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Male mating history and female fecundity in the Lepidoptera: do male virgins make better partners?

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Abstract In insects, large ejaculate and associated materials, including spermatophores, appear to have evolved via sexual selection acting on males to either delay female remating or to increase the rate of egg-laying. It is also possible, however, that females use nutrients transferred during mating to increase their lifetime fecundity. If so, male ejaculate size may also have evolved under natural selection as a form of paternal investment. In Lepidoptera, males with a greater number of prior matings tend to produce smaller spermatophores. However, the reported effects of male mating history on female fecundity vary widely among species. We therefore performed a meta-analysis using data from 29 studies of 25 species. Overall, the reproductive output of females mated to virgin males was significantly higher than that of females mated to sexually experienced males (Hedges' $d=0.33$, $P<0.01$). A sample size of around 145 females per male mating type is required to detect an effect of this size with 80% statistical power at $\alpha=0.05$ (two-tailed). There was no difference in mean effect size between butterflies/skippers and moths. After controlling for any effect of taxonomic group, however, the mean effect size for polyandrous species was significantly greater than that for monandrous species (Hedges' $d=0.45$ vs 0.25 , $P=0.01$). We then discuss possible reasons why male mating history, presumably acting through its effect on spermatophore size, might have a stronger effect in polyandrous than monandrous species.

Keywords Lepidoptera · Mating effort · Paternal investment · Polyandry · Sexual selection

Introduction

Multiple mating by males is widespread in insects, including the Lepidoptera. A male's reproductive output is closely linked with the number of females he is able to inseminate, so it is widely accepted that the best male strategy to maximise fitness is generally to acquire as many mates as possible (Trivers 1972; Thornhill and Alcock 1983). This argument may not always hold, however, because mating can impose substantial costs on males, particularly in cases where males provide material resources. In a landmark paper, Dewsbury (1982) showed that the cost of producing ejaculate, often considered to be very cheap, is not trivial. If ejaculate, and any associated nuptial gifts or resources, are a potentially limiting resource, then males may have to allocate sperm strategically depending on the relative value of different females and the opportunities to remate in the time it will take to replenish sperm supplies. A considerable amount of recent experimental work has therefore addressed: the extent to which cryptic male choice occurs in the form of strategic allocation of ejaculate among potential mates (review of insects: Simmons 2001); whether females discriminate among males based on their ability to provide material resources upon mating or fertilize all their eggs; and the effect a male's mating history has on a female's reproduction output. In this paper we focus on the last issue.

In the Lepidoptera, the effect of male mating history on female fecundity is unclear. Some studies show that females that mate with sexually experienced males have lower lifetime fecundity than those that mate with virgin males, while other studies do not find this relationship (for a full list see Table 1). This inconclusive scenario may occur because male mating history is affected by a number of factors. The size, quality and number of spermatophores delivered by males have been shown to

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Table 1 Published research on the effect of male mating history on female reproductive output in Lepidoptera species. Mating pattern (percentage of polyandry) and the effect of male mating history on spermatophore size are also given for each study

