

1 **Selection on sperm size in response to promiscuity and variation in female sperm storage organs**

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14

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21 **Abstract.** – Sperm cells are exceptionally morphologically diverse across taxa. However, morphology
22 can be quite uniform within species, particularly for species where females copulate with many
23 males per reproductive bout. Strong sexual selection in these promiscuous species is widely
24 hypothesized to reduce intraspecific sperm variation. Conversely, we hypothesize that intraspecific
25 sperm size variation may be maintained by high among-female variation in the size of sperm storage
26 organs, assuming that paternity success improves when sperm are compatible in size with the sperm
27 storage organ. We use individual-based simulations and an analytical model to evaluate how
28 selection on sperm size depends on promiscuity level and variation in sperm storage organ size
29 (hereafter, female preference variation). Simulated species with high promiscuity showed stabilizing
30 selection on sperm when female preference variation was low, and disruptive selection when female
31 preference variation was high, consistent with the analytical model results. With low promiscuity (2-3
32 mates per female), selection on sperm was stabilizing for all levels of female preference variation in
33 the simulations, contrasting with the analytical model. Promiscuity level, or mate sampling, thus has
34 a strong impact on the selection resulting from female preferences. Further, for species with low
35 promiscuity, disruptive selection on male traits will occur under more limited circumstances than
36 many previous models suggest. Variation in female sperm storage organs likely has strong
37 implications for intraspecific sperm variation in highly promiscuous species, but likely does not
38 explain differences in intraspecific sperm variation for less promiscuous taxa.

39 **Key words:** sperm morphology, sperm storage, mate choice, cryptic female choice, sperm length

40 **Introduction**

41 Sperm cells have exceptional morphological diversity across species (Pitnick *et al.*, 2009). This
42 diversity is partly driven by fertilization environment (internal vs. external; Kahrl *et al.*, 2021b), and is
43 also hypothesized to be driven by sexual selection, which can arise when a female copulates with
44 multiple males in a single reproductive bout. With such female promiscuity, sperm from different

45 males may compete to fertilize the egg(s) (Parker, 1970) and/or the female may exert cryptic choice
46 for particular sperm or male characteristics (Eberhard, 1996). How (and whether) such post-
47 copulatory sexual selection processes result in selection on sperm morphology requires more study
48 in most study systems (Lüpold & Pitnick, 2018), but two patterns are quite robust across studies.
49 Specifically, sperm cell morphology co-evolves with the morphology of female sperm storage organs
50 both in comparative studies (Dybas & Dybas 1981; Briskie & Montgomerie 1992; Higginson *et al.*
51 2012; reviewed in Lüpold & Pitnick 2018) and in experimental evolution studies (e.g., Hosken *et al.*
52 2001; Miller & Pitnick 2002). These studies suggest that sperm evolve to “fit” sperm storage organs
53 (and/or vice versa) in internally fertilizing species. In addition, among-male variation in sperm length
54 is lower in more promiscuous taxa, suggesting stronger selection for an optimal sperm phenotype
55 (sperm total length: birds, Calhim *et al.* 2007; Lifjeld *et al.* 2010; rodents, Varea-Sánchez *et al.* 2014;
56 and social insects, Fitzpatrick & Baer 2011; flagellum length: sharks, Rowley *et al.* 2019). In this paper,
57 we use simulations and an analytical model to explore how promiscuity level and among-female
58 variability in the sperm storage organs interact in driving selection on sperm.

59 Female sperm storage organs represent an important selective environment for sperm cells
60 in many species. Correlations between individual males’ proportion of sperm stored and proportion
61 of eggs fertilized can be high, reinforcing the idea that successful interaction with the female is
62 important (Bretman *et al.* 2009; Manier *et al.* 2010; Hemmings & Birkhead 2017; though note that
63 females do not necessarily use stored sperm from all males, e.g. Simmons & Beveridge 2010; Turnell
64 & Shaw 2015). Many factors may impact the successful storage of sperm, including motility as the
65 sperm enter the sperm storage organ (Mendonca *et al.*, 2019), mating order (Hellriegel & Bernasconi,
66 2000; Manier *et al.*, 2010; Hemmings & Birkhead, 2017), complex biochemical interactions among
67 ejaculates and with the female (den Boer *et al.*, 2010), and genetic compatibility of the male and
68 female (Simmons *et al.* 2006; Gasparini & Pilastro 2011; though genetic compatibility may be
69 assessed in the male rather than directly from the sperm, Løvlie *et al.* 2013). Here we focus on the
70 potential impact of morphological compatibility between the sperm cell and the sperm storage

71 organ, which is suggested by the co-evolution of morphology of sperm and sperm storage organs
72 across taxa (reviewed in Lüpold & Pitnick 2018). There are notable exceptions to the idea of
73 morphological compatibility; for example, García-González and Simmons (2007) find stronger
74 selection for short sperm in females with larger sperm storage organs in the dung beetle
75 *Onthophagus taurus*, so the mechanism we outline here will not be applicable in all systems.

76 In addition to being important selective environments for sperm, female sperm storage
77 organs likely vary among individuals, following several lines of evidence. First, since genetic variation
78 is a pre-requisite for evolution, the fact that sperm storage organ morphology evolves suggests that
79 it varies (Jennions & Petrie, 1997). Genetic variation in sperm storage organ morphology has also
80 been directly documented (Miller & Pitnick, 2002; Lüpold *et al.*, 2013). In addition, environmental
81 and social conditions during development can affect sperm storage organ morphology (Amitin &
82 Pitnick, 2007; Berger *et al.*, 2011; Farrow *et al.*, 2022). Within-female variation is also known, for
83 example, in birds, where each female has hundreds of sperm storage tubules, whose lengths vary in
84 a gradient across the utero-vaginal junction (where these structures occur), and with stage of the
85 egg-laying cycle (Briskie, 1996).

