

1 Mate choice intensifies motor signalling in *Drosophila*

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13 Mate choice has the potential to act on the evolution of motor performance via its direct
14 influence on motor sexual signals. However, studies demonstrating this are rare. Here, we
15 perform an in-depth analysis of *Drosophila pseudoobscura* courtship song rate, a motor signal
16 under mate choice in this species, and analyse the response of this signal to sexual selection
17 manipulation using experimental evolution. We show that manipulating the opportunity for
18 sexual selection led to changes in song production rate and singing endurance, with males
19 from the polyandrous populations producing faster song rates over longer time periods than
20 males from monogamous populations. We also show that song rate is correlated with
21 estimates of overall courtship vigour. Our results suggest that the action of mate choice on a
22 motor signal has affected male motor performance displayed during courtship. We consider
23 potential selective benefits associated with changes in motor performance, including
24 condition-dependent signalling, and discuss the implications of these results for the study of
25 motor signals under sexual selection.

26 Keywords: courtship song, *Drosophila pseudoobscura*, experimental evolution, interpulse
27 interval, mate choice, motor performance, sexual selection

28 Motor performance, or *vigour* (Darwin, 1859; Darwin, 1871), is the ability of an individual to
29 repeatedly perform energetically-costly motor acts (Byers, Hebets, & Podos, 2010). As this
30 ability often has drastic fitness consequences (e.g. determining the ability to escape
31 predators, forage or capture preys), its evolution is often driven by natural selection (Byers et
32 al., 2010; Irschick & Garland, 2001). Yet, sexual selection also has the potential to affect the
33 evolution of motor performance, when mate choice or mate competition targets motor signals
34 (i.e. signals involving any kind of sustained muscular activity such as threat displays,
35 courtship displays such as dances, or acoustic and vibratory signals; Bonduriansky, 2011;
36 Husak & Fox, 2008). Because such signals typically require high-speed muscle contractions
37 that are energetically-costly to produce (Lailvaux & Irschick, 2006), they have the potential to
38 be reliable indicators of a signaller's overall motor capacities, and thus of the individual's
39 current condition (Byers et al., 2010; Clark, 2012; Lailvaux & Irschick, 2006; Oufiero &
40 Garland, 2007). Hence, by directly influencing the evolution of a given motor signal, sexual
41 selection may lead to a correlated increase of the overall motor capacities of signallers (Byers
42 et al., 2010; Clark, 2012; Mowles & Ord, 2012; Ryan, 1988).

43 Although potential links between motor sexual signals and motor performance have received
44 significant attention in the recent literature (Byers et al., 2010; Irschick, Meyers, Husak, & Le
45 Gaillard, 2008; Mowles & Ord, 2012), their investigation has so far been restricted to two
46 issues; the link between motor signals involved in mate competition and overall motor
47 performance (Andersson, 1996; Byers et al., 2010; Lailvaux & Irschick, 2006) and the link
48 between motor signals involved in mate choice and non-motor measures of mate condition
49 (e.g. offspring production, growth rate, etc.; Irschick et al., 2008). For example, a link between
50 male dominance display and running endurance has been shown in *Anolis* lizards (Perry,
51 Levering, Girard, & Garland, 2004), and a correlation between male song structure and

52 offspring survival was found in the zebra finch *Taeniopygia guttata* (Woodgate, Mariette, &
53 Bennett, 2012). Yet, mate choice for motor signals may also affect the evolution of overall
54 mate motor performance (Byers et al., 2010; Clark, 2012; Mowles & Ord, 2012; Ryan, 1988;
55 Ryan & Keddy-Hector, 1992). Numerous studies have shown that mate choice could drive the
56 evolution of motor signals, but evidence for a correlated effect on overall motor performance
57 is still lacking (Byers et al., 2010; Fusani, Barske, Day, Fuxjager, & Schlinger, 2014; Mowles
58 & Ord, 2012).

59 A suitable approach to investigate this question is to determine how mate choice affects a
60 motor signal over evolutionary time, and then examine whether these changes also results in
61 changes in aspects of overall motor performance. As a widely studied acoustic mating signal,
62 the pulse production rate of *Drosophila* male courtship song is a prime candidate for such a
63 study, for multiple reasons. First, *Drosophila* courtship song consists of a series of repeated
64 pulses created by rapid wing vibrations, obtained via high-speed contractions of thoracic
65 muscles (Ewing, 1979; Ewing, 1977; Shirangi, Stern, & Truman, 2013). The rate at which
66 these pulses are produced (commonly reported as the interpulse interval, or IPI, representing
67 the inverse of pulse rate) is thus likely to be a physically challenging motor trait. Next, the
68 song pulse rate is a key target of female choice in several *Drosophila* species. It is involved in
69 the sexual isolation between *D. melanogaster* and *D. simulans*, and in intraspecific mate
70 choice in *D. melanogaster*, *D. montana* and *D. pseudoobscura* (Bennet-Clark & Ewing, 1969;
71 Debelle, Ritchie, & Snook, 2014; Kyriacou & Hall, 1982; Ritchie, Halsey, & Gleason, 1999;
72 Veltsos, Wicker-Thomas, Butlin, Hoikkala, & Ritchie, 2012; Williams, Blouin, & Noor, 2001).
73 Then, the fact that song pulse rate is a target of female choice has been further demonstrated
74 by showing the coevolution of pulse rate and female preference for pulse rate in experimental
75 populations of *D. pseudoobscura* (Debelle et al., 2014). Finally, a direct action of male-male

76 competition on pulse rate evolution is improbable. Courtship song is a near-field acoustic
77 signal produced within 2.5-5mm of the female's head (Bennet-Clark, 1971, 1998), rendering
78 its accurate reception by surrounding male competitors unlikely (Morley, Steinmann, Casas, &
79 Robert, 2012). Hence, and although playing artificial courtship songs to males in playback
80 experiments triggers male locomotion (Eberl & Tauber, 2002; von Schilcher, 1976), varying
81 pulse rate does not have an effect on male courtship behaviour (Talyn & Dowse, 2004).
82 Therefore, *Drosophila* song pulse rate has all the necessary characteristics to be a suitable
83 candidate for this study.

84 Yet, how song pulse rate relates to the evolution of male motor performance has so far not
85 been investigated. This may be because the rate of *Drosophila* courtship song is commonly
86 considered to be static, i.e. stable in time and independent of male motor capacities. Like
87 many other acoustic signals, pulse rate is thus usually measured at a single time point or
88 averaged over the entire courtship sequence (Tauber & Eberl, 2003; but see a notable
89 exception in Arthur, Sunayama-Morita, Coen, Murthy, & Stern, 2013). That restricted view of
90 this motor signal makes it impossible to know how much this trait depends on a male's motor
91 capacities (Irschick & Garland, 2001). Another important aspect in studying the action of mate
92 choice on motor performance evolution is to measure how the trait under mate choice is
93 associated with other traits. By targeting pulse rate, mate choice could lead to a correlated
94 response on other motor traits (Gerhardt & Brooks, 2009; Lande & Arnold, 1983), and
95 therefore investigating these associations is essential to understand how mate choice may
96 influence the evolution of motor performance beyond pulse rate.

97 In this study, we examine closely the production of a motor signal involved in mate choice,
98 and quantify how manipulating the opportunity for sexual selection (Jones, 2009) influences
99 the evolution of motor performance that is displayed during courtship. For that purpose, we

100 study the production of *D. pseudoobscura* pulse rate over the duration of courtship, and
101 explore its response to a long-term experimental manipulation of the opportunity for sexual
102 selection in *D. pseudoobscura* populations (>100 generations of experimental evolution of
103 elevated polyandry or enforced monogamy). An analysis of these experimental lines
104 performed after 30 generations of selection has found that mean pulse rate had responded to
105 sexual selection manipulation, and had become faster in males from polyandrous lines
106 compared to monogamous lines (Snook, Robertson, Crudgington, & Ritchie, 2005). This
107 previous study was however performed on a restricted number of individuals and limited to
108 the examination of average pulse rate. As a consequence, this study did not allow
109 investigating potential differences in pulse rate production over time, which is necessary to
110 study overall motor performance. Here, we perform an in-depth study of pulse rate production
111 over time in our experimental lines after much longer evolution, and analyse the effect of
112 sexual selection manipulation on motor signalling.

113 Our main prediction is that an increased opportunity for sexual selection will lead to the
114 evolution of more intense male signalling characteristics and thus to an increased motor
115 performance. For this end, we look at the detailed structure of pulse rate production over
116 courtship time, to uncover potential sources of motor performance difference between males.
117 We then compare pulse rate production between the sexual selection treatments, to study
118 whether pulse rate production responded to sexual selection manipulation. Finally, we
119 examine associations between pulse rate and other motor courtship traits, to test whether
120 pulse rate may be correlated with overall courtship vigour.

121 **METHODS**

122 *Courtship Song Description*

123 The courtship behaviour of *D. pseudoobscura* has been described in detail elsewhere (Brown,
124 1964; Ewing & Bennet-Clark, 1968). Courtship song is produced by the vibration of one or
125 both male wing(s), and consists of two main components: a low-repetition rate song (LRR)
126 and a high-repetition rate song (HRR) (Fig. 1). LRR consists of high-amplitude polycyclic
127 pulses and is generally produced first, while the male orients in the direction of the female and
128 approaches her, by flicking one or both wing(s) in a scissoring movement. Once the male has
129 reached the female, he extends the wing that is nearest the female's head to 90° and vibrates
130 it rapidly, producing a burst of HRR, characterised by a high number of low-amplitude
131 polycyclic pulses and an increase in pulse rate (i.e. a shorter duration between two
132 consecutive pulses in a burst of song, and thus a shorter interpulse interval). The male will
133 then generally attempt to mount the female and copulate. If the female refuses to mate, the
134 male will start another courtship sequence, including another round of song bursts. As HRR
135 pulse rate is the main target of female preference in this species (Debelle et al., 2014; Snook
136 et al., 2005; Williams et al., 2001), we focus on HRR song in this study (but provide a similar
137 analysis of LRR song in Appendix A).

138 *Sexual Selection Treatments*

139 An ancestral wild-caught population of *D. pseudoobscura*, a naturally polyandrous species
140 (more than 80% of wild-caught females have been shown to be inseminated by up to two
141 males at any given time; Cobbs, 1977), was used to create the selection lines. The
142 establishment and maintenance of the selection lines are described in detail elsewhere
143 (Crudgington, Beckerman, Brüstle, Green, & Snook, 2005). In brief, from an ancestral
144 population derived from Tucson (Arizona, US), 4 replicates (replicate 1, 2, 3 and 4) of two

145 sexual selection treatments were initiated. To modify the opportunity for sexual selection at
146 each generation, adult sex-ratio in vials is manipulated by either confining one female with a
147 single male ('monogamy' treatment; **M**) or one female with 6 males ('elevated polyandry'
148 treatment; **E**) in vials. Both intra- and inter- sexual selection are relaxed in the monogamy
149 treatment whereas both types of sexual selection are increased in the polyandry treatment.
150 As previously reported, effective population size was successfully equalized between the
151 treatments ($N_e > 100$ for all the populations; Snook, Brüstle, & Slate, 2009). At each
152 generation and in each population independently, offspring are collected and then pooled
153 together. A random sample of this pool is used to establish the next generation using the
154 appropriate sex-ratios. This protocol thus proportionally reflects the relative offspring
155 production across all families. Standard vials (2.5mm x 80mm) are used to maintain the
156 selection lines, with a 28-day generation time. Bottles (57 mm x 132 mm) are used to
157 maintain the ancestral population, with an equal sex-ratio of adult flies. Therefore, a total of 8
158 selection lines (M1, M2, M3, M4 and E1, E2, E3, E4) and one ancestral population are
159 maintained and kept at 22°C on a 12L:12D cycle, using standard food media and added live
160 yeast.

161 *Experimental Flies*

162 The flies used in this experiment were from the following generations: 111 and 112 for E1 and
163 M1, 110 and 111 for E2 and M2, 109 and 110 for E3 and M3, 107 and 108 for E4 and M4. To
164 generate the experimental flies, 50 reproductively mature adults of each selection line (25
165 males and 25 females) were used as parents and kept in mass-cultures, providing a common
166 mating set up for the parents of both sexual selection treatments. The resulting larvae were
167 raised in controlled density vials (100 first instar larvae per food vial), to standardize the larval
168 rearing environment and relax selection. The flies were collected and sexed on the day of

169 emergence, using CO₂ anaesthetisation. Males from each population were kept in yeasted
170 food vials of 10 individuals from the day of emergence to day 4, and then transferred to
171 individual yeasted food vials the day before the recording. We used ancestral females for
172 male courtship song recording to standardise female response. Ancestral females were
173 collected and kept in vials of 10 individuals until used for the song recording experiment.
174 Ancestral females were mated to ancestral males the day before the experiment to reduce
175 their receptivity and prevent them from mating with the focal recorded male within the 5
176 minutes of the trial. Female receptivity is drastically reduced in the 24 hours following a
177 mating, and thus the probability of remating for the ancestral females used in this experiment
178 would be nearly zero (Crudgington et al., 2005; Snook, 1998). This method forces males to
179 continuously court females, therefore facilitating detailed study of song production over time.
180 All males and females used in this experiment were 5 days old and thus reproductively
181 mature (Snook & Markow, 2001). Henceforth, reference to polyandrous or monogamous does
182 not mean current mating situation in any experiments, but refers to the experimental sexual
183 selection treatment from which flies are derived.

184 *Courtship Song Recording*

185 Recordings were performed during the flies' morning photoperiod (Noor, 1998). Courtship
186 song was recorded by confining one virgin selection line male with a mated ancestral female
187 for 5 minutes in a transparent chamber (15 mm x 4 mm) in an Insectavox (Gorczyca & Hall,
188 1987). Recordings took place over the course of 12 days. All 8 lines were randomised across
189 and within days of recording. Each male was only recorded once, and 60 males were
190 recorded per selection line. The Insectavox was connected to a Toshiba Satellite Pro S300-
191 117 laptop, and sound was recorded using Audacity (v. 1.3.11). All songs were digitised after
192 filtering with a Fern EF5-04 filter, band-passed between 100 and 800 Hz. After the

193 experiments, recordings were manually prepared for software analysis by silencing parts of
194 the recording without song using Audacity (v. 1.3.11). Recordings were then analysed using a
195 custom script from the software DataView (Heitler, 2007), allowing the detection of the
196 position of each 'song event' (pulses and bursts) in a recording. Intrapulse frequency for both
197 LRR and HRR songs was obtained using a fast Fourier Transform (FFT) in DataView (FFT
198 duration=16ms, FFT window=hamming, percentage overlap=50%).

199 *Temperature and Body Size*

200 To understand more extensively how pulse rate is related to male motor capacities, it is
201 informative to examine how it covaries with two key bioenergetic factors that can affect
202 acoustic communication in insects: temperature and body size (Bailey, 1991; Bennett, 1990;
203 Gillooly & Ophir, 2010). Temperature – which strongly influences muscle contraction rate - is
204 tightly associated with motor power and endurance in ectotherms via its effect on metabolic
205 rate (Gillooly, Brown, West, Savage, & Charnov, 2001), and has a major impact on the
206 temporal components of acoustic signals in insects (Bailey, 1991), including *Drosophila*
207 courtship song traits (Noor & Aquadro, 1998; Ritchie & Kyriacou, 1994; Ritchie & Gleason,
208 1995; Ritchie, Saarikettu, Livingstone, & Hoikkala, 2001). Likewise, body size – a target of
209 sexual selection – is positively correlated with motor performance, notably due to the
210 increased power provided by larger muscles (Biewener, 2003; Carrier, 1996). Thus, including
211 these variables in our analyses will give a better understanding of how much pulse rate
212 production depends on male motor capacities, and thus of how the physiological properties of
213 *Drosophila* courtship song can have an impact on its evolution as a sexual signal.

