



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE ECOLOGÍA
BIOLOGÍA EVOLUTIVA
PROYECTO
EFFECTOS DE CÓPULAS CONSECUTIVAS EN LA LONGEVIDAD
Y TAMAÑO DE ESPERMATÓFOROS EN EL ORDEN
LEPIDOPTERA: UN METANÁLISIS
TESIS

POR ARTÍCULO CIENTÍFICO

MALE MATING COSTS IN LEPIDOPTERA: A META-ANALYSIS

QUE PARA OPTAR POR EL GRADO DE:

MAESTRO EN CIENCIAS BIOLÓGICAS

PRESENTA:

RICARDO RAÑA MENDOZA

TUTOR(A) PRINCIPAL DE TESIS: DR. CARLOS RAFAEL CORDERO MACEDO
INSTITUTO DE ECOLOGÍA, UNAM

COMITÉ TUTOR: DR. JOSÉ JAIME ZÚÑIGA VEGA
FACULTAD DE CIENCIAS, UNAM

M. EN C. MOISÉS ARMANDO LUIS MARTÍNEZ
FACULTAD DE CIENCIAS, UNAM

CIUDAD UNIVERSITARIA, CDMX, DICIEMBRE, 2023



Universidad Nacional
Autónoma de México

Dirección General de Bibliotecas de la UNAM

Biblioteca Central



UNAM – Dirección General de Bibliotecas
Tesis Digitales
Restricciones de uso

DERECHOS RESERVADOS ©
PROHIBIDA SU REPRODUCCIÓN TOTAL O PARCIAL

Todo el material contenido en esta tesis esta protegido por la Ley Federal del Derecho de Autor (LFDA) de los Estados Unidos Mexicanos (México).

El uso de imágenes, fragmentos de videos, y demás material que sea objeto de protección de los derechos de autor, será exclusivamente para fines educativos e informativos y deberá citar la fuente donde la obtuvo mencionando el autor o autores. Cualquier uso distinto como el lucro, reproducción, edición o modificación, será perseguido y sancionado por el respectivo titular de los Derechos de Autor.



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE ECOLOGÍA
BIOLOGÍA EVOLUTIVA
PROYECTO
EFFECTOS DE CÓPULAS CONSECUTIVAS EN LA LONGEVIDAD
Y TAMAÑO DE ESPERMATÓFOROS EN EL ORDEN
LEPIDOPTERA: UN METANÁLISIS
TESIS

POR ARTÍCULO CIENTÍFICO

MALE MATING COSTS IN LEPIDOPTERA: A META-ANALYSIS

QUE PARA OPTAR POR EL GRADO DE:

MAESTRO EN CIENCIAS BIOLÓGICAS

PRESENTA:

RICARDO RAÑA MENDOZA

TUTOR(A) PRINCIPAL DE TESIS: DR. CARLOS RAFAEL CORDERO MACEDO
INSTITUTO DE ECOLOGÍA, UNAM

COMITÉ TUTOR: DR. JOSÉ JAIME ZÚÑIGA VEGA
FACULTAD DE CIENCIAS, UNAM

M. EN C. MOISÉS ARMANDO LUIS MARTÍNEZ
FACULTAD DE CIENCIAS, UNAM

CIUDAD UNIVERSITARIA, CDMX, DICIEMBRE, 2023

Agradecimientos Institucionales

Agradezco al Posgrado de Ciencias Biológicas de la UNAM por la oportunidad para llevar a cabo mis estudios de maestría, al Instituto de Ecología de la misma universidad por permitirme trabajar en mi proyecto de titulación en sus instalaciones dentro del Laboratorio de Ecología y Conducta de Artrópodos y de forma remota y a CONAHCYT por su apoyo económico. (Proyecto PAPIIT IN214120, Selección sexual en mariposas)

Por su asesoría, apoyo y enorme paciencia agradezco a mi tutor el Dr. Carlos Rafael Cordero Macedo

Por su consejo y observaciones sobre este proyecto de tesis y examen profesional, agradezco a mi Comité Tutor compuesto por el Dr. José Jaime Zúñiga Vega y el M. en C. Moisés Armando Luis Martínez.

Agradecimientos a título personal

Quiero agradecer a mi papá por su constante apoyo, te lo debo todo y más.

A Angee, Alice, Juan Carlos y Julio por su incansable ayuda, los amo como a hermanos.

A Andrés, por tu cariño y acompañamiento, te amo incansablemente.

A Marcos, por su enorme ayuda,

Y a Zephyr y Pirita, que para bien o para mal, nunca dejan mi lado.

Índice

Resumen	1
Abstract.....	2
Introducción.....	3
Abstract.....	6
Introduction	7
Methodology.....	8
Selection criteria	8
Data extraction.....	10
Statistical analysis	10
Results.....	11
Search results	11
Effect on spermatophore size	12
Effect on longevity	17
Publication bias	20
Discussion	20
Conclusions	23
Acknowledgments.....	23
References	24
Supporting Information.....	28
Discusión	29
Conclusiones	31

Resumen

Uno de los principios básicos de los roles sexuales darwinianos es que la inversión de un macho en el eyaculado es insignificante mayor en comparación con la inversión de la hembra en los óvulos fertilizados por dicho eyaculado. Sin embargo, la historia natural y la investigación experimental han demostrado que esto no es cierto, al menos para algunos grupos de animales. Se ha mencionado con frecuencia que los machos de lepidópteros subvierten los roles sexuales tradicionales porque producen eyaculados en forma de espermatozoides que pueden ser muy costosos. Sin embargo, una mirada detallada a la literatura muestra que algunos estudios experimentales no han logrado demostrar costos masculinos. Realizamos un metanálisis de estudios experimentales que miden el efecto del número de cópulas consecutivas de mariposas y polillas macho sobre el tamaño de los espermatozoides y la longevidad de los machos. Estas dos últimas variables son componentes importantes de la adecuación de los lepidópteros machos y una relación negativa con el número de cópulas indicaría costos para los machos. Encontramos un efecto negativo global significativo en el tamaño del espermatozoide. El efecto global sobre la longevidad no fue significativamente diferente de cero, pero hubo un efecto negativo sobre la longevidad de los machos cuando el análisis se realizó a nivel de especie. También encontramos una amplia variación entre estudios y especies en los tamaños del efecto para ambos componentes de adecuación. Esto es de esperar teniendo en cuenta la enorme diversidad de lepidópteros y la heterogeneidad de las metodologías experimentales; sin embargo, no hay un muestreo suficiente entre los taxones de lepidópteros para descartar el sesgo de publicación. Nuestros resultados respaldan la idea de que la cópula y la producción de eyaculados en el orden Lepidoptera tienen costos significativos de adecuación para los machos.

Abstract

One of the basic tenets of Darwinian sex roles is that a male's investment in the ejaculate is negligible compared to the female's investment in the eggs fertilized by that ejaculate. However, natural history and experimental research have shown that this does not hold true, at least for some animal groups. Male Lepidoptera have been frequently mentioned as subverting traditional sex roles because they produce ejaculates in the form of spermatophores that can be very costly. However, a detailed look at the literature shows that some experimental studies have failed to demonstrate male costs. We performed a meta-analysis of experimental studies that measure the effect of the number of consecutive copulations by male butterflies and moths on spermatophore size and male longevity. These last two variables are important fitness components of male Lepidoptera, and a negative relationship with the number of copulations would indicate male costs. We found a significant global negative effect on spermatophore size. The global effect on longevity was not significantly different from zero, but there was a negative effect on male longevity when the analysis was performed at the species level. We also found wide variation among studies and species in the effect sizes for both fitness components. This is expected considering the enormous diversity of the Lepidoptera and the heterogeneity of experimental methodologies, however, there is insufficient sampling across Lepidopteran taxa to rule out publication bias. In conclusion, our results support the idea that copulation and ejaculate production in the order Lepidoptera has significant fitness costs for males.

Introducción

La mayoría de los biólogos consideran que la producción de espermatozoides por parte de los machos es menos costosa en comparación con la producción de óvulos por parte de las hembras (1,2). De acuerdo con este punto de vista, los principales costos de reproducción para los machos son los relacionados con la búsqueda de pareja y las exhibiciones de cortejo. Este síndrome de rol sexual darwiniano (3) se ha utilizado para predecir los efectos de la selección sexual en los fenotipos masculinos y femeninos y los sistemas de apareamiento. Sin embargo, cada vez hay más evidencia que apunta a diferentes estrategias y realidades que difieren de estos roles sexuales tradicionales en formas importantes (4,5), así como inconsistencias en su uso y definición (6,7). Como tal, existe la necesidad de reexaminar las estrategias sexuales de los machos en diferentes grupos para medir más cuidadosamente qué tan costoso es para ellos producir eyaculados.

