Behav Ecol Sociobiol (1993) 33:25–33

Behavioral Ecology and Sociobiology
© Springer-Verlag 1993

Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.)

Christer Wiklund, Arja Kaitala, Virpi Lindfors, and Johan Abenius

Department of Zoology, University of Stockholm, S-10691 Stockholm, Sweden

Received September 24, 1992 / Accepted in revised form March 15, 1993

Summary. In many insects nutrients transferred by the male to the female at mating are later incorporated into both the eggs and soma of the mated females. Accordingly, it has been suggested that female insects can use these male-derived nutrients both for somatic maintenance and to increase both the number and quality of their offspring. Moreover, much discussion is presently devoted to whether the male nuptial gift represents paternal investment, defined as "any increase in given male's total surviving progeny by increasing the reproductive output by a given female", or mating effort which obtains "if a male gains by increasing the proportion of eggs he fertilizes from a given female" (Parker and Simmons 1989). If the male nuptial gift represents parental investment it should be expected to benefit predominantly the offspring sired by the donor, whereas the "physiological fate" of the male nuptial gift is somewhat irrelevant under the mating effort explanation. In this paper we test these issues by studying the lifetime fecundity, egg weights and longevity of two groups of females of the polyandrous green-veined white butterfly, *Pieris napi*, one group of which was allowed to mate only once and the other of which was allowed to mate at liberty, the latter group of females mating on average 2.28 times. Moreover, to test the incorporation rate of male-derived nutrients, we performed a second set of experiments where females were allowed to mate with radioactively labelled males. The results showed that polyandrous females had higher lifetime fecundity compared to monandrous females, laying on average 1.61 as many eggs, and that the difference in cumulative fecundity between the two groups was statistically significant from the 5th day of egg-laying onwards. Polyandrous females also lived longer and maintained egg weight at a high level for longer than monandrous females. Largely concomitant with egg-laying rate, incorporation rate of male-derived nutrients peaked 3–4 days after mating, subsequently tapering off to stabilize at about 40% of the maximum. Given the opportunity, female *P. napi* remated after 3–5 days, the duration of the refractory period being positively correlated with ejaculate mass. Hence, although the nutrient investment of the first male to mate with a female "subsidizes" the progeny of later-mating males, the male nuptial gift in *P. napi* clearly qualifies as both paternal investment and mating effort.

Correspondence to: C. Wiklund

Introduction

In butterflies female mating systems vary between species, from strict monandry, where all females mate only once during their life-time, to strong polyandry, where most females mate several times during their life-time (Burns 1968; Ehrlich and Ehrlich 1978; Drummond 1984; Svärd and Wiklund 1989; Wiklund and Forsberg 1991). Although females may vary within species in the degree of polyandry, it is generally possible to characterise individual species as being essentially monandrous or polyandrous. This is because a number of adaptations have been identified among males of polyandrous species which increase the number of matings males can perform. Hence, it is not only the size of the ejaculate that is larger in more polyandrous species, but also the rate at which males are able to produce sperm and accessory substances (Svärd and Wiklund 1989). Moreover, relative male size is positively correlated with the degree of female polyandry in both satyrid and pierid butterflies (Wiklund and Forsberg 1991). Because females that have received a large ejaculate wait longer before remating than do females that have received a small ejaculate (Oberhauser 1989; A. Kaitala and C. Wiklund in preparation), these male adaptations, which increase the size and production rate of ejaculates, can be seen as resulting from sperm competition.

With the exception of the monarch, *Danaus plexippus*, female butterflies usually seem able to resist male mating attempts (Rothschild 1978; Svärd and Wiklund 1988), and so female polygamy has generally been assumed

to yield some benefit to females. Since Boggs and Gilbert's (1979) discovery that nutrients from the spermatophore delivered from males to females at mating were incorporated into the soma and eggs of female butterflies, polyandry has been viewed as an adaptation whereby females can obtain these male-derived nutrients for egg production and somatic maintenance (Boggs 1981a, b; Boggs and Watt 1981; Parker and Simmons 1989; Boggs 1990). The best evidence to date for female benefits from repeated matings comes from Orthoptera where females of *Requena verticalis* and *Gryllus bimaculatus* have been shown to increase fecundity and egg size as a result of procuring nutrients from spermatophores (Gwynne 1984; Simmons 1988; but see Wedell and Arak 1989, for data from *Decticus verrucivorus* in which female reproductive output was not influenced by male nutrient donations). This is surprising in view of the fact that adult female orthopterans feed on items that are as rich in protein as food items consumed in the larval instars. In butterflies, where adults usually do not feed on food rich in protein [with the exception of the pollen-feeding *Heliconius* spp.; Gilbert 1972; Boggs et al. 1981, and possibly ithomiines that feed on bird droppings, C.L. Boggs (personal communication)] and where the need for proteinaceous nutrients is more apparent, studies of the utility of repeated matings and the importance of male-derived nutrients have yielded somewhat contradictory results. In *Colias eurytheme*, where recently mated males produce only about 40% of the quantity of spermatophore material produced by males that have not recently mated (Rutowski and Gilchrist 1986), females that mated with recently mated males died sooner, had a lower lifefime fecundity, and laid fewer eggs per day overall, especially during the first 2 days after mating (Rutowski et al. 1987). In *Papilio xuthus* Watanabe (1988) showed that multiply mated females had higher lifetime fecundity than singly mated females. However, in *Euphydryas editha* and *E. chalcedona* spermatophore weight did not influence reproductive output (Jones et al. 1986). Likewise, in *Papilio machaon* females that mated with recently mated males (that delivered small spermatophroes) did not have reduced reproductive output compared with females that mated with virgin males (Svärd and Wiklund 1991). The ambiguity of butterfly data is highlighted by two studies on *D. plexippus*, where one study did show and effect of spermatophore weight on female reproductive output (Oberhauser 1989), whereas another study failed to find any evidence for such an effect (Svärd and Wiklund 1988). Recently Boggs (1990) presented a general model of the role of male-derived nutrients in female insects' reproduction which may help explain this variation. Her model predicted patterns of association among the proportion of eggs with yolks at adult female eclosion, the amount of male nutrient donation relative to the female's nutrient budget, and adult foraging habits. Of particular relevance here is the model's prediciton that male nutrients cannot be important for egg production in species in which females eclose with a large proportion of eggs already with yolks, and that the importance of male nutrient donation is inversely related to nutrient acquisition through female foraging. In this study of the reproductive biology of the highly polyandrous green-veined white butterfly, *Pieris napi*, in which the females do eclose from the pupa with a large proportion of the eggs already with yolks, we show that multiply mated females have considerably higher life-time fecundity, maintain relatively large egg size for longer and live longer than singly mated females.

