

Sexual conflict and cooperation in butterfly reproduction: a comparative study of polyandry and female fitness

Christer Wiklund*, Bengt Karlsson and Olof Leimar

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

Most butterfly species can be characterized as capital breeders, meaning that reproductive output is strongly coupled to the amount of resources they have procured during the larval stage. Accordingly, female fecundity is generally correlated with female mass, both within and across species. However, the females of some species can be partly characterized as income breeders, in the sense that their reproductive output is dependent not only on larval-derived capital but also on resources acquired during the adult stage. These adult resources can be derived from female feeding or from male-transferred nuptial gifts. Recent studies on the within-species effects of multiple matings on female fitness show that females generally gain directly from multiple matings in terms of increased lifetime offspring production. Here, we test whether the positive effects of multiple mating on female fitness also hold at a comparative level, by conducting a laboratory study of female reproductive output in eight pierid species that differ in lifetime female mating frequency. Female reproductive output, measured as cumulative egg mass divided by female mass, increased significantly with polyandry ($r = 0.942$, $p < 0.001$), demonstrating that the positive effect of mating rate on female reproductive fitness also holds between species. The positive effect of male nutrient contribution is substantial, and the per capita reproductive output is more than twice as high in the most polyandrous species as in the most monandrous ones. Hence, the positive net effect of the ejaculates is highly substantial, although males and females can have sexual interests that run counter to each other, setting the stage for sexually antagonistic coevolution, so that the various component parts of the male ejaculate—sperm, nutrients, anti-aphrodisiacs, and gonadotrophic hormones—may each correspond to a separate conflict–cooperation balance between the sexes. Two scenarios for the evolution of nuptial gifts in butterflies are discussed, one arguing that variation in larval food is the underlying factor and the other arguing that sexually antagonistic coevolution is the driving force. The two views are complementary rather than mutually exclusive, although the former hypothesis predicts that variation in female mating rate depends on variation in larval food availability, whereas the latter suggests that variation in female mating rate between species results from species-specific idiosyncrasies.

Keywords: nuptial gifts; mating frequency; sexual conflict; lifetime fecundity

1. INTRODUCTION

The realization that males and females often have reproductive interests that are adversary to each other, as eloquently exemplified by Dawkins' (1976) chapter 'Battle of the sexes', has led to the current view that sexual conflict is pervasive (Arnqvist & Rowe 1995; Chapman *et al.* 1995; Chapman & Partridge 1996; Rice 1996, 1998; Brown *et al.* 1997; Rice & Holland 1997; Holland & Rice 1998; Arnqvist & Nilsson 2000). However, at the same time, it is obvious that sexual reproduction also incorporates an element of cooperation between the sexes. The challenge for the evolutionary biologist is to understand different aspects of mating systems in terms of conflict and cooperation.

In the Lepidoptera the females of most species can be characterized as capital breeders (*sensu* Boggs 1992; Stearns 1992), meaning that reproductive output is strongly coupled to the amount of resources they have procured during the larval stage. In agreement with this scenario, female fecundity in the Lepidoptera is generally correlated with female mass, both within and across

species (cf. Haukioja & Neuvonen 1985; Wickman & Karlsson 1989; Karlsson & Wickman 1990). However, in some butterfly species females can be partly characterized as income breeders, in the sense that their reproductive output is determined not only by the larval-derived resources but also by resources acquired during the adult stage. These adult resources can be the result either of female feeding (e.g. females of *Heliconius* spp. that feed on protein-rich pollen (Gilbert 1972, 1991)) or of females having received nutrients from males during mating (cf. Boggs & Gilbert 1979).

In butterflies, the male transfers an ejaculate to the female during mating, which contains sperm, nutrients, anti-aphrodisiacs and gonadotrophic hormones (Boggs & Gilbert 1979; Boggs 1990, 1995, 1997*a,b*; Ramaswamy *et al.* 1997). In many species it has been shown that the male ejaculate functions as a nuptial gift and that multiply mated females benefit from male-derived nutrients in terms of fecundity, egg size and longevity (Boggs 1990; Wiklund *et al.* 1993). The contribution from the males can be quite substantial, and in *Pieris napi* multiply mated females attain almost twice the fecundity of females that have mated only once. Hence, females often forage for matings (Kaitala & Wiklund 1994), and in the light of the benefits accrued monandry as a female strategy can

*Author for correspondence (christer.wiklund@zoologi.su.se).

be more problematic to explain than polyandry (Arnqvist & Nilsson 2000; Wedell *et al.* 2001).

