

Nuptial gifts and the use of body resources for reproduction in the green-veined white butterfly *Pieris napi*

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Reproduction in butterflies, as in many holometabolous insects, is usually constrained by the amount of nutrients the animals can collect as juveniles. In polyandric species the females can also supplement their larval-derived reserves with protein-rich donations, so-called nuptial gifts, delivered by the males at mating. Recent findings also indicate that females have access to nitrogen from the histolysis of flight muscles in the thorax. This field study examined how butterflies of the polyandric gift-giving species *Pieris napi* (Lepidoptera: Pieridae) use body resources in their reproduction and how the male donations affect the females use of stored reserves. The results support earlier studies, indicating that females use resources from the breakdown of thorax muscles to increase their reproductive potential and the results also indicate that males also use thorax material in their reproduction. The study also supports recent findings that the male donation increases the breakdown of body resources and thereby boosts the reproductive output of the female.

Keywords: nuptial gifts; polyandry; resource allocation; reproductive reserves; *Pieris napi*; mating success

1. INTRODUCTION

Butterflies are holometabolous insects and the juvenile and adult diets differ markedly in composition. The larvae feed on the green parts of plants which are rich in both carbon and proteins. The adults, on the other hand, usually feed on nectar which also contains carbon but only small amounts of nitrogenous compounds (Baker & Baker 1973; Watt *et al.* 1974). Butterflies belonging to the genus *Heliconius* are an exception to this pattern in that the adults complement larval resources by feeding on pollen, which is rich in nitrogen (Gilbert 1972). Since eggs and spermatophores contain significant amounts of protein, it is speculated that nitrogen is limiting to reproduction. Both experiments and comparative studies with pollen- and nectar-feeding species support this idea (Boggs 1981; Svård & Wiklund 1986; Karlsson & Wickman 1989). Apart from the juvenile diet, females also gain nitrogen and other nutrients from male ejaculates delivered at mating, often referred to as nuptial gifts. Radiotracer studies by Boggs & Gilbert (1979) and Wiklund *et al.* (1993) showed that male material is incorporated into both the eggs and soma of females. Furthermore, Karlsson (1998) and Wiklund *et al.* (1993) found that male donations significantly increase female fecundity and life span. The amount of nutrients that the females receive from males depends on the mating system adopted by the species (Svard & Wiklund 1989) and the mating status of the male (Kaitala & Wiklund 1995). There are two generally recognized mating systems in butterflies. A species is either monandric where the females usually mate only once, or polyandric where the females mate several times. In monandrous species, males transfer relatively small spermatophores constituting up to a few per cent of their total body weight. On the other hand, polyandrous males transfer larger spermatophores,

which, on average, make up 15% of the body weight in the most polyandric species (Svard & Wiklund 1989).

Spermatophores consist of sperm and accessory gland secretions, which are rich in nutrients, particularly proteins and carbon, but which also contain sodium, lipids and hormones (Marshall 1982; Bissoondath & Wiklund 1995; Karlsson 1995). Spermatophores are costly to produce and it takes several days after mating for a male to produce a new full-sized spermatophore (Svard 1985; Svård & Wiklund 1986). Recently mated males therefore transfer considerably smaller spermatophores than virgin males. Polyandrous males also produce a new full-size spermatophore faster than monandrous males (Svard & Wiklund 1989). Kaitala & Wiklund (1995) and Oberhauser (1989) found that the female refractory period increases with an increase in the size of the male donation. This is interpreted as a female foraging strategy for male material, whereby females enhance their stores of nutrients. It turns out that females of *Pieris napi* most likely need at least two full-sized spermatophores in order to realize their potential fecundity (Karlsson 1998). In monandric species mating only seems to entail a transfer of sperm, but not any significant amount of nutrients (Svard & Wiklund 1989).