Ref. no.	Species	Family	Percentage polyandry ^a	Previous male mating reduces:		References
				Reproductive output ^b	Spermatophore size ^c	
1	<i>Acrolepia assectella</i>	Yponomeutidae	38	No ^{d, p}	Yes	Thibout (1978); Thibout and Rhan (1972)
2	<i>Agrotis segetum</i>	Noctuidae	21–34	Yes	Yes	Svensson et al. (1998)
3	<i>Busseola fusca</i>	Noctuidae	[2.0]	No	?	Unnithan and Paye (1990)
4	<i>Colias eurytheme</i>	Pieridae	47 ^e	Yes	Yes	Rutowski et al. (1987)
5	<i>Chilo partellus</i>	Pyralidae	0–10	No	?	Unnithan and Paye (1991)
6	<i>Choristoneura fumiferana</i>	Tortricidae	18–37	No	Yes	Outram (1971)
7	<i>Choristoneura rosaceana</i>	Tortricidae	45	Yes ^f	Yes	Delisle and Bouchard (1995)
8	<i>Diatraea considerata</i>	Pyralidae	7–8	No	?	Osorio-Osorio and Cibrián-Tovar (2000)
9	<i>Epiphyas postvittana</i>	Tortricidae	10–15 ^g	No	Yes	Foster and Ayers (1996)
10	<i>Eurema hecabe</i>	Pieridae		No	Yes	Hiroki and Obara (1997)
11	<i>Helicoverpa armigera</i>	Noctuidae	68	Yes	?	Hou and Sheng (1999)
12	<i>Jalmenus evagoras</i>	Lycaenidae	“very low”	No	Yes	Hughes et al. (2000)
13	<i>Lobesia botrana</i>	Tortricidae	20–35 ^h	No	Yes	Torres-Vila et al. (1995)
14	<i>Ostrinia nubilalis</i>	Pyralidae	7–38 ⁱ	Yes ^j	Yes	Royer and McNeil (1993)
15	<i>Papilio glaucus</i>	Papilionidae	65 ^k	No	Yes	Lederhouse et al. (1990)
16	<i>Papilio machaon</i>	Papilionidae	[1.16] ^l	No	Yes	Svärd and Wiklund (1991)
17	<i>Pectinophora gossypiella</i>	Gelechiidae	74–88 ^m	Yes	?	LaChance et al. (1978)
18	<i>Pieris napi</i>	Pieridae	100?	Yes	Yes	Karlsson (1998)
19	<i>Pieris napi</i>	Pieridae	47–93 ⁿ	Yes	Yes	Wiklund et al. (1998)
20	<i>Pieris napi</i>	Pieridae	47–93 ⁿ	Yes ^p	Yes	Wedell and Karlsson (2003)
21	<i>Plodia interpunctella</i>	Pyralidae	[2.0–2.5]	No	?	Brower (1975)
22	<i>Plodia interpunctella</i>	Pyralidae	58	No	Yes	Cook (1999)
23	<i>Plodia interpunctella</i>	Pyralidae	17	No	Yes	Ryne et al. (2001)
24	<i>Pseudaletia unipuncta</i>	Noctuidae	55 ^o	No	Yes	Fitzpatrick and McNeil (1989)
25	<i>Sitotroga cerealella</i>	Gelechiidae	65–72	Yes ^p	Yes	Stockel (1973a, 1973b)
26	<i>Spodoptera littoralis</i>	Noctuidae	50–73	No ^q	?	Sadek (2001)
27	<i>Thymelicus lineola</i>	Hesperiidae	22	No	Yes	Pivnick and McNeil (1987)
28	<i>Trichoplusia ni</i>	Noctuidae	81	No	?	Ward and Landolt (1995)
29	<i>Zeiraphera canadiensis</i>	Tortricidae	8 ^r	No	Yes	Carroll (1994)
Additional studies ^s						
30	<i>Antheraea mylitta</i> ^t	Saturniidae	–	No	–	Dash et al. (1993)
31	<i>Antheraea mylitta</i> ^t	Saturniidae	–	No ^p	–	Ravi Kumar et al. (1995)
32	<i>Danaus plexippus</i> ^u	Danaidae	95	No	–	Oberhauser (1989)
33	<i>Epiphyas postvittana</i> ^v	Tortricidae	10–15 ^g	No	–	Foster and Ayers (1996)
34	<i>Lobesia botrana</i> ^v	Tortricidae	20–35 ^h	No	–	Torres-Vila et al. (1995)
35	<i>Polygonia c-album</i> ^w	Nymphalidae	[2.4] ^l	No	–	Wedell (1996)
36	<i>Trichoplusia ni</i> ^v	Noctuidae	81	No	–	Ward and Landolt (1995)

^a Percentage of polyandrous females. Mean number of spermatophores per female is given in square brackets when percent polyandry was unknown.

^b Effect of male mating history on female reproductive output; fecundity (total eggs) was used as estimator of reproductive output except in six studies (seven contrasts, refs.: 2, 8, 9, 22, 23, 29 and 33) where just data on fertility (viable eggs or progeny number) were available.

^c Effect of male mating history on spermatophore size; ? indicates no data from that study.

^d Variable effect depending on trial, data were weighted for our analysis.

^e See Rutowski and Gilchrist (1986).

^f Pooled data from three larval regimes.

^g Polyandry was underestimated in this study; Danthanarayana and Gu (1991) report 60% polyandry.

^h See Torres-Vila et al. (1997).

ⁱ See Gohari and Hawlitzky (1986); Fadamiro and Baker (1999).

