

1 This is a pre-copyedited, author-produced version of an article accepted for
2 publication in [Behavioral Ecology] following peer review. The published article is
3 available online at:
4 <https://academic.oup.com/beheco/article/30/1/20/5230134?searchresult=1>, or
5 <https://doi.org/10.1093/beheco/ary161>

6

7 Title: Pale by comparison: Competitive interactions between signalling female
8 glow-worms

9

10 Short title: Competition between signalling female glow-worms

11

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25 Funding

26 This work was supported by Academy of Finland (grant number 294664 to AK and
27 AMB).

28

29 Acknowledgements

30 We want to thank Tvärminne Zoological Station for facilities, Maiju Kemppainen for
31 help with experiments, Ulrika Candolin, Jukka Forsman, Jenni Kesäniemi, Olli
32 Loukola, Lumi Viljakainen, Phill Watts, Pirita Anttila and Antti Yrjölä for advice and
33 support, and anonymous reviewers for valuable advice.

34

35 Data Accessibility

36 Analyses reported in this article can be reproduced using the data provided by
37 Anna-Maria Borshagovski.

38

39 Lay summary

40 When individuals differ in attractiveness, less attractive ones may fail to mate. In
41 the common glow-worm, females glow to attract flying males, which prefer brighter
42 females. We show that females move away from more attractive competitors,
43 possibly to avoid comparison. In nature, females glowed far from each other. As
44 distance between females may affect how males perceive female attractiveness,
45 dim females may appear bright if close by and if comparison is not possible for
46 males.

47

48 Title

49 Steer clear of beauty! Female sexual signaling and female-female competition

50

51

52 Abstract

53 When individuals differ in their abilities to compete for a mate, weaker competitors
54 may evolve tactics to increase their mating success. Exploiting attractiveness of
55 others to get mates is a common tactic in many taxa, although examples of this
56 behavior in females are scarce. In glow-worms (*Lampyrus noctiluca* L., Coleoptera:
57 Lampyridae), females attract males by glowing and males prefer the brightest
58 female. How unattractive females succeed in competition for mates is largely
59 understudied. We hypothesize that less attractive female glow-worms may
60 succeed in competition over mates by parasitizing glow of more attractive
61 competitors. We tested our hypothesis with a combination of field and laboratory
62 experiments. Contrary to our expectations, we found that females move away from

63 brighter competitors. This behavior may explain our field observation that females
64 are often more than one meter apart from each other. Increasing distance to a
65 brighter female may make comparison on brightness difficult for males and
66 increase attractiveness of dimmer females. Our study provides evidence of
67 behavior by which less attractive competitors may actively avoid competition and
68 therefore affect female distribution in nature. This behavior may explain
69 maintenance of variation in attractiveness of sexual signals, even in species where
70 mates are selected by ornaments. We conclude that sexual competition may play a
71 crucial role in spatial distribution. Spatial distribution of competing sex affects
72 choosing individuals' ability to compare mates and thus affects mate choice.

73

74 **Keywords**

75 Sexual competition, female mate attraction, reproductive tactics, *Lampyris*
76 *noctiluca*

77

78 INTRODUCTION

79 Ornaments are specialized phenotypic traits, which increase sexual attractiveness
80 of their bearer and give better access to reproductive opportunities (more matings
81 and better mates) (Andersson 1994; Clutton-Brock 2007). Individuals with less
82 attractive ornaments may fail to mate (Andersson 1994; Rhainds 2010). Sexual
83 ornaments and competition over mates are more common in males than in females
84 (Andersson 1994; Tobias et al. 2012). However, sometimes females compete to
85 attract mates, for example, when mating opportunities are not guaranteed for every
86 female (Kokko and Mappes 2005; Clutton-Brock 2009; Rosvall 2011). As a result
87 of competition, females may evolve ornaments to attract mates and increase
88 mating success (Clutton-Brock 2009; Rhainds 2010).

89 When individuals differ in their abilities to compete for mates, weaker
90 competitors may develop ways to increase their probabilities to mate (Oliveira et al.
91 2008). A common tactic is to be parasitic and exploit attractiveness of other
92 competitors (Gross 1996; Oliveira et al. 2008). Parasitic individuals intercept mates
93 attracted by better competitors and may succeed in mating without costs of mate
94 attraction. For example, in field crickets males normally chirp to attract females,
95 whereas some males do not chirp (Clade 1975; Zuk et al. 2006). Instead of
96 chirping, the silent males move close to attracting males, wait for females to arrive
97 and mate with them. Parasitizing allows them to get mating partners while they
98 avoid costs of mate attraction, such as losing resources or attracting predators.
99 Parasitic mating tactics are relatively rare in females and are mostly evolved to
100 avoid costs of parental care and male harassment (for example, Lyon 1993; Van

101 Gossum et al. 2001; Oliveira et al. 2008), rather than to increase mate attraction.
102 One example of females exploiting attractiveness of others comes from a fish,
103 where females that fail to attract males fertilize their eggs by sneaking to nests of
104 spawning pairs and laying their own eggs among the eggs of the attractive female
105 (Johnston 1996, cited in Henson and Warner 1997). Examples of parasitic females
106 increasing access to mates are scarce. How unattractive females succeed in
107 competition over males is largely unknown (Although see Wijk et al. 2017).

108 In our study species, the common glow-worm (*Lampyris noctiluca* L.,
109 Coleoptera: Lampyridae), wingless females glow to attract flying males (Grassé
110 1949). Males are more attracted to brighter and thereby larger and more fecund
111 females when given a choice (Hopkins et al. 2015). Females glow until they either
112 mate or die (Tyler 2002). In nature, about half of females glow for only one night
113 while some continue for a couple of weeks (Hickmott and Tyler 2011). Adults do
114 not eat, so they have a limited amount of resources for survival, mate attraction
115 and reproduction (Tyler 2002; Hopkins et al. unpublished). It is important to mate
116 as soon as possible, as delay of mating reduces number of eggs (Wing 1989;
117 Hopkins et al., unpublished).