86 Thus we hypothesize that females vary in their sperm storage organ morphology, and that
87 the morphological fit between these organs and sperm cells is a mechanism of cryptic female choice,
88 because it biases storage success (and therefore fertilization success) towards well-fitted sperm. We
89 model a scenario where all females have the same preference function, whereby the sperm that best
90 fit their sperm storage organs is more likely to fertilize their eggs. However, females' preferences
91 (i.e., the sperm trait values that best fit individual females) vary because the preference function is
92 self-referential against a variable morphological trait. This hypothesis is supported by Hemmings *et*
93 *al.* (2016), who allowed females to copulate with one male and then compared the morphology of
94 ejaculated cells and of sperm cells that reached the ovum after sperm storage. Re-analysis of their
95 data (see Supplemental file) indicates that the mean sperm length at the egg differed significantly

96 from the mean ejaculated sperm in 10 of 27 females (Table S1). Sperm at the egg was longer than
97 ejaculated sperm for half the females and shorter in the other half, consistent with variable female
98 preferences for sperm size. Furthermore, under this hypothesis, we can expect that males may have
99 different relative fertilization success when they copulate with different females. Several studies do
100 indeed find that the combination of male and female identities (or genetic lines) has a strong impact
101 on fertilization success (Wilson *et al.*, 1997; Clark, 2002; Birkhead *et al.*, 2004; Bjork *et al.*, 2007;
102 Simmons *et al.*, 2014; Reinhart *et al.*, 2015) (although we note that a combinatorial effect of male
103 and female may also arise due to other processes, for example, variation in copulation duration, Eady
104 and Brown 2017, or sperm swimming speed, Urbach *et al.* 2005; Cramer *et al.* 2014; Cramer *et al.*
105 2016).

106 Because we view the fit of sperm and sperm storage organ as a mechanism of cryptic female
107 choice (Lüpold & Pitnick, 2018), we can expect some parallels between this process and mate choice.
108 However, to our knowledge, no theoretical work on mate choice models the conditions most
109 relevant for sperm-female interactions. Specifically, most mate choice models assume that females
110 copulate with a single male in the population, while empirical data show that females often copulate
111 with multiple males, who then share paternity of their offspring (e.g., Gage, 1994; Simmons *et al.*,
112 2007; Simmons & Beveridge, 2010; Turnell & Shaw, 2015; Brouwer & Griffith, 2019; Kahrl *et al.*,
113 2021a). In addition, we assume that females copulate with fewer males than they assess during mate
114 choice, implying that females sample the sperm of relatively few males. The number of sampled
115 partners is known to impact resulting selection strength (Janetos, 1980; Gomulkiewicz, 1991; Muniz
116 & Machado, 2018). Finally, in species where eggs are ovulated in batches, female sperm storage
117 organs have already gathered all the sperm cells that potentially can fertilize the eggs, making cryptic
118 female choice best represented by a simultaneous assessment model. Under a simultaneous
119 assessment strategy, the female evaluates all individuals in a set of potential males before choosing
120 among them. Simultaneous assessment strategies can give different results from other assessment
121 strategies (Janetos, 1980; Jennions & Petrie, 1997; Muniz & Machado, 2018), and to the best of our

122 knowledge, continuous variation in female preferences has not been modeled with simultaneous
123 assessment with a reasonable number of copulation partners (for an internally fertilizing species).
124 See Millan *et al.* (2020) for relevant work with a different assessment model, and Van Doorn *et al.*,
125 (2001) and van Doorn & Weissing (2002) for models relevant for broad-cast spawners with high mate
126 sampling. Further work is thus needed to understand how variation in female sperm storage organs
127 impacts selection on sperm.

128 Here, we use individual-based simulations and an analytical model to investigate how
129 among-female variation in sperm storage organs affects the resulting selective pressure on sperm,
130 and we assess whether this relationship depends on the level of female promiscuity, ie., number of
131 copulation partners. We predict that selection will be stronger with higher promiscuity (Janetos,
132 1980; Gomulkiewicz, 1991; Muniz & Machado, 2018). We further hypothesize that where female
133 preference is less variable than sperm, there will be stronger stabilizing selection on sperm as female
134 trait variation is further reduced. Conversely, where female preference is more variable than sperm,
135 we predict that there will be stronger disruptive selection on sperm as variation in the female trait
136 increases (Jennions & Petrie, 2000; Van Doorn *et al.*, 2001; van Doorn & Weissing, 2002; Weissing *et*
137 *al.*, 2011).

138

139 **Methods**

140 *Assumptions*

141 We assume a closed population with an equal sex ratio, where copulations occur randomly with
142 respect to the sperm and preference traits. All eggs are fertilized, so that preferences are selectively
143 neutral for females. This assumption is similar to the “last-chance” option of Janetos (1980), whereby
144 females accept any male rather than not mate.