Before eclosion, the larval-derived resources are allocated to either the reproductive reserves in the abdomen or to the soma. The proportion of the resources allocated to each should depend on the future reproductive output and expectations of adult nutrient income (Boggs 1981). Karlsson (1995) showed that the resources allocated to reproduction covary positively with the degree of polyandry in males and negatively in females. Generally, abdominal reserves have been approximated with reproductive reserves (Boggs 1981; Karlsson & Wickman 1989). The abdomen contains the nutrients which are earmarked for reproduction and are stored in fat bodies, haemolymph and, to some extent, the reproductive organs (Boggs 1981). In some other insects nutrients from the histolysis

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Table 1. *Properties of P. napi in the different age classes*

(Age based on wing wear. *F*-values from ANOVA. Means are followed by standard deviations. The capture date denotes the number of days after the first butterfly of the respective sex was caught. For females the capture date is in days after 1 June. For males the capture date is in days after 12 May.)

age (wing wear)	capture date	wing length (mm)	number of spermatophores	thorax mass (mg)	thorax nitrogen (mg)	abdomen mass (mg)	abdomen nitrogen (mg)
females							
1	13.39 ± 6.64	21.14 ± 1.08	1.43 ± 0.63	5.19 ± 1.00	0.61 ± 0.10	7.71 ± 2.18	0.72 ± 0.23
2	22.87 ± 7.51	21.44 ± 1.34	2.24 ± 0.77	4.72 ± 1.17	0.56 ± 0.14	5.57 ± 1.93	0.47 ± 0.18
3	24.82 ± 4.69	21.53 ± 1.09	2.36 ± 0.93	4.06 ± 0.86	0.47 ± 0.10	4.30 ± 1.71	0.34 ± 0.10
<i>n</i>	116	116	116	116	116	116	116
<i>F</i>	26.23	0.84	12.74	9.07	10.58	23.91	36.04
<i>p</i>	< 0.00001	0.43	< 0.0001	< 0.002	< 0.0001	< 0.00001	< 0.00001
males							
1	20.75 ± 13.35	22.00 ± 1.10	—	6.03 ± 0.86	0.73 ± 0.09	7.00 ± 1.89	0.79 ± 0.27
2	36.33 ± 10.00	21.42 ± 1.73	—	5.22 ± 1.13	0.62 ± 0.13	4.92 ± 1.34	0.49 ± 0.18
3	43.29 ± 6.02	22.29 ± 1.28	—	5.96 ± 0.77	0.70 ± 0.09	4.50 ± 1.32	0.38 ± 0.15
<i>n</i>	51	51	—	51	51	51	51
<i>F</i>	20.28	1.68	—	4.67	4.87	11.48	16.51
<i>p</i>	< 0.00001	0.196	—	0.014	0.012	< 0.0001	< 0.00001

of flight muscles contribute to reproduction (e.g. Tanaka 1993; see also Kobayashi & Ishikawa (1993) and references therein). Recent studies have indicated that butterflies can also use nutrients from the thorax, presumably the flight muscles, for reproduction (Karlsson 1994, 1998). The flight muscles are reported to constitute 85–95% of the thorax mass in butterflies (Hocking 1958; Marden 1989). Some of these resources should be available for reproduction when the animals have used some of the reserves in the abdomen and the muscle mass needed for flight is reduced.

How butterflies are expected to use the resources in the thorax should depend on the balance of their nitrogen budgets. In species where the animals do not have access to nitrogen as adults, the thorax resources could conceivably boost their reproductive potential. For females the nutritional value of the thorax resources should perhaps vary somewhat with the contribution of the male donation. The difference is not expected to be very pronounced since reproduction is also very costly for polyandric females despite their male-derived nutrients. For males, the use of thorax resources should vary with the number and size of the spermatophores produced. Monandric males produce small spermatophores and mate on average only once in their lifetimes which means that the need for resources for reproduction is relatively low. For polyandric males the reproductive cost increases with the degree of polyandry and in the most polyandric species reaches the level of the females (Leimar *et al.* 1994; Karlsson *et al.* 1997; Karlsson 1998; Wiklund *et al.* 1998). This means that the males could also benefit from the ability to use nutrients from the flight muscles in reproduction.

Karlsson (1998) showed that the amount of male nutrients received by the female at mating affects her ability to use her own reproductive reserves. Multiply mated females used more of the resources in their thorax compared to singly mated females, and male material increases the females' life span. This could explain the increase in resource use found for multiply compared to singly mated females in several studies (e.g. Oberhauser 1989; Karlsson 1998).

In this investigation we studied how wild butterflies of the polyandrous species *Pieris napi* use the resources stored in their thoraxes and abdomens and how transferred male material affects female use of their stored reserves.