^j Fecundity diminished significantly only after males had three previous matings.

^k See Lederhouse and Scriber (1987).

^l See Svård and Wiklund (1989).

^m See Henneberry and Clayton (1983); *P. gossypiella* has also been shown to be polyandrous in studies by Henneberry and Leal (1979) (“high polyandry”) or Bartlett and Lewis (1985) (1.9 spermatophores/female), but monandrous in others: 18% polyandry in Graham et al. (1965), 28–29% in Lukefahr and Griffin (1957) and 29% in Ouye et al. (1964).

ⁿ See Wiklund et al. (1993), Bergström and Wiklund (2002).

^o See Callahan and Chapin (1960).

^p Partial reproductive output as it was not recorded for all female lifetime.

^q There were significant differences in Sadek study when fertility (viable eggs) was used in lieu of fecundity as the estimator of reproductive output.

^r See Turgeon (1985).

^s These seven studies related female reproductive output to factors other than mating history known to affect spermatophore size or the amount of spermatophore content transferred:

^t mating duration (pairs were physically decoupled at different times after copulation began),

^u mating history plus male age together,

^v male age or

^w larval host plant. These studies were not included in the main meta-analysis.

be highly sensitive to such factors as male age at mating, body weight, larval and adult feeding regime, mating order and the time that elapses between consecutive matings (see Torres-Vila et al. 1995 and references therein). It may also be the case that the effect of male mating history on female fecundity is modest and that many studies that report no significant relationship do so because of small sample sizes and low statistical power (Jennions and Møller 2003).

In this study, we have therefore performed a meta-analysis to explore the effect of male mating history on female reproductive output to ascertain whether a general trend across Lepidoptera species can be detected. We also tested whether this relationship is influenced by the species' mean level of polyandry or taxonomic group (i.e. phylogeny). We compared polyandrous and monandrous species because, in an earlier paper (Torres-Vila et al. 2004), we found that multiple mating tended to have a more beneficial effect on fecundity in polyandrous than monandrous species. Here we wanted to investigate whether the same relationship held when considering the effect of spermatophore size (which tends to decrease with male mating history). That is, the degree of multiple mating and mating with males with different recent mating histories are two quite different factors that determine the amounts of ejaculate females obtain. They should, however, have similar effects on female fecundity if it is strongly influenced by the contents of spermatophores. We then discuss our results in the light of the generally accepted statement that spermatophore size is primarily driven by sexual selection on males to reduce female remating rather than selection on males to provide paternal investment.

Methods

We consulted scientific literature databases, the Internet and previous reviews on this and related topics (Ridley 1988; Vahed 1998; Arnqvist and Nilsson 2000; Torres-Vila et al. 2004) to compile data from 29 studies of 25 Lepidopteran species that related female reproductive output to male mating history (Table 1). Studies were included if two basic pieces of information were available: the species' mating pattern and the effect of male mating history on female reproductive output. We excluded the well-known study of Oberhauser (1989) as it conflated male mating history and male age.

We classified species on the basis of mating pattern and taxonomic group (Table 2). Species were scored as either Rhopalocera (butterflies and skippers) or Heterocera (moths). Following Torres-Vila et al. (2004), we classified species according to the percentage of females that were reported to have mated more than once in the focal publication. If this figure was unreported or unclear, an estimate of female remating frequency was taken from another source. Monandrous (<25% polyandry) or mildly polyandrous (25–40%) species were simply labelled “monandrous”, while species in which polyandry was higher (>40%) were labelled “polyandrous”. This criterion is somewhat arbitrary as there is no clear discontinuity in the species-level distribution of mean mating frequencies (Ridley 1988; Torres-Vila et al. 2002). We have, however, adopted a conservative approach in labelling species as monandrous. Under the laboratory conditions that applied in most studies, female remating is often unnaturally elevated due to a high population density, forced proximity of the sexes and/or a male-biased sex

ratio. The >40% value to define polyandrous species broadly agrees with frequencies other authors use to categorise their target species.