118 We investigated how less attractive females succeed in competition
119 over mates. We hypothesized that females may parasitize better competitors by
120 getting close to an attractive female and stopping glowing. We tested our
121 hypothesis with a combination of four field and laboratory experiments: 1) We
122 conducted a field experiment to investigate whether females stop glowing in
123 response to a brighter competitor close by. As we noticed that females did not stop
124 glowing, 2) we tested in the laboratory if females succeed to mate in presence of a

125 more attractive female (“Mating experiment”). We predicted that males would mate
126 with both females but start with the larger one. 3) We also conducted a laboratory
127 experiment (“Competition experiment”) to investigate if females parasitize brighter
128 females by going close to them. We predicted that females move towards a
129 brighter competitor to get a mate. 4) Finally, to see if female spatial distribution
130 supports our laboratory results, we observed female distribution in nature.

131

132

133 MATERIALS AND METHODS

134 Study species

135 Females glow on average for one to two hours each night (Tyler 2002). In Finland,
136 most females glow when summer nights are the shortest (personal observation).
137 They have only approximately two hours of total darkness and two hours of dusk to
138 attract males. Females stop glowing during or soon after mating, lay their eggs in
139 one or two clutches and die (Tyler 2002). As females do not fly, encounter rates
140 with males are constrained (Rhains 2010), increasing the possibility of delayed
141 mating and importance of female visibility and attractiveness. Female brightness
142 correlates with female size with large females being the brightest and most fecund
143 (Hopkins et al., 2015). As males select females by glow, dim females may not
144 acquire a mate and may be forced to attract males the following night. Mating delay
145 is costly especially for dim (and thus small) females as they may lose up to 20% of
146 their eggs each day (Hopkins et al., unpublished), whereas large females do not
147 suffer a significant loss of eggs with time. As small females have fewer eggs in the

148 first place (Hopkins et al. 2015), even a five-day delay in mating may cause
149 reproductive failure (Hopkins et al., unpublished).

150 Males have a short period during a night to find a mate. Copulation may
151 take from a couple of minutes to several hours (Tyler 2002, personal observations).
152 Mating multiply is possible (Tyler 2002), but if females are far from each other,
153 males are unlikely to take off during the same night and search for a new female.
154 Males may guard the female instead of searching for a new one as several males
155 are sometimes found around the same female (Tyler 2002).

156

157 Collection of individuals

158 We collected individuals at nights in June and July 2016 in Hanko (N59°51',
159 E23°12'), Lohja (N60°15', E24°04'), Särkisalo (N60°06', E22°57') and Kemiö
160 (N60°10', E22°44'), southern Finland. We visited known glow-worm habitats to
161 collect females. Collection was started just before breeding season and before
162 females attracted males or mated. We collected glowing females by hand and
163 males using traps with green LEDs (Hopkins et al. 2015). We kept individuals in
164 separate vials with some fresh grass or moss and transferred them to Tvärminne
165 Zoological Station during the same night. Glow-worms were kept in a laboratory in
166 a light/dark rhythm (20h L/4h D) similar to the natural rhythm during summer in
167 Finland at Helsinki latitude. Dark periods in the lab started at 10 pm and lasted until
168 2 am. The following day after collecting individuals, we measured the widths of
169 female pronotums as this is a good proxy of size (Tyler 2002; Hopkins et al. 2015)
170 and we placed each individual separately into an experimental arena for
171 acclimatization.

172

173 Experimental setups

174 *Field experiment*

175 To test if females stop glowing in response to a close brighter competitor in nature,
176 we used green light emitting diodes (LED) of 5 mm diameter to simulate brighter
177 females. The wavelength of the LEDs (562 nm) corresponded with the wavelength
178 of female light (546-570 nm) (De Cock 2004; Bird and Parker 2014). The LEDs
179 were at least twice as bright as the brightest females measured (unpublished data
180 from spectrophotometric measurements), so every female was dimmer than the
181 LEDs. We placed a LED 10 cm from glowing females, facing directly towards them.
182 We switched LEDs on (“LED treatment”) for arbitrarily chosen females while the
183 others had LEDs switched off (“control treatment”). We left females for 5 minutes,
184 after which we observed if females still glowed. We also estimated female
185 brightness on a scale from 1 (barely visible) to 5 (brightest seen in nature) before
186 and after exposing females to LEDs to see if females change their brightness in
187 response to the LEDs. Even if the method is crude, estimations derived with the
188 method correlate well with female size and thus are reliable (Hopkins et al. 2015).

189

190 *Mating experiment*

191 As females neither stopped glowing nor changed their brightness when close to a
192 brighter LED in the field experiment, we brought the same females to the
193 laboratory and continued to test experimentally if females succeed to mate despite
194 having a brighter female in close proximity. We used one male and two females of
195 different size in each trial of the experiment. To ensure clear size difference within

196 female pairs, we chose females so that size differences were large enough for
197 even humans to see the difference (differences within $n = 23$ female pairs in
198 pronotum widths were on average $0.61 \text{ mm}, \pm 0.07 \text{ mm}$ (mean \pm SE)). We placed
199 females in the middle of an experimental arena (38 x 28 x 16 cm box containing
200 soil layer) under transparent cups 5 cm apart. We noticed during a pre-experiment
201 that males stop paying attention to females that do not glow, and thus we waited
202 until both females glowed before we placed a male under a third cup next to the
203 females. To ensure that the males had a possibility to notice both females, we kept
204 them under transparent cups for 5 minutes. The experiment started when we
205 removed the cups. We observed the individuals in the dark for approximately one
206 hour and recorded which female was chosen as the first mate and whether males
207 mated with both females. The experiment was conducted in the laboratory with just
208 enough natural light for a human eye to follow what the glow-worms were doing.

