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**Attendance, but not performance, predicts good genes in a lek-breeding treefrog**

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(Article begins on next page)

1 ATTENDANCE, BUT NOT PERFORMANCE, PREDICTS GOOD GENES IN A  
2 LEK-BREEDING TREEFROG

3 Botto Valentina, Castellano Sergio

4 Dipartimento di Scienze della Vita e Biologia dei sistemi.

5 Via Accademia Albertina, 13. 10123 TORINO, ITALY

6 RUNNING TITLE:

7 Lek attendance, display performance and good genes in treefrogs

8

9 LAY SUMMARY:

10 The "good genes" hypothesis predicts a positive correlation between offspring fitness  
11 and paternal attractiveness and, in the lekking Italian treefrog, males that call more are  
12 more attractive to females. However, we found offspring fitness to correlate with the  
13 seasonal lek attendance of fathers, but not with their nightly calling effort. We suggest  
14 that female preferences ensure that only high-quality males can attend the lek  
15 frequently; females thus get genetic benefits despite preferring poorly reliable signals.

16

17 ABSTRACT

18 According to the good-genes hypothesis, females prefer males with costly displays  
19 because the costs are reliable indicators of genetic quality. In leks, mating costs result  
20 from a multiplicative interaction between the number of display performances (lek  
21 attendance) and the energetic expenditure associated with each display performance. If  
22 males differ in their allocation strategies between the two components of mating effort,  
23 the reliability of display performance as an indicator of genetic quality may be  
24 disrupted. Here, we investigate the association between male genetic quality and both  
25 lek attendance and display performance in the Italian treefrog, *Hyla intermedia*. We  
26 recorded lek attendance and display performance (nightly calling effort) during two  
27 breeding seasons, and we set up a breeding experiment to evaluate sire effects on three  
28 larval fitness-related traits (growth rate, age and size at metamorphosis). Attendance and  
29 calling effort were positively correlated with each other and both with male mating  
30 success, providing no evidence for a performance-attendance trade-off at the population  
31 level. In the breeding experiment, we found some evidence for an effect of sire identity  
32 and attendance on both growth rate and age at metamorphosis, but no evidence for an  
33 effect of sire calling effort. We conclude that female preference, by imposing high  
34 quality standards for calling males, strengthens the role of endurance rivalry in male  
35 mating competition and, thus, it indirectly favors males of higher-than-average mating  
36 effort. Under this scenario, although male displays are unreliable indicators of mating  
37 quality, females nevertheless gain benefits because of the reduced risk of coming upon  
38 low quality males.

39 **Keywords:** endurance rivalry, female choice, sexual selection, good genes, attendance-  
40 performance trade-off.

41 INTRODUCTION

42 In lek-breeding species, males aggregate in areas from where they perform their  
43 courting displays to attract females and may engage in physical or acoustical contests to  
44 defend their display arenas from intruders (Höglund and Alatalo, 2014). In these  
45 species, the most important determinants of male mating success are two distinct but not  
46 mutually exclusive mechanisms of selection: endurance rivalry and female choice  
47 (Andersson, 1994; Koenig and Albano, 1986). Both these mechanisms are expected to  
48 favor males of higher-than-average mating effort. Endurance rivalry, which is an  
49 interaction-independent selective mechanism (Murphy, 1998), selects for males with  
50 higher-than-average lek attendance; whereas female choice, which depends on the  
51 female-male interaction, selects for males with the most attractive (and costly) display  
52 performances. Since mating effort is constrained by the amount of resources available to  
53 males, which, in turn, depends on males' physical conditions and genetic quality, both  
54 endurance rivalry and female choice are expected to select for males of higher-than-  
55 average genetic quality.

56

57 Lek attendance and display performance, however, interact multiplicatively in  
58 determining male mating effort and thus, when assessed independently, these two  
59 factors may convey unreliable information about their combined effect. This is  
60 particularly relevant for mate choice in lekking species in which females visit the lek  
61 only when they are ready to mate, because, in these species, females can assess  
62 prospective mates only on the basis of their display performance, but are unaware of  
63 their lek attendance. If individual males vary in the attendance-performance tradeoff,  
64 then the reliability of males' displays as indicators of good-genes would inevitably

65 decrease (Kokko et al., 1999). Castellano (2009) used game theory to investigate the  
66 effect of the attendance-performance tradeoff on mate choice. According to his model,  
67 strong selection on males' display performance would force low-quality males to invest  
68 proportionally more on performance than high-quality males, making the assessable  
69 mating signals much less reliable indicators of signalers' quality than the non-assessable  
70 lek attendances. In contrast, weak selection on males' performance would have the  
71 opposite effect of shifting the tradeoff in the direction that maximizes attendance to the  
72 detriment of performance, making the latter a much more reliable indicator of good  
73 genes than the former. The model suggests that female choice can be favored even when  
74 mating signals are only poorly reliable indicators of mating effort (and good genes), as  
75 long as the risk of choosing low-quality males is more than compensated by the effects  
76 of endurance rivalry, which reduces the frequency of low-quality males at leks  
77 (Castellano, 2009).

78

79 Most empirical tests of the good-genes hypothesis focus on the predicted positive  
80 association between male display performance and genetic quality (Prokop et al., 2012),  
81 without considering the effects of the performance-attendance trade-off. In this paper,  
82 we focus on this trade-off. Using the Italian treefrog (*Hyla intermedia*) as a model, we  
83 describe the effects of both lek attendance and display performance on male mating  
84 success in a small natural lek, and we estimate their covariation with male genetic  
85 quality in a half-sibling breeding experiment. As in most anurans with prolonged  
86 breeding seasons, male Italian treefrogs spend a variable number of nights at the chorus,  
87 where they engage in highly energetically-demanding acoustic competitions consisting  
88 of long bouts of short, amplitude-modulated calls (Castellano et al., 2002). Females visit

89 the lek only when they are ready to reproduce, and mate preferentially with males  
90 whose calls have higher-than-average call-rate and longer-than-average call-group  
91 duration (Castellano et al., 2009). For these reasons, the number of nights spent in the  
92 chorus is a good descriptor of male attendance, whereas the nightly calling effort (either  
93 the number of calls or the time spent calling) is a good descriptor of male display  
94 performance. In this species, males provide no apparent direct benefits to females;  
95 therefore, the Italian treefrog is a good candidate to test the good-genes hypothesis for  
96 the evolution of female choice.