2. MATERIAL AND METHODS

The butterflies used in this study were collected at Rådmanö, 90 km north-east of Stockholm, during the period 12 May to 3 July 1998. The study was performed on animals from the first of two generations in central Sweden.

In order to determine how the body constitution and nitrogen content change with age for both sexes and also with number of matings for the females, the butterflies were treated as follows. After the animals were sexed, their age was estimated from wing wear using a ranking from 1 to 3, where 1 is virtually intact wings, 2 is intermediately worn wings and 3 is animals with heavily worn wings.

In order to determine how many times females had mated, their abdomens were separated from their thoraces and dissected for spermatophores. The number of spermatophores in the bursa copulatrix was counted and used as a measure of the number of matings (Burns 1968). The thoraxes and abdomens were dried to a constant weight at 60 °C. The dry weights of the abdomen without the bursa and the thorax excluding the wings, legs and head were measured to the nearest 0.01 mg on a Cahn 28 automatic electrobalance (CAHN Instruments, Milan, Italy). Throughout this work the term thorax is used to denote the thorax excluding the wings, legs and head. The nitrogen contents in the abdomens and thoraces were analysed by flash combustion with a Carlo Erba NA 1500 (CE Instruments, Milan, Italy). Ecdysis mass is estimated from the length of the right forewing measured to the nearest 0.5 mm. The data were analysed with STATISTICA 5.0.

3. RESULTS

Table 1 presents the data from the butterflies sampled. Age (as indicated by wing wear) affected the remaining body resources among both females and males. As

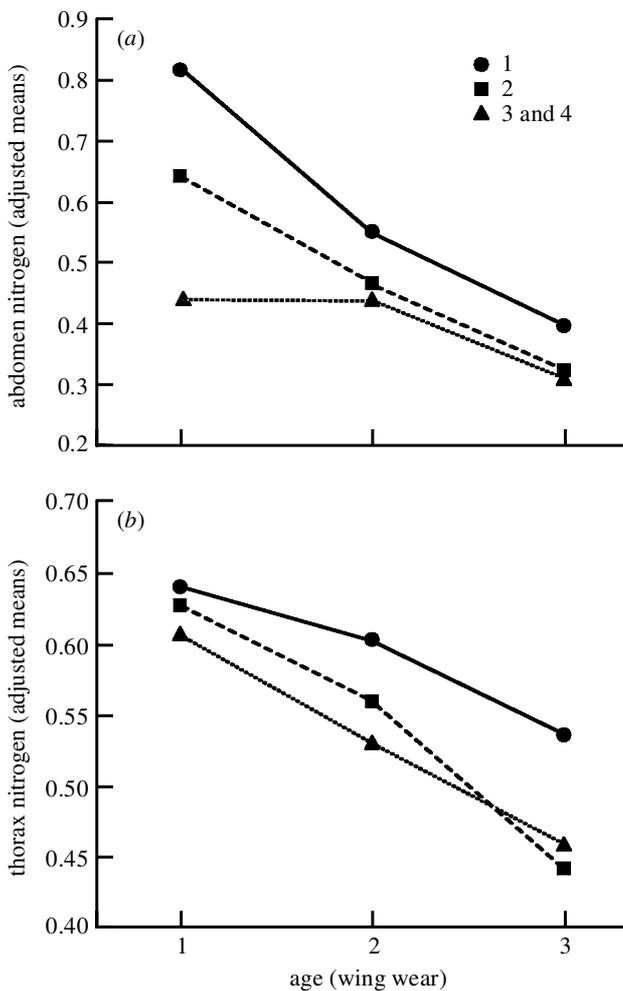


Figure 1. Nitrogen content in (a) the abdomen and (b) the thorax in relation to age in three mating groups of female *P. napi*. The nitrogen content was adjusted for differences in eclosion size (estimated by wing length). Mating group 1 (circles) contained one spermatophore, group 2 (squares) contained two spermatophores and group 3 (triangles) contained three or four spermatophores. An analysis of covariance, with eclosion size (wing length) as the covariate, detected a significant effect of age on the abdomen and thorax nitrogen contents ($F_{4,106} = 17.64$ and $p < 0.0001$ and $F_{4,106} = 16.67$ and $p < 0.0001$, respectively). There was also a significant effect of the number of matings on the abdomen and thorax nitrogen contents ($F_{4,106} = 7.84$ and $p = 0.0007$ and $F_{4,106} = 3.53$ and $p = 0.033$, respectively).

expected, the number of spermatophores found in the bursa increased with age. Figure 1 shows how the female nitrogen contents in the abdomen and thorax decrease with both age and the number of spermatophores found in the bursa.