For 23 of the 29 studies, female reproductive output was measured as lifetime fecundity (total number of eggs laid). In six studies, fecundity records were unavailable so we used the number of viable eggs or progeny produced (“fertility”) (see Table 1). Unless there is a strong effect of male mating history on fertility, this should yield a difference in means for the two classes of females similar to that based on fecundity. The available evidence indicates, however, that egg viability may decline with a male's number of prior matings. This would, if anything, increase the reported effect size. We therefore directly tested whether effect sizes calculated using “fecundity” and “fertility” measures differed. They did not. Those for “fertility” were actually slightly smaller (see Results).

Finally, we compiled data from seven studies that related female reproductive output to factors known to effect spermatophore size. In general, spermatophore size decreases with each successive mating by a male (see Table 1). These studies were not included in the main meta-analyses.

Data analysis

In 26 of the 29 studies, we found information on means, a statistical measure of dispersion (standard deviation or error) and sample sizes for the reproductive output of females mating with males with different prior mating histories (Table 2). Data were either retrieved from the text or tables or indirectly obtained by measuring figures. We compared the group of females that mated with virgin males to those that mated with previously mated (experienced) males. If data from experienced males were presented in separate classes (e.g. one prior mating, two prior matings and so on), we calculated the weighted mean and standard deviation for the pooled set of experienced males (up to five prior matings). For each study, we then calculated Hedges' unbiased effect size estimator (d) where:

$$d = \left(1 - \frac{3}{4(n_1 + n_2) - 9}\right)g$$

and g is the standardised mean difference between the two groups $g = [(M_1 - M_2)/SD]$, n_1 and n_2 are the sample sizes, M_1 and M_2 the mean reproductive outputs of females mated to virgin or experienced males, respectively, and SD is the pooled standard deviation for the two groups (see Rosenberg et al. 2000).

In two studies (Carroll 1994; Svensson et al. 1998), the data were presented as the correlation between female reproductive output and the number of previous matings by their mate. In one study (Wedell and Karlsson 2003), the correlation was between female reproductive output and a measure of spermatophore size. Variation in spermatophore size was, however, generated by mating some males prior to their being used in the experiment and recently mated males produce smaller spermatophores (see Table 1). We converted these correlations to Hedges' d using the MetaWin 2.0 calculator (Rosenberg et al. 2000) and setting the sample sizes for the two groups as half the total sample size (Table 2).

We calculated mean effect sizes weighted for sample size (sampling variance) using MetaWin 2.0 (Rosenberg et al. 2000). We used one effect size per species. For *Pieris napi* (three studies) and *Plodia interpunctella*, (three studies) we first calculated the weighted mean effect sizes for the available studies and estimated its standard deviation using the mean sample sizes. There was only a single study available for the other 23 species. We ran random effects models that allow for a true random component, in addition to sampling error, as a source of variation in effect size among studies (Hedges and Olkin 1985). We report the mean effect size and 95% confidence intervals calculated using a bias-corrected bootstrap approach (1,000 replicates), as well as the within-group heterogeneity (Q_w) for each group of studies, assuming Q_w follows a chi-square distribution, with $df = \text{number of studies} - 1$.

We tested whether the mean effect size differed between groups (e.g. Rhopalocera versus Heterocera) by testing for significance in between-group heterogeneity (Q_b) using randomisation tests based

Table 2 Effect sizes (Hedges' *d*) for the effect of male mating history on female reproductive output for each species as they were used as grouping variables for meta-analysis (n_1 and n_2 , M_1 and M_2 , and SD_1 and SD_2 are sample sizes, means and standard deviations of reproductive output for virgin and experienced male groups, respectively)

