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How sexual and natural selection shape sexual size dimorphism: evidence from multiple evolutionary scales

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Abstract

1. Sexual size dimorphism (SSD) is pervasive across taxa and reflects differences in the effects of sexual and natural selection on body size between the sexes. However, disentangling the complex eco-evolutionary interactions between these two mechanisms remains a major challenge for biologists.

2. Here, we combine macro-evolutionary (between-species), local evolutionary (between-population) and fine-scale evolutionary (within-population) patterns of SSD to explore how sexual and natural selection interact and shape the evolution of SSD in Australian agamid lizards. Australian agamid lizards show substantial variation in SSD, ecological traits and species density making them an ideal study system to address this question.

3. At the between-species level, population density, ecological generalism and mean species size significantly predict SSD variation, however, only ecological generalism was found to significantly explain variation in larger than average male-biased SSD. At the population level, density positively correlated with SSD in native habitats, but not city park habitats. Last, agonistic behaviour acted as the primary driver of SSD at the within-population level.

4. Our results indicate how sexual and natural selection can interact at different evolutionary scales, and show the importance of considering both selective mechanisms when investigating patterns of SSD.

53   **Keywords**

54   Sexual size dimorphism, natural selection, sexual selection, Agamid lizards, eastern water

55   dragon, city landscapes, eco-evolutionary interactions.

56   **Introduction**

57   Sexual size dimorphism (SSD) is common in nature and often reflects the conflicting

58   demands and selective pressures operating on the sexes (Wikelski and Trillmich 1997). Many

59   vertebrate taxa display male-biased SSD (Abouheif and Fairbairn 1997), which is usually

60   attributed to sexual selection (Darwin 1883), but can also result from natural selection

61   through ecological divergence (Darwin 1859). Although these mechanisms (natural and

62   sexual selection) are often studied in isolation, they are not necessarily mutually exclusive,

63   with their interaction often being complex (Andersson 1994, Krüger 2005, Krüger, Davies,

64   and Sorenson 2007) and underappreciated. This has resulted in the evolution of male-biased

65   SSD remaining poorly understood for most taxa.

66

67   Male-biased SSD is primarily associated with sexual selection since larger male body size

68   can confer an advantage for territory defence and mate acquisition (Stamps 1983, Carothers

69   1984, Andersson 1994, Cox et al. 2003, Cox, Butler, and John-Alder 2007). Larger males,

70   therefore, obtain a greater lifetime reproductive success (Clutton-Brock et al. 1987, Owen-

71   Smith 1993, Baird, Fox, and McCoy 1997, McElligott et al. 2001, Isaac 2005). Both mating

72   system and mating competition have been shown to positively influence SSD (Dunn,

73   Whittingham, and Pitcher 2001, Szekely, Lislevand, and Figuerola 2007), indicated by

74   correlations between male-biased SSD and the sex ratio of the population (Stamps 1983,

75   Poulin 1997), and the frequency of agonistic behaviour (Stamps 1983, Carothers 1984, Cox et

76   al. 2003). In terrestrial turtles, for example, male-biased SSD is predominantly determined by

77   male-male combat (Berry and Shine 1980), whilst in ungulates and cervids, SSD is largely

78   driven by adult sex ratio (Alexander et al. 1979, Clutton-Brock, Albon, and Harvey 1980).

79   However, a growing body of literature suggests that natural selection through ecological

80   niche partitioning between the sexes may also result in the evolution of SSD (Fisher 1958,

81   Clutton-Brock, Harvey, and Rudder 1977, Ralls 1977, Lande 1980, Slatkin 1984, Shine 1989,

82   Fairbairn 1997). Ecological niche partitioning can occur in order to reduce resource

83   competition between the sexes (Schoener 1967, Shine 1991, Pearson, Shine, and How 2002,

84   Losos, Butler, and Schoener 2003, Butler, Sawyer, and Losos 2007), or can occur due to

85   differences in life histories between males and females (Wikelski and Trillmich 1997).

86   Quality, dispersion and density of food have also been shown to influence SSD as food often

87   determines whether territorial mating systems are energetically feasible (Jarman 1974).

