What are you lookin' at?: A role for sexual conflict in coevolution of female display signals and male sensory adaptations?

R. Axel W. Wiberg[1,3*], Rosalind L. Murray[1,2], Elizabeth Herridge[1,4], Darryl T. Gwynne[2], Luc F. Bussière[1,5,6*]

1. Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA, United Kingdom

2. Department of Biology, University of Toronto at Mississauga, Ontario, L5L 1C6, Canada

3. Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, Uppsala, Sweden, 752 36

4. Health and Social Care Analytical Services, St Andrews House, Regent Road, Edinburgh, EH1 3DG, United Kingdom

5. Biologi och Miljövetenskap, University of Gothenburg, Box 451, Göteborg, Sweden, 405 30

6. Gothenburg Global Biodiversity Centre, University of Gothenburg, Box 451, Göteborg, Sweden, 405 30

* Corresponding authors: **R. Axel W. Wiberg**; raw.wiberg@protonmail.com, **Luc F. Bussière**; luc.bussiere@bioenv.gu.se

ORCID Numbers:

RAWW	0000-0002-8074-8670
RLM	0000-0001-6464-0072
LFB	0000-0001-8937-8381

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Abstract

The prevalence and diversity of female ornaments poses a challenge to evolutionary theory because males should prefer mates that spend resources on offspring rather than ornaments. Among dance flies, there is extraordinary variation in sexual dimorphism. Females of many species have conspicuous ornaments (leg scales and inflatable abdominal sacs). Meanwhile males of some species have regions of their eyes with larger ommatidial facets, common traits in many arthropod taxa that allow for regionally elevated photosensitivity and/or acuity. Here, we conduct a comparative study of these traits using both species descriptions available from the literature, as well as quantitative measures of eyes and ornaments from wild-caught flies. We show a conspicuous covariance between exaggerated male dorsal eye regions and the extent of female ornaments: species with highly ornamented females have males with more exaggerated eyes. We discuss this pattern in the context of competing hypotheses for their evolution.

Introduction

36 Female secondary sexual traits, such as ornaments, remain poorly understood in evolutionary biology. This is, in part, because female fecundity is often resource-limited, and males should avoid females that divert resources away from offspring towards extravagant 39 morphologies (Fitzpatrick *et al.,* 1995; Clutton-Brock 2009). Although some examples of such traits exist (Dougherty 2021), female ornamentation remains rare in nature.

The dagger flies of the subfamily Empidinae (Diptera: Empididae) are a speciose 42 group with a global distribution, and exhibit remarkable diversity in mating systems and sexual dimorphisms among both sexes (Collin 1961; Cumming 1994; Chvala 2005). The ancestral mating behaviour appears to have involved the aerial transfer of a nuptial gift 45 (typically, an exogenous oral nuptial gift, *sensu* Lewis *et al.,* 2014) from the male upon which the female feeds during copulation (Downes 1970; Cumming 1994). Females generally do not hunt as adults (Newkirk 1970), and, in many species, contests for nuptial gifts are so intense 48 that the operational sex ratio (OSR; Emlen and Oring 1977; Ahnesjö *et al.,* 2002) in aerial mating swarms is heavily female-biased. Intense contests for nuptial gifts seem to have promoted the evolution of several secondary sexual characters in females (Cumming 1994), 51 including enlarged or coloured wings, inflatable abdominal sacs and, most frequently, pinnate scales on one or more pairs of legs (see figure 1A) which serve to attract and secure a male with a nuptial gift (Murray *et al.,* 2020).

54 Meanwhile, males of some species have dichoptic compound eyes, with dorsal eye regions showing enlarged ommatidia in comparison to females (Collin 1961; see figure 1A). Such regions increase photosensitivity and are referred to as “bright zones”; when combined 57 with a flatter curvature of the eye these regions can also increase acuity (“acute zones”; Land

1989; 1997). These adaptations can locally adjust the optimal facet size, which is otherwise constrained by the size and resolving power of the full compound eye (Land 1989; 1997).
60 Previous hypotheses to explain variation in male eye morphology have explicitly or implicitly invoked selection among males for faster mate acquisition or choice of more ornamented females (Downes 1970; Thornhill and Alcock 1983; Cumming 1994). For example, selection
63 might favour adaptations to "scramble-like" male competition as in other systems (e.g. Eichorn *et al.,* 2017). Alternatively, they have been proposed as adaptations to assess honest female ornaments (LeBas et al., 2003).