In butterflies, mating systems vary from monandry to polyandry, and across eleven species of Swedish Pieridae the mean number of matings per female varies from a low of 1.11 in the orange tip, *Anthocharis cardamines*, to a high of 2.13 in the small cabbage white, *Pieris rapae* (Wiklund & Forsberg 1991). Numerically, these mean figures may appear unimpressive but in monandrous species such as *A. cardamines* and *Leptidea sinapis* females never seem to mate more than twice, and these twice-mated females, as a rule, contain one small and one large spermatophore, suggesting that the second mating may have been triggered by the female receiving an unusually small first spermatophore. In contrast, females belonging to polyandrous species such as *P. rapae* and *P. napi* mate up to five times in the wild; moreover, the average degree of polyandry typically increases with time, and in *P. napi* the majority of females collected in the third week of the flight season had mated three times or more (Forsberg & Wiklund 1989).

Returning to the issue of conflict and cooperation between the sexes, Rice & Holland (1997) have argued that sexually antagonistic coevolution is all pervasive and that internal fertilization in insects fundamentally extends the range of opportunity for male–female conflict because it constrains the sexes to interact directly. They point out that in order to receive sperm a female must also accept seminal-fluid proteins, airborne pheromones and male behaviour, all of which can act to modify her behavioural and physiological phenotype in ways that increase male fitness at the expense of female fitness. However, this sets the scene for female counter-adaptations to neutralize such male-induced impediments to female fitness. In line with this scenario, it might be expected that substances transferred from males to females during mating could influence female fitness in some way, and have negative, neutral or positive effects.

Recently, Arnqvist & Nilsson (2000) performed a meta-analysis of 122 experimental studies of the effects of multiple matings on female fitness in insects and concluded that females generally gain directly from multiple matings in terms of increased lifetime offspring production. However, they noted a negative effect of re-mating on female longevity in species without nuptial gifts, and concluded that there exists an intermediate optimal female mating rate beyond which a further elevated mating rate is deleterious. Conversely, in species with nuptial gifts no such negative effects were noted and female lifetime offspring production increased linearly with female mating rate. These two scenarios give rise to different predictions concerning the effect of female mating rate on female fitness across species. In species without nuptial gifts, increased female reproductive output is effected through male manipulation of female reproductive behaviour, e.g. through male-transferred gonadotrophic hormones, and reproductive output by females should not be expected to increase with female mating rate across species. However, in species with nuptial gifts, increased female reproductive output is effected through male-derived nutrient transfer (which does not rule out male manipulation in addition to nutrient transfer) and so female reproductive output should be expected to increase with female mating rate across species. In this study, we

test the latter prediction by performing a comparative analysis to study the relationship between female mating frequency and reproductive output in eight species of pierid butterflies that exhibit variation in natural mating system from monandry to polyandry and in which the males appear to transfer nuptial gifts.