214 As the light within the Insectavox generates inevitable random small variations in
215 temperature, we examine in detail how song traits vary with these minor changes in

216 temperature. Temperature was measured within the chamber every 10 seconds ($\pm 0.01\text{C}$)
217 using a Testo 735-1 thermometer (Testo Limited, United Kingdom) and recorded for each
218 burst of song in each recording. This temperature variation was then included as a covariate
219 in the song analyses (temperature was either calculated for each burst in the case of HRR
220 pulse rate, or averaged over all bursts for the other traits).

221 To estimate how body size could associate with pulse rate production, the size of the singing
222 male was included in the analyses. The length of wing vein IV of each individual was
223 measured after the experiment (wing vein length has been shown to be a good estimator of
224 body size in *Drosophila* species, (e.g. Crudginton et al., 2005; Gilchrist, Huey, & Serra,
225 2001; Robertson & Reeve, 1952; Sokoloff, 1966). Wings were mounted in a 30% glycerol-
226 70% ethanol medium, images taken using a Motic camera and Motic Images Plus 2.0
227 software (Motic Asia, Hong Kong) and then measured with ImageJ (v. 1.44e; Abramoff,
228 Magalhães, & Ram, 2004).

229 *Courtship Traits Analysis*

230 The different courtship traits analysed in this study are represented in Fig. 1. All the statistical
231 analyses were performed in R (v. 3.3.2; R Development Core Team, 2005).

232 We first tested for differences between the sexual selection treatments in body size and in
233 their probability of producing song. As HRR interpulse interval (i.e. the inverse of pulse rate) is
234 not constant over time but lengthens as courtship time increases (see Fig. B1 in Appendix B),
235 we then conducted a detailed analysis of pulse rate production over courtship time, and
236 compared pulse rate production between treatments. Finally, we performed multivariate
237 analyses on all courtship traits to study phenotypic correlations between pulse rate and other

238 courtship traits, and to test whether sexual selection manipulation modified these
239 associations.

240 *Differences In Body Size and Singing Probability Between The Sexual Selection Treatments:*

241 Potential differences in body size between the sexual selection treatments were analysed
242 using a univariate linear mixed model (LMM), in which the sexual selection treatment of the
243 recorded male (E or M) was included as a fixed effect, and the male replicate population (M1,
244 M2, M3, M4, E1, E2, E3 or E4) included as a Gaussian random effect nested within sexual
245 selection treatment. The model was fitted using maximum likelihood estimation, with a
246 Gaussian error distribution. The difference in the probability of singing (i.e. the probability of a
247 male producing at least one burst of HRR during the 5-min recording) between the sexual
248 selection treatments was analysed using the same model structure but fitted a generalized
249 linear mixed model (GLMM) for the binomial family. Both models were fitted using the
250 package *spaMM* (Rousset & Ferdy, 2014) and estimates were compared to zero using
251 parametric bootstraps which were consistent with results from model comparison using
252 asymptotic likelihood ratio tests.

253 *Detailed Analysis Of Pulse Rate Production Over Time:* To distinguish between HRR

254 interpulse and interburst interval (i.e. the interval of time between the last pulse of a burst and
255 the first pulse of the following burst), an upper threshold was determined visually by plotting
256 the distribution of the duration between two pulses (threshold = 55 ms; the average HRR
257 interpulse interval is approximately 38 ms in *D. pseudoobscura*, Noor & Aquadro, 1998;
258 Snook et al., 2005). To allow sufficient HRR interpulse interval values for each burst, we only
259 included recordings with at least 10 interpulse interval values (i.e. the overall average number
260 of HRR pulses per burst for both E and M males is 17; see Figure B2 for more details).

261 Variation in individual interpulse interval values along the courtship sequence was analysed
262 by fitting a univariate LMM, using the function `glmmPQL()` of the *MASS* package (Venables &
263 Ripley, 2002). This enables correcting for temporal autocorrelation between consecutive
264 interpulse interval values within a burst. We thus included in the model a fourth-order
265 autoregressive moving-average (*corARMA*) function for autocorrelation, using the pulse
266 position in a burst (i.e. 1, 2, 3, etc.) as a time covariate, and the burst identity (1735 levels)
267 nested within replicate (8 levels) as a grouping factor (*nlme* package; Pinheiro, Bates,
268 DebRoy, Sarkar, & R Core Team, 2016). We also included two covariates indicating the
269 position of the interpulse interval value within the courtship sequence, the burst position in the
270 recording (i.e. 1, 2, 3, etc.) and the pulse position within a burst (i.e. 1, 2, 3, etc.), to test for a
271 lengthening of interpulse interval over courtship duration (both within bursts and along bursts;
272 see Fig. 1 for more details). The interactions between sexual selection treatment and the two
273 event position covariates (burst position and pulse position) were included in the model, as
274 well as their three-way interaction. This allowed us to assess how interpulse interval variation
275 changes depending on the quantity of song already produced, and also to test whether
276 interpulse interval variation over time is consistent between the two treatments. The
277 interaction between temperature and burst position was also added, to test for an effect of
278 temperature on interpulse interval lengthening over time (the interaction between temperature
279 and pulse position in the burst could not be included in the model, as the mean duration of a
280 burst, <3s, did not allow enough time for the recorded temperature to vary). The significance
281 of the different fixed effects was extracted from the summary table of the *glmmPQL* fit, which
282 provides the t-test results comparing estimates to zero. The same pulse rate production
283 model was also fitted while including individual body size as an additional covariate (see
284 Table D1 in Appendix D).

285 *Multivariate Response Of Courtship Song To Sexual Selection Treatment:* Because
286 multivariate analyses require the different dependent variables to present the same number of
287 observations, we performed the multivariate analysis using only the mean interpulse interval
288 value of the first HRR burst produced (E and M males produced on average 14 bursts of HRR
289 song in a recording; see Figure B2 for more details). Performing such averaging also
290 precludes the need to consider the temporal autocorrelation that exists between successive
291 pulses. To analyse whether interpulse interval and the other courtship traits jointly responded
292 to sexual selection manipulation, we fitted a multivariate LMM on song data. In a multivariate
293 LMM, the different response variables are transformed into a single univariate response
294 variable by creating a vector that considers all observations across the different response
295 variables sequentially (Christensen, 2001). A fixed effect factor is then used to indicate the
296 correspondence between these observations and the original response variables. We
297 assessed the fixed effects of the mean temperature during a recording and sexual selection
298 treatment on five courtship traits: the mean interpulse interval of the first burst of song, the
299 mean amplitude, the mean intrapulse frequency, the total number of bursts produced, and the
300 singing latency (i.e. the time it took a male to produce its first burst of song). All response
301 variables were log-transformed for normalization and then converted to z-scores, to facilitate
302 model convergence. The estimates we provide in the tables of this study correspond to the
303 direct output from the model fit. In the text, we untransformed the estimates back to the
304 original scale of the response variable. To do this, we calculated the exponential of the sum of
305 (1) the product of the standard deviation of the log of the original variable by the
306 corresponding estimate (2) the log of the mean of the original variable.

307 The model was fitted using the *MCMCglmm* package (Hadfield, 2010). We ran MCMC chains
308 for 100,000 iterations (burn-in phase), followed by five million iterations during which

309 parameter estimates were sampled every 5000 iterations. This sampling scheme resulted in
310 1000 recorded estimate values for each parameter and for each model. This was sufficient to
311 ensure that the autocorrelation between successive estimates was always lower than ± 0.07 .
312 All tests on estimates or quantities derived from estimates (e.g. correlations, see below) for
313 this model are based on the analysis of the distribution of the 1000 records associated with a
314 given parameter. Details about the specification of the prior distributions are given in
315 Appendix C.

316 We allowed for the effects of the different covariates to differ between courtship traits. The
317 number of estimated fixed-effect parameters was thus 15 ($(1+2) \times 5$). We estimated the
318 variances and covariances between the response courtship traits using random effects. We
319 computed these covariance matrices for each selection treatment (i.e. $[5 \text{ variances} + 10$
320 $\text{different covariances}] \times 2 = 30$ (co)variance). We also estimated the variance between
321 replicates separately for each courtship trait (i.e. 5 variances) considering the identity of the
322 replicate as a random effect. We assumed the covariance between model residuals to be null,
323 as no dependence between observations is expected with the random structure considered.
324 The significance of the different fixed effects was extracted from the summary table of the
325 MCMCglmm fit. Here, the p-value is computed as twice the minimum between the
326 probabilities that estimates sampled along the MCMC chains are either greater or lower than
327 zero. The same model was also run while including individual body size as an additional
328 covariate (see Table D2 in Appendix D), with 20 estimated fixed-effect parameters
329 ($[1+1+2] \times 5$).

330 Estimating the variances and covariances of courtship traits allowed us to calculate the
331 correlations between courtship traits for each treatment. Using this approach offers the
332 advantage of estimating correlations that are not confounded by the variables included in the

333 model as fixed (e.g. temperature) or random effects (e.g. the replicate). We then examined
334 the significance of each individual correlation estimate, and tested for differences between the
335 treatments, to examine whether the associations between courtship traits have changed as a
336 result of sexual selection treatment. The significance test of these correlations was based on
337 the analysis of estimates along the MCMC chains, as explained previously.

338 In all figures, the mean fixed effect estimates, hereafter referred as “predicted values” of the
339 mixed models, are represented. Predicted values were adjusted to 22°C, the temperature at
340 which all populations are maintained. The 95% confidence intervals were computed for the
341 two univariate LMMs as ± 1.96 standard errors around the predicted values, with the standard
342 error being derived from the covariance matrix of parameter estimates for fixed effects. For
343 the GLMM, confidence intervals were computed similarly, but at the scale of the linear
344 predictor (i.e. before the transformation from logit to probabilities). For the multivariate LMM,
345 confidence intervals are computed as quantiles of the posterior distribution of parameter
346 estimates along the MCMC chains. Although technically, intervals obtained this way present
347 statistical properties that can differ from confidence intervals (e.g. Rousset, Gouy, Martinez-
348 Almoyna, & Courtiol, 2017; they are called credibility intervals), we will refer to both types as
349 being confidence intervals.

350 *Predictions*

351 First, given that energetically-costly repeated motor signals are predicted to advertise the
352 signaller’s condition (Mowles & Ord, 2012), we expect pulse rate to depend on courtship
353 effort, and thus on the quantity of song already produced by a male. For similar reasons, as
354 motor performance should correlate positively with both temperature and body size,
355 particularly for traits likely to act as indicators of mate condition (Clark, 2012), we also expect

356 pulse rate to be associated with temperature and body size. Then, if pulse rate production has
357 been affected by sexual selection manipulation, we expect to observe faster pulse rates and a
358 shallower slope of decline in pulse rate (i.e. a less pronounced lengthening in interpulse
359 interval) in polyandrous males compared to monogamous males. Finally, for pulse rate to be
360 used as an indicator of motor performance, fast pulse rates should be positively correlated
361 with overall courtship vigour estimates (i.e. here estimated by the other motor courtship traits
362 measured).

363 *Ethical Note*

364 Our design minimised the stress imposed to the individuals used in this experiment. Stress at
365 the larval stage was prevented by controlling for larval density. At adulthood, individuals were
366 transferred in new vials with fresh food and medium adult density. A mouth aspirator was
367 used to gently handle live individuals throughout all the steps of the experiment. The
368 experimental time was only 5 minutes long, after which flies were anaesthetised with CO₂ and
369 rapidly killed in ethanol for wing measurement.

370 **RESULTS**

371 *Differences In HRR Singing Probability Between The Sexual Selection Treatments*

372 The probability of singing differs significantly between the sexual selection treatments (Table
373 1), with monogamous males having a lower probability of singing than polyandrous males
374 (Fig. 2).

375 *Detailed Analysis Of HRR Pulse Rate Production Over Time*

376 This analysis, based on all bursts produced, identifies changes in interpulse interval variation
377 between, and also within, bouts of courtship. The interpulse interval value lengthens between

378 consecutive bursts, meaning that the rate at which males produce pulses decreases more
379 and more as the male produces song (Table 2 and Fig. 3). Interpulse interval also lengthens
380 within a burst, meaning that pulse rate progressively decreases during a burst too (Table 2
381 and Fig. 4).

382 *Temperature:* As expected, temperature strongly associates with courtship traits (Tables 2
383 and 3). Interpulse interval is negatively associated with temperature, meaning that interpulse
384 interval is longer at lower temperatures, as indicated by both the pulse rate production LMM
385 and the multivariate LMM. In the latter case, interpulse interval shortens by 0.32ms (95% C.I.
386 = -0.07 – -0.57, $P=0.012$) when temperature increases by 1°C (Table 3).

387 The pulse rate production LMM (Table 2, and Table D1 for its equivalent with body size
388 included) also shows that the progressive shortening observed in interpulse interval is
389 strongly correlated with temperature, with colder recording temperatures being associated
390 with an even more pronounced lengthening in interpulse interval over courtship time (i.e. a
391 steeper decrease in pulse rate; Fig. 5a).

392 *Body Size:* Males from polyandrous lines are larger on average than males from
393 monogamous lines (Table 1 and Fig. 5b). When body size is included in the pulse rate
394 production LMM (Table D1), we observe a negative effect of body size on interpulse interval.
395 Body size significantly influences interpulse interval both within and between bursts, meaning
396 that larger males produce song with a shorter interpulse interval and maintained this short
397 interpulse interval for a longer time than smaller males (Table D1 and Fig. 5c).

398 The multivariate LMM also reveals that, when body size is included in the model, interpulse
399 interval shortens with increasing body size, with an increase in wing size of 1 standard
400 deviation being associated with a reduction of 0.31ms in interpulse interval (95% C.I. = -0.03

401 – -0.62, $P=0.046$; Table D2). Amplitude increases with increasing body size as well, with an
402 increase in wing size of 1 standard deviation being associated with an increase in amplitude
403 of 11.8 units (95% C.I. = 2.2 – 21.4, $P=0.024$; Table D2).

404 *Evolutionary Response to Sexual Selection Manipulation*

405 The pulse rate production LMM shows a significant effect of sexual selection treatment on
406 interpulse interval, with polyandrous males producing a shorter interpulse interval (i.e. a faster
407 pulse rate) than monogamous males (Table 2 and Fig. 3 & 4). The model also shows a
408 significant interaction between sexual selection treatment and the quantity of song already
409 produced by a male (i.e. the burst and pulse positions in the courtship sequence), showing
410 that the decrease in pulse rate in polyandrous males is shallower than in monogamous males.
411 Therefore, pulse rate differs between the sexual selection treatments, and this difference
412 gradually widens the more males beat their wings to produce song.

413 Although body size is significantly different between the sexual selection treatments, including
414 body size in the pulse rate production LMM shows a difference between the sexual selection
415 treatments that is independent from the effect mediated by body size, with polyandrous males
416 showing again more endurance than monogamous males (Table D1).

417 *Analysis of the Associations Between Courtship Traits*

418 The multivariate LMM shows that polyandrous males start to produce song earlier than
419 monogamous males (mean difference in song latency: 5.7s; 95% C.I.= 3.5 – 7.7, $P=0.001$;
420 Table 3). All other courtship traits do not show a significant difference between the sexual
421 selection treatments (Table 3).

422 Table 4 presents the correlations (r) between courtship traits for each sexual selection
423 treatment extracted from the fit of the multivariate LMM (Table 3), while Table 5 examines
424 whether these associations differ between the sexual selection treatments ($r_E - r_M$). The
425 equivalent of these two tables for the multivariate LMM with body size included as a covariate
426 are shown in the Appendix D (Table D3 and D4). Two out of the 10 correlations between
427 courtship traits changed as a result of selection (Fig. 6 and Table 5), and both of them are
428 associated with interpulse interval. Interpulse interval is correlated with almost all other
429 courtship traits in the polyandry treatment (i.e. with amplitude, latency and the total number of
430 bursts produced; Table 4), but only with amplitude in the monogamy treatment. Faster pulse
431 rates are thus associated with shorter singing latencies, louder songs, and more bursts
432 produced.

433 The multivariate LMM identified three significant associations between courtship traits that do
434 not differ between sexual selection treatments (compare Table 4 to Table 5). In addition to the
435 correlation between interpulse interval and amplitude, it found similar associations between
436 the sexual selection treatments between intrapulse frequency and amplitude, and between
437 latency and the total number of bursts produced (Table 4). The last associations found were
438 a small positive correlation between amplitude and the total number of bursts produced, and
439 between amplitude and latency, however these were only significant for polyandrous males,
440 and did not significantly differ between the sexual selection treatments (Table 5).

