1   **Sexual selection on multiple female ornaments in dance flies**

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18 **Abstract**

19 Sex-specific ornaments typically occur in males, but they can also develop in

20 females. While there are several models concerning the evolution of male-specific

21 ornaments, it is not clear how, or under what circumstances, those models apply to

22 female-specific ornament evolution. Here, we present a manipulative field

23 experiment that explores the theoretical 'trait space' of multiple female-specific

24 ornaments to study how these unusual traits evolved. We measured the

25 attractiveness of two female-specific ornaments (pinnate leg scales and inflatable

26 abdominal sacs) in the dance fly *Rhamphomyia longicauda* in a wild mating swarm.

27 We found significant directional preferences for larger ornaments of both types,

28 however, variation in one of the ornaments (abdominal sacs) was almost three

29 times more effective at improving attractiveness. The abdominal ornament was

30 consistently effective in increasing attractiveness to males regardless of leg

31 ornament expression, while leg ornament size was only effective if abdominal

32 ornaments were very small. These results are consistent with predictions from a

33 sexual conflict model of ornament expression in supporting the probable role of

34 deception in the evolution of female-specific ornaments among dance flies. Sexual

35 conflict can be an important force in generating elaborate sex-specific ornaments in

36 females as well as males.

37

38   **Key Words**

39   multiple ornaments, sexual conflict, sexual selection, female ornamentation, dance

40   fly, honest signaling

Introduction

Sexually selected ornaments are among the most fantastic and bizarre traits found in nature. While extravagant ornaments undoubtedly can improve an individual's reproductive success [1], they might also trade off with other important life history traits. Benefits from increased reproductive success must, therefore, outweigh any costs in order for an ornament to persist. The trade-off between sexual displays and other characters may be particularly important in explaining the rarity of female ornaments [2, 3]. Compared to males, female reproductive fitness is typically more resource limited [4], so investing in costly sexual traits might decrease fecundity in females more often than it constrains reproductive success in males [5].

Given their potential costs, the persistence of female ornaments in a few rare taxa is puzzling [6-9] and remains understudied. The problem is probably resolved in some taxa because what appear to be ornaments have evolved for reasons other than for improving sexual attraction [9-13]; in such cases the costs of investing in elaborate traits are outweighed by advantages in other aspects of life history. For example, female ornaments might evolve primarily for the purposes of intrasexual competition for resources rather than for access to mates [6, 7, 13, 14].

If, however, female ornaments arise through adaptive mate choice (e.g., via male choice for honest signals of fecundity) mediated by sexual competition, the resource investment required to express ornaments must be compensated by the subsequent payoff of winning contests for mates. One clear scenario in which this is

63   true is if resources used to construct ornaments are not limiting for offspring

64   production [5]; in that case spending resources on ornaments need not compromise

65   a female's reproductive effort. However, such a scenario underlines a further

66   problem for female ornament evolution via male choice: the ornaments must not

67   only compensate for their expression costs, but also honestly provide information to

68   males about female reproductive value [7, 15, 16].

69   A further, and potentially more serious, constraint on the signal value of

70   ornaments could occur if females store sperm from multiple partners. In such cases,

71   the expected benefit of ornamentation to females (in terms of heightened

72   attractiveness) is frequently associated with a cost to their mates; males should

73   generally prefer relatively unattractive but monandrous females over attractive

74   ones that present higher risks or intensities of sperm competition [5, 8, 17, 18]. In

75   other words, the heightened attractiveness of adorned females dilutes the share of

76   eggs to which any individual male has access, which should undermine the

77   usefulness of any attractive trait for signaling reproductive value. However, the cost

78   to a male of mating with an attractive female would be reduced if males were able to

79   identify females in which the conditions of sperm competition are most favourable.

80   In many insects, the last male to mate before oviposition often has a distinct

81   paternity advantage, and males could conceivably identify females that are ready to

82   oviposit by preferring mates with large abdomens that indicate late stages of

83   vitellogenesis [8, 19].

