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Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo como parte dos requisitos para obtenção do título de Mestre em Ciências, na área de Ecologia de Ecossistemas Terrestres e Aquáticos.

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Resumo

SOLANO BRENES, D. E. **Alocação diferencial em uma aranha com presente nupcial: machos ajustam seu investimento reprodutivo em resposta à condição da fêmea.** 2021. Dissertação (Mestrado) - Instituto de Biociências, Universidade de São Paulo, SP.

Quando machos são seletivos, eles podem rejeitar fêmeas de baixa qualidade ou podem ajustar seu investimento em reprodução em resposta a características femininas que indiquem qualidade (e.g., tamanho ou condição corporal). Segundo a *hipótese de alocação diferencial*, machos podem incrementar o investimento reprodutivo quando se acasalam com fêmeas de alta qualidade (*alocação diferencial positiva*) ou podem incrementar o investimento reprodutivo quando se acasalam com fêmeas de baixa qualidade (*alocação diferencial negativa*). Esta hipótese foi proposta para explicar comportamentos de espécies monogâmicas com cuidado biparental e a maioria dos testes empíricos foram feitos com aves. Neste trabalho, utilizamos *Paratrechalea ornata*, uma aranha polígama em que os machos oferecem presas envoltas em seda como presente nupcial, para testar se os machos ajustam o investimento reprodutivo em tamanho do presente nupcial, intensidade do cortejo pré-copulatório e copulatório e transferência de esperma em resposta à condição corporal da fêmea. Encontramos que machos expostos a fêmeas em boa condição corporal agregam mais moscas ao presente nupcial, estimulam mais intensamente as fêmeas com toques no abdômen durante o cortejo pré-copulatório e fazem inserções dos pedipalpos mais duradouras do que machos expostos a fêmeas com má condição corporal. A condição corporal da fêmea não afetou o investimento em seda no presente nupcial nem a quantidade de esperma transferido. Finalmente, fêmeas em boa condição corporal colocam mais ovos e ovipositam mais rápido do que fêmeas em má condição corporal. Com estes resultados demonstramos que machos com presentes nupciais apresentam alocação diferencial positiva em três aspectos do seu investimento reprodutivo: tamanho do presente nupcial, intensidade de cortejo pré-copulatório e a duração das inserções dos pedipalpos (cortejo copulatório). A alocação diferencial positiva está associada aos benefícios de se acasalar com fêmeas em boa condição corporal. Estas fêmeas são mais fecundas e ovipõem mais rápido que as fêmeas em má condição corporal. Estas características provavelmente podem reduzir o risco de múltiplos acasalamentos das fêmeas na natureza e, portanto, a competição espermática enfrentada pelos machos seria menor. Finalmente, nossos resultados indicam que a hipótese de alocação diferencial também aplica para espécies que investem em presentes nupciais, mas não têm cuidado parental à prole.

Palavras-chaves. Condição corporal, cortejo copulatório, seleção críptica masculina, escolha masculina de parceiras, esforço de acasalamento, esforço parental, cortejo pré-copulatório, transferência de esperma.

Abstract

SOLANO BRENES, D. E. **Differential allocation in a gift-giving spider: males adjust their reproductive investment in response to female condition.** 2021. Dissertação (Mestrado) - Instituto de Biociências, Universidade de São Paulo, SP.

When males are selective, they can either reject low-quality females or adjust their reproductive investment in response to traits that indicate female quality (e.g., body size or condition). According to the *differential allocation hypothesis*, males increase their reproductive investment when paired with high-quality females (*positive differential allocation*) or increase their reproductive investment when paired with low-quality females (*negative differential allocation*). This hypothesis has been proposed for monogamous species with biparental care, and most empirical studies focus on birds. Here we used the polygamous spider *Paratrechalea ornata,* in which males offer prey wrapped in silk as nuptial gifts, to test whether males adjust their reproductive investment in gift size, pre-copulatory and copulatory courtship intensity, and sperm transfer in response to female body condition. We found that males exposed to females in good body condition added more flies to the gift, stimulated these females more intensively with abdominal touches during pre-copulatory courtship, and had longer pedipalp insertions than males exposed to females in poor body condition. Female condition affected neither silk investment in nuptial gift wrapping nor the quantity of sperm transferred by males. Finally, females in good body condition laid more eggs and oviposited faster after copulation than females in poor body condition. We provide experimental evidence that males of a gift-giving spider exhibit positive differential allocation in three key aspects of their reproductive investment: the size of the nutritious gift, pre-copulatory courtship intensity, and duration of pedipalp insertions, which is regarded as a form of genital courtship by males in spiders. This positive differential allocation is likely associated with the benefits of copulating with females in good body condition. These females are more fecund and oviposit faster after copulation than females in poor body condition, which under natural field conditions probably reduce the risk of multiple matings and thus the level of sperm competition faced by the males. As a final remark, our findings indicate that the hypothesis of differential allocation also applies to species with a scramble competition mating system, in which males heavily invest in nuptial gift construction, but not in parental care.

Keywords: Body condition, Copulatory courtship, Cryptic male choice, Male mate choice, Mating effort, Parental effort, Pre-copulatory courtship, Sperm transfer.

Differential allocation in a gift-giving spider: males adjust their reproductive investment in response to female condition*

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Background

The ability to choose the best mating partner is a critical factor for the reproductive success of an individual [1, 2]. Due to early assumptions about sex roles, mate choice has been historically studied mainly in females [3]. However, there is increasing evidence that males of many species also choose their mating partners (reviewed in [4] and [5]). Current theory predicts that male mate choice should evolve when: (i) the mate encounter rate is high, (ii) there is great variation among females in traits associated with quality (e.g., fecundity), (iii) mate searching effort is relatively inexpensive for males, and (iv) males perform substantial reproductive investment or suffer from sperm depletion so that they are unable to mate with many available females [5, 6]. For instance, in the pipefish *Syngnathus typhle* (Syngnathidae), a species with exclusive paternal care and limited mating opportunities, males are selective when there are more females than males in the population, preferring the larger and more fecund females. However, males mate indiscriminately when there are fewer females than males in the population, probably because the mate searching costs are too high [7].

In species showing male mate choice, males can reject females of low-quality or, in a more subtle way, adjust their reproductive investment according to female quality, a behavior known as cryptic male choice [4]. When individuals increase their reproductive investment in response to high-quality mates, we call it *positive differential allocation* [8, 9]. For instance, there is empirical evidence for some species of insects, crustaceans, fish, and birds showing that males increase ejaculate volume or the quantity of sperm cells when mating with high-quality females, which may be more fecund or more ornamented [e.g., 10-12]. In contrast, when males increase their reproductive investment in response to low-quality mates, we call it *negative differential allocation* [9]. For instance, in the blue tit *Cyanistes caeruleus* (Paridae), a species with biparental care, males increase the investment in paternal care when paired with low-quality females [e.g., 13]. In this case, the higher paternal effort

may be regarded as a form reproductive compensation that increases offspring production and/or survival when males are paired with females of low genetic quality, which are those with less intense ultra-violet plumage [9, 13].

Species in which males offer nuptial gifts are good models to investigate male mate choice and differential allocation for at least two main reasons. First, edible gifts with nutritive value are usually expensive and represent substantial reproductive investment for males, which may reduce their mating opportunities [e.g., 14-17]. The costs associated with the production of a single gift in some insect species are so high that males take as much as five days to replenish it, and during this period they are prevented from mating [e.g., 18]. Considering that the cost of producing nuptial gifts limits the number of copulations, males should mate mainly with high-quality females if the availability of potential mating partners is high and female quality shows great variation in the population [e.g., 14, 19, 20]. Second, the nutrients of nuptial gifts may be regarded as a form of paternal effort (*sensu* [21]) if they provide direct benefits to females, increasing their fecundity [22]. Considering that paternal effort is subject to differential allocation [8, 9], males should adjust their investment in the size and/or content of the nuptial gifts in response to the quality of the mating partners [23]. In case of a positive differential allocation, males should offer larger and/or more nutritive gifts when paired with high-quality females, thus increasing their mating chances. In turn, if males exhibit negative differential allocation, they should offer larger and/or more nutritive gifts when paired with low-quality females, thus providing resources to be used in egg production. In both cases, larger and/or more nutritive gifts can also prolong copulation, which may increase the amount of sperm transferred to the female [e.g., 24].

Most empirical studies on male mate choice in gift-giving arthropods focus on orthopterans that produce endogenous gifts, known as spermatophylax, which is released together with the spermatophore (reviewed in [25]). Given that the spermatophylax is

73 already formed when a male finds a potential mating partner [22], he is probably unable to
74 adjust the size and/or content of the gift in response to female quality (but see [26]).
75 However, not all endogenous gifts are pre-formed, opening the possibility that their size
76 and/or content can be adjusted by the males in response to female quality. In fact, males of
77 the scorpionfly *Panorpa cognata* (Panorpidae) adjust the size of the salivary mass (an
78 endogenous gift) based on female body condition, but only when they are in poor body
79 condition [27]. Thus, the investment in some types of endogenous gifts can be flexible,
80 responding to both female and male condition. This finding raises the question of whether
81 the investment on exogenous gifts, such as prey items [25], can also be adjusted adaptively
82 by the males. This is one of the gaps we intend to fill in our study.

83 Several spider species construct exogenous gifts consisting of items wrapped in silk
84 that include either nutritive prey or inedible prey leftovers [e.g., 28-31]. One of these species
85 is *Paratrechalea ornata* (Trechaleidae), in which males construct prey-gifts when they perceive
86 chemical cues of the draglines left by conspecific females on the substrate [32]. Experimental
87 evidence shows that prey-gift construction is costly because males in poor body condition
88 consistently eat the prey instead of wrapping it in silk to construct a gift [33]. Despite the
89 costs, the production of nuptial gifts is necessary for the male to be accepted by the female
90 [34, 35]. Moreover, chemicals deposited by the male on the silk layer surrounding the gift
91 entice the female to grab it [36], and the larger the gift, the longer copulation duration is [37].
92 Finally, the consumption of nutritive prey-gifts by females increases their fecundity,
93 indicating that the offspring receives part of the nutrients contained in the gift [38]. Taken
94 together, these findings indicate that prey-gifts in *P. ornata* entice females to copulate (i.e.,
95 mating effort) and provide food resources that enhance offspring production (i.e., paternal
96 effort). Thus, if males can adjust the size of their nuptial prey-gifts and the quantity of silk

97 added on them in response to female phenotypic traits, males could exhibit either positive or
98 negative differential allocation.

99 Here we explored if *P. ornata* males adjust their reproductive investment in response to
100 female quality, measured as body condition, which is known to have a marked effect on
101 female fecundity (e.g., [39, 40]) and offspring performance in spiders (e.g., [41, 42]). We
102 created two experimental groups of females: high-quality females (i.e., those in *good body*
103 *condition*) and low-quality females (i.e., those in *poor body condition*). Then, we paired these
104 females with males in good body condition and quantified their reproductive investment.
105 We used three measurements of male reproductive investment: (1) the quality of the prey-
106 gift, measured as the quantity of both prey and silk added to the gift, (2) the duration of pre-
107 copulatory courtship, which is a crucial component of male mating effort in *P. ornata* and
108 other gift-giving spiders [30], and (3) the copulation duration and the quantity of sperm
109 transferred to the female, which are associated with post-copulatory processes in spiders,
110 such as cryptic female choice [e.g., 43, 44] and sperm competition (reviewed in [45]). If males
111 exhibit positive differential allocation, we expect higher investment in high-quality females
112 than low-quality females. In turn, if males exhibit negative differential allocation, we expect
113 the opposite response, with higher investment in low-quality females than high-quality
114 females. Finally, to evaluate the potential benefits of male differential allocation, we
115 quantified the latency between copulation and oviposition and the number and mass of eggs
116 laid by low- and high-quality females. We expected that high-quality females would have a
117 shorter latency between copulation and oviposition and would lay a larger number of eggs
118 with higher mass than low-quality females.