88   Furthermore, habitat type can be an important ecological factor influencing SSD

89   (Kaliontzopoulou, Carretero, and Adams 2015) as habitats can vary in food availability,

90   visibility and density of competitors (Selander 1966). *Anolis* lizards in Puerto Rico and

91   Jamaica, for instance, exhibit habitat-specific SSD (Butler, Schoener, and Losos 2000).

92

93   Although the influences of sexual and natural selection on the evolution of SSD are

94   commonly tested in isolation, they are likely to interact in multiple ways (Shine 1989, Krüger

95   2005, Krüger, Davies, and Sorenson 2007). First, the proliferation of 'good genes' via strong

96   sexual selection not only drives traits that confer a reproductive advantage (e.g. larger male

97   body sizes), but also traits that improve viability (Proulx 1999). For example, species that

98   occur in a variety of environments (ecological generalists) often display greater SSD (Östman

99   and Stuart-Fox 2011). Second, natural selection can constrain the evolution of exaggerated

100   sexual traits, including body size (Hosken and House 2011). Third, effects of sexual and

101   natural selection on SSD may be mediated by population density. Population density can

102   result in, and intensify, male sexual selection (Ghiselin 1976, Emlen and Oring 1977, Kokko

103   and Rankin 2006), where larger male size is favoured at high density (increased male-male

104   competition) due to the fitness advantage larger size confers (Ghiselin 1976, Stamps 1983).

105   However, at extremely high population densities, competition for mates may become too

106   great, resulting in the evolution of alternative reproductive tactics (Gross 1996), where larger

107   males guard females and defend territories whilst smaller males sneak copulations (Wirtz

108   1982, Forsyth and Montgomerie 1987, Zimmerer and Kallman 1989, Eadie and Fryxell 1992,

109   Berard et al. 1994, Lucas and Howard 1995, Reichard, Smith, and Jordan 2004, Tomkins and

110   Brown 2004). The evolution of alternative reproductive tactics within a population results in

111   greater variation in male body size and thus SSD. High population densities can also

112   exacerbate competition for food, favouring niche partitioning of the sexes by natural selection

113   (Wikelski and Trillmich 1997). Resource density and habitat type ultimately modulate

114   population density, and can therefore, in turn, influence the degree of competition over mates

115   (Kokko and Rankin 2006). However, effects of population density on SSD have rarely been

116   examined.

117

118   Here, we investigate the interaction between sexual selection (mating interactions) and

119   natural selection (ecological interactions) on the evolution of SSD in agamid lizards across

120   different evolutionary scales (species, populations and individuals). We focus on effects of

121   density and habitat on SSD at each scale using i) 43 Australian agamid species (1069

122   individuals), ii) nine populations of a single species, the eastern water dragon (*Intellegama*

123   *lesueurii*; 264 individuals), and iii) 137 eastern water dragon individuals from a single

124   population. In addition, to further investigate the mechanisms influencing the evolution of

125   SSD, we examine the effect of agonistic behaviour and sex ratio on SSD at the within-

126   population level. Agamid lizards show substantial variation in SSD, ecological traits and

127   density within and between species making them an ideal system in which to study the

128   evolution of SSD. Specifically, eastern water dragons show male-biased SSD and occur at

129   varying densities in a broad range of riparian and human-dominated landscapes. By

130   integrating multiple evolutionary scales, our study provides empirical insights into the ways

131   sexual and natural selection interact to drive the evolution of male-biased SSD in agamid

132   lizards.

133   **Materials and Methods**

134   *Study design*

135   Table 1 shows the number of males and females that ecological and morphological data were

136   collected for at each evolutionary level. Supporting Information Table S1, Fig. S1 and S2

137   show the sex differences between morphological traits at the between-species, between-

138   population and within-population level analyses.

139

140   i.   *Between-species level*

141   Morphological data were compiled using museum specimens and published literature for 43

142   agamid species (Thompson and Withers 2005, Thompson et al. 2009, Littleford‐Colquhoun

143   et al. 2017). Where possible, data were collected for at least five individuals of each sex, for

144   each species (see Table S2 for species numbers).

145

146   ii.   *Between-population level*

147   At the between-population level, ecological and morphological data were collected for nine

148   genetically independent (Littleford‐Colquhoun et al. 2017) populations of the eastern water

149   dragon. The eastern water dragon is a semi-aquatic arboreal agamid lizard that is native to

150   Australia. They are found in abundance across both their native riparian habitat (ranging from

151   Northern Queensland to north eastern Victoria) and across human-dominated landscapes

152   (including urban and suburban locations (Baird, Baird, and Shine 2012, Gardiner et al. 2014)).