84   Sexual ornaments might therefore provide males with cues of female value,

85   including egg number and egg development stage (i.e., egg size) that are difficult to

86   perceive externally. Previous studies of female-specific ornaments [15, 20-22] have

87   assessed the value of female ornaments as honest indicators of fecundity or egg

88   maturity providing mixed support both across and within species. Ornaments are

89   often positively correlated with female egg numbers or size, but the degree to which

90   the signal improves male abilities to discern female reproduction (e.g., relative to

91   the situation in an unadorned ancestor) is not clear. For pipefish, temporary female

92   ornaments served to honestly signal female mating success and fecundity [20]. In a

93   study of dance flies [8], abdomen size predicted 23% of variation in egg size in

94   *Rhamphomyia longicauda*, a species with multiple ornaments, but the unadorned

95   female abdomens of a closely related species (*R. sociabilis*) predicted much more

96   (72%) of the variation in egg size [8]. The authors argued that female ornaments

97   were deceptive traits rather than honest signals, which served to improve female

98   access to food gifts provided by males during copulation, even though the males

99   themselves gained relatively little information from the ornaments [8]. Further, the

100   relatively small amount of egg size variation predicted by abdomen size in *R.*

101   *longicauda* [8] could be indicative of a cost associated with producing ornaments in

102   some females that are forced to trade off egg quality (in this case, egg size) in order

103   to attract mates, as predicted by Fitzpatrick *et al* in [5].   In another study of *R.*

104   *longicauda* [22], abdominal ornament size predicted only 6% of variation in

105   fecundity, but 49% of variation in egg size; however, ornaments on the legs had no

106   significant relationship with either egg number or size. In *R. tarsata* (a congener of

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107   *R. longicauda* and a species that displays only leg ornamentation), leg ornament

108   expression predicted female fecundity better than other morphological traits, but

109   did not significantly predict egg size [15]. In fact, LeBas et al [15] argue that

110   positioning of legs during courtship displays may actually impair male assessments

111   of egg size. A clear constraint on the signal value of any insect exoskeleton trait

112   (including the ornaments of dance flies) is that such characters are fixed in size at

113   eclosion, and cannot therefore track the development of ovaries that occurs during

114   an adult's lifespan. While such characters might conceivably indicate overall size,

115   and therefore larval resource acquisition, it is hard to imagine how they might

116   improve male detection of female ovarian condition. Clearly, the role of female

117   ornaments as signals of female reproductive fecundity or ovarian maturity is, at

118   best, unclear.

119   Dance flies from the subfamily Empidinae (Diptera: Empididae) display

120   considerable interspecific variation in mating system. Roughly a third of the species

121   from the group feature female ornaments, including species with some of the most

122   extravagant female-specific ornaments yet described [8, 15, 23]. Empidine

123   ornaments can take several forms, including darkened, patterned or enlarged wings,

124   feathery "pinnate" leg scales and, more rarely, inflatable abdominal (pleural) sacs

125   [23, 24]. Males of many species provide direct benefits to females by offering a prey

126   item as a nuptial gift during copulation. In most species, females are not known to

127   hunt as adults, and seem to rely on protein from male-provisioned gifts to

128   supplement their nutritional reserves [25]. As is typical for insects, female dance

129   flies can store sperm from many males, and eggs are only fertilized immediately

130 prior to oviposition. Although the precise mechanics of sperm precedence are not

131 yet known with certainty, two lines of evidence suggest that last male sperm

132 precedence is operating. First, the spermathecae are broadly spherical and

133 sclerotized (our own observations), which means that they cannot expand in

134 volume indefinitely, but rather facilitate displacement of one ejaculate by another

135 through flushing. Second, the mean proportion of paternity assigned to the most

136 successful genotype is not sensitive to total mate number, as predicted if the last

137 mate displaces rival ejaculates [26].

138   Females of the long-tailed dance fly, *R. longicauda*, possess two extravagant

139 ornaments: pinnate scales over the length of all femora and tibia, and abdominal

140 pleural sacs that are inflated just prior to swarming. Relatively few empidine dance

141 fly species have abdominal ornaments, whereas pinnate leg scales are reasonably

142 common throughout the group (see [23, 24, 27]). Both ornaments appear to

143 exaggerate a female's apparent size and to improve female attractiveness [8] in the

144 highly competitive context of *R. longicauda* mating swarms (which are usually

145 heavily female-biased; [8, 22, 28]).