119

Methods

Collection and maintenance

We visited two rivers belonging to the same basin between August 22nd and 24th, 2019, in the municipality of Picada Café, state of Rio Grande do Sul, southern Brazil (29°27′8.64″ S; 51°7′7.42″ W and 29°27′10.82″ S; 51°2′37.30″ W). On the riverbanks, we collected juvenile and subadult males and females of *Paratrechalea ornata* and placed each spider in individual centrifuge tubes (50 mL) with wet cotton as a water source. Then, we transported them to our laboratory at Universidade de São Paulo (São Paulo, Brazil), where we kept the temperature around 25 °C and an inverted light-dark cycle of 12:12 hours during the entire period of the experiment. In the laboratory, we placed the spiders individually inside larger plastic pots (200 mL) covered with a fabric mesh. While the spiders were juveniles, we fed them three times a week with one cricket nymph (*Gryllus* sp.) about 3–5 mm long.

Conditioning period

A week after individuals molted to adulthood, we photographed each of them (n = 197) in dorsal view and measured the cephalothorax width in its wider portion using the software *ImageJ* [46]. The repeatability of the cephalothorax width measurements for both males and females was higher than 90% (Table S1 in Supplementary Material). After measuring all individuals, we divided unmated females into two experimental groups: females in good body condition (i.e., *high-quality females*, hereafter 'GOOD females') and females in poor body condition (i.e., *low-quality females*, hereafter 'POOR females'). We fed GOOD females and males with one cricket nymph (about 5–10 mm long) three times a week for three weeks. In contrast, we fed POOR females with a single cricket nymph (about 5–10 mm long) once a week for three weeks. Although the size of the crickets showed great variation due to weekly

availability of nymphs in our stock population, the nymphs offered to POOR and GOOD females had always similar sizes in any given week. We also divided unmated males (n = 60) into two experimental groups: those exposed to GOOD females (n = 27) and those exposed to POOR females (n = 33). To avoid undesirable differences in the mean size of the individuals (females and males) between groups, we considered the cephalothorax width when we split them into the two experimental groups (Table S2 in Supplementary Material).

After three weeks of conditioning, we weighed males and females using a digital scale (Shimazu AUW220) to the nearest 0.0001 g. Using body weight and cephalothorax width, we performed a linear regression for females and males independently. The residuals of this linear regression are a good proxy of body condition in spiders, so that positive values indicate individuals in good body condition whereas negative values indicate individuals in poor body condition [47, 48]. In fact, GOOD females showed positive residual values that were significantly higher than the values of POOR females, which showed negative residual values. For the males, we found no difference in residual values between individuals exposed to females of each experimental group (Table S2 in Supplementary Material).

Experimental setup

All individuals used in the experiment (both males and females) were 35 ± 4 (mean ± SD) days old after maturation molt. The couples were paired assortatively according to their size, so that the difference in cephalothorax width between males and females was similar for all couples in both experimental groups (Table S2 in Supplementary Material). The experiments were conducted during October 2019, and the trials started at 14:00 h (3 hours after the beginning of the dark cycle) and finished at 23:00 h (just before the beginning of the light cycle). One to four trials were conducted each day, alternating the order of the experimental groups. The trials occurred in a circular arena with 15 cm diameter and 3 cm depth, with the

floor covered with a single sheet of filter paper. The arena was divided into two halves by a removable glass barrier: the *male half* and the *female half*. During the trials, we kept the arena covered with a glass lid that allowed us to record the trials from above using a video camera (Sony HDR-CX405). To obtain more details of the behavioral interactions between males and females, we also recorded the trials laterally with another camera (Olympus Tough TG-6). Using the video recordings, we extracted all behavioral data described in the following topics. After each trial, we cleaned the entire arena with 70% ethanol and replaced the filter paper.

Male investment in prey-gift

Three days before the beginning of the experiment, we stopped feeding males and females of both experimental groups. Then, we placed a female in the male half of the arena for 24 hours before the beginning of the trial. During this period, the female adds silk threads on the filter paper and this silk stimulates the male to construct the gift [32]. A few minutes before the beginning of the trial, we moved the female to the female half and placed the male in the male half of the arena. At this point, the male and the female could not touch each other, but they were able to see each other through the glass barrier and perceive substrate-borne vibrations that are used in pre-copulatory interactions of some spider species [49], including the gift-giving *Pisaura mirabilis* (Pisauridae) [50].

After 5 min of acclimation of the male in the arena, we placed approximately 40 (mean ± SD = 41.7 ± 4.6) fruit flies (*Drosophila melanogaster*) inside the male half. Once the male captured the first fly, we allowed him to catch other flies and add them to the gift for 1 hour. After this period, we removed the remaining flies that were not added to the gift and allowed the male to add silk to the gift for another 10 min. We counted the number of flies captured by each male and recorded the time invested in adding silk to the gift. We also

recorded the time spent by the female close to the glass barrier (i.e., when 100% of her cephalothorax and abdomen were less than 3 cm away from the barrier). When females were close to the barrier, we assume that males could visually evaluate female size and/or perceive short-range vibratory signals, which are known to be a condition dependent trait in other gift-giving spiders [50]. We selected the value of 3 cm away from the barrier because it was the longest distance a female was observed moving in response to male movements on the other side of the glass barriers.

Male investment in pre-copulatory courtship

After the end of the first phase of the trial (i.e., *Male investment in prey-gift*), we removed the glass barrier allowing physical contact between male and female. If neither the female nor the male moved during 10 min, we gently touched the female with a brush so that she approached the male and the pre-copulatory courtship initiated. We recorded the time spent by the male touching the female abdomen with his first pair of legs ('quick touching' *sensu* [30]), a behavior that characterizes male investment in pre-copulatory courtship in *P. ornata*. The total time spent by the male touching the female abdomen was estimated as the sum of all touching bouts during pre-copulatory courtship. We also recorded the total duration of the mating interaction, from gift acceptance by the female until the couple's separation. Finally, as part of the male investment in prey-gift (previous topic), we recorded the time spent by the male adding more silk to the gift after he established physical contact with the female.

Male investment in copulation duration and sperm transfer

We recorded the duration of each successful pedipalp insertion, defined as any instance in which the tip of one of the male pedipalps was in contact with the female epigyne (genital opening), and the increase in the male's internal hydraulic pressure kept erected his leg spines for a few seconds. During the copulatory phase, a male may perform multiple insertions with both pedipalps, and we summed the time of all individual insertions to have a variable called 'total duration of pedipalp insertions'. Although the total duration of pedipalp insertions may be positively correlated with the total quantity of sperm transferred by the males, this is not a general pattern in spiders (reviewed in [45]). In some species, for instance, the total duration of pedipalp insertions shows no correlation with the total quantity of sperm transferred by the males (e.g., [44]). Because we had no *a priori* information on whether the total duration of pedipalp insertions was correlated or not with the total quantity of sperm transferred by *P. ornata* males [51], we decided to use these two variables independently in our analyses (see below).

After copulation, we sacrificed the experimental males and photographed both their pedipalps in ventral view under a stereomicroscope. Based on these photographs, we used the software *ImageJ* [46] to measure some morphological traits that could explain the quantity of sperm stored in each pedipalp: (1) the area of the bulb, (2) the area of the median apophysis, (3) the area of the tegulum, and (4) the area of the subtegulum (Fig. S1 in Supplementary Material). For each trait, we estimated the repeatability of the measurements in a sample of 20 males using three measurements of each pedipalp (Table S1 in Supplementary Material). If a trait had repeatability higher than 90% in this sample, we measured this trait only once in all other males; otherwise, we measured the trait three times and then calculated the mean of these values.

After photographing the pedipalps, we conserved them individually under -80 °C. We quantified the sperm stored in each pedipalp following the procedure proposed by Bukowski & Christenson [52]. In summary, we first placed the dissected bulb of each pedipalp into a centrifuge tube with 100 µL of a solution containing 1 ml of 0.9% saline solution and 10 µL of 10% triton-x detergent. Then, we grinded them with metal forceps for approximately 90 s. Next, we did three cycles of 90 s in a vortex and 25 min of centrifugation at 1,000 g and 25 °C. Finally, we placed two samples of 10 µL in a Neubauer improved double-chamber hemocytometer. For each of the two samples, we used a microscope at 200x to count the sperm cells in the four corners of the chamber (16 squares in each corner) and summed them to obtain the number of sperm cells in the sample. Finally, we used the mean cell count of the two samples to estimate the number of sperm cells in 1 mL using the equation: (1 mL × number of sperm cells counted)/0.004 mL.

We repeated all the procedures described above with a sample of 20 unmated males that were not included in the experiment. The data obtained for these males provided us an estimation of the quantity of sperm present in each pedipalp *before* copulation. Thus, considering the size of some pedipalpal and body traits, we could infer the total quantity of sperm transferred by the males during the experiment (see details of this procedure in the topic *Statistical analyses* below).

Potential benefits of male mate choice

After copulation, we fed both POOR and GOOD females with one cricket nymph (about 5–10 mm long, depending on their weekly availability in our stock population) three times a week until they laid their eggs. After oviposition, we waited 15 days to allow the eggs to develop so that we could distinguish fertilized from unfertilized eggs. Then, we preserved the females and their egg-sacs in 70% ethanol. Under a stereomicroscope, we counted the total

number of eggs inside each egg-sac. To weigh the eggs, we placed them on filter paper for 24 h to dry the ethanol. Finally, we weighed separately the fertilized and unfertilized eggs using a digital scale (Shimazu AUW220) to the nearest 0.0001 g. Unfertilized eggs have a diameter similar to fertilized eggs, but their content includes only a homogenous yellow yolk, and not a pre-formed spiderling, which can be easily seen through the chorion of the egg. Dividing the total mass of fertilized eggs by the total number of fertilized eggs, we obtained the mean mass of fertilized eggs laid by each female.

Statistical analyses

Male investment in prey-gift

To test whether males adjust the number of flies added to the prey-gift in response to female body condition, we used a generalized linear model (GLM) with quasibinomial distribution of errors and logit as link function. The response variable was the proportion of captured flies in relation to the total number of flies offered to each male. Given that the number of flies offered to all males was approximately 40, the higher the proportion of captured flies and added to the gift, the greater its size. As predictor variables, we used the experimental groups (POOR x GOOD), the time the female spent close to the glass barrier (which represents the visual and/or short-range vibratory stimulus received by the male), and the interaction between these two variables.

To test whether males adjust the time adding silk to the gift (i.e., another measurement of male investment in the prey-gift) in response to female body condition, we performed three analyses. First, we used a GLM with gamma distribution of errors and identity as link function to test whether the time adding silk to the prey-gift *before* any physical interaction with the female was affected by the experimental groups, the time spent by the female close

to the glass barrier, and the interaction between these two variables. Second, we used a GLM with binomial distribution of errors and logit as link function to estimate the probability the male adding more silk to the prey-gift *after* physical contact with the female. The predictor variables were the experimental groups, the time spent adding silk to the gift before physical contact with the female, and the interaction between these two variables. Finally, we used a GLM with gamma distribution of errors and identity as link function to test whether the *total* time spent adding silk to the gift (i.e., *before* and *after* physical contact with a female) was affected by the experimental groups. As the gamma distribution does not accept zero values, we added an infimum value (1^{-16}) to the observed values of the response variable. As the number of flies in the prey-gift was not correlated with the silk response variables of the three models (Table S3 in Supplementary Material), we did not include it as a predictor variable in the models.

Male investment in pre-copulatory courtship

To test whether males adjust the duration of pre-copulatory courtship in response to female body condition, we used a GLM with gamma distribution of errors and identity as link function. Our proxy for the duration of pre-copulatory courtship was the total time invested by a male touching a female's abdomen (hereafter 'abdominal touches'). As predictor variables, we used the experimental groups, the duration of the mating interaction (starting with gift acceptance and finishing with the couple's separation), and the interaction between these two variables. Finally, as an exploratory analysis, we investigated whether male investment in pre-copulatory courtship is affected by differences between experimental groups in female receptivity. We used a GLM with negative binomial distribution of errors and log as link function. The experimental groups were the predictor variable, and the response variable was the latency between the first abdominal touch performed by the male

and the abdominal twist performed by the female that exposes her genital opening to allow copulation (hereafter 'latency to pedipalp insertion').

Male investment in copulation duration and sperm transfer

To test whether males adjust the duration of pedipalp insertions in response to female body condition, we used a GLM with gamma distribution of errors and identity as link function. The total duration of pedipalp insertions was the response variable, and the predictor variables were the experimental groups, the duration of the mating interaction (starting with gift acceptance and finishing with the couple's separation), and the interaction between these two variables.