97

## 98 MATERIAL AND METHODS

### 99 *Field activities*

100 The study was carried out over two consecutive breeding seasons, from April 22<sup>nd</sup> to  
101 June 27<sup>th</sup> in 2013 and from April 20<sup>th</sup> to June 20<sup>th</sup> in 2014. The breeding site, situated in a  
102 sparsely inhabited rural area in central Piedmont (NW Italy), was a 5x1x0.3 m artificial  
103 pond obtained by aligning five 1-m<sup>2</sup> pools (Bestway mod. 56217B, Shanghai, China).  
104 The pond had been regularly used by treefrogs as a breeding site for a few years before  
105 the onset of the experiments described in this paper and supported a stable population of  
106 about 35 males during both years of the experiment.

107 Each night, we checked the lek every 45 minutes to record the identity and the position  
108 of all males and females at the breeding site. Untagged treefrogs were kept overnight in  
109 individual fauna-boxes. On the following morning, they were anesthetized with a 2%  
110 solution of tricaine methanosulfonate (MS222 Sandoz, Sandoz Inc., Princeton, NJ,  
111 USA), their snout-vent length (SVL) was measured ( $\pm 0.01$  mm) with a digital calliper  
112 (Mitutoyo Absolute CD-15DCX, Mitutoyo Corporation, Kawasaki, Kanagawa, Japan),

113 and they were marked with unique subcutaneous alphanumeric tags (Northwest  
114 Marine Technology Inc., Shaw Island, WA, USA) injected in the tarsal area on the left  
115 hind leg.

116

117 Male mating success was assessed during the regular nightly visits at the breeding pond  
118 Each amplexed couple was removed from the breeding site, and the identity of both  
119 partners was checked without separating them. A part of the captured couples were then  
120 separated and used in the breeding experiment described below; the remaining couples  
121 were instead housed in separate fauna boxes to complete mating and egg laying. Since  
122 the breeding pond was relatively isolated from nearby water bodies (min. distance  $\approx 0.4$   
123 km) and males usually show, within a breeding season, a high-degree of site-fidelity  
124 (pers. obs.), we exclude that the males involved in our observation visited other ponds;  
125 therefore, we are reasonably convinced that we observed all the matings gained by each  
126 experimental male.

127

128 *Recording apparatus, acoustic analyses and the estimate of males' calling effort*

129 For the whole duration of the two breeding seasons, we recorded the calling activity at  
130 the lek using a multichannel recording apparatus. We set up four fixed recording points.  
131 By representing the pond as a rectangle with vertices at (0,0), (0,1), (5,0), (5,1), the  
132 recording points had coordinates (2,0), (4,0), (1,1) and (3,1). At each point, at about 50  
133 cm above the ground, we placed two Sony ECM-T6 condenser microphones (Sony  
134 Corporation, Tokyo, Japan) pointing toward the center of the pond, respectively, with a  
135 45° and a 135° orientation. To improve directionality, we placed a 25x25 cm wood  
136 panel covered with a 30-mm thick pyramid polyurethane acoustic foam between, above,

137 and behind the two microphones. The eight microphones were connected to an 8-  
138 input/8-output audio interface M-Audio Fast Track 8R (Avid Technology Inc.,  
139 Burlington, MA, USA). Multi-channel recording (sample rate = 44.1 kHz) was carried  
140 out using the open source software KRISTAL Audio Engine (Kreatives.org). Each  
141 night, we monitored the lek from dusk to the end of male activity, and we carried out 92  
142 eight-channel recordings (49 in 2013 and 43 in 2014), during which the chorus activity  
143 lasted from 11 to 190 min (median = 79 min).

144

145 The analysis of the recordings followed a three-step procedure. First, after a 11025-Hz  
146 resampling, we visualized the waveforms of all eight channels of a recording session  
147 using the free software Audacity 2.0.6 (<http://audacity.sourceforge.net/>). In most cases,  
148 a channel had its own focal male, which was recorded at a much higher intensity than  
149 the chorus background noise. In these cases, by using the field notes (see above), we  
150 could unequivocally identify the male calling on that channel. Sometimes, however, two  
151 or more males happened to call close to each other and equally distant from the nearest  
152 microphone, at least for a portion of the recording session. In these cases, by taking  
153 advantage of the partial overlapping of the recording space of different microphones, we  
154 were able to assign the calls to different males (see below). When this was unfeasible,  
155 the portion of the recording session was considered as ‘time-out’ and excluded from the  
156 calculation of the male’s calling effort. Once the assignment was completed, we used a  
157 custom-designed Matlab program (The Mathworks Inc., Asheboro, NC, U.S.A.), to  
158 automatically cut in separate, 60s wav files, whose name retained information of their  
159 temporal position relative to the start of the recording session.

160



161 In the second phase of acoustic analyses, we used a Matlab graphical-user-interface  
162 (GUI) program to process the 60-s files. The program allowed the operator to analyze  
163 up to three wav files simultaneously, in separate panels. In this way, the operator could  
164 visualize and compare the calling activity recorded simultaneously at three distinct  
165 recording points. In each panel, the program computed the amplitude envelope and  
166 derived a binary peak array (a vector that assigned “one ” to the points of within-call  
167 maximum intensity and “zero” otherwise), showing the position of single calls. To  
168 select the calls of the focal male, the operator set the intensity threshold at a value that  
169 was halfway between the intensity of the focal male and the intensity of the other  
170 interfering males and chose a gate function of appropriate length. In this way, the  
171 operator was able to exclude all calls that were below the intensity threshold or that fell  
172 in the same gate window of previously identified calls. When two males (e.g. m1 and  
173 m2) were recorded at similar intensity on a channel (e.g. C1), the intensity threshold did  
174 not allow us to discriminate between them. However, if one of these males (e.g. m2)  
175 was recorded also on a second channel (e.g. C2), at an intensity that provided effective  
176 discrimination, then the program allowed us to subtract from the C1 peak array (m1 +  
177 m2) the C2 peak array (m2) and, thus, to filter m2 out of C1. In this way, we could  
178 discriminate up to three males on a single channel, provided that at least one of them  
179 was recorded with an appropriate signal-to-noise ratio on another channel. After visual  
180 inspection, the program allowed the operator to delete (or to add) those peaks, which the  
181 previous analyses erroneously included (or excluded), to modify the setting and repeat  
182 the analysis, or to conclude. In the latter case, the program saved a txt file listing the  
183 temporal position (sample number) of all the peaks for each 60s portion.