209

210 *Competition experiment*

211 We brought females to the laboratory to study whether females parasitize brighter
212 competitors by moving close to them. We used green light emitting diodes (LED) to
213 simulate bright females, similar to the ones used in the field experiment. Every
214 female in this experiment was dimmer than the LEDs. We used 90-100 cm long
215 and 15 cm wide boxes with damp soil layer on the bottom as experimental arenas
216 (Figure 1). We attached an LED to one end wall, 5 cm above the soil surface. As
217 females seek higher places to glow (Tyler, 2002; personal observations), we
218 placed two 5 cm high pieces of cardboard upright and lengthwise into the soil,
219 spanning from end to end (about one third of each cardboard buried in soil), to

220 allow females to climb but still freely choose their location between the ends.
221 During the day after a collection night, each female was placed alone in the middle
222 of an arena under a transparent cup with a mussel shell. Shells provided shelter
223 and cups prevented females from escaping from the arenas. Females were left in
224 the arenas under the cups to acclimate and to wait for the experiment to start the
225 following night. They were unable to see each other from the experimental arenas.

226 Each female was in the experiment for two consecutive nights.
227 Females were arbitrarily and equally assigned a treatment. During the first night,
228 we switched the LEDs on (“LED treatment”) for half of the females. The other half
229 had the LEDs switched off (“control treatment”). During the second night, we
230 swapped treatments for each female. We switched the LEDs on just before the
231 dark period started at 10pm and before any of the females had started to glow.
232 Females were kept under their cups for half an hour (for a related experiment) after
233 which we removed the cups and mussel shells.

234 We observed female locations in the arenas for one hour after the cups
235 had been removed. We define female location in the experimental arena as the
236 location where females were in the end of the observation time in relation to the
237 starting point (The starting point is in the middle of the arena, defined as 0 cm.
238 Positive values mean distances towards the LED and negative values distances
239 away from the LED, Figure 1). After the observation time, we switched the LEDs off
240 and returned females and mussel shells to the middle of the arenas under the
241 original cups to wait for the second experiment night. Each female remained in the
242 same arena for both nights.

243

244 *Female distribution in nature*

245 To study female distribution in nature, we estimated distances between glowing
246 females in three locations in Hanko, Southern Finland, 2017. We took all females
247 to the laboratory after distance estimations each night to make sure we estimated
248 distance for the same individuals only once. We collected females during several
249 nights (four nights in Bengtsårbryggan, three nights in Lappohja Beach and six
250 nights in Tvärminne Village harbor).

251

252 *Statistical analysis*

253 To test if females ($n = 106$) stop glowing when a brighter competitor is near in the
254 nature, we used Fisher's exact test and compared the number of females that
255 stopped and did not stop glowing between control ($n = 51$) and LED ($n = 55$)
256 treatments. To test if the presence of a brighter LED affects the intensity of female
257 glow, we used Fisher's exact test and compared the number of females that
258 increased brightness, decreased brightness and did not change brightness in the
259 control and LED treatments.

260 We used a Chi-squared test to analyze whether males preferred larger
261 females as their first mates. We excluded 14 cases (females did not glow ($n = 12$)
262 and males did nothing or escaped ($n = 2$)), having 28 trials in the end.

263 To study whether presence of a brighter competitor (LED) affects
264 where females move in relation to the LEDs, we built a linear mixed-effects model
265 (LMM), having location as a continuous dependent variable, treatments as an
266 independent variable and individuals as a random factor. Unexpectedly, the order
267 of the experimental nights affected how females glowed (Baudry et al.,

268 unpublished). Therefore, as we cannot be sure whether the order of the
269 experimental nights affects other aspects of female behavior as well, such as
270 moving, we added it as another independent variable. As we suspected an effect of
271 female size (pronotum width) on location in the first experimental night in the LED
272 treatment (Figure 3), we included size to the LMM as a new independent variable.
273 Therefore, we formed a full factorial model with female location as the dependent
274 variable, treatments, order of the experimental nights and female size as
275 dependent variables and individuals as a random factor. We simplified the model
276 by backwards stepwise removal of non-significant terms, eventually having a
277 model with location as the dependent variable, interaction between treatments and
278 order of the experimental nights and interaction between female size and order of
279 the experimental nights as independent variables, and individuals as a random
280 factor (Table 1). We calculated the marginal (R^2_m) and conditional (R^2_c)
281 coefficients of determination for the LMM.

282 Further examination revealed differences between female behaviors.
283 We found 50 females, which settled down during both experimental nights (called
284 “settled females”) while the rest settled down during only one night or did not settle
285 down at all (called “wandering females”). We define settled females as individuals,
286 which stayed at the same location in the experimental arenas at least 10 minutes.
287 Given that the results did not differ between settled and wandering females, all the
288 data were retained in the analyzes, but we offer more details about the analyzes
289 and results of settled females as supplementary material.

290 Females which died ($n = 5$), laid eggs ($n = 3$), did not glow at all during
291 both experimental nights ($n = 5$) or were lacking size data ($n = 6$) were excluded

292 from the analyzes. Exclusion criteria were decided prior to the experiments. We
293 had data of 80 females in the end. We conducted the analyzes with R v 2.12.1 with
294 packages nlme for building the LMM and lme4 and MuMIn to calculate the R^2_m
295 and R^2_c .

296 We investigated whether female size difference affects distances
297 between closest neighbors in nature. Instead of studying individual females, we
298 studied pairs of females so that each female in each population (Lappohja Beach,
299 Tvärminne Village harbor, Bengtsårsbryggan) was paired with its nearest neighbor.
300 Some females were in more than one pair. We excluded any pairs where we
301 lacked data in the size of either female ($n = 8$) and included 34 pairs of females for
302 the analysis. If females were more than 10 m apart, we did not consider them as a
303 pair as it was very unlikely that they would have been able to see each other either
304 at the time of study or when choosing where to signal from. We used an ANCOVA
305 with distance between females in the pair as the dependent variable, female size
306 difference (difference between pronotum widths) as the independent variable, and
307 the population the females came from as the fixed factor. As interaction between
308 female size difference and population was highly non-significant ($p = 0.488$), we
309 excluded it from the analysis.