146   Previous work using plastic models of females within the mating swarm

147 showed that *R. longicauda* males are more attracted to larger females, consistent

148 with directional selection on ornament size [8]. However, Wheeler et al. [16] found

149 that females with intermediate levels of ornamentation were more likely to mate

150 than either extreme. This inconsistency of selection across episodes remains

151 unexplained. One possibility is that patterns of selection are inconsistent across

152 populations of dance flies, while another is that the initial attraction during male

153 approaches is only part of what determines eventual mate choice. In order to clarify

154 these possibilities, we experimentally manipulated the relative size of both leg and

155 abdominal ornaments using plastic models similar to those employed by Funk and

156 Tallamy [8] and quantified both the independent effect of each trait on

157 attractiveness, and the combined effects of both.

158

159 **Methods**

160 *Study system*

161   In Northeastern North America, courtship swarms of *R. longicauda* form

162 annually along riverbanks and occur from the end of May until the beginning of July

163 [25, 29]. Swarms are crepuscular, form beneath gaps in the forest canopy, and are

164 typically strongly female-biased [8, 23, 30]. Before they enter the swarm, female *R.*

165 *longicauda* swallow air to inflate abdominal sacs that exaggerate their body size.

166 Within the swarm, females fly parallel to the ground and position their pinnate-

167 scaled legs laterally around their inflated abdomen, which further exaggerates their

168 size when they are viewed from below.

169 *Female silhouette creation*

170   We independently manipulated both female ornaments and mating swarm

171 position in order to disentangle the partial effects of selection for attractiveness on

172 each of the ornamental modules (abdomens and leg scales). There is strong natural

173 covariance between both ornament types in wild female flies (because females who

174   accumulate more resources as larvae are likely to invest heavily in both ornaments;

175   [31], which makes separating the effects of selection on each ornament in real

176   specimens difficult [16]. Our artificial silhouettes break apart the natural covariance,

177   and display combinations of ornaments that do not exist in nature. This

178   manipulation improves both our ability to visualize the whole fitness landscape, as

179   well as our statistical power for measuring partial selection on each character; it

180   also allows us to measure the combined effects of variation in both ornaments (i.e.,

181   to determine if the signals are complementary or otherwise).

182   We created 25 artificial female silhouettes (Figure. 1) using a template

183   provided by David Funk (Figure. 3 in [8]). We manipulated the abdomen size

184   independently of leg scales such that we had five different abdomen widths: mean

185   +/-2SD, mean +/-SD, and population mean (estimates of population means and

186   standard deviations come from Wheeler [16]). Although we initially attempted to

187   similarly restrict our models' pinnate scales to the range of natural variation, we

188   could not precisely and consistently control the apparent size of the legs across

189   models, which made such fine scale variation impractical. Consequently for leg

190   scales we used a larger range of sizes including legs similar to males (the ancestral

191   condition) and legs twice as large as the largest found in nature: mean +/-10.8SD,

192   mean +/-5.4SD, and population mean.

193   We printed the artificial silhouettes on plastic transparencies and attached

194   each of them to a 30cm length of fishing line. To simulate the positioning of female

195   dance flies in the mating swarm, we fastened fishing weights above the models to

196   keep the silhouettes parallel with the ground. We placed a stake on either side of

197   the swarm site (1.5m apart) with a piece of fishing line stretched between them 1m

198   above the ground. For each sampling interval (e.g., each swarming event), we then

199   chose five silhouettes at random (without replacement) from the panel of 25 and

200   spaced them 15cm apart across the centre of the line such that the flanking

201   silhouettes were approximately 37cm from a stake. This design ensured that our

202   artificial silhouettes were usually greatly outnumbered by wild females; natural

203   swarms vary in size over time and space, but during peak swarming can feature

204   hundreds of flies packed rather densely into the swam space (with gaps of a few cm

205   between adjacent flies). Our initial trial date includes observations for only two

206   silhouettes, rather than five. Three silhouettes, and their associated male approach

207   data, were discarded when they were found (after the trial) to have errors

208   associated with their printing. As a consequence, ten days of observations yielded

209   data for 47 silhouettes being approached by males.