The idea that males transfer nutrients, as well as sperm, to females at mating has sparked off a discussion as to whether the male gift represents paternal investment or mating effort. According to Simmons and Parker (1989) "any increase in a given male's total surviving progeny by increasing the reproductive output by a given female represents paternal investment", whereas mating effort obtains "if a male gains by increasing the proportion of eggs he fertilizes from a given female". Hence, if the male gift represents paternal investment the male nutrient investment should be expected to benefit primarily the offspring of the donating male (Bowen et al. 1984; Gwynne 1984, 1986, 1988; Wickler 1985; Rutowski et al. 1987; Simmons and Parker 1989). However, if the male gift represents mating effort, the main funciton should be to increase the proportion of offspring sired, which presumably is best effected by delaying the female's remating. In this case the "physiological fate" of the male's nutrients is irrelevant and would be expected not to benefit exclusively the offspring sired by the donor male (Wickler 1985; 1986; Wedell and Arak 1989; Wedell 1992; Simmons and Parker 1989). As should be obvious, paternal-investment and mating-effort explanations for the function of the male's nutrient gift are not mutually exclusive. To test the duration of a female's usage of a male's nutrient investment, and whether the female's utilization pattern of a first male's nutrient contribution is affected by subsequent matings by the female, we studied how radioactively labelled materials from two males, labelled with different isotopes and both mated with the same female, appeared in the eggs laid throughout the life time of that female.

Materials and methods

Female reproduction in relation to number of matings. In order to test whether male-derived nutrients influence fecundity, egg weight and longevity of females of *P. napi* we performed three replicates of one experiment. The data from these replicates are presented separately because both fecundtiy and longevity of females differed between replicates precluding the pooling of data for statistical analysis. (The most likely reason why results differed between replicates is that the butterflies were maintained in greenhouses in which temperatures covaried with outdoor conditions, thus affecting female reproductive output which is known to be strongly dependent on temperature). The animals used in this study were derived from animals collected in the vicinity of Stockholm, where *P. napi* has two generations per year and hibernates in the pupal stage. The first replicate took place in May/June 1989 using adults eclosing from overwintering pupae, whereas the remaining two replicates used adults from directly developing pupae and were performed in September/October 1990 and 1991.

On the day of eclosion from the pupa all adults were marked individually with a Staedler permanent pen on the underside of

the hindwing and weighed to the nearest tenth of milligram on a Sauer electrobalance. Then males and females were transferred to a mating cage which was checked for matings at least every 15 min. Since matings involving virgin males and females last for an average of about 1.5 h, no matings are likely to have passed unnoticed. Pairs in copula were transferred to a transparent 0.5-l plastic cup, to prevent them from being disturbed by conspecifics during mating. When mating had ended the females were transferred to a 0.5-m high egg-laying cage with the sides measuring 0.8×0.8 m. After mating females were randomly assigned to one of two groups: polyandrous females which had one virgin male present in their cage throughout their life time, and monandrous females which were completely alone in the cage throughout their life time. Altogether 20 egg-laying cages were available for simultaneous use, and they were located in two rows in each of two greenhouses. To avoid systematic effects on female performance due to the location of the egg-laying cage, polyandrous and monandrous females were placed in the rows of egg-laying cages in an A-B-A-B pattern, so that a polyandrous female was always put in a cage next to a monandrous female, and vice versa.

Females of the two groups were approximately equal in size, the mean weight of the monandrous females being slightly, but not significantly, higher than that of the polyandrous females. The bottoms of the egg-laying cages were covered with cleansing paper, which was wetted once or twice daily to maintain high humidity. The butterflies were provided with flowers for nectar, *Tussilago farfara* and potted *Chrysanthemum* spp. in the spring, and *Cirsium arvense* in late summer and autumn. These flowers were supplied with extra food for the butterflies once or twice every day in the form of 25% sucrose solution which was added to the flowers with a syringe. The females were provided with leaves of the crucifer *Alliaria petiolata* for egglaying. The *Alliaria* leaves were supplied with water from a bottle in which the petiole of the leaf was inserted, and after each day of egg-laying the eggs laid were counted and a new leaf added to the cage. In the first two experiments eggs were weighed every 3rd day, but in the third experiment eggs were weighed every day. Before weighing, eggs were removed from the leaf with the aid of a fine brush. During the first two experiments 10 eggs from each female were weighed, whereas 20 eggs from each female were weighed every day in the third experiment. If fewer eggs were laid, all of the female's eggs laid that day were weighed. Eggs from the same female laid on the same day were pooled before being weighed to the nearest microgram on a Cahn 26 electrobalance.

The egg-laying cages were inspected at least every hout to check for rematings of females assigned to the polyandrous group. After death all females were dissected to assess the number of times they had mated. The result of the spermatophore count tallied well with the rematings observed by visual inspection, and only 1 mating out of the 31 matings over the total of 94 days through which the three experiments were run went unnoticed, as inferred from the spermatophore count (i.e. one female in the polyandrous group contained three spermatophores although she had only been observed to remate once). In total 22 females were assigned to the monandrous group, whereas 27 females were assigned to the polyandrous group. However, among females assigned to the polyandrous group two failed to remate and were therefore excluded from analysis. Of the 25 females that did remate, 18 females mated twice and 7 females mated three times.

Female reproduction in relation to male presence/absence. Because monandrous females, once they had mated, were kept alone in their cages during the remainder of their life whereas the group of polyandrous females always had the company of a male, we wanted to control for the stimulatory effect a male cage-companion might have on female egg-laying pattern. We therefore compared the cumulative fecundity of two groups of monandrous females, one group of 18 females that were kept alone in their egg-laying cages after mating, and the other group of 23 females that were kept with a male in the egg-laying cages throughout their lifetime. This experiment was performed during May/June and August/September 1991, and since previous results had shown that the difference in cumulative fecundity between monandrous and polyandrous females was apparent within the first 5 days of egg-laying, the experiment was terminated after 14 days of egg-laying for each individual female. The virgin males used in this experiment vigorusly courted the females throughout their lives, but were prevented from mating because their genital claspers were glued together by using Casco RX Liima glue. As before, the egg-laying cages were checked every hour for matings, and four matings were observed, indicating that the gluing of those males had been unsuccessful. All females were dissected after death, and only 1 of the 19 females that had only been observed to mate once in fact had two spermatophores, showing that she had remated unnoticed. Thus five females assigned to the "male-accompanied" monandrous group of females had actually mated twice and were as a consequence excluded from analysis, leaving 18 females each in the two experimental groups. The females were treated like the females in the first three experiments, and were also placed in the egg-laying cages alternately as in the previous experiments.