441 **DISCUSSION**

442 In this study, we tested the hypothesis that mate choice influences the evolution of motor
443 performance and predicted improved motor performance in populations subjected to more
444 intense sexual selection. We performed a detailed analysis of the production of *Drosophila*

445 *pseudoobscura* song pulse rate, a motor signal under mate choice in this species. We also
446 analysed the response of this motor signal to sexual selection manipulation via experimental
447 evolution. We showed that song pulse rate decreases with the amount of song a male has
448 already produced, and is associated with body size and recording temperature, indicating a
449 potential for pulse rate to act as an indicator of male condition. Consistent with this,
450 manipulating the opportunity for sexual selection led to the evolution of faster pulse rates and
451 improved song production endurance in males from polyandrous lines compared to males
452 from monogamous lines. Finally, we showed that pulse rate is correlated with estimates of
453 overall courtship vigour, particularly in polyandrous males. In total, these results suggest that
454 selection on song pulse rate by females led to the evolution of increased courtship vigour
455 displayed during courtship, indicating a potential correlated response of overall male motor
456 capacities.

457 *Pulse Rate Production*

458 Our results show that pulse rate is not constant over courtship duration, but progressively
459 declines as a male continues to beat his wings. Although this pattern has previously been
460 reported in the courtship songs of two other *Drosophila* species (*D. melanogaster*: Bernstein,
461 Neumann, & Hall, 1992; Dow, 1978; Ewing, 1983; Wilson, Burnet, Eastwood, & Connolly,
462 1976; *D. simulans*: Bernstein et al., 1992), its relevance and implications for sexual selection
463 have not yet been considered. In *D. pseudoobscura*, pulse rate appears to decrease
464 progressively both within a burst of song, and also along the burst sequence. The pattern
465 observed in our study suggests that males start producing song with a fast pulse rate but
466 cannot sustain this as courtship progresses (and particularly for males who evolved under
467 monogamy conditions). Repetitive signals are thought to provide a useful measure of mate
468 quality to the receivers, both via the average rate at which they are produced and the

469 variation of this rate (i.e. increase or decrease) over courtship time (Kotiaho et al., 1998;
470 Mowles & Ord, 2012). As a song with a fast pulse rate can be a physically challenging task,
471 requiring both sustained motor power and motor endurance and thus pushing males to their
472 maximum capacities, variation in the ability of males to maintain a given pulse rate over time
473 has the potential to accurately reflect mate condition.

474 Pulse rate production is associated with both temperature and body size variation. The effect
475 of temperature on acoustic signalling is common through its effect on metabolic rate and has
476 already been shown in many species (Gillooly et al., 2001; Gillooly & Ophir, 2010), including
477 *Drosophila* species (Noor & Aquadro 1998; Ritchie & Gleason, 1995; Ritchie & Kyriacou,
478 1994; Ritchie et al., 2001). In addition to this effect, we show that temperature is not only
479 associated with mean pulse rate, but also with pulse rate decrease over time, indicating that
480 both power output and endurance are temperature-dependent. These results suggest that
481 pulse rate probably strongly relies on male physiological state (Lailvaux & Irschick, 2006).
482 Pulse rate also positively correlates with body size, this effect being unsurprising as motor
483 power often positively covaries with body size (Biewener, 2003; Carrier, 1996). The influence
484 of body size on pulse rate has rarely been investigated in *Drosophila* species, sometimes only
485 via correlations between body size and 'raw' pulse rate data (i.e. not temperature-corrected),
486 which failed to find an association between size and rate (Hoikkala, Aspi, & Suvanto, 1998;
487 Partridge, Ewing, & Chandler, 1987). The positive influence of body size on pulse rate found
488 here indicates that larger males are able to produce a faster pulse rate than smaller males,
489 suggesting that body size influences motor power. This effect could potentially be due to
490 variation in thoracic muscle size, and lead to a higher power output (i.e. a faster pulse rate) of
491 larger males. Indeed, thorax volume is positively correlated with flight wing-beat frequency in
492 *D. melanogaster* (Curtsinger & Laurie-Ahlberg, 1981). Overall, these results suggest pulse

493 rate has the ability to reflect both male motor power and endurance to females, potentially
494 making it an evolutionary driver of overall male motor capacities (Clark, 2012).

495 *Effect of Sexual Selection on Courtship Song Evolution*

496 Males from polyandrous lines are not only more likely to produce song and produce song
497 faster, but they are also able to maintain a fast rate for longer than males from the
498 monogamous lines, demonstrating that manipulating sexual selection had a significant impact
499 on male motor performance during courtship. Our results are consistent with previous work
500 suggesting that polyandrous females prefer faster male pulse rates (DeBelle et al., 2014;
501 Williams et al., 2001). Signals with an increased energy content have been shown to be under
502 directional female preference in several species (e.g. in frogs: Gerhardt & Brooks, 2009;
503 Ryan, 1988; in wolf spiders: Shamble, Wilgers, Swoboda, & Hebets, 2009; in crickets:
504 Simmons, Thomas, Simmons, & Zuk, 2013), with females typically preferring louder song,
505 higher calling rate and higher pulse repetition rate (Clark, 2012; Mowles & Ord, 2012). The
506 fact that the ability to sustain a fast pulse rate has been affected by sexual selection
507 manipulation suggests that selection by females towards fast pulse rates led to the evolution
508 of males delivering songs with increased motor power and sustained intensity (i.e. more
509 endurance), and indicates that pulse rate may be used as an indicator of male motor
510 performance by females.

511 We find, after c.a. 110 generations of selection, a difference in average pulse rate in the same
512 direction than in the preliminary song study (conducted after 30 generations of selection;
513 Snook et al., 2005). The comparable difference in pulse rate between males from
514 polyandrous and monogamous lines after a further 80 generations of selection (1.54 ms
515 between the polyandry and monogamy treatments in Snook et al., 2005; 1.57 ms in the

516 current study, see Table 2) could indicate that pulse rate evolution has reached stable
517 equilibrium conditions between sexual and viability selection (Hine, McGuigan, & Blows,
518 2011; Kirkpatrick, 1996), but could also mean that genetic variation for faster pulse rates has
519 been depleted in the polyandrous lines. Two studies using artificial selection on pulse rate in
520 *D. melanogaster* showed a lower evolutionary response towards faster pulse rates (Ritchie &
521 Kyriacou, 1996; Turner & Miller, 2012), suggesting reduced expressed genetic variation for
522 fast pulse rates in this environment, an expected result if selection has persistently acted in
523 this direction.

524 Male body size responded to the variation in sexual selection opportunity, with males from the
525 polyandrous lines being overall larger than males from the monogamous lines. Body size
526 commonly responds to pre-copulatory sexual selection among species (Andersson, 1996;
527 Blanckenhorn, 2000; Thornhill & Alcock, 1983) and affects male mating success in several
528 *Drosophila* species (including *D. pseudoobscura*), with larger males winning more aggressive
529 encounters, delivering more courtship and mating faster (Ewing, 1961; Partridge, Hoffmann, &
530 Jones, 1987; Partridge et al., 1987; Partridge & Farquhar, 1983). As body size also influences
531 pulse rate, any pulse rate difference between the experimental evolution treatments could
532 thus be explained by size differences. However, even after controlling for the effects of body
533 size (cf. Appendix A and D), sexual selection treatments still differ in their pulse rate
534 production pattern, indicating that traits other than body size have diverged between the
535 treatments and contribute to the differences in motor signalling between the treatments.

536 *Mate Choice As An Evolutionary Mechanism Driving The Evolution Of Motor Performance*

537 Males from polyandrous lines are more vigorous than males from monogamous lines. Indeed,
538 males from polyandrous lines have an enhanced mating capacity and a higher courtship

539 frequency relative to males from monogamous lines (Crudgington, Fellows, & Snook, 2010;
540 Crudgington, Fellows, Badcock, & Snook, 2009). Our study also shows that males from
541 polyandrous lines start producing song faster, produce a faster pulse rate, and have a higher
542 endurance than males from monogamous lines. In theory, male-male competition could
543 participate to this observed increase in male motor capacities. A direct effect of male-male
544 competition on the evolution of pulse rate seems however unlikely, as courtship song is a
545 near-field sound (Bennet-Clark, 1971, 1998) and pulse rate value does not affect other males
546 behaviour (Talyn & Dowse, 2004). Conversely, pulse rate affects male mating success in no-
547 choice assays in this species (Williams et al., 2001), pulse rate coevolved with female
548 preference for pulse rate in our experimental lines (Debelle et al., 2014), and pulse rate is
549 correlated with other courtship motor traits. This suggests that the action of mate choice on
550 pulse rate is actively involved in the observed evolutionary motor changes in our experimental
551 lines.

552 *Drosophila* courtship song has so far only been linked to non-motor selective benefits (i.e.
553 high intrapulse frequency in *D. montana* is associated with a higher male mating success and
554 a higher offspring survival; Hoikkala et al., 1998; Ritchie et al., 2001). Our results support the
555 idea that *Drosophila* courtship song could also signal motor performance. Our analysis
556 focused on courtship-related traits, however motor performance expressed during courtship is
557 likely to reflect an individual's overall motor performance (Byers et al., 2010; Clark, 2012;
558 Lailvaux & Irschick, 2006; Oufiero & Garland, 2007). In a context of strong sexual selection,
559 the selection of fast singing males by females could thus also influence the evolution of other
560 motor characteristics (e.g. flying ability, competitive ability, etc.; Byers et al., 2010).

561 Contrary to what we observe in the polyandrous lines, reducing the opportunity for sexual
562 selection in the monogamous lines is associated with lower singing probability, a longer

563 singing latency, and the inability to maintain a fast pulse rate. This suggests that these traits
564 are costly and could be selected against in the absence of mating competition. Males from
565 monogamous lines also have a lower courtship frequency compared to males from
566 polyandrous lines (Crudgington et al., 2010). As courting (without mating) has been shown to
567 reduce male longevity in *D. melanogaster* (Cordts & Partridge, 1996), these results overall
568 suggest that intense courtship song could impose an important fitness cost to males in a
569 monogamous context, which may have resulted in the reduced investment in courtship song
570 observed in populations under relaxed sexual selection (Crudgington et al., 2010;
571 Crudgington et al., 2005).

572 In conclusion, our results suggest that the pulse rate has the potential to be an indicator of
573 male condition to females, and that the action of female choice on this motor signal affected
574 male motor performance during courtship in our replicated experimental populations. In
575 natural populations, female selection of male courtship motor performance could thus have an
576 impact on the evolution of motor performance exhibited in contexts other than courtship. This
577 work contributes to the limited number of studies providing evidence that sexual selection via
578 mate choice of motor signals may also drive the evolution of mate motor performance (Byers
579 et al., 2010; Mowles & Ord, 2012). Further work in this and other systems should quantify the
580 selective benefits gained by an increased motor performance in mating and non-mating
581 contexts (e.g. standard locomotion, foraging, escaping predators), and investigate what
582 evolutionary changes lead to enhanced motor signals (e.g. morphological, anatomical,
583 physiological), to gain a better understanding of the influence of sexual selection on the
584 evolution of motor performance.

585

586 **REFERENCES**

- 587 Abramoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ.
588 *Biophotonics International*, 11(7), 36–42.
- 589 Albert, A., & Anderson, J. A. (1984). On the existence of maximum likelihood estimates in
590 logistic regression models. *Biometrika*, 71(1), 1–10. <http://doi.org/10.1093/biomet/71.1.1>
- 591 Andersson, M. B. (1996). Sexual selection. *Trends in Ecology & Evolution*, 11(2), 53–58.
592 [http://doi.org/10.1016/0169-5347\(96\)81042-1](http://doi.org/10.1016/0169-5347(96)81042-1)
- 593 Arthur, B. J., Sunayama-Morita, T., Coen, P., Murthy, M., & Stern, D. L. (2013). Multi-channel
594 acoustic recording and automated analysis of *Drosophila* courtship songs. *BMC Biology*,
595 11, 11. <http://doi.org/10.1186/1741-7007-11-11>
- 596 Bailey, W. J. (1991). *Acoustic Behaviour of Insects. An Evolutionary Perspective*. London:
597 Chapman and Hall.
- 598 Bennet-Clark, H. C. (1971). Acoustics of insect song. *Nature*, 255–259.
599 <http://doi.org/10.1038/234255a0>
- 600 Bennet-Clark, H. C. C. (1998). Size and scale effects as constraints in insect sound
601 communication. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
602 353(1367), 407–419. <http://doi.org/10.1098/rstb.1998.0219>
- 603 Bennet-Clark, H. C., & Ewing, A. W. (1969). Pulse interval as a critical parameter in the
604 courtship song of *Drosophila melanogaster*. *Animal Behaviour*, 755–759.
605 [http://doi.org/doi:10.1016/S0003-3472\(69\)80023-0](http://doi.org/doi:10.1016/S0003-3472(69)80023-0)

606 Bennett, A. F. (1990). Thermal dependence of locomotor. *American Journal of Physiology*,
607 259, 253–258.

608 Bernstein, A. S., Neumann, E. K., & Hall, J. C. (1992). Temporal analysis of tone pulses
609 within the courtship songs of two sibling *Drosophila* species, their interspecific hybrid,
610 and behavioral mutants of *D. melanogaster* (Diptera: Drosophilidae). *Journal of Insect*
611 *Behavior*, 5(1), 15–36. <http://doi.org/10.1007/BF01049155>

612 Biewener, A. A. (2003). *Animal locomotion*. New York: Oxford University Press.

613 Blanckenhorn, W. U. (2000). The evolution of body size: what keeps organisms small? *The*
614 *Quarterly Review of Biology*, 75(4), 385–407. <http://doi.org/10.1086/393620>

615 Bonduriansky, R. (2011). Sexual selection and conflict as engines of ecological diversification.
616 *The American Naturalist*, 178(6), 729–745. <http://doi.org/10.1086/662665>

617 Brown, R. G. B. (1964). Courtship behavior in the *Drosophila obscura* group. I: *D.*
618 *pseudoobscura*. *Behaviour*, 23(1–2), 61–106. <http://doi.org/10.1163/156853964X00094>

619 Byers, J., Hebets, E., & Podos, J. (2010). Female mate choice based upon male motor
620 performance. *Animal Behaviour*, 79(4), 771–778.
621 <http://doi.org/10.1016/j.anbehav.2010.01.009>

622 Carrier, D. R. (1996). Ontogenetic limits on locomotor performance. *Physiological Zoology*,
623 69(3), 467–488. <http://doi.org/10.2307/30164211>

624 Christensen, R. (2001). *Advanced Linear Modeling : Multivariate, Time Series, and Spatial*
625 *Data ; Nonparametric Regression and Response Surface Maximization*. New York:
626 Springer.