En muchos insectos, el macho transfiere espermatozoides a la hembra dentro del espermatóforo, un saco producido por el macho que contiene espermatozoides y nutrientes importantes (8). Este saco puede contener recursos que son difíciles o incluso imposibles de obtener en su dieta normal, como el sodio obtenido del encharcamiento (9) o las proteínas del néctar (10,11). Este costoso regalo nupcial (12) ejerce una presión evolutiva sobre los machos para garantizar que sea su espermatozoides (no el de otro macho) el que se utilice para fertilizar los óvulos de la hembra. Esto frecuentemente resulta en medidas para promover o asegurar el uso del espermatozoides del macho, como la producción de tapones de apareamiento (13), la transferencia en el eyaculado de compuestos inhibidores de la receptividad (14–16) o antiafrodisíacos que disuaden a otros machos de cortejar a la hembra apareada (17). A su vez, esto genera presión sobre las hembras para que contrarresten estas medidas y puedan aparearse con tantos machos como sea óptimo para ellas (18), por ejemplo, produciendo sustancias para disolver el tapón de apareamiento (19).

Entre los miembros del orden Lepidoptera, esta coevolución sexualmente antagónica implica la inhibición de la receptividad sexual experimentada por las hembras después de recibir un espermatozoido en su bursa copulatrix (20). Al recibir un espermatozoido, este órgano se contrae repetidamente y secreta enzimas para digerir y absorber el espermatozoido y su contenido (21,22). Como un estómago, produce señales de su plenitud para inhibir la recepción de otro espermatozoido. Para los machos que producen espermatozoidos más grandes esto resulta en una clara ventaja ya que estos producen un efecto inhibitor más duradero (23). Como una respuesta contradaptativa en las hembras, la selección sexual ha favorecido la evolución de los signa: estructuras afiladas dentro de la bursa copulatrix donde se recibe el espermatozoido y que permiten romper el espermatozoido y eliminar la inhibición de la receptividad más rápidamente. En respuesta, en los machos se ha favorecido la evolución de envolturas de espermatozoidos más gruesas, lo que perpetúa el antagonismo entre los sexos (24). Este proceso coevolutivo sugiere que los machos pagan un costo fisiológico importante para producir espermatozoidos. Este costo ha sido estimado de dos maneras. La primera es buscando una relación negativa entre la cantidad de eyaculado producida (normalmente medida por el número de eyaculados transferidos en cópulas consecutivas) y la longevidad de los machos. La segunda es buscando un tamaño decreciente en los eyaculados tras cópulas consecutivas. Estas se consideran medidas apropiadas de costos porque indican un agotamiento de las sustancias utilizadas contenidas en el eyaculado, y varios estudios han identificado un efecto positivo del tamaño del eyaculado en la adecuación de los machos (11,25–27). Aunque varios estudios han encontrado estos costos (28–31), otras investigaciones no muestran los efectos esperados (32,33). Para abordar esta controversia, en este trabajo realizamos un metanálisis sobre el efecto que tienen las cópulas repetidas en el tamaño de los espermatozoidos y la longevidad de los machos en el orden Lepidoptera.

2

3 Male mating costs in Lepidoptera: A meta-analysis

4

5

6

7 Ricardo Raña Mendoza¹, Carlos Cordero ^{2*}

8

9

10

11 ¹ Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Mexico City, México

12 ²Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de

13 México, Mexico City, Mexico

14 *Corresponding author

15 E-mail: cordero@ecologia.unam.mx (CRCM)

16

17

18 **Abstract**

19 One of the basic tenets of Darwinian sex roles is that a male's investment in the ejaculate is
20 negligible compared to the female's investment in the eggs fertilized by that ejaculate. However,
21 natural history and experimental research have shown that this does not hold true, at least for some
22 animal groups. Male Lepidoptera have been frequently mentioned as subverting traditional sex
23 roles because they produce ejaculates in the form of spermatophores that can be very costly.
24 However, a detailed look at the literature shows that some experimental studies have failed to
25 demonstrate male costs. We performed a meta-analysis of experimental studies that measure the
26 effect of the number of consecutive copulations by male butterflies and moths on spermatophore
27 size and male longevity. These last two variables are important fitness components of male
28 Lepidoptera, and a negative relationship with the number of copulations would indicate male costs.
29 We found a significant global negative effect on spermatophore size. The global effect on longevity
30 was not significantly different from zero, but there was a negative effect on male longevity when
31 the analysis was performed at the species level. We also found wide variation among studies and
32 species in the effect sizes for both fitness components. This is expected considering the enormous
33 diversity of the Lepidoptera and the heterogeneity of experimental methodologies, however, there
34 is insufficient sampling across Lepidopteran taxa to rule out publication bias. In conclusion, our
35 results support the idea that copulation and ejaculate production in the order Lepidoptera has
36 significant fitness costs for males.

37

38

39 Introduction

40 Most biologists consider that sperm production by males is inexpensive compared to the production
41 of eggs by females (1,2). According to this view, the main costs of reproduction for males are those
42 involved in mate searching and courtship displays. This Darwinian sex-role syndrome (3) has been
43 used to predict the effects of sexual selection on male and female phenotypes and mating systems.
44 However, there is growing evidence pointing to different strategies and realities that differ from
45 these traditional sex roles in important ways (4,5), as well as inconsistencies in their use and
46 definition (6,7). As such, there is a need to reexamine the sexual strategies of males in different
47 groups to measure more carefully how costly it is for them to produce ejaculates.

48 In many insects, sperm is offered by the male to the female within the spermatophore, a
49 sac produced by the male that contains both sperm cells and valuable nutrients (8). This sac can
50 contain resources that are difficult or even impossible to obtain in their normal diet, like sodium
51 from puddling (9), or proteins from nectar (10,11). This costly nuptial gift (12) puts an evolutionary
52 pressure on males to ensure that it is their sperm (not that of another male) that is being used to
53 fertilize the female's eggs. This frequently results in measures to promote or ensure the use of the
54 male's sperm, such as the production of mating plugs (13), the transfer in the ejaculate of
55 receptivity-inhibiting compounds (14–16) or anti-aphrodisiacs that deter other males from courting
56 the mated female (17). In turn, this places pressure on females to counteract these measures so
57 that they can mate with as many males as is optimal for them (18), for example, producing substances
58 to dissolve a mating plug (19).

59 Among members of the order Lepidoptera, this sexually antagonistic coevolution involves
60 the sexual receptivity inhibition experienced by the females after receiving a spermatophore in their
61 bursa copulatrix (20). Upon receiving a spermatophore, this organ contracts repeatedly and secretes

62 enzymes to digest and absorb the spermatophore and its contents (21,22). Like a stomach, it signals
63 its fullness to inhibit the reception of another spermatophore. This results in a clear advantage for
64 males that produce larger spermatophores ,and therefore produces a longer-lasting inhibitory effect
65 (23). To remove this inhibition faster, females evolved signa—sharp structures within the bursa
66 copulatrix where the spermatophore is received. In response, males may develop thicker
67 spermatophore envelopes, which perpetuates the antagonism (24). This coevolutionary process
68 suggests that males pay an important physiological cost for producing spermatophores. This cost
69 has been estimated in two ways. The first is by looking for a negative relationship between the
70 amount of ejaculate produced (usually measured by the number of ejaculates transferred in
71 consecutive copulations) and male longevity. The second is by looking for decreasing ejaculate size
72 over consecutive copulations. This is considered a measure of costs because it indicates a depletion
73 of ejaculate substances, and several studies have identified a positive effect of ejaculate size on
74 male fitness (11,25–27). Although several studies have found these costs (28–31), other
75 investigations did not show the predicted effects (32,33). To address this controversy, we conducted
76 a meta-analysis on the effect of repeated copulations on spermatophore size and male longevity in
77 the order Lepidoptera.

78 **Methodology**

79 **Selection criteria**

80 We searched for relevant studies between November 2020 and March 2021 using the search
81 engines of SCOPUS, Science Direct, Web of Science, Biological Abstracts, ProQuest, and SciELO. We
82 used keyword combinations, both in English and Spanish, that included “Lepidoptera”, “Male”,
83 “Investment”, “Mating Frequency”, “Polyandry”, “Polygyny”, “Longevity”, “Survivorship”,
84 “Ejaculate Size”, and “Spermatophore” (Table 1). We also included studies from a previously

85 compiled list (made “by hand” by CC) and several works recommended by colleagues. All of these
 86 were listed and managed using the reference manager Mendeley (<https://www.mendeley.com>).

87 **Table 1. A meta-analysis of male mating costs in Lepidoptera:** Results for the literature search for
 88 each search engine and with each keyword combination used.