2. MATERIAL AND METHODS

In six species, namely *P. rapae*, *P. napi*, *Pieris brassicae*, *Pontia daplidice*, *A. cardamines* and *L. sinapis*, the butterflies used in the experiments were the offspring of females collected in the wild. These butterflies were reared on natural host plants as larvae—hence, *P. brassicae* were reared on *Armoracia rusticana*, *P. rapae* and *P. napi* on *Alliaria petiolata*, *P. daplidice* on *Reseda lutea* and *A. cardamines* on *Cardamine pratensis* and *L. sinapis* on *Lathyrus pratensis*. The black-veined white, *Aporia crataegi*, were collected as egg batches on *Sorbus aucuparia*, and larvae, which overwinter in the second instar, were reared both before and after hibernation on this host plant. The eighth species, the brimstone, *Gonepteryx rhamni*, which eclose as butterflies in July but do not mate until after hibernation, were collected during the first two weeks of September and brought to an underground storehouse where they hibernated until mid-April of the following spring, after which they were used in the experiments. All of these butterflies mate readily in flight cages, as long as they are kept close to large windows under lamps that emit ultraviolet radiation, except for *L. sinapis*; accordingly, newly eclosed females of *L. sinapis* were taken to their natural habitats, released and mated with wild males, and then brought back to the laboratory for egg-laying (hence, all female *L. sinapis* used in the experiments had mated only once). All other females were maintained together with two conspecific virgin males throughout their lives, and when a male had mated with a female he was replaced by a new virgin male. This procedure was followed in an attempt to maintain natural levels of female polyandry under the laboratory conditions. The mean number of matings performed by the females in these experiments was assessed by spermatophore counts on females after they had died. The flight cages measured 0.7 m × 0.7 m × 0.5 m and had tops made of transparent plastic; they were supplied with the above-mentioned natural host plants for egg-laying and a *Chrysanthemum* sp. into which 20% sucrose solution was dropped several times per day to permit feeding. Host-plant leaves were replaced every day and the eggs laid were counted at the end of each day. The bottoms of the cages were covered with paper towels, which were kept humid throughout the experiment. The cages were located immediately adjacent to windows and approximately below 400 W halogen mercury vapour (HQIL) lamps, which were lit between 09.00 and 17.00.

Female mass for all species was assessed by weighing females on the day of eclosion on a Kern 410 Electrobalance to the nearest 0.1 mg. Individual egg mass was assessed by weighing ten eggs from each female on the first day of egg laying; the eggs were collected individually with a fine brush and were weighed to the nearest 0.001 g on a Cahn Electrobalance (Cahn, Madison, WI, USA). Cumulative egg mass was assessed for each female by multiplying the number of eggs laid by the mean egg weight (from eggs laid on the first day).

To control for the effects of shared ancestry we used a computer program called PDAP developed by Garland *et al.* (1993) (the original name of the method is FLIP). The basis for these methods derives from Felsenstein (1985).

3. RESULTS

The mean degree of polyandry under laboratory conditions was as follows: *A. crataegi*, 1.60; *P. brassicae*, 1.22; *P. rapae*, 2.43; *P. napi*, 2.28; *P. daphidice*, 1.83; *A. cardamines*, 1.17; and *G. rhamni*, 1.20. These values are very similar to those assessed by spermatophore counts on wild-caught females (table 1). In the eight pierids, mean female body mass varied by an order of magnitude from 37 mg in *L. sinapis* to 303 mg in *A. crataegi*; the mean egg mass showed relatively little variation across species, from a low of 0.109 mg in *G. rhamni* to a high of 0.185 mg in *P. brassicae* (table 1). These two species were the third and second largest, respectively, as judged by mean female mass; in fact there was no significant correlation between female mass and egg mass in these eight pierids. However, mean lifetime fecundity was positively correlated with female mass ($r = 0.85$, $p < 0.01$) and varied from a low of 95 eggs in the smallest species, *L. sinapis*, to a high of 1044 eggs in the largest species, *A. crataegi* (table 1). Mean female longevity varied from 11 days in *P. daphidice* to 24 days in *P. napi*, but there was no correlation between longevity and fecundity.

Reproductive output, measured as cumulative egg mass divided by female mass, was positively correlated with mean polyandry across the eight pierids (figure 1; $r = 0.942$, $p < 0.001$). Assuming phylogenies for the eight species in accordance with Geiger (1981), seven independent contrasts were extracted. The slope of the regression through the origin for these contrasts was $k = 0.545$ ($r = 0.930$, $F = 38.34$, $p < 0.001$). Thus, female reproductive output increases with mean polyandry even when shared ancestry is controlled for.