153   Eastern water dragons display male-biased sexual dimorphism with males having larger

154   heads and jaws and displaying red ventral colouration (Baird, Baird, and Shine 2013,

155   Thompson 1993, Cuervo and Shine 2007). In addition, males exhibit alternative mating

156   strategies by either aggressively defending a territory or assuming satellite behaviour (Baird,

157   Baird, and Shine 2012), whilst female dragons are polyandrous (Frère, Chandrasoma, and

158   Whiting 2015). Both male and female dragons display agonistic behaviours including tail

159   slapping and arm waving, however, males also display head bobbing and push-ups (Baird,

160   Baird, and Shine 2012). Eastern water dragons are regarded as an omnivorous generalist

161   species, with their diet including insects, native/exotic flowers, fruits and seeds which can

162   extend to anthropogenic food sources within the city. They show no niche differentiation.

163

164   Here, we sampled four city park populations, and five isolated native habitat (hereafter native

165   habitat) populations. The four city park populations were enclosed habitats surrounded by an

166   impermeable/impenetrable urban matrix. These city parks were manmade and highly curated

167   spaces located within or adjacent to the central business district of Brisbane, Queensland

168   (QLD), Australia (CP1: -27.474366, 153.029116; CP2: -27.475915, 152.978495; CP3: -

169   27.462811, 153.019148; CP4: -27.482239, 153.029533). Fig. S3 shows the location of all

170   city park populations within the central business district of Brisbane. City park 1 (CP1; 18 ha),

171   CP2 (52 ha) and CP3 (16 ha) are botanical showcases and CP4 (17.5 ha) is a manicured

172   leisure space. The five native habitat populations were found along un-fragmented native

173   (without human modification) riverside habitats in south-east QLD (INH1: -27.525913,

174   152.92557; INH2: -26.622728, 152.960120; INH3: -26.564226, 152.973053; INH4: -

175   26.759938, 152.852996; INH5: -26.674471, 153.114576). See Table S3 for sample sizes for

176   each city park and native habitat populations. Satellite images of all city park and native

177   habitat populations are displayed in Fig. S4. Only adult eastern water dragons were used in

178   between-population SSD analyses (male SVL > 226mm, female SVL > 187mm). As we do

179   not currently have growth trajectories for the eastern water dragon, we used SVL cut offs to

180   differentiate between adult and sub adult dragons. These cut offs were verified using known

181   age records and hatchling data from a larger longitudinal morphological dataset on CP3. All

182   individuals (male and female) with SVL < 150mm were considered juveniles, whilst the

183   mean SVL of all individuals with SVL > 150mm was used to differentiate between sub adult

184   and adult eastern water dragons. This mean SVL was calculated separately for males and

185   females.

186

187   *iii.   Within-population level*

188   Ecological and morphological data were collected for 102 adult individuals (51 males, 51

189   females) as part of an ongoing behavioural and genetic study of CP3 eastern water dragons.

190   Only adult individuals which had been sighted a minimum of 25 times were used in the

191   analysis at this evolutionary scale. City Park 3 comprises a range of discrete microhabitats

192   (Fig. S5), including a lake area (Lake), rainforest area (Rainforest), a tropical bromeliad

193   garden (Broms), a baobab garden (Baobabs), a children's playground area (Playground) and a

194   densely planted ornamental garden named the Spectacular Garden (Spec). It accommodates a

195   large population of dragons, estimated at 336 individuals (Strickland et al. 2017). City Park 3

196   is an enclosed population, surrounded by residential and commercial buildings, busy roads,

197   and a train line (Strickland et al. 2014). This prevents the immigration and emigration of

198   eastern water dragons from the parkland (Gardiner et al. 2014). Behavioural surveys were

199   conducted twice daily (between 0730 and 1030 hours and between 1300 and 1500 hours)

200   between August and April (2014 to 2016). Methodology for how dragons were identified and

201   how behavioural surveys were conducted can be found in Gardiner et al. (2014) and

202   Strickland et al. (2014).

203

204   *Morphology, sexual dimorphism index (SDI) and sexual size dimorphism (SSD)*

205   Morphological measurements taken for all individuals in this study included: jaw width (JW),

206   jaw length (JL), upper forelimb length (UFL), lower forelimb length (LFL), upper hindlimb