184

185 In the third step of analysis, we concatenated all the txt files of a male recording session  
186 and derived a quantitative description of his nightly calling activity. We assigned each  
187 call to a new call-group if the interval from the previous call was longer than 1 s. We  
188 calculated the duration of both the call-groups and the inter call-groups (time lag  
189 between two successive call-groups). Notice that, when there was a ‘time-out’ in the  
190 recording session, inter-callgroup duration was a missing value. Finally we calculated  
191 the nightly calling effort by dividing the sum of all the call-group durations by the  
192 effective duration of the entire recording session, which was defined as  $T_{session}=T_{end}-T_0-$   
193  $T_{out}$ .  $T_{end}$  and  $T_0$  were the times, respectively, of the last and the first call recorded on any  
194 one of the eight channels, whereas  $T_{out}$  was the total time-out duration of that male.  
195 When a calling male was later observed mating, we considered his  $T_{end}$  as the time of his  
196 last call.

197

### 198 *Breeding experiment*

199 In order to analyze the effects of parental identity on offspring development, we set up a  
200 breeding experiment using an incomplete factorial design, which allowed us to  
201 disentangle maternal and paternal effects (Roff, 1997). Over the two years, we used nine  
202 quartets composed by two males and two females. Within each quartet, we randomly  
203 assigned a male to each female; the amplexed couples were then allowed to mate in  
204 separate tanks until a sufficient amount the eggs had been laid; then, the couples were  
205 separated and the partners were switched and moved to different tanks, to avoid mixing  
206 of eggs fertilized by different parents. This approach allowed us to produce four  
207 families for each quartet. Since in only seven quartets (five in 2013 and two in 2014)  
208 were we able to obtain enough tadpoles from all the four possible families, our breeding

209 experiment involved 28 full-sib families, each with two replicates of 15 randomly  
210 selected tadpoles. Full-sib tadpoles that were excluded from the experiment were kept at  
211 a similar density in separate tanks, to be eventually used as a reserve if some of their  
212 experimental sibs accidentally died. Since even slight variations in tadpole density  
213 might have had dramatic effects on tadpole growth and development (e.g., Wilbur,  
214 1976; Cadeddu & Castellano, 2012), the number of tadpoles in each replicate was  
215 checked daily. During the first two weeks of the 2013 breeding experiment, a few  
216 tadpoles died because of a predation event, and five others died because of accidental  
217 errors during the manipulation. Those tadpoles were immediately replaced with full-sibs  
218 from the reserve stock.

219

220 In the two years, experimental matings involved randomly selected males and were  
221 carried out during the first two-three days of the first peak of breeding activity, in order  
222 to ensure comparable rearing conditions to all the tadpoles in the experiment. Indeed,  
223 egg hatching of all families was almost perfectly synchronous and tadpoles developed  
224 under similar environmental conditions. The 15 tadpoles from each replicate were  
225 transferred to 40l plastic tanks and fed ad libitum until metamorphosis. Since larval  
226 growth is affected by sun irradiation (Smith-Gill and Breven, 1979), we placed the tanks  
227 outdoor in a sunny lawn and, once a week, when we changed the water, we also  
228 randomly swapped the position of the tanks.

229

230 Every week we randomly captured eight tadpoles from each tank, we placed them in a  
231 Petri dish, lined with graph paper, and photographed them using a Sony Cyber-Shot  
232 DSC-HX200V camera (Sony Corporation, Tokyo, Japan). From the pictures, after

233 calibration, we measured tadpoles' SVL with the open source software ImageJ  
234 (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA,  
235 <http://imagej.nih.gov/ij/>, 1997-2014). These weekly measures were then used to fit  
236 growth curves and estimate growth rate for each replicate (see below). At the  
237 completion of metamorphosis, froglets were captured, anesthetized with a 1% solution  
238 of MS222 Sandoz and measured to the nearest 0.01 mm. Due to logistical constraints, in  
239 both breeding seasons, the rearing experiment was ended in late July, 75 days after egg  
240 hatching. This time frame is adequate to observe complete tadpole development in the  
241 model species at our latitudes; indeed 765 tadpoles out of 840 (91.1%) completed  
242 metamorphosis by the end of the experiment. All the tadpoles that failed to complete  
243 metamorphosis were still alive at the end of the experiment and they were released in  
244 the breeding site to complete the larval development.

245

246 We considered three inter-related life-history traits that are known to affect fitness. The  
247 first two, body size (SVL) and age at metamorphosis (AGE), were directly measured on  
248 the first 10 froglets that completed metamorphosis within each replicate; whereas the  
249 third trait, the larval growth rate (GROWTH), was estimated by fitting the within-  
250 replicate averages of weekly tadpoles' SVL ( $l_t$ ) and age ( $t$ ) to the von Bertalanffy  
251 growth equation,  $L_t = L_\infty \cdot (1 - e^{GROWTH \cdot (t-t_0)})$ , where  $L_\infty$  was set at 1.688 cm  
252 (observed maximum body size) and  $t_0 = 0$ .

253

254 *Ethic statement*

255 The “Regione Piemonte – Direzione Ambiente – Settore Sostenibilità e recupero  
256 ambientale, bonifiche” provided the authorization for tree frog capture and handling  
257 (permits n° 152 and 153 issued on February 25<sup>th</sup>, 2013).

258

259 *Testing hypotheses and statistical analyses*

260

261 Our study had two main aims: to identify the effect of mating effort on male mating  
262 success and to estimate the parental (in particular, paternal) effects on offspring fitness.