145 *Simulation procedure*

146 For each iteration of the simulation, we created a population of 200 individuals of each sex, breeding
147 for one season. Each female produced one set of 25 offspring. This value was chosen to enable us to
148 exploring a relatively high number of copulation partners, while still allowing a substantial probability
149 for each partner to sire offspring. Males are assigned a sperm trait from a normal distribution with
150 mean 0 and SD = 1. Females are assigned a preference (i.e., sperm storage organ size) on the same
151 scale, such that the fit between sperm and preference is best when the trait values are equal. We
152 varied population-level SD in female preference (values of 0.5, 1, 1.5, and 2; comparable to the
153 variation explored by Millan *et al.* 2020), but, for simplicity, the population mean preference was
154 always equal to the sperm preference mean.

155 All individuals copulated with members of the opposite sex 1, 2, 3, 5, 10, or 25 times.
156 Detailed information on number of copulation partners is poorly known for many species, and is
157 often inferred from genotyping stored sperm in the female or determining paternity of offspring.
158 Empirical data thus provides a minimum estimate of number of individual partners (Cramer *et al.*,
159 2020a). For many species, an average number of copulation partners less than 5 appears realistic
160 (Gage, 1994; Brommer *et al.*, 2007, 2010; Simmons *et al.*, 2007; Simmons & Beveridge, 2010; Turnell
161 & Shaw, 2015; Cramer *et al.*, 2020a; Kahrl *et al.*, 2021a), though in eusocial insects the average can
162 be over 50 (Tarpay *et al.*, 2004). The values we chose to investigate were also informed by the
163 expectation that selection strength should asymptote with > about 10 copulation partners
164 (Gomulkiewicz, 1991; Muniz & Machado, 2018). We include 1 copulation partner to confirm the
165 expectation of no selection on sperm under this condition. Copulation partners were assigned
166 randomly by shuffling the list of individual identities for each copulation event. This could result in
167 a pair of individuals copulating with each other more than once. Since that presumably occurs in
168 nature and represents a limited proportion of copulations, we do not control for such repeated
169 copulations in statistical analysis.

170 Following copulation, the fertilizing sperm for each egg was determined using R's *sample*
171 function. Weighting the *sample* function requires positive, non-zero values; it then sums all
172 individuals' weight values, and the probability that an individual is drawn is proportional to its
173 contribution to the sum of the weight values across all individuals. Thus, a male's success depends on
174 his relative weight (i.e., fit) for the female preference compared to the other copulation partners, not
175 his absolute fit. To calculate absolute fit, we modeled the fit quality as a Gaussian function which
176 attains its maximum when the male's trait value, y , matches the female's preference, x . The
177 parameter σ_U , akin to standard deviation, controls the strength of the preference (sensu Millan *et*
178 *al.*, 2020). For the sake of simplicity, we use a value of $\sigma_U=1$ in all simulations. We therefore
179 calculated the fit score between the sperm size, y , and the female preference, x , as:

$$180 \quad e^{\frac{-(x-y)^2}{2\sigma_U^2}} \quad \text{Eq 1}$$

181 This equation represents the preference function used by all females. After calculating the fit for all
182 copulation partners, we assigned fertilization by drawing male identities from a list of the individual
183 female's copulation partners, weighted according to the fit scores.

184 After counting all offspring sired for each male, the selection gradient on the sperm trait was
185 calculated. To do so, reproductive success was standardized by dividing by the population mean
186 reproductive success. Sperm trait values were standardized to have a population mean of 0 and
187 standard deviation of 1 (following Lande & Arnold, 1983). Standardized reproductive success was
188 then regressed on the standardized sperm trait, including both a linear and a quadratic term (Lande
189 & Arnold, 1983). Negative values of the quadratic term indicate stabilizing selection, and positive
190 values indicate disruptive selection. We extracted the quadratic selection gradient parameter from
191 each replicate population.

192 After performing 1000 replicate populations with the same set of conditions, we compared
193 how the quadratic selection gradient changed with the treatments (variation in female preference

194 and number of copulation partners). To facilitate interpretation, we treat each predictor as
195 categorical rather than continuous. Following the logic outlined in White *et al.* (2014), we rely on
196 effect size estimates rather than p-values in interpreting our results (since simulations can make
197 sample size be arbitrarily high and p-values correspondingly low). Following Richardson (2011), we
198 use η^2 as the effect size estimate, with values of 0.1, 0.25, and 0.5 considered small, medium, and
199 large, respectively. These were calculated via sjstats (Lüdecke, 2021). We further directly calculated
200 the 95% confidence limits on each simulation condition, as the 2.5% and 97.5% quantiles of the
201 observed values.

202 All simulations and statistics were performed in R using base functions and the tidyverse
203 package (Wickham *et al.*, 2019). We ran a modified set of simulations to assess the impact of sharing
204 paternity (Table S2) and of having larger clutch size relative to copulation partner count in an open
205 population (Table S3). Overall patterns were highly similar.

206 *Analytical model*

207 Among-female variation in female preference had strong impacts on the shape of selection (see
208 Results), which depended also on the number of copulation partners. To better understand when
209 disruptive or stabilizing selection should be expected when the female could sample all males, we
210 used an analytical model that parallels the simulation. Similar to the simulations, among-female
211 variation in preference is modeled as normally distributed with mean of 0 and standard deviation σ_F .
212 Among-male variation in sperm is modeled as normally distributed with mean 0 and standard
213 deviation σ_M . The probability density function of the female preference across all females is then

$$214 \quad F(x) = \frac{1}{\sqrt{2\pi}\sigma_F} e^{-x^2/2\sigma_F^2} \quad (\text{Eq. 2})$$

215 and the probability density function for the sperm trait across all males is

$$216 \quad M(y) = \frac{1}{\sqrt{2\pi}\sigma_M} e^{-y^2/2\sigma_M^2} \quad (\text{Eq. 3})$$

217 Denoting the preference function as $U(x,y)$, the probability distribution function of fertilization
218 success for all males with trait value y , given a female with preference x , can be expressed as

$$219 \quad S(y | X = x) = \frac{M(y)U(x,y)}{\int_{-\infty}^{\infty} M(y)U(x,y) dy} = \frac{M(y)U(x,y)}{V(x)} \quad (\text{Eq. 4})$$

220

221 Intuitively, the denominator, $V(x)$, can be thought of as the total of the female's fit scores across all
 222 males in the population, and the numerator expresses the contribution of males with trait value y to
 223 the total of the female's fit scores. This is analogous to the *sample* function if all males were
 224 sampled.

