased their egg production following each additional mating (Watanabe, 1988). The reproductive contribution of *P. glaucus* males may entail more than just sperm. Older *P. glaucus* females observed puddling (Berger & Lederhouse, 1985) may be attempting to supplement their sodium and/or nitrogen budgets directly when previous matings have been inadequate and an additional mating fails to occur. We are presently unable to discriminate between this and the alternative hypothesis that sodium and nitrogen supplementation in the male simply increases the number, longevity and viability of sperm cells. Pursuant studies are investigating the effect on reproductive output of directly supplementing the female diet with these nutrients.

Since *P. glaucus* males vary in the size and quality of their spermatophores depending on their puddling and mating histories, females that are able to discriminate among males and mate preferentially with virile males, should be favoured. Female solicitation of courtship has been documented in *P. glaucus* (Krebs & West, 1988). In other butterfly species, female solicitation has been related to inadequate previous mating (Sugawara, 1979; Rutowski, 1980; Rutowski *et al.*, 1981). The importance of sodium to male coupling behaviour in this study suggests that *P. glaucus* males may court as 'honest salesman' (*sensu* Rutowski, 1979) reflecting their sodium and nitrogen accumulations.

Acknowledgments

We thank R. Cowles, J. Nitao, J. Spencer and two anonymous reviewers for helpful comments on this manuscript. David Grossmueller provided unpublished data. Erika Dutcher provided valued technical support. This work was supported in part by NSF grants BSR-8718448 and BSR-8801184 and the Michigan State University College of Natural Sciences and the Michigan Agricultural Experiment Station (project 8051).

References

Adler, P.H. (1982) Soil- and puddle-visiting habits of moths. *Journal of the Lepidopterists' Society,* **36,** 161–173.

Adler, P.H. & Pearson D.L. (1982) Why do male butterflies visit mud puddles? *Canadian Journal of Zoology,* **60,** 322–325.

Ae, S.A. (1979) The phylogeny of some *Papilio* species based on interspecific hybridization data. *Systematic Entomology,* **4,** 1–16.

Arms, K., Feeny, P. & Lederhouse, R.C. (1974) Sodium: stimulus for puddling behavior by tiger swallowtail butterflies, *Papilio glaucus. Science,* **185,** 372–374.

Baker, H. G. & Baker, I. (1975) Studies of nectar-constitution and pollinator-plant coevolution. In *Coevolution of Animals and Plants* (eds. L.E. Gilbert & P.H. Raven), pp. 100–140. University of Texas Press, Austin, Texas, USA.

Berger, T.A. (1986) Habitat use and reproductive ecology of the eastern tiger swallowtail, *Papilio glaucus* L. Dissertation. Rutgers University, Newark, New Jersey, USA.

Berger, T.A. & Lederhouse, R.C. (1985) Puddling by single male and female tiger swallowtails, *Papilio glaucus* L. (Papilionidae). *Journal of the Lepidopterists' Society,* **39,** 339–340.

Blanchard, R. & Descimon, H. (1988) Hybridization between two species of swallowtails, meiosis mechanism, and the genesis of gyandromorphs. *Journal of the Lepidopterists' Society,* **43,** 94–102.

Blum, M.S. (1985) *Fundamentals of Insect Physiology.* John Wiley & Sons, New York.

Boggs, C.L. (1981a) Nutritional and life-history determinants of resource allocation in holometabolous insects. *American Naturalist,* **117,** 692–709.

Boggs, C.L. (1981b) Selection pressures affecting male nutrient investment at mating in heliconiine butterflies. *Evolution,* **35,** 931–940.

Boggs, C.L. & Gilbert, L.E. (1979) Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science,* **206,** 83–84.

Boggs, C.L. & Watt, W.B. (1981) Population structure of pierid butterflies IV. Genetic and physiological investment in offspring by male *Colias. Oecologia,* **50,** 320–324.

Boppre, M. (1986) Insects pharmacophagously utilizing defensive plant chemicals (pyrrolizidine alkaloids). *Naturwissenschaften,* **73,** 17–26.