Ref. no.	Species	Mating pattern ^a	Taxa ^b	Virgin males		Experienced males		<i>n</i> (total)	Hedges' <i>d</i>	Var- <i>d</i>
				n_1	M_1	SD_1	n_2			
1	<i>Acrolepia assectella</i>	M	H	47	208	44	183	94	0.54	0.04
2	<i>Agrotis segetum</i>	M	H	$r=0.187$				191	0.38 ^c	0.02
3	<i>Bassella fusca</i>	P	H	16	675	278	539	72	0.47	0.08
4	<i>Colias eurytheme</i>	P	R	11	490	123	314	22	1.01	0.21
5	<i>Chilo partellus</i>	M	H	25	399	220	407	107	-0.04	0.05
6	<i>Choristoneura fumiferana</i>	M	H	50	145	45	139	86	0.14	0.05
7	<i>Choristoneura rosaceana</i>	P	H	45	549	139	407	135	1.03	0.04
8	<i>Diatraea considerata</i>	M	H	16	517	146	450	31	0.46	0.13
9	<i>Epiphyas postvittana</i>	M?	H	15	360	271	391	59	-0.12	0.09
10	<i>Eurema hecabe</i>	M?	R	10	246	85	234	20	0.11	0.20
11	<i>Helicoverpa armigera</i>	P	H	13	913	839	709	33	0.30	0.13
12	<i>Jalmenus evagoras</i>	M	R	28	70	91	43	57	0.37	0.07
13	<i>Lobesia botrana</i>	M	H	29	165	51	161	114	0.07	0.05
14	<i>Ostrinia nubilalis</i>	M	H	101	754	194	688	246	0.36	0.02
15	<i>Papilio glaucus</i>	P	R	40	18	29	12	95	0.23	0.04
16	<i>Papilio machaon</i>	M	R	13	499	130	461	26	0.25	0.16
17	<i>Pectinophora gossypiella</i>	P	H	83	134	76	77	184	0.81	0.02
18	<i>Pieris napi</i>	P	R	7	322	102	233	14	1.01	0.32
19	<i>Pieris napi</i>	P	R	10	342	152	252	19	0.58	0.22
20	<i>Pieris napi</i>	P	R	$r=0.73$				22	1.96 ^c	0.27
21	<i>Plodia interpunctella</i>	P	H	37	178	79	157	87	0.20	0.05
22	<i>Plodia interpunctella</i>	P	H	18	70	87	76	55	-0.08	0.08
23	<i>Plodia interpunctella</i>	P ^d	H	24	285	80	275	120	0.14	0.05
24	<i>Pseudaletia unipuncta</i>	P	H	27	998	556	1074	117	-0.15	0.05
25	<i>Sitotroga cerealella</i>	P	H	105	142	61	110	192	0.51	0.02
26	<i>Spodoptera littoralis</i>	P	H	28	1715	519	1663	42	0.10	0.11
27	<i>Thymelicus lineola</i>	M	R	150	99	31	95	164	0.11	0.08
28	<i>Trichoplusia ni</i>	P	H	20	1931	367	1846	61	0.26	0.08
29	<i>Zeiraphera canadiensis</i>	M	H	$r=0.008$				60	0.02 ^c	0.07
Additional studies										
30	<i>Antheraea mylitta</i>	P?	H	60	204	9	204	120	0.00	0.03
31	<i>Antheraea mylitta</i>	P?	H	30	215	17	207	60	0.43	0.07
32	<i>Danaus plexippus</i>	P	R	10	884	133	882	23	0.01	0.18
33	<i>Epiphyas postvittana</i>	M?	H	11	506	250	515	22	-0.04	0.18
34	<i>Lobesia botrana</i>	M	H	21	172	56	165	50	0.13	0.08
35	<i>Polytonia c-album</i>	P	R	14	356	92	321	28	0.34	0.14
36	<i>Trichoplusia ni</i>	P	H	8	2140	201	1931	28	0.61	0.18

^a M monandrous, P polyandrous, using a 40% polyandry criterion (see Table 1 and text).

^b Lepidoptera species were scored as H Heterocera (moths) or R Rhopalocera (butterflies and skippers).

^c In these correlational studies g was estimated from the weighted Pearson correlation coefficient $r=g/[g^2+4]^{0.5}$; g was then converted to Hedges' d .

^d In this study *Plodia interpunctella* appeared to be monandrous; however, for the analysis it was considered polyandrous in concordance with most other studies of this species.

Table 3 Summary of mean effect sizes (Hedges' d with 95% bias-corrected bootstrapped confidence intervals) for the relationship between male mating history and female reproductive output in Lepidoptera species. Data were grouped on the basis of mating pattern (monandry vs polyandry) and higher taxa (Heterocera:

moths vs Rhopalocera: butterflies and skippers). Within-group heterogeneity in effect sizes (Q_w), Rosenthal's fail-safe number and the Begg-Mazumdar correlation between standardised effect size and sample size (r -bias) are also given (n_e number of effect sizes)