210   *Experimental set up*

211   We carried out male mate choice trials from June 13-22, 2012 at the study

212   site (used for previous studies of this species [16, 30, 32]), located near Glen

213   Williams, Ontario, Canada on an island in the Credit River (43o41'11"N,

214   79o55'34"W). A trial consisted of a single swarming event at dawn (roughly

215   4:45am) lasting approximately one hour. We set up female silhouettes the evening

216   before a trial began (at least eight hours in advance) so that they were already *in*

217   *situ* when the swarm started. Each trial began when a male first approached one of

218   the female models, by which time wild females had always already joined the

219   simulated swarm, and outnumbered the artificial flies. We recorded data by directly

220   scoring male approaches to silhouettes within the swarm. We used one observer

221   who was blind to the phenotypes of the female silhouettes being scored. Following

222   methods described in [8], an approach to a model was recorded when a male fly

223   carrying a nuptial gift hovered approximately less than 5cm beneath a female

224   silhouette for more then 3 seconds. We did not record rejections (i.e. males that did

225   not pause beneath silhouettes).   Swarm position, ornament sizes and number of

226   male approaches were tallied for each silhouette on each date. We concluded a trial

227   when five minutes passed without observing a male approach (typically between

228   5:45 and 6am). All raw data are available as electronic supplementary material

229   (ESM1).

230   *Statistical Analyses*

231   We computed all analyses using R statistical software [33]. To investigate the

232   relationship between female silhouette morphology and male attraction, we fit all

233   models with the number of approaches by courting males as the response variable

234   and ornament expression levels and the swarm position of silhouettes (distance

235   from the centre of the swarm; position within the swarm is thought to affect

236   attractiveness [32]) as predictor variables. We scaled morphological predictors in

237   phenotypic standard deviations to facilitate comparisons between traits. Because

238   our standardized coefficients are not strictly equivalent to selection gradients,

239   (attraction is only the first stage of mating success), they cannot be

240   straightforwardly translated into fitness [16].

241   The nature of selection varies as a function of swarm composition, which can

242   differ substantially from day to day [22]. Therefore we built generalized linear

243   mixed models with Poisson error and log-link (because attraction is measured in

244   male approaches and is a count variable), including "date" as a random effect (to

245   represent the potentially distinct composition of each swarm), and using the lme4

246   package in R [34]. Pinnate leg scale size, abdomen inflation and position within

247   swarm were fit as fixed effects. We included each predictor variable as well as its

248   square and cross-products in order to assess curvilinear and correlational effects of

249   morphology on attractiveness [35]. We did not fit a three-way interaction for these

250   data because we wanted to mainly focus on correlational selection. We illustrate the

251   partial effects of each ornamental trait by plotting the fit effects after setting the

252   other covariates to their mean value.

253   One of the ways in which the two ornaments might conceivably combine to

254   affect attractiveness is if males simply perceive the overall actual size of legs and

255   abdomens together, irrespective of whether the ornament is located on the

256   abdomen or legs. To test this hypothesis, we performed a separate analysis in which

257   we regressed male approaches on the total area (mm^2) contributed by each

258   ornament type to the silhouette area (instead of the standardized trait size). If males

259   are primarily concerned with the total size of ornaments, we expect to see similar

260   improvements in attractiveness for an additional unit of female silhouette area,

261   regardless of whether that increase in area comes from leg scales or abdominal

262   inflation. By contrast, if the abdominal ornament represents a recent evolutionary

263   innovation brought about by increasing levels of male resistance to deceptive leg

264   pinnation, we predict that abdominal ornaments should be more effective at

265   improving attractiveness than leg ornaments, whether these ornaments are

266   computed in terms of absolute area or phenotypic standard deviations. All code is

267   available as electronic supplementary material (ESM2).