66 Although a link between male eye morphology and female ornamentation in dance flies has been proposed (e.g. LeBas et al., 2003), the degree of variation in eye dimorphism and whether these traits covary across species has never been explicitly investigated. Here we
69 conduct a comparative study investigating the co-evolution of female ornaments and male eye morphology in dance flies. We first make use of a survey of species descriptions on morphology to assess broad patterns of association between eye dimorphism and ornament
72 dimorphism among many British dance flies. We then take a quantitative approach and quantify male and female eye morphology as well as the degree of female ornamentation for a subset of species. We discuss our findings in the context of competing hypotheses on the
75 evolution of female ornamentation and male eye adaptations in these species.

Methods

78 We examined species descriptions in Collin (1961) to document possible associations between female ornaments and eye sexual dimorphism among the 88 British dance fly species of the subfamily Empidinae. We noted the presence or absence of four main categories of

81 sexually dimorphic traits in females that are likely to be sexually selected ornaments: enlarged wing size, darker wing colouration, pinnate scales on legs and inflatable abdominal sacs. We also noted any descriptions of sexual dimorphism in eye facets. We tested for an association 84 between eye dimorphism and female ornamentation using several Chi-squared tests to evaluate whether ornamental traits were associated with eye dimorphism (table S1). The first test considered multiple ornaments (enlarged or darkened wings, inflatable abdominal sacs, 87 pinnate leg scales). We also tested for association between a) just pinnate leg scales and eye exaggeration or b) all ornaments excluding pinnate leg scales and eye exaggeration. This final contingency table has expected frequencies < 5 in some cells (table S1C), we therefore used a 90 Fisher's Exact Test in that analysis.

Sampling

93 We collected dance fly samples during the summers 2009, 2010 and 2011 on the eastern side of Loch Lomond between the Scottish Centre for Ecology and the Natural Environment (SCENE) and Rowardennan, Scotland, and during the summer of 2012 near 96 Glen Williams in Ontario, Canada. We caught flies from mating swarms or when resting on vegetation using sweep nets (Murray *et al.,* 2017). Of the sampled species, we selected eight common ones that represent a range of variation in female ornaments, male eyes, and mating 99 systems (see figure 1 and table S2).

Morphological Measures

102 Differences in eye morphology were quantified from nail varnish casts (see figure 1; Ribi *et al.,* 1986; Narendra *et al.,* 2010). The diameters of seven neighbouring ommatidia on

the dorsal and ventral portions of the eyes were measured from micrographs using tpsUtil (v 1.58; Rholf 2017) and tpsDig2 (v 2.17; Rholf 2017) in a manner similar to Döring and Spaethe (2009). The difference between mean dorsal and ventral diameters was standardised by thorax length and taken as a measure of exaggeration. Thus, a positive value of exaggeration indicates larger dorsal ommatidia and a negative value indicates larger ventral ommatidia.

Female ornamentation was quantified as the total leg area of the four posterior legs of males and females for each species using the software ImageJ (v 1.48; Rasband 2016). While these ornament indices underestimate ornament expression for some species (*e.g.,* for species where females have multiple ornament, such as inflated abdominal sacs and/or darker wings in addition to pinnate scales), they nevertheless allowed us to measure trait expression in an analogous way across multiple taxa. Because the most heavily adorned species also had elaborate pinnate scales, ignoring the other ornament types makes our assessment of covariance between eyes and ornament expression conservative. Moreover, our results from the literature search of Collin's (1961) key indicate that pinnation itself may be a strong predictor (see Results below). These measures were scaled by the total length of the femora and tibia of all four legs. Finally, the mean scaled male leg area was subtracted from each individual female measure for each species to give individual measures of ornamentation for each female.