4. DISCUSSION

The results show that cumulative egg mass divided by female mass increases with the mean degree of polyandry in the eight studied pierid species. A quantitative association between reproductive output and male-ejaculate mass has been previously demonstrated within species for *P. napi* (Karlsson 1998; Wiklund *et al.* 1998), and the result from this comparative study is consistent with the ideas that the females can use male-derived nutrients to increase their reproductive output and that this ability increases gradually with the degree of polyandry. Hence, these results are concordant with the hypotheses that female fitness increases with mating rate in gift-giving species and that this applies both within and between species. However, although this comparative study demonstrates a positive effect of mating rate on female fitness, this does not mean that male and female interests are wholly congruent. Although the net outcome of the association between the sexes is positive, it is likely that the reproductive interests of males and females are characterized by both conflict and cooperation in these eight gift-giving pierid butterfly species.

(a) Sexual conflict and cooperation

In species where females generally mate more than once there is an obvious conflict of interest between the female and the first male to mate with her: whereas the female benefits from re-mating as soon as physiologically,

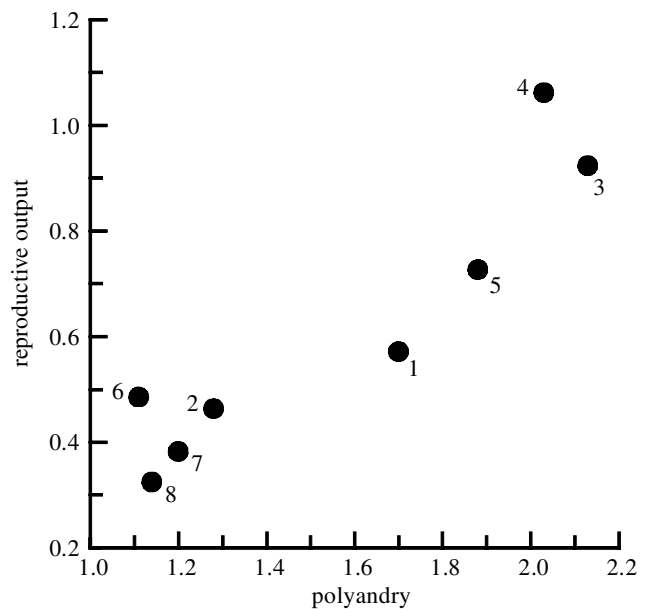


Figure 1. Reproductive output, measured as lifetime cumulative egg mass divided by female mass, as a function of mean polyandry, measured as lifetime number of matings in wild-caught females (as assessed by spermatophore count), in eight pierid butterflies. Numbers refer to species as in table 1.

ecologically and behaviourally possible, the male would benefit from preventing the female from ever re-mating. In this context, it should be pointed out that the size of the ejaculate transferred by a male to a female during mating is strongly dependent on male mating history: a recently mated male transfers an ejaculate that is, on average, five times smaller than the ejaculate transferred by a previously unmated male. Butterflies as a rule transfer large ejaculates, which, in pierids, correspond on average to between 5% and 15% of the male body mass, but which can correspond to up to 23% of an individual male's mass (Forsberg & Wiklund 1989; Svård & Wiklund 1989). Moreover, in polyandrous species the duration of a female's unreceptivity period is strongly dependent on the mass of the ejaculate; females have stretch receptors in the mating bursa and can thereby assess the size of the ejaculate received during mating (Sugawara 1979). In *P. napi* the mean duration of the post-mating unreceptivity period is about three to five days for females that have mated with virgin males but only one to three days for females that have mated with a previously, and recently, mated male (Kaitala & Wiklund 1994). The bottom line here supports the conclusion above: although females benefit nutritionally from re-mating, a female does not re-mate very soon after a first mating, and so the male's expenditure in a large and nutritious ejaculate when mating with a virgin female may pay off; it seems that the male can exercise some degree of control over the female's unreceptivity period, with females being unreceptive for longer if the male has transferred a large ejaculate.

This cooperation–conflict between a female and her first mate is also tangible with respect to another component in the ejaculate: it has recently been shown that male *P. napi* transfer an anti-aphrodisiac, methylsilylate, to females during mating and that this

Table 1. Degree of polyandry, female mass, individual egg mass, lifetime fecundity, cumulative egg mass and longevity for eight Swedish pierid butterfly species.