		SCOPUS	Science Direct	WoS	Bio Abstracts	ProQuest	SciELO	Total
#1	Lepidoptera AND Male AND Investment	102	559	175	125	1079	0	2040
#2	Lepidoptera AND Macho AND Inversión	0	8	0	0	26	0	34
#3	Lepidoptera AND Male AND "Mating Frequency"	78	124	76	92	756	2	1128
#4	Lepidoptera AND Macho AND "frecuencia de cópula"	0	0	0	0	1	0	1
#5	Lepidoptera AND Male AND Polyandry	73	90	112	76	641	2	994
#6	Lepidoptera AND Macho AND Poliandria	0	0	0	0	3	1	4
#7	Lepidoptera AND Male AND Polygyny	13	65	11	4	436	2	531
#8	Lepidoptera AND Macho AND Poliginia	0	0	0	0	6	1	7
#9	#1 AND Longevity	22	179	39	25	1201	0	1466
#10	#2 AND Longevidad	0	0	0	0	3	0	3
#11	#1 AND Survivorship	3	55	4	2	813	0	877
#12	#2 AND Supervivencia	0	0	0	0	7	0	7
#13	#1 AND "ejaculate size"	9	24	27	13	158	0	231
#14	#2 AND "tamaño del eyaculado"	0	0	0	0	0	0	0
#15	#1 AND Spermatophore	36	103	82	36	391	0	648
#16	#2 AND Espermatóforo	0	0	0	0	1	0	1
	Total	336	1207	526	373	5522	8	7972

89

90 We established our eligibility criteria using the PICO structure (34), where studies that were
91 to be included had to [1] use males of the Lepidoptera order [2] that were subjected to regular
92 consecutive copulations, [3] reported different numbers of copulations, and [4] measured an effect
93 on longevity and/or spermatophore size. Results reported in text, tables or figures were included.
94 Different units of measure for the spermatophore size were also included if they considered its full
95 size, such as length, volume, or ejaculate mass relative to body mass (35). Following these criteria,
96 and after removing duplicates, we first screened the articles by reading their title and abstract, then
97 selected the relevant articles, reading them in full, following the PRISMA guideline (36).

98 **Data extraction**

99 The primary data extracted from the selected studies were the species and family, the number of
100 consecutive copulations and the sample size of each experimental group, the time interval between
101 consecutive copulations, whether feeding was *ad libitum*, restricted as larvae or restricted as adults,
102 the mean and standard error of spermatophore size and/or longevity of each group and the units
103 of measurement used. We also extracted the title, year, and the corresponding author's
104 information. If the necessary measurements were not provided directly in the text or tables, we
105 extracted them from the figures using the program WebPlotDigitizer (37). All the extracted data was
106 encoded in a Microsoft Excel (2018) spreadsheet.

107 **Statistical analysis**

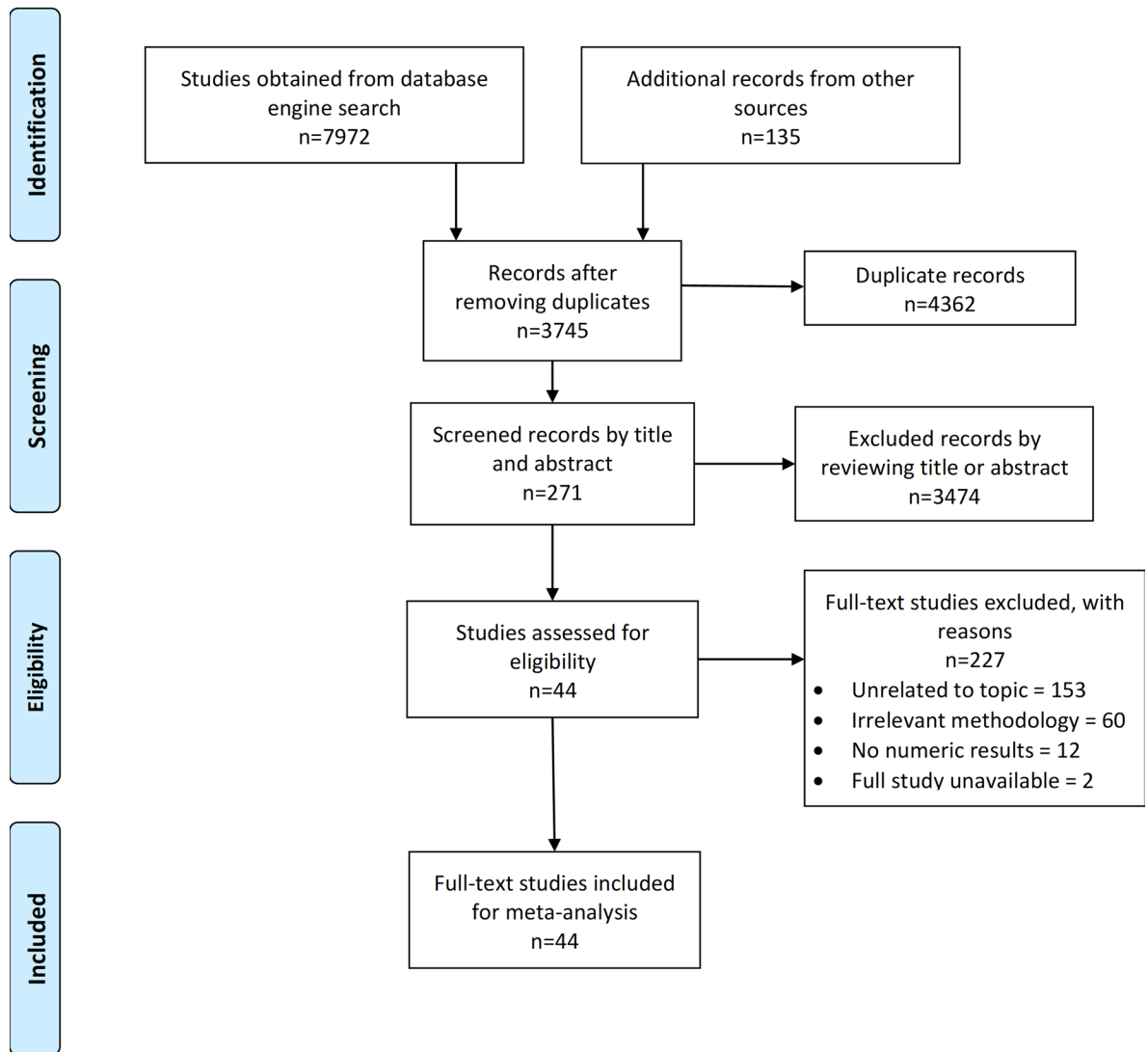
108 We used the R software (38) to analyze our data using the meta (39), dmetar (40), and metafor (41)
109 packages. The full reproducible code is available in S1 File. For both the spermatophore size and
110 longevity analyses, standardized mean differences were obtained from each article by calculating a
111 rate of change from the first measurement to each consecutive replicate and relating these scaled
112 measurements to the number of copulations using a linear model. The pooled effect was obtained

113 with a random-effects model utilizing the DerSimonian and Laird method (42) to estimate variation.
114 The Higgins I^2 statistic was used to quantify heterogeneity (43). Subgroup analyses using family and
115 species as moderators were used to test for phylogenetic correlations in the measured effects.
116 Finally, we checked for publication bias using separate funnel plots for the spermatophore size and
117 longevity datasets. We also tested these plots for asymmetry with Begg's (44) and Egger's (45) tests,
118 where a significant asymmetry would show a bias.

119 **Results**

120 **Search results**

121 A total of 8017 results were obtained from our literature search, with 3745 results remaining after
122 removing duplicates. Of these, 3474 were removed after title and abstract screening, and 271 were
123 subjected to an eligibility assessment. After this, we were left with 44 studies, ranging from 1985 to
124 2020 amongst 25 different publications with 104 authors (Fig.1). The full data set is available in S2
125 File.