Statistical Analyses

The relationship between ornamentation, sex and eye exaggeration was tested in a mixed model framework, fitting ornamentation, sex, and their interaction to predict eye exaggeration. We included species as a random effect to control for non-independence of

measures within species. The species studied here are from two genera, but recent molecular phylogenetic work shows that these genera are not monophyletic and that female ornamental
129 traits have evolved independently several times (Watts *et al.,* 2016; Murray *et al.,* 2020). To account for phylogenetic pseudoreplication arising from including closely related species in our analysis, we also fitted models that control for phylogeny. We used the consensus tree
132 from Murray et al., (2020), pruned to contain the eight species of interest. In addition to the main analysis on the consensus tree, we also marginalised our analyses over a random sample of 2,000 trees from the posterior distribution of trees (Murray *et al.,* 2020; Hadfield and
135 Nakagawa 2010; Hadfield 2010; Ross *et al.,* 2013). Continuous predictors were scaled (the mean subtracted from each value) prior to analysis (Schielzeth *et al.,* 2010). All analyses were performed in R (v.3.6.3; R Development Core Team 2020) using the packages "ape" (v. 5.4;
138 Paradis *et al.,* 2004), "lme4" (v. 1.1-23; Bates *et al.,* 2015), "pbkrtest" (v. 0.4-8.6; Halekoh *et al.,* 2014), and "MCMCglmm" (v. 2.29; Hadfield and Nakagawa 2010; Hadfield 2010). Figures were plotted with "ggplot2" (v. 3.3.1; Wickham 2016).

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Results

We found strong positive associations between ornament expression and eye
144 exaggeration from taxonomic descriptions of female ornaments and male eyes (Collin 1961; table S1). Dimorphism in eye morphology was more strongly associated with pinnate leg scales than other ornament characters (table S1A, all ornaments together: Chi-squared = 9.91,
147 d.f. = 1, $p < 0.01$; table S1B, pinnate scales only: Chi-squared = 15.06, d.f. = 1, $p < 0.001$; table S1C, other ornaments excluding pinnate scales: Fisher's Exact Test: Odds Ratio (95% CI) = 0.72 (0.14, 3.14) , $p = 0.75$).

150 For a subset of eight species for which we collected wild specimens, we found a strong association between continuous measures of female ornamentation and eye exaggeration, with more exaggerated eyes occurring in taxa with more ornate females. 153 Notably, female eyes in ornamented species tended to show the opposite pattern; females had larger ventral facets than dorsal ones, and more ornamented species tended to have more exaggerated ventral facets (partial slopes for females were negative, but non-significant; table 156 1A, figure 1). These associations remained significant in models that controlled for relatedness (table 1B), and models that accounted for phylogenetic uncertainty (table 1C).

159 **Discussion**

A number of hypotheses involving conventional sexual selection or sexual conflict have been proposed for female ornamentation and male eye exaggerations in Empidine 162 dagger flies. We show that species with more heavily ornamented females also have males with more exaggerated dorsal eye facets. These findings strongly suggest a process of co-evolution between these two traits.

165 Previous hypotheses to explain variation in male eye morphology have explicitly or implicitly invoked selection *among males* for faster mate acquisition or choice of more ornamented females (Downes 1970; Thornhill and Alcock 1983; Cumming 1994). First, 168 exaggerated eye morphologies could be adaptations to "scramble-like" male competition as in other systems (Eichorn *et al.,* 2017). However, this hypothesis predicts more exaggeration in species with strong competition among males, which would produce negative associations 171 between eye dimorphism and female ornament extravagance, since strong pre-mating competition among males is not expected to co-occur with female ornaments (Murray et al.,

2020). This is the opposite of our finding of positive covariance between ornament expression and male eye dimorphism.

Alternatively, eye adaptations could result from competition among males to facilitate appraisal and identification of the highest quality females. Under this scenario, exaggerated eye morphologies facilitate assessments of female quality. Thus, these adaptations could serve as alternative routes for honest signals, with large ornaments being particularly useful in species with poor male visual acuity, and heightened vision being especially useful in species with less exaggerated ornaments. However, the coincidence of both ornaments and heightened vision would be surprising under this hypothesis, since large, honest ornaments should facilitate perception on their own.