310

311

312 RESULTS

313 Field experiment

314 Most females (96%, $n = 106$) continued to glow after being exposed to a LED.

315 There was no difference between the LED and control treatments: in the control,

316 one stopped and 50 continued to glow, whereas in the LED treatment three
317 stopped and 52 continued glowing (Fisher's exact test: $n = 106$, $p = 0.619$, Table
318 2). From the females that continued to glow, most did not change their brightness
319 when exposed to a bright LED. LEDs affected female brightness: in the control, 45
320 did not change, three increased and two decreased brightness, whereas in the
321 LED treatment, the numbers were 34, 5 and 13 respectively (Fisher's exact test: n
322 $= 102$, $p = 0.0049$).

323

324 Mate choice experiment

325 Out of all 28 males, 23 (82%) mated with small females. 21 males out of 28 (75%)
326 mated with both small and large females. In the seven cases where males mated
327 with only one female, they mated with five large and two small females. Males did
328 not prioritize large females as their first mate: they mated first with the large female
329 in 15 and first with the small female in 13 cases ($n = 28$, $X^2 = 0.143$, $df = 1$, $p =$
330 0.705).

331

332 Competition experiment

333 LEDs significantly affected female location in the arena at the end of the
334 observation time (lme: $n = 80$, $t = -3.902$, $df = 78$, $p = 0.0002$, R^2_m and $R^2_c =$
335 0.123 ; Table 1, Figure 2). In the LED treatment, females moved on average $12.9 \pm$
336 2.5 cm (mean \pm SE) away from the LEDs, whereas in the control treatment, they
337 moved on average 1.0 ± 2.7 cm towards the LEDs. Size, order of the experimental
338 nights or interaction between treatments and order of the experimental nights did
339 not affect female location (lme: $t = 0.696$, $df = 76$, $p = 0.489$; $t = 1.805$, $df = 76$, $p =$

340 0.075; $t = 1.741$, $df = 78$, $p = 0.086$ respectively). However, interaction between
341 female size and order of the experimental nights was significant (lme: $t = -1.997$, df
342 $= 76$, $p = 0.049$).

343

344 Female distribution in nature

345 In the three populations together (combined $n = 34$ female pairs), average distance
346 to the closest neighbor was 4 m (range from 0.3 m to 10 m. Note that we defined
347 10 m as the upper limit for a population). 79% of the distances within female pairs
348 was larger than one meter, and 53% was larger than three meters (Supplementary
349 material, Figure S3). Size difference or population had no effect on distance
350 between females in each pair ($F = 0.236$, $df = 1$, $p = 0.631$; $F = 2.876$, $df = 2$, $p =$
351 0.072 respectively, Figure 4).

352

353

354 DISCUSSION

355 Our results support part of our hypothesis. Small females managed to mate in
356 presence of a more attractive female, as 82% of males mated with small females
357 when both small and large females were present. The result indicates that being
358 parasitic may be a successful tactic to increase mating probability. However, most
359 females moved away from LEDs (brighter competitors) instead of moving close to
360 them, which is against our hypothesis. Females moved on average 13.00 ± 2.5 cm
361 (mean + SE) away from the LEDs in the LED treatment, whereas in control they
362 stayed on average at the starting point. Females avoiding brighter competitors may
363 at least partly explain female distribution in nature, as 79% of glowing females

364 were more than one meter apart from each other. Our study suggests that mate
365 attraction and sexual competition may have a role in spatial distribution. While our
366 results show that female glow-worms avoid brighter competitors, this does not
367 exclude the possibility that they show similar avoidance towards weaker or equal
368 competitors. Further experimentation would reveal if aversion were only towards
369 stronger competitors or all competitors.

370 Optimally, males should always fertilize as many eggs as possible and
371 thus mate with several females, either prioritizing (or mating first with) the most
372 fecund female or the female of higher genetic quality (Andersson 1994). Therefore,
373 it was not surprising that males mated with both females in our mating experiment.
374 However, as one would expect males to mate with a larger female first, as they are
375 more fecund (Honěk 1993; Tyler 2002; Hopkins et al. 2015), it was surprising that
376 our males did not prioritize larger females. One reason for this behavior may be
377 lack of male competition in the experiment, as both a large and a small female
378 were presented to each male without competing males. However, sometimes male
379 glow-worms have to compete over females in nature as there may be several
380 males around one female (Tyler 2002). Another reason for not prioritizing larger
381 females in this experiment may be the short distance from which males perceive
382 females. Usually males fly in search of females (Tyler 2002) and select the
383 brightest female (Hopkins et al., 2015). The eyes of male glow-worms have
384 developed to find glowing females from a distance and from above while flying, so
385 perceiving female brightness from very short distances on ground may be
386 challenging. The results of our mating experiment suggest that being attractive
387 may not always guarantee superiority in competition over mates, and that less

388 attractive females succeed to mate despite being close to more attractive
389 competitors. Surprisingly, in our competition experiment, we did not find evidence
390 of females generally moving actively close to better competitors.

391 A correct choice of the best mate requires comparison between
392 potential mates. However, mate choice may be context-dependent and affected by,
393 for example, environmental conditions (e.g. Wiley 1994; Jennions and Petrie 1997;
394 Cotton et al. 2006). For example, how an observer perceives the attractiveness of
395 a mate is dependent on the distances and angles between attracting and choosing
396 individuals (Hopkins and Kaitala, unpublished). Mate choice based on
397 attractiveness is thus in many cases made on the basis of proportional instead of
398 absolute differences between competitors (Bateson and Healy 2005; Hopkins and
399 Kaitala, unpublished). Therefore, with enough distance between an observer and
400 competitors, the observer may not detect which mate is actually the most
401 attractive, but selects the one it perceives to be the most attractive. In cases where
402 attractiveness is advertised with sexual ornaments, a large ornament may appear
403 small from a distance and small appear large close by, and thus the possibility to
404 select “wrong” mate is higher. Confusion in mate choice due to distances would not
405 be the case in glow-worms if glow-worms estimate distances between objects
406 reliably in dark. However, females of some firefly species are not able to
407 discriminate between one distant but bright and one near but weak light sources
408 (Fister et al. 2013, original reference unknown). If the same applies for males,
409 moving away from better competitors may well alter male mate choice. It may also
410 reduce local competition and thereby increase weaker competitors’ probabilities to

411 mate. Less attractive individuals may take advantage of this and increase their
412 relative attractiveness by increasing distance to other competitors.