207   length (UHL), lower hindlimb length (LHL), and snout-vent length (SVL) as described in

208   Littleford - Colquhoun et al. (2017).

209

210   To estimate overall SSD, a sexual dimorphism index (SDI) was first calculated for each of

211   the seven morphological measurements taken. The SDI was calculated as: *[(larger sex /*

212   *smaller sex) -1]*, arbitrarily set to positive when males are larger and negative when females

213   are larger (Lovich and Gibbons 1992). Whilst there are multiple ways in which the above

214   calculation can be applied to morphological data (SDI can be calculated for each of the seven

215   morphological traits using all female-male pairs of a species/population/territory, or SDI can

216   be calculated for each of the seven morphological traits using male and female averages for

217   each species/population/territory), we found a high correlation between these two

218   methodologies (Fig. S6), and therefore used all female-male pairs to calculate overall SSD

219   rather than using male and female averages.

220

221   At the between-species level, for each species, SDI measurements were calculated for each

222   male to every female. The mean SDI for each morphological measurement was then used in a

223   phylogenetic principal component analysis (pPCA; corrects for non-independence among

224   observations; Revell (2009)). Species principal component (PC) scores from the first axis of

225   the pPCA were used as a measure of overall SSD between males and females. The pPCA was

226   carried out using the *phyl.pca* function in R package *phytools* (Revell 2012) and utilised the

227   phylogeny of Pyron and Burbrink (2014) which was pruned to only include the 43 agamid

228   species used in this study. In our dataset, sample sizes per species were highly variable,

229   however, we did not find that subsampling significantly influenced mean SDI measurements

230   (correlation between subsampled and full dataset: R^2 = 0.99), and therefore the full dataset

231   was used in subsequent analyses.

232

233   At the between-population level, SDI measurements were calculated for every male to every

234   female within each population. All pairwise SDI measurements were used in a principal

235   component analysis (PCA) with the PC scores from the first axis used as a measurement of

236   overall SSD between males and females (similar methods used in Östman & Stuart-Fox

237   2011).

238

239   At the within-population level, SDI measurements were calculated for each focal male

240   (minimum of 25 sightings) to every female within its 95% home range. Home range size of

241   each individual was calculated following the methodology outlined in Gardiner *et al*., (2014).

242   The size of the home range was calculated using kernel utilisation distribution methods in the

243   adehabitat package (Calenge 2006) in R version 3.3.1 (R Development Core Team 2013),

244   estimating contours of 95% to represent an individual's outermost boundary. The smoothing

245   factor was visually selected, *h* = 7, to control for variation around density estimates

246   (Strickland et al. 2017). It should be noted that all males, regardless of mating tactic

247   (territorial or satellite behaviour), occupy a home range. All pairwise SDI measurements were

248   used in a PCA with the PC scores from the first axis used as a measurement of overall SSD

249   between males and females.

250

251   For each evolutionary scale (between-species, between-populations and within-population), a

252   PC score of zero was indicative of the average SSD, whereas a more positive SSD PC score

253   was indicative of a larger than average male-biased SSD, whilst a more negative PC score

254   was indicative of a smaller than average male-biased SSD, which also included female-biased

255 SSD. Table 1 outlines how SDI and SSD were calculated at each evolutionary scale (see

256 Table S4 for pPCA/PCA loadings for each evolutionary level). SSD values were used in

257 subsequent analyses.

258

259 *Predictor variables*

260 *1. Density*

261   *i.   Between-species level*

262 To estimate species density (number of individuals per square kilometre), we used the Atlas

263 of Living Australia (ALA) records for each species (ALA website at http://www.ala.org.au.

264 Accessed 28 July 2017) divided by the species' Australian geographical range in square

265 kilometres (calculated in Stuart-Fox and Owens 2003). Whilst we acknowledge there are

266 caveats using this estimate, unfortunately there are no current density estimates available for

267 these species. We therefore acknowledge that this estimate of species density can only be

268 used as a coarse proxy measure.

269

270   *ii.   Between-population level*

271 Population density was estimated as the number of individuals per square meter and was

272 calculated by slowly walking transects through each population, recording all lizards

273 encountered (Anderson et al. 1979, Kacoliris, Berkunsky, and Williams 2009, de Infante

274 Anton et al. 2014). Lizards were not captured during density transects. At least three 30 metre

275 (m) transects were walked in each population, with each transect replicated three times.