Grouping	n_e	Effect size		Effect size heterogeneity			Rosenthal's fail-safe no.	r -bias
		Hedges' d	95% CI	Q_w	df	P -value		
All	25	0.33	0.21–0.47	23.0	24	0.52	440 ^a	–0.03
Polyandrous	12	0.45	0.25–0.68	10.6	11	0.48	188 ^a	0.04
Monandrous	13	0.25	0.12–0.34	9.2	12	0.69	42	0.12
Heterocera	18	0.32	0.17–0.48	16.1	17	0.51	255 ^a	0.28
Heterocera Monandrous	9	0.25	0.10–0.36	8.0	8	0.43	25	0.33
Heterocera Polyandrous	9	0.41	0.17–0.66	7.2	8	0.51	106 ^a	0.47
Rhopalocera	7	0.34	0.20–0.62	6.1	6	0.42	18	–0.75
Rhopalocera Monandrous	4	0.23	0.11–0.34	0.5	3	0.91	–	–
Rhopalocera Polyandrous	3	0.68	0.23–1.11	1.7	2	0.43	–	–

^a Rosenthal's fail-safe number was greater than $5n_e+10$.

on 1,000 replicates. To test for an interaction between taxonomic group and mating pattern type, and their independent influence on effect sizes, we performed weighted two-way ANOVAs following the method of Cooper and Hedges (1994). In brief, the weight given to the effect estimate is the reciprocal of its sampling variance. The sums of squares for each term in the ANOVA then have chi-square distributions with the degrees of freedom associated with that term in the standard ANOVA. F -statistics are not used.

In addition to dividing the data into Rhopalocera and Heterocera, to partially correct for a lack of phylogenetic independence, we also calculated the difference in mean effect size for monandrous and polyandrous species in families where both mating types occurred. We did this by converting the mean Hedges' d to the effect size r and then testing whether the mean value of Cohen's q ($=Z_{r1}-Z_{r2}$) was greater than zero.

We calculated Rosenthal's fail-safe number to assess the robustness of our results. This is the number of additional, unpublished studies with a mean effect of zero required to turn a significant mean effect size into one that does not differ significantly from zero given $\alpha=0.05$. By convention, a value of $5n_e+10$ is considered robust (where n_e =number of studies used to calculate the mean effect size).

To indirectly test for publication bias, we calculated the Begg-Mazumdar correlation between standardised effect size and sample size (r -bias). This should be viewed with caution, however, as this test has low statistical power with sample sizes less than 25 (review: Møller and Jennions 2001). We also used the "trim and fill" method of Duval and Tweedie (2000) to estimate the number of "missing" studies based on a funnel plot of the data (i.e. effect size vs sample size) and then calculated the mean effect if these hypothetical "missing" studies are included. Finally, we tested whether there was a relationship between year of publication and effect size or sample size.

Results

In 22 of 25 lepidopteran species, the reproductive output of females mated to virgin males was higher than that of females mated to experienced males. The mean effect size was Hedges' $d=0.33$ (95% CI: 0.21–0.47); that is a third of a standard deviation. The mean effect sizes and related information for studies divided on the basis of species' mating pattern and taxonomic group are presented in Table 3.

There was no difference in mean effect size between butterflies and moths (randomisation test, $Q_b=0.08$,

$P=0.78$). There was, however, a marginally non-significant trend towards a greater effect size in polyandrous than monandrous species ($Q_b=3.43$, $P=0.084$). We therefore performed a two-way ANOVA to compare mating types while controlling for the effect of taxonomic grouping. There was no significant interaction between taxonomic grouping and mating pattern ($\chi^2=0.002$, $P=0.96$) so we removed the interaction from the final model. Controlling for the non-significant effect of taxonomic group, the mean effect size for polyandrous species was significantly greater than that for monandrous species ($\chi^2=6.60$, $df=1$, $P=0.010$). There was still no effect of taxonomic grouping after controlling for mating pattern ($\chi^2=0.03$, $df=1$, $P=0.86$). The difference in mean effect size between polyandrous and monandrous species within families was Cohen's $q=0.12$ (95% CI: –0.08 to 0.38), which did not differ significantly from zero. There were, however, only five families that contained both mating types, so this test has very low statistical power.

There was no difference in the strength of the effect size calculated using fecundity versus fertility data (Hedges' $d=0.36$ and 0.17, respectively, $n=21$, 5, randomisation test, $Q_b=1.41$, $P=0.28$). The same was true even after controlling for the influence of mating pattern ($\chi^2=1.03$, $df=1$, $P=0.31$). For this analysis, we used one data point per measure per species.