413 Spacing of attracting individuals may affect mate choice dramatically.
414 Maybe one of the most distinct ways of mate distribution affecting mate choice is
415 lekking, where males aggregate to court and compete over females and where
416 females have a great assemblage of males to select from (Emlen and Oring 1977).
417 However, in male bush crickets (*Tettigonia viridissima*) and painted reedfrog
418 (*Hyperolius marmoratus*), wide spacing between attracting males ensure better
419 mating success compared to when males were clumped (Telford 1985; Arak et al.
420 1990). In glow-worms, greater distance to a better competitor may increase female
421 success to attract males. As our results show, females are widely distributed in
422 nature, which may be at least partly due to females moving away from better
423 competitors. If a scattered distribution of competing individuals can increase mating
424 success, it may cause selective pressure for less attractive individuals to move
425 actively further away from other competitors to attract mates.

426 Some animals recognize conspecifics by olfactory cues. If this is true
427 with glow-worms, it might be possible that females do not consider LEDs as other
428 females, as LEDs lack the characteristic smell of glow-worms. However, use of
429 olfactory cues has been reported mostly in diurnal firefly species, although females
430 of some Lampyrid species use both pheromones and bioluminescence to attract
431 males (Lewis and Cratsley 2008). Although the scent of female glow-worms
432 intensifies male sexual behavior when males are very close to females, it does not
433 seem to contribute to mate attraction otherwise (Schwalb 1961). Glow alone seems
434 to be enough for males as males are attracted by green LEDs (for example,

435 Schwalb 1961; Booth et al. 2004; De Cock 2009; Ineichen and Rüttimann 2012;
436 Hopkins et al. 2015) and even try to mate with them (personal observations). To
437 make sure that LEDs are at least visually similar as real females in our
438 experiments, we chose LEDs so that they would match to the spectral range of
439 female glow and would be of similar size.

440 We noticed differences between females that faced a competitor
441 during their first night compared to those that faced a competitor during their
442 second night in the competition experiment (Figure 3). We found that large females
443 moved further away from LEDs than small females if they faced a competitor
444 during their first night. When females faced a brighter competitor during their
445 second night, there was no such trend. On average, all females moved away from
446 LEDs, but it seems that in some cases large females may be more prone to move
447 away from better competitors. For example, females may move away from
448 competitors of similar quality, but move closer to less attractive competitors when
449 they might benefit from parasitizing better competitors. However, as the trend was
450 dependent on experimental nights, more studies are needed to clarify this subject.

451 In addition to escape in space, animals might avoid competition with
452 more attractive individuals in time, as is the case in most insects (Morbey and
453 Ydenberg 2001). Emerging earlier in the breeding season allows less attractive
454 individuals to overcome the disadvantage of small size in sexual competition, such
455 as in Dawson's burrowing bee (Alcock 1997). By emerging earlier in the season,
456 less successful individuals would have the advantage of being the first ones mates
457 can choose from. Although emerging before better competitors in season may be a
458 valid tactic for many species, it may not work for glow-worms because males

459 emerge later in season than females (Tyler 2002). However, whether less
460 attractive females could emerge earlier in nights to be the first ones to attract
461 males has yet to be studied.

462 We tested if females succeed to mate when close to a more attractive
463 competitor and if they parasitize more attractive females by moving close to them.
464 Our results show that less attractive females succeed to mate close to a more
465 attractive female, which indicates that parasitizing could be a functional tactic for
466 less attractive females to secure mating. However, according to our results, it is
467 clear that relatively less attractive females do not parasitize brighter competitors. In
468 contrast, they move away from better competitors, which may at least partly
469 explain female distribution in nature. As distance affects how an observer
470 perceives attractiveness of mates, less attractive competitors may use the effect of
471 distance to succeed in sexual competition. Our study provides evidence that mate
472 attraction and sexual competition may have a role in spatial distribution of
473 competing individuals.

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566

567 **FIGURE LEGENDS**

568

569 Figure 1. Experimental arena to study female locations in response to a green
570 LED. The LED was switched on in the LED treatment (above) and switched off in
571 the control treatment (below). A female was placed in the middle under a
572 transparent cup. 0 is the starting point in the middle of the arena, plus (+) end is
573 where the LEDs are and minus (-) end is without an LED.

574

575 Figure 2. The mean female locations (\pm SE) in the end of the observation time in
576 the experimental arena: 0 means the middle of the arena, negative values away
577 from and positive values towards the LEDs.

578

579 Figure 3: Female size (pronotum width) and location in the experimental arena
580 during both experimental nights in the LED treatment. 0 means the middle of the
581 arena, negative values away from and positive values towards the LEDs.

582

583 Figure 4. Size differences and distances between the closest neighbours in
584 Tvärminne Village harbor, Bengtsårsbryggan and Lappohja Beach populations.

585

586 TABLES AND TABLE LEGENDS

587

588 Table 1. Results of linear mixed-effects model of location in the end of the
589 observation time in relation to treatments, female size and order of the
590 experimental nights. The model and parameter estimates after backwards
591 stepwise removal of non-significant terms.