276 Population density was then calculated as the average of these transect densities. At least a

277 30-minute gap was kept between transect replications so dragons could return to the area if

278 disturbed. Only dragons that occurred within seven to 10m (depending on habitat) of transect

279 were recorded. The total searched area of each transect was then calculated, with the number

280 of individuals recorded divided by the transect area. In native habitat populations, transects

281 were walked along river banks, and hence very linear. In city park populations, which are

282 non-linear, transects were walked within a defined plot, with the area of each plot calculated

283 for density estimates.

284

285   *iii.   Within-population level*

286 Home range density was calculated as the number of individuals (adult males and adult

287 females) that occurred within the focal male's 95% home range, divided by its home range

288 size (i.e. individuals per m^2).

289

2. Habitat

   i.   Between-species level

Climate zones were measured at the between-species level. Here, the number of discrete

climate zones (equatorial, tropical, subtropical, desert, grassland or temperate) each species'

range extends across was tallied using ALA presence records mapped over the Köppen major

climate classifications of Australia (acquired from the Bureau of Meteorology, Australia).

   ii.   Between-population level

At the between-population level, habitat type was classified as city park (CP) or native

habitat (INH) for each individual.

   iii.   Within-population level

At the within-population level, microhabitat was recorded as lake area (Lake), rainforest area

(Rainforest), tropical bromeliad garden (Broms), baobab garden (Baobabs), children's

playground area (Playground) or Spectacled Garden (Spec).

3. Size

Size was used as a predictor variable at the between-species level analysis to test for

allometry in SSD (Rensch 1959). Morphological measurements for all individuals from all 43

species were used in a PCA. We used the first PC (95% of variance; see Table S5 for PCA

loadings) to calculate species size as *[(mean male PC1 + mean female PC1) / 2]* for each

species.

4. Agonistic displays

The frequency of agonistic displays (per male) were used as a predictor variable at the

within-population level analysis and was calculated as the number of times a focal male was

sighted during the behavioural surveys displaying agonistic behaviours (head bob, tail slap,

arm wave, push-ups; Baird *et al*. 2012), divided by his total number of sightings. Therefore,

agonistic display frequencies represent an average over the entire field season.

5. Sex ratio

Sex ratio within a male's home range was used as a predictor variable at the within-

population level analysis only. Of the total number of adult individuals that occurred within a

323   focal male's 95% home range, the sex ratio was calculated by dividing the number of adult

324   males by the number of all adult individuals.

325

326   *Statistical analyses*

327   Table 1 outlines the statistical tests and model structures used at each evolutionary scale to

328   test for associations between SSD and predictor variables. Prior to all analyses, at all

329   evolutionary scales, density was log10 transformed (logDensity) so that the distribution of

330   density better approximated normality. At each level, collinearity among predictor variables

331   was visually assessed prior to analysis, however, none of the variables were strongly

332   correlated. Model residuals were checked for assumptions of normality and homoscedasticity.

333   All statistical analyses were performed in R 3.3.1 (R Development Core Team 2013).

334

335   At the between-species level, we first checked for a phylogenetic signal in SSD PC scores

336   using the *phylosig* function in R package *phytools* to obtain a value of Pagel's λ (Pagel 1999)

337   and its corresponding *P*-value. Pagel's λ is a parameter that estimates the size of phylogenetic

338   signal in trait data, where low λ (close to zero) indicates a weak phylogenetic signal, and a

339   high λ (close to one) indicates a strong phylogenetic signal. The significance of λ was

340   assessed based on the comparison of the likelihood a model accounting for the observed λ

341   with the likelihood of a model that assumes complete phylogenetic independence using

342   1,000,000 simulations. We then ran a phylogenetic generalised least square regression (PGLS)

343   to determine the predictors of SSD (Table 1). A PGLS was run regardless of whether a

344   phylogenetic signal was detected in SSD PC scores as it is important to check whether a

345   phylogenetic signal is present in the residuals of the regression as well as the dependent

346   variable itself. In addition, the PC scores provided by pPCA are in the original,

347   phylogenetically dependent state (not in a phylogenetically independent state), therefore,

348   subsequent analyses using these scores should be analysed using phylogenetic methods

349   (Revell 2009). In the PGLS, we used the maximum likelihood value of λ to adjust the

350   strength of phylogenetic non-independence, implemented in the R package *caper* (Orme

351   2013). In addition to the PGLS models ran for all 43 agamid species, individual PGLS

352   models were run for 1) those species which showed a smaller than average male-biased SSD

353   (negative PC scores), including species which displayed female-biased SSD (species which

354   displayed female-biased SSD are shown with an asterisks (*) in Table S2 and were identified

355   using raw morphology data), and 2) those species which displayed a larger than average

356   male-biased SSD (positive PC scores).