- 627 Clark, C. J. (2012). The role of power versus energy in courtship: what is the “energetic cost”
628 of a courtship display? *Animal Behaviour*, 84(1), 269–277.
629 <http://doi.org/10.1016/j.anbehav.2012.04.012>
- 630 Cobbs, G. (1977). Multiple insemination and male sexual selection in natural population of
631 *Drosophila pseudoobscura*. *The American Naturalist*, 111(2), 641–656.
632 <http://dx.doi.org/10.1086/283197>
- 633 Cordts, R., & Partridge, L. (1996). Courtship reduces longevity of male *Drosophila*
634 *melanogaster*. *Animal Behaviour*, 52(2), 269–278. <http://doi.org/10.1006/anbe.1996.0172>
- 635 Crudginton, H. S., Beckerman, A. P., Brüstle, L., Green, K., & Snook, R. R. (2005).
636 Experimental removal and elevation of sexual selection: does sexual selection generate
637 manipulative males and resistant females? *The American Naturalist*, 165, S72–87.
638 <http://doi.org/10.1086/429353>
- 639 Crudginton, H. S., Fellows, S., Badcock, N. S., & Snook, R. R. (2009). Experimental
640 manipulation of sexual selection promotes greater male mating capacity but does not
641 alter sperm investment. *Evolution*, 63(4), 926–938. [http://doi.org/10.1111/j.1558-
642 5646.2008.00601.x](http://doi.org/10.1111/j.1558-5646.2008.00601.x)
- 643 Crudginton, H. S., Fellows, S., & Snook, R. R. (2010). Increased opportunity for sexual
644 conflict promotes harmful males with elevated courtship frequencies. *Journal of*
645 *Evolutionary Biology*, 23(2), 440–446. <http://doi.org/10.1111/j.1420-9101.2009.01907.x>
- 646 Curtsinger, J. W., & Laurie-Ahlberg, C. C. (1981). Genetic variability of flight metabolism in
647 *Drosophila melanogaster*. I. Characterization of power output during tethered flight.
648 *Genetics*, (6655), 549–564. <http://dx.doi.org/10.1111/j.1570-7458.1995.tb01943.x>

- 649 Darwin, C. (1859). *The Origin of Species by Means of Natural Selection Or the Preservation*
650 *of Favoured Races in the Struggle for Life*. London: Murray. London: Murray.
- 651 Darwin, C. (1871). *The Descent of Man, and Selection in Relation to Sex*. London: Murray.
- 652 Debelle, A., Ritchie, M. G., & Snook, R. R. (2014). Evolution of divergent female mating
653 preference in response to experimental sexual selection. *Evolution*, 68(9), 2524–2533.
654 <http://doi.org/10.1111/evo.12473>
- 655 Dow, M. A. (1978). *Function and Organization of Courtship Behaviour in Drosophila*
656 *melanogaster*. PhD thesis, University of Edinburgh.
- 657 Eberl, D. F., & Tauber, E. (2002). The effect of male competition on the courtship song of
658 *Drosophila melanogaster*. *Journal of Insect Behavior*, 15(1), 109–120.
659 <http://doi.org/10.1023/A:1014488330548>
- 660 Ewing, A. (1961). Body size and courtship behaviour in *Drosophila melanogaster*. *Animal*
661 *Behaviour*, 9(1–2), 93–96. [http://doi.org/10.1016/0003-3472\(61\)90055-0](http://doi.org/10.1016/0003-3472(61)90055-0)
- 662 Ewing, A. W. (1977). The neuromuscular basis of courtship song in *Drosophila*: The role of
663 the indirect flight muscles. *Journal of Comparative Physiology*, 119(3), 249–265.
664 <http://doi.org/10.1007/BF00656637>
- 665 Ewing, A. W. (1979). The neuromuscular basis of courtship song in *Drosophila*: the role of the
666 direct and axillary wing muscles. *Journal of Comparative Physiology*, 130(1), 87–93.
667 <http://doi.org/10.1007/BF02582977>
- 668 Ewing, A. W. (1983). Functional aspects of *Drosophila* courtship. *Biological Reviews*, 58(2),
669 275–292. <http://doi.org/10.1111/j.1469-185X.1983.tb00390.x>

- 670 Ewing, A. W., & Bennet-Clark, H. C. (1968). The courtship songs of *Drosophila*. *Behaviour*,
671 31(3/4), 288–301. <http://doi.org/10.1163/156853968X00298>
- 672 Fusani, L., Barske, J., Day, L. D., Fuxjager, M. J., & Schlinger, B. A. (2014). Physiological
673 control of elaborate male courtship: female choice for neuromuscular systems.
674 *Neuroscience and Biobehavioral Reviews*, 46(P4), 534–546.
675 <http://doi.org/10.1016/j.neubiorev.2014.07.017>
- 676 Gerhardt, H. C., & Brooks, R. (2009). Experimental analysis of multivariate female choice in
677 gray treefrogs (*Hyla versicolor*): evidence for directional and stabilizing selection.
678 *Evolution*, 63(10), 2504–2512. <http://doi.org/10.1111/j.1558-5646.2009.00746.x>
- 679 Gilchrist, G. W., Huey, R. B., & Serra, L. (2001). Rapid evolution of wing size clines in
680 *Drosophila subobscura*. *Genetica*, 112–113:273–286.
681 <http://doi.org/10.1023/A:1013358931816>
- 682 Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. B., & Charnov, E. L. (2001). Effects of
683 size and temperature on metabolic rate. *Science*, 293(5538), 2248–2251.
684 <http://doi.org/10.1126/science.1061967>
- 685 Gillooly, J. F., & Ophir, A. G. (2010). The energetic basis of acoustic communication.
686 *Proceedings of the Royal Society B: Biological Sciences*, 277(1686), 1325–1331.
687 <http://doi.org/10.1098/rspb.2009.2134>
- 688 Gorczyca, M., & Hall, J. C. (1987). The INSECTAVOX, an integrated device for recording and
689 amplifying courtship songs of *Drosophila*. *Dros Inf Serv*, 66, 157–160.
- 690 Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models:

- 691 The MCMCglmm R Package. *Journal of Statistical Software*, 33(2), 1–22.
692 <http://doi.org/10.18637/jss.v033.i02> R package version 2.24 *cran.r-*
693 *project.org/package=MCMCglmm*.
- 694 Heitler, W. J. (2007). DataView: a tutorial tool for data analysis. template-based spike sorting
695 and frequency analysis. *Journal of Undergraduate Neuroscience Education*, 6(1), A1–7.
- 696 Hine, E., McGuigan, K., & Blows, M. W. (2011). Natural selection stops the evolution of male
697 attractiveness. *Proceedings of the National Academy of Sciences of the United States of*
698 *America*, 108(9), 3659–3664. <http://doi.org/10.1073/pnas.1011876108>
- 699 Hoikkala, A., Aspi, J., & Suvanto, L. (1998). Male courtship song frequency as an indicator of
700 male genetic quality in an insect species, *Drosophila montana*. *Proceedings of the Royal*
701 *Society B: Biological Sciences*, 265(1395), 503–508.
702 <http://doi.org/10.1098/rspb.1998.0323>
- 703 Husak, J. F., & Fox, S. F. (2008). Sexual selection on locomotor performance. *Evolutionary*
704 *Ecology Research*, 213–228.
- 705 Irschick, D. J., & Garland, T. (2001). Integrating function and ecology in studies of adaptation:
706 investigations of locomotor capacity as a model system. *Annual Review of Ecology and*
707 *Systematics*, 32, 367–396. <http://doi.org/10.1146/annurev.ecolsys.32.081501.114048>
- 708 Irschick, D. J., Meyers, J. J., Husak, J. F., & Le Gaillard, J. F. (2008). How does selection
709 operate on whole-organism functional performance capacities? A review and synthesis.
710 *Evolutionary Ecology Research*, 10, 177–196.
- 711 Jones, A. G. (2009). On the opportunity for sexual selection, the Bateman gradient and the

712 maximum intensity of sexual selection. *Evolution*, 63(7), 1673–1684.
713 <http://doi.org/10.1111/j.1558-5646.2009.00664.x>

714 Kirkpatrick, M. (1996). Good genes and direct selection in the evolution of mating
715 preferences. *Evolution*, 50(6), 2125–2140. <http://doi.org/10.2307/2410684>

716 Kotiaho, J. S., Alatalo, R. V., Mappes, J., Nielsen, M. G., Parri, S., & Rivero, A. (1998).
717 Energetic costs of size and sexual signalling in a wolf spider. *Proceedings of the Royal*
718 *Society B: Biological Sciences*, 265(1411), 2203–2209.
719 <http://doi.org/10.1098/rspb.1998.0560>

720 Kyriacou, C. P., & Hall, J. C. (1982). The function of courtship song rhythms in *Drosophila*.
721 *Animal Behaviour*, 30(3), 794–801. [http://doi.org/10.1016/S0003-3472\(82\)80152-8](http://doi.org/10.1016/S0003-3472(82)80152-8)

722 Lailvaux, S. P., & Irschick, D. J. (2006). A functional perspective on sexual selection: insights
723 and future prospects. *Animal Behaviour*, 72(2), 263–273.
724 <http://doi.org/10.1016/j.anbehav.2006.02.003>

725 Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters.
726 *Evolution*, 37(6), 1210–1226.

727 Morley, E. L., Steinmann, T., Casas, J., & Robert, D. (2012). Directional cues in *Drosophila*
728 *melanogaster* audition: structure of acoustic flow and inter-antennal velocity differences.
729 *The Journal of Experimental Biology*, 215(14), 2405–2413.
730 <http://doi.org/10.1242/jeb.068940>

731 Mowles, S. L., & Ord, T. J. (2012). Repetitive signals and mate choice: insights from contest
732 theory. *Animal Behaviour*, 84(2), 295–304. <http://doi.org/10.1016/j.anbehav.2012.05.015>

- 733 Noor, M. A. F. (1998). Diurnal activity patterns of *Drosophila subobscura* and *D.*
734 *pseudoobscura* in sympatric populations. *The American Midland Naturalist*, 140(1), 34–
735 41. [http://doi.org/10.1674/0003-0031\(1998\)140\[0034:DAPODS\]2.0.CO;2](http://doi.org/10.1674/0003-0031(1998)140[0034:DAPODS]2.0.CO;2)
- 736 Noor, M. A. F., & Aquadro, C. F. (1998). Courtship songs of *Drosophila pseudoobscura* and
737 *D. persimilis*: analysis of variation. *Animal Behaviour*, 56(1), 115–125.
738 <http://doi.org/10.1046/j.1365-2540.2001.00811.x>
- 739 Oufiero, C. E., & Garland, T. (2007). Evaluating performance costs of sexually selected traits.
740 *Functional Ecology*, 21(4), 676–689. <http://doi.org/10.1111/j.1365-2435.2007.01259.x>
- 741 Partridge, L., Ewing, A., & Chandler, A. (1987). Male size and mating success in *Drosophila*
742 *melanogaster*: the roles of male and female behaviour. *Animal Behaviour*, 35(2), 555–
743 562. [http://doi.org/10.1016/S0003-3472\(87\)80281-6](http://doi.org/10.1016/S0003-3472(87)80281-6)
- 744 Partridge, L., & Farquhar, M. (1983). Lifetime mating success of male fruitflies (*Drosophila*
745 *melanogaster*) is related to their size. *Animal Behaviour*, 31(3), 871–877.
746 [http://doi.org/10.1016/S0003-3472\(83\)80242-5](http://doi.org/10.1016/S0003-3472(83)80242-5)
- 747 Partridge, L., Hoffmann, A., & Jones, J. S. (1987). Male size and mating success in
748 *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. *Animal*
749 *Behaviour*, 35(2), 468–476. [http://doi.org/10.1016/S0003-3472\(87\)80272-5](http://doi.org/10.1016/S0003-3472(87)80272-5)
- 750 Perry, G., Levering, K., Girard, I., & Garland, T. (2004). Locomotor performance and social
751 dominance in male *Anolis cristatellus*. *Animal Behaviour*, 67(1), 37–47.
752 <http://doi.org/10.1016/j.anbehav.2003.02.003>
- 753 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2016). nlme: Linear and

754 Nonlinear Mixed Effects Models. R package version 3.1-131 *cran.r-*
755 *project.org/package=nlme*.

756 R Development Core Team. (2005). R: A language and environment for statistical computing.
757 *R Foundation Statistical Computing*.

758 Ritchie, M. G., & Gleason, J. M. (1995). Rapid evolution of courtship song pattern in
759 *Drosophila willistoni* sibling species. *Journal of Evolutionary Biology*, 8(4), 463–479.
760 <http://doi.org/10.1046/j.1420-9101.1995.8040463.x>

761 Ritchie, M. G., Halsey, E. J., & Gleason, J. M. (1999). *Drosophila* song as a species-specific
762 mating signal and the behavioural importance of Kyriacou & Hall cycles in *D.*
763 *melanogaster* song. *Animal Behaviour*, 58(3), 649–657.
764 <http://doi.org/10.1006/anbe.1999.1167>

765 Ritchie, M. G., & Kyriacou, C. P. (1996). Artificial selection for a courtship signal in *Drosophila*
766 *melanogaster*. *Animal Behaviour*, 603–611.

767 Ritchie, M. G. M. G., & Kyriacou, C. P. (1994). Genetic variability of courtship song in a
768 population of *Drosophila melanogaster*. *Animal Behaviour*, 48(2), 425–434.
769 <http://doi.org/10.1006/anbe.1994.1256>

770 Ritchie, M. G., Saarikettu, M., Livingstone, S., & Hoikkala, A. (2001). Characterization of
771 female preference functions for *Drosophila montana* courtship song and a test of the
772 temperature coupling hypothesis. *Evolution*, 55(4), 721–727.
773 <http://doi.org/10.1111/j.0014-3820.2001.tb00808.x>

774 Robertson, F. W., & Reeve, E. C. R. (1952). Heterozygosity, environmental variation and

775 heterosis. *Nature*, 170(4320), 286. <http://doi.org/10.1038/170286a0>

776 Rousset, F., & Ferdy, J.-B. (2014). Testing environmental and genetic effects in the presence
777 of spatial autocorrelation. *Ecography*, 37(8), 781–790. <http://doi.org/10.1111/ecog.00566>
778 R package version 1.11-64 cran.r-project.org/package=spaMM.

779 Rousset, F., Gouy, A., Martinez-Almoyna, C., & Courtiol, A. (2017). The summary-likelihood
780 method and its implementation in the Infusion package. *Molecular Ecology Resources*,
781 17(1), 110–119. <http://doi.org/10.1111/1755-0998.12627>

782 Ryan, M. J. (1988). Energy, calling, and selection. *American Zoologist*, 28, 885–898.
783 <http://doi.org/10.1093/icb/28.3.885>

784 Ryan, M. J. M. J., & Keddy-Hector, A. (1992). Directional patterns of female mate choice and
785 the role of sensory biases. *American Naturalist*, 139, S4--S35.
786 <http://doi.org/10.1086/285303>

787 Shamble, P. S., Wilgers, D. J., Swoboda, K. A., & Hebets, E. A. (2009). Courtship effort is a
788 better predictor of mating success than ornamentation for male wolf spiders. *Behavioral*
789 *Ecology*, 20(6), 1242–1251. <http://doi.org/10.1093/beheco/arp116>

790 Shirangi, T. R., Stern, D. L., & Truman, J. W. (2013). Motor control of *Drosophila* courtship
791 song. *Cell Reports*, 5(3), 678–686. <http://doi.org/10.1016/j.celrep.2013.09.039>

792 Simmons, L. W., Thomas, M. L., Simmons, F. W., & Zuk, M. (2013). Female preferences for
793 acoustic and olfactory signals during courtship: male crickets send multiple messages.
794 *Behavioral Ecology*, 24(5), 1099–1107. <http://doi.org/10.1093/beheco/art036>

795 Snook, R. R. (1998). The risk of sperm competition and the evolution of sperm

796 heteromorphism. *Animal Behaviour*, 56(6), 1497–1507.
797 <http://doi.org/10.1006/anbe.1998.0930>

798 Snook, R. R., Brüstle, L., & Slate, J. (2009). A test and review of the role of effective
799 population size on experimental sexual selection patterns. *Evolution*, 63(7), 1923–1933.
800 <http://doi.org/10.1111/j.1558-5646.2009.00682.x>

801 Snook, R. R., & Markow, T. A. (2001). Mating system evolution in sperm-heteromorphic
802 *Drosophila*. *Journal of Insect Physiology*, 47(9), 957–964. [http://doi.org/10.1016/S0022-](http://doi.org/10.1016/S0022-1910(01)00070-1)
803 1910(01)00070-1

804 Snook, R. R., Robertson, A., Crudgington, H. S., & Ritchie, M. G. (2005). Experimental
805 manipulation of sexual selection and the evolution of courtship song in *Drosophila*
806 *pseudoobscura*. *Behavior Genetics*, 35(3), 245–255. [http://doi.org/10.1007/s10519-005-](http://doi.org/10.1007/s10519-005-3217-0)
807 3217-0

808 Sokoloff, A. (1966). Morphological Variation in Natural and Experimental Populations of
809 *Drosophila pseudoobscura* and *Drosophila persimilis*. *Evolution*, 20(1), 49–71.
810 <http://doi.org/10.2307/2406148>

811 Talyn, B. C., & Dowse, H. B. (2004). The role of courtship song in sexual selection and
812 species recognition by female *Drosophila melanogaster*. *Animal Behaviour*, 68(5), 1165–
813 1180. <http://doi.org/10.1016/j.anbehav.2003.11.023>

814 Tauber, E., & Eberl, D. F. (2003). Acoustic communication in *Drosophila*. *Behavioural*
815 *Processes*, 64(2), 197–210. [http://doi.org/10.1016/S0376-6357\(03\)00135-9](http://doi.org/10.1016/S0376-6357(03)00135-9)

816 Thornhill, R., & Alcock, J. (1983). *The evolution of insect mating systems*. Cambridge:

817 Harvard University Press.

818 Turner, T. L., & Miller, P. M. (2012). Investigating natural variation in *Drosophila* courtship
819 song by the evolve and resequence approach. *Genetics*, 191(2), 633–642.
820 <http://doi.org/10.1534/genetics.112.139337>

821 Veltsos, P., Wicker-Thomas, C., Butlin, R. K., Hoikkala, A., & Ritchie, M. G. (2012). Sexual
822 selection on song and cuticular hydrocarbons in two distinct populations of *Drosophila*
823 *montana*. *Ecology and Evolution*, 2(1), 80–94. <http://doi.org/10.1002/ece3.75>

824 Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (Fourth). New York:
825 Springer. R package version 7.3-45 cran.r-project.org/package=MASS.