Female reproduction: incorporation rate of male-derived nutrients in eggs. In order to study how females used nutrients from male ejaculates, females were assigned to one of four procedures. Three females were allowed to mate only once with radioactively labelled males, after which their eggs were collected every day for a period of 11–17 days, and analysed for presence of radioactivity. In order to study if incorporation rate of radioactive male-derived materials was influenced by whether the females first mated with a radioactively labelled male or if the female mated with a radioactively labelled male after first having mated with an unlabelled male, four females first mated with unlabelled males and then remated with radioactively labelled males. As before the eggs of these females were collected every day for 7–23 days after the day of remating, and analysed for radioactivity.

In order to study how repeated matings influenced the incorporation of materials from males, one female was first mated with a radioactively labelled male, and later allowed to remate with an unlabelled male. The eggs of this female were collected for 19 days after the first mating, and 12 days after the second mating, (the female remated 7 days after the first mating), and analysed for presence of radioactivity. In addition, four females were first mated with males who were labelled with ^{14}C, and later allowed to remate with males labelled with ^{3}H. As before, the eggs laid by these females were collected every day for 19–22 days after the first mating, and analysed for ^{14}C and ^{3}H radioactivity, respectively. The females treated according to all of the first three procedures above all mated with males labelled with ^{3}H.

Males in these experiments were radioactively labelled by first allowing them to mate once, and then transferring them to a cage where they were kept for a minimum of 4 days during which they were given a 25% sucrose solution into which a ^{3}H amino acid mixture (37.0 Mbq/ml, 1.0 mCi/ml in 2% ethanol; Amersham) or a U-^{14}C protein hydrolysate (specific activity 57 mCi/mAtom, concentration 50 microCi/ml in 2% ethanol; Amersham) was added. Previous experiments have shown that males of *P. napi* recuperate quickly and are able to transfer an ejaculate as large as the one transferred by virgin males after refraining from mating for a period of at least 4 days (Svärd and Wiklund 1989).

From each female a maximum of 20 eggs were collected every day and weighed to the nearest microgram on a Cahn 26 Electrobalance. After being transferred to a glass vial the eggs were dissolved using a tissue solubilizer (Soluene 350 Packard), whereafter they were tested for radioactivity by adding 5 ml of scintillating fluid (HiSafe, Fermenta) and counting radio-activity with a 1214 Rack-Beta LKB Wallac scintillation counter. Each sample was counted for 10 min and one replicate was performed. Resulting disintegrations per minute were corrected for background activity and for quenching using a standatd quench curve. Because our aim was to assess the incorporation rate of male-derived nutrients into eggs, we calculated radioactive counts per minute in relation to egg weight for each female every day, and give values on radioactivity

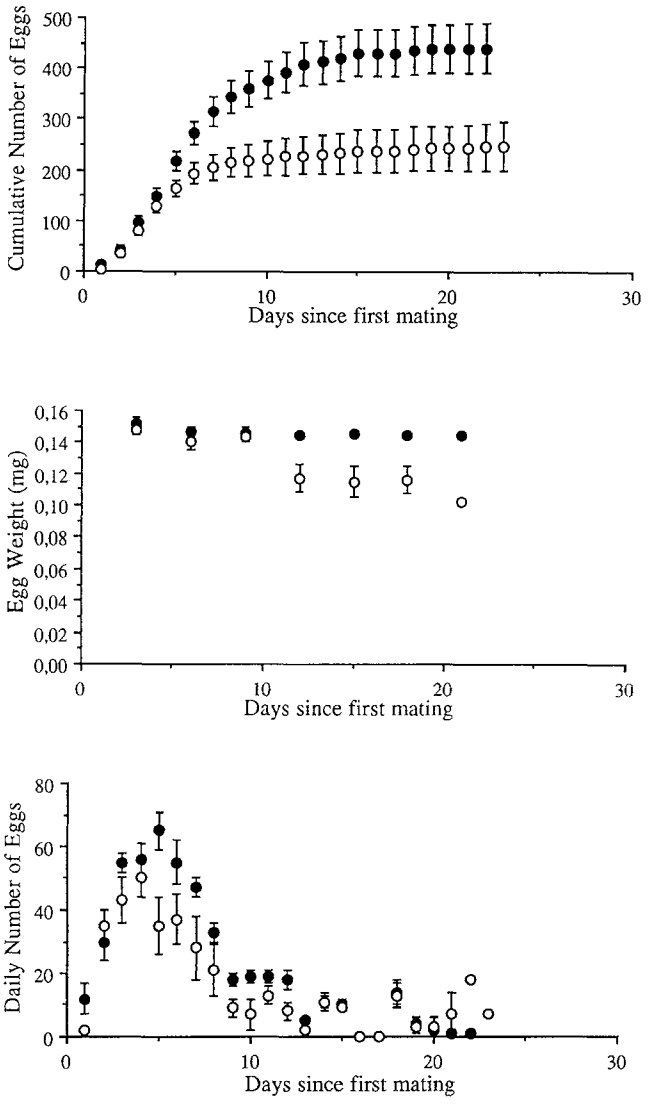


Fig. 1. Cumulative fecundity, mean daily fecundity, and mean daily egg weight for polyandrous (*filled circles*) and monandrous (*open circles*) first-generation females (that had hibernated as pupae) in replicate. 1. SE is indicated by *bars*