225 Fertilization success for all males with trait value y can be calculated as the integral of their
 226 fertilization success across all females:

$$227 \quad S(y) = \int_{-\infty}^{\infty} F(x)S(y | X = x) dx = M(y) \int_{-\infty}^{\infty} \frac{F(x)U(x,y)}{V(x)} dx = M(y)R(y) \quad (\text{Eq. 5})$$

228 where

$$229 \quad R(y) = \int_{-\infty}^{\infty} \frac{F(x)U(x,y)}{V(x)} dx = \frac{S(y)}{M(y)} \quad (\text{Eq. 6})$$

230 gives the fertilization success of males with value y , relative to their representation in the population.

231 In the simulations, we assumed that $U(x,y)$ was given by Eq. 1. Under this condition, we can
 232 explicitly calculate the function $R(y)$. By substituting Eq 1- 3 into the more general form equations 4-
 233 6, we have

$$234 \quad V(x) = \frac{1}{\sqrt{2\pi}\sqrt{\sigma_M^2 + \sigma_U^2}} e^{-\frac{x^2}{2(\sigma_M^2 + \sigma_U^2)}} \quad (\text{Eq 7})$$

$$235 \quad S(y | X = x) = \frac{\sqrt{\frac{\sigma_M^2 + \sigma_U^2}{2\pi\sigma_M^2\sigma_U^2}}}{\sqrt{\frac{\sigma_M^2 + \sigma_U^2}{2\pi\sigma_M^2\sigma_U^2}}} e^{\frac{x^2}{2(\sigma_M^2 + \sigma_U^2)} - \frac{y^2}{2\sigma_M^2} - \frac{(x-y)^2}{2\sigma_U^2}} \quad (\text{Eq. 8})$$

$$236 \quad R(y) = \frac{\sigma_M \sqrt{\frac{1}{\sigma_M^2} + \frac{1}{\sigma_U^2}}}{\sigma_F \sqrt{\frac{1}{\sigma_F^2} - \frac{1}{\sigma_M^2 + \sigma_U^2} + \frac{1}{\sigma_U^2}}} e^{-\frac{y^2(-\sigma_F^2 + \sigma_M^2 + \sigma_U^2)}{2(\sigma_F^2\sigma_M^2 + \sigma_M^2\sigma_U^2 + \sigma_U^4)}} \quad (\text{Eq. 9})$$

237 We can re-write Eq. 9 as follows:

$$238 \quad = \frac{\sigma_M^2 + \sigma_U^2}{\sqrt{\sigma_F^2\sigma_M^2 + \sigma_M^2\sigma_U^2 + \sigma_U^4}} e^{-\frac{y^2(-\sigma_F^2 + \sigma_M^2 + \sigma_U^2)}{2(\sigma_F^2\sigma_M^2 + \sigma_M^2\sigma_U^2 + \sigma_U^4)}} \quad (\text{Eq 10})$$

$$239 \quad = \frac{\sigma_M^2 + \sigma_U^2}{\sqrt{\sigma_M^2(\sigma_F^2 - \sigma_M^2 - \sigma_U^2) + (\sigma_M^2 + \sigma_U^2)^2}} e^{-\frac{y^2(-\sigma_F^2 + \sigma_M^2 + \sigma_U^2)}{2(\sigma_F^2\sigma_M^2 + \sigma_M^2\sigma_U^2 + \sigma_U^4)}} \quad (\text{Eq 11})$$