Another alternative is that eye exaggerations might be required as part of a male preference trait for female ornaments that honestly provide signals of reproductive value (Bloch 2015). In this case the preference trait (exaggerated eye morphology) is expected to covary positively with the degree of female ornamentation. This pattern of positive covariation is a standard prediction of sexual selection theory where preferences and ornamental traits coevolve (Andersson 1994). However, this hypothesis relies on female ornaments being an honest indicator, which is problematic on several grounds (see below).

Hypotheses to explain the evolution of female dance fly ornaments, either invoke honest signalling (LeBas et al., 2003) or deception (Funk and Tallamy 2000; Hockham and Ritchie 2000) both of which are discussed in more detail below. Conventional sexual selection on honest signals predicts a negative association between female ornament expression and the proportion of males in mating swarms (Murray *et al.,* 2020), because choice is most likely when receptive females outnumber males (Bonduriansky 2001).

Ornamented females could for example be advertising direct (material) fitness benefits to mates, or indirect (genetic) gains for pairing with them. Direct benefits are much more likely than indirect ones to explain male mating preferences; they can act immediately rather than awaiting expression in offspring, and are not dependent on strong inter-sexual genetic correlations (Kirkpatrick and Barton 2001). Furthermore, female variation in reproductive value should almost always exceed variation in genetic quality (Kirkpatrick and Barton 2001).

In dance flies, males might prefer females with ornaments that signal greater fecundity or a later stage of ovarian development. Being later in ovarian development may be a particularly valuable indicator of reproductive value among insects with sperm storage, because it should indicate that a female is less likely to mate again prior to oviposition (remating should generally decrease a focal male's paternity by displacing his ejaculate within sperm stores *e.g.* Boorman and Parker 1976). However, several facts speak against this hypothesis. First, while some studies suggest that males prefer females with larger ornaments, the presence and intensity of sexual selection is inconsistent across dance fly species and episodes of mating (Funk and Tallamy 2000; LeBas *et al.,* 2003; Murray et al., 2018). In some species, there is no evidence that larger or more ornamented females acquire more mates (Hockham and Ritchie 2000; Bussière *et al.,* 2008; Wheeler *et al.,* 2012; Herridge 2016; Murray *et al.,* 2019); such inconsistency is not predicted by hypotheses of honest signalling. Moreover, covariances between female ornamentation and fecundity or egg size are generally low compared to covariances between fecundity or ovarian maturity and other indices of body size (*e.g.* thorax length; LeBas *et al.,* 2003; Bussière *et al.,* 2008; Wheeler *et al.,* 2012; Murray *et al.,* 2019; Murray *et al.,* 2020). Indeed, female ornaments are an unreliable signal of reproductive value since ornaments like pinnate leg scales and wing colour are fixed at

219 eclosion, while the number and developmental maturity of eggs changes throughout adult life. For example, in some ornamented species ovarian development appears to be entirely arrested prior to an initial mating and nuptial feeding event (Hunter & Bussière 2019), which means 222 that signals of fecundity from virgin females are by necessity deceptive. Together, these facts suggest that ornaments do not always arise to improve male assessments of female reproductive quality.

225 What if these ornaments were instead indicators of genetic benefits? Addressing this possibility requires knowledge of the relative costs (e.g., in paternity share) and benefits (in enhanced fitness of offspring) for mating with attractive females. There is some evidence of 228 increased sperm competition among males in more ornamented species (Murray *et al.,* 2020), but we altogether lack information on heritable benefits of female attractiveness. Given equivocal support for good-genes benefits for mating attractive males in many systems, the 231 presence of substantial variation in direct benefits undercuts the probability that good-genes could favour male preferences that incur substantial direct costs (Kirkpatrick and Barton 1997). Consequently, neither good-genes nor direct benefits honest signalling seem able to 234 satisfyingly explain the extraordinary diversity of dance fly ornaments.