Parameter	Estimate	SE	df	t	p	95% confidence interval	
						Lower	Upper
<hr/>							
Location ~ Treatment * Night + Size * Night							
Intercept	-11.727	23.205	78	-0.505	0.615	-57,905	34,451
Treatments	-20.632	5.287	78	-3.902	0.0002	-31,153	-10,111
Female size	4.581	6.582	76	0.696	0.489	-8,517	17,679
Order of the experimental nights	63.617	35.241	76	1.805	0.075	-6,513	133,747
Treatments x Order of the experimental nights	12.703	7.296	78	1.741	0.086	-1,816	27,222
Female size x Order of the experimental nights	-19.940	9.987	76	-1.997	0.049	-39,814	-0,066

592

593 Table 2. Effects of a bright LED on female glow in nature.

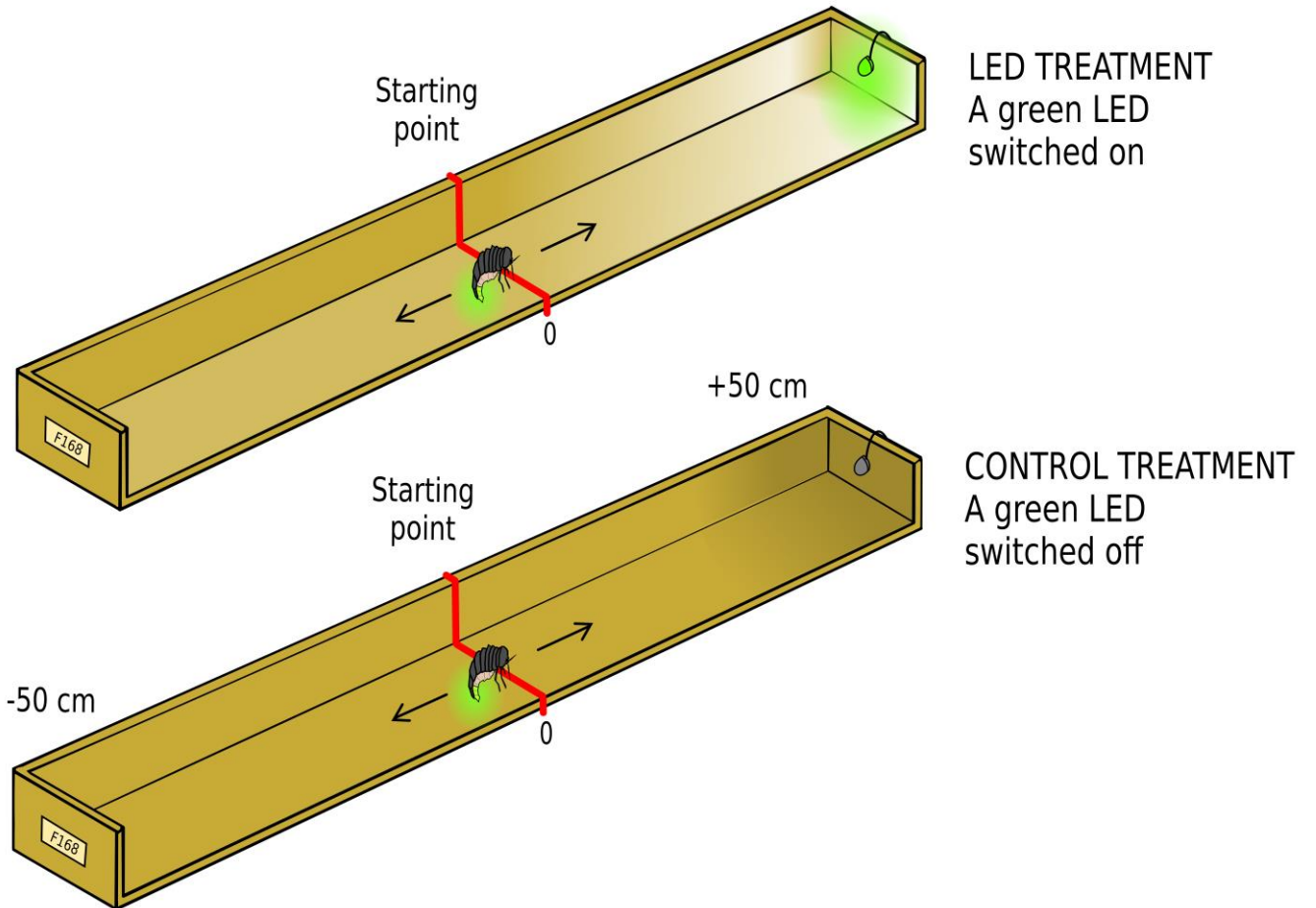
	Stopped glowing	No brightness change	Brightening	Dimming
Control	1	45	3	2
LED	3	34	5	13