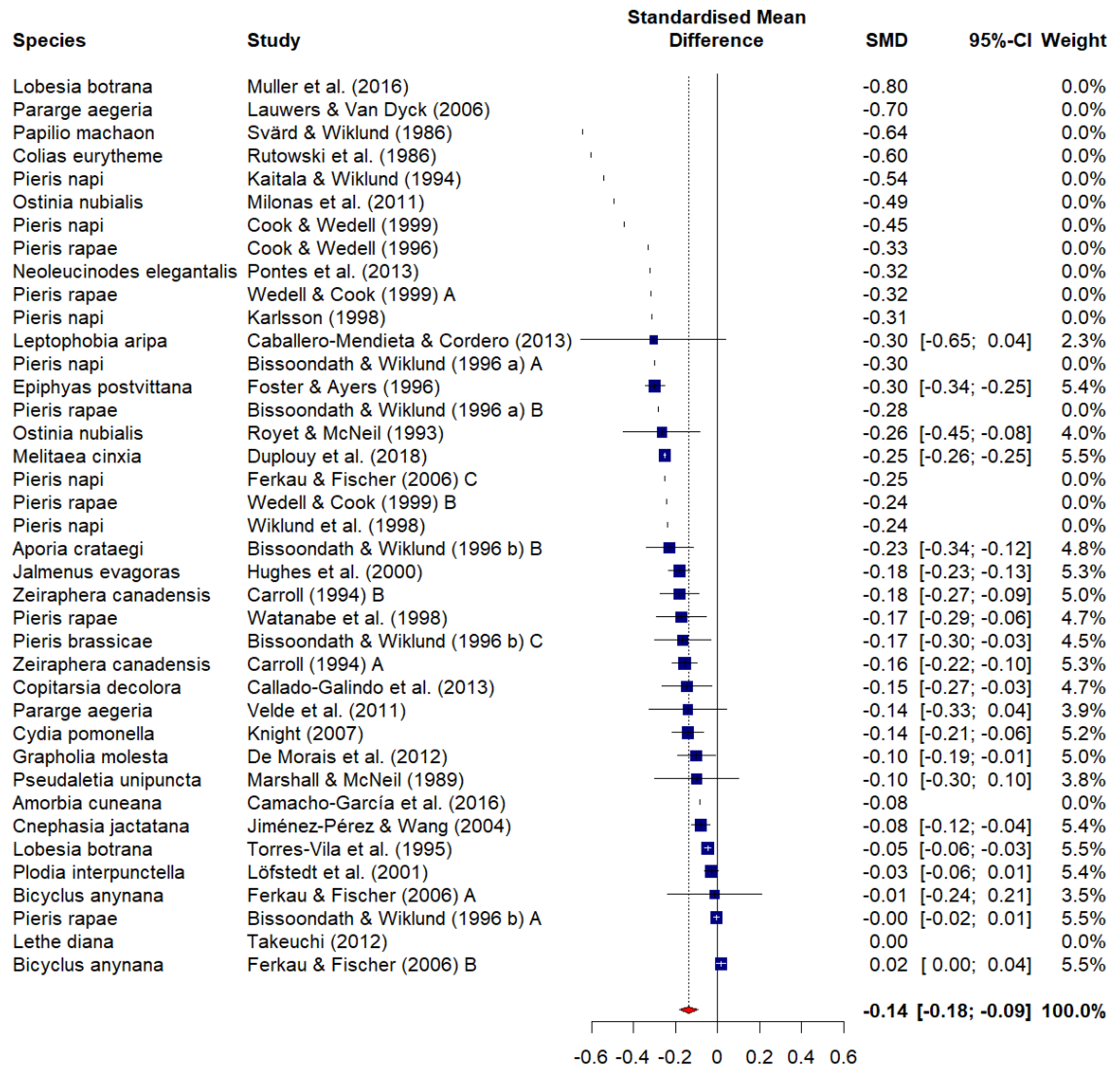
126

127 **Fig 1. PRISMA diagram.** We show the flow of information through the different phases of the
 128 systematic review and the number of records identified, included, or excluded, and the reasons for
 129 exclusions.

130 **Effect on spermatophore size**

131 The combined data from 21 studies showed a significantly negative pooled effect of the number of
 132 consecutive copulations on spermatophore size (SMD = -0.1373, 95% CI: [-0.1798; -0.0948], p-value
 133 < 0.0001; Fig 2). Among all studies, the effect size obtained ranged from -0.80 to a fully neutral 0.

134 Tested heterogeneity was very high ($I^2 = 99\%$, $\tau^2 = 0.0225$, d.f. = 20, $Q = 2239.75$, p-value = 0),
 135 indicating high variation in effect sizes among studies.

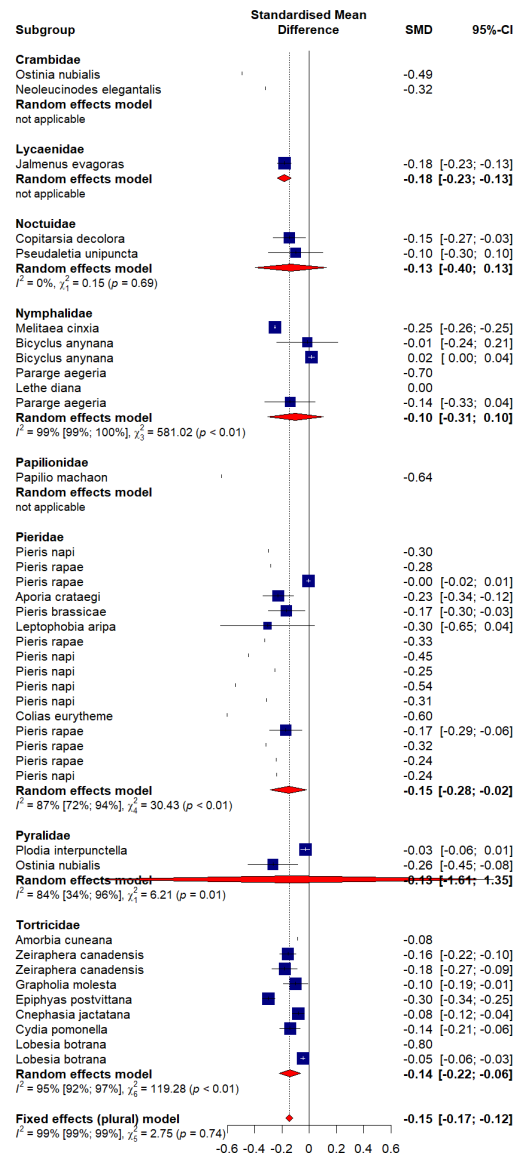


136

137 **Figure 2. Global effect of consecutive matings on spermatophore size.** Squares with horizontal
 138 lines represent the mean effect size with associated confidence intervals for each of the studies.
 139 Single vertical lines indicate the effect size of studies with only two points of data, which makes their
 140 variation and weight zero. The diamond at the bottom indicates the pooled effect.

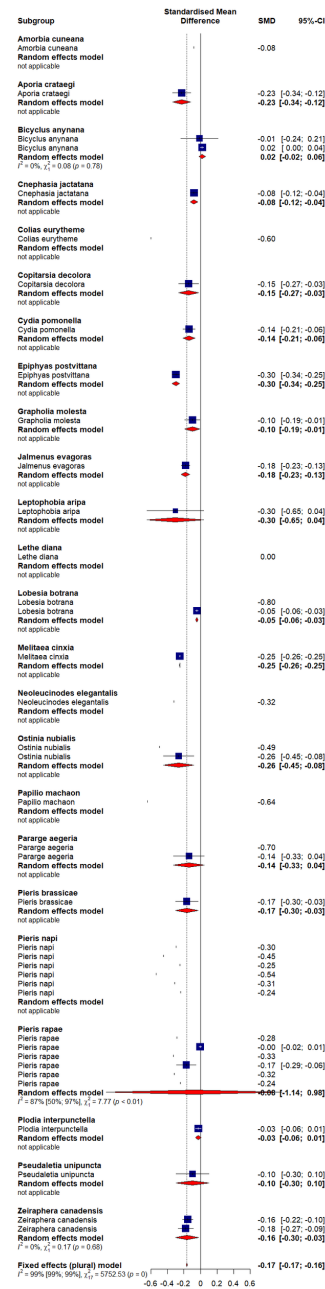
141 The subgroup analysis by family showed no reduction in overall heterogeneity and no
142 significant difference among subgroups ($I^2=99%$, d.f.= 5, $Q = 2.75$, p-value = 0.7379), suggesting that
143 the negative effect of the number of consecutive copulations on spermatophore size is similar
144 among all families (Fig 3). However, the analysis grouped by species reduced the heterogeneity and
145 showed significant between-species difference ($I^2=63%$, d.f.= 17, $Q = 5753$, p-value=0), indicating
146 high variation in investment strategies at the species level (Fig 4).

147



148

149 **Figure 3. Subgroup analysis by family of the effect of consecutive matings on spermatophore**
 150 **size.** Squares with horizontal lines represent the mean effect size with associated confidence
 151 intervals for each of the studies. Single vertical lines indicate the effect size of studies with only two
 152 points of data, which makes their variation and weight zero. The diamond at the bottom of each
 153 family grouping indicates the summary measure for that family, and the diamond at the bottom of
 154 the figure, the overall effect.

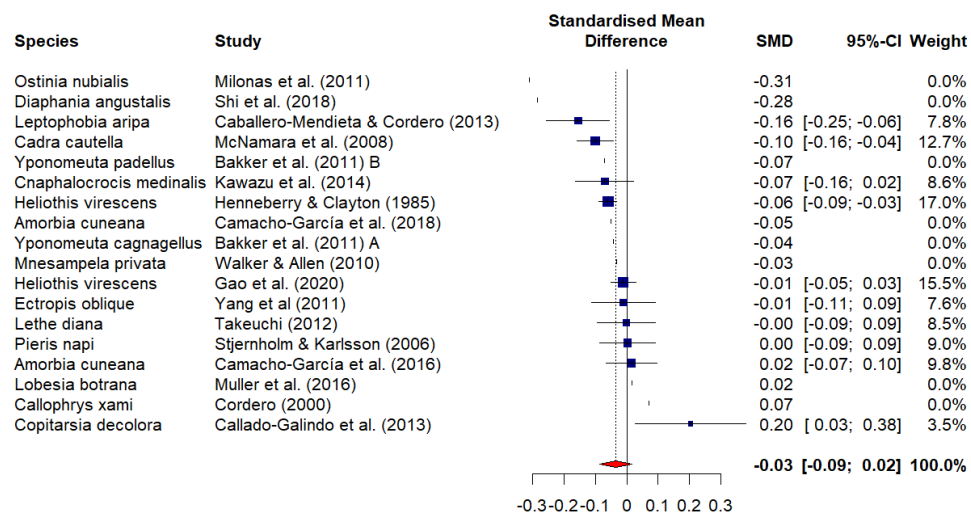


155

156 **Figure 4. Subgroup analysis by species of the effect of consecutive matings on spermatophore**
 157 **size.** Squares with horizontal lines represent the mean effect size with associated confidence
 158 intervals for each of the studies. The diamond at the bottom of each species grouping indicates the
 159 summary measure for that species, and the diamond at the bottom of the figure, the overall effect.