As an alternative to honest signalling, female ornaments in dance flies could be generally deceptive adaptations that exploit historical male preferences for larger females. 237 Deceptive ornaments would allow females to obtain nuptial gifts regardless of their current fecundity or stage of egg development (Funk and Tallamy 2000; Hockham and Ritchie 2000; Arnqvist and Rowe 2005; Arnqvist 2006), even if males prefer highly gravid females to avoid 240 increased sperm competition risk (Herridge *et al.,* 2016). If female ornaments are in fact deceptive signals (Funk and Tallamy 2000; Hockham and Ritchie 2000) designed to exploit

male preferences for large females, we might expect resistance traits in some well-adorned
243 species (Arnqvist and Rowe 2005). Although no resistance traits have ever been described among male dance flies, adaptations that augment visual perception and acuity, such as the exaggerated male eye regions observed here, are exactly the sorts of traits that are expected.

246 Sexual conflict and sexually antagonistic coevolution (SAC) are relatively recent models proposed to explain the origin and exaggeration of some sexually dimorphic traits (Parker 1979; Holland and Rice 1998; Arnqvist and Rowe 2005). Sensory exploitation of one
249 sex by the other can play a key role in this model, with deceptive signal traits selecting for resistance in the receiver sex; the resulting arms race drives the diversification of both signal and receiver traits (Holland and Rice 1998; Arnqvist and Rowe 2005). When sensory
252 exploitation via a seductive signal induces a suboptimal mating rate in the receiver, it increases the receiver's exposure to costs of mating (Arnqvist 2006). To avoid costs, receivers should evolve increased discriminatory power to distinguish manipulative signals from honest
255 ones, or to reduce susceptibility to seductive stimuli altogether. Repeated bouts of exploitation and resistance should lead to cycles of antagonistic co-evolution (Holland and Rice 1998). More generally, SAC could explain seductive female traits and male resistance in rare species
258 possessing strong male choice (Funk and Tallamy 2000; Murray *et al.,* 2018). In such species male preference evolution should be constrained because female fecundity is often resource-limited, and males should avoid females diverting resources away from offspring towards
261 extravagant morphologies (Fitzpatrick *et al.,* 1995; Clutton-Brock 2009). Additionally, if females store their mates' sperm, heavily ornamented females might acquire more mates, and consequently provide each male with a lower paternity share than less ornamented (and less
264 polyandrous) females (Herridge *et al.,* 2016). There is good evidence that sexually

antagonistic co-evolution drives diversification in traits that mediate physical mating interactions (Arnqvist and Rowe 2002; Rowe and Arnqvist 2002; Bergsten and Miller 2007; Tatarnic and Cassis 2010). While sexually antagonistic sensory exploitation has been offered as an explanation for the origins of nuptial gifts and some signal traits (Sakaluk 2000; Amcoff and Kolm 2015), to our knowledge, no studies have shown comparative evidence for antagonistic co-evolution between signal and receiver traits.

SAC, therefore, offers a compelling alternative hypothesis that overcomes several difficulties with the evolution of female ornaments, and can at once explain variation in both eye and ornamental traits across species. Female ornaments might exploit pre-existing male preferences for larger but unadorned females (because large females tend to be more gravid or more fecund; Funk and Tallamy 2000; Arnqvist 2006), so that females can obtain nutritious nuptial gifts regardless of their current state of ova development. Males respond to female deception by developing resistance traits (increased visual perception) to avoid low-value females. According to SAC, exaggerations in male eye morphology reflect selection for increased discriminatory power to "see past" increasingly deceptive ornaments of hungry females, and identify the most gravid or fecund mates despite female disguises. This hypothesis predicts a positive association between exaggerations in eye morphology and ornamentation across species, as is seen in our data. This positive covariation arises because exploitative adaptations that increase mating frequency for females (increased ornamentation) should be countered by increased investment in discriminatory power by males (exaggerated dorsal facet morphologies). The SAC hypothesis also predicts a negative association between ornamentation and the proportion of males in the swarm (the swarm sex ratio) because large deceptive ornaments should be most favoured in environments where females compete more

288 intensely for resources and experience stronger selection to deceive males (Murray *et al.,* 2020).