(Mean values \pm s.e.m. in the upper row, maximum and minimum values in brackets below.)

	polyandry ^a (number of spermatophores per female)	female mass (mg)	individual egg mass (mg)	lifetime fecundity (number of eggs)	cumulative egg mass (mg)	longevity (days)	<i>n</i>
1. <i>Aporia crataegi</i>	1.70 (3–1)	303 \pm 9 (330–282)	0.168 \pm 0.003 (0.180–0.163)	1044 \pm 223 (1631–411)	173 \pm 35 (266–69)	20 \pm 1 (23–16)	6
2. <i>Pieris brassicae</i>	1.28 (4–1)	177 \pm 6 (204–146)	0.185 \pm 0.004 (0.203–0.164)	435 \pm 71 (749–244)	82 \pm 15 (148–40)	18 \pm 2 (25–11)	9
3. <i>Pieris rapae</i>	2.13 (5–1)	65 \pm 5 (88–54)	0.112 \pm 0.002 (0.120–0.103)	514 \pm 45 (699–464)	60 \pm 4 (78–51)	13 \pm 2 (21–7)	7
4. <i>Pieris napi</i> ^b	2.03 (5–1)	66 \pm 2 (84–51)	0.143 \pm 0.002 (0.168–0.105)	489 \pm 32 (787–186)	70 \pm 5 (104–20)	24 \pm 1 (32–6)	25
5. <i>Pontia daplidice</i>	1.88 (3–1)	62 \pm 3 (72–52)	0.120 \pm 0.002 (0.127–0.114)	374 \pm 67 (532–144)	45 \pm 8 (65–16)	11 \pm 1 (15–8)	6
6. <i>Anthocharis cardamines</i>	1.11 (2–1)	66 \pm 4 (79–53)	0.181 \pm 0.003 (0.191–0.170)	177 \pm 30 (317–117)	32 \pm 6 (58–20)	12 \pm 1 (16–10)	6
7. <i>Gonepteryx rhamni</i>	1.20 (2–1)	110 \pm 3 (122–101)	0.109 \pm 0.002 (0.125–0.102)	382 \pm 55 (655–128)	42 \pm 6 (70–15)	21 \pm 2 ^c (25–20)	10
8. <i>Leptidea sinapis</i>	1.14 (2–1)	37 \pm 1 (44–28)	0.128 \pm 0.005 (0.171–0.096)	95 \pm 12 (196–51)	12 \pm 2 (24–7)	18 \pm 2 (29–9)	14

^aData from Wiklund & Forsberg (1991).^bData from Wiklund *et al.* (1993).^cLongevity refers to length of life after hibernation.

substance makes females strongly unattractive to courting males, which curtail their courtship within a second or two of perceiving the anti-aphrodisiac that is emitted by females in response to courtship (Andersson *et al.* 2000). Although both the female and her first mate benefit from the anti-aphrodisiac rendering the female unattractive by reducing potentially harmful male harassment of egg-laying females, it is as yet unresolved which sex controls the subsequent lowering of the anti-aphrodisiac titre that presumably accompanies the process that makes the female more attractive to males with time passed since the first mating (cf. Forsberg & Wiklund 1989).

Although males, in many respects, lose control over the ejaculate once it has been transferred to the female, males do have control over the component parts of the ejaculate itself. In several pierid species it has been demonstrated that males can distinguish between virgin and previously mated females (*A. cardamines*, Wiklund & Forsberg 1985; *P. napi*, Forsberg & Wiklund 1989; Andersson *et al.* 2000; *P. brassicae*, *P. rapae* and *P. daplidice*, J. Andersson *et al.* unpublished data), and so males could design their ejaculate components in line with whether they mate with a virgin or a mated female. In butterflies males produce and transfer not only fertilizing, eupyrene, sperm but also non-fertilizing, apyrene, sperm. The function of these non-fertilizing sperm, which customarily outnumber fertilizing sperm by nine to one, has been under substantial debate (Silberglied *et al.* 1984) but recent evidence suggests that they are produced as filler substance and function to delay female re-mating by filling the bulk of the female mating receptacle (Cook & Wedell 1999). (Cook & Wedell argue that apyrene sperm are 'cheaper' than eupyrene sperm, but this has yet to be shown; we feel that a 'division of labour' is sufficient to explain the