160 **Effect on longevity**

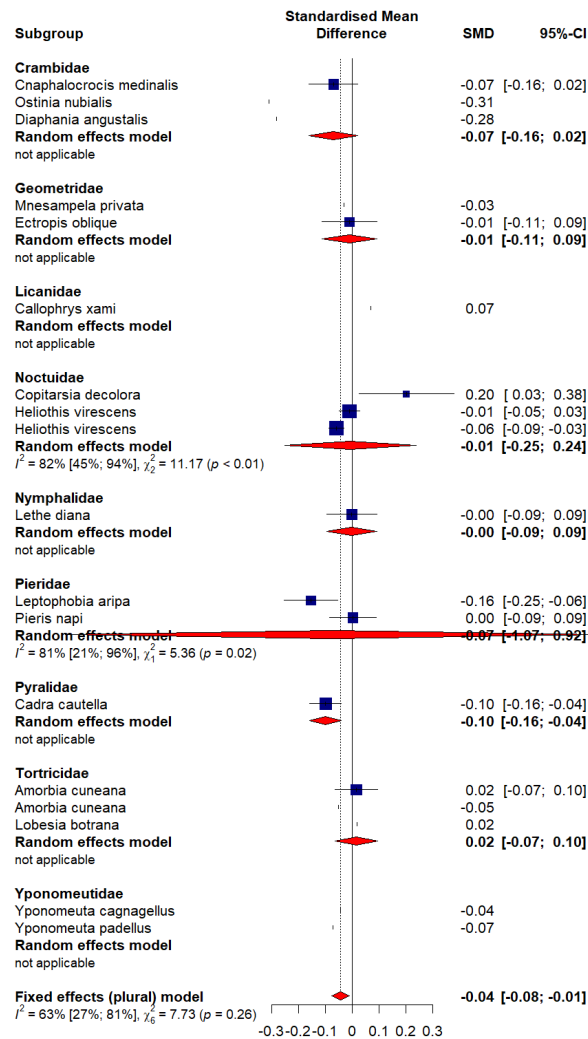
161 The combined data of 10 studies showed a non-significant negative pooled effect size of the number
 162 of consecutive copulations on longevity (SMD = -0.03, 95% CI: [-0.09; 0.02], p-value = 0.15). Amongst
 163 all studies, the effect size ranged from -0.31 to 0.2. The tested heterogeneity (I^2) was 63% ($\tau^2 =$
 164 0.0018, d.f. = 9, $Q = 24.34$, p-value = 0.004), indicating moderate variation among the studies (Fig
 165 5).



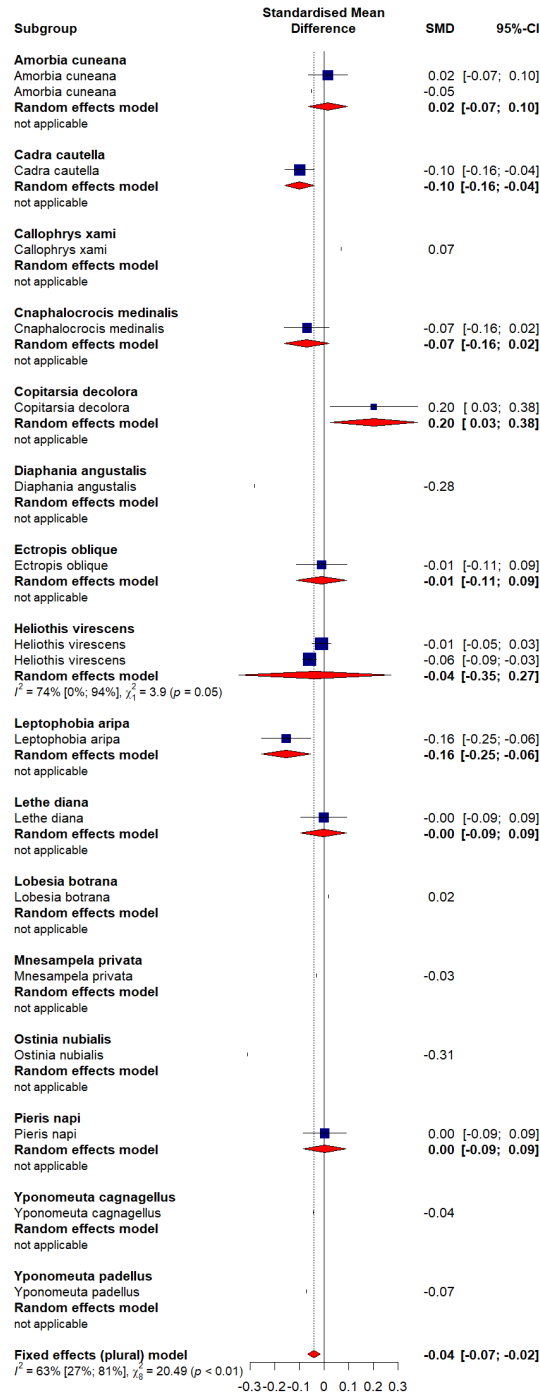
166
 167 **Figure 5. Global effect of consecutive matings on longevity.** Squares with horizontal lines represent
 168 the mean effect size with associated confidence intervals for each of the studies. The diamond at
 169 the bottom indicates the pooled effect.

170 The subgroup analysis by family showed a significant negative pooled effect of the number
 171 of consecutive copulations on longevity (SMD = -0.04, 95% CI: [-0.08; -0.01]), but no reduction in
 172 overall heterogeneity and no significant difference among subgroups ($I^2=63%$, d.f.= 6, $Q = 7.727$, p-
 173 value = 0.258) This suggests an uneven representation of studies for each family (Fig 6). The
 174 subgroup analysis by species also showed a significant difference among species, with a significant
 175 negative effect (SMD = -0.04, 95% CI: [-0.07; -0.02], $I^2=63%$, d.f. = 8, $Q = 20.49$, p-value = 0.008),

176 although it also showed the same heterogeneity as the global analysis. This indicates that an overall
 177 negative effect occurs at the species-level, but that there is wide variation in the size of that effect
 178 (Fig 7).



179
 180 **Figure 6. Subgroup analysis by family of the effect of consecutive matings on longevity.** Squares
 181 with horizontal lines represent the mean effect size with associated confidence intervals for each of
 182 the studies. The diamond at the bottom of each family grouping indicates the summary measure for
 183 that family, and the diamond at the bottom of the figure, the overall effect.



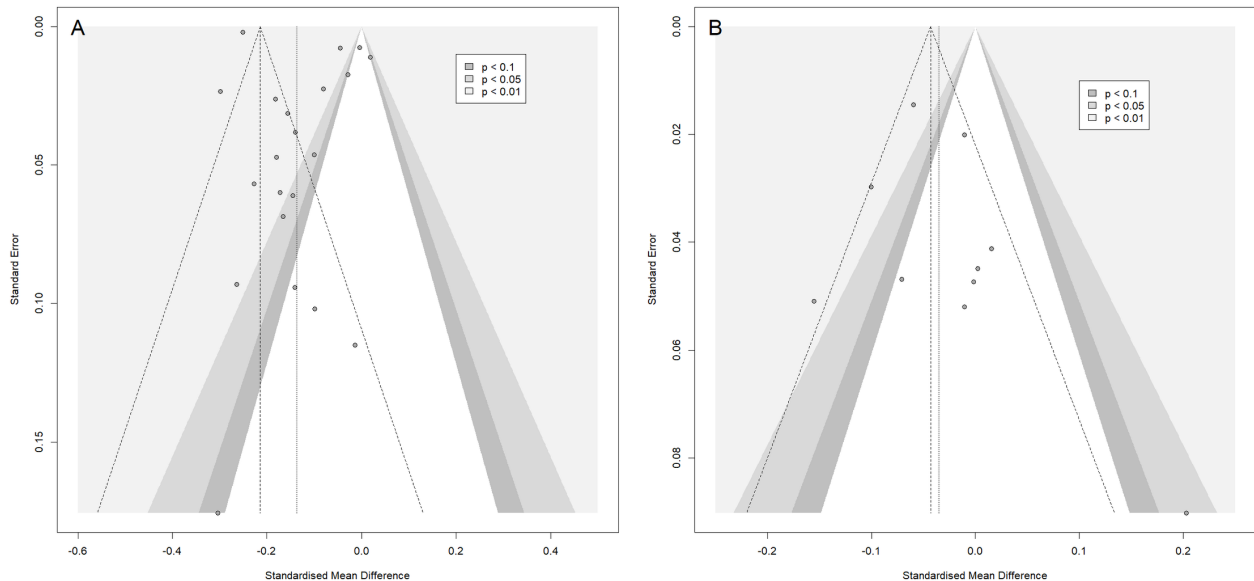
184

185 **Figure 7. Subgroup analysis by species of the effect of consecutive matings on longevity.** Squares
 186 with horizontal lines represent the mean effect size with associated confidence intervals for each of
 187 the studies. The diamond at the bottom of each species grouping indicates the summary measure
 188 for that species, and the diamond at the bottom of the figure, the overall effect.