The decreases in the abdomen and thorax nitrogen contents are *ca.* 62 and 28%, respectively (figure 2).

It appears that female use of the resources in the abdomen and thorax not only depends on female age but also directly on male material, since the nitrogen contents in these parts decrease with increasing number of matings irrespective of age (figure 1). The thorax and abdomen mass and nitrogen contents also decrease with age for males (table 1). Older males have significantly lower amounts of nitrogen in their abdomens and thoraces

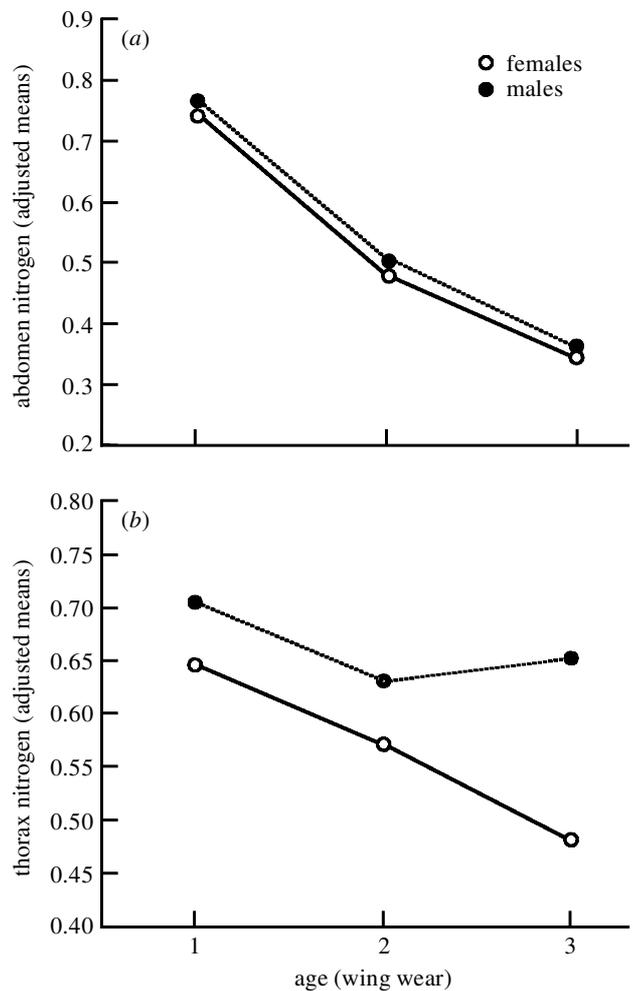


Figure 2. Nitrogen content in (a) the abdomen and (b) the thorax in relation to age in females (open circles) and males (filled circles). The nitrogen content was adjusted for differences in eclosion size (estimated by wing length). An analysis of covariance, with eclosion size (wing length) as the covariate, detected a significant effect of age on the abdomen and thorax nitrogen contents ($F_{2,160} = 57.31$ and $p < 0.0001$ and $F_{2,160} = 20.10$ and $p < 0.0001$, respectively). There was no effect of sex on the abdomen nitrogen content ($F_{2,160} = 0.506$ and n.s.), but a significant effect of sex on the thorax nitrogen content ($F_{2,160} = 47.80$ and $p < 0.0001$). There was also a significant interaction between sex and age for the thorax nitrogen content ($F_{2,160} = 7.23$ and $p = 0.001$).

compared to younger males (one-way ANCOVA with eclosion size estimated by wing length as the covariate, $F_{2,47} = 16.55$ and $p < 0.0001$ and $F_{2,47} = 5.07$ and $p = 0.010$, respectively).

There seems to be no difference in how males and females use nitrogen in the abdomen (figure 2). Although both sexes show a decrease in their thorax nitrogen content with age, females show a more pronounced decrease.