263

264 To accomplish the first of these objectives, we analyzed male mating success both at a  
265 seasonal and at a nightly level. At seasonal level, we regressed the seasonal mating  
266 success of a male against his SVL, chorus attendance (number of nights), and mean  
267 nightly calling effort (Poisson error distribution, log-link function). At nightly level, we  
268 considered the recording sessions in which there were two or more calling males and in  
269 which one of them mated. For each mating episode matching these requirements (N =  
270 20), we measured the calling effort (sum of all call-group durations) of all males at the  
271 lek either 5, 15 or 30 minutes before mating occurred (which we established as the time  
272 of the last call uttered by the male later observed mated) and we used generalized linear  
273 models with binomial error distribution and logit-link function to assess the effect of  
274 SVL and calling effort on mating success. In these models the mating episode appeared  
275 as a random factor. If a male was at the lek during a mating episode (because he was  
276 recorded before and after the mating occurred), but his call was not recorded during the  
277 time window considered, we assigned him a zero calling effort. To investigate the effect  
278 of chorus size on male mating success, for each of the 20 mating episodes we analyzed

279 the chorus activity during the 30-min before mating occurred and ranked all calling  
280 males on the basis of their calling effort (silent males were excluded). Then, we used a  
281 randomization procedure to test the hypothesis that mating success was independent of  
282 male calling rank. Specifically, we calculated on the entire sample of the 20 mating  
283 episodes the sum of percentile ranks of successful males,  $S = \sum_{i=2} \sum_j^i R(i, j) * f(i, j)$ ,  
284 where  $i$  is the number of males in the chorus,  $j$  is the absolute rank,  $R$  is the percentile  
285 rank of the male with absolute rank  $j$  in a chorus of size  $i$  ( $R(i, j) = \frac{j-0.5}{i} * 100$ ), and  $f$  is  
286 the number of males with rank  $R(i, j)$  that mated successfully. Then, we randomly  
287 assigned the mating success within a chorus and recalculated the resulting sums of  
288 percentile ranks of successful males  $S'$ . After 100,000 randomizations, we estimated the  
289 probability of the null hypothesis as the relative frequency of randomizations with  $S' \geq$   
290  $S$ . The randomization test was carried out, first, on the entire sample. In addition, we  
291 computed average chorus size ( $\bar{x} = 4.65$ ) and, we repeated the test on both smaller-  
292 than-average and larger-than-average choruses.

293

294 We analyzed differences in mating success, lek attendance and calling effort between  
295 sires and not-sires with the Mann-Whitney statistics, and we tested for between-group  
296 differences in the regression coefficients of these three variables by means of general or  
297 generalized linear models. To estimate the sires' and dams' effects on offspring life-  
298 history traits we used two types of linear models. When the dependent variable was  
299 either SVL or AGE, in order to obtain a fully balanced dataset and to minimize  
300 systematic error in the estimate of the mean age at metamorphosis (see Supplementary  
301 Materials), we considered the first 10 froglets reaching metamorphosis in each replicate  
302 (i.e. the minimum number of froglets obtained from all replicates). The models had five

303 main random factors and one interaction term: (i) the breeding season, (ii) the quartet  
304 nested within the year; (iii) male identity, (iv) female identity and (v) the male-female  
305 interaction nested within quartet and year, and (vi) the replicate nested within male-  
306 female interaction, quartet and year. When the dependent variable was GROWTH (for  
307 which we had only one value in each replicate), the replicate could not be included in  
308 the model and was therefore omitted.

309

310 To analyze the effect of sires' phenotype on each of the three offspring traits, we used a  
311 series of general linear models, whose independent categorical factors were year,  
312 quartet, and dam identity, as described in the previous paragraph, and which included as  
313 covariates either the SVL, the attendance or the calling effort of sires. To avoid  
314 pseudoreplication, these analyses were carried out using the mean values in each family.

315

## 316 RESULTS

317

### 318 *Male mating effort and reproductive success*

319 Calling activity occurred on 42 nights in 2013 and 37 nights in 2014. During these  
320 nights, the average number of males at the lek was 4.55 in 2013 (range: 1-16) and 3.49  
321 in 2014 (range: 1-15). On 15 nights in 2013 and on 9 night in 2014, only one male was  
322 calling at the breeding site. Gravid females were observed at the lek on 13 nights in  
323 2013 and 11 in 2014. In total, we observed 32 matings in 2013 and 20 matings in 2014.

324

325 Table 1 shows the descriptive statistics of three predictors of mating success (male  
326 SVL, lek attendance and calling effort) in 2013 and 2014. In both years, attendance was

327 highly skewed towards lower values and was almost twice as variable as male calling  
328 effort. The frequency distribution of mating success (Fig. 1) differed significantly from  
329 a Poisson distribution ( $\chi^2_2 = 9.419, p = 0.009$ ), providing evidence that individual males  
330 differed in mating probability. Independent of study year, seasonal male mating success  
331 was positively associated with both attendance ( $b = 0.085, \text{s.e.} = 0.023, \chi^2_1 = 13.266, p <$   
332  $0.001$ ) and calling effort ( $b = 5.561, \text{s.e.} = 1.635, \chi^2_1 = 11.566, p = 0.001$ ), whereas it  
333 was positively, but not significantly, associated with SVL ( $b = 0.077, \text{s.e.} = 0.0463, \chi^2_1$   
334  $= 2.79, p = 0.095$ ). Independent of the breeding season, Generalized Linear Models  
335 showed positive associations between the three predictors of male mating success, but  
336 the association was statistically significant only between attendance and calling effort ( $b$   
337  $= 2.912, \text{s.e.} = 0.620, \chi^2_1 = 22.065, p < 0.001$ ).

338

339 At the nightly level, binomial regressions showed that mating success was significantly  
340 affected by calling effort, when chorus activity was measured over both a 30-min ( $b =$   
341  $0.241, \text{s.e.} = 0.115, z = 2.091, p = 0.036$ ) and a 15-min ( $b = 0.333, \text{s.e.} = 0.159, z =$   
342  $2.098, p = 0.036$ ) time window, but not over the shorter 5-min interval ( $b = 0.407, \text{s.e.} =$   
343  $0.297, z = 1.372, p = 0.17$ ). The effect of calling effort at the nightly level, however, was  
344 not very strong, because when the analyses were repeated excluding males that were at  
345 the breeding site but that did not call during the examined time window, the effect of  
346 calling effort was significant only over the 30-min time frame ( $b = 0.239, \text{s.e.} = 0.118, z$   
347  $= 2.026, p = 0.043$ ). Male SVL showed no significant effect on mating success in any of  
348 the time windows considered (results not shown).