raison d'être of apyrene sperm—given that they are designed to have greater 'staying power' in the female receptaculum seminis.) Moreover, since a virgin female that has received a full ejaculate usually does not re-mate until some five days later, a male mating with a virgin female could benefit from transferring an ejaculate where the ratio of fertilizing to non-fertilizing sperm is strongly biased towards non-fertilizing sperm, in line with the current belief that these sperm function to delay female re-mating. On the other hand, a male that mates with a non-virgin female 'knows' that his sperm will compete with those of the female's previous mate, and so he could benefit from transferring an ejaculate where the ratio of fertilizing to non-fertilizing sperm is biased towards fertilizing sperm. In *P. rapae* there is evidence that males do have the ability to control the relative numbers of fertilizing and non-fertilizing sperm in this manner, and, indeed, design their first ejaculates to delay female re-mating and their second ejaculates for sperm competition (Wedell & Cook 1999a,b).

Presently, little is known about the male contribution of gonadotrophic hormones in ejaculates among butterflies but it is well known that this occurs in other Lepidoptera (Ramaswamy *et al.* 1997; Arnqvist & Nilsson 2000). However, from a theoretical viewpoint, it would seem that the degree of sexual conflict would increase with the degree of polyandry in accordance with the scenario of interlocus contest competition developed by Rice & Holland (1997). In so far as male butterflies do transfer gonadotrophic hormones that can influence the rate of female oviposition, the male and female share a common interest under monogamy. However, in polyandrous mating systems males should benefit from exploiting females and might benefit from inducing a higher rate of

female oviposition than that which would be beneficial from the female's point of view, leaving the field open for an arms race where males might be selected to increase gonadotrophic-hormone content in their ejaculates and females might be selected to synthesize lower amounts of gonadotrophic hormones as a countermeasure. This scenario is presently speculative but is suggested by the observation that the polyandrous females of *P. napi* occasionally decrease their oviposition rate relatively early in life and do not pick up their egg-laying rate until after re-mating (C. Wiklund, unpublished data). This 'voluntary' drop in egg-laying rate seems puzzling but could be understood in mechanistic terms as the result of a potentially polyandrous female having synthesized sub-optimal levels of gonadotrophic hormone, thereby becoming dependent on male-transferred egg-laying stimulants.

(b) *Male benefit from nutrient transfer*

Earlier studies have shown that male investment in reproductive reserves increases with the degree of polygamy (Karlsson 1995, 1996), as does the relative nitrogen content of the male ejaculate (Bissoondath & Wiklund 1995). This suggests that the nutrient content of male-transferred ejaculates increases with polygamy and that female ability to make use of these nutrients underlies the positive association between female polygamy and reproductive output shown here. The fact that males of polyandrous species allocate more resources to ejaculates may seem surprising in the light of sperm competition, which should, on average, increase with the degree of female polygamy. However, the evolution of nuptial gifts in polyandrous species may be explained both in terms of conflict and cooperation between the sexes and in terms of conflicts of interest between a female's successive mates.

Under polyandry, females can exercise more control than males over the ejaculate and its contents once it has been delivered. Hence, given that the nutrients transferred from the male to the female during mating benefit a female's first mate in terms of increased quantity and/or quality of offspring, compared with what would have resulted had he not transferred any nutrients during mating, it would seem that male butterflies mating with virgin females could benefit from transferring nutrients. This holds even in polyandrous species, where the nutrients transferred by a female's first mate will later be used to increase the reproductive output of successive males that mate with the female (given that the latest male to mate with a female enjoys sperm precedence, a pattern that seems quite general in insects and has been demonstrated for two of the pierids in this study, namely *P. napi* (Bissoondath & Wiklund 1997) and *P. rapae* (Wedell & Cook 1998); moreover, in cases where latest-male sperm precedence is not applicable, nutrient delivery by a female's first mate would not pose a problem). Our point here is that even though the first male risks having the nutrients that he transfers to a female used later to produce eggs that are fertilized by another male, male nutrient transfer might be beneficial. This will be especially true in systems where the operational sex ratio is strongly male-biased, and where females can control whether to mate or not—both conditions hold generally in butterflies (Wiklund *et*

al. 1998). We also point out that these conditions mean that the benefit of male nutrient transfer holds not only for a female's first mate but also for her successive mates.