826 von Schilcher, F. (1976). The role of auditory stimuli in the courtship of *Drosophila*
827 *melanogaster*. *Animal Behaviour*, 18–26. [http://doi.org/10.1016/S0003-3472\(76\)80095-4](http://doi.org/10.1016/S0003-3472(76)80095-4)

828 Williams, M. A., Blouin, A. G., & Noor, M. A. F. (2001). Courtship songs of *Drosophila*
829 *pseudoobscura* and *D. persimilis*. II. Genetics of species differences. *Heredity*, 86(1), 68–
830 77. <http://doi.org/10.1046/j.1365-2540.2001.00811.x>

831 Wilson, R., Burnet, B., Eastwood, L., & Connolly, K. (1976). Behavioural pleiotropy of the
832 yellow gene in *Drosophila melanogaster*. *Genetical Research*, 28(1), 75–88.
833 <http://doi.org/10.1017/S0016672300016748>

834 Woodgate, J. L., Mariette, M. M., & Bennett, A. T. D. (2012). Male song structure predicts
835 reproductive success in a wild zebra finch population. *Animal Behaviour*, 83(3), 773–781.
836 <http://doi.org/10.1016/j.anbehav.2011.12.027>

838 **APPENDIX A – LRR Song Analyses**

839 **Methods**

840 LRR song was analysed similarly to HRR song. To allow distinguishing between interpulse
841 and interburst intervals, an upper threshold was also determined visually by plotting the
842 distribution of the duration between two pulses (LRR threshold = 482ms, the mean LRR
843 interpulse interval is approximately 220 ms in our populations; Snook, Robertson,
844 Crudgington, & Ritchie, 2005)

845 *Differences In Singing Probability Between The Sexual Selection Treatments*

846 The difference in the probability of singing LRR (i.e. the probability of a male producing at
847 least a single pulse of LRR) between the sexual selection treatments was investigated using a
848 Fisher's exact test on the pooled replicates, due to the distribution of LRR data (as
849 polyandrous males always produced LRR in all replicates, but monogamous males do not,
850 model parameters could not be estimated by a linear model as maximum likelihood estimates
851 do not exist for this particular pattern of data; Albert & Anderson, 1984).

852 *Multivariate Response Of Courtship Song To Sexual Selection Treatment*

853 LRR interpulse interval does not vary over the length of courtship (see Table A1), and
854 therefore values were averaged over the entire length of each recording, and the resulting
855 mean LRR interpulse interval was used for statistical modelling. We fitted a multivariate LMM
856 on LRR song traits with the same structure as the one for HRR song, to test for a response of
857 the mean LRR interpulse interval (of the entire recording in this case), the mean LRR
858 intrapulse frequency, the total number of LRR pulses produced and the LRR singing latency
859 to sexual selection manipulation (Table A2). The number of estimated fixed-effect parameters

860 were 12 ($[1+2] \times 4$), with $[4+6] \times 2 = 20$ (co)variance parameters. We also estimated the
861 variance between replicates separately for each trait (4 variances) as random effects.

862 We also tested the significance of correlations between song traits (i.e. LRR interpulse
863 interval, LRR intrapulse frequency, the total number of LRR pulses produced and LRR
864 latency), as well as the differences in song trait correlations between sexual selection
865 treatments.

866 The same model was also run while including individual body size as a covariate (Table A3).
867 The number of estimated fixed-effect parameters was this time 16 ($[1+1+2] \times 4$), with $[4+6] \times 2 =$
868 20 (co)variance parameters.

869 **Results**

870 *Differences In LRR Singing Probability Between The Sexual Selection Treatments*

871 The probability of singing LRR song differs between treatments, with monogamous males
872 having a lower probability than polyandrous males (Fisher's exact test; polyandrous males: 0
873 recordings without LRR song out of 231 recordings; monogamous males: 11 recordings
874 without LRR song out of 230 recordings; $P < 0.001$).

875 *Evolutionary response to sexual selection manipulation*

876 The multivariate LMM does not identify any significant response of LRR traits to selection
877 sexual treatment (Table A2).

878 *Temperature*: The multivariate LMM shows that LRR interpulse interval shortens with
879 temperature (Table A3). Increasing temperature by one degree reduces the interpulse interval
880 by 3.28ms (95% C.I. = -0.67 – -6.05, $P = 0.02$). Both LRR singing latency and LRR intrapulse
881 frequency significantly increase with temperature, with an increase in temperature of one

882 degree resulting in a latency increase of 14.5s (95% C.I. = 302 – 2558, $P=0.01$), and an LRR
883 intrapulse frequency increase of 4.18Hz (95% C.I. = 1.20 – 7.18, $P=0.01$).

884 *Body Size*: Including body size in the multivariate LMM does not change the results of the
885 model (Table A3). LRR interpulse interval shortens with body size, with an increase of 1
886 standard deviation in wing size being associated with an interpulse interval reduction of
887 4.72ms (95% C.I. = -1.52– -7.53, $P=0.004$).

888 *Analysis of the Associations Between Courtship traits*

889 The multivariate LMM also revealed that LRR interpulse interval was positively correlated with
890 the total number of LRR pulses produced (Table A4), but the correlation reached significance
891 only for monogamous males. As with HRR song, we found a significant negative association
892 between LRR latency and the total number of LRR pulses produced for both treatments
893 (Table A4). There was no significant impact of the sexual selection treatment on the
894 correlations between LRR traits (Table A5, Fig. A1).

895 Including body size in the multivariate LMM generated very similar results (Tables A6, A7).

896

897 **APPENDIX B – HRR IPI Variation Over Time**

898 Figure B1 shows a pattern in pulse rate production over courtship time, by illustrating how
899 HRR interpulse interval lengthens as courtship time increases. Figure B2 shows the range of
900 the distribution of the burst and pulse numbers, depending on sexual selection treatment and
901 recording temperature.

902 **APPENDIX C – Prior Definition For Fitting The Multivariate LMM.**

903 In both MCMCglmm models (the one with body size and the one without), we retained the
904 default settings for the prior distributions for fixed effects. In contrast, we set identity matrices
905 as prior specification for all other prior distributions and we used a degree of belief of 0.001
906 for the priors used in variance estimations only (i.e. weakly informative improper prior) and a
907 degree of belief equal to the number of response variables plus one (i.e. proper prior) for the
908 prior used in covariance matrix estimations. This structure follows the recommendations of
909 the package instructions.

910 **APPENDIX D – HRR Song Analyses (With Body Size Included)**

911 Running analyses of HRR interpulse interval while including body size as a covariate shows
912 that, although body size has a significant effect on courtship song production, the effect of
913 sexual selection treatment also remains significant (see Tables D1-4).

914 **TABLES**

915 **Table 1.** Summary tables for the fitted GLMM analysing HRR singing probability and the
 916 univariate LMM analysing body size.

Model	Factor	HRR singing probability				Body size			
		β^*	Lower CI	Upper CI	P	β	Lower CI	Upper CI	P
Treatment	E	1.20	0.56	1.85	<0.001	0.023	0.010	0.035	<0.001
Intercept		1.35	0.98	1.71	<0.001	1.08	1.07	1.09	<0.001
Inter-replicate variance			0.038				0.000066		
Residual variance			-				0.00062		

In both models, sexual selection treatment was tested as a fixed effect, and replicate was included as a random effect. The following elements are specified: the model estimate of each variable (β), the lower and upper limit of the estimate's 95% confidence interval (CI), and p-value of the test comparing the estimate to zero (P). HRR = high-repetition rate song, Treatment = sexual selection treatment (E = polyandrous; the monogamous treatment M was used as the reference level). N = 471 recordings for HRR probability and N = 355 recordings for body size. * Given that the HRR singing probability GLMM used a binomial error distribution, the given estimates for this model are on a logit scale.

917 **Table 2.** Summary table for the fitted univariate LMM analysing the HRR pulse rate
 918 production between and within HRR bursts.

Model parameters	Factor level	β	Lower CI	Upper CI	P
Treatment	E	-1.57	-2.64	-0.501	0.026
Temperature		-0.71	-0.81	-0.62	<0.001
BP		0.69	0.51	0.87	<0.001
PP		0.92	0.87	0.98	<0.001
Treatment * BP		-0.10	-0.32	0.11	0.339
Temperature * BP		-0.30	-0.40	-0.20	<0.001
Treatment * PP		-0.093	-0.17	-0.018	0.013
BP * PP		0.11	0.039	0.17	0.002
Treatment * BP * PP		-0.16	-0.24	-0.076	<0.001
Intercept		39.27	38.52	40.01	<0.001
Inter-replicate variance				0.56	
Inter-burst variance (nested within replicate)				3.47	
Residual variance				4.92	

919
 920 The following elements are specified: the model estimate of each variable (β), the lower
 921 and upper limit of the estimate's 95% confidence interval (CI), and the p-value of the test
 922 comparing the estimate to zero (P). BP = Burst position, PP = Pulse position, HRR = high-
 923 repetition rate song, IPI = interpulse interval, Treatment = sexual selection treatment (E =
 924 polyandrous; the monogamous treatment M was used as the reference level), burst
 925 position = the position of the burst in the recording, pulse position = the position of the
 926 pulse in the HRR burst. The autocorrelation parameters are $\varphi_1 = 0.23$, $\varphi_2 = 0.12$, $\varphi_3 =$

927 0.058 and $\phi_4 = 0.031$. N = 35206 individual interpulse interval values. The same model
928 was fitted while including body size as a covariate (Table D1).

929

930 **Table 3.** Summary table for the fitted multivariate LMM analysing HRR traits.

Trait	Model parameters	Factor level	β	Lower CI	Upper CI	P
IPI	Treatment	E	-0.49	-1.10	0.12	0.092
	Temperature		-0.14	-0.24	-0.028	0.012
	Intercept		3.41	1.14	6.10	0.010
Amplitude	Treatment	E	-0.21	-0.57	0.18	0.262
	Temperature		0.083	-0.032	0.188	0.158
	Intercept		-1.79	-4.24	0.78	0.176
Frequency	Treatment	E	-0.196	-0.771	0.43	0.446
	Temperature		0.076	-0.024	0.20	0.172
	Intercept		-1.65	-4.20	0.89	0.218
Total number of bursts	Treatment	E	0.21	-0.31	0.74	0.398
	Temperature		0.047	-0.078	0.15	0.414
	Intercept		-1.16	-3.84	1.45	0.378
Latency	Treatment	E	-0.51	-0.77	-0.25	0.001
	Temperature		0.080	-0.061	0.19	0.192
	Intercept		-1.56	-4.25	1.39	0.254

931

932 The following elements are specified: the model estimate of each variable (β ; here the

933 posterior mean), the lower and upper limits of the estimate's 95% credibility interval (CI),

934 and the p-value of the test comparing the estimate to zero (P). IPI = interpulse interval,

935 Treatment = sexual selection treatment (E = polyandrous; the monogamous treatment M
936 was used as the reference level), Latency = the time taken to sing the first burst of HRR
937 song. Covariances between the response variables of the model are provided as
938 correlations in Table 4. Estimated variances between replicates were $\sigma^2 = 0.17$ for IPI, σ^2
939 = 0.04 for amplitude, $\sigma^2 = 0.14$ for intrapulse frequency, $\sigma^2 = 0.12$ for the total number of
940 bursts and $\sigma^2 = 0.01$ for latency. Note that all responses are expressed as z-scores of the
941 log transformed value of the original measurements, but temperature was not altered.
942 Estimates in the table are thus not expressed on the original data scale, but in z-scores of
943 log values (see Methods). The means and standard deviations of the log of the original
944 variables are as following: IPI (mean = 3.63, $\sigma = 0.06$), amplitude (mean = 5.85, $\sigma = 0.20$),
945 frequency (mean = 5.56, $\sigma = 0.09$), total number of bursts (mean = 2.35, $\sigma = 0.84$), latency
946 (mean = 9.38, $\sigma = 1.32$). N = 280 recordings. The same model was fitted while including
947 body size as a covariate (Table D2).

948 **Table 4.** Correlation matrix between courtship traits for the two sexual selection
 949 treatments.

	IPI		Amplitude		Frequency		Bursts		Latency	
	r	P	r	P	r	P	r	P	r	P
IPI	1	-	-0.41	<0.001	-0.11	0.192	-0.38	<0.001	0.28	0.002
Amplitude	-0.31	<0.001	1	-	-0.29	<0.001	0.24	0.008	-0.17	0.038
Frequency	0.07	0.470	-0.42	<0.001	1	-	0.20	0.028	-0.09	0.298
Bursts	0.09	0.354	0.07	0.410	-0.04	0.656	1	-	-0.29	<0.001
Latency	-0.05	0.594	-0.06	0.504	-0.06	0.544	-0.39	<0.001	1	-

950
 951 The following elements are specified: the correlation coefficient (r) and the p-value (P).
 952 HRR = high-repetition rate song, IPI = interpulse interval, Bursts = the total number of
 953 HRR bursts produced, Latency = the time taken to sing the first burst of HRR song. These
 954 correlations were derived from the variances and covariances estimated by the
 955 multivariate LMM (see Table 3). Since correlation matrices are symmetric, correlation
 956 values for polyandrous males and monogamous males are shown above and below the
 957 diagonal, respectively.

958 **Table 5.** Differences in courtship trait correlations between the sexual selection treatments
 959 ($r_E - r_M$ from Table 4).

	IPI		Amplitude		Frequency		Bursts		Latency	
	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P
IPI	-	-								
Amplitude	-0.10	0.358	-	-						
Frequency	-0.18	0.144	0.13	0.242	-	-				
Bursts	-0.47	<0.001	0.16	0.188	0.24	0.090	-	-		
Latency	0.33	0.008	-0.11	0.344	-0.03	0.814	0.10	0.402	-	-

960
 961 The following elements are specified: the corresponding correlation coefficients for
 962 polyandrous (r_E) and monogamous males (r_M) and the p-value (P). HRR = high-repetition
 963 rate song, IPI = interpulse interval, Bursts = the total number of HRR bursts produced,
 964 Latency = the time taken to sing the first burst of HRR song.

965

966 **Table A1.** Correlations between LRR IPI and courtship duration in 8 randomly chosen
967 songs, one from each of the 8 replicated populations.

Song	r	P
M1	0.081	0.438
M2	0.19	0.365
M3	0.36	0.113
M4	-0.17	0.437
E1	-0.30	0.161
E2	0.023	0.904
E3	0.28	0.235
E4	-0.16	0.395

968 The Spearman correlation coefficient (r) and its associated p-value (P) are given. E =
969 polyandrous, M = monogamous, 1-4 refers to the replicate population of the song
970 example.