There was no significant correlation between standardised effect size and sample size in any of the grouping of the data presented in Table 3 (r -bias, all $P>0.20$). This suggests there is no publication bias, but this conclusion is weak because of low statistical power. There was also no correlation between year of publication and effect size ($r_s=0.012$, $P=0.95$, $n=29$). There was, however, a significant decline in sample size with year of publication ($r_s=-0.54$, $P=0.003$, $n=29$).

A "Trim and Fill" analysis suggested that there were five "missing" studies. This was, however, due to a left skew in the funnel plot of effect size on sample size. The "corrected" effect size was in fact larger than that observed at Hedges' $d=0.43$ (95% CI: 0.30–0.57).

Of the seven studies that related female fecundity to factors known to affect spermatophore size, five showed that fecundity increased with presumed spermatophore size. The mean effect was Hedges' $d=0.17$ (95% CI: 0.03–0.37).

Discussion

Our results support the claim that male mating history strongly affects female reproductive output in Lepidoptera. Females mated to virgin males had higher fecundities than those mated to previously mated, experienced males. This was true irrespective of the species' polyandry level or taxonomic background. The reported mean effect size was $d=0.33$. The sample size per group needed to detect an effect of this size with 80% power using a two-sample t -test with $\alpha=0.05$ (two-tailed) is 145 (Cohen 1988). This is considerably larger than that of any of the 29 studies investigating the relationship. The likelihood of type II errors should therefore be taken into account for species where there was no significant effect of male mating history on female fecundity.

The most obvious proximate explanation for the effect of male mating history on female fecundity is that mating history affects spermatophore size. In all the studies in Table 1 in which the topic was addressed, virgin males produced larger spermatophores than experienced males. This suggests that lepidopteran males suffer a depletion of spermatophore precursors after each mating. It should be noted, however, that this decline is not irreversible. There is a trend for spermatophore size to increase the longer the interval between consecutive matings. In some species, subsequent spermatophores may never reach the full size of the first one produced, but in other species they do (e.g. Torres-Vila et al. 1995). Svård and Wiklund (1986) have further proposed that this trend may vary quantitatively depending on the species' polyandry level. It is also noteworthy that in the seven studies that related female reproductive output to factors known to effect spermatophore size, fecundity increased significantly with presumed spermatophore size (mean Hedges' $d=0.17$).

Two non-mutually exclusive hypotheses have been proposed to explain the proximate effect of male donations at mating (in particular spermatophore size) on female reproductive output. These are the so-called *parental investment* and *mating effort* hypotheses (reviews: Simmons and Parker 1989; Eberhard 1996; Simmons 2001). The parental investment hypothesis, developed from studies using radio-active labelling, showed that male-donated seminal substances were incorporated by females and reassigned to egg production and somatic maintenance (Boggs and Gilbert 1979; Greenfield 1982). This hypothesis assumes that nuptial gifts increase male fitness by provide extra nutritional resources that enhance females' reproductive output. In general, multiple mating, which increases the net amount of seminal product received, elevates the lifetime fecundity of female lepidopterans (Torres-Vila et al. 2004). This suggests there is

a beneficial effect of male-transferred substances. The extent to which nuptial gifts enhance fitness may, however, be constrained by larval and adult feeding experience and/or the species-specific egg production pattern (Boggs 1990; 1995). In addition, some studies offer evidence that larger spermatophores do not increase paternal investment as spermatophore size and nutritional content are uncorrelated (Marshall and McNeil 1989; Delisle and Bouchard 1995; Bissoondath and Wiklund 1996).

The mating effort hypothesis assumes that larger ejaculates reduce post-mating female receptivity and/or increase oviposition rate (see Table 4.1 in Simmons 2001). Both effects will increase a male's share of paternity if females mate multiply. A longer refractory period or more intense period of egg-laying might be triggered by: (1) the amount of sperm transferred or, more subtly, the eupyrene to apyrene sperm ratio (Watanabe et al. 1998; Cook and Wedell 1999); (2) the amount of male-transferred hormones or hormonal-like substances (e.g. Cordero 1995; Eberhard 1996). In insects, there is more evidence to support the mating effort hypothesis than the parental investment hypothesis (Vahed 1998; Simmons 2001). The case is less clear for lepidopterans though (Torres-Vila et al. 2004), especially polyandrous species where males invest relatively more nutrients into spermatophore production (Karlsson 1996).