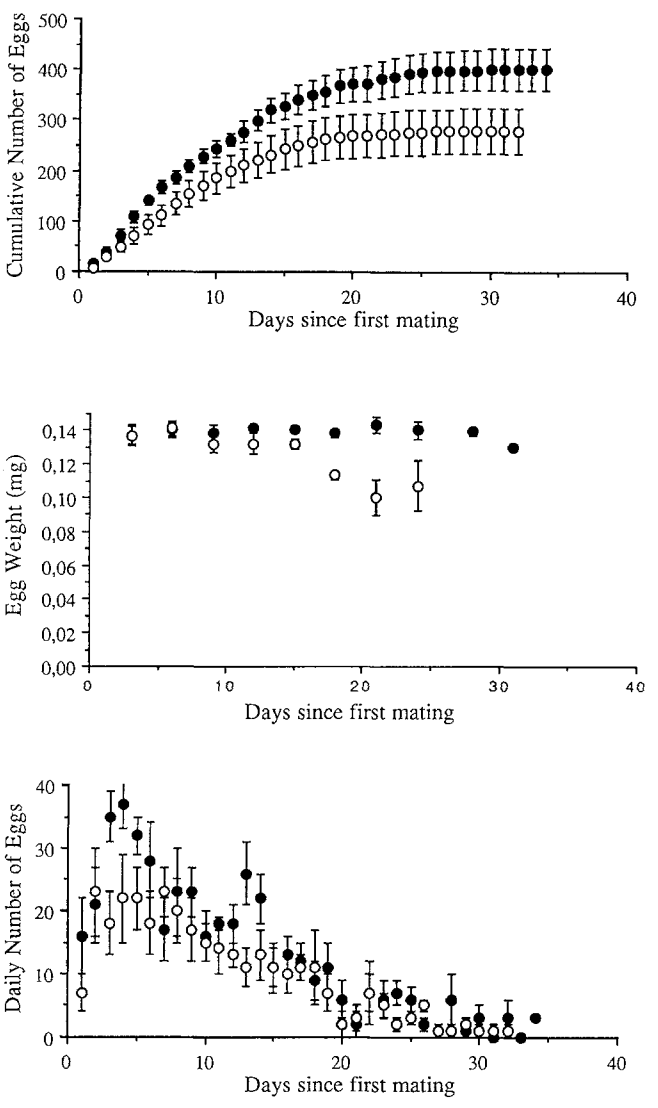


Fig. 2. Cumulative fecundity, mean daily fecundity, and mean daily egg weight for polyandrous (*filled circles*) and monandrous (*open circels*) second-generation females, that had developed directly, in replicate 2. SE is indicated by *bars*

of eggs as proportions in relation to the maximum value for each female. Hence all values on radioactivity of eggs are given as percentages of the maximum weight-related value.

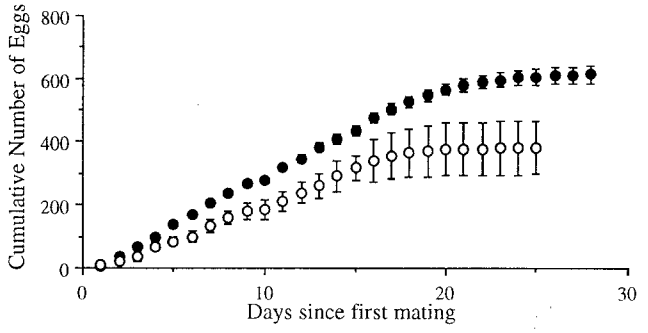
Results

Polyandrous females had substantially higher lifetime fecundity than did monandrous females in all three replicates (Table 1; Figs. 1–3). The quotient between the polyandrous and monandrous groups ranged from 1.44 (403/280) to 1.62(611/378) to 1.78(440/247) in the three replicates, the mean of the three ratios showing that polyandrous females laid a mean of 1.61 as many eggs as monandrous females. Polyandrous females also lived longer, on average, than monandrous females, but this difference was statistically significant only in the third replicate (Table 1). However, a combined analysis shows that the positive effect of multiple mating on female longevity was statistically significant when all three replicates are considered ($\chi^2 = 6.93$; df = 6; $P < 0.05$; combined probabilities from independent tests of significance; Sokal and Rohlf 1981). Polyandrous females also maintained egg weight at a high level for longer than monandrous females, and the difference in relative egg weight was statistically significant after 15 days of egg-laying (Fig. 1–4).

The difference in cumulative fecundity was significant beginning with the 5th day of egg-laying (Table 1); this was surprising in view of the fact that only 11 out

Table 1. Fecundity and longevity in relation to monandry/polyandry in *Pieris napi*. Results are given as means ± SE

Experiment		Females		*df*	*t*	*P*
		Monandrous	Polyandrous			
1	Fecundity during first 5 days	164 ± 17	219 ± 19	15	2.18	0.04
	Lifetime fecundity	247 ± 47	440 ± 49	15	2.85	0.01
	Longevity	11 ± 3	18 ± 2	15	1.91	0.08
2	Fecundity during first 5 days	92 ± 19	140 ± 9	11.6	2.26	0.04
	Lifetime fecundity	280 ± 45	403 ± 40	15	2.03	0.06
	Longevity	22 ± 3	26 ± 3	15	1.05	0.31
3	Fecundity during first 5 days	85 ± 14	137 ± 11	11	2.78	0.02
	Lifetime fecundity	378 ± 83	611 ± 46	11	2.61	0.02
	Longevity	20 ± 3	26 ± 2	11	2.31	0.04



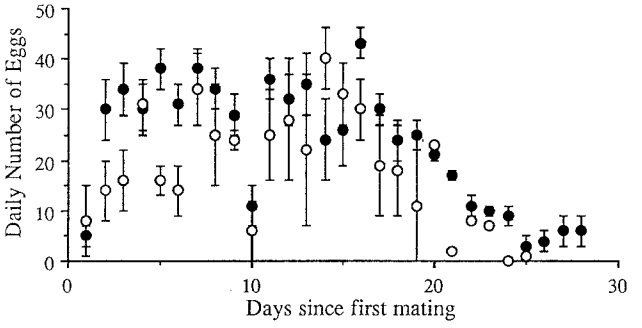
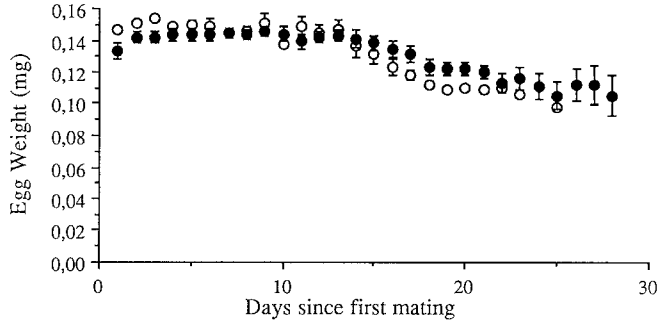