189 **Publication bias**

190 The funnel plot for the effect of the number of consecutive copulations on spermatophore size (Fig
191 8A) showed significant asymmetry in Begg's test ($z = -2.23$, p -value = 0.025), whereas Egger's test
192 was close to significance ($\beta_0 = 4.905$, 95% CI: [0.05; 9.76], $t = 1.98$, p -value = 0.062). In the case of
193 the effect on longevity (Fig 8B), neither Begg's ($z = 0.09$, p -value = 0.9287) nor Egger's ($\beta_0 = 0.997$,
194 95% CI: [-1.08; 3.07], $t = 0.942$, p -value = 0.373) showed significant funnel plot asymmetry.

195



196 **Figure 8. Contour-enhanced funnel plots of the studies included.** In the plot, each paper's precision
197 is plotted against its effect size. Contours of statistical significance are overlaid on the plots (i.e.,
198 shading). (A) Spermatophore size. (B) Longevity.

199 **Discussion**

200 Our results supported the hypothesis that copulation and ejaculate production in males of the order
201 Lepidoptera has significant fitness costs. Our analysis showed that spermatophore size decreases in

202 consecutive copulations. This is probably a result of the fact that the content of the spermatophores
203 frequently includes sugars, fats, proteins, and salts (46,47) that are difficult or impossible to recover
204 for the adult male. The transfer of a small spermatophore reduces the fitness accrued by the male
205 from a copulation for two main reasons. First, copulation induces a period of reduced sexual
206 receptivity in the female that is positively correlated with spermatophore size; and second, the
207 reduced amount of nutrients transferred could result in a smaller number of eggs laid or a reduction
208 in the resources invested in each egg (48). Furthermore, although the global effect of copulation on
209 longevity was not significant, the analysis by species shows a significant negative effect. This
210 suggests that the materials in ejaculates come not only from the accessory reproductive glands and
211 testicles, but also are sequestered from other reservoirs of the male body, and that these materials
212 are essential for increasing male survival.

213 Our findings showing that copulation and ejaculate production are substantially costly for
214 male Lepidoptera have broad evolutionary implications. Since a large male investment of resources
215 during copulation is one of the factors that could favor the evolution of male mate choice (49,50),
216 we predict that male mate choice (probably combined with female mate choice, i.e., mutual mate
217 choice) is common in Lepidoptera. In agreement with this prediction, Bondurianski's review of male
218 mate choice in insects (49) identified studies of 12 species belonging to seven families of butterflies
219 and moths in which male mate choice was demonstrated. In a brief informal internet search of
220 studies published after Bondurianski's review, we found further demonstrations of male mate
221 choice in additional species (*Ephestia kuehniella* (Pyralidae) (51), *Bicyclus anynana* (Nymphalidae)
222 (52) and *Achroia grisella* (Pyralidae) (53)). The case of *B. anynana* is fascinating because male mate
223 choice shows developmental plasticity, being present only in dry-season males, while female choice
224 is observed during the wet season (52,54), and the hormonal and genetic mechanisms responsible
225 for this behavioural plasticity are known (55). Other researchers uncovered male preferences for

226 certain types of females (some of these studies include females of closely related species in the
227 experimental design) in other butterflies (*Papilio glaucus* and *P. canadensis*, Papilionidae; (56)) and
228 moths (*Therapsimima ampellophaga*, Zygaenidae; (57)). In a broader context, a recent comparative
229 study found that male mate choice is apparently very common in arthropods (58), and Lepidoptera,
230 being one of the most diverse groups of arthropods, contributes significantly to this estimate.
231 Tuschhoff & Wiens (58) report data from 114 families of Lepidoptera, and in 46 of them (40%) they
232 found at least suggestive evidence of male mate choice. In all of these cases they also found
233 evidence of female mate choice (i.e. mutual mate choice could be very common in Lepidoptera)
234 (58). Thus, our results are also relevant for the controversy about the “reality” and importance of
235 the Darwinian sex-roles (3,6,50). We agree with the opinion expressed by Schlupp (50) that the term
236 is restrictive and limited, considering that the degree of investment of each sex in mating and
237 parental effort varies continuously and, in part, independently, thus having multiple effects on the
238 strength of intrasexual competition, mate choice and sexual conflict in each sex and in each species.

239 Finally, we consider the caveats of our literature review and meta-analysis. The tests of
240 funnel plot asymmetry for the effect of successive copulations on spermatophore size were
241 significant (and near significant). This could be a product of the small number of species studied
242 (relative to the tens of thousands of Lepidoptera species) and the large number of articles on just
243 two closely related species (*Pieris rapae* and *P. napi*), rather than true publication bias. In the case
244 of the effect on longevity, the funnel plot symmetry analyses did not produce significant results.
245 However, in this case, to the problem of the small number of species studied we must add the fact
246 that most of the species included in our review were represented by just one or two studies. Thus,
247 publication bias cannot be ruled out. Despite the limited number of species and papers included in
248 our meta-analyses, our results suggest that there is wide variation in the magnitude of the effects
249 of multiple copulations on spermatophore size and longevity within the Lepidoptera.

250 Another problem is the methodological heterogeneity among articles. We did not include
251 several interesting papers because their methodologies were too different, or their results were
252 presented in such a way that they could not be compared to the rest of the articles. For example,
253 Oberhauser's study of the monarch butterfly (59) and Svård & Wicklund's study of 25 species
254 belonging to three families of butterflies (60) do not report data on each consecutive mating, but
255 only correlation coefficients. Thus, we consider that it is necessary to have replicate studies for most
256 of the species and recommend that future studies use standardized methodologies and make raw
257 data easily accessible to the scientific community.

258 **Conclusions**

259 Our meta-analyses identified significant negative effects of multiple copulations on spermatophore
260 size and longevity of the males in the order Lepidoptera, indicating that copulation is costly for the
261 males of one of the most diverse animal taxa. This is an important insight that runs against the still
262 predominant view of the traditional Darwinian sex roles. Our results led us to predict that male mate
263 choice in Lepidoptera is common, probably the rule. Future studies should focus on replicating the
264 experiments carried out in each previously studied species, as well as using different species from
265 the enormous diversity of taxa that have yet to be studied.

266 **Acknowledgments**

267 We thank Dr. Marcos Rosetti Sciutto for his crucial help with the statistical analysis and Raúl Iván
268 Martínez for technical help. We thank Dr. Lynna Kiere for commentaries on a previous version of
269 the manuscript. This paper is part of the master's thesis of Ricardo Raña Mendoza (RRM) in the
270 Posgrado en Ciencias Biológicas of the Universidad Nacional Autónoma de México. RRM was
271 supported by a CONACYT scholarship.