594

595

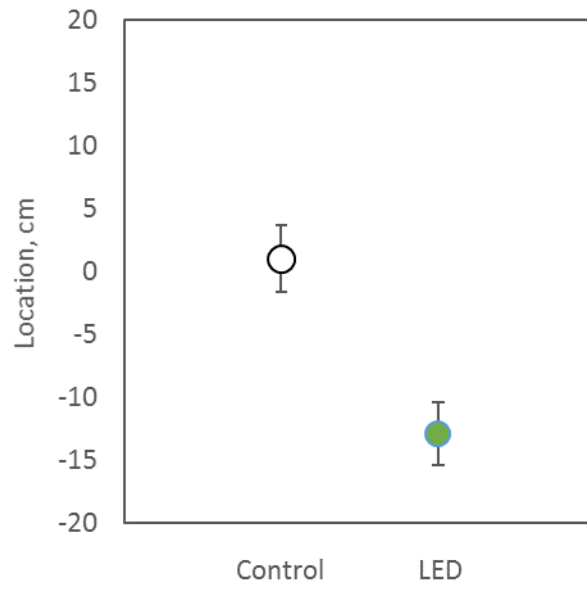
596 FIGURES

597



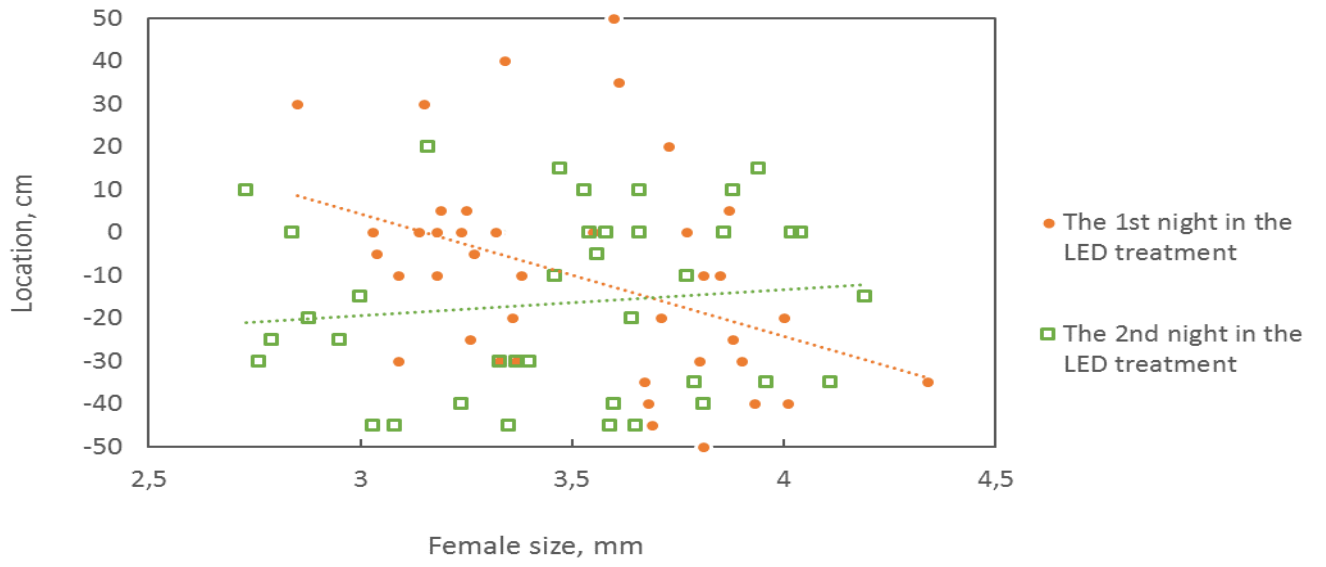
598

599 Figure 1.



600

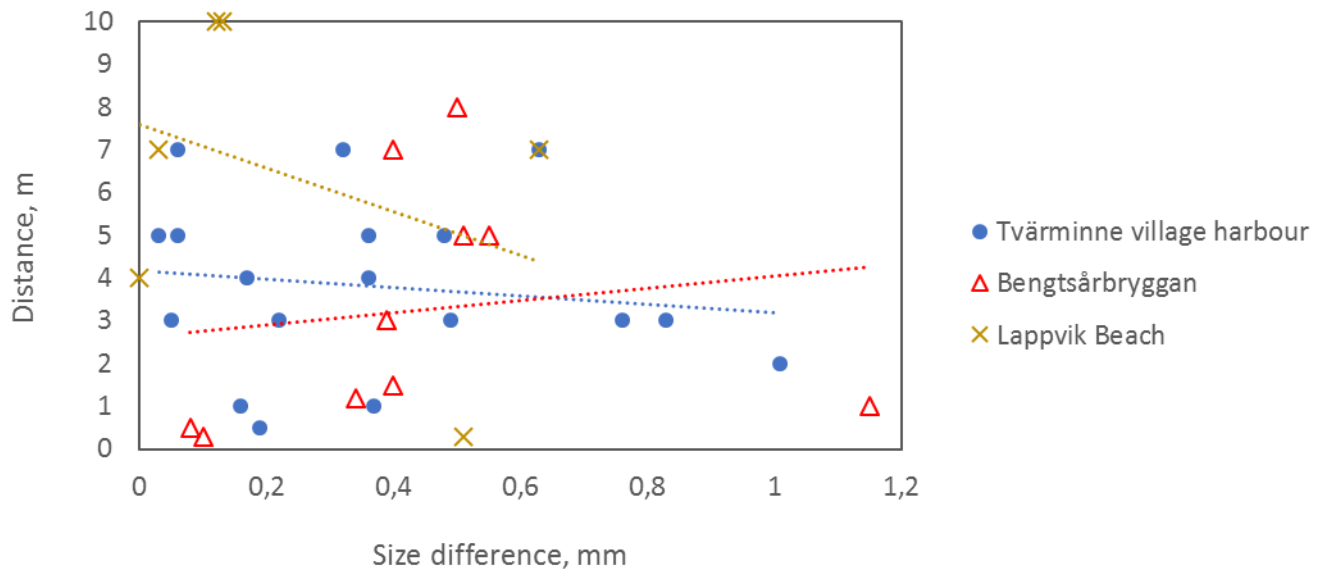
601 Figure 2.



602

603 Figure 3.

604



605

606 Figure 4.

607

608 SUPPLEMENTARY MATERIAL

609

610 Expanded methods: settled versus wandering females

611 Statistical analysis

612 We conducted paired t-test to compare settled locations between control and LED
613 treatments to study if the presence of a stronger competitor (LED) affected where
614 females settled down in experimental arenas. We define settled females as
615 individuals, which stayed at the same location (locations rounded to the closest 5
616 cm) at least 10 minutes. To analyze if female size (width of pronotum) affected the
617 choice of a location in relation to the LEDs, we built linear regression for the
618 females in the LED treatment, having pronotum width as the independent variable
619 and female location in the end of the experiment as the dependent variable.
620 Because the number of settled females is small ($n = 49$), instead of mixed-effects
621 model we conducted the analysis separately for both treatment nights.

622 As there are two distinct female groups (“settled females”, $n = 49$, and
623 “wandering females”, $n = 31$), we built a full-factorial linear mixed-effects model
624 (LMM) to study if the female groups differed in their locations in the end of the
625 observation time. We had location as a continuous dependent variable, treatments
626 and groups as independent variables and individuals as a random factor. Highly
627 non-significant interaction between treatments and female groups ($p = 0.693$)
628 allowed us to exclude the interaction from the model (Table S1).