Fig. 3. Cumulative fecundity, mean daily fecundity, and mean daily egg weight for polyandrous (*filled circles*) and monandrous (*open circles*) second-generation females, that had developed directly, in replicate 3. During this replicate the outdoor temperature dropped to below freezing point when the females were some 10 days old. The temperature also dropped below that necessary for butterfly activity in the green house, and all butterflies were transferred to individual egg-laying cages in the laboratory whereafter they recommenced egg-laying without apparently having suffered any harm. The drastic decrease in the daily number of eggs laid can be seen on day *10*. SE is indicated by *bars*



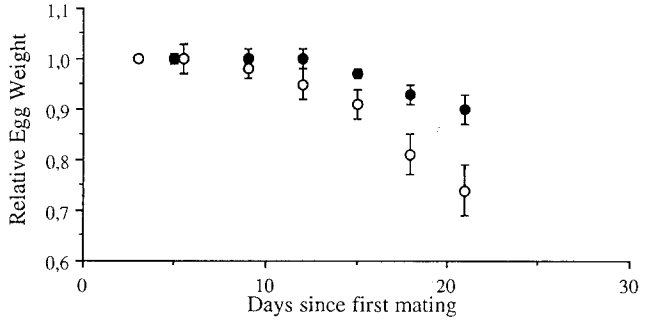


Fig. 4. Change over time in mean daily relative egg weight (mean egg weight relative to mean egg weight of eggs laid during the 3rd day of egg-laying) for polyandrous (*filled circles*) and monandrous (*open circles*) females. SE is indicated by *bars*. The difference in mean weight of eggs laid by monandrous and polyandrous females, respectively, was statistically significantly different at the 0.05 level on day 15, and at the 0.01 level on days 18 and 21

of 25 females in the polyandrous group had remated before the 5th day of egg-laying (Fig. 5). Since females in the polyandrous group were accompanied by one virgin male throughout their lifetime, we tested if male presence per se had any effect on female fecundity. However, there was no statistically significant difference in cumulative fecundity during the first 5 days between solitary females (90 ± 13 mean ± SE) and those accompanied by males (96 ± 10 mean ± SE) (Fig. 6). Neither was there any significant difference in cumulative fecundity over the whole 14 days of the experiment (253 ± 21 mean ± SE) in the male-accompanied group and 229 ± 26 mean ± SE in the solitary group, values which correspond well with those of solitary monandrous females in the first experiment (Table 1 and Figs. 1–3). Hence we conclude that male presence does not seem to have a strong effect on female fecundity, and that higher fecundity of polyandrous females is most likely due to the effect of male-derived nutrients transferred to females at mating.

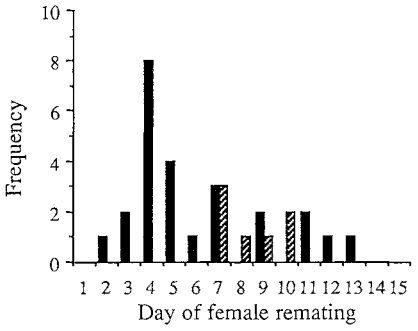


Fig. 5. The time distribution of first (*black bars*) and second matings (*shadded bars*) for the polyandrous group of females, i.e. females that were accompanied by a virgin male throughout their life-time

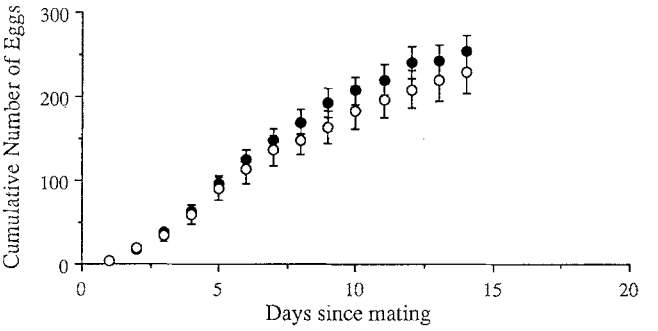


Fig. 6. Cumulative fecundity over 14 days for monandrous females that were accompanied by a virgin "mating-incapacitated" male during egg-laying (*black circles*) and monandrous females that were alone during egg-laying (*open circles*). SE is indicated by *bars*