(c) *The evolution and maintenance of nuptial gifts*

Our results demonstrate that female reproductive output increases with polygamy, which begs the question why do not females of all of these pierid butterflies practise polyandry? This question needs to be analysed in its two component parts: under what circumstances are female polygamy and male nutrient transfer expected to evolve, and under what circumstances will natural selection favour the maintenance of such a mating system?

We have argued that the underlying factor in the evolution of nuptial gifts might be variation in food availability for the growing larvae, which causes some males to have much to provide and some females to be in great need, in terms of nutrients (Leimar *et al.* 1994). Hence, males with larger gifts should be rewarded, in effect trading the larger gift for more offspring, and there is some evidence to support this idea. First, in a sample of 16 butterfly species, relative male ejaculate mass increases with variation in female wing length (Leimar *et al.* 1994). Moreover, the degree of female polygamy also increases with variation in female wing length (taken to be an indicator of variation in larval food availability) in the same sample of 16 butterfly species (Karlsson *et al.* 1997). Second, the observation that polyandry increases with variation in female wing length is also in accordance with the general observation that polyandry is more common in pierid butterflies, where the larvae of most species feed on ephemeral crucifers, than in satyrid butterflies, where the larvae feed on abundant grasses (Wiklund & Forsberg 1991). A different explanation for the evolution of nuptial gifts is based on the scenario of sexually antagonistic coevolution (Hammerstein & Parker 1987; Rice & Holland 1997; Holland & Rice 1998; Parker & Partridge 1998; Arnqvist & Nilsson 2000). According to this scenario, male induction of female unreceptivity by means of transferred accessory substances is basically dose dependent, which selects for large ejaculates. Given that male induction of female unreceptivity becomes overly effective and compromises female interests, female resistance to male refractory-inducing signals could be achieved by increasing female ability to neutralize the deleterious effects of male signals by metabolizing the transferred substances. Male ejaculates may thus become exaggerated by an antagonistic and perpetual coevolutionary arms race between the sexes. Hence, Arnqvist & Nilsson (2000) argue that 'nuptial gifts may be a very misleading term for the protein-rich and voluminous ejaculates of many insects, since the evolutionary origin and maintenance of greatly enlarged and elaborate ejaculates may be the result of sexual conflict, manipulation and extortion rather than sexual confluence'. Although the two explanations for the evolution of nuptial gifts described above are complementary rather than mutually exclusive, they present somewhat different scenarios and whereas the sexually antagonistic view should be generally applicable to all species it offers little understanding of the variation in female mating rate, which is provided by Leimar *et al.*'s (1994) nutrition-based explanation for this variance.

In conclusion, we have demonstrated that there is a strong positive association between the degree of polyandry and the reproductive output across species, which underscores the generality of previous experimental studies demonstrating a positive association between female mating rate and reproductive fitness within species. The positive effect of male nutrient contribution is substantial and the per capita reproductive output is more than twice as high in the most polyandrous species as in the most monandrous ones (figure 1). Hence, although the various component parts of male ejaculates—sperm (eupyrene and apyrene), nutrients, anti-aphrodisiacs and gonadotrophic hormones—may each correspond to a separate conflict–cooperation balance between the sexes, we argue that the positive net effect of these ejaculates justifies the retention of the term nuptial gift in polyandrous butterfly mating systems. The variation in butterfly mating systems in the face of the potential benefits that can be harvested under a system of male nutrient gifts may be explained by variation in ecological and life-history variables, or may be an idiosyncratic outcome of sexually antagonistic coevolution.

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