268   **Results**

269   We recorded a total of 1479 male approaches over the course of ten mating

270   swarms. We cannot be sure of exactly how many males this represents, but given

271   the large number of receptive females, this number is unlikely to represent many

272   repeated approaches by the same male. Consistent with Funk and Tallamy [8], and

273   our own predictions, males preferentially approached female silhouettes displaying

274   larger ornaments (leg scale pinnation B = 1.35 ± SE 0.379; z = 3.57; P < 0.0001;

275   abdomen size B = 3.72 ± 0.438; z = 8.50; P < 0.0001; Table 1). Males were also more

276   likely to approach and court a female silhouette if it was positioned near the centre,

277   rather than the periphery, of the swarm (swarm position B = -2.94 ± 0.216; z = -

278   13.6; P < 0.0001; Table 1, Figure 2).

279   Figure 3 illustrates the partial effects of abdomen and leg scale size on

280   numbers of male approaches. We have superimposed the raw data to assist with

281   visualization, but note that much of the variation in attractiveness is explained by

282   orthogonal dimensions of the silhouette phenotype that are accounted for in the

283 estimation of the partial effects. We also note that although the effects illustrated in

284 Figure 3 appear curvilinear, this is due to the back-transformation from loglinear

285 phenotypic space; on the log scale there was no evidence of significant quadratic

286 selection on either of the ornaments (leg scale pinnation B = -0.278 ± 0.252; z =

287 1.11; P = 0.269; abdomen size B = -0.0731 ± 0.433; z = -0.169; P = 0.866; Table 1).

288 Instead, we found straightforward directional selection for both ornamental traits:

289 males are more likely to approach female silhouettes with larger pinnate leg scales

290 and larger abdomens. Although the variation in silhouette pinnate leg scales was

291 larger than variation in abdominal ornamentation, (+/- 10.8SD compared with +/-

292 2SD, respectively), male dance flies responded much more strongly to variation in

293 abdomens than in pinnate leg scales (Figure 3).

294 We also found a significant negative coefficient associated with the term

295 describing an interaction between abdominal and leg ornamentation (B = -0.0136 ±

296 0.0031; z = -4.36; P < 0.0001; Table 1). Figure 4 helps to illustrate this nonlinear

297 correlational effect: the convex curvature near the apex shows that the two

298 ornaments combine in a less than additive way. Furthermore, while abdominal

299 ornaments are always important for attracting mates, pinnate leg scales are only

300 important if a female's abdomen is small.

301 In order to compare the effects of both ornaments on attractiveness as a

302 function of overall signal area (rather than in terms of phenotypic variation), we

303 performed the same analysis as that described in Table 1 except using the area (in

304 mm^2) of each ornament as a predictor. Table 2 illustrates that abdominal area still

305 has a stronger effect on attractiveness than leg scales; a given unit of silhouette area

306 is nearly twice as effective at improving attractiveness if it contributes to the

307 abdomen (B = 11.29 ± 1.86; z = 6.07; P < 0.0001) rather than the leg (B = 6.98 ±

308 1.59; z = 4.38; P < 0.0001).

309 **Discussion**

310   We measured male attraction to two female-specific ornaments in *R.*

311 *longicauda* and show that males are attracted to both ornaments, but that variation

312 in inflatable abdominal sacs has a stronger effect on attractiveness than variation in

313 pinnate leg scales. Furthermore, rather than combining additively, as predicted for

314 multiple ornaments that reinforce an honest signal of quality [36, 37], we found that

315 large leg ornaments are only favoured when abdominal ornaments are small. This

316 difference in selection is sustained whether we regress attractiveness on units of

317 ornament area or phenotypic standard deviations, which suggests that the two

318 ornaments are not simply acting together to reinforce a single signal of overall size.

319 Below we discuss the implications of our study for understanding the nature of

320 sexual selection on female dance flies.

321 ***The effect of mating swarm position on female attractiveness***

322   Our results show that a female silhouette is more likely to attract courting

323 males if it is displayed closer to the centre of the lek-like mating swarm (Figure 2).