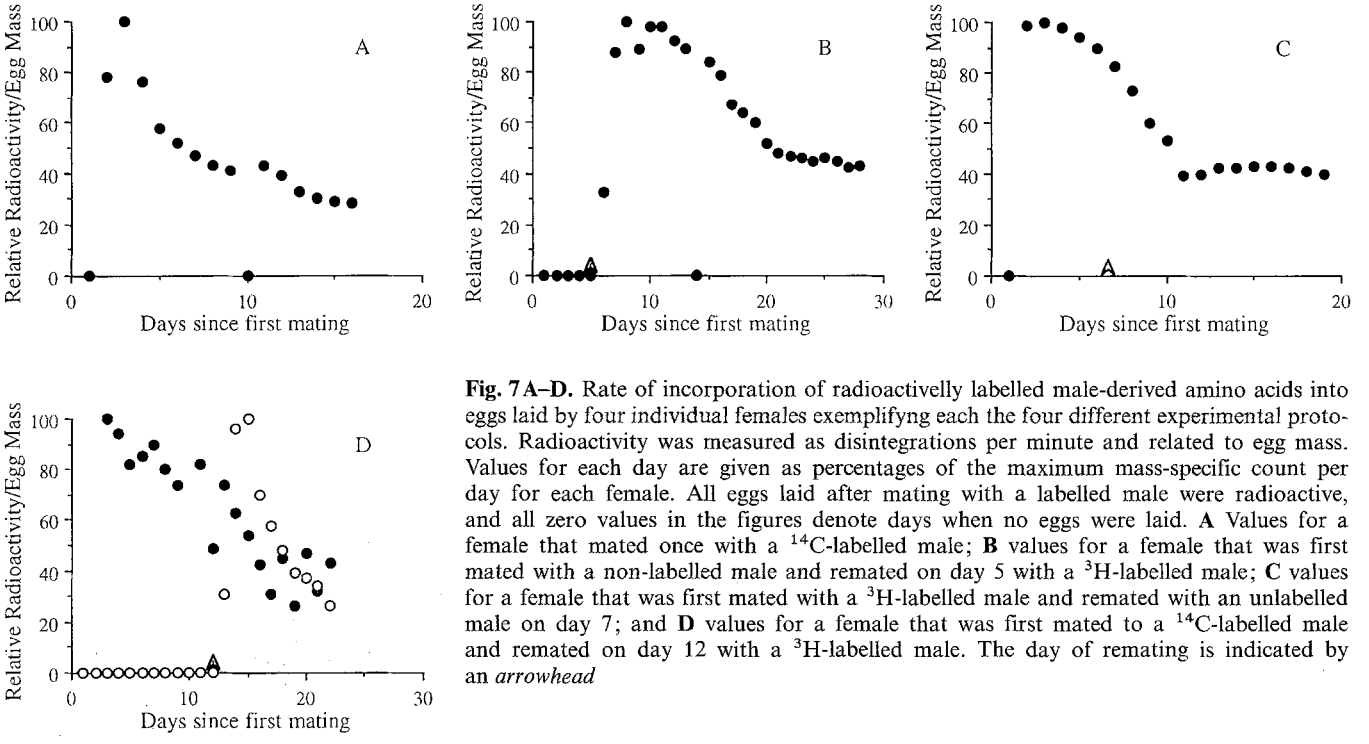


Fig. 7A–D. Rate of incorporation of radioactivelly labelled male-derived amino acids into eggs laid by four individual females exemplifyng each the four different experimental protocols. Radioactivity was measured as disintegrations per minute and related to egg mass. Values for each day are given as percentages of the maximum mass-specific count per day for each female. All eggs laid after mating with a labelled male were radioactive, and all zero values in the figures denote days when no eggs were laid. **A** Values for a female that mated once with a ^{14}C-labelled male; **B** values for a female that was first mated with a non-labelled male and remated on day 5 with a ^{3}H-labelled male; **C** values for a female that was first mated with a ^{3}H-labelled male and remated with an unlabelled male on day 7; and **D** values for a female that was first mated to a ^{14}C-labelled male and remated on day 12 with a ^{3}H-labelled male. The day of remating is indicated by an *arrowhead*

The experiments designed to examine the rate of incorporation of male-derived nutrients by females suggest that incorporation rate does not differ for materials derived from a female's first and second mating. Hence, incorporation of male-derived nutrients into eggs of the three females that had radioactively labelled males as their first mates, reached a peak after an average of 4.0 ± 1.0 days (mean $\pm$ SE), whereas that of the four females that had radioactively labelled males as their second mates reached a peak after an average of 3.5 ± 0.3 days (mean $\pm$ SE) after a second mating (Fig. 7). Moreover, there seems to be no evidence to suggest that female incorporation rate of a fist male's nutrients is affected by subsequent matings. This follows from Fig. 7, which shows that females incorporated nutrients derived from the first male they mated with throughout their lives, and even the very last eggs laid by these five females contained some 40% of the peak value of some 3 weeks earlier.

This extended period of female utilization of male-derived nutrients is interesting in view of the fact that all female rematings took place within the first 2 weeks of the female's life (Fig. 5). This is noteworthy because females were intensively courted by males throughout their lives and the average longevity in the three experiments varied from 18 to 26 days, with some females living to be 36 days old.

Discussion

Female benefit from multiple mating

The results show that polyandrous females have higher lifetime fecundity, live longer and maintain egg weight

for longer than monandrous females. On the assumption that incorporation of radioactively labelled amino acids into eggs reflects the incorporation rate of male-derived nutrients in general, the radiotracer study shows that the peak of female incorporation of male-derived nutrients into eggs occurs after 3–4 days, irrespective of whether the female receives the male-derived material when mating for the first or the second time. Moreover, the incorporation rate of male-derived nutrients into eggs decreases slowly, and appears to be unaffected by whether the female remates or not.

Under what circumstances should male nutrient contribution of female reproduction be expected? The model of Boggs (1990) predicts that females would be unlikely to benefit from male nutrient investment in species in which they eclose with a large proportion of their eggs already yolked, but also that there ought to be an inverse relationship between the importance of male nutrients and female ability to acquire proteins from their own feeding activity. At face value, the observation that females of *P. napi* do increase their reproductive output as a result of male nutrient contribution at mating seems to contradict the prediction of Boggs (1990), because females of *P. napi* do eclose with a large proportion of their eggs already yolked. However, the results are in agreement with the second part of the prediction because female *P. napi* feed on nectar, a food source which generally has a low protein content (Baker and Baker 1973; Watt et al. 1974).

In general, the importance of male nutrients for female reproduction has been hypothesized to be associated with (1) the degree of female polyandry, (2) the mass of the ejaculate transferred by the male to the female at mating, and (3) female longevity (Svärd and Wiklund 1988, 1991). In the study of Boggs (1990) females of the polyandrous *Heliconius cydno* were much more dependent on male nutrients than females of the largely monandrous *H. charitonius*. Likewise, females of *Colias eurytheme* (Rutowski et al. 1987) and *P. napi* (this study), which have been shown to use male-derived nutrients to increase their reproductive output, are both strongly polyandrous, whereas no evidence has been found for female utilization of male-derived nutrients in the largely monandrous *Euphydryas editha*, *E. chalcedona* (Jones et al. 1986) or *Papilio machaon* (Svärd and Wiklund 1991).

In a comparative study of 25 butterfly species from three different families, Svärd and Wiklund (1989) showed that the mass of the male ejaculate was positively correlated with the degree of female polyandry, which may be interpreted as circumstantial evidence in agreement with the hypothesis that there should be a positive association between ejaculate mass and female utilization of male-derived nutrients for their reproduction.

Little is known about longevity of female butterflies, and so it is difficult to test the hypothesis about a positive association between female longevity and use of male-derived nutrients. Moreover, the association between the two parameters is such that relatively long female life-expectancy is a necessary, but not sufficient, condition for female ability to use male-derived nutrients. However, the results from the experiments on *P. napi* are of interest with respect to the issue of longevity. First, the radiotracer study showed that incorporation rate of male-derived nutrients peaked after 3–4 days, and subsequently tapered off to stabilize at about 40% of the maximum value. This relatively long-term use of male-derived nutrients is in agreement with results from other insects (cf. Markow 1988; Markow et al. 1990; Pitnick et al. 1991), and may indicate a physiological constraint on female capacity to use up male-derived materials rapidly (cf. Oberhauser 1992). This may explain both why females are not more polygamous than they are (i.e. set a limit to female polyandry) and why females cease to mate after 2 weeks of adult life (Fig. 5), in spite of their being constantly courted throughout their lives in our experiments. This suggests that the cost/benefit balance for further matings changes at that point in the female's life, perhaps because there is too low a likelihood that the female will live long enough to have time to reap the benefit in terms of enhanced future reproduction set against the more immediate time cost of additional matings.