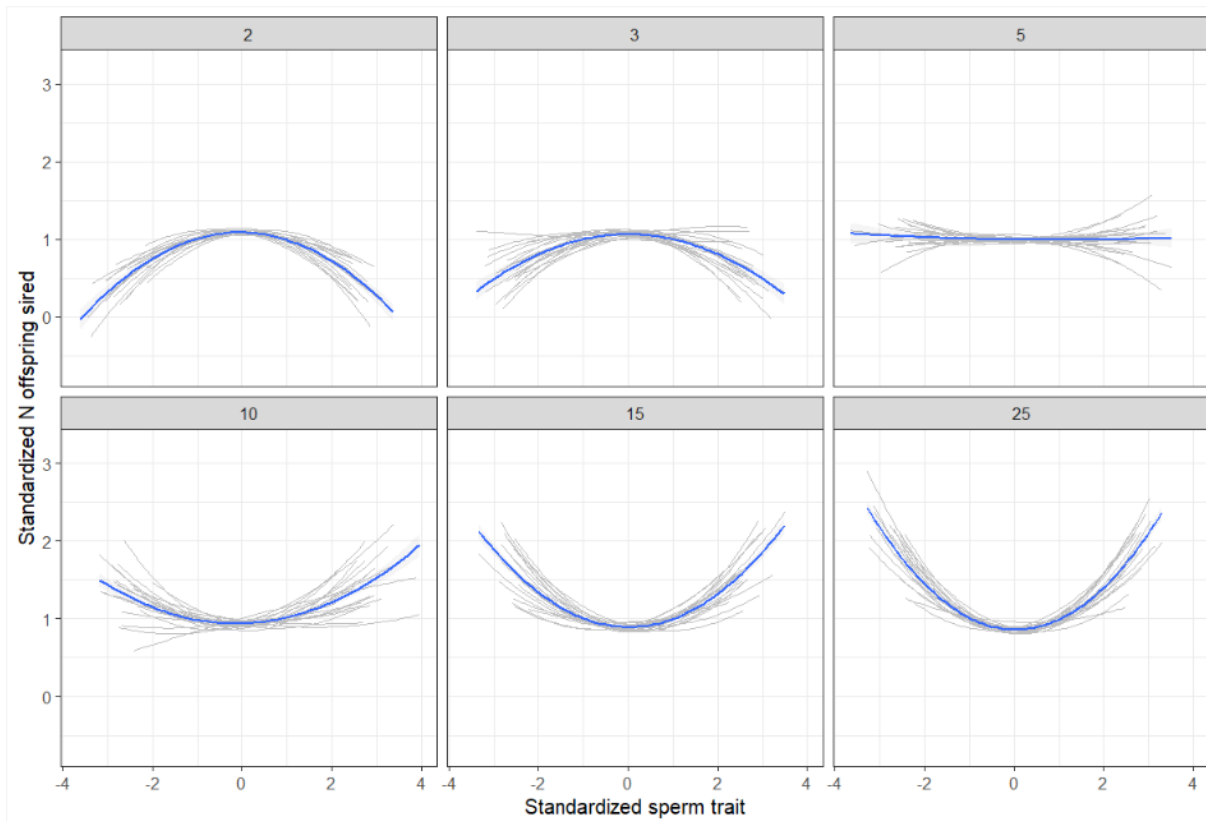
240 This model is similar to models used by several authors (e.g., Lande, 1981; Dieckmann &
241 Doebeli, 1999), but those authors did not explicitly describe conditions predicting stabilizing and
242 disruptive selection.

243

244 Results

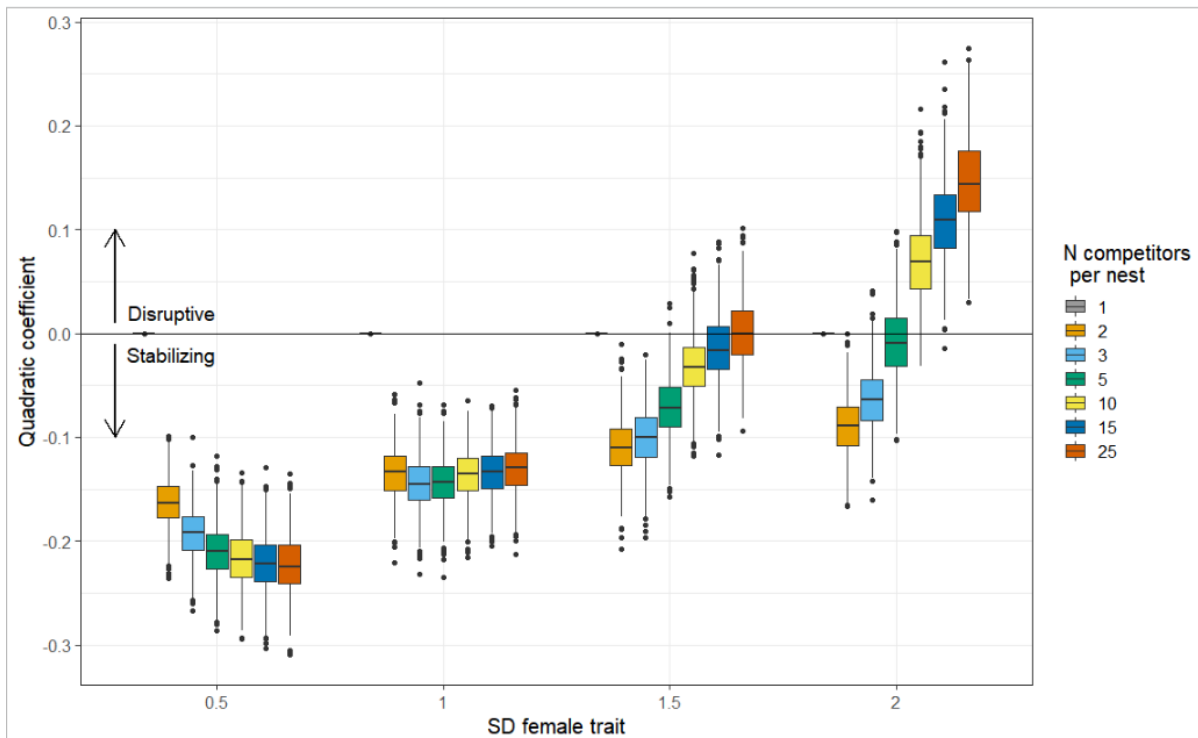
245 Simulations

246 The value of the quadratic selection gradient term depended on among-female variation in
247 preference ($F_{3,27972} = 74073$, $\eta^2 = 0.54$), number of copulation partners ($F_{6,27972} = 11557$, $\eta^2 = 0.17$), and
248 the interaction between the two variables ($F_{18,27972} = 5169$, $\eta^2 = 0.23$; Fig. 1, Fig. 2). Quadratic
249 selection estimates changed dramatically with variation in female preference when the number of
250 copulation partners was high. However, when females copulated only twice, stabilizing selection was
251 only slightly weakened by higher variation in female preference (Table 1). There was no selection on
252 sperm when the female copulated with only one male (Table 1, Fig 2). Patterns were similar in the
253 additional simulation conditions tested (e.g., larger number of offspring per female; see
254 supplementary information).