272 References

- 273 1. Bateman AJ. Intra-sexual selection in *Drosophila*. *Heredity (Edinb)*. 1948;2(3):349–68.
- 274 2. Parker GA. The sexual cascade and the rise of pre-ejaculatory (Darwinian) sexual selection,
275 sex roles, and sexual conflict. *Cold Spring Harb Perspect Biol*. 2014 Oct 1;6(10).
- 276 3. Janicke T, Häderer IK, Lajeunesse MJ, Anthes N. Darwinian sex roles confirmed across the
277 animal kingdom. *Sci Adv*. 2016 Feb 12;2(2).
- 278 4. Stockley P, Bro-Jørgensen J. Female competition and its evolutionary consequences in
279 mammals. *Biological Reviews*. 2011;86(2):341–66.
- 280 5. Olsson M, Madsen T, Shine R. Is sperm really so cheap? Costs of reproduction in male
281 adders, *Vipera berus*. *Proceedings of the Royal Society B: Biological Sciences*.
282 1997;264(1380):455–9.
- 283 6. Ah-King M, Ahnesjö I. The “sex role” concept: an overview and evaluation. *Evol Biol*. 2013
284 Mar 19;40(1):461–70.
- 285 7. Pollo P, Kasumovic MM. Let’s talk about sex roles: what affects perceptions of sex
286 differences in animal behaviour? *Anim Behav*. 2022 Jan 1;183:1–12.
- 287 8. Vahed K. The function of nuptial feeding in insects: A review of empirical studies. *Biological
288 Reviews*. 1998;73(1):43–78.
- 289 9. Molleman F. Puddling: from natural history to understanding how it affects fitness.
290 *Entomol Exp Appl*. 2010 Jan 11;134(2):107–13.
- 291 10. Baker HG, Baker I. Studies of nectar-constitution and pollinator-plant coevolution. In:
292 *Coevolution of Animals and Plants*. University of Texas Press; 1975. p. 100–40.
- 293 11. Xochipiltecatl D, Baixeras J, Cordero CR. Atypical functioning of female genitalia explains
294 monandry in a butterfly. *PeerJ*. 2021 Nov 22;9:e12499.
- 295 12. Lewis S, South A. The evolution of animal nuptial gifts. In: Brockmann HJ, Roper TJ, Naguib
296 M, Mitani JC, Simmons LW, editors. *Advances in the study of behavior*. Academic Press;
297 2012. p. 53–97. (*Advances in the Study of Behavior*; vol. 44).
- 298 13. Drummond BA III. Multiple mating and sperm competition in the Lepidoptera. In: Sith RL,
299 editor. *Sperm Competition and the Evolution of Animal Mating Systems*. Academic Press;
300 1984. p. 291-370.
- 301 14. Gwynne DT. Courtship feeding in katydids (Orthoptera: Tettigoniidae): Investment in
302 offspring or in obtaining fertilizations? *Am Nat*. 1986 Sep;128(3):342–52.
- 303 15. Engqvist L. Nuptial gift consumption influences female remating in a scorpionfly: Male or
304 female control of mating rate? *Evol Ecol*. 2007 Jan;21(1):49–61.

- 305 16. Cordero C. Ejaculate substances that affect female insect reproductive physiology and
306 behavior: honest or arbitrary traits? *J. Theor. Biol.* 1995;174:453–461.
- 307 17. Estrada C, Schulz S, Yildizhan S, Gilbert LE. Sexual selection drives the evolution of
308 antiaphrodisiac pheromones in butterflies. *Evolution (N Y)*. 2011 May 20;65(10):2843–54.
- 309 18. Arnqvist G, Nilsson T. The evolution of polyandry: Multiple mating and female fitness in
310 insects. *Anim Behav.* 2000;60(2):145–64.
- 311 19. Giglioli MEC, Mason GF. The mating plug in anopheline mosquitoes. *Proceedings of the*
312 *Royal Entomological Society of London Series A, General Entomology.* 1966;41(7–9):123–9.
- 313 20. Sánchez V, Hernández-Baños BE, Cordero C, Sanchez V, Estela Hernandez-Banos B, Cordero
314 C. The evolution of a female genital trait widely distributed in the Lepidoptera: comparative
315 evidence for an effect of sexual coevolution. *PLoS One.* 2011 Aug;6(8):e22642.
- 316 21. Plakke MS, Deutsch AB, Meslin C, Clark NL, Morehouse NI. Dynamic digestive physiology of
317 a female reproductive organ in a polyandrous butterfly. *Journal of Experimental Biology.*
318 2015 May 15;218(10):1548–55.
- 319 22. Meslin C, Plakke MS, Deutsch AB, Small BS, Morehouse NI, Clark NL. Digestive organ in the
320 female reproductive tract borrows genes from multiple organ systems to adopt critical
321 functions. *Mol Biol Evol.* 2015 Jun;32(6):1567–80.
- 322 23. Sugawara T. Stretch reception in the bursa copulatrix of the butterfly, *Pieris rapae*
323 *crucivora*, and its role in behaviour. *Journal of comparative physiology* 1979 130:3. 1979
324 Sep;130(3):191–9.
- 325 24. Sánchez V, Cordero C. Sexual coevolution of spermatophore envelopes and female genital
326 traits in butterflies: Evidence of male coercion? *PeerJ.* 2014 Jan 30;2014(1).
- 327 25. Bissoondath CJ, Wiklund C. Effect of male body size on sperm precedence in the
328 polyandrous butterfly *Pieris napi* L. (Lepidoptera: Pieridae). *Behavioral Ecology.* 1997 Sep
329 1;8(5):518–23.
- 330 26. Murphy AF, Krupke CH. Mating success and spermatophore composition in western corn
331 rootworm (Coleoptera: Chrysomelidae). *Environ Entomol.* 2011;40(6):1585–94.
- 332 27. Cardoso MZ, Silva ES. Spermatophore quality and production in two *Heliconius* butterflies
333 with contrasting mating systems. *J Insect Behav.* 2015 Nov 13;28(6):693–703.
- 334 28. Svärd L. Paternal Investment in a Monandrous Butterfly, *Pararge Aegeria*. *Oikos.* 1985
335 Aug;45(1):66.
- 336 29. Cordero C. Trade-off between fitness components in males of the polygynous butterfly
337 *Callophrys xami* (Lycaenidae): the effect of multiple mating on longevity. *Behav Ecol*
338 *Sociobiol.* 2000 Nov;48(6):458–62.
- 339 30. Caballero-Mendieta N, Cordero C. Male mating costs in a butterfly that produces small
340 ejaculates. *Physiol Entomol.* 2013 Dec;38(4):318–25.

- 341 31. Takeuchi T. Cost of reproduction in males of a satyrine butterfly *Lethe diana*. *Physiol*
342 *Entomol.* 2012 Jun 1;37(2):171–6.
- 343 32. Jones KN, Odendaal FJ, Ehrlich PR. Evidence against the spermatophore as paternal
344 investment in checkerspot butterflies (Euphydryas: Nymphalidae). *American Midland*
345 *Naturalist.* 1986 Jul;116(1):1.
- 346 33. Ferkau C, Fischer K. Costs of reproduction in male *Bicyclus anynana* and *Pieris napi*
347 butterflies: Effects of mating history and food limitation. *ETHOLOGY.* 2006
348 Nov;112(11):1117–27.
- 349 34. Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to
350 improve searching PubMed for clinical questions. *BMC Med Inform Decis Mak.* 2007;7:1–6.
- 351 35. Bissoondath CJ, Wiklund C. Male butterfly investment in successive ejaculates in relation to
352 mating system. *Behav Ecol Sociobiol.* 1996;39(5):285–92.
- 353 36. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting
354 items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.*
355 2009;6(7).
- 356 37. Marin F, Rohatgi A, Charlot S. WebPlotDigitizer, a polyvalent and free software to extract
357 spectra from old astronomical publications: application to ultraviolet spectropolarimetry.
358 2017 Aug 7;237–41.
- 359 38. R Core Team. R: A language and environment for statistical computing. Vienna, Austria;
360 2021.
- 361 39. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical
362 tutorial. *Evidence Based Mental Health.* 2019 Nov;22(4):153–60.
- 363 40. Harrer M, Cuijpers P, Furukawa T, Ebert DD. dmetar: Companion R package for the guide
364 “Doing meta-analysis in R.” 2019.
- 365 41. Viechtbauer W. Conducting meta-analyses in R with the metafor. *J Stat Softw.* 2010 Aug
366 5;36(3):1–48.
- 367 42. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7(3):177–
368 88.
- 369 43. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses.
370 *BMJ.* 2003 Sep 4;327(7414):557–60.
- 371 44. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication
372 bias. *Biometrics.* 1994 Dec;50(4):1088.
- 373 45. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple,
374 graphical test. *BMJ.* 1997 Sep 13;315(7109):629–34.