Brownell, P.F. & Crossland, C.J. (1972) The requirement for sodium as a micronutrient by species having the C_4 dicarboxylic photosynthetic pathway. *Plant Physiology,* **49,** 794–797.

Clarke, C.A. & Sheppard, P.M. (1956) Hand-pairing of butterflies. *Lepidopterists' News,* **10,** 47–53.

Downes, J.A. (1973) Lepidoptera feeding at puddle-margins, dung, and carrion. *Journal of the Lepidopterists' Society,* **27,** 89–99.

Drummond, B.A. (1984) Multiple mating and sperm competition in the Lepidoptera. In *Sperm Competition and the Evolution of Animal Mating Systems* (ed. R.L. Smith), pp. 291–370. Academic Press, New York.

Dunlap-Pianka, H., Boggs, C.L. & Gilbert, L.E. (1977) Ovarian dynamics in heliconiine butterflies: programmed senescence versus eternal youth. *Science,* **197,** 487–490.

Gilbert, L.E. (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proceedings of the National Academy of Science USA,* **69,** 1402–1407.

Greenfield, M.D. (1982) The question of paternal investment in Lepidoptera: male-contributed proteins in *Plodia interpunctella. International Journal of Invertebrate Reproduction,* **5,** 323–330.

Hiebert, S.M. & Calder III, W.A. (1983) Sodium, potassium, and chloride in floral nectars: energy-free contributions to refractive index and salt balance. *Ecology,* **64,** 399–402.

Jones, K.N., Odendall, F.J. & Ehrlich P.R. (1986) Evidence against the spermatophore as paternal investment in checkerspot butterflies (*Euphydryas*: Nymphalidae). *American Midland Naturalist,* **116,** 1–6.

Klots, A.B. (1958) *The World of Butterflies and Moths.* Harrap, London.

Krebs, R.A. & West, D.A. (1988) Female mate preference and the evolution of female-limited Batesian mimicry. *Evolution,* **42,** 1101–1104.

Lederhouse, R.C. (1981) The effect of female mating frequency on egg fertility in the black swallowtail, *Papilio polyxenes asterius* (Papilionidae). *Journal of the Lepidopterists' Society,* **35,** 266–277.

Lederhouse, R.C. (1982) Factors affecting equal catchability in two swallowtail butterflies, *Papilio polyxenes* and *P. glaucus. Ecological Entomology,* **7,** 379–383.

Lederhouse R.C. & Scriber, J.M. (1987) Ecological significance of a postmating decline in egg viability in the tiger swallowtail. *Journal of the Lepidopterists' Society,* **41,** 83–93.

Lederhouse, R.C., Ayres, M,.P. & Scriber, J. M. (1989) Evaluation of spermatophore counts in studying mating systems of Lepidoptera. *Journal of the Lepidopterists' Society,* **43,** 93–101.

Lorkovick, Z. (1986) Enzyme electrophoresis and interspecific hybridization in Pieridae (Lepidoptera). *Journal of Research on the Lepidoptera,* **24,** 334–358.

Mattson Jr, W.J. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics,* **11,** 119–161.

Mattson, W.J. & Scriber, J.M. (1987) Nutritional ecology of insect folivores of woody plants: nitrogen, water, fiber, and mineral consideration. In *Nutritional Ecology of Insects, Mites, and Spiders* (eds. F. Slansky Jr & J.G. Rodriguez), pp. 105–146. John Wiley & Sons, New York.

Morris, W.A. (1953) The chipmunk as a predator of the adult yellow swallowtail butterfly. *Journal of Mammology,* **34,** 510–511.

Neter, H., Wasserman, W. & Kutner, M. (1985) *Applied Linear Statistical Models.* Richard D. Irwin, Inc., Homewood, Illinois.

Norris, M.J. (1936) The feeding habits of the adult Heteroneura. *Transactions of the Royal Entomological Society of London,* **85,** 61–90

Oberhauser, K.S. (1988) Male monarch butterfly spermatophore mass and mating strategies. *Animal Behaviour,* **36,** 1384–1388.

Oliver, C.G. (1979) Genetic differentiation and hybrid viability within and between some Lepidoptera species. *American Naturalist,* **114,** 681–694.