To determine the total quantity of sperm transferred to the females, we first estimated the quantity of sperm stored in each pedipalp *before* copulation. To do that, we used only the unmated males to find the best combination of morphological traits to describe the quantity of sperm stored in each pedipalp. We divided our morphological traits into body-related traits, including cephalothorax width and body condition, and pedipalp-related traits, including the areas of the bulb, median apophysis, tegulum, and subtegulum. We ran models with different distributions of errors (Gaussian, Poisson, and negative binomial), always using the quantity of sperm (number of sperm cells/mL) stored in each pedipalp as the response variable. As predictor variables, we included combinations of three or fewer morphological traits (body- and/or pedipalp-related) with additive and interactive effects between them (for further details see *Methods of sperm quantification* in Supplementary Material). In total, we had 174 concurrent models and used the Akaike Information Criterion corrected for small samples (AIC_c) to select the best model, i.e., the one that includes the morphological trait(s) that better predict the quantity of sperm stored in each pedipalp (Table S4 in Supplementary Material).

As an alternative approach, we performed a Principal Components Analyses (PCA) to reduce the dimensionality of the morphological traits. Using combinations of one to three Principal Components (PCs), we ran models with different distributions of errors and including both additive and interactive effects between predictor variables (Tables S5-S7 in Supplementary Material). Given that the best model using PCs explained less variability of the data (17.7%) than the best model using morphological traits (20%), the procedure we used to estimate the quantity of sperm transferred by the males is based on the data obtained with morphological traits.

According to the analysis performed with morphological traits, we found two models with $\Delta AIC_c < 2$: (1) *sperm stored* = *cephalothorax width*, and (2) *sperm stored* = *cephalothorax width* + *subtegulum area* (Table S4 in Supplementary Material). For the sake of simplicity, we used the model (1), which has only one predictor variable, also included in the model (2). Based on the equation obtained using the model (1), we estimated the quantity of sperm stored in each pedipalp of the experimental males before copulation. By subtracting the quantity of sperm in the pedipalp *after* copulation (i.e., mated males) from the estimation of sperm stored in the pedipalp *before* copulation (obtained with unmated males), we had an estimation of the quantity of sperm transferred to the female by each pedipalp. In only one case, the estimation of the total sperm transferred had a slightly negative value, and we approximated it to zero, considering that there was no sperm transference. For each male, we summed the quantity of sperm transferred by each pedipalp and used this value as our response variable.

To test whether males adjust the quantity of sperm transferred in response to female quality, we used a generalized least square (GLS) model. The predictor variables were the experimental groups, the total duration of pedipalp insertion, and the interaction between these two variables. As an exploratory analysis, we also tested whether the quantity of sperm

transferred by the males was related to the number of fertilized eggs laid by the females. To do so, we used a GLM with gamma distribution of errors and identity as link function.

Potential benefits of male mate choice

To determine whether GOOD females provide greater benefits to males than POOR females, we used three proxies: (1) the latency between copulation and oviposition, (2) the total number of eggs laid by the female, and (3) the mean mass of fertilized eggs. The rationale for the first proxy is that the lower the latency between copulation and oviposition, the lower the risk of the female copulating with other males, and thus the lower the sperm competition risk and/or intensity faced by the male. We lost information about some POOR (n = 8) and GOOD females (n = 14) that either died before oviposition or ate their egg-sac immediately after oviposition. Therefore, our sample size was 25 POOR females and 18 GOOD females for the test on the latency between copulation and oviposition, and 25 POOR females and 13 GOOD females for the tests on the total number of eggs and the mean mass of fertilized eggs.

To test whether GOOD females lay eggs faster than POOR females, we ran models with different distributions of errors (Gaussian, Poisson, and gamma), always using the number of days between copulation and oviposition as the response variable. We ran five models to each distribution of errors with different combinations of predictor variables: (1) experimental groups, (2) number of flies added to the gift, (3) additive effect of experimental groups and number of flies added to the gift, (4) interactive effect of experimental groups and number of flies added to the gift, and (5) null model. We used the Akaike Information Criterion corrected for small samples (AIC_c) to select the best fitted model. We used the same approach to test whether GOOD females lay more eggs and heavier fertilized eggs than POOR females. In these two analyses, we used as response variables the total number of eggs laid by the females and the mean mass of fertilized eggs (mg), respectively. As the mean

mass of fertilized eggs is a continuous variable, we only used Gaussian and gamma distributions of errors in the model selection.

Software and packages

We ran all analyses in the software R 4.0.3 [53], using the packages *stats* [53] and *MASS* [54] to perform the GLMs, the package *bbmle* [55] to perform the model selection, and the package *nlme* [56] to perform the GLS.

Results

Male investment in prey-gift

Males captured 1 to 27 flies to construct the gift. There was an interaction between the experimental groups and the time females spent close to the glass barrier (Table 1). When males were exposed to POOR females, the proportion of flies added to gift decreased with the time females spent close to the glass barrier. In turn, when males were exposed to GOOD females, the proportion of flies added to the gift increased with time females spent close to the glass barrier (Fig. 1a).

Fourteen males (23% of the total) did not add silk to the gift *before* physically interacting with the female, but 10 of these males added silk to the gift *after* physically interacting with the female. Males exposed to POOR females invested a total of 3.8 ± 3.1 min (mean ± SD) adding silk to the gift, whereas males exposed to GOOD females invested a total of 3.0 ± 2.1 min. There was no difference in the time males invested adding silk to the gift (both *before* the physical interaction and *total*) when they were exposed to POOR or GOOD females (Table 1). However, the probability of the male adding more silk *after* physically

412 interacting with the females increased when the previous investment in silk was low (Fig. 1b,
413 Table 1).

414

415 **Male investment in pre-copulatory courtship**

416 All males stimulated the females with abdominal touches (n = 60). Males exposed to POOR
417 females spent 9.2 ± 4.4 s (mean ± SD) stimulating the abdomen of the partner, whereas males
418 exposed to GOOD females spent 18.2 ± 10.7 s. Males exposed to GOOD females spent more
419 time stimulating females with abdominal touches than males exposed to POOR females (Fig.
420 2, Table 2). Moreover, the longer the mating interaction, the more abdominal touches the
421 males performed (Fig. 2, Table 2). There was no difference between experimental groups in
422 the latency to pedipalp insertion, i.e., between the first abdominal touch performed by the
423 male and the abdominal twist performed by the female that exposes her genital opening to
424 allow copulation (Fig. 3, Table 2).

425

426 **Male investment in copulation duration and sperm transfer**

427 Males performed from zero to five pedipalp insertions during the copulatory phase. They
428 usually alternated pedipalps between insertions, and each insertion lasted from 0.33 to 12.22
429 min. The total duration of pedipalp insertions was longer for males exposed to GOOD
430 females than males exposed to POOR females (Fig. 4, Table 2). Moreover, the total duration
431 of pedipalp insertions decreased in longer mating interactions, but only for males exposed to
432 GOOD females (Fig. 4). For males exposed to POOR females, the total duration of pedipalp
433 insertion increased in longer mating interactions (Fig. 4). Regarding the total quantity of
434 sperm transferred to females, there was no difference between males exposed to POOR and
435 GOOD females (Table 2). Moreover, there was no correlation between the total duration of

436 pedipalp insertions and the quantity of sperm transferred to the females (Table 2). Finally,
437 there was also no correlation between the quantity of sperm transferred to the females and
438 the number of fertilized eggs laid by these females ($t = 0.90$, $df = 32$, $p = 0.375$).

439

440 **Potential benefits of male mate choice**

441 We found two models with $\Delta AIC_c < 2$ to explain the latency between copulation and
442 oviposition. Both models include an interaction between the experimental groups and the
443 number of flies added to the gift, differing only in the type of distribution of errors (Table S8
444 in Supplementary Material). Because the Akaike weight of the best fitted model with Poisson
445 distribution of errors is almost two times higher than that of the model with gamma
446 distribution of errors, we used the former to estimate the parameters presented in Table 3. In
447 general terms, the latency between copulation and oviposition was shorter for GOOD
448 females when the males added a greater number of flies to the gift (Fig. 5a). For POOR
449 females, the number of flies added to the gift did not affect the latency between copulation
450 and oviposition (Fig. 5a).

451 We also obtained two models with $\Delta AIC_c < 2$ to explain the number of eggs laid by the
452 females (both with Gaussian distribution of errors): model (1) includes the additive effect of
453 the experimental groups and number of flies added to the gift, and model (2) includes only
454 the experimental groups (Table S8 in Supplementary Material). Although the Akaike weight
455 of the model (1) is two times higher than that of model (2), the explanatory power of the
456 number of flies is low and this variable is not significant in model (1) (Table 3). Thus, based
457 on the model (2), which includes only the effect of experimental groups, the number of eggs
458 laid was higher for GOOD females (mean ± SD = 123 ± 18 eggs) compared with POOR
459 females (103 ± 24 eggs) (Fig. 5b).

One of 13 GOOD females and five of 25 POOR females laid only unfertilized eggs. The number of fertilized eggs laid by females of both experimental groups was positively correlated with the total number of eggs laid ($r = 0.65$, $t = 5.076$, $df = 35$, $p < 0.001$). Finally, the best fitted model to explain the mean mass of fertilized eggs was the null model (Table S8 in Supplementary Material). The mean mass (± SD) of fertilized eggs in both experimental groups was 0.122 ± 0.063 mg.

Discussion

Here, we provide experimental evidence that males of the gift-giving spider *Paratrechalea ornata* adjust their reproductive investment in response to females' body condition, which was our proxy of quality. Our findings show that, during gift construction, males exposed to high-quality females (i.e., those in good body condition) capture more flies than males exposed to low-quality females (i.e., those in poor body condition). However, silk investment in the nuptial gift does not differ between males exposed to low- and high-quality females. During pre-copulatory courtship, males exposed to high-quality females stimulate longer their partners with abdominal touches compared with males exposed to low-quality females. In the copulatory phase, males exposed to high-quality females perform longer pedipalp insertions than males exposed to low-quality females, but the quantity of sperm transferred does not vary according to female quality. Finally, high-quality females have shorter latency between copulation and oviposition and lay more eggs than low-quality females.

Our results suggest that the adjustment in gift size is mediated by visual and/or short-range vibrational stimuli acquired by the male in the pre-contact phase of the mating interaction. When females are close to the glass barrier of the experimental arena, males increase the number of flies added to the gift in response to high-quality partners, while decrease the number of flies added to the gift in response to low-quality partners (Fig. 1a).

485 The use of visual cues to access the size and body condition of potential partners has already
486 been reported for visually oriented spiders. In wolf spiders [e.g., 57, 58], for instance, females
487 select males in good body condition using both visual and tactile cues. Moreover, in the gift-
488 giving spider *Pisaura mirabilis*, females distinguish males in poor and good body condition
489 using short-range vibrational signals emitted during pre-copulatory and copulatory
490 courtship [50]. In the case of *P. ornata*, males may use visual cues, such as the volume of the
491 female's abdomen, and/or short-range vibrational signals, such as tremulation of legs or
492 abdomen, to distinguish mating partners in poor and good body condition. Although the
493 presence of female silk is important to trigger gift construction by *P. ornata* males [32],
494 chemical cues alone are probably ineffective in inducing adjustments in gift size because
495 when females are far from the glass barrier, the number of flies added to the gift is similar
496 between males exposed to low- and high-quality females (Fig. 1a). Despite extensive
497 empirical evidence showing that males use chemical cues in female silk to access information
498 about her mating status [e.g., 59–62], male's ability to access female body condition using silk
499 cues seems to be relatively rarer in spiders [e.g., 63].