971

972 **Table A2.** Summary table for the fitted multivariate LMM analysing LRR song.

Song trait	Model parameters	Factor level	β	Lower CI	Upper CI	P
	Treatment	E	-0.080	-0.71	0.54	0.798
IPI	Temperature		-0.11	-0.20	-0.016	0.022
	Intercept		2.59	0.39	4.68	0.020
	Treatment	E	-0.27	-0.85	0.24	0.246
Frequency	Temperature		0.14	0.044	0.23	0.001
	Intercept		-2.95	-5.47	-0.92	0.006
	Treatment	E	0.14	-0.083	0.39	0.220
Total number of pulses	Temperature		-0.077	-0.16	0.027	0.094
	Intercept		1.69	-0.68	3.63	0.11

	Treatment	E	-0.25	-0.60	0.19	0.206
Latency	Temperature		0.13	0.019	0.21	0.010
	Intercept		-2.75	-4.80	-0.35	0.014

The following elements are specified: the model estimate of each variable (β ; here the posterior mean), the lower and upper limits of the estimate's 95% credibility interval (CI), and the p-value of the test comparing the estimate to zero (P). IPI = interpulse interval, Treatment = sexual selection treatment (E = polyandrous; the monogamous treatment M was used as the reference level), Latency = the time taken to sing the first pulse of LRR song. Covariances between the response variables of the model are provided as correlations in Table A4. Estimated variances between replicates were $\sigma^2 = 0.20$ for IPI, $\sigma^2 = 0.12$ for intrapulse frequency, $\sigma^2 = 0.01$ for the total number of bursts and $\sigma^2 = 0.06$ for latency. Note that all responses are expressed as z-scores of the log transformed value of the original measurements, but temperature was not altered. Estimates in the table are thus not expressed on the original data scale, but in z-scores of log values (see Methods). The means and standard deviations of the log of the original variables are as following: IPI (mean = 5.34, $\sigma = 0.14$), frequency (mean = 6.19, $\sigma = 0.06$), total number of pulses (mean = 3.36, $\sigma = 0.78$), latency (mean = 8.90, $\sigma = 1.38$). N = 415 recordings. The same model was fitted while including body size as a covariate (Table A3).

973 **Table A3.** Summary table for the fitted multivariate LMM analysing LRR song (with body size
 974 included in the model).

Song trait	Model parameters	Factor level	β	Lower CI	Upper CI	P
IPI	Treatment	E	0.056	-0.61	0.67	0.838
	Temperature		-0.11	-0.21	-0.023	0.020
	Body size		-0.16	-0.26	-0.052	0.004
	Intercept		2.58	0.30	4.72	0.026
Frequency	Treatment	E	-0.24	-0.82	0.26	0.298
	Temperature		0.14	0.039	0.23	0.010
	Body size		0.0040	-0.096	0.11	0.922
	Intercept		-3.01	-5.29	-0.84	0.012
Total number of	Treatment	E	0.068	-0.17	0.31	0.592

pulses	Temperature		-0.077	-0.17	0.019	0.096
	Body size		0.10	-0.0090	0.20	0.082
	Intercept		1.71	-0.58	3.72	0.106
Latency	Treatment	E	-0.26	-0.63	0.13	0.192
	Temperature		0.13	0.029	0.22	0.001
	Body size		0.014	-0.087	0.12	0.814
	Intercept		-2.82	-4.83	-0.58	0.008

The following elements are specified: the model estimate of each variable (β ; here the posterior mean), the lower and upper limits of the estimate's 95% credibility interval (CI), and the p-value of the test comparing the estimate to zero (P). IPI = interpulse interval, Treatment = sexual selection treatment (E = polyandrous; the monogamous treatment M was used as the reference level), Latency = the time taken to sing the first pulse of LRR song. Covariances between the response variables of the model are provided as correlations in Table A6. Estimated variances between replicates were $\sigma^2 = 0.23$ for IPI, $\sigma^2 = 0.13$ for intrapulse frequency, $\sigma^2 = 0.01$ for the total number of bursts and $\sigma^2 = 0.06$ for latency. Note that all responses are expressed as z-scores of the log transformed value of the original measurements. Wing size was also transformed into z-scores, but temperature was not

altered. Estimates in the table are thus not expressed on the original data scale, but in z-scores of log values (see Methods). The means and standard deviations of the log of the original variables are as following: IPI (mean = 5.34, σ = 0.14), frequency (mean = 6.19, σ = 0.06), total number of pulses (mean = 3.36, σ = 0.78), latency (mean = 8.90, σ = 1.38). N = 415 recordings.

975

976 **Table A4.** Correlation matrix between LRR song traits for the two sexual selection treatments.

	IPI		Frequency		Pulse number		Latency	
	r	P	r	P	r	P	r	P
IPI	1	-	-0.14	0.088	0.07	0.38	0.06	0.428
Frequency	-0.14	0.06	1	-	0.06	0.466	-0.01	0.900
Pulse number	0.22	0.002	0.03	0.650	1	-	-0.23	0.001
Latency	0.07	0.358	-0.06	0.512	-0.35	<0.001	1	-

977 The following elements are specified: the correlation coefficient (r) and the p-value (P). LRR =
 978 low-repetition rate song, IPI = interpulse interval, Latency = the time taken to sing the first
 979 pulse of LRR song. These correlations were derived from the variances and covariances
 980 estimated by the multivariate LMM (see Table A2). Since correlation matrices are symmetric,

981 correlation values for polyandrous males and monogamous males are shown above and
 982 below the diagonal, respectively.

983 **Table A5.** Differences in LRR song trait correlations between the sexual selection treatments
 984 ($r_E - r_M$ from Table A4).

	IPI		Frequency		Pulse number		Latency	
	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P
IPI	-	-						
Frequency	0.00	0.988	-	-				
Pulse number	-0.15	0.152	0.03	0.802	-	-		
Latency	-0.01	0.978	0.04	0.684	0.12	0.224	-	-

985 The following elements are specified: the corresponding correlation coefficients for
 986 polyandrous (r_E) and monogamous males (r_M) and the p-value (P). LRR = low-repetition rate
 987 song, IPI = interpulse interval, Latency = the time taken to sing the first pulse of LRR song.

988

989 **Table A6.** Correlation matrix between LRR song traits for the two sexual selection treatments
 990 (with body size included in the model)

	IPI		Frequency		Pulse number		Latency	
	r	P	r	P	r	P	r	P
IPI	1	-	-0.13	0.100	0.09	0.238	0.06	0.450
Frequency	-0.15	0.052	1	-	0.06	0.482	-0.01	0.900
Pulse number	0.21	0.004	0.03	0.682	1	-	-0.23	0.004
Latency	0.09	0.256	-0.05	0.556	-0.35	<0.001	1	-

991 The following elements are specified: the correlation coefficient (r) and the p-value (P). LRR =
 992 low-repetition rate song, IPI = interpulse interval, Latency = the time taken to sing the first
 993 pulse of LRR song. These correlations were derived from the variances and covariances
 994 estimated by the multivariate LMM (see Table A3). Since correlation matrices are symmetric,
 995 correlation values for polyandrous males and monogamous males are shown above and
 996 below the diagonal, respectively.

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1000 **Table A7.** Differences in LRR song trait correlations between the sexual selection treatments
 1001 ($r_E - r_M$ from Table A6) (with body size included in the model)

	IPI		Frequency		Pulse number		Latency	
	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P
IPI	-	-						
Frequency	0.01	0.868	-	-				
Pulse number	-0.12	0.262	0.02	0.886	-	-		
Latency	-0.03	0.808	0.04	0.722	0.12	0.252	-	-

1002 The following elements are specified: the corresponding correlation coefficients for
 1003 polyandrous (r_E) and monogamous males (r_M) and the p-value (P). LRR = low-repetition rate
 1004 song, IPI = interpulse interval, Latency = the time taken to sing the first pulse of LRR song.

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1007 **Table D1.** Summary table for the fitted univariate LMM analysing the HRR pulse rate
 1008 production between and within HRR bursts (with body size included in the model).

Model parameters	Factor level	β	Lower CI	Upper CI	P
Wing size		-0.42	-0.53	-0.31	<0.001
Treatment	E	-1.17	-2.39	0.046	0.102
Temperature		-0.75	-0.85	-0.65	<0.001
BP		0.69	0.51	0.87	<0.001
PP		0.92	0.86	0.98	<0.001
Treatment * BP		-0.055	-0.27	0.16	0.609
Temperature * BP		-0.3	-0.40	-0.20	<0.001
Treatment * PP		-0.09	-0.16	-0.015	0.015
BP * PP		0.11	0.042	0.18	0.001
Treatment * BP * PP		-0.16	-0.24	-0.079	<0.001
Intercept		39.04	38.20	39.89	<0.001

Inter-replicate variance	0.73
Inter-burst variance (nested within replicate)	3.32
Residual variance	4.91

The following elements are specified: the model estimate of each variable (β), the lower and upper limit of the estimate's 95% confidence interval (CI), the p-value of the test comparing the estimate to zero (P). BP = Burst position, PP = Pulse position, HRR = high-repetition rate song, IPI = interpulse interval, Treatment = sexual selection treatment (E = polyandrous; the monogamous treatment M was used as the reference level), burst position = the position of the burst in the recording, pulse position = the position of the pulse in the HRR burst. The autocorrelation parameters are $\varphi_1 = 0.23$, $\varphi_2 = 0.12$, $\varphi_3 = 0.058$ and $\varphi_4 = 0.031$. N = 35206 individual interpulse interval values.

1010 **Table D2.** Summary table for the fitted multivariate LMM analysing HRR song (with body size
 1011 included in the model)

Song trait	Model parameters	Factor level	β	Lower CI	Upper CI	P
IPI	Treatment	E	-0.41	-1.014	0.26	0.178
	Temperature		-0.14	-0.26	-0.045	0.016
	Body size		-0.13	-0.27	-0.014	0.046
	Intercept		3.51	1.032	5.89	0.008
Amplitude	Treatment	E	-0.32	-0.74	0.11	0.128
	Temperature		0.095	-0.018	0.21	0.106
	Body size		0.16	0.042	0.30	0.024
	Intercept		-2.02	-4.63	0.57	0.138
Frequency	Treatment	E	-0.11	-0.63	0.52	0.678

	Temperature		0.069	-0.057	0.19	0.266
	Body size		-0.092	-0.22	0.040	0.180
	Intercept		-1.52	-4.12	1.38	0.296
Total number of bursts	Treatment	E	0.21	-0.37	0.74	0.432
	Temperature		0.050	-0.064	0.16	0.440
	Body size		0.010	-0.10	0.14	0.862
	Intercept		-1.22	-3.52	1.58	0.404
Latency	Treatment	E	-0.47	-0.74	-0.18	0.002
	Temperature		0.076	-0.041	0.19	0.202
	Body size		-0.054	-0.17	0.073	0.392
	Intercept		-1.48	-4.18	1.12	0.246

1012 The following elements are specified: the model estimate of each variable (β ; here the
1013 posterior mean), the lower and upper limits of the estimate's 95% credibility interval (CI), and

1014 the p-value of the test comparing the estimate to zero (P). IPI = interpulse interval, Treatment
1015 = sexual selection treatment (E = polyandrous; the monogamous treatment M was used as
1016 the reference level), Latency = the time taken to sing the first burst of HRR song. Covariances
1017 between the response variables of the model are provided as correlations in Table D3.
1018 Estimated variances between replicates were $\sigma^2 = 0.2$ for IPI, $\sigma^2 = 0.05$ for amplitude, $\sigma^2 =$
1019 0.13 for intrapulse frequency, $\sigma^2 = 0.12$ for the total number of bursts and $\sigma^2 = 0.01$ for
1020 latency. Note that all responses are expressed as z-scores of the log transformed value of the
1021 original measurements. Wing size was also transformed into z-scores, but temperature was
1022 not altered. Estimates in the table are thus not expressed on the original data scale, but in z-
1023 scores of log values (see Methods). The means and standard deviations of the log of the
1024 original variables are as following: IPI (mean = 3.63, $\sigma = 0.06$), amplitude (mean = 5.85, $\sigma =$
1025 0.20), frequency (mean = 5.56, $\sigma = 0.09$), total number of bursts (mean = 2.35, $\sigma = 0.84$),
1026 latency (mean = 9.38, $\sigma = 1.32$). N = 280 recordings.

1027

1028 **Table D3.** Correlation matrix between HRR courtship traits for the two sexual selection
 1029 treatments (with body size included in the model).

	IPI		Amplitude		Frequency		Bursts		Latency	
	r	P	r	P	r	P	r	P	r	P
IPI	1	-	-0.39	<0.001	-0.12	0.138	-0.37	<0.001	0.26	0.002
Amplitude	-0.3	<0.001	1	-	-0.27	<0.001	0.23	0.008	-0.15	0.076
Frequency	0.06	0.560	-0.41	<0.001	1	-	0.21	0.018	-0.1	0.260
Bursts	0.07	0.436	0.09	0.314	-0.04	0.650	1	-	-0.29	0.002
Latency	-0.04	0.614	-0.07	0.490	-0.05	0.584	-0.39	<0.001	1	-

1030 The following elements are specified: the correlation coefficient (r) and the p-value (P). HRR =
 1031 high-repetition rate song, IPI = interpulse interval, Bursts = the total number of HRR bursts
 1032 produced, Latency = the time taken to sing the first burst of HRR song. These correlations
 1033 were derived from the variances and covariances estimated by the multivariate LMM (see
 1034 Table D2). Since correlation matrices are symmetric, correlation values for polyandrous males
 1035 and monogamous males are shown above and below the diagonal, respectively.

1036 **Table D4.** Differences in HRR courtship trait correlations between the sexual selection
 1037 treatments ($r_E - r_M$ from Table D3) (with body size included in the model).

	IPI		Amplitude		Frequency		Bursts		Latency	
	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P	$r_E - r_M$	P
IPI	-	-								
Amplitude	-0.09	0.426	-	-						
Frequency	-0.18	0.142	0.14	0.218	-	-				
Bursts	-0.44	<0.001	0.14	0.290	0.25	0.042	-	-		
Latency	0.31	0.012	-0.09	0.484	-0.05	0.700	0.10	0.360	-	-

1038 The following elements are specified: the corresponding correlation coefficients for
 1039 polyandrous (r_E) and monogamous males (r_M) and the p-value (P). HRR = high-repetition rate
 1040 song, IPI = interpulse interval, Bursts = the total number of HRR bursts produced, Latency =
 1041 the time taken to sing the first burst of HRR song.