349



350 To further analyze the effect of calling effort and chorus size on male mating success,  
351 for each of the 20 mating episodes, we assigned to all calling males a percentile rank on  
352 the basis of their calling effort in the 30 minutes before mating occurred. In Figure 2,  
353 we plot the percentile ranks of successful males against chorus size. A randomization  
354 test was carried out to test whether mating success was independent of male percentile  
355 ranks. When we considered the entire sample, the association between mating success  
356 and percentile rank was not statistically significant, although the null-hypothesis  
357 probability was very close to the rejection threshold ( $p = 0.05237$ , after 100,000  
358 randomizations). However, when we restricted the analysis to choruses of lower-than-  
359 average size (2-4 calling males), we found that high-ranking males were significantly  
360 more likely to mate than low-ranking rivals ( $p = 0.0085$ , after 100,000 randomizations).  
361 In larger-than-average choruses (5 or 6 calling males), in contrast, the association  
362 between percentile ranks and mating success was no longer significant ( $p = 0.3546$ ,  
363 after 100,000 randomizations), suggesting that the strength of selection on calling effort  
364 decreased with increasing chorus size.

365

#### 366 *The subset of sires*

367

368 Although the males involved in the breeding experiment were chosen randomly at the  
369 beginning of the seasons, actually they were not a random sample of the entire male  
370 population (Figure 3). Indeed, by the end of the breeding seasons, the sire sample  
371 showed a higher-than-average mating success ( $U = 151, p < 0.001$ ), a higher-than-  
372 average attendance ( $U = 94, p < 0.001$ ) and a higher-than-average seasonal calling effort  
373 ( $U = 150, p = 0.01$ ) (see Tab. 2), and there was no evidence for a positive association

374 either between attendance and calling effort ( $b = -0.122$ ,  $s.e. = 1.196$ ,  $\chi_1^2 = 0.10$ ,  $p =$   
375  $0.918$ ) or between mating success and attendance ( $b = 0.048$ ,  $s.e. = 0.041$ ,  $\chi_1^2 = 1.371$ ,  $p =$   
376  $= 0.242$ ) or mating success and calling effort ( $b = 4.886$ ,  $s.e. = 2.828$ ,  $\chi_1^2 = 2.985$ ,  $p =$   
377  $0.084$ ). Furthermore, by comparing the linear relationships of sires and not-sires, we  
378 found significant differences in the regression coefficients between attendance and both  
379 calling effort ( $\chi_1^2 = 4.823$ ,  $p = 0.028$ ) and mating success ( $\chi_1^2 = 4.054$ ,  $p = 0.044$ ),  
380 whereas the differences in the regression coefficients between mating success and  
381 calling effort were not significant, but close to the significance statistical threshold ( $\chi_1^2$   
382  $= 3.731$ ,  $p = 0.053$ ).

383

#### 384 *Offspring life-history traits*

385 Table 3 shows results of the general linear models used to infer the parental effects on  
386 offspring SVL and AGE, as well as the variance estimates ( $s^2$ ) for each factor. Our  
387 experiment provided no evidence for either a sire or a dam effect on offspring SVL. In  
388 contrast, AGE was significantly affected by both the dam and sire identity, with the  
389 former somewhat stronger than the latter (Tab. 3). It is important to underline, however,  
390 that the sire effect on age at metamorphosis lost significance when unbalanced datasets  
391 of 12 or more tadpoles were used for the analyses (see Supplementary Materials, Fig.  
392 S2) Similarly, we observed a significant effect of both dam ( $F_{7,35} = 12.330$ ,  $p < 0.001$ )  
393 and sire ( $F_{7,35} = 3.417$ ,  $p = 0.007$ ) on GROWTH.

394

395 To analyze the effect of sires' phenotype on offspring life-history traits, we carried out a  
396 series of general linear models, in which SVL, attendance and calling effort were the  
397 covariates and year, quartet and dam were the controlling factors. Both SVL and calling

398 effort showed no significant effects on offspring traits (results shown in the  
399 Supplementary Materials – Tab. S2 and Tab. S3), whereas sire attendance was  
400 associated positively with GROWTH ( $b = 0.036$ ,  $s.e. = 0.011$ ,  $p = 0.004$ ) and negatively  
401 with AGE ( $b = -0.244$ ,  $s.e. = 0.051$ ,  $p < 0.001$ ). Remarkably, the negative association  
402 between paternal attendance and age at metamorphosis was robust to the number of  
403 tadpoles included in the analysis (see Supplementary Materials, Tab. S2).

404

## 405 DISCUSSION

406 The “good genes” hypothesis for the evolution of female mating preferences makes  
407 three assumptions. First, males compete against each other to occupy the highest  
408 attractiveness ranks. Second, attractiveness is positively associated with male genetic  
409 quality. Third, male genetic quality is heritable and affects offspring fitness (Hunt et al.,  
410 2004; Kokko et al., 2002). In lek-breeding species, however, males not only compete  
411 for the highest ranks in attractiveness (display performance), but also for the largest  
412 number of competitive episodes they take part (lek attendance) and it has been  
413 suggested that, under strong female choice, lek attendance may be a better predictor of  
414 male genetic quality than display performance (Castellano 2009). Our results are  
415 consistent with this hypothesis. In fact, in the study population, we observed (i) strong  
416 selection on both lek attendance and nightly performance, (ii) heritable variation in male  
417 genetic quality, and (iii) a positive association between offspring fitness and the lek  
418 attendance (but not the nightly performance) of sires. Specifically, two of the three traits  
419 used to describe offspring quality (growth rate and age at metamorphosis) showed a  
420 significant paternal effect (at least when using the ten-tadpole balanced dataset ), but  
421 none of them covaried with male calling effort (nightly performance). In contrast,

422 growth rate and age at metamorphosis did covary significantly with lek attendance,  
423 independent of the number of tadpoles included in the analysis. Since lek attendance  
424 and nightly performance correlate positively and interact multiplicatively to define male  
425 mating effort, our results support the hypothesis that sexual selection, by favoring males  
426 with higher-than-average mating effort, provides females with indirect genetic benefits.  
427 However, the results also suggest that these benefits are associated with male traits that  
428 cannot be assessed by females and arise mostly from interaction-independent  
429 (endurance rivalry) rather than from interaction-dependent (female choice) mechanisms  
430 of sexual selection.