Male benefit from nuptial gifts: paternal investment

Multiply mated females have much higher lifetime fecundity (Fig. 1–3). Although the ejaculate in *D. plexippus* has been shown to contain hormones that stimulate female oviposition (Herman and Barker 1977), as in many other insects (cf. Chen 1984; Gromko et al. 1984), this can only influence the *rate* of female egg-laying, not the *number* of eggs that a female can produce during her lifetime, at least under laboratory conditions when females usually die with very few eggs remaining in the abdomen. Moreover, the egg-laying rate of young females reached a peak of 3–4 days after mating, and was largely concomitant with the peak of the incorporation rate of male-derived nutrients (Fig. 1–3, and Fig. 7). Hence, the male nuptial gift increased his progeny by enhancing the reproductive output by the female mated with, and so qualifies as paternal investment *sensu* Parker and Simmons (1989).

Male benefit from nuptial gifts: mating effort

In *D. plexippus* Oberhauser (1988) has shown that females that have received a small ejaculate at mating remate sooner than do females that have received a large ejaculate. Likewise, A. Kaitala and C. Wiklund (submitted) have shown that females of *P. napi* mated to virgin males (that deliver large ejaculates) on average remate after 5 days, whereas females mated to recently mated males (that deliver small ejaculates) on average remate after 3 days. Because the last male to mate with a female usually fertilizes the majority of the remaining eggs in *P. napi* as in the majority of insects (A. Kaitala and C. Wiklund, in preparation; Labine 1966; Drummond 1984), the size of the ejaculate transferred to the female at mating has a strong influence on the proportion of

eggs sired. Hence, the male ejaculate also qualifies as mating effort *sensu* Parker and Simmons (1989).

The mechanism by which females become unreceptive in *P. napi* has not been studied, but in the Lepidoptera in general two factors are thought to control female receptivity: (1) the mechanical pressure of the ejaculate in the bursa copulatrix, detected by stretch receptors that relay the information either by afferent nerve impulses (Labine 1964; Obara et al. 1975; Sugawara 1979; Drummond 1984) or by release of a hormone into the blood (Obara 1982), or (2) the presence of sperm in the spermatheca (for references see Drummond 1984).

Male nutrient gift as both paternal investment and mating effort

Discussion about whether to regard male nupital gifts as paternal investment or mating effort has been rather heated, perhaps because the issue has been formulated as a dichotomy, giving the impression that the two functions are mutually exclusive. Clearly this is not the case. The general picture that emerges from our experiments on *P. napi* is that the male ejaculate contains a substantial amount of nutrients, which are used by females to increase not only the number (and perhaps quality) of offspring sired by the donor male, but also to "subsidize" the offspring of males that mate with the female at a later time. Although empirical data indicate that a substatantial part of a mating male's nuptial gift is used to favour his own offspring (Rutowski et al. 1987; this study) and theoretical models also predict that females should benefit from using male nuptial gifts rapidly (Simmons and Parker 1989) the utilization pattern we found is consistent with the view that males "lose control" over their gift once it has been transferred to the female.

It has been argued that the evolution of male nupital gifts would be difficult to explain if the nutrients from the first male to mate with a female are later used for subsidizing the progeny of later-mating males. However, in a mating system where all females mate twice, all males should, on average, also mate twice. Given that the distribution of matings with virgins and once-mated females is equal between males, so that all males, on average, mate with one virgin female and one previously mated female, this means that males will have their nutrient gift partly usurped by later-mating males when mating with a virgin female. On the other hand, when mating with a previously mated female, they will have their own progeny partly subsidized by the first male that had mated with that given female. Hence, in a polygamous insect mating system it is likely that males mating with young females will have their nuptial gifts partly usurped by later-mating males, whereas their nutrient investment is more likely to exclusively benefit their own progeny when mating with older females. It could be argued that such a system is not stable against male cheating, e.g. with males withholding from nutrient investment when mating with young females. However, in view of the strongly male-biased operational sex-ratio exhibited by virtually all butterfly populations studied, matings are hard to come by for males. This means that it is doubtful whether males would ever benefit from withholding resources when mating; all available data seem compatible with the view that butterfly males "empty themselves" at each mating (Svärd and Wiklund 1986, 1989).

Emprical data from *P. napi* (Forsberg and Wiklund 1989) and *Anthocharis cardamines* (Wiklund and Forsberg 1985) show that male butterflies clearly distinguish between virgin and previously mated females, and that virgin males are preferentially courted. This male preference for virgin females, in spite of the likelihood of having their nuptial gift usurped by later-mating males, is understandable in terms of young females having a much higher reproductive value. This is evidenced by the pattern common in female butterflies where the daily number of eggs laid usually peaks at 2–4 days of age, and later declines rather rapidly (Svärd and Wiklund 1989, 1991; this study). In fact male nutrient investment at mating helps explain why mating with old females is beneficial to males at all, since in so doing the males actually boost the reproductive value of the female mated with quite substantially, whereas, in systems without male nuptial gifts, mating with an old female with relatively few eggs remaining to be laid may be unprofitable.

Acknowledgements. We thank Peter Abrams, Carol Boggs, Charlene Higgins, Leigh Simmons, Birgitta Tullberg and Nina Wedell for comments on the MS. Moreover, our views on the role of male nuptial gifts were influenced by views espoused by Robin Baker when acting as faculty opponent for Nina Wedell's disputation. This study was supported by the Swedish Natural Science Research Council.

References

Baker HG, Baker I (1973) Amino acids in nectar and their evolutionary significance. Nature 241:543–545

Boggs CL (1981a) Selection pressures affecting male nutrient investment at mating in Heliconiine butterflies. Evolution 35:931–940

Boggs CL (1981b) Nutritional and life-history determinants of resource allocation in holometabolous insects. Am Nat 117:692–709

Boggs CL (1990) A general model of the role of male-donated nutrients in female insect's reproduction. Am Nat 136:598–617

Boggs CL, Gilbert LE (1979) Male contribution to egg production in butterflies evidence for transfer of nutrients at mating. Science 206:83–84

Boggs CL, Watt WB (1981) Population structure of pierid butterflies. IV. Genetic and physiological investment in offspring by male *Colias*. Oecologica 50:320–324

Boggs CL, Smiley JT, Gilbert LE (1981) Patterns of pollen exploitation by *Heliconius* butterflies. Oecologia 48:284–289