- 375 46. Blanco CA, Rojas MG, Groot AT, Morales-Ramos J, Abel CA. Size and chemical composition
376 of *Heliothis virescens* (Lepidoptera: Noctuidae) Spermatophores. Ann Entomol Soc Am.
377 2009 Jul 1;102(4):629–37.
- 378 47. Bissoondath CJ, Wiklund C. Protein content of spermatophores in relation to
379 monandry/polyandry in butterflies. Behav Ecol Sociobiol. 1995 Dec;37(6):365–71.
- 380 48. Watanabe M, Wiklund C, BoN'No M. The effect of repeated matings on sperm numbers in
381 successive ejaculates of the cabbage white butterfly *Pieris rapae* (Lepidoptera: Pieridae). J
382 Insect Behav [Internet]. 1998 [cited 2023 Mar 15];11(4):559–70. Available from:
383 <https://link.springer.com/article/10.1023/A:1022319514424>
- 384 49. Bonduriansky R. The evolution of male mate choice in insects: a synthesis of ideas and
385 evidence. Biol Rev Camb Philos Soc. 2001 Aug;76(3).
- 386 50. Schlupp I. Male Choice, Female Competition, and Female Ornaments in Sexual Selection.
387 Male Choice, Female Competition, and Female Ornaments in Sexual Selection. 2021 Jan
388 1;1–164.
- 389 51. Xu J, Wang Q. Male moths undertake both pre- and in-copulation mate choice based on
390 female age and weight. Behav Ecol Sociobiol. 2009 Apr 4;63(6):801–8.
- 391 52. Prudic KL, Jeon C, Cao H, Monteiro A. Developmental plasticity in sexual roles of butterfly
392 species drives mutual sexual ornamentation. Science. 2011 Jan 7;331(6013):73–5. Available
393 from: <https://pubmed.ncbi.nlm.nih.gov/21212355/>
- 394 53. Goubault M, Burlaud R. Do males choose their mates in the lekking moth *Achroia grisella*?
395 Influence of female body mass and male reproductive status on male mate choice. Insect
396 Sci. 2018 Oct 1;25(5):861–8.
- 397 54. Ng SY, Bhardwaj S, Monteiro A. Males become choosier in response to manipulations of
398 female wing ornaments in dry season *Bicyclus anynana* butterflies. Journal of Insect
399 Science. 2017 Jul 1;17(4).
- 400 55. Connahs H, Tan EJ, Ter YT, Dion E, Matsuoka Y, Bear A, et al. The yellow gene regulates
401 behavioural plasticity by repressing male courtship in *Bicyclus anynana* butterflies. Proc
402 Biol Sci. 2022;289(1972).
- 403 56. Deering MD, Scriber JM. Field bioassays show heterospecific mating preference asymmetry
404 between hybridizing North American *Papilio* butterfly species (Lepidoptera: Papilionidae). J
405 Ethol. 2002 Feb 1;20(1):25–33.
- 406 57. Toshova TB, Subchev MA, Toth M. Role of olfactory and visual stimuli in the mating
407 behaviour of male vine bud moths, *Theresimima ampellophaga* (Lepidoptera: Zygaenidae).
408 Eur J Entomol. 2007;104(1):57.
- 409 58. Tuschhoff E, Wiens JJ. Evolution of sexually selected traits across animals. Front Ecol Evol.
410 2023 Jan 19;11:14.

411 59. Oberhauser KS. Male monarch butterfly spermatophore mass and mating strategies. Anim
412 Behav. 1988 Sep;36(5):1384–8.

413 60. Svärd L, Wiklund C. Mass and production rate of ejaculates in relation to
414 monandry/polyandry in butterflies. Behav Ecol Sociobiol. 1989 Jun;24(6):395–402.

415

416 **Supporting Information**

417 **S1 File. R code.** Full R code used for the information analysis and figure generation. (R)

418 **S2 File. Raw data file.** Full compiled data extracted from the selected papers. (CSV)

419 **S3 File. PRISMA checklist.** (DOCX)

420

Discusión

Nuestros resultados respaldaron la hipótesis de que la cópula y la producción de eyaculados en machos del orden Lepidoptera tienen costos de adecuación significativos. Nuestro análisis mostró que el tamaño del espermátforo disminuye tras experimentar cópulas consecutivas. Esto probablemente se deba a que el contenido de los espermátforos frecuentemente incluye azúcares, grasas, proteínas y sales (46,47) que son difíciles o imposibles de recuperar para el macho adulto. La transferencia de un espermátforo pequeño reduce la adecuación obtenida por el macho tras copular por dos razones principales. Primero, la cópula induce un período de receptividad sexual reducida en la hembra que se correlaciona positivamente con el tamaño del espermátforo; y segundo, la cantidad reducida de nutrientes transferidos podría resultar en una menor cantidad de huevos puestos o una reducción en los recursos invertidos en cada huevo (48). Además, aunque el efecto global de la cópula sobre la longevidad no fue significativo, el análisis por especies muestra un efecto negativo significativo. Esto sugiere que los materiales en los eyaculados provienen no solo de las glándulas reproductivas accesorias y los testículos, sino que también son secuestrados de otros depósitos del cuerpo del macho y estos materiales son esenciales para aumentar su supervivencia. Nuestros hallazgos muestran que la cópula y la producción de eyaculados son sustancialmente costosas para los machos de lepidópteros, lo cual tienen amplias implicaciones evolutivas. Dado que una gran inversión de recursos por parte del macho durante la cópula es uno de los factores que podría favorecer la evolución de la elección de pareja masculina (49, 50), predecimos que la elección de pareja masculina (probablemente combinada con la elección de pareja femenina, es decir, la elección recíproca de pareja) es común en lepidópteros. De acuerdo con esta predicción, la revisión de Bondurianski sobre la elección de pareja masculina en insectos (49) identificó estudios de 12 especies pertenecientes a siete familias de mariposas y polillas en las que se demostró la elección masculina de pareja. En una breve búsqueda informal en Internet de

estudios publicados después de la revisión de Bondurianski, encontramos más demostraciones de elección de pareja masculina en especies adicionales (*Ephestia kuehniella* (Pyralidae) (51), *Bicyclus anynana* (Nymphalidae) (52) y *Achroia grisella* (Pyralidae) (53)). El caso de *B. anynana* es fascinante porque la elección masculina de pareja muestra plasticidad en el desarrollo, estando presente solo en los machos de la estación seca, mientras que la elección de las hembras se observa durante la estación húmeda (52,54), y los mecanismos hormonales y genéticos responsables de esta plasticidad del comportamiento son conocidas (55). Otros investigadores descubrieron preferencias masculinas por ciertos tipos de hembras (algunos de estos estudios incluyen hembras de especies estrechamente relacionadas en el diseño experimental) en otras mariposas (*Papilio glaucus* y *P. canadensis*, Papilionidae; (56)) y polillas (*Theresimima amellophaga*, Zygaenidae; (57)). En un contexto más amplio, un estudio comparativo reciente encontró que la elección de pareja masculina aparentemente es muy común en los artrópodos (58), y los lepidópteros, al ser uno de los grupos de artrópodos más diversos, contribuyen significativamente a esta estimación. Tuschhoff & Wiens (58) informan datos de 114 familias de lepidópteros, y en 46 de ellas (40%) encontraron al menos evidencia sugestiva de elección de pareja masculina. En todos estos casos, también encontraron evidencia de elección de pareja femenina (es decir, la elección mutua de pareja podría ser muy común en los lepidópteros) (58). Por lo tanto, nuestros resultados también son relevantes para la controversia sobre la “realidad” y la importancia de los roles sexuales darwinianos (3,6,50). Coincidimos con la opinión expresada por Schlupp (50) de que el término es restrictivo y limitado, considerando que el grado de inversión de cada sexo en el apareamiento y el esfuerzo parental varía de forma continua y, en parte, independiente, teniendo así múltiples efectos sobre la fuerza de la competencia intrasexual, elección de pareja y conflicto sexual en cada sexo y en cada especie. Finalmente, consideramos los problemas en nuestra revisión y metanálisis de la literatura. Las pruebas de asimetría del gráfico en embudo para el efecto de las cópulas sucesivas sobre el tamaño

del espermatóforo fueron significativas (y casi significativas). Esto podría ser producto de la pequeña cantidad de especies estudiadas (en relación con las decenas de miles de especies de lepidópteros) y la gran cantidad de artículos sobre solo dos especies estrechamente relacionadas (*Pieris rapae* y *P. napi*), en lugar de un verdadero sesgo de publicación. En el caso del efecto sobre la longevidad, los análisis de simetría del gráfico de embudo no arrojaron resultados significativos. Sin embargo, en este caso, al problema del escaso número de especies estudiadas hay que añadir el hecho de que la mayoría de las especies incluidas en nuestra revisión estaban representadas por uno o dos estudios. Por lo tanto, no se puede descartar el sesgo de publicación. A pesar del número limitado de especies y artículos incluidos en nuestros metanálisis, nuestros resultados sugieren que existe una amplia variación en la magnitud de los efectos de las cópulas múltiples sobre el tamaño y la longevidad de los espermatóforos dentro de los lepidópteros. Otro problema es la heterogeneidad metodológica entre los artículos. No incluimos varios artículos interesantes porque sus metodologías eran demasiado diferentes o sus resultados fueron presentados de tal manera que no pudieron ser comparados con el resto de los artículos. Por ejemplo, el estudio de Oberhauser de la mariposa monarca (59) y el estudio de Svärd & Wicklund de 25 especies pertenecientes a tres familias de mariposas (60) no reportan datos sobre cada apareamiento consecutivo, sino solo coeficientes de correlación. Por lo tanto, consideramos que es necesario contar con estudios réplica para la mayoría de las especies y recomendamos que los estudios futuros utilicen metodologías estandarizadas y hagan que los datos sin procesar sean fácilmente accesibles para la comunidad científica.

Conclusiones

Nuestros metanálisis identificaron efectos negativos significativos de cópulas consecutivas sobre el tamaño de los espermatóforos y la longevidad de los machos del orden lepidóptera, lo que indica

que la cópula es costosa para los machos de uno de los taxones animales más diversos. Esta es una idea importante que va en contra de la visión aún predominante de los roles sexuales darwinianos tradicionales. Nuestros resultados nos llevaron a predecir que la elección de pareja masculina en lepidópteros es común, probablemente la regla. Futuros estudios deberían centrarse en replicar los experimentos realizados en cada especie previamente estudiada, así como utilizar diferentes especies de la enorme diversidad de taxones que quedan por ser estudiados.