500 Gift-giving is regarded as a form of mating effort when it increases male's mating
501 probability [22]. In *P. ornata*, the silk layer surrounding the gift contains chemicals deposited
502 by the male that entice the female to accept the prey-gift [36]. However, our results show that
503 males do not adjust the quantity of silk deposited on the prey-gift in response to female
504 quality. Considering that silk production in spiders is costly [64], males may invest the
505 minimum amount of silk to properly wrap the prey-gift and adjust the quality of the prey-
506 gift by changing the number of prey added to it. In fact, we found that males construct larger
507 gifts when exposed to high-quality females and smaller gifts when exposed to low-quality
508 females (Fig. 1a). An experimental study with the scorpionfly *Panorpa cognata* has shown that
509 males adjust the size of their *endogenous* gift (i.e., salivary mass) in response to female quality

510 [27]. Our study is perhaps the first demonstration that males can also adjust the size of an
511 *exogenous* gift (i.e., prey wrapped in silk) in response to female quality. Our findings expand
512 previous studies on modulation of gift size in arthropods because we show that high-quality
513 females indeed offer more fitness benefits to males than low-quality females. By increasing
514 their mating effort in response to high-quality females, males have access to more eggs (Fig.
515 5b). Moreover, males probably face lower levels of sperm competition because the more flies
516 they add to the gift offered to high-quality females, the lower the latency to oviposition (Fig.
517 5a). For the largest gifts recorded in our experiment, the latency to oviposition for high-
518 quality females is slightly shorter than the mean remating interval (5.5 days) previously
519 reported for well-fed females of *P. ornata* [65]. In turn, low-quality females are non-
520 responsive to the number of flies added to the gift and their latency to oviposition is
521 consistently longer than the mean remating interval (Fig. 5a).

522 Theoretical and empirical studies on differential allocation indicate that individuals
523 can either increase or decrease their parental effort in response to the quality of their mating
524 partners [9, 13, 66]. Previous studies with *P. ornata* show that the nuptial gift also functions as
525 paternal effort because well-fed females that receive a nutritious gift lay more eggs than
526 well-fed females that receive a worthless gift [38]. Thus, males could construct larger gifts
527 when exposed to low-quality females to increase the fecundity of their partners and gain
528 more fitness benefits. However, our results show that males construct smaller gifts when
529 exposed to low-quality females (Fig. 1a). We suggest that these females use most of the gift's
530 nutrients for self-maintenance rather than egg production. In support of this suggestion,
531 starved males of *P. ornata* prioritize self-maintenance and feed on the prey instead of
532 constructing a nuptial gift when exposed to cues of an unmated female [33]. Our results also
533 show that males exposed to high-quality females construct large gifts (Fig. 1a). However, the
534 possibility that these females capitalize the nutrients of the gift for egg production is unlikely

because the interval between copulation and oviposition is too short (Fig. 5b). In fact, we found that the number of flies added to the gift has no relevant effect on the number of eggs laid by the females, even when they were in good body condition. Although the gift's nutrients could also be used to increase egg size, we found no difference in the mean mass of the fertilized eggs laid by low- and high-quality females. Thus, we argue that the adjustment in gift size reported here is more related to its function as *mating* effort than *parental* effort.

The modulation of males' behavior in response to female quality extends to the pre-copulatory courtship, in which males exposed to high-quality females invest more in abdominal touches compared with low-quality females. Pre-copulatory courtship is an essential source of information for females to evaluate potential mates and a key factor influencing male mating success in arthropods (examples in [67]). In *P. ornata*, the abdominal touches performed by males during pre-copulatory courtship are followed by a female abdominal twist that exposes her genital opening to allow copulation [30]. In the absence of abdominal touches, copulation does not happen, suggesting that male tactile stimulation is crucial for male mating success. Males spend more time stimulating high-quality females because these females may be choosier than low-quality females. For instance, in wolf spiders of the genus *Schizocosa* and in the beetle *Callosobruchus maculatus,* females in poor body condition mate with males no matter their quality, but females in good body condition only mate with high-quality males [68, 69]. However, we found no effect of female condition in the latency to accept the first pedipalp insertion, suggesting that high-quality females of *P. ornata* are not choosier than low-quality females (Fig. 3). Thus, we interpret that the higher investment in pre-copulatory courtship is mainly related to the greater fitness benefits males may acquire by stimulating more fecund females (Fig. 5b). In the cricket *Gryllus bimaculatus*, for instance, males invest more time courting larger and probably more fecund females [70]. In *P. ornata* males, specifically, the longer pre-courtship of high-quality females compared

560 with low-quality females can increase their chances of fertilizing a greater number of eggs, a
561 post-copulatory process not investigated here.

562 Males of *P. ornata* also adjust the duration of pedipalp insertions in response to female
563 quality, with males exposed to high-quality females showing longer insertions than males
564 exposed to low-quality females. However, there is no difference in sperm quantity
565 transferred to low- and high-quality females. Moreover, the duration of pedipalp insertions
566 is not correlated with the sperm quantity transferred to the females, suggesting that the
567 biological meaning of these two variables is not the same. In fact, a recent experimental study
568 with the spider *Holocnemus pluchei* (Pholcidae) has shown that males with prolonged
569 copulation have more stored sperm in the female reproductive tract, even though males with
570 shorter copulation transfer the same quantity of sperm [44]. The authors suggest that
571 prolonged copulation is related to the stimulation of the female reproductive tract by the
572 male (see also [71] and [72] for additional examples with damselflies and soldier flies,
573 respectively). This stimulation during copulation could be under cryptic female choice, so
574 that males that provide longer stimulation are favored by females and thus increase their
575 fertilization success [73]. If the quantity of sperm stored by *P. ornata* females is also
576 cryptically selected based on male stimulation during copulation, sexual selection should
577 favor males that increase the investment in copulation duration when exposed to more
578 fecund females (i.e., females in good body condition), as we found here. Why males do not
579 adjust the quantity of sperm transferred to the females is an open question that deserves
580 further investigation. An interesting possibility is that females have control over the fate of
581 the sperm inside their reproductive tract (see discussion in [51]). We argue that the lack of
582 correlation between the quantity of sperm transferred by the males and the number of
583 fertilized eggs laid by the females supports this suggestion. Thus, the best male strategy to

584 increase fertilization success may be to invest in copulatory stimulation rather than in the
585 quantity of sperm transferred to the mating partner [51].

586

587 **Conclusion**

588 There is growing evidence that males can select their mating partners, but the subject of
589 differential allocation in males remains poorly explored, especially in species with nuptial
590 gifts (but see [26, 27]). Here we provide experimental evidence that males of the gift-giving
591 spider *P. ornata* exhibit positive differential allocation in three key aspects of their mating
592 effort: nuptial gift size, duration of pre-copulatory courtship, and duration of pedipalp
593 insertions. This positive differential allocation is likely associated with the benefits of
594 copulating with high-quality females, which lay approximately 20% more eggs than low-
595 quality females and produce a larger number of fertilized eggs. Moreover, high-quality
596 females probably represent a lower level of sperm competition because they (i) oviposit
597 faster after copulation and (ii) may be less prone to remating than low-quality females, as
598 reported for other gift-giving spiders [74, 75]. Both the benefits in terms of more eggs that
599 can be fertilized, and the decreased level of sperm competition may favor the evolution of
600 positive differential allocation by the males. As a final remark, our findings reinforce the
601 notion that the hypothesis of differential allocation, which has been originally proposed to
602 monogamous mating systems with biparental care [76], also applies to species with a
603 scramble competition mating system, in which males invest in nuptial gift construction, but
604 not in parental care.

605

606 **Ethics approval**

607 Our experiments meet the Animal Behaviour Society guidelines for ethical treatment of
608 animals [77]. Both the collection and maintenance of the individuals in captivity were
609 conducted with proper permits of the Brazilian Government (SISBIO/ICMBio, permit
610 68883/2).

611 **Availability of data and material**

612 The data used in this study are available at Mendeley Repository.

613 **Competing interests**

614 The authors declare no competing or financial interests.

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621 **Authors' contributions**

622 Conceptualization: DSB and GM; Methodology: DSB, GM, and MJA; Data collection: DSB
623 and LECS (field) and DSB (laboratory); Formal analysis: DSB and LECS; Data curation: DSB;
624 Writing (original draft): DSB and GM; Writing (review & editing): DSB, LECS, MJA, and GM;
625 Visualization: DSB and GM; Supervision: GM; Project administration: DSB; Funding
626 acquisition: DSB and GM.

627

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806

807 **Table 1** Summary of the statistical models used to explain male investment in flies and silk
808 added to the nuptial gift. For the models of *Proportion of flies added to the gift* and *Probability of*
809 *adding more silk before physical interaction*, we report the estimate in logit units and present the
810 original units in parentheses. Moreover, the model of *Probability of adding more silk before*
811 *physical interaction* uses a z-value instead of a t-value as in the other models. Significant
812 results are highlighted in bold. SE = standard error.

813

Predictors	**Estimate**	**SE**	**t- or z-value**	**p-value**
Proportion of flies added to the gift				
Intercept (POOR females)	-0.28 (0.43)	0.18	-1.54	0.130
GOOD females	-0.41 (0.40)	0.24	-1.71	0.093
Time near the barrier	-0.01 (0.50)	0.005	-2.080	**0.042**
GOOD females × Time near the barrier	0.02 (0.50)	0.007	2.498	**0.016**
Time adding silk before physical interaction				
Intercept (POOR females)	3.05	1.06	2.878	0.006
GOOD females	0.01	0.02	0.236	0.814
Time near the barrier	-1.59	1.18	-1.35	0.182
GOOD females × Time near the barrier	0.03	0.03	0.897	0.373
Probability of adding more silk after physical interaction				
Intercept (POOR females)	0.57 (0.64)	0.58	0.99	0.051
GOOD females	-0.38 (0.41)	0.87	-0.435	0.658
Time previously invested in silk	-0.52 (0.37)	0.22	-2.411	**0.016**
GOOD females × Previous invest in silk	0.01 (0.50)	0.35	0.038	0.970
Total time adding silk				
Intercept (POOR females)	3.79	0.50	7.597	< 0.001
GOOD females	-0.77	0.67	-1.149	0.255

814

Table 2 Summary of the statistical models used to explain pre-copulatory (*Abdominal touches* and *Latency to pedipalp insertion*) and copulatory (*Total duration of pedipalp insertions* and *Total sperm transferred*) investment by males exposed to females in POOR and GOOD condition. In the model of *Abdominal touches*, the total duration of the mating interaction (starting with gift acceptance and finishing with the couple's separation) was included as continuous predictor. For the model of *Latency to pedipalp insertion*, we report the estimate in log units and the original units are presented in parentheses. Significant results are highlighted in bold. SE = standard error.

Predictors	**Estimate**	**SE**	**t-value**	**p-value**
Abdominal touches				
Intercept (POOR females)	6.17	1.26	4.905	< 0.001
GOOD females	8.43	2.79	3.022	**0.004**
Duration of the mating interaction	0.49	0.22	2.215	**0.031**
GOOD females × Duration of the mating interaction	-0.27	0.26	-1.047	0.300
Latency to pedipalp insertion				
Intercept (POOR females)	3.18 (24.15)	0.20	15.715	< 0.001
GOOD females	0.51 (1.67)	0.30	1.719	0.085
Total time of pedipalp insertion				
Intercept (POOR females)	0.65	0.25	2.63	< 0.001
GOOD females	2.04	0.46	4.41	**< 0.001**
Duration of interaction	0.16	0.05	2.98	**0.004**
GOOD females × Duration of interaction	-0.19	0.05	-3.51	**< 0.001**
Total sperm transferred				
Intercept (POOR females)	50,846.11	7,262.34	7.00	< 0.001
GOOD females	2,889.66	8,627.38	0.33	0.739
Total time of pedipalp insertion	4,881.56	3,669.48	1.33	0.189
GOOD females × Total time of pedipalp insertion	-2,599.45	4,033.99	-0.64	0.522

825 **Table 3.** Summary of the statistical models used to evaluate the potential benefits provided
826 by females in POOR and GOOD condition. For the model of *Latency of oviposition*, we report
827 the estimate in log units and present the original units in parentheses. Moreover, the model
828 of *Latency of oviposition* uses a z-value instead of a t-value as in the other models. For the
829 model of *Total number of eggs*, we present the results of the two best fitted models ($\Delta AIC_c <$
830 2): model (1) includes the additive effect of the experimental groups and number of flies
831 added to the gift, and model (2) includes only the effect of the experimental groups.
832 Significant results are highlighted in bold. SE = standard error.