4. DISCUSSION

(a) Use of body resources

Like most butterflies, the females of *P. napi* use the resources stored in their abdomen for reproduction (Karlsson 1987, 1994, 1998). In addition, Karlsson (1998)

found that females of *P. napi* are also able to use resources from their thorax in reproduction. Thus, it is possible that some of the decrease in the thorax mass and nitrogen content in this study reflects the breakdown of thorax muscles.

In pollen-feeding *Heliconius* spp., which have access to nitrogen as adults, both their abdomen and thorax masses may instead increase, contrary to what is the case for nectar-feeding species (Karlsson 1994). This is expected considering that these butterflies can easily replenish the nutrients they lose in reproduction and stresses the importance of the adult diet in how stored nutrients are used.

The use of flight muscles for reproduction in insects is not uncommon and is most often found in association with migratory morphs; it is generally referred to as the oogenesis-flight syndrome (Johnson 1969). These insects can histolyse their flight muscles and reallocate the nutrients to reproduction. Some species even lose their wings before they start to reproduce. Although butterflies do not lose their wings, the use of resources from the abdomen increases the thorax ratio (mass of the muscles compared to the rest of the body) and renders the flight muscles more powerful than necessary (cf. Srygley & Chai 1990). Since reproduction in most butterflies seems to be limited by the availability of proteins, these animals have an incentive to break down their surplus muscles and re-use the nutrients.

Even if butterflies histolyse and use their flight muscles in reproduction, the breakdown should be constrained by the demands of flight. The animals need to find mates as well as avoid predators. In some species males are territorial and engage in intense flight interactions (Wickman 1992). The use of flight muscles for these males should be more limited than for males where flight is of less importance for acquiring mates.

The fact that the use of thorax resources is a trade-off between reproductive needs and the requirements on somatic function probably means that the use of the thorax muscles can at best be viewed as a supplement to the reproductive reserves in the abdomen and not as resources primarily meant for reproduction.

Female butterflies are usually the larger sex and invest more of their nutrients in reproduction than males (Wiklund & Forsberg 1991; Karlsson 1995). With increasing male nutrient investment in offspring the difference in size decreases (Leimar *et al.* 1994). Males of *P. napi* are slightly larger than the females (table 1) (Wiklund & Forsberg 1991) and it seems that the use of resources in the abdomen is similar between the sexes (figure 2). Since males seem to have a higher demand on flight properties than females because of their mate-searching strategies, they should be more conservative in the breakdown of their thorax muscles. This could explain why the use of thorax resources differs between the sexes in this study (figure 2). It is also interesting to note that Karlsson (1994) found that thorax weight decreased for females but not males of two monandrous species in which reproduction is costly in terms of nutrient investment for the females but not for the males. This difference between mating systems probably reflects the importance of their reproductive output in how resources are used.

(b) *Function of male material*

Karlsson (1998) and Wiklund *et al.* (1993) found that male material increases female longevity in *P. napi*. Thus, since the use of female resources depends on age it thereby also indirectly depends on the amount of male material received. This study indicates that male material may also play a more direct role in the dynamics of female resource use, since the use of nitrogen in the abdomen and perhaps also the thorax depends directly on the number of matings when the effect of life span is controlled for. This could basically be explained in two ways. The male could provide the female with some substance which increases her reproductive investment. In *Danaus plexippus* the male transfers a hormone with the spermatophore which stimulates oviposition (Herman & Barker 1977). This has probably evolved to increase female investment in the progeny of the donor male.

The alternative explanation is that the female can access the amount of male nutrients she has received and change her use of stored reserves accordingly. Since there are differences in the quality of the male gifts, the females should benefit from the ability to assess mate quality. Insect females can exercise mate choice before, during or after mating (Thornhill 1983). Females of *P. napi* do not seem to exhibit pre-copulatory mate choice (Kaitala & Wiklund 1995). However, it is possible that the females change their reproductive effort to correspond to the amount of nutrients they receive from males. Wedell (1996) found that females of *Polygonia c-album* changed their investment of resources according to the quality of the male gift, using proportionally more of their stored resources in egg production when mated to a high-quality male which transfers large amounts of nutrients at mating.

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