324 This finding is consistent with previous work on a male-lekking insect species,

325 *Ceratitis capitata*, which found that male lek position was an important indicator of

326 attractiveness [38]. Further, many studies investigating diverse taxa with lek

327   mating systems have shown that centrally positioned males are the most attractive

328   [39-41]. In many male leks, intra- as well as intersexual selection for a central

329   position is described, however, in *R. longicauda* mating swarms, while we have

330   compelling evidence for intersexual selection on swarm position (Figure 2), there is

331   no evidence that females physically engage with one another [22, 32]. Previous

332   work on *R. longicauda* swarm position [32] showed that female flies at the bottom of

333   the swarm (where males enter) were larger than females higher up in the swarm.

334   Our study suggests that the swarm may also be structured horizontally; with

335   centrally located females possessing an advantage due to their proximity to the

336   entry point for swarming males. Additionally or alternatively, being close to the

337   periphery of the swarm might make individuals more vulnerable to predation.

338   *Tetragnatha* spiders build webs around the periphery of *R. longicauda* mating

339   swarms and dance flies are frequently preyed upon [42]. It is likely that being in the

340   centre of the swarm means that both sexes are safer from spider predation, which

341   could confer an advantage to centrally positioned silhouettes in the absence of any

342   intraspecific competition for position.

343   ***How did multiple female ornaments evolve in dance flies?***

344 	Several hypotheses could explain how multiple female-specific ornaments

345   arise in *R. longicauda*, including nonadaptive mate choice models (involving

346   Fisherian processes, e.g., via sensory biases), adaptive models (e.g., honest signaling

347   of direct or indirect benefits), and sexually antagonistic coevolution via sexual

348   conflict.

349   Nonadaptive models [43, 44] could conceivably have contributed to the

350   origin of female ornaments in dance flies, because larger objects are easier to

351   perceive from a distance. Although these models may well have been crucial for the

352   initial evolution or ornaments, however, the extravagance of pinnate leg scales and

353   inflatable abdomens (and the presumably large costs that accompany their

354   expression, (see[30, 45])) suggests that they are probably maintained by other

355   mechanisms.

356   Adaptive models based on direct benefits are frequently invoked in systems

357   featuring male choice, because variation among females in fecundity or sperm

358   competition intensity is expected to be most important for choosing males [19, 46].

359   In dance flies, if ornaments can communicate the remaining time required for

360   vitellogenesis, then males might favour females primarily because their chosen

361   mates would be less likely to mate again prior to oviposition (assuming a last male

362   paternity advantage in sperm competition; [8, 46-48]). Under this "honest

363   signaling" hypothesis, female ornaments evolve in spite of their costs because they

364   clarify or exaggerate an aspect of female phenotype that is difficult for males to

365   discern from the unadorned female's phenotype [21].

366   Many previous studies investigating female ornamentation have concluded

367   that ornaments serve to honestly signal female fecundity or egg maturity [15, 20-

368   22]. In one sample from our study site, *R. longicauda* abdominal ornaments

369   predicted 49% of the variation in egg size, but did not covary with fecundity;

370   furthermore, leg ornaments did not predict egg size or number [22]. If female

371   ornaments are generally serving to honestly signal mate quality to males, then we

372   would expect that all ornamental traits should have evolved to correlate with some

373   measure of female quality and that many would continue to do so. While it is

374   possible that the two ornaments in *R. longicauda* evolved for different purposes

375   [37], (e.g. maybe pinnate leg scales are important in intrasexual competition, while

376   abdominal ornaments are important for intersexual competition) this explanation

377   seems unlikely. First, there is no evidence of physical competition between

378   swarming females [32] and second, in other dance fly species leg scales are known

379   to be involved in intersexual selection [15]. In addition, the fact that dance fly

380   ornaments are fixed in size at eclosion (and therefore cannot accurately reflect

381   differences in ovarian development as vitellogenesis progresses) undercuts the

382   potential for ornaments to be honest signals of female quality related to egg

383   maturity. Finally, if exaggerated ornaments were strong signals, it is unclear why

384   there would be such a striking divergence in selection between patterns of

385   attraction to large ornaments (Figure 3; [8]) and copulation with individuals

386   displaying moderate ornaments [22] in this species.