833

Predictors	**Estimate**	**SE**	**z- or t-value**	**p-value**
Latency of oviposition				
Intercept (POOR females)	2.55 (12.81)	0.19	13.122	< 0.001
GOOD females	0.74 (2.09)	0.54	1.357	0.175
Number of flies added	0.01 (1.01)	0.01	1.105	0.269
GOOD females x Number of flies added	-0.09 (0.91)	0.03	-2.629	**0.008**
Total number of eggs				
Model (1)				
Intercept (POOR females)	127.77	13.53	9.296	< 0.001
GOOD females	23.80	7.72	3.084	**0.004**
Number of flies added	-1.54	0.83	-1.854	0.073
Model (2)				
Intercept (POOR females)	102.18	4.78	21.385	< 0.001
GOOD females	20.97	7.84	2.675	**0.011**
Mean mass of fertilized eggs				
Intercept	0.12	0.01	11.27	< 0.001

834

835 **FIGURE LABELS**

836

837 **Fig. 1.** Investment in (a) flies and (b) silk added to the nuptial gift by males of the spider
838 *Paratrechalea ornata*. (a) Proportion of flies captured by males exposed to females in POOR
839 condition (white dots) and GOOD condition (black dots). Given that we offered
840 approximately 40 flies for each male, the higher the proportion of flies added to gift, the
841 larger it is. The time spent by the females close to the glass barrier in the experimental arena
842 was included a continuous predictor variable because it represents the visual and/or short-
843 range, substrate-borne vibratory stimulus received by the males during the pre-copulatory
844 phase. Lines indicate the tendency predicted for males exposed to each experimental group:
845 dashed = females in POOR condition; solid = females in GOOD condition. (b) Probability of
846 a male adding more silk to the gift *after* physically interacting with the female in response to
847 his previous investment in silk (i.e., the time spent adding silk to the gift *before* physically
848 interacting with the female). Circle sizes are proportional to the number of superimposed
849 data points. The shaded area indicates the 95% confidence interval.

850

851 **Fig. 2.** Time spent by males of the spider *Paratrechalea ornata* touching the female abdomen
852 according to the total duration of the mating interaction. White dots represent males exposed
853 to females in POOR condition and black dots represent males exposed to females in GOOD
854 condition. Lines indicate the tendency predicted for males exposed to each experimental
855 group: dashed = females in POOR condition; solid = females in GOOD condition. The
856 shaded area indicates the 95% confidence interval.

857

Fig. 3. Latency to pedipalp insertion in the spider *Paratrechalea ornata* according to female body condition. The latency is the interval between the first abdominal touch performed by the male and the abdominal twist performed by the female that exposes her genital opening to allow copulation. The boxes contain 50% of the data and the line inside each box represents the median.

Fig. 4. Time spent by males of the spider *Paratrechalea ornata* in pedipalp insertions into the female epigyne (genital opening) according to the total duration of the mating interaction. White dots represent males exposed to females in POOR condition and black dots represent males exposed to females in GOOD condition. Lines indicate the tendency predicted for males exposed to each experimental group: dashed = females in POOR condition; solid = females in GOOD condition. The shaded area indicates the 95% confidence interval.

Fig. 5. Potential benefits of differential allocation in reproductive investment when males of the spider *Paratrechalea ornata* are exposed to females in POOR and GOOD condition. (a) Latency between copulation and oviposition according to female condition and the number of flies added to the gift. White dots represent males exposed to females in POOR condition and black dots represent males exposed to females in GOOD condition. Lines indicate the tendency predicted for males exposed to each experimental group: dashed = females in POOR condition; solid = females in GOOD condition. The shaded area indicates the 95% confidence interval. (b) Boxplot of the total number of eggs laid by females according to their condition. The boxes contain 50% of the data and the line inside each box represents the median.

FIGURE 1

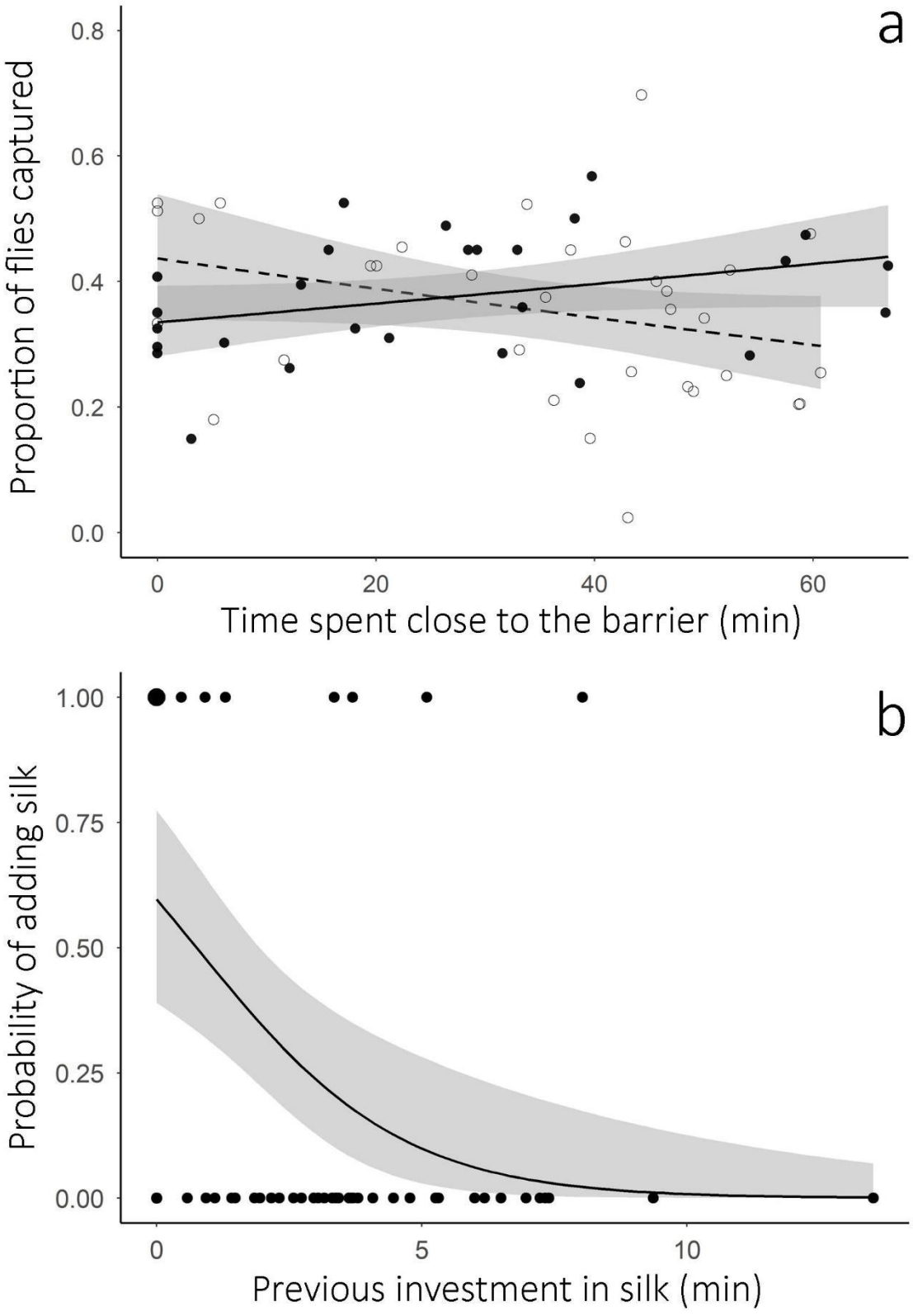

a
Proportion of flies captured
0.8
0.6
0.4
0.2
0.0
0
20
40
60
Time spent close to the barrier (min)
b
Probability of adding silk
1.00
0.75
0.50
0.25
0.00
0
5
10
Previous investment in silk (min)

FIGURE 2

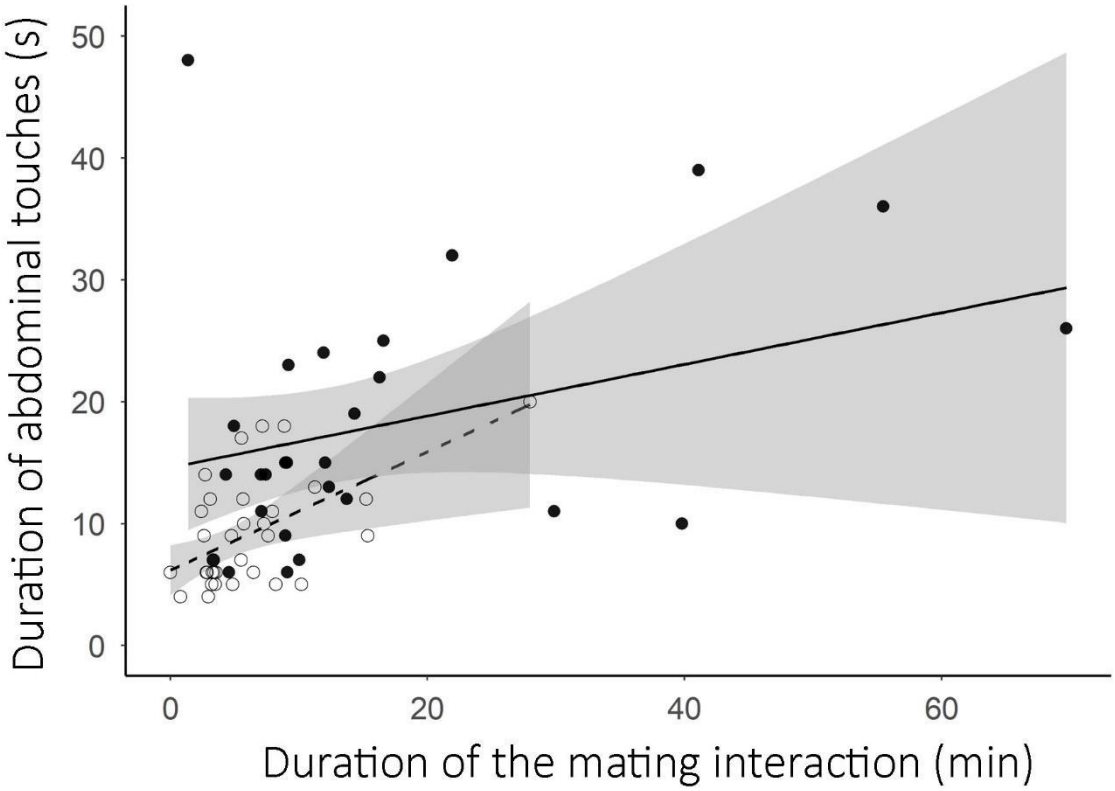

Duration of abdominal touches (s)
50
40
30
20
10
0
0
20
40
60
Duration of the mating interaction (min)

FIGURE 3

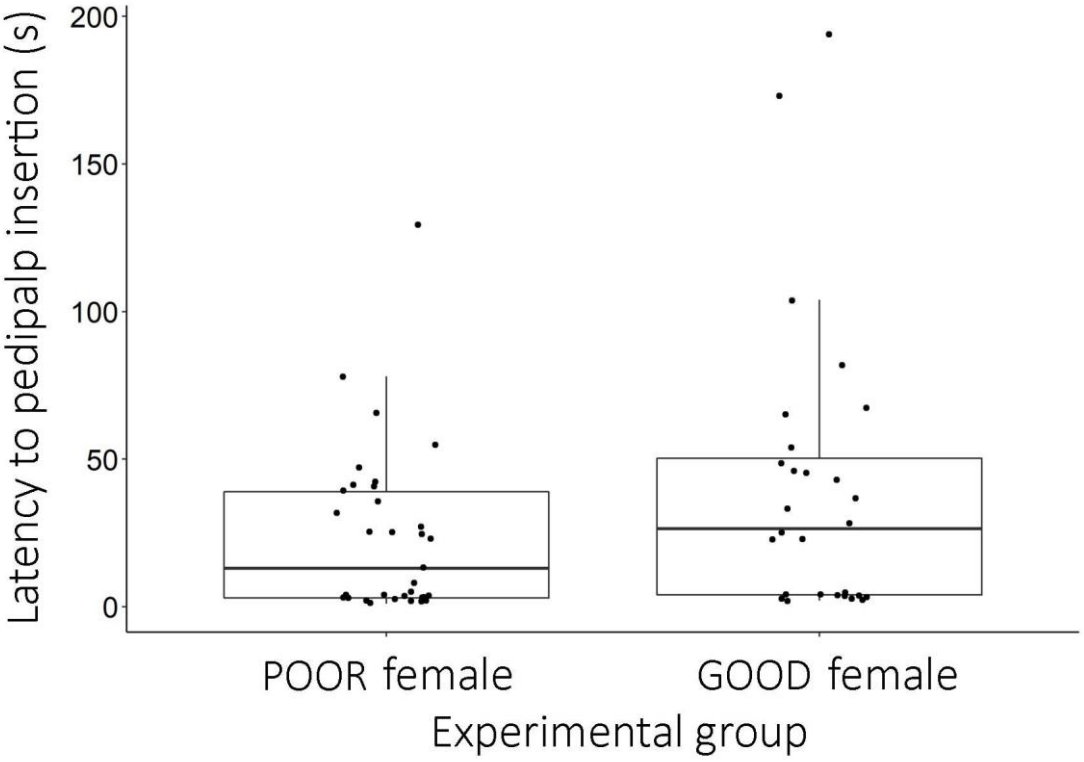

Latency to pedipalp insertion (s)
200
150
100
50
0
POOR female
GOOD female
Experimental group