431

432 *Lek attendance and interaction-independent sexual selection via endurance rivalry.*

433 Attendance positively affects male mating success in several lekking species (Höglund  
434 and Alatalo, 2014), including many anurans (Friedl and Klump, 2005) and, in particular,  
435 species of the *H. arborea* group (Castellano et al., 2009; Friedl and Klump, 2005;  
436 Jaquierey et al., 2010). In frogs and toads, the major constraint on lek attendance is  
437 individual capability to build up energy reserves both before and during the breeding  
438 season, with mortality and migration playing only a minor role (Marler and Ryan, 1996;  
439 Murphy, 1994a). Therefore, it is reasonable to assume that male attendance is a good  
440 indicator of male quality (condition and stamina). Even though the correlation between  
441 lek attendance and individual genetic quality, to our knowledge, has not been  
442 specifically investigated so far, some tempting results indicate that it may exist. In  
443 yearling black grouse *Tetrao tetrix*, for example, a negative correlation has been  
444 demonstrated between lek attendance and parasite load (Kervinen et al., 2012). In the  
445 little bustard *Tetrax tetrax*, meanwhile, a positive correlation was found between lek

446 attendance and body condition (Jiguet and Bretagnolle, 2014). In addition, Dyson et al.  
447 (1998) showed that male reedfrogs (*Hyperolius marmoratus*) who were successful in  
448 mating were more likely to return to the chorus than those that did not, and Murphy  
449 showed that male condition in *Hyla gratiosa* was positively related to chorus tenure  
450 (1994a) and found a positive correlation between chorus tenure and mating efficiency  
451 (nightly probability of mating) (Murphy, 1994b). In the European treefrog, *H. arborea*,  
452 Friedl and Klump (2005) found a positive correlation between lek attendance and inter-  
453 annual survival, and highlighted the absence of trade-off between chorus attendance and  
454 male growth rate.

455

456 Our results not only confirm the important role of lek attendance as a determinant of  
457 male mating success, but also suggest a strong association between paternal attendance  
458 and some fitness-related tadpole traits. In fact, males with longer lek attendance  
459 produced offspring that grew faster and reached metamorphosis at an earlier age. This is  
460 a clear benefit for the offspring, since an early metamorphosis decreases tadpole  
461 exposure to predators, minimizes the risk of death due to pond drying and positively  
462 affects short-term and long-term post-metamorphic survival (Altwegg and Reyer, 2003),  
463 as well as adult fecundity (Smith, 1987). Our findings, however, contrast with those  
464 obtained by Jaquiéry et al. (2010) in a population of European treefrogs, where  
465 offspring fitness was associated not with their father's attendance, but with his nightly  
466 probability of mating (i.e. total number of matings divided by attendance), an indicator  
467 of male attractiveness. This discrepancy suggests that the relative importance of display  
468 performance vs. lek attendance as an indicator of male genetic quality may vary across  
469 years and environments, and between closely related species.

470

471 *Nightly calling effort and the direct and indirect effects of female choice*

472

473 Unlike females of some lekking birds, which are known to visit repeatedly the display  
474 arenas and to remember the identity and quality of different prospective mates (DuVal,  
475 2013; Uy et al., 2001), female treefrogs usually visit the lek only when they are ready to  
476 mate, and they were rarely observed spending more than one night at the breeding site.

477 For this reason, male attendance cannot be directly assessed by females and, thus, it  
478 cannot be the target of mate choice.

479

480 Male features that can be more realistically assessed over the short time window of a  
481 female mating decision are likely to be found among calling traits (Gerhardt and Huber,  
482 2002) and, possibly, among visual cues (Gomez et al., 2009). Previous playback  
483 experiments on female Italian treefrogs showed directional preferences for both call rate  
484 and the number of calls per call group (Castellano, 2010; Castellano and Rosso, 2007),  
485 and there is evidence that these preferences can result in directional selection in natural  
486 populations (Castellano et al., 2009). This study provides further evidence for  
487 directional selection on male calling activity. In fact, we found that calling effort  
488 positively affected male mating success both at the nightly and at the seasonal level. At  
489 the nightly level, we found that males that were the most active in the chorus during the  
490 15 and 30 minutes preceding a mating episode were the most likely to mate. This was  
491 not true, however, when calling activity was assessed over a shorter 5-min interval,  
492 suggesting that females were not simply and passively attracted by male calling (Arak,  
493 1988; Parker, 1983), but that they integrated acoustic perceptions over a time window

494 much longer than that estimated for other hylids (Schwartz et al., 2004). Interestingly,  
495 however, we found evidence that female preferences for high calling effort might have  
496 been affected by chorus size. Indeed, in smaller-than-average choruses, the mating  
497 success of males with high ranks of calling effort was much larger than the mating  
498 success of low-ranking males. In contrast, in larger-than-average chorus sizes, the  
499 mating success of low- and high-ranking males did not differ significantly, suggesting  
500 that high level of background noise might impair female discrimination capability  
501 (Bishop et al. 1995). At the seasonal level, we found that, independent of chorus  
502 attendance, the mean nightly calling effort correlated positively to mating success. We  
503 also found no evidence for a trade-off between attendance and calling effort at the  
504 population level: in fact, these two components of male mating effort were found to  
505 correlate positively rather than negatively with each other. The calling of treefrogs is  
506 one of the most expensive activities ever encountered in the natural world (Wells,  
507 2001), and there is experimental evidence that, under energetic constraints, male  
508 treefrogs become less attractive, because they reduce either their calling activity or their  
509 call rate (Brepson et al., 2013). For these reasons, the observed positive association  
510 between calling effort and attendance suggests a large among-male variation in mating  
511 effort, which is more likely due to variation in the total rather than in the relative  
512 amount of resources that males allocate to reproduction (Van Noordwijk and Dejong,  
513 1986).