Moreover, our results from the literature survey suggest that exaggerations of dorsal 291 eye morphologies in males may be specific responses to deception *via* leg pinnation. The association between ornaments and male eye exaggerations were strongest when ornamentation was defined as the presence of leg pinnation alone. However, the prevalence of 294 pinnate scales relative to other kinds of ornaments makes strong conclusions difficult (31/88 British Empidinae species have females with pinnate leg scales; while only 11/88 species have other forms of female ornamentation). Nevertheless, the possibility that leg ornaments in 297 particular select for dichoptic eyes is intriguing because the heightened photosensitivity of large eye facets may be particularly effective at detecting stray photons that betray the difference between deceptively-positioned scaly legs that exaggerate abdomen size and egg-300 inflated large abdomens. In this context it is also noteworthy that the most extremely ornamented species, with the most exaggerated male dorsal acute zones, also have other ornamental features, *R. longicauda,* for example, has inflatable abdominal sacs that can serve 303 to further increase a female's apparent size to the male (Funk and Tallamy 2000; Murray *et al.,* 2018; Murray *et al.,* 2020). These inflatable sacs might also be more difficult for the male to resist by increases in photosensitivity or visual acuity. In this way, additional ornamental 306 features, such as abdominal sacs or darkened wings may represent more recent or sequential innovations in the historical arms race between seductive female characters and discriminatory male sensory structures.

309 Naturally, visual perception is an important part of dance fly ecology in many other contexts apart from mate assessment. For example, hunting ability probably depends heavily

on visual acuity. The sex-difference in hunting nuptial gifts could therefore conceivably lead
312 to differences in eye dimorphism. However, there are no obvious cross-species differences in prey choice that align with variation in ornament expression or eye exaggeration (almost all empidines present nuptial gifts, including the eight species we measured quantitatively;
315 Laurence 1952; Collin 1961; Chvala 2005). Furthermore, if hunting ability did drive the evolution of eye exaggerations, it might be strongest and most obvious in species where male competition for females is intense, to favour rapid return to mating swarms, whereas we found
318 the converse. We lack any evidence that hunting efficiency covaries strongly with ornament expression, and a more systematic survey of dance fly foraging ecology would help shed light on these questions.

321 The light environment also selects strongly on visual systems (Land 1989; 1997; Eichorn *et al.,* 2017), and some theory suggests that female ornaments are more likely in environments where male discrimination is difficult (Chenoweth *et al.,* 2006). However, no
324 great differences in light environment (*e.g.* time of day, canopy cover) are obvious among these species from field observations (Downes 1970; Cumming 1994; Murray *et al.,* 2017 Murray *et al.,* 2018). It is also not clear why the light environment would select for both better
327 visual perception *and* large ornaments in females because either trait on its own would presumably improve discrimination. Nevertheless, subtle differences might play a role in adaptations toward greater visual acuity.

330 Our study establishes a strong signal of covariation between male eye-exaggeration and female ornamentation. We suggest that this signal is inconsistent with sexual selection in male biased swarms and models relying on honest female ornaments are problematic. We
333 outline a plausible and intriguing alternative hypothesis involving sexual conflict and a co-

evolutionary diversification of female ornaments and male visual systems. These traits provide several exciting opportunities to study the emergence of novel phenotypes, as it may be possible to use female (in the case of eye morphology) or phylogenetically "basal" morphologies as ancestral states or starting points. It also provides a novel system in which to study the inherent sexual conflicts that arise in producing sexually dimorphic phenotypes from a common genome (Pennell and Morrow 2013).

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Ethics

Specimens of male and female Empidine dance flies were captured in the field and sacrificed by freezing until morphological measurements were made. We obtained ethical approval for all sampling and measurement procedures from the University of Stirling research ethics committee.

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Data and analysis code availability

All data and scripts used for the statistical analyses and for producing the figures in this manuscript have been deposited in a dryad repository (doi:https://doi.org/10.5061/dryad.rr4xgxd5z).

Competing interests

The authors declare no competing interests.

Author contributions

RAWW and LFB conceived the study. RAWW, RLM, EH, DTG, and LFB all contributed to

363 the collection of specimens and data. RAWW performed the data analysis with help from RLM and LFB. RAWW led the writing of the manuscript with input from RLM, EH, DTG and LFB. All authors have approved the publication of the final manuscript.