FIGURE 4

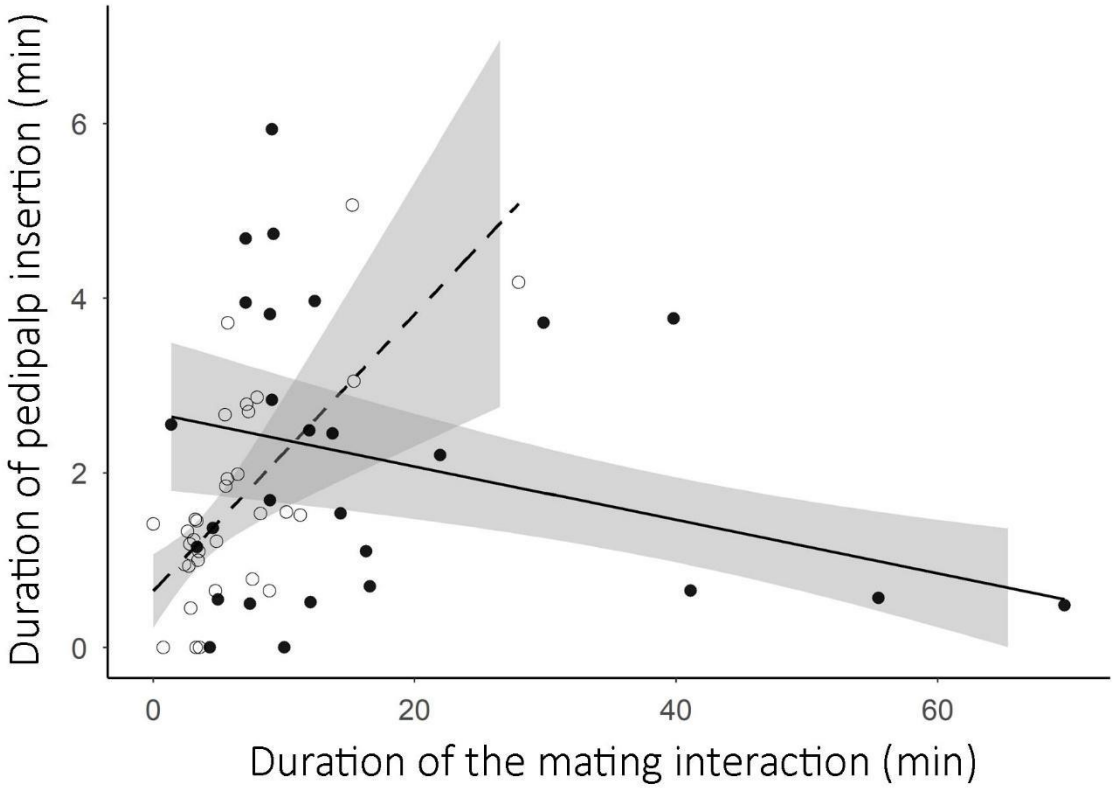
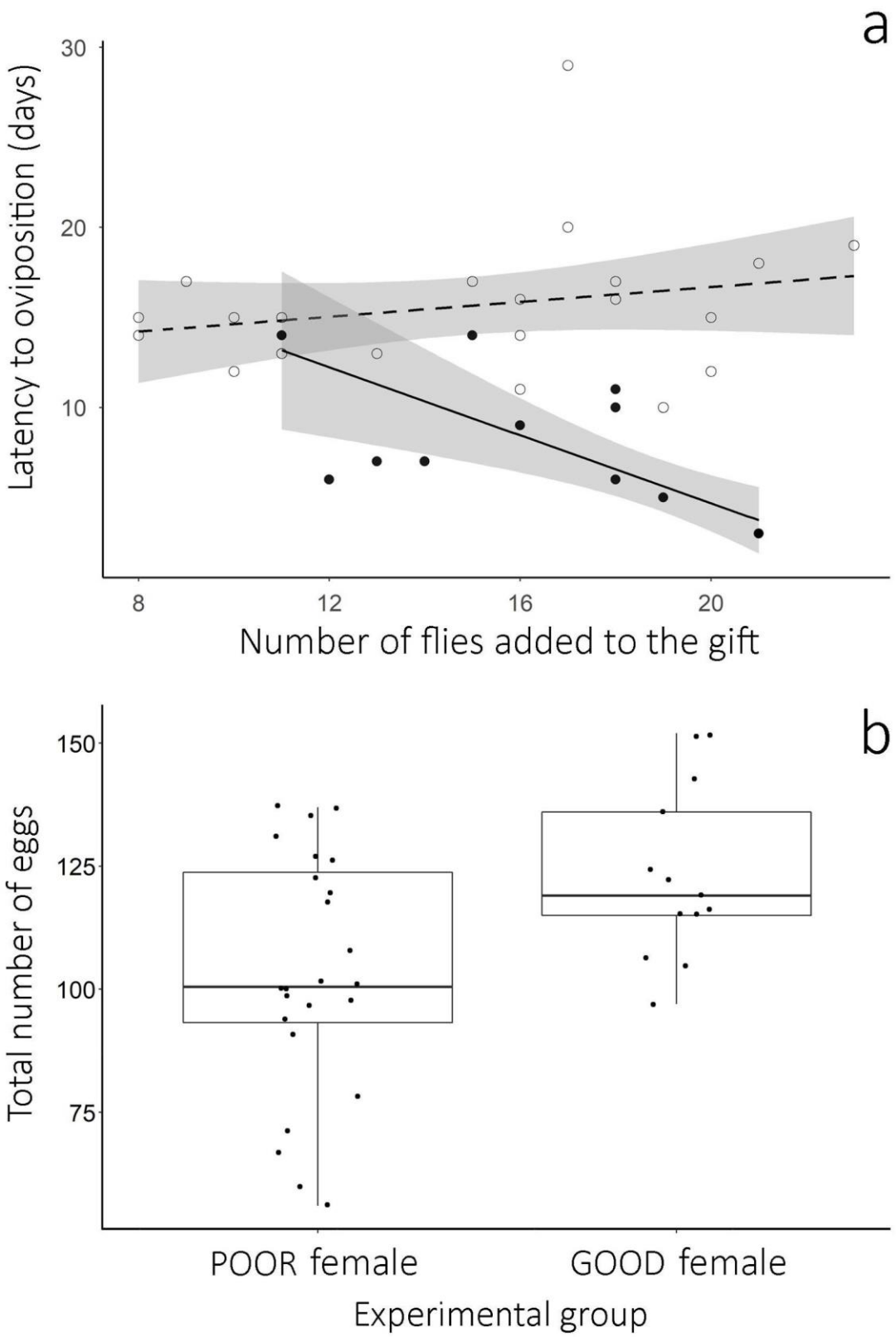

Duration of pedipalp insertion (min)
0
2
4
6
0
20
40
60
Duration of the mating interaction (min)

FIGURE 5


a
Latency to oviposition (days)
30
20
10
8
12
16
20
Number of flies added to the gift
b
Total number of eggs
150
125
100
75
POOR female
GOOD female
Experimental group

Supplementary Material

Table S1. Repeatability of the measurements of cephalothorax width of males and females of the spider *Paratrechalea ornata*. The table also includes measurements of the areas of the bulb, median apophysis, tegulum, and subtegulum of males' pedipalps. We followed Lessells & Boag (1987) to calculate the repeatability using three measurements of each structure in a sample of 20 individuals.

Structure	**Repeatability**
Female cephalothorax width	0.984
Male cephalothorax width	0.964
Area of the bulb	0.962
Area of the median apophysis	0.979
Area of the tegulum	0.601
Area of the subtegulum	0.498

Table S2. Tests of some key assumptions of the experimental protocol. Values are presented as mean ± standard deviation.

Assumption	Experimental group		Statistics
	POOR	GOOD	
Difference in female body condition after conditioning	-0.020 ± 0.009	0.024 ± 0.014	$t = 14.498$, $df = 58$, $p < 0.001$
No difference in male body condition	0.0014 ± 0.0082	-0.0016 ± 0.0053	$t = -1.685$, $df = 58$, $p = 0.097$
No difference in female size	3.924 ± 0.271	3.958 ± 0.231	$t = 0.515$, $df = 58$, $p = 0.609$
No difference in male size	3.720 ± 0.205	3.768 ± 0.176	$t = 0.971$, $df = 58$, $p = 0.336$
No difference in the difference between female and male size	-0.0008 ± 0.215	0.0009 ± 0.225	$t = 0.030$, $df = 58$, $p = 0.976$

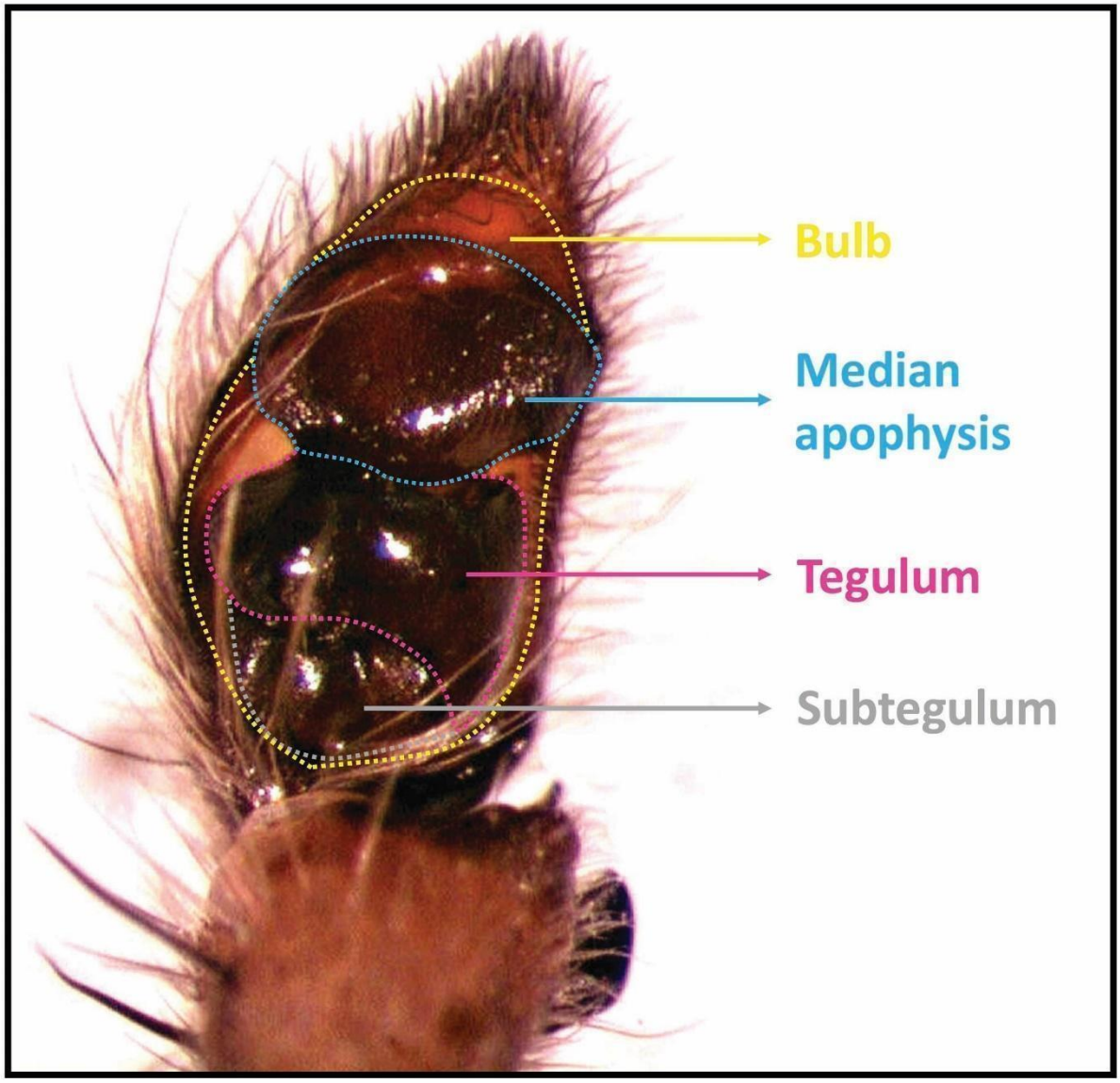


Figure S1. Pedipalp of a *Paratrechalea ornata* male showing the structures measured to estimate the sperm stored *before* copulation and then the quantity of sperm transferred to the female *after* copulation.