514

515 The positive association between calling effort and attendance makes both these traits  
516 reliable indicators of male mating effort and, consequently, of male condition and,  
517 possibly, of male genetic quality. Intriguingly, however, attendance (but not calling

518 effort) correlated positively to offspring fitness-related traits. This result is consistent  
519 with the hypothesis that mate choice can provide females with indirect genetic benefits  
520 even when mating signals convey unreliable information about mate quality (Castellano  
521 2009). However, we cannot exclude the possibility that the lack of a positive association  
522 between sire's nightly calling effort and offspring fitness was a statistical artifact of our  
523 experimental design. In fact, for logistic reasons (see methods), the breeding experiment  
524 started at the first peak of breeding activity. Consequently, the subset of males involved  
525 in the experiment was not fully representative of the entire male population. Sires  
526 showed mating success, lek attendance and calling effort significantly higher than  
527 average, suggesting that they were likely to be of higher-than-average genetic quality.  
528 We provide evidence that, at least in our sire subsample, male genetic quality was more  
529 accurately predicted by lek attendance than by nightly performance; however, we  
530 recognize that it may not be possible to extend this finding to the entire male  
531 population, which was likely to include a large proportion of low-quality males.

532

533 In conclusion, our results show that females did not mate randomly and preferred males  
534 with higher-than average nightly calling effort. But they also suggest that nightly calling  
535 effort was a poor indicator of male-genetic quality and that selection for high quality  
536 males was more likely to arise from endurance rivalry rather than from female choice.  
537 In the same way as lek attendance interacts with nightly performance in determining  
538 male mating effort, endurance rivalry and female choice interact synergistically in  
539 determining the strength of selection on male mating effort. Choosy females, by  
540 imposing highly competitive standards to calling males, boost endurance rivalry and,  
541 even if male attractiveness is an unreliable indicator of genetic quality, they



542 nevertheless increase the probability of mating with males of higher-than-average  
543 genetic quality.

544

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547

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635

636 FIGURE LEGENDS

637

638 Figure 1 – Distribution of male mating success

639

640 Figure 2 – Calling effort and mating probability in choruses of different size. The figure  
641 shows the results of 20 mating episodes. For each episode, all calling males within the  
642 chorus were ranked on the basis of their calling effort measured over the 30 min prior to  
643 mating. The circles show the percentile rank of successful males, whereas the numbers  
644 into the circles indicate the number of mating episodes. The figure shows that female  
645 discrimination is sufficiently accurate and rewards high-ranking males at moderate  
646 chorus sizes (2-4 males), but becomes much less so when chorus size increases (5+  
647 males).

648

649 Figure 3 – Calling effort versus attendance in males either involved (squares) or  
650 excluded (circles) from the breeding experiment. Filled markers refer to males that  
651 mated at least once in the breeding season, whereas open markers refer to males that  
652 were never observed mated. At the beginning of both breeding seasons, males were  
653 randomly assigned to the sire and not-sire groups. However, by the end of the season,  
654 the sire subsample showed higher-than-average calling effort and attendance (dashed  
655 lines).

656

657

658

659 Table 1. Descriptive statistics of the three predictors of male mating success (SVL, lek  
 660 attendance and calling effort) in the two breeding seasons.

661

	2013			2014		
	SVL (mm)	Attendance (days)	Calling effort	SVL (mm)	Attendance (days)	Calling effort
N	37	37	25	36	36	29
Mean	35.44	5.16	0.166	33.16	3.58	0.139
Median	35.63	3	0.151	32.09	2	0.113
Min	29.88	1	0.006	26.94	1	0.004
Max	42.80	16	0.271	40.23	22	0.466
CV (%)	8.4	95.2	42.6	10.7	121.4	75.2

662

663 Table 2 – Comparison of the mating effort and mating success of males that were either  
 664 included (Sires) or excluded (Non-sires) from the breeding experiment.

	calling effort		Attendance (days)		Mating success	
	Sires	Non-sires	Sires	Non-sires	Sires	Non-sires
N	14	40	14	59	14	59
Mean	0.194	0.136	9.571	3.152	1.714	0.475
Median	0.209	0.116	9	2	2	0
Min	0.057	0.004	2	1	0	0
Max	0.328	0.466	17	22	4	3
S.D.	0.077	0.091	5.244	3.605	1.069	0.838

665

666



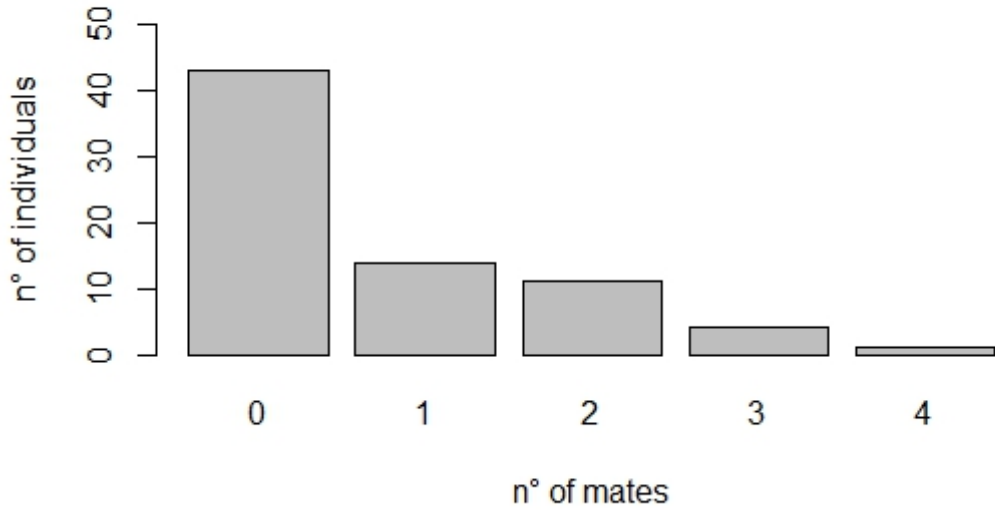
667 Table 3. Components of variance ( $s^2$ ) of offspring SVL and age at metamorphosis as  
668 inferred from the incomplete factorial breeding experiment. In the models we included  
669 five random factors and one interaction term: (i) year, (ii) quartet nested within the year;  
670 (iii) sire identity, (iv) dam identity and (v) sire-dam interaction nested within quartet  
671 and year; and (vi) replicate nested within sire-dam interaction, quartet and year. Degrees  
672 of freedom are approximated with the Satterthwaite method.