In an earlier meta-analysis, we showed that remating significantly increases females' long-term reproductive output in polyandrous but not in monandrous species (Hedges' $d=0.77$ vs 0.27; Torres-Vila et al. 2004). Here we have shown that prior male mating, which decreases spermatophore size, significantly reduces female reproductive output in polyandrous and, albeit less strongly, monandrous species (Hedges' $d=0.45$ and 0.25, respectively). In combination, these results support the claim that in polyandrous species, female fecundity is closely linked with the amount of male donations at mating irrespective of whether these vary due to the degree of multiple mating or the size of the spermatophore. One interpretation of this result is that spermatophores are a form of paternal investment. However, this is not a robust conclusion and the mating effort hypothesis cannot be rejected because smaller spermatophores may simply contain lesser amounts of substances that stimulate oviposition (see discussion of Morrow and Gage 2000 by Simmons 2001, p 140). Alternatively, for example, increased production of apyrene sperm by recently mated males (e.g. Cook and Wedell 1996) may reduce female fecundity even though the main function of apyrene sperm appears to be delaying female remating (Cook and Wedell 1999). In contrast, our results suggest that spermatophore size in monandrous species may have been primarily shaped by sexual selection on males to increase their share of paternity. This statement may, at first, seem strange but it is important to remember that our definition of "monandrous species" includes those where up to 40% of females remate. There is, therefore, still strong selection on males to increase their share of paternity. It is also important to remember that an evolutionary consequence

of strong selection on males to reduce female remating may be that spermatophore size has been subject to the strongest sexual selection in species that presently show the lowest levels of polyandry.

A major finding of our studies is that the species' polyandry level should be considered when investigating the relationship between male donations and fecundity. In our survey, the effect of male mating history on fecundity for polyandrous species was nearly double that for monandrous species. One could explain this purely in terms of species differences in the size of nuptial gifts, but a number of studies of Lepidoptera also demonstrate a positive correlation between body-size corrected testis size and the level of polyandry (e.g. Gage 1994), a pattern that supports the mating effort hypothesis. Oberhauser (1989) suggested that there was initially selection on males to produce larger spermatophores to delay female remating (i.e. selection for mating effort). As male seminal investment increased, however, females secondarily evolved the ability to take advantage of male donations (i.e. paternal investment). This could ultimately lead to sexual selection for female preferences for males that provided higher-quality ejaculates and natural selection for males that increased their parental investment (Simmons and Parker 1989).

The fact that the effect of spermatophore size on female fecundity depends on the species' mating system could also be explained *without* evoking differences in paternal investment across species. Our alternative explanation draws on recent work showing that polyandrous and monandrous female phenotypes (Torres-Vila et al. 2002), or "alternative lifestyles" (Wedell et al. 2002), coexist within a species. The observed effect size difference between polyandrous and monandrous species could simply reflect the fact that the relative proportion of females that regain sexual receptivity (and/or experience shorter refractory periods) is greater in polyandrous than monandrous species when females receive a small spermatophore. Greater spermatophore size increases the female refractory period in most species, be they polyandrous or monandrous (Table 4.1 in Simmons 2001). Moreover, females' investment in egg production can vary depending on male quality. Females receiving small spermatophores often decrease their own reproductive output, exercising cryptic female choice through differential allocation that depends on the quality of her mate (Wedell 1996; Wedell and Karlsson 2003).

We make three assumptions: (1) the number of females that become receptive after mating is, obviously, higher in polyandrous species; (2) if females receive a small spermatophore, the number that become receptive again will be proportionally higher in polyandrous species; (3) females receiving a small spermatophore are more likely to show diminished oviposition rates because of the time and energy they devote to attracting another mate and/or because of differential allocation. From these assumptions it follows that the effect of a small spermatophore on fecundity will be more detrimental in a polyandrous species because there are proportionately more females pursuing

a multiple mating strategy in these species. To test this alternate explanation, we need more data from an array of polyandrous and monandrous species, which measure the propensity of singly mated females to remate, and data on the effect of having to attract a male to remate on fecundity (Torres-Vila et al. 2004).

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