629

630 Results

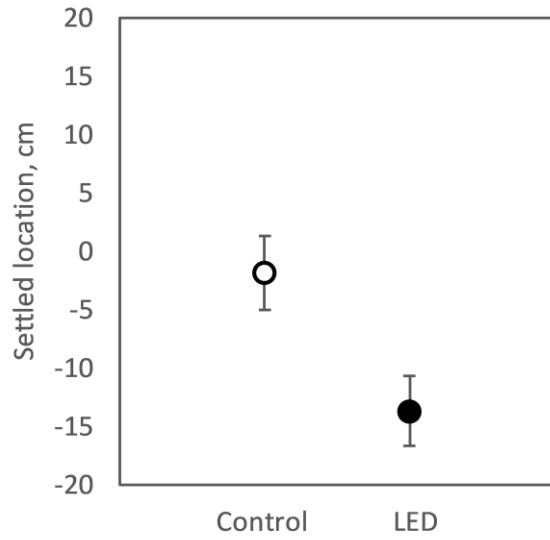
631 Females that settled down during both experimental nights (n= 49) moved on
632 average -13.4 ± 3.0 centimeters away from the LEDs in the LED treatment and -1.9
633 ± 3.1 centimeters away from the LEDs in the control treatment (Figure S1). The
634 difference was statistically significant (paired t-test: $t = 2.459$, $df = 48$, $p = 0.018$).
635 Female size did not affect where females settled down in the LED treatment in
636 relation to the LEDs ($n = 49$, $t = -0.863$, $df = 48$, $r^2 = 0.016$, $p = 0.393$), except
637 when locations were analyzed separately for each experimental night. During the
638 first night, the larger females were, the further away from the LEDs they were in the
639 arena ($n = 25$, $t = -2.135$, $df = 24$, $r^2 = 0.165$, $p = 0.044$). Size had no effect during
640 the second experimental night ($n = 24$, $t = 1.810$, $df = 23$, $r^2 = 0.029$, $p = 0.426$).
641 Whether females were settled or wandering did not affect female locations (lme: n
642 $= 80$, $df = 78$, $t = -0.997$, $p = 0.328$), (Table S1, Figure S2).
643

644 Table S1. Results of linear mixed-effects model of location in the end of the
 645 observation time in relation to treatments and female groups.

646

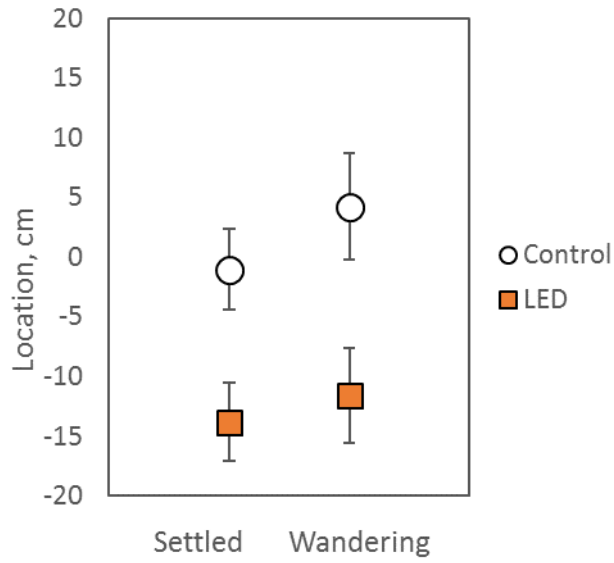
Parameter	Estimate	SE	df	t	p	95% confidence interval	
						Lower	Upper
<hr/> Location ~ Treatment * Groups							
Intercept	4.258	4.198	78	1.014	0.314	-4.097	12.613
Treatments	-15.806	5.938	78	-2.662	0.009	-27,622	-3.991
Groups	-5.278	5.365	78	-0.984	0.328	-15.954	5.397
Treatments x Groups	3.011	7.587	78	0.397	0.693	-12.087	18.108
<hr/> Location ~ Treatments + Groups							
Intercept	3.336	3.488	79	0.957	0.342	-3,604	10,276
Treatments	-13.963	3.686	79	-3.788	0.0003	-21,298	-6,627
Groups	-3.773	3.783	78	-0.997	0.328	-11,302	3,755

647



648

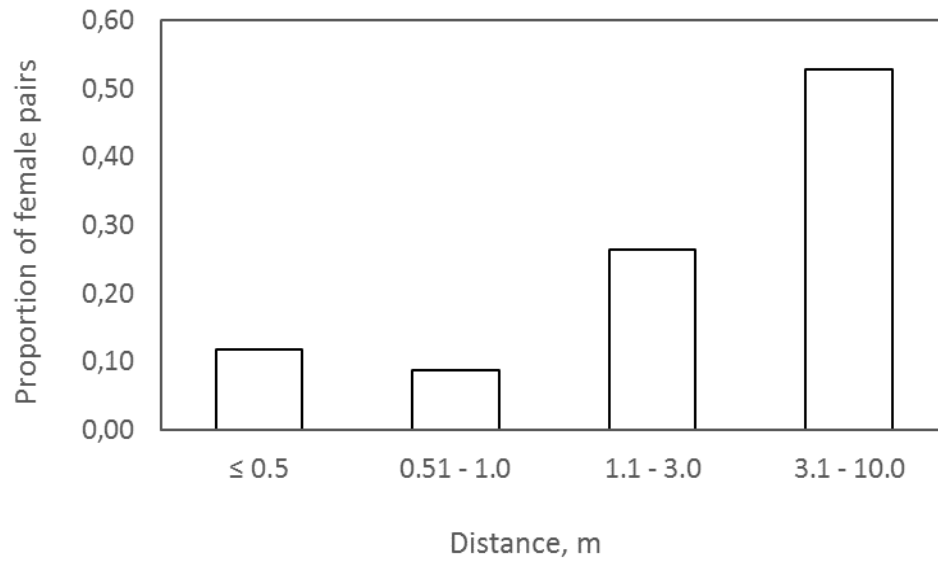
649 Figure S1. The mean female locations (+ SE) that settled down during both
650 experimental nights in the experimental arena: 0 means the middle of the arena,
651 negative values away from and positive values towards the LEDs.



652

653 Figure S2. The mean female locations (+ SE) in the end of the observation time in
654 the experimental arena: 0 means the middle of the arena, negative values away
655 from and positive values towards the LEDs.

656



657

658 Figure S3. Distances between closest neighbors (three populations together).

659