Bowen BJ, Codd CG, Gwynne DT (1984) The katydid spermatophore (Orthoptera: Tettigoniidae): Male nutritional investment and its fate in the mated female. Aust J Zool. 32:23–31

Burns JM (1968) Mating frequency in natural populations of skippers and butterflies as determined by spermatophore counts. Proc Natl Acad Sci USA 61:852–859

Chen PS (1984) The functional morphology and biochemistry of insect male accessory glands and their secretions. Annu Rev Entomol 29:233–255

Drummond BA III (1984) Multiple mating and sperm competition in the Lepidoptera. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic, New York, pp 291–370

Ehrlich AH, Ehrlich PH (1978) Reproductive strategies in the butterflies: I. Mating frequency, plugging, and egg number. J Kansas Entomol Soc 51:666–697

Forsberg J, Wiklund C (1989) Mating in the afternoon: time saving in courtship and remating by females of a polyandrous butterfly, *Pieris napi*. Behav Ecol Sociobiol 25:349–356

Gilbert LE (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies. Proc Natl Acad Sci USA 69:1403–1407

Gromko MH, Gilbert DG, Richmond RC (1984) Sperm transfer and use in the multiple mating system of *Drosophila*. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic, New York, pp 371–426

Gwynne DT (1984) Courtship feeding increased female reproductive success in bush crickets. Nature 307:361–363

Gwynne DT (1986) Stepfathers in insects and their pseudo-paternal investment. Ethology 71:74–77

Gwynne DT (1988) Courtship feeding benefits the mating male's offspring. Behav Ecol Sociobiol 23:373–377

Herman WS, Barker JF (1977) Effect of mating on monarch butterfly oogenesis. Experentia 33:688–689

Jones KN, Odendaal FJ, Ehrlich PR (1986) Evidence against the spermatophore as paternal investmant in the checkerspot butterflies (*Euphydryas*: Nymphalidae) Am Midl Nat 116:1–6

Labine P (1964) Population biology of the butterfly, *Euphydryas editha*. I. Barriers to multiple inseminations. Evolution 18:335–336

Labine P (1966) The population biology of the butterfly, *Euphydryas editha*. IV. Sperm precedence – a preliminary report. Evolution 20:580–586

Markow TA (1988) *Drosophila* males provide a material contribution to offspring sired by other males. Funct Ecol 2:77–79

Markow TA, Gallagher PD, Krebs RA (1990) Ejaculate-derived nutritional contribution and female reproductive success in *Drosophila mojavensis*. Funct Ecol 4:67–73

Obara Y (1982) Mate refusal hormone in the cabbage white butterfly? Naturwissenschaften 69:551–552

Obara Y, Tateda H, Kuwabara M (1975) Mating behaviour of the cabbage white butterfly, *Pieris rapae crucivora*. V. Copulatory stimuli inducing changes of female response patterns. Zool Mag 84:71–76

Oberhauser K (1988) Male monarch butterfly spermatophore mass and mating strategies. Anim Behav 36:1384–1388

Oberhauser K (1989) Effects of spermatophores on male and female monarch butterfly reproductive success. Behav Ecol Sociobiol 25:237–246

Oberhauser K (1992) Rate of ejaculate breakdown and intermating intervals in monarch butterflies. Behav Ecol Sociobiol 31:367–373

Parker G, Simmons L (1989) Nuptial feeding in insects: Theoretical models of male and female interests. Ethology 82:3–26

Pitnick S, Markow TA, Riedy MF (1991) Transfer of ejaculate and incorporation of male-derived substances by females in the *nannoptera* species group (Diptera: Drosophilidae). Evolution 45:774–780

Rothschild M (1978) Hell's angels. Antenna 2:38–39

Rutowski RL, Gilchrist GW (1986) Copulation in *Colias eurytheme* (Lepidoptera: Pieridae): patterns and frequency. J Zool 209:115–124

Rutowski RL, Gilchrist GW, Terkanian B (1987) Female butterflies mated with recently mated males show reduced reproductive output. Behav Ecol Sociobiol 20:319–322

Simmons L (1988) The contribution of multiple mating and spermatophore consumption to the lifetime reproductive success of female field crickets (*Gryllus bimaculatus*). Ecol Entomol 13:57–69

Simmons L, Parker G (1989) Nuptial feeding in insects: Mating effort versus paternal investment. Ethology 81:332–343

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, New York

Sugawara ((1979) Stretch reception in bursa copulatrix of the butterfly, *Pieris rapae crucivora*, and its role in behaviour. J Comp Physiol 130:191–199

Svärd L, Wiklund C (1986) Different ejaculate delivey strategies in first versus subsequent matings in the swallowtail butterfly *Papilio machaon*. Behav Ecol Sociobiol 18:325–330

Svärd L, Wiklund C (1998) Fecundity, egg weight and longevity in relation to multiple matings in the monarch butterfly. Behav Ecol Sociobiol 23:39–43

Svärd L, Wiklund C (1989) Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. Behav Ecol Sociobiol 24:395–402

Svärd L, Wiklund C (1991) The effect of ejaculate mass on female reproductive output in the European swallowtail butterfly *Papilio machaon*. J Insect Behavior 4:33–41

Watanabe M (1988) Multiple matings increase the fecundity of the yellow swallowtail butterfly, *Papilio xuthus*, in summer generations. J Insect Behavior 1:17–30

Watt WB, Hoch PC, Mills SG (1974) Nectar resource use by *Colias* butterflies. Chemical and visual aspects. Oecologia 14:353–374

Wedell N, Arak A (1989) The wartbiter spermatophore and its effect on female reproductive output (Orthoptera: Tettigoniidae, *Decticus verrucivorus*). Behav Ecol Sociobiol 24:117–125

Wedell N (1992) Protandry, spermatophore size and mate assessment in the wartbiter *Decticus verucivorus* (Orthoptera: Tettigoniidae). Behav Ecol Sociobiol 31:301–308

Wickler W (1985) Stepfathers in insects and their pseudo-paternal investment. Z Tierpsychol 69:72–78

Wickler W (1986) Mating costs versus paternal investment: a reply to Gwynne. Ethology 71:78–79

Wiklund C, Forsberg J (1985) Courtship and mate discrimination between virgin and mated females in the orange tip butterfly, *Anthocharis cardamines*. Anim Behav 34:328–332

Wiklund C, Forsberg J (1991) Sexual size dimorphism in relation to female polygamy and protandry in butterflies: a comparative study of Swedish Pieridae and Satyridae. Oikos 60:373–381