Table S3. Correlation between the quantity of flies added to the gift and three different variables of investment in silk added to the gift by males of the spider *Paratrechalea ornata*. For each variable we show the value of the Pearson's correlation (*r*) and its t-value, the degree of freedom (df), and the p-value.

Variable	***r***	**t-value**	**df**	**p-value**
Silk before (min)	0.056	0.426	58	0.672
Silk after (min)	0.028	0.217	58	0.829
Total silk (min)	0.069	0.524	58	0.602

Methods of sperm quantification

In our first approach, we conducted a model selection using morphological variables to predict the sperm stored in the pedipalps before copulation. The predictor variables included body traits, such as the cephalothorax width (Cephalothorax) and the residuals of a linear regression between cephalothorax width and body weight (Condition), and pedipalpal traits, such as the median apophysis area (Apophysis), tegulum area (Tegulum), subtegulum area (Subtegulum), and bulb area (Bulb). The concurrent models included all possible combinations of three or less predictor traits, with additive and interactive effects between them. To keep our capacity to detect reasonable-size effects with acceptable power, we did not include models with a combination of more predictor variables (following Harrel, 2015). For each combination we constructed models using Gaussian, Poisson, and negative binomial (NB) distribution of errors. To select the best combination of predictors to explain the quantity of sperm stored in the pedipalps, we used the Akaike Information Criteria corrected for small samples (AIC_c).

The results of the model selection are presented in Table S4. The two best models (i.e., those with $\Delta AIC_c < 2$) contained "Cephalothorax" and "Cephalothorax + Subtegulum" as predictor variables. Among the 10 top ranked models, which accounted together for 0.528 of the Akaike Weight, none of them included three predictor variables. Moreover, the Akaike weight of all models with three variables (0.185) is lower than the Akaike weight of the models including one (0.237) or two variables (0.568). Taken together, these findings suggest that the inclusion of many predictor variables in the models does not increase their explanatory power. In general, models with a Gaussian error distribution had higher Akaike weight than models with a negative binomial or a Poisson error distribution (Table S4). The model including only Cephalothorax (i.e., the best fitted model) explains 20% of the variation in the quantity of sperm stored in the pedipalps ($F_{1,37} = 9.269$, $p = 0.004$; $R^2 = 0.200$).

In our second approach, we conducted a model selection using Principal Components Analysis (PCA) to reduce the dimensionality of all morphological variables and predict the sperm stored in the pedipalps before copulation. Before the PCA, each morphological variable was standardized, i.e., the cephalothorax width, the residuals of a linear regression between cephalothorax width and body weight, and the areas of the median apophysis area, tegulum, subtegulum, and bulb were centered to their mean and scaled to their standard deviation. The three first principal components (PCs) accumulated 73% of the variance of the six morphological variables (Tables S5-S6). Then, we created models with all the possible combinations of the three PC, with additive and interactive effects between them. For each combination, we constructed models using Gaussian, Poisson and negative binomial (NB) distribution of errors. To select the best combination of predictors to explain the quantity of sperm stored in the pedipalps, we used the Akaike Information Criteria corrected for small samples (AIC_c).

The results of the model selection are presented in Table S7. We obtained three models with ΔAICc lower than 2: one including PC1, one including PC1+PC3, and one including PC1+PC2. For the sake of simplicity, we used the model including only PC1 as predictor variable of the quantity of sperm stored in the pedipalp. This model explained 17.7% of the variability in the sperm stored ($F_{1,37} = 7.95$, $p = 0.008$, $R^2 = 0.177$). Thus, the results obtained with the PCA, which synthesizes the variation of the six morphological traits, do not improve our predictive power when compared with the individual morphological traits. Based on this conclusion, we used the results obtained with the individual morphological traits (first approach) in the analyses reported in the main text.

Table S4. List of concurrent models including all possible combinations of three or less morphological variables to find the best predictors of the quantity of sperm stored in pedipalps of *Paratrechalea ornata* males before copulation. We also show the number of slopes estimated (*k*) and the Akaike weight (Weight) of each model. The symbol × represents interaction between variables and the symbol + represents additive effects between variables.

Predictor variables	**Distribution**	**ΔAIC_c**	***k***	**Weight**
Cephalothorax	Gaussian	0	1	0.134
Cephalothorax + Subtegulum	Gaussian	0.70	2	0.094
Cephalothorax + Bulb	Gaussian	2.18	2	0.045
Cephalothorax + Tegulum	Gaussian	2.28	2	0.043
Cephalothorax × Apophysis	Gaussian	2.41	3	0.040
Cephalothorax + Apophysis	Gaussian	2.49	2	0.038
Cephalothorax	NB	2.49	1	0.038
Cephalothorax × Tegulum	Gaussian	2.60	3	0.036
Cephalothorax × Subtegulum	Gaussian	2.87	3	0.032
Cephalothorax + Subtegulum	NB	3.10	2	0.028
Cephalothorax + Apophysis + Subtegulum	Gaussian	3.24	3	0.026
Cephalothorax + Tegulum + Subtegulum	Gaussian	3.30	3	0.026
Cephalothorax + Condition + Subtegulum	Gaussian	3.34	3	0.025
Subtegulum	Gaussian	3.72	1	0.021
Cephalothorax × Bulb	Gaussian	3.79	3	0.020
Apophysis × Subtegulum	Gaussian	3.90	3	0.019
Cephalothorax × Apophysis	NB	4.06	3	0.017
Tegulum + Subtegulum	Gaussian	4.14	2	0.017
Cephalothorax × Tegulum	NB	4.21	3	0.016
Tegulum	Gaussian	4.23	1	0.016
Cephalothorax + Bulb	NB	4.49	2	0.014
Cephalothorax + Tegulum	NB	4.70	2	0.013
Cephalothorax + Condition + Bulb	Gaussian	4.70	3	0.013

Cephalothorax × Subtegulum	NB	4.73	3	0.013
Cephalothorax + Condition + Tegulum	Gaussian	4.77	3	0.012
Cephalothorax + Apophysis + Tegulum	Gaussian	4.87	3	0.012
Cephalothorax + Condition + Apophysis	Gaussian	4.97	3	0.011
Cephalothorax + Apophysis	NB	4.99	2	0.011
Tegulum × Subtegulum	Gaussian	5.36	3	0.009
Apophysis + Subtegulum	Gaussian	5.38	2	0.009
Cephalothorax + Tegulum + Subtegulum	NB	5.64	3	0.008
Cephalothorax + Apophysis + Subtegulum	NB	5.66	3	0.008
Cephalothorax + Condition + Subtegulum	NB	5.72	3	0.008
Apophysis	Gaussian	5.91	1	0.007
Bulb	Gaussian	5.92	1	0.007
Cephalothorax × Bulb	NB	5.99	3	0.007
Apophysis × Subtegulum	NB	6.13	3	0.006
Condition + Subtegulum	Gaussian	6.15	2	0.006
Null model	Gaussian	6.40	0	0.005
Apophysis + Tegulum	Gaussian	6.55	2	0.005
Condition + Tegulum	Gaussian	6.59	2	0.005
Condition + Tegulum + Subtegulum	Gaussian	6.77	3	0.005
Apophysis + Tegulum + Subtegulum	Gaussian	6.77	3	0.005
Subtegulum	NB	6.83	1	0.004
Cephalothorax + Condition + Bulb	NB	6.89	3	0.004
Tegulum + Subtegulum	NB	6.97	2	0.004
Cephalothorax + Condition + Tegulum	NB	7.06	3	0.004
Tegulum × Subtegulum	NB	7.09	3	0.004
Cephalothorax + Apophysis + Tegulum	NB	7.25	3	0.004
Tegulum	NB	7.28	1	0.004
Cephalothorax + Condition + Apophysis	NB	7.37	3	0.003
Condition × Tegulum	Gaussian	7.72	3	0.003
Condition + Apophysis + Subtegulum	Gaussian	8.01	3	0.002

Condition + Apophysis	Gaussian	8.14	2	0.002
Condition + Bulb	Gaussian	8.20	2	0.002
Apophysis + Subtegulum	NB	8.41	2	0.002
Condition × Subtegulum	Gaussian	8.55	3	0.002
Condition	Gaussian	8.58	1	0.002
Apophysis × Tegulum	Gaussian	8.94	3	0.002
Bulb	NB	8.97	1	0.002
Apophysis	NB	9.01	1	0.001
Condition + Apophysis + Tegulum	Gaussian	9.01	3	0.001
Null model	NB	9.17	0	0.001
Condition + Subtegulum	NB	9.28	2	0.001
Condition × Apophysis	Gaussian	9.34	3	0.001
Condition + Tegulum	NB	9.56	2	0.001
Apophysis + Tegulum + Subtegulum	NB	9.61	3	0.001
Condition + Tegulum + Subtegulum	NB	9.61	3	0.001
Apophysis + Tegulum	NB	9.64	2	0.001
Condition × Tegulum	NB	10.48	3	0.001
Cephalothorax × Condition × Subtegulum	Gaussian	10.58	7	0.001
Condition × Bulb	Gaussian	10.80	3	0.001
Condition + Apophysis + Subtegulum	NB	11.05	3	0.001
Condition + Apophysis	NB	11.17	2	0.001
Cephalothorax × Apophysis × Tegulum	Gaussian	11.24	7	<0.001
Condition + Bulb	NB	11.28	2	<0.001
Condition	NB	11.39	1	<0.001
Condition × Subtegulum	NB	11.60	3	<0.001
Cephalothorax × Condition × Apophysis	Gaussian	11.64	7	<0.001
Cephalothorax × Condition × Apophysis	NB	11.67	7	<0.001
Cephalothorax × Apophysis × Subtegulum	Gaussian	11.70	7	<0.001
Condition + Apophysis + Tegulum	NB	12.01	3	<0.001
Apophysis × Tegulum	NB	12.02	3	<0.001
Condition × Apophysis	NB	12.17	3	<0.001