387   Our experimental design allowed us to assess how the ornaments combined

388   to improve attractiveness. Both the combined effect of ornaments in standardized

389   phenotypic space (Table 1, Figure 4) and our analysis of ornament area (Table 2)

390   suggest that males are not simply attending to the overall size of a silhouette. This

391   finding suggests that the multiple female-specific ornaments displayed by *R.*

392   *longicauda* are not providing complementary information that additively

393   contributes to male perceptions of mate quality [37].

394   An alternative to adaptive mate choice is that sexual conflict [49] helps to

395   maintain ornament expression in this species (7). In fact, an arms race resulting

396   from sexual conflict might explain the presence of two extravagant female

397   ornaments in *R. longicauda* without requiring adaptive male choice: female

398   ornaments are favoured because they improve female access to male-provisioned

399   nuptial gifts, while males try to resist seduction by deceptive females by more

400   closely inspecting mates prior to passing over prey. Cyclic bouts of sexually

401   antagonistic coevolution [50] (in which females develop deceptive ornaments, and

402   males evolve to resist them) would result in the development of a series of

403   ornamental traits, of which some are only marginally effective thanks to selection

404   for resistance by the opposite sex. As we found for pinnate scales, weaker selection

405   is expected for more ancestral forms of ornamentation (pinnate leg scales are a

406   prevalent form of ornamentation that is presumably relatively ancient among dance

407   flies, although further phylogenetic study is needed [51]) compared to more

408   recently derived ornaments (possibly including inflatable abdominal sacs).

409   Moreover, pinnate leg scales are only effective at increasing attractiveness when the

410   abdominal ornament is small; when abdominal sacs are large there is no discernible

411   effect of pinnate leg scales (Figure 4).

412   Our results indicate that abdomens currently have a bigger influence on male

413   impressions of female attractiveness than legs do. Perhaps there is an advantage for

414   males that attend more closely to the details of a female's phenotype. We predict

415   that inspections prior to pair formation (such as are observed in *R. longicauda*)

416   should be especially prolonged in taxa featuring female ornaments that might

417   otherwise disguise a female's ovarian condition. Similarly, any trait (such as visual

418   acuity) that allows males to better distinguish between potentially deceptive

419   ornaments and cues of actual fecundity should be favoured, and increase selection

420   for novel ornaments that circumvent male resistance. The inflatable abdomens

421   found in *R. longicauda* (and which may have evolved independently in a few other

422   dance flies) may be more effective disguises because the degree to which they

423   exaggerate fecundity may be difficult to detect by male sensory systems (i.e., it is

424   probably harder to distinguish egg-filled from air-filled abdomens than it is to

425   separately assess ovarian condition and pinnate leg ornament expression). It may

426   also be easier to differentiate a deceptive fecundity signal that evolves on the legs

427   compared to the abdomen simply because abdomen size is more closely associated

428   with fecundity [19]. Interestingly, it is unknown whether *R. longicauda* females

429   display variation in the amount they inflate their abdomens. While their maximum

430   inflation size is fixed during the moult to adulthood, it is possible that the amount

431   females inflate their abdominal sacs could vary with changes in environmental

432   conditions, through space or time; it is possible that variable inflation could provide

433   a mechanism by which egg development and ornament were temporally related.

434   We note that episodes of selection beyond initial attraction are undoubtedly

435   important for determining mating success in dance flies. Following male approach, a

436   female might undergo further assessment by her mate, resulting in discrimination

437   against (overinvesting) females displaying the largest ornaments that are a greater

438   cost to fecundity [5, 21]. In fact, Wheeler et al [16] showed that *R. longicauda*

439   females displaying the largest ornaments are less likely than intermediately

440   ornamented females to mate. An alternative explanation for the intermediate female

441   advantage predicted by Chenoweth [21] and observed by Wheeler [16] is that

442   females displaying the largest ornaments are actually too cumbersome (e.g.

443   awkward for males to carry females with large, inflated abdomens) or heavy (e.g.

444   larger pinnate scales) for some males to carry while flying united. Further study

445   investigating the biomechanics of aerial copulation as well as quantifying the total

446   load a male carries (female and nuptial gift) and traits related to a male's own load-

447   lifting ability (e.g. wing load, aspect ratio) is required to fully assess the role of

448   constraints in the evolution of female ornaments [52].