255

256 **Figure 1.** Examples of selection gradients on the sperm trait for 20 randomly selected populations
257 with the SD for among-female variation in preference = 2. Each panel shows a different level of
258 number of copulation partners. The male trait had a standard deviation of 1 in all treatments. Grey
259 lines show 20 randomly selected individual populations and the blue line shows the overall pattern.



260

261 **Figure 2.** Values of the quadratic selection gradient for all simulation conditions. Colors indicate the
 262 number of copulation partners. A value of 0 (heavy horizontal line) indicates no quadratic selection;
 263 negative values indicate stabilizing selection; and positive values indicate disruptive selection. Results
 264 include 1000 replicates per simulation condition.

265

Table 1. Median and 95% quantile limits on the quadratic term from selection gradients

N copulation partners	Among-female SD in preference			
	0.5	1	1.5	2
1	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)
2	-0.16 (-0.21, -0.12)	-0.13 (-0.18, -0.09)	-0.11 (-0.16, -0.06)	-0.09 (-0.14, -0.03)
3	-0.19 (-0.24, -0.14)	-0.15 (-0.20, -0.10)	-0.10 (-0.15, -0.05)	-0.06 (-0.12, 0.00)
5	-0.21 (-0.26, -0.16)	-0.14 (-0.19, -0.10)	-0.07 (-0.13, -0.02)	-0.01 (-0.07, 0.06)
10	-0.22 (-0.27, -0.17)	-0.14 (-0.18, -0.09)	-0.03 (-0.09, 0.03)	0.07 (-0.00, 0.15)
15	-0.22 (-0.28, -0.17)	-0.13 (-0.18, -0.09)	-0.02 (-0.07, 0.05)	0.11 (0.03, 0.19)
25	-0.22 (-0.28, -0.17)	-0.13 (-0.18, -0.09)	-0.00 (-0.05, 0.06)	0.14 (0.07, 0.23)

266

267 With female preference SD 0.5, there was stabilizing selection on sperm (negative quadratic selection
 268 coefficients, with 95% quantiles not overlapping 0), and the median strength of stabilizing selection

269 increased with the number of copulation partners between 2 and 5, but did not increase between 5
270 and 25 (Table 1, Figure 2). With female preference SD 1, i.e., equal to male trait SD, selection on
271 sperm was again stabilizing, but here there was little variation depending on number of copulation
272 partners. When the SD of the female preference was 1.5, selection was stabilizing with 2, 3, or 5
273 copulation partners, and there was no selection with 10, 15 or 25 copulation partners (95% quantiles
274 include 0). When the SD of the female preference was 2, selection was stabilizing with 2 copulation
275 partners; there was no selection with 3, 5, or 10 partners, and there was disruptive selection with 15
276 or 25 partners (95% quantiles not overlapping 0, positive quadratic selection coefficients). The
277 impact of increasing the number of copulation partners appeared to asymptote here, as the
278 difference in median disruptive selection between 5 and 15 partners was 0.1, while the difference in
279 median disruptive selection between 15 and 25 partners was only 0.03 (Table 1).

280 *Analytical model*

281 From Eq 11, we see that the shape and intercept of $R(y)$ is determined by the expression
282 $-\sigma_F^2 + \sigma_M^2 + \sigma_U^2$. In particular, if $\sigma_F^2 < \sigma_M^2 + \sigma_U^2$, $R(y)$ is bell-shaped and has $R(0) > 1$ as its
283 maximum value, indicating that males with average trait values gain greater fertilization success than
284 would be expected given their frequency in the population. This implies stabilizing selection. If $\sigma_F^2 >$
285 $\sigma_M^2 + \sigma_U^2$, $R(y)$ is U-shaped and $R(0) < 1$, indicating that males with average trait values gain less
286 fertilization success than expected and implying disruptive selection. No selection is expected where
287 $\sigma_F^2 = \sigma_M^2 + \sigma_U^2$ as this results in the constant function $R(y) = 1$. We evaluated whether this result
288 agreed with model results by arbitrarily choosing several sets of values for the three variances that
289 should give no quadratic selection (see supplementary materials, Table S4).

290

291 **Discussion**

292 Stabilizing selection on the sperm trait when there is less variation in female preference than
293 in sperm is intuitive: all females share a preference for sperm with a phenotype close to the male

294 population mean. Disruptive selection when female preference is more variable than sperm is
295 similarly intuitive: many females have preferences outside the sperm trait distribution, thus the most
296 extreme males in the population obtain high fertilization success after copulating with a matching
297 female (Van Doorn *et al.*, 2001; van Doorn & Weissing, 2002; Millan *et al.*, 2020). Our analytical
298 model indicates that the change from stabilizing to disruptive selection should occur when the
299 among-female preference variance is greater than the sum of the among-male variance in sperm
300 traits and the variance parameter in the female preference function (Eq 1, which we do not vary in
301 this simulation). Our simulation results, however, show stabilizing selection under more conditions
302 than expected, also compared to previous models where females sampled a large subset of males
303 (Millan *et al.*, 2020). Specifically, when the number of copulation partners is low, stabilizing selection
304 can occur even with high among-female variation in preference. We suggest that this stabilizing
305 selection occurs because males with relatively extreme sperm values are relatively unlikely to
306 copulate with females with a matching preference, and their fertilization advantage when they do
307 achieve these matching copulations is insufficient to offset the rarity of the copulations. The
308 importance of sampling number is also evident in the empirical literature, where mating preferences
309 are expressed more strongly in studies where individuals can choose among two mating options,
310 compared to studies where individuals have a single option and can mate or not (Dougherty &
311 Shuker, 2015).