357

358   At the between-population and within-population level analyses, REML based linear mixed-

359   effect models (LME) were used to test for associations between SSD and predictor variables

360   (Table 1) using the lmer function of the lme4 package (version 1.1-1.4). To deal with the

361   non-independent nature of pairwise data points (all male to female pairs used to calculate SDI

362   measurements and thus SSD PC scores), significance was assessed using a randomised null

363   model. We randomly shuffled observed SSD PC scores across male-female pairs at each level,

364   regardless of habitat or population at the between-population level, and home range or

365   microhabitat at the within-population level. We repeated this randomisation procedure 1000

366   times to generate a distribution of random t values. We estimated the significance of the

367   effect of predictor variables by calculating a P value as the proportion of times the random t

368   estimate of each predictor variable was larger (if positive estimate) or smaller (if negative

369   estimate) than the observed (Ruxton and Neuhäuser 2013).

370

371   In order to assess whether the body size of a particular sex was driving variation in SSD PC

372   scores, at the between-population level, we tested for associations between predictor

373   variables and variability around the mean (coefficient of variation; CV) of male and female

374   body size using linear regressions (lm; Table 1). Identifying whether male or female body

375   size (or both) is driving SSD variation allows us to better understand the mechanisms

376   underpinning SSD. For example, the increased frequency of alternative reproductive tactics

377   in males would translate into a higher coefficient of variation in male body size compared to

378   female body size. In addition, we also tested for associations between predictor variables and

379   body size at the within-population level using linear regressions (Table 1). At both the

380   between-population and within-population level, all morphological measurements for all

381   individuals were used in PCA. We used the first PC as a measure of body size (86% of

382   variance for between-population and 84% for within-population level; see Table S5 for PCA

383   loadings).

Results

Between-species level

We assessed the effect of species' density (number of individuals per km^2 estimated from locality records), ecological generalism (the number of climate zones occupied), and species mean size on the extent and direction of SSD among 43 Australian agamid species. Overall, we found variation in SSD PC scores among species (2.7E-06 ± 0.31; mean ± s.d), ranging from negative PC scores which were indicative of species with smaller than average male-biased SSD, including those species which displayed female-biased SSD (species which displayed female-biased SSD are identified with an asterisks (*) in Table S2), to positive PC scores which were indicative of species with larger than average male-biased SSD. We found a lack of phylogenetic signal in SSD PC scores at the species level (Pagel's λ =0.00072, P = 1; Fig. S7). All 43 species occupied more than one Australian climate zone (3.93 ± 1.44), with a mean species' density of 0.003 ± 0.003 individuals per km^2, and an average body size PC score of -1.12 ± 1.86 (negative scores represent smaller species whilst positive scores represent larger species).

The full PGLS model accounted for 31% of inter-species variation in SSD, with density, number of climate zones and species size all found to be significant predictors (Table 2a, Fig 1a and Fig. S8). Phylogenetic analysis of the regression parameters suggested that there was no phylogenetic effect in the residual error of the regression model (Pagel's λ = 0; Table 2a; Fig. 1ai). When split by species with a larger than average male-biased SSD and a smaller than average male-biased SSD (including those species which displayed female-biased SSD), only the number of climate zones a species inhabits was a significant predictor of species with a larger than average male-biased SSD (*P* = 0.009; Table 2b; Fig. 1 aii). Conversely, no variables significantly predicted smaller than average male-biased SSD and female-biased SSD (Table 2c).

Between-population level

At the between-population level, we assessed the extent and effect of habitat type and density on male-biased SSD in eastern water dragons using nine distinct populations. The nine populations comprised of four city park populations (CP1, CP2, CP3 and CP4) found in enclosed human-engineered habitats and five native habitat populations (INH1, INH 2, INH 3, INH 4 and INH 5) found in continuous native habitats. On average, city park populations displayed larger male-biased SSD (0.064 ± 1.972; mean ± s.d; Fig. 1bi) than native habitat

418 populations (-0.168 ± 1.949). Overall, city park populations and native habitat populations
419 showed similar density estimates (City park populations: 0.029 ± 0.013, native habitat
420 populations: 0.027 ± 0.018), however, it should be noted that INH1 and INH3 had a lower
421 population density than INH2 and INH4.