673

	SVL			AGE		
	$s^2$	$F$ ( $df$ )	$p$	$s^2$	$F$ ( $df$ )	$P$
Year	-0.012	0.027 (1, 5)	0.875	1.035	3.542 (1, 5)	0.119
Quartet	-0.040	0.458 (5, 4.837)	0.794	-0.878	0.570 (5, 10.999)	0.722
Sire	-0.017	0.695 (7, 7)	0.678	1.363	4.524 (7, 7)	0.032
Dam	0.110	2.983 (7, 7)	0.086	2.334	7.034 (7, 7)	0.010
Sire*Dam	0.045	1.673 (7, 28)	0.157	-0.567	0.577 (7, 28)	0.768
Replicate	0.076	2.310 (28, 504)	<0.001	1.811	3.083 (28, 504)	<0.001
Residuals	0.577			8.693		

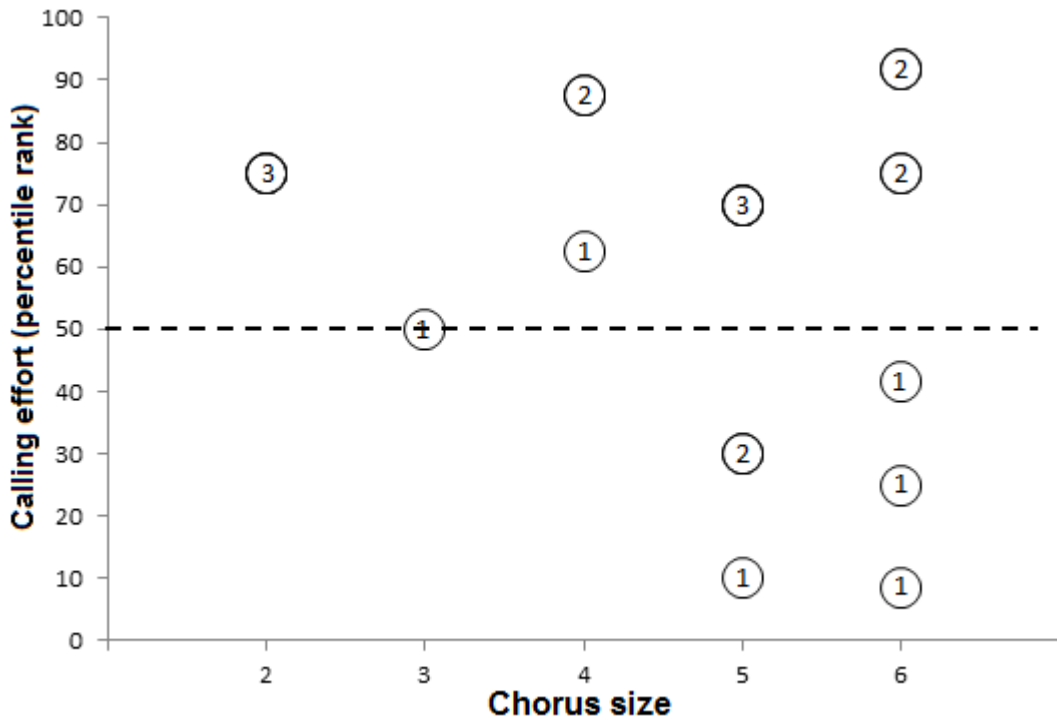
674

675 Fig. 1



676

677 Fig. 2



678

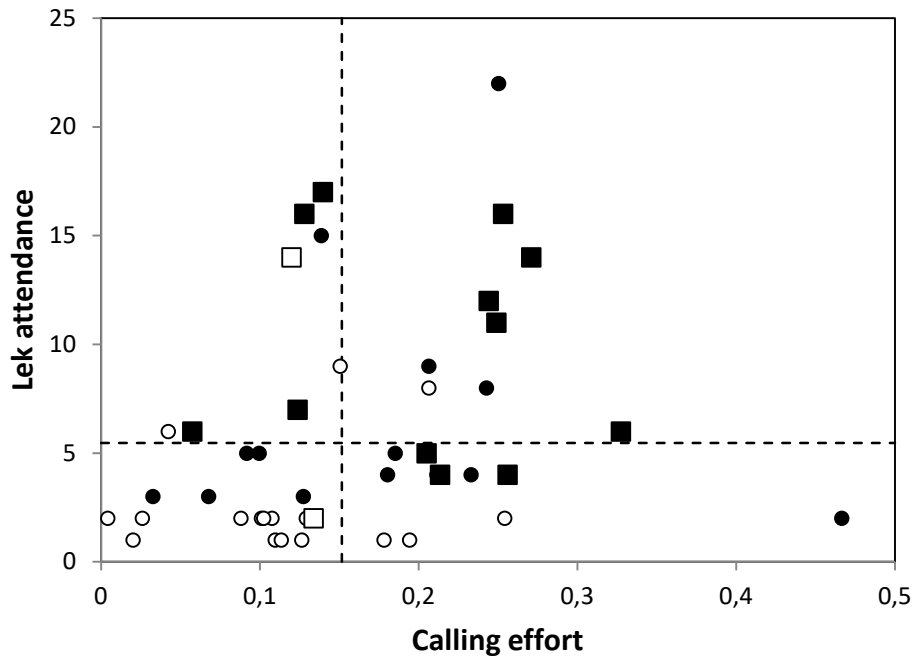
679

680 Fig. 3

681

682

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684