312 *Implications for sperm evolution*

313 This study shows that variation in cryptic female preferences and variation in number of
314 copulation partners each can have a strong impact on the strength, or even shape, of selection on
315 sperm morphology. Perhaps surprisingly, there are conditions where number of copulation partners
316 does not impact the strength of selection (i.e., where sperm and preference variation are equal), and
317 there are conditions where no selection on sperm is expected even when there are a large number of
318 copulations. It is difficult to know which combination of variables is likely to be most biologically

319 relevant, since variation in female genital morphology is under-studied relative to male genital traits
320 (Ah-King *et al.*, 2014), and copulation behavior is difficult to observe in the wild. However, we can
321 draw some generalizations. With low levels of promiscuity (2-3 copulations), selection is expected to
322 be stabilizing, and it is similar across levels of variation in female preference. In contrast, with high
323 numbers of copulations (10-25), selection on sperm is stabilizing, null, or disruptive, depending on
324 the level of variation in the female preference.

325 *Low to moderate promiscuity systems.*-For many species, we suspect that the number of
326 copulation partners is low enough that stabilizing selection is broadly expected. Inferences of
327 copulation rate from paternity patterns suggest in socially monogamous passerine birds that females
328 on average copulate with fewer than 3 males (Brommer *et al.*, 2007, 2010; Cramer *et al.*, 2020a).
329 Genotyping remnants of stored sperm in the female reproductive tract indicates that mean number
330 of mates is between 2 and 6 for several invertebrates (including butterflies, crickets, and beetles;
331 Gage, 1994; Simmons *et al.*, 2007; Simmons & Beveridge, 2010; Turnell & Shaw, 2015). In such
332 species, assuming heritability of sperm morphology (reviewed by Edme *et al.*, 2019), the sperm-
333 female fit function modeled here would then often be expected to erode variation in sperm
334 morphology over time. Why, then, are sperm cells still variable, and why does the level of variability
335 correlate with promiscuity rates?

336 Stabilizing selection imposed by the need to fit the female's sperm storage organs may be
337 countered by diverse other selective pressures. For example, different sperm morphology may
338 confer a fertilization advantage depending on whether the sperm are the first-inseminated (ie., in a
339 defensive position relative to competitors) or are later-inseminated (in an offensive role) (Clark *et al.*,
340 1995; Calhim *et al.*, 2011). The most advantageous sperm morphology may also depend on the
341 phenotype of the male himself (Ålund *et al.*, 2018). Sperm morphology may correlate with other
342 ejaculate traits that are also under selection, such as sperm number and sperm swimming speed,
343 resulting in complex multivariate selection patterns (Snook, 2005; Fitzpatrick *et al.*, 2012; Lüpold *et*

344 *al.*, 2012b). Sperm morphology may correlate with pre-copulatory traits under selection (e.g.
345 Simmons *et al.*, 2017), creating indirect selection on sperm morphology (Cramer, 2021). Finally,
346 selection for genetically compatible sperm (Simmons *et al.*, 2006; Fossøy *et al.*, 2008; Bretman *et al.*,
347 2009; Gasparini & Pilastro, 2011; Rekdal *et al.*, 2019) is expected to be independent of sperm
348 morphology, since it depends on the genotypes of the male and female. As these examples show, it is
349 most appropriate to consider the sperm-sperm storage organ fit as one component of a complex
350 selective landscape.

351 At an ontogenetic level, variation in sperm morphology may arise due to various
352 environmental factors, including but not limited to age (e.g., Cramer *et al.*, 2020b), seasonal changes
353 in sperm morphology (Lüpold *et al.*, 2012a; Cramer *et al.*, 2013; Edme *et al.*, 2019), larval rearing
354 conditions and timing (Vermeulen *et al.*, 2009), differences in the social environment as an adult
355 (Immler *et al.*, 2010; Rojas Mora *et al.*, 2018), and condition-dependence of sperm phenotypes
356 (which has been documented in some studies but is not generally expected; Macartney *et al.*, 2019).

357 Persistence of variation in sperm morphology may also depend on the genetic and genomic
358 underpinnings of the trait. In zebra finches, for example, a genomic inversion on the sex
359 chromosome allows many loci to act as a super gene influencing sperm morphology (Kim *et al.*,
360 2017), and this supergene shows heterozygote advantage that could sustain genetic variation over
361 time (Knief *et al.*, 2017). Maternal genetic effects on sperm traits have been found in several studies
362 (e.g., Ward, 2000; Morrow & Gage, 2001; Froman *et al.*, 2002). If the genes causing these maternal
363 effects are X-linked or on the mitochondria, they may be protected to some extent from selection
364 acting on the sperm phenotype (Gemmell *et al.*, 2004). Genetic underpinnings of sperm morphology
365 are poorly known for most species, although substantial heritability of sperm morphology indicates
366 strong genetic effects (reviewed in Edme *et al.*, 2019). However, heritability is less directly relevant to
367 how quickly a trait is expected to evolve in response to selection than is evolvability (Hansen *et al.*,
368 2011). Evolvability for sperm morphological traits is comparable to values for other linear trait

369 measurements (median 0.1% for linear traits in Hansen *et al.*, 2011; range for total sperm length
370 0.02% - 0.26% in Edme *et al.*, 2019, recalculated from CV_A to I_A for comparability to Hansen *et al.*,
371 2011).