Cephalothorax × Apophysis × Tegulum	NB	12.61	7	<0.001
Cephalothorax × Condition × Subtegulum	NB	12.81	7	<0.001
Cephalothorax × Condition × Bulb	Gaussian	12.82	7	<0.001
Cephalothorax × Subtegulum × Tegulum	Gaussian	13.01	7	<0.001
Cephalothorax × Condition × Tegulum	Gaussian	13.64	7	<0.001
Cephalothorax × Apophysis × Subtegulum	NB	13.73	7	<0.001
Condition × Bulb	NB	13.89	3	<0.001
Cephalothorax × Condition × Bulb	NB	14.71	7	<0.001
Cephalothorax × Condition × Tegulum	NB	14.98	7	<0.001
Apophysis × Subtegulum × Tegulum	Gaussian	15.12	7	<0.001
Cephalothorax × Subtegulum × Tegulum	NB	15.23	7	<0.001
Condition × Apophysis × Subtegulum	Gaussian	15.40	7	<0.001
Condition × Apophysis × Tegulum	Gaussian	15.71	7	<0.001
Condition × Subtegulum × Tegulum	Gaussian	15.90	7	<0.001
Condition × Apophysis × Tegulum	NB	16.92	7	<0.001
Apophysis × Tegulum × Subtegulum	NB	17.05	7	<0.001
Condition × Apophysis × Subtegulum	NB	17.29	7	<0.001
Condition × Subtegulum × Tegulum	NB	18.29	7	<0.001
Cephalothorax × Apophysis × Tegulum	Poisson	72388.34	7	<0.001
Cephalothorax × Condition × Apophysis	Poisson	72526.73	7	<0.001
Cephalothorax × Condition × Subtegulum	Poisson	72657.31	7	<0.001
Cephalothorax × Subtegulum × Apophysis	Poisson	74520.56	7	<0.001
Cephalothorax × Subtegulum × Tegulum	Poisson	76693.23	7	<0.001
Cephalothorax × Condition × Bulb	Poisson	76725.25	7	<0.001
Cephalothorax × Condition × Tegulum	Poisson	77263.50	7	<0.001
Cephalothorax × Tegulum	Poisson	79964.50	3	<0.001
Cephalothorax × Apophysis	Poisson	80324.40	3	<0.001
Apophysis × Subtegulum × Tegulum	Poisson	80928.09	7	<0.001
Cephalothorax × Subtegulum	Poisson	81267.68	3	<0.001
Condition × Apophysis × Subtegulum	Poisson	81420.67	7	<0.001
Condition × Apophysis × Tegulum	Poisson	82207.16	7	<0.001

Condition × Tegulum × Subtegulum	Poisson	83186.32	7	<0.001
Cephalothorax × Bulb	Poisson	83288.39	3	<0.001
Cephalothorax + Apophysis + Subtegulum	Poisson	83386.31	3	<0.001
Cephalothorax + Tegulum + Subtegulum	Poisson	83526.82	3	<0.001
Cephalothorax + Subtegulum	Poisson	83661.14	2	<0.001
Cephalothorax + Condition + Subtegulum	Poisson	83662.41	3	<0.001
Apophysis × Subtegulum	Poisson	83820.61	3	<0.001
Tegulum × Subtegulum	Poisson	86296.82	3	<0.001
Cephalothorax + Condition + Bulb	Poisson	86801.07	3	<0.001
Cephalothorax + Condition + Tegulum	Poisson	86940.54	3	<0.001
Cephalothorax + Bulb	Poisson	87077.97	2	<0.001
Cephalothorax + Tegulum + Apophysis	Poisson	87085.07	3	<0.001
Cephalothorax + Tegulum	Poisson	87231.37	2	<0.001
Cephalothorax + Condition + Apophysis	Poisson	87476.85	3	<0.001
Cephalothorax	Poisson	87804.76	1	<0.001
Cephalothorax + Apophysis	Poisson	87806.89	2	<0.001
Condition + Tegulum + Subtegulum	Poisson	91388.96	3	<0.001
Apophysis + Tegulum + Subtegulum	Poisson	91419.69	3	<0.001
Tegulum + Subtegulum	Poisson	91420.21	2	<0.001
Condition × Tegulum	Poisson	93282.83	3	<0.001
Condition + Apophysis + Subtegulum	Poisson	94414.18	3	<0.001
Apophysis + Subtegulum	Poisson	94423.34	2	<0.001
Condition × Subtegulum	Poisson	95481.32	3	<0.001
Condition + Subtegulum	Poisson	96165.02	2	<0.001
Apophysis × Tegulum	Poisson	96266.63	3	<0.001
Subtegulum	Poisson	96288.34	1	<0.001
Condition +Apophysis + Tegulum	Poisson	96879.58	3	<0.001
Apophysis + Tegulum	Poisson	97215.05	2	<0.001
Condition + Tegulum	Poisson	97353.18	2	<0.001
Condition × Apophysis	Poisson	97570.00	3	<0.001
Tegulum	Poisson	97626.05	1	<0.001

Condition + Apophysis	Poisson	101109.72	2	<0.001
Condition × Bulb	Poisson	101224.92	3	<0.001
Condition + Bulb	Poisson	101280.73	2	<0.001
Apophysis	Poisson	101693.42	1	<0.001
Bulb	Poisson	101801.31	1	<0.001
Condition	Poisson	108645.73	1	<0.001
Null model	Poisson	109021.93	0	<0.001

Table S5. Eigenvectors of the used Principal Components (PC). The values represent the covariance between each morphological variable and the corresponding PC.

Morphological variable	**PC1**	**PC2**	**PC3**
Bulb	0.533	-	0.287
Subtegulum	0.320	0.223	-0.625
Tegulum	0.262	-0.473	-0.625
Apophysis	0.511	-	0.111
Cephalothorax	0.509	0.246	0.219
Condition	0.156	-0.811	0.276

Table S6. Variance explained by the Principal Components Analysis of six morphological traits of the pedipalps of *Paratrechalea ornata* males. For each Principal Component (PC) we present the standard deviation, the proportion of variance explained, and the cumulative proportion of variance.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Standard deviation	1.56	1.03	0.92	0.89	0.68	0.57
Proportion of variance	0.41	0.18	0.14	0.13	0.08	0.06
Cumulative proportion	0.41	0.59	0.73	0.87	0.94	1.00

Table S7. List of concurrent models including all the possible combinations of three Principal Components (PC1, PC2, and PC3) to find the best predictors of the quantity of sperm stored in pedipalps of *Paratrechalea ornata* males before copulation. We also show the number of slopes estimated (*k*) and the Akaike weight (Weight) of each model. The symbol × represents interaction between variables and the symbol + represents additive effects between variables.

Predictor variables	**Distribution**	**ΔAIC_c**	***k***	**Weight**
PC1	Gaussian	0	1	0.265
PC1+PC3	Gaussian	1.3	2	0.138
PC1+PC2	Gaussian	1.8	2	0.109
PC1×PC2	Gaussian	2.7	3	0.069
PC1	NB	3	1	0.059
PC1×PC3	Gaussian	3.2	3	0.055
PC1+PC2+PC3	Gaussian	3.9	3	0.038
PC1+PC3	NB	4	2	0.035
PC1+PC2	NB	4.5	2	0.027
PC1×PC2+PC3	Gaussian	4.9	4	0.023
PC1×PC2	NB	5	3	0.021
PC2×PC3	Gaussian	5.2	3	0.019
NULL	Gaussian	5.2	0	0.019
PC1×PC3	NB	5.4	3	0.017
PC1+PC2×PC3	Gaussian	5.6	4	0.016
PC2	Gaussian	5.9	1	0.014
PC1×PC2+PC2×PC3	Gaussian	6.5	5	0.010
PC1+PC2+PC3	NB	6.6	3	0.010
PC1×PC2+PC3	NB	7.1	4	0.008
PC3	Gaussian	7.3	1	0.007
PC1×PC2+PC1×PC3	Gaussian	7.9	5	0.005
NULL	NB	8	0	0.005
PC2×PC3	NB	8.2	3	0.005
PC1×PC3+PC2×PC3	Gaussian	8.2	5	0.004

PC2+PC3	Gaussian	8.4	2	0.004
PC1+PC2×PC3	NB	8.5	4	0.003
PC1×PC2+PC2×PC3	NB	8.8	5	0.003
PC2	NB	8.8	1	0.003
PC1×PC2+PC1×PC3+PC2×PC3	Gaussian	9.3	6	0.002
PC1×PC2+PC1×PC3	NB	10.1	5	0.002
PC3	NB	10.1	1	0.001
PC1×PC3+PC2×PC3	NB	10.6	5	<0.001
PC2+PC3	NB	11.3	2	<0.001
PC1×PC2+PC1×PC3+PC2×PC3	NB	11.8	6	<0.001
PC1×PC2×PC3	Gaussian	11.9	7	<0.001
PC1×PC2×PC3	NB	15	7	<0.001
PC1×PC2×PC3	Poisson	77411	7	<0.001
PC1×PC2+PC1×PC3+PC2×PC3	Poisson	78226	6	<0.001
PC1×PC2+PC2×PC3	Poisson	78877	5	<0.001
PC1×PC2+PC1×PC3	Poisson	82140	5	<0.001
PC1×PC2+PC3	Poisson	82143	4	<0.001
PC1×PC3+PC2×PC3	Poisson	83277	5	<0.001
PC1×PC2	Poisson	83716	3	<0.001
PC1+PC2×PC3	Poisson	84973	4	<0.001
PC1×PC3	Poisson	85012	3	<0.001
PC1+PC2+PC3	Poisson	87529	3	<0.001
PC1+PC3	Poisson	87595	2	<0.001
PC1+PC2	Poisson	88791	2	<0.001
PC1	Poisson	90222	1	<0.001
PC2×PC3	Poisson	90526	3	<0.001
PC2+PC3	Poisson	104675	2	<0.001
PC2	Poisson	104758	1	<0.001
PC3	Poisson	108133	1	<0.001
NULL	Poisson	109020	0	<0.001

Table S8. List of concurrent models to explain three potential benefits of differential allocation in reproductive investment by males of the spider *Paratrechalea ornata*: latency to oviposition, total number of eggs, and mean mass of fertilized eggs. The list includes all possible combinations of the predictor variables (experimental groups and number of flies added to the gift). We used three distributions of errors (Gaussian, Poisson, and gamma) to model the latency to oviposition and the total number of eggs, and two distributions of errors (Gaussian and gamma) to model the mean mass of fertilized eggs. We also show the number of slopes estimated (*k*) and the Akaike weight (Weight) of each model. The symbol × represents interaction between variables and the symbol + represents additive effects between variables.

Predictor variables	**Distribution**	**ΔAIC_c**	***k***	**Weight**
Latency to oviposition				
Experimental groups × Number of flies	Poisson	0.0	3	0.467
Experimental groups × Number of flies	Gamma	1.3	3	0.244
Experimental groups	Poisson	2.0	1	0.171
Experimental groups + Number of flies	Poisson	4.4	2	0.052
Experimental groups	Gaussian	5.6	1	0.028
Experimental groups × Number of flies	Gaussian	6.0	3	0.023
Experimental groups + Number of flies	Gaussian	8.2	2	0.008
Experimental groups	Gamma	9.5	1	0.004
Experimental groups + Number of flies	Gamma	9.8	2	0.004
Null	Gaussian	26.5	0	<0.001
Number of flies	Gaussian	28.8	1	<0.001
Null	Gamma	28.8	0	<0.001
Number of flies	Gamma	31.1	1	<0.001
Null	Poisson	39.3	0	<0.001
Number of flies	Poisson	41.3	1	<0.001
Total number of eggs				
Experimental groups + Number of flies	Gaussian	0.0	2	0.459
Experimental groups	Gaussian	1.0	1	0.276
Experimental groups × Number of flies	Gaussian	2.7	3	0.118

Experimental groups + Number of flies	Gamma	4.6	2	0.045
Experimental groups	Gamma	5.2	1	0.033
Null	Gaussian	5.5	0	0.030
Number of flies	Gaussian	6.5	1	0.017
Experimental groups × Number of flies	Gamma	7.3	3	0.012
Null	Gamma	8.6	0	0.006
Number of flies	Gamma	9.9	1	0.003
Experimental groups + Number of flies	Poisson	60.7	2	<0.001
Experimental groups × Number of flies	Poisson	62.9	3	<0.001
Experimental groups	Poisson	73.1	1	<0.001
Number of flies	Poisson	98.4	1	<0.001
Null	Poisson	103.0	0	<0.001
Mean mass of fertilized eggs				
Null	Gamma	0.0	0	0.586
Experimental groups	Gamma	2.4	1	0.176
Flies	Gamma	2.4	1	0.176
Experimental groups + Number of flies	Gamma	5.0	2	0.049
Experimental groups × Number of flies	Gamma	7.7	3	0.013
Null	Gaussian	238.3	0	<0.001
Experimental groups	Gaussian	240.7	1	<0.001
Number of flies	Gaussian	240.7	1	<0.001
Experimental groups + Number of flies	Gaussian	243.3	2	<0.001
Experimental groups × Number of flies	Gaussian	246.0	3	<0.001

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