1042

1043 **FIGURES**

Figure 1. *D. pseudoobscura* courtship song representation. Both LRR (low-repetition rate) and HRR (high-repetition rate) song are represented. Each burst of song is composed of multiple pulses, each separated by a certain time interval, the interpulse interval (IPI; i.e. the inverse of pulse rate). As the interpulse interval represents the amount of time between two consecutive pulses, a short interpulse interval means that a male rapidly beats his wings (i.e. fast pulse repetition rate), whereas that a long interpulse interval means that a male slowly beats his wings (i.e. slow pulse repetition rate). HRR frequency represents the intrapulse frequency of a pulse of HRR. In our analysis, we will refer to ‘burst position’ as the position of an HRR burst in the courtship sequence (i.e. the 1st burst produced, the 2nd burst produced, the 3rd burst produced), and to ‘pulse position’ as the position of a pulse within a burst of HRR (i.e. the 1st pulse of a burst, the 2nd pulse of a burst, the 3rd pulse of a burst). **|| in colour on the Web and in black-and-white in print - 2-column fitting image ||**

1044 **Figure 2.** Differences between the sexual selection treatments in singing probability (the
1045 probability of singing HRR). Model estimates are given in Table 1. The letters represent the
1046 fitted values predicted by the mixed model depending on male sexual selection treatment (E =
1047 polyandrous males, M = monogamous males). HRR = high-repetition rate song. 95%
1048 confidence intervals are represented in dashed lines. **|| in black-and-white colour on the**
1049 **Web and in print - 1-column fitting image ||**

1050

Figure 3. Changes in HRR interpulse interval production along bursts in the courtship sequence, depending on sexual selection treatment, as predicted by the fitted univariate pulse rate production LMM. The figure shows the changes of the mean interpulse interval

value along bursts in a 40-burst courtship sequence of song for monogamous (grey) and polyandrous (black). Model estimates are given in Table 2. The letters represent the fitted values predicted by the mixed model depending on male sexual selection treatment (E = polyandrous males, M = monogamous males). HRR = high-repetition rate song, IPI = interpulse interval. 95% confidence intervals are represented in dashed lines. **|| in black-and-white colour on the Web and in print - 1-column fitting image ||**

1051 **Figure 4.** Changes in HRR interpulse interval production along pulses within a burst,
1052 depending on sexual selection treatment, as predicted by the fitted univariate pulse rate
1053 production LMM. The figure shows the changes of individual interpulse interval values along
1054 pulses at the beginning of courtship (burst 1; grey) and after 40 bursts of song (burst 40;
1055 black), for males of polyandrous (a) and monogamous (b) males. Model estimates are given
1056 in Table 2. The letters represent the fitted values predicted by the mixed model depending on
1057 male sexual selection treatment (E = polyandrous males, M = monogamous males). HRR =
1058 high-repetition rate song, IPI = interpulse interval. 95% confidence intervals are represented
1059 in dashed lines. **|| in black-and-white colour on the Web and in print - 2-column fitting
1060 image ||**

1061
1062 **Figure 5.** Body size and temperature effects on HRR interpulse interval, as predicted by the
1063 fitted univariate pulse rate production and body size LMMs: (a) the effect of recording
1064 temperature variation on interpulse interval variation along bursts (estimated for 4 different
1065 recording temperatures: 21, 22, 23, and 24°C); (b) the average body size difference between
1066 the treatments; and (c) the effect of body size on interpulse interval. Model estimates for
1067 figure (a) and (c) were extracted from the univariate pulse rate production LMM that included
1068 body size as a covariate (Table D1), while figure (b) is based on the univariate body size LMM

1069 presented in Table 1. The symbols represent the fitted values predicted by the mixed models
1070 depending on male sexual selection treatment (E = polyandrous males, M = monogamous
1071 males), body size (circles) or temperature (21, 22, 23 and 24). IPI = interpulse interval. 95%
1072 confidence intervals are represented in dashed lines. **|| in black-and-white colour on the
1073 Web and in print - 1-column fitting image ||**

1074

1075 **Figure 6.** Correlations ellipses between courtship traits for polyandrous (black) and
1076 monogamous (dark grey) males. This figure is a graphical representation of the correlation
1077 values provided in Table 4. The dotted light grey circle represents a null correlation ($r = 0$).
1078 The stronger the correlation, the narrower the ellipse becomes. **|| in black-and-white colour
1079 on the Web and in print - 2-column fitting image ||**

1080

1081 **Figure A1.** Correlations ellipses between LRR song traits for polyandrous (black) and
1082 monogamous (dark grey) males. This figure is a graphical representation of the correlation
1083 values provided in Table A4. The dotted light grey circle represents a null correlation ($r = 0$).
1084 The stronger the correlation, the narrower the ellipse becomes.

1085

Figure B1. Example of HRR interpulse interval lengthening along HRR bursts, over courtship duration. Three random songs are represented (the three types of symbols in black, dark grey and grey), with each data point showing the mean HRR interpulse interval value of a single burst of song. A trend line showing the relationship between HRR interpulse interval and courtship duration was added for each song, for illustration purposes only. The Spearman correlation coefficient (r) and its associated p -value (P) are given for of each song as follows:

black squares ($r = 0.69$, $P = 0.002$), grey circles ($r = 0.59$, $P = 0.057$), crosses ($r = 0.57$, $P = 0.001$). HRR = high-repetition rate song, IPI = interpulse interval.

Figure B2. Violin plots showing the distribution of: a) burst number and b) pulse number depending on sexual selection treatment, and c) burst number and d) pulse number depending on recording temperature distribution. The means (grey circles) \pm 1 standard deviation (vertical grey bars) are represented. E = polyandrous males, M = monogamous males.

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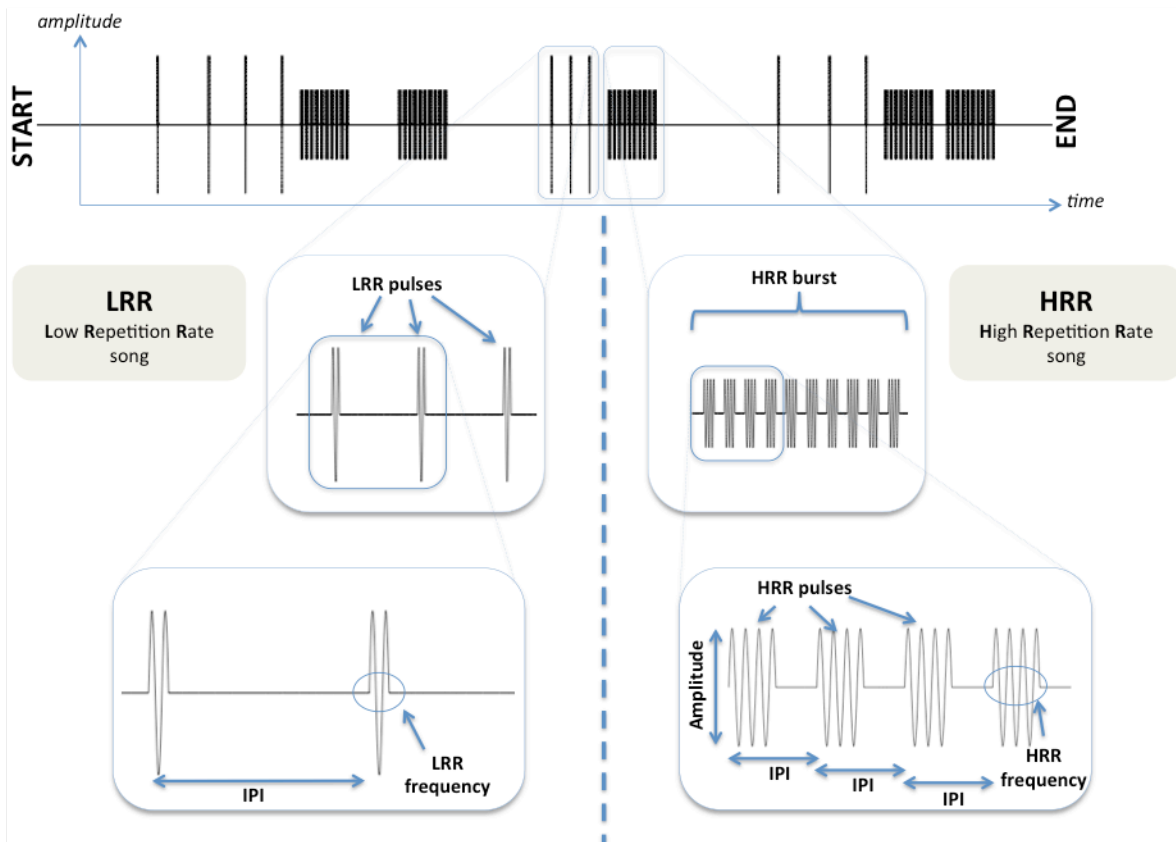
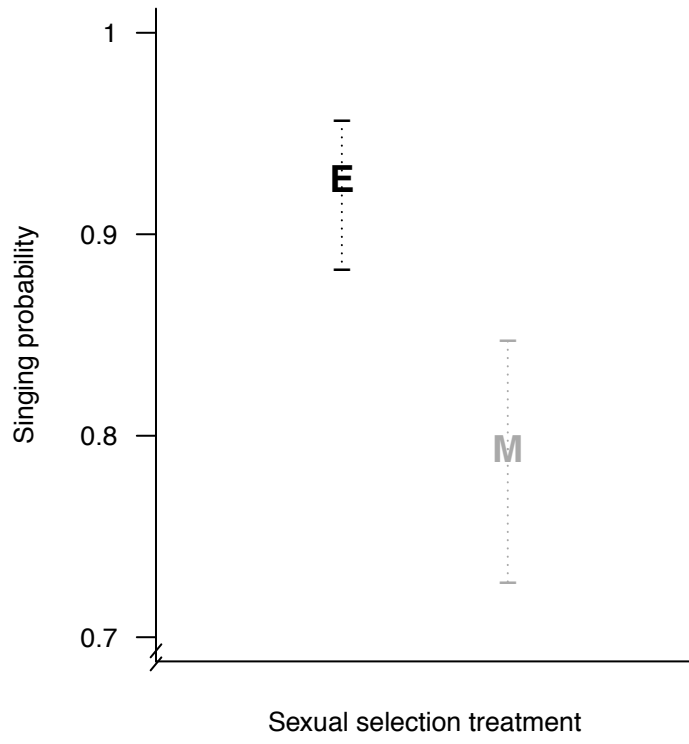


Figure 1

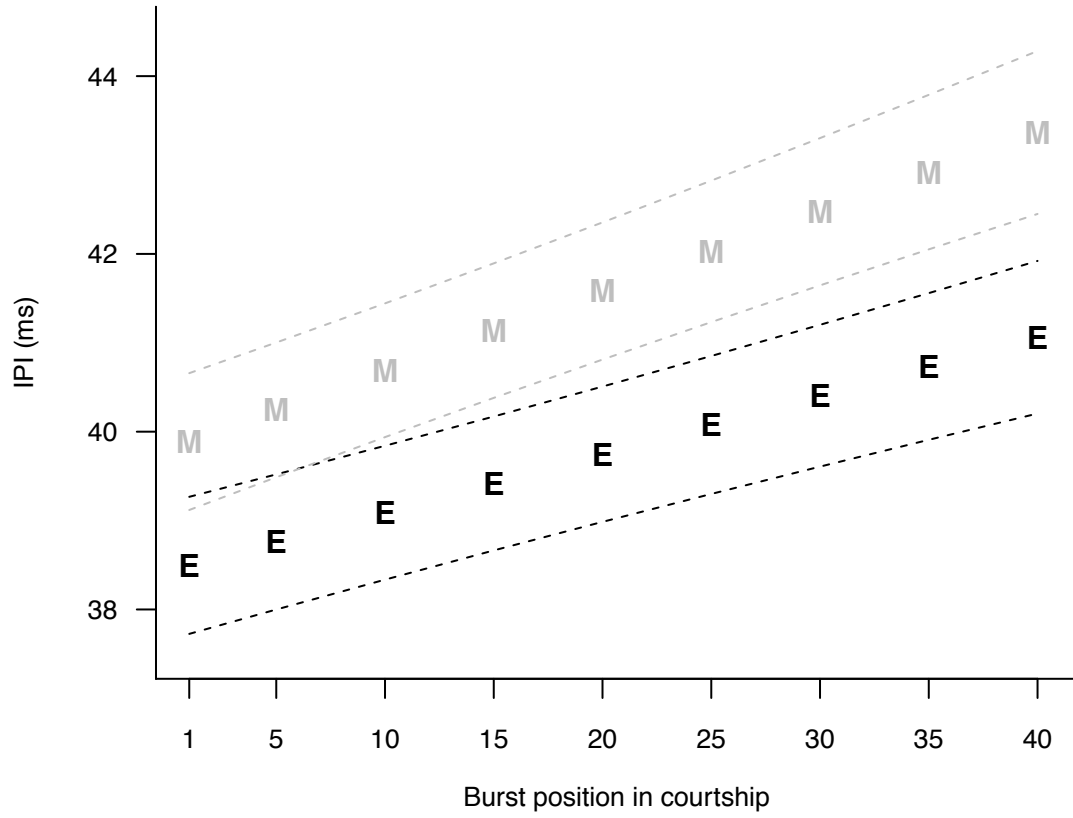


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Figure 2

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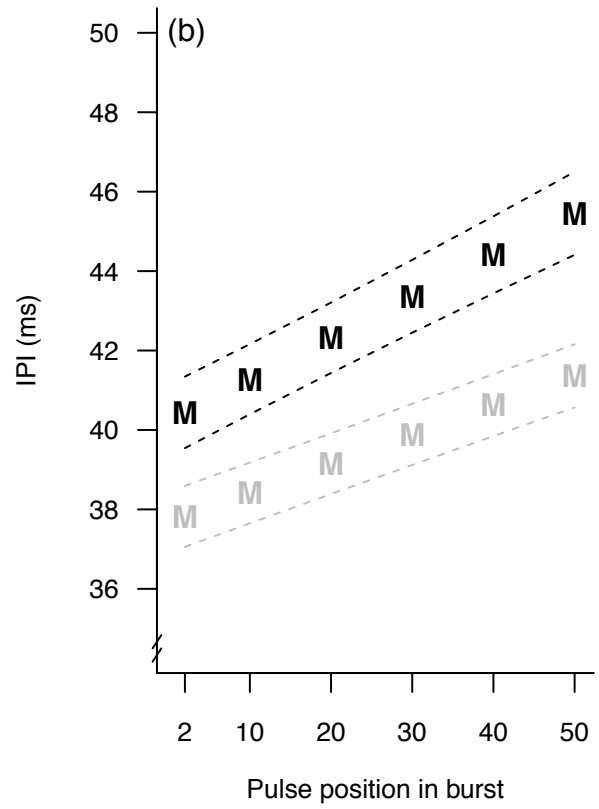
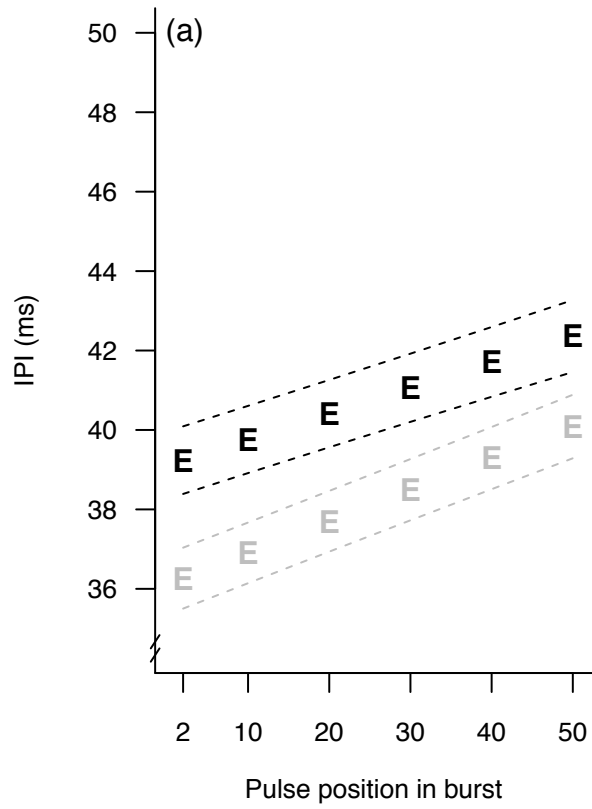