372 The above examples may help to explain why sperm remain variable despite stabilizing
373 selection, but they do not immediately explain the among-species correlation between promiscuity
374 level and intraspecific sperm morphological variation. Here, at least for social monogamy with extra-
375 pair paternity, we argue that the among-species pattern is likely driven by the proportion of non-
376 promiscuous females in the population. Most socially monogamous species with extra-pair paternity
377 probably include some females that copulate only with their social mate, for example due to
378 successful mate-guarding by that male (e.g., Chuang-Dobbs *et al.*, 2001; Brylawski & Whittingham,
379 2004; Johnsen *et al.*, 2008). When a female copulates with only one male and his sperm fertilize all
380 her eggs, she exerts no selection on his sperm morphology. If social mates also copulate much more
381 frequently than extra-pair mates, such that the social male has many more sperm in competition
382 with extra-pair males, selection in the morphology of the social male's sperm may also be weakened.
383 If we assume that monogamous females exert no selection on sperm and females copulating with 2-
384 3 males exert stabilizing selection (as indicated in the model), then the total strength of stabilizing
385 selection should depend on the proportion of monogamous versus promiscuous females. Assuming
386 that the proportion of monogamous females is lower, or that social males contribute a smaller
387 proportion of sperm, in species with higher extra-pair paternity rates, we then can expect stronger
388 overall stabilizing selection on sperm in those species. Strong stabilizing selection due to high
389 proportions of females obtaining 2-3 copulation partners may result in faster evolution of sperm in
390 these lineages (as seen in Rowe *et al.*, 2015), if mean preferences become different from mean
391 sperm traits, for example due to genetic drift.

392 *High promiscuity systems.*- For some groups, for example some eusocial insects, the number
393 of copulation partners can be quite high (Tarpay *et al.*, 2004). Here, we expect the shape of selection

394 to depend strongly on the degree of variation in female preference, ranging from stabilizing to
395 disruptive selection. Assuming similar levels of variation in female preference within these taxa,
396 increases in the number of mating partners from an already-high baseline may not result in strong
397 increases in selection strength, due to asymptotic effects. Perhaps in contrast to these expectations,
398 Fitzpatrick & Baer (2011) found a between-species correlation between intraspecific variation in
399 sperm morphology and promiscuity level in eusocial insects, even when excluding monogamous
400 species. However, only five species in one genus (*Apis*) had more than 10 copulation partners in their
401 dataset, limiting the power to test whether promiscuity level correlates with sperm morphological
402 variation only within highly promiscuous species. Our simulation results suggest that selection on
403 sperm will be highly dependent on the degree of variation in the female sperm storage organs in
404 such taxa, although the myriad other factors influencing sperm variation discussed above may also
405 be at play in high-promiscuity systems. The combination of high promiscuity and high variation in
406 female sperm storage organs creates an expectation of disruptive sexual selection, which in turn can
407 play a role in the splitting of lineages to form separate species (Lande, 1981; van Doorn & Weissing,
408 2002; Weissing *et al.*, 2011; see also Van Doorn *et al.*, 2001; Howard *et al.*, 2009).

409 *Implications for previous work on mate choice*

410 Our observation that limited mate sampling causes stabilizing selection even with substantial
411 among-female preference variation has important implications for interpreting previous models of
412 sympatric speciation. Previous models have highlighted a broad female preference distribution as a
413 key element in generating disruptive selection on male traits as one step that can lead to sympatric
414 speciation (Higashi *et al.*, 1999; Van Doorn *et al.*, 2001; van Doorn & Weissing, 2002; Weissing *et al.*,
415 2011). Our results suggest that disruptive selection will occur under more limited circumstances than
416 was previously appreciated, as females generally are expected to be somewhat limited in the number
417 of males they can sample (Jennions & Petrie, 1997). We thus support Servedio & Boughman (2017)'s
418 assertion that novel insights may be obtained in the sympatric speciation literature by further

419 exploring closed-ended preference functions and limited female searches, similar to what we have
420 simulated here.

421 As expected from previous models (Janetos, 1980; Gomulkiewicz, 1991; Muniz & Machado,
422 2018), increasing the number of partners generally increased the strength of selection, and the
423 relationships was asymptotic, with the asymptote reached at a lower value in the conditions with
424 stabilizing selection than the conditions with disruptive selection. We further find that selection is
425 generally weaker when paternity is shared within each batch of offspring, compared to when the
426 best-fit male sires all offspring (Table S2). Models of mate choice should therefore use realistic values
427 for number of males sampled and number of males succeeding (in copulating or fertilizing) to obtain
428 the most biologically relevant results.

429 *Conclusions.*- Despite broad interest in sperm morphology, relatively few studies have
430 evaluated selection on sperm morphology in the wild (Lüpold & Pitnick, 2018), and even fewer have
431 evaluated the presence and effect of variation in female sperm storage organs. Under our model
432 where the sperm storage organs bias paternity success towards sperm of a similar size, the level of
433 variation in the female sperm storage organs determines whether selection on sperm is stabilizing or
434 disruptive for highly promiscuous species, whereas selection is stabilizing for species with only 2-3
435 copulations per female.

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