422

423 We found a significant interaction between habitat type and density (logDensity:Habitat: P =
424 0.007; Table 3a; Fig. 1 bii), indicating that the effect of density on male-biased SSD differed
425 between habitat types. Male-biased SSD PC scores were positively associated with density in
426 native habitat but not city park populations (Fig. 1 bii). To check that this trend was not
427 primarily driven by smaller SSD PC scores in the two low density native habitat populations
428 (INH1 and INH3), we also ran LME models using populations with logDensity > -1.8.
429 Regardless of the number of populations used, we found a significant interaction between
430 habitat type and density (logDensity:Habitat: P = 0.028). Moreover, for populations with a
431 high density (> -1.8 logDensity), we found that city park populations displayed smaller SSD
432 PC scores (0.064 ± 1.972; mean ± s.d) than native habitat populations (0.071 ± 1.736).

433

434 In addition, the interaction between habitat type and density predicted male but not female
435 body size variation (Table 3b, c). For native habitat populations, male body size variation
436 decreased with density, whilst variation increased with density for city park populations.

437

438 *Within-population level*
439 Previous observational studies suggested that population density may vary between
440 microhabitats within study site CP3. We therefore assessed the effect of microhabitat, home
441 range density (density of adult individuals sighted within a focal male's 95% home range),
442 the interaction between microhabitat and density, sex ratio and the frequency of agonistic
443 displays (agonistic frequency) on within-population SSD, estimated for each focal male
444 relative to females within his home range. On average, CP3 dragons showed large variation
445 in SSD PC scores (2.11E-12 ± 1.687; mean ± s.d; see Fig. 1ci for an example of the variation
446 in SSD PC scores for two different focal males and the females that occur within their 95%
447 home range), however, only frequency of agonistic displays (per male) significantly
448 correlated with within-population male-biased SSD PC scores (P < 0.001; Table 3d; Fig. 1
449 cii), where the frequency of agonistic displays increased with overall male-biased SSD.
450 Microhabitat, home range density (number of individuals within each focal male's home
451 range), their interaction, and sex ratio did not show a significant correlation with SSD PC

452   scores. In addition, the frequency of male agonistic displays was not significantly associated

453   with home range density ($P = 0.089$) but was significantly associated with male body size (P

454    $= 0.001$). Larger agonistic males were also found to have, on average, smaller females within

455   their home range ($P = 0.047$). Male body size was not significantly associated with the

456   number of males or females within a male's home range, or variation in female body size

457   (standard deviation of female size).

Discussion

459 **Discussion**

460 Here, we combine macro-evolutionary (between-species), local evolutionary (between-

461 population) and fine-scale evolutionary (within-population) patterns of SSD to provide

462 insight into the ways by which sexual and natural selection interact to drive and shape the

463 evolution of SSD in agamid lizards. At the between-species level we found that density,

464 ecological generalism (the number of climate zones a species inhabits) and mean species size

465 all significantly predict variation in SSD, however, only ecological generalism significantly

466 explained variation in larger than average male-biased SSD. At the between-population level,

467 we found that density influences the extent of male-biased SSD in eastern water dragons, but

468 the effect of density on SSD depends on habitat type. Specifically, SSD was positively

469 associated with population density in native but not city park habitats. Furthermore, within a

470 single city park population (within-population level), males that were larger relative to

471 females within their home range (higher SSD) showed a higher frequency of agonistic

472 displays but did not have relatively more females or a higher density within their home range.