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1092 **Figure 3**

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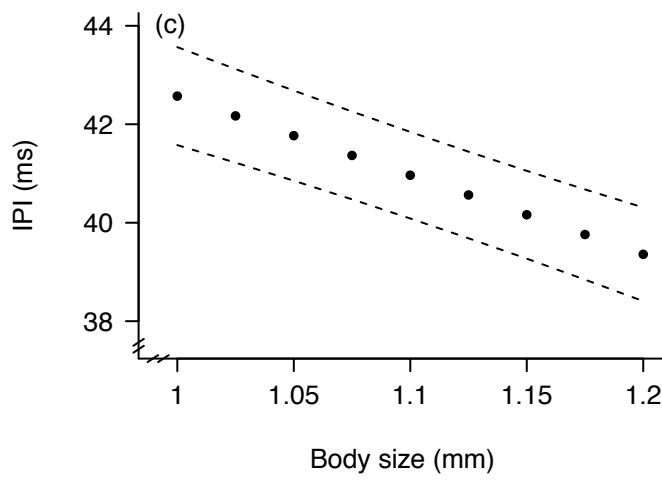
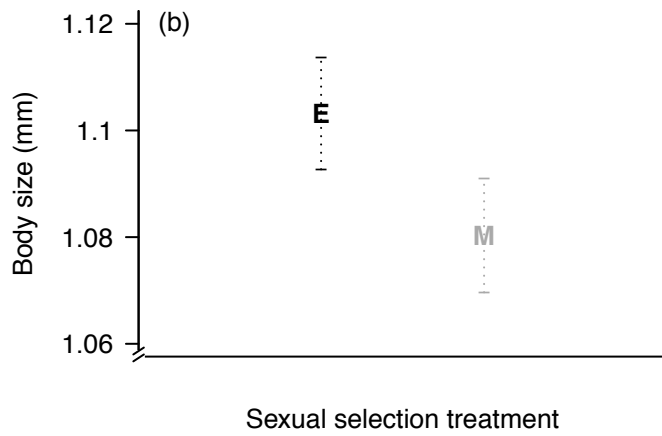
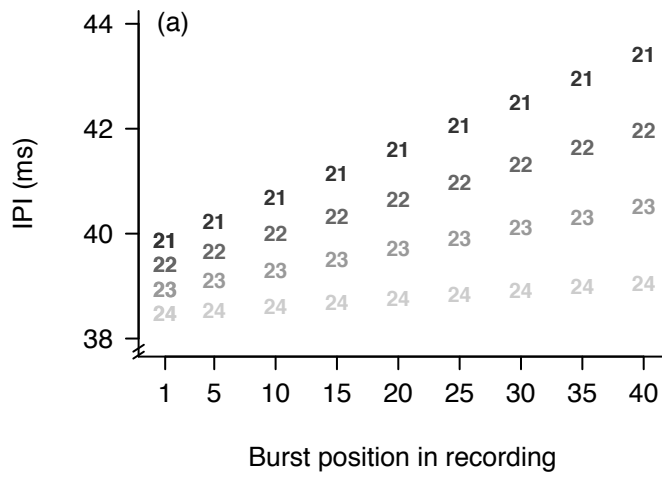
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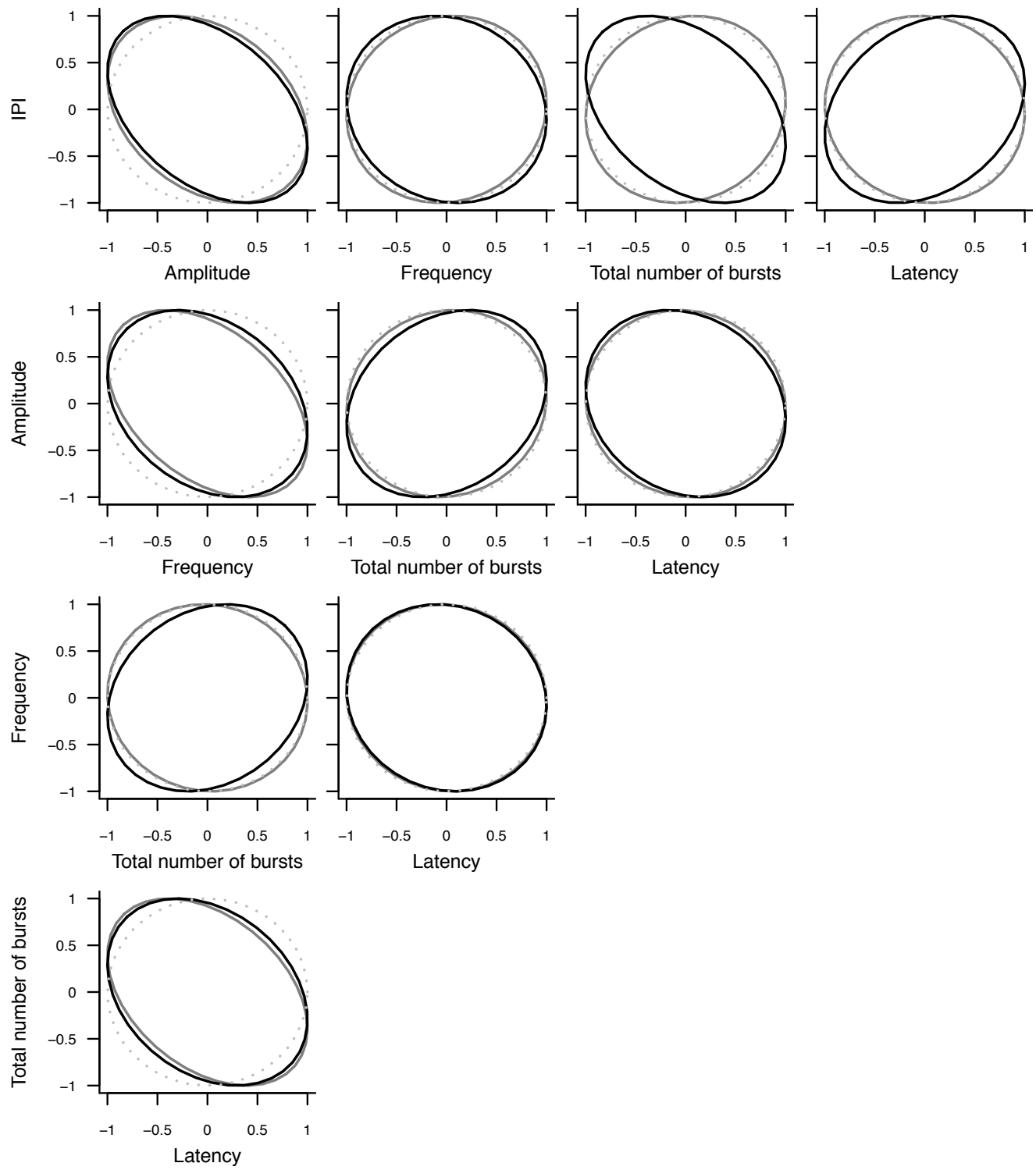
1096 **Figure 4**

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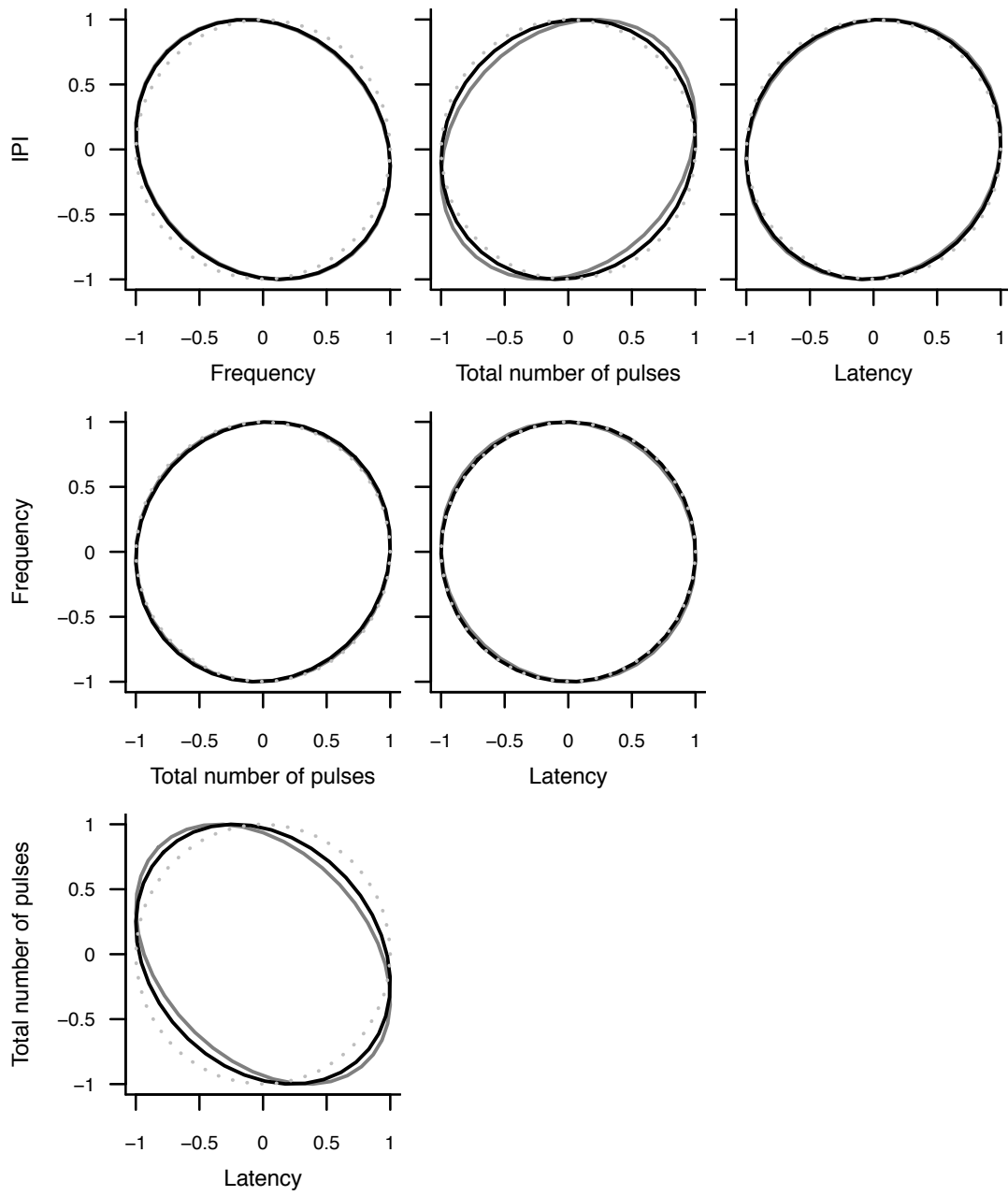
1099 **Figure 5**



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1101 **Figure 6**

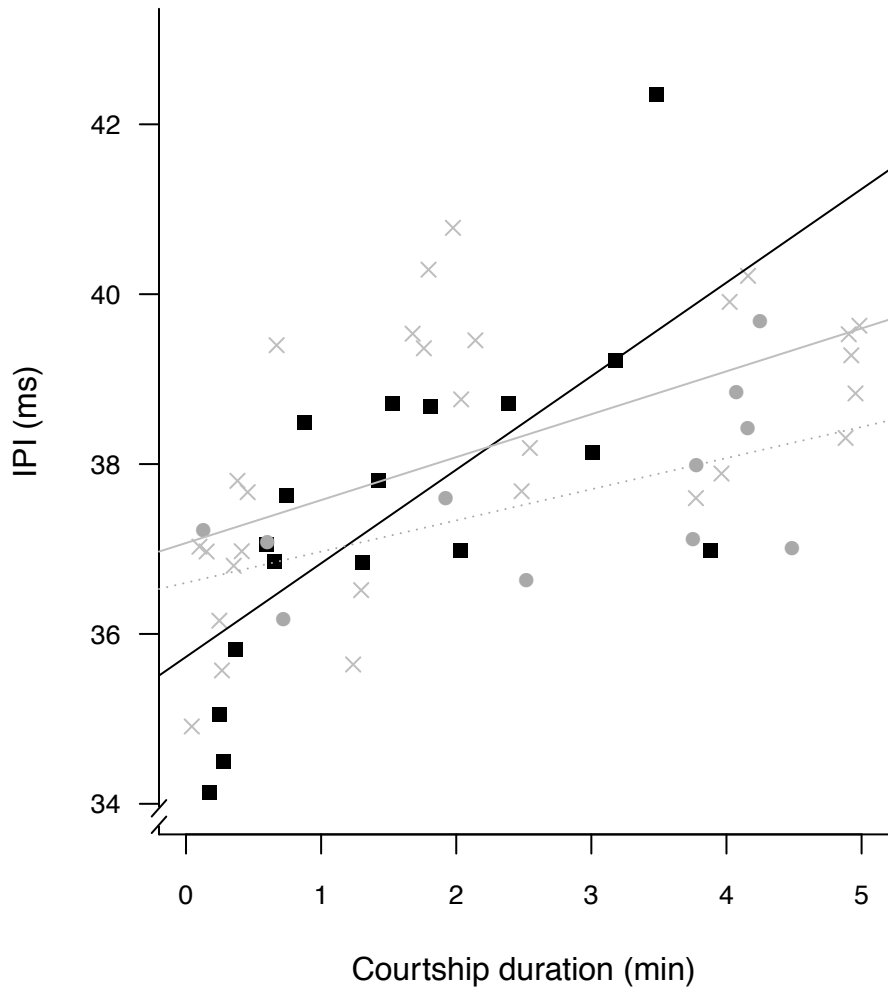
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1104 **Figure A1**

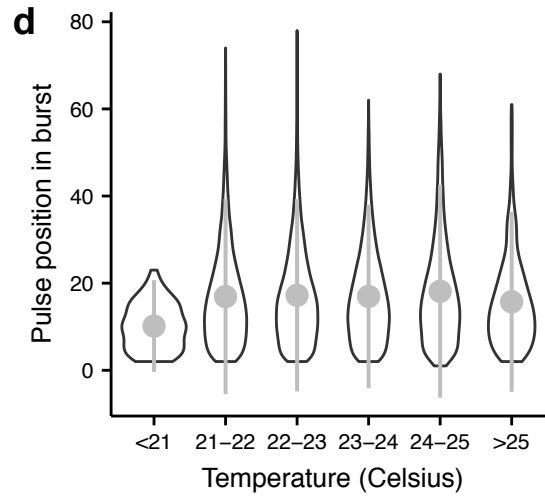
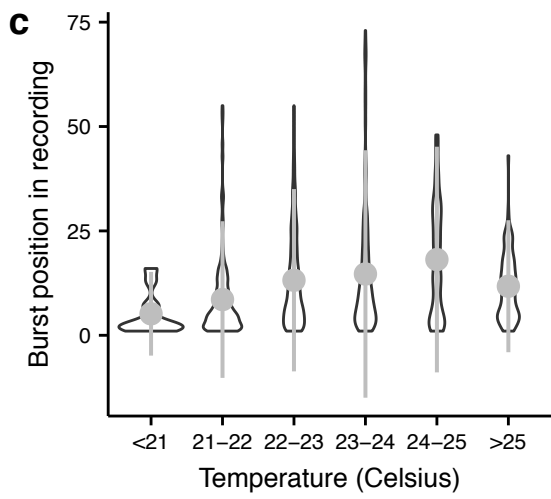
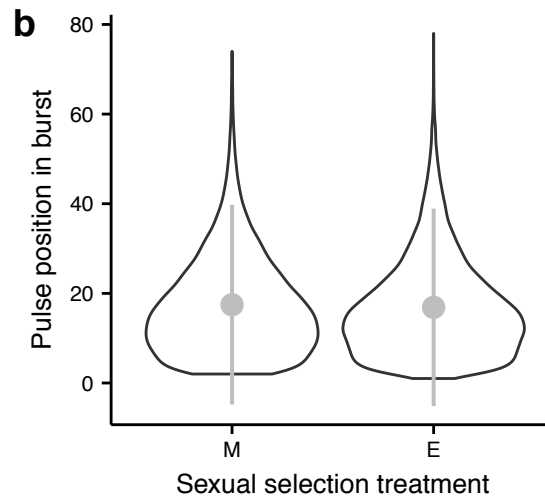
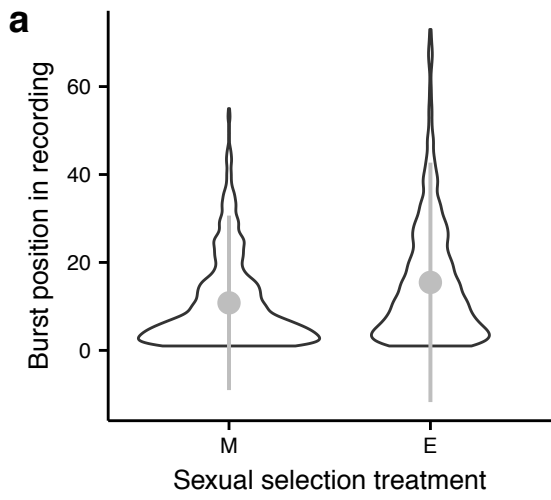
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1107 **Figure B1**

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1110 **Figure B2**