Pivnick, K.A. & McNeil, J.N. (1987) Puddling in butterflies: sodium affects reproductive success in *Thymelicus lineola. Physiological Entomology,* **12,** 461–472.

Rawson, G.W. & Bellinger, P.F. (1953) Sparrows feeding on congregating *Papilio. Lepidopterists' News,* **7,** 27.

Rutowski, R.L. (1979) The butterfly as an honest salesman. *Animal Behavior,* **27,** 1269–1270.

Rutowski, R.L. (1980) Courtship solicitation by females of the checkered white butterfly, *Pieris protodice. Behavioral Ecology and Sociobiology,* **7,** 113–117.

Rutowski, R.L., Long, C.E., Marshall, L.D. & Vetter, R.S. (1981) Courtship solicitation by *Colias* females (Lepidoptera: Pieridae). *American Midland Naturalist,* **105,** 334–340.

Rutowski, R.L., Gilchrist, G.E. & Terkanian B. (1987) Female butterflies mated with recently mated males show reduced reproductive output. *Behavioral Ecology and Sociobiology,* **20,** 319–322.

Scriber, J.M. (1987) Puddling by female Florida tiger swallowtail butterflies, *Papilio glaucus australis* (Lepidoptera: Papilionidae). *Great Lakes Entomologist,* **20,** 21–23.

Scriber, J.M. & Slansky Jr, F. (1981) The nutritional ecology of immature insects. *Annual Review of Entomology,* **26,** 183–211.

Scudder, S.H. (1889) *The Butterflies of the Eastern United States and Canada with Special Reference to New England.* Published by author. Cambridge, Massachusetts, 3 volumes.

Seastedt, T.R. & Crossley, D.A. (1981) Sodium dynamics in forest ecosystems and the animal starvation hypothesis. *American Naturalist,* **117,** 1029–1034.

Sevastopulo, D.G. (1974) Lepidoptera feeding at puddle-margins, dung, and carrion. *Journal of the Lepidopterists' Society,* **28,** 167–168.

Siegel, S. (1956) *Nonparametric Statistics for the Behavioral Sciences.* McGraw-Hill, New York.

Sims, S.R. (1979) Aspects of mating frequency and reproductive maturity in *Papilio zelicaon. American Midland Naturalist,* **102,** 36–50.

Slansky Jr, F. & Feeny, P. (1977) Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological Monographs,* **47,** 209–288.

Sugawara, T. (1979) Stretch reception in the bursa copulatrix of the butterfly, *Pieris rapae crucivora,* and its role in behaviour. *Journal of Comparative Physiology A,* **130,** 191–199.

Svard, L. (1985) Paternal investment in a monandrous butterfly, *Pararge aegeria. Oikos,* **45,** 66–70.

Svard, L. & Wiklund, C. (1986) Different ejaculate delivery strategies in first versus subsequent matings in the swallowtail butterfly *Papilio machaon* L. *Behavioral Ecology and Sociobiology,* **18,** 325–330

Waldbauer, G.P. (1978) Phenological adaptation and the polymodal emergence patterns of insects. In *Evolution of Insect Migration and Diapause* (ed. H. Dingle), pp. 127–144. Springer, New York.

Waller, G.D., Carpenter, E.W. & Ziehl, O.A. (1972) Potassium in onion nectar and its probable effect on attractiveness of onion flowers to honey bees. *Journal of the American Society for Horticultural Science,* **97,** 535–539.

Watanabe, M. (1988) Multiple matings increase the fecundity of the yellow swallowtail butterfly, *Papilio xuthus* L., in summer generations. *Journal of Insect Behavior,* **1,** 17–29.

Watt, W.B., Hoch, P.C. & Mills, S.G. (1974) Nectar resource use by *Colias* butterflies: chemical and visual aspects. *Oecologia,* **14,** 353–374.

Zar, J. H. (1984) *Biostatistical Analysis,* 2nd edn. Prentice-Hall, Englewood Cliffs, New Jersey.

Received 3 April 1989; revised 12 March 1990; accepted 21 May 1990