473 Overall, this study highlights that the interacting effects of sexual and natural selection on

474 SSD can shift depending on evolutionary scale.

475

476 Density, ecological generalism and mean species size significantly influenced SSD at the

477 between-species level. However, the relationship between SSD and ecological generalism

478 was primarily driven by species with a larger than average male-biased SSD. This suggests

479 that ecological generalism may predict the extent of male-biased SSD. This relationship may

480 arise through the proliferation of 'good genes' (Proulx 1999, Östman and Stuart-Fox 2011) or

481 as a by-product of density (Borregaard and Rahbek 2010), although we found no correlation

482 between density and ecological generalism. In addition, our results suggest that density may

483 influence the overall directionality of SSD at the between-species level, with SSD switching

484 from female-biased SSD (and smaller than average male-biased SSD) to larger than average

485 male-biased SSD at higher densities. Shine (1989) proposed that sexual selection drives the

486 directionality of SSD (female-biased to male-biased), while natural selection may influence

487 the extent of the SSD. Our results are consistent with this hypothesis, given that higher

488 densities generally result in stronger sexual selection (i.e. competition over mates; Ghiselin

489 1976, Emlen and Oring 1977, Kokko and Rankin 2006). However, the influence of natural

490 selection cannot be discounted since higher density can also increase competition over other

491 resources. Additionally, we interpret our data cautiously because species density estimates

492   from locality records may be biased towards certain species and/or certain parts of their

493   distribution (e.g. more urbanised versus more rural locations).

494

495   At the between-population level, we found that the relationship between density and SSD was

496   influenced by habitat type (Fig. 1 bii). Similar to other lizard species and pseudo-scorpions

497   (Stamps 1983, Zeh 1987, Stamps, Losos, and Andrews 1997), we found that, in native habitat,

498   SSD was positively correlated with population density. Further, for high density populations (>

499   -1.8 logDensity), we found that city park populations, on average, displayed smaller SSD

500   with larger variation than native habitat populations. In addition, compared to native habitat

501   populations, we found that male body size variation increased with density for city park

502   populations. Together, these results suggest that these trends within the city may be linked to

503   an increased frequency of alternative male reproductive tactics (previously described in the

504   eastern water dragon (Baird, Baird, and Shine 2012)), where both large and small males

505   coexist. This could be because, unlike native habitat populations, city park populations are

506   enclosed habitats where males are unable to disperse and are therefore faced with a limited

507   number of available territories. In these highly competitive environments (high density and

508   limited territories), larger males may be better able to hold and maintain territories, compared

509   with smaller males. Smaller males are then left to either forgo reproduction or adopt satellite

510   mating tactics and sneak copulations in order to reproduce (Baird, Baird, and Shine 2012).

511   The presence of alternative male reproductive tactics, and thus the existence of both large and

512   small males (increased male body size variation), creates variation in the extent of male-

513   biased SSD, thus 'diluting' the extent of SSD we observe.

514

515   Our analyses of a single city park population suggest that sexual selection is the primary

516   driver of within-population male-biased SSD. We found a significant positive relationship

517   between SSD and agonistic frequency. Agonistic behaviour is often a sexually selected trait

518   as it is tightly linked to an individual's fitness since it plays an important role in territory

519   defence and male-male competition (Carpenter 1978, Trivers 1976). Consistent with

520   alternative reproductive tactics, male eastern water dragons that were larger relative to

521   females within their home ranges (larger SSD), and had a larger overall body size compared

522   to other males, displayed a higher frequency of agonistic displays than smaller males (Fig. 1

523   cii). In larger territorial males, agonistic behaviours are displayed in order to defend females

524   and/or resources, whereas smaller satellite males do not defend territories and therefore tend

525   to display fewer agonistic behaviours (Baird, Baird, and Shine 2012). This aligns with

526   previous studies in other lizard species, where male-biased SSD has been linked to male

527   agonistic behaviour (Carothers 1984). For instance, using 497 lizard populations representing

528   302 species and 18 families, Cox et al. (2003) found that, as predicted by the intrasexual

529   selection hypothesis (sexual selection arises from variance in mating success and can act via

530   intrasexual processes e.g. male aggression), agonistic male behaviour was significantly

531   correlated with male-biased SSD. We expected that larger agonistic males may have more

532   females or males within their home range and potentially a higher home range density.

533   However, this was not the case, possibly because the frequency of a male's agonistic

534   behaviour largely depends on his reproductive tactic (dominance or satellite tactics) rather

535   than the number of individuals that occur within his home range (home range density). In

536   addition, we found that larger agonistic males have, on average, smaller females within their

537   home range compared to other smaller less agonistic males. Whilst further research is

538   required to better understand this result, it highlights that SSD at the within-population level

539   is driven by relatively larger (agonistic) males having relatively smaller females within their

540   home range (increased SSD).

541

542   Overall, our results suggest that although sexual selection may drive SSD at the within-

543   population level, effects of density on variation in SSD between populations