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Figure 1.

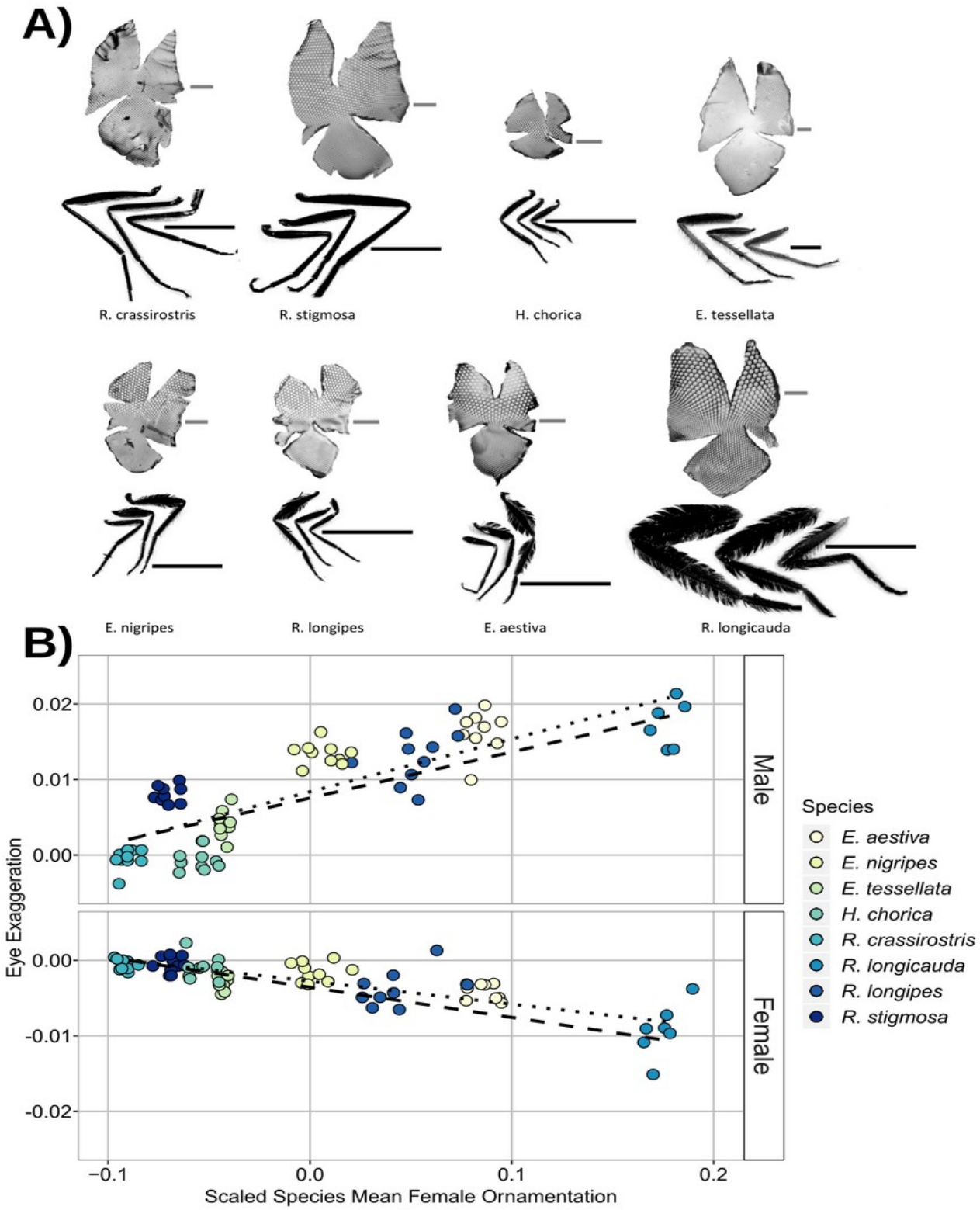
A)
R. crassirostris
R. stigmosa
H. chorica
E. tessellata
E. nigripes
R. longipes
E. aestiva
R. longicauda
B)
Male
Female
Eye Exaggeration
0.02
0.01
0.00
-0.01
-0.02
−0.1
0.0
0.1
0.2
Scaled Species Mean Female Ornamentation
Species
E. aestiva
E. nigripes
E. tessellata
H. chorica
R. crassirostris
R. longicauda
R. longipes
R. stigmosa