449   Although our findings are compellingly consistent with a partial role of

450   sexual conflict, it is important to recognize that the alternative models of ornament

451   evolution need not be exclusive, and that patterns from one species may not reflect

452   the evolutionary trajectories for its entire subfamily. We need more work

453   comparing selection on ornaments in other dance fly species, including taxa with

454   different numbers of ornaments and levels of ornament expression. Such work will

455   need to be creative to overcome the currently limited capacity for experimental

456   work on these systems.

457

458   **Tables**

459   **Table 1**. Parameter estimates for a generalized linear mixed effects model

460   describing how standardized ornamentation and swarm position traits affect female

461   attractiveness. Variance component: 0.768.

	trait	estimate	standard error	z value	p value
linear	swarm position	-2.94	0.216	-13.6	<0.0001
	leg scale pinnation	1.35	0.379	3.57	<0.0001
	abdomen size	3.72	0.438	8.50	<0.0001
quadratic	swarm position	-1.02	0.187	-5.43	<0.0001
	leg scale pinnation	-0.278	0.252	-1.11	0.269
	abdomen size	-0.0731	0.433	-0.169	0.866
interaction	swarm position * leg scale	0.0078	0.0067	1.16	0.246
	swarm position * abdomen	-0.0196	0.0294	-0.667	0.505
	leg scale * abdomen	-0.0136	0.0031	-4.36	<0.0001

462

463

Table 2. Parameter estimates for a generalized linear mixed effects model describing how area (mm^2) of ornamentation and swarm position affect female attractiveness. Variance component: 0.782.

	trait	estimate	standard error	z value	p value
linear	swarm position	-4.39	2.44	-1.8	0.0725
	leg scale pinnation	6.98	1.59	4.38	<0.0001
	abdomen size	11.29	1.86	6.07	<0.0001
quadratic	swarm position	-1.00	0.188	-5.32	<0.0001
	leg scale pinnation	-0.273	0.235	1.161	0.246
	abdomen size	-0.0894	0.43	-0.208	0.835
interaction	swarm position * leg scale	0.114	0.118	0.966	0.339
	swarm position * abdomen	-0.0284	0.069	-0.412	0.68
	leg scale * abdomen size	-0.532	0.119	-4.47	<0.0001

467

468

Figure captions

470

471 **Figure 1**. Silhouettes of 25 plastic models of females created to display to males
472 within the mating swarm. Silhouettes vary in the amount of two female-specific
473 ornaments on display. Abdomen ornament sizes are population mean, mean ± 1.5
474 SD and mean ± 2.5 SD. Leg scale ornament sizes are population mean, mean ± 5.4
475 SD and mean ± 10.8 SD.

476 **Figure 2**. The partial quadratic effect of female swarm position on male visitation
477 from a linear mixed effects model. Swarm position was measured as the distance
478 from the centre of the mating swarm in increments of 15cm. The shaded area
479 represents the standard error around the measure, values for the mode are
480 reported in Table 1.

481 **Figure 3**. The partial effect of manipulated female ornamentation (abdomen width
482 and pinnate leg scale length) on male courtship attempts overlaid on the raw data.
483 Abdomen size was partitioned from the population's natural size variation ranging
484 from two standard above and below the mean population size (-2 to 2 on the x-axis).
485 The leg scale length was manipulated such that males were presented with
486 ornament sizes from outside the natural population range (no scales (mean male leg
487 size), 0.5×, 1.5× and 2× mean female scale length).

488 **Figure 4**. The mate attraction landscape incorporating linear and correlational
489 attraction on abdominal ornaments and pinnate leg scales in *R. longicauda* females,
490 as visualized using a nonparametric thin plate spline. Values on contour lines
491 indicate the predicted number of male visitors for the trait space that each line
492 occupies.

493

Data accessibility

All data and code used in this manuscript are available as supplementary materials

Competing interests

We have no competing interests.

Authors' contributions

RM and LB conceived the experiment; RM, JW, DG carried out the field experiment; RM and LB carried out the statistical analysis; all authors contributed to the writing of the manuscript. All authors gave final approval for publication.

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