Figure 1. (previous page) A) Micrographs of nail varnish eye casts and female front, middle
507 and hind legs. Each of the 8 species included in the comparative analysis is represented. Images are taken at different magnifications; black scale bars to the right of the respective female legs represent 1 mm, while grey scale bars to the right of the respective eye casts
510 represent 0.1 mm. **B)** The relationship between eye exaggeration (the standardised difference between dorsal facet sizes and ventral facet sizes), for males and females, and scaled species level mean female ornamentation (as measured by standardised leg area) for 8 species of
513 dance flies (Diptera: Empididae). Regression lines represent fitted lines; dotted lines are from a generalised linear mixed model (table 1A), dashed lines are from a phylogenetically controlled generalised linear mixed model (PC-GLMM) using a consensus tree for the
516 relatedness matrix (table 1B). The points represent individual values for male eyes; to help visualise the standard error around the mean of female ornamentation for each species, we have horizontally spread points for each species in proportion to the uncertainty in species
519 mean.

Table 1. Parameter estimates from **A)** generalised linear mixed model using Restricted Maximum Likelihood (REML), **B)** phylogenetically controlled generalised linear mixed model in a Bayesian framework and using Markov Chain Monte-Carlo (MCMC) and **C)** phylogenetically-controlled generalised linear mixed model marginalised over 2,000 phylogenetic trees in the posterior distribution of tree topologies. Predictors included were Sex (M = male, F = female) and scaled species mean female ornamentation as well as their interaction. The response value in each model is the eye exaggeration, quantified as dorsal facet size minus ventral facet size. *p-values were derived using parametric bootstrap sampling using the "pbkrtest" R package; because this requires removing terms sequentially from the minimal model, for some models only a p-value for the highest order term was calculated. N = 146 in each case.

A) Generalised linear mixed model parameter estimates				
Parameter	***Estimate***	***SE***	***t***	***p-value****
Intercept	-0.0028	0.00085	-3.24	-
SexM	0.011	0.00041	27.45	-
MeanOrnamentation	-0.03	0.010	-3.05	-
SexM:MeanOrnamentation	0.10	0.0051	19.72	< 0.001
Variance of species = 0.0000051, residual variance = 0.000006				
B) Phylogenetically controlled linear mixed model parameter estimates (using a consensus tree)				
Parameter	***Estimate***	***CI***	**-**	***MCMC p-value***
Intercept	-0.0082	(-0.37, 0.39)	-	0.98
SexM	0.011	(0.0097, 0.013)	-	<0.001

MeanOrnamentation	-0.041	(-1.54, 1.28)	-	0.97
SexM:MeanOrnamentation	0.10	(0.082, 0.12)	-	<0.001
Variance of species (mean [95%CI]) = 0.03 [$1.3x10^{-8}$, 0.15], residual variance = $2.1x10^{-5}$ [$1.6x10^{-5}$, $2.6x10^{-5}$]				
C) Phylogenetically controlled linear mixed model parameter estimates (marginalised over 2,000 trees in the posterior distribution of tree topologies)				
Parameter	***Estimate***	***CI***	**-**	***MCMC p-value***
Intercept	-0.024	(-2.47, 2.47)	-	0.64
SexM	0.011	(0.010, 0.013)	-	<0.001
MeanOrnamentation	0.053	(-7.08, 10.02)	-	0.62
SexM:MeanOrnamentation	0.10	(0.08, 0.12)	-	<0.001
Variance of species (mean [95%CI]) = 5.2 [$5.6x10^{-10}$, 28.58], residual variance = $2.1x10^{-5}$ [$1.6x10^{-5}$, $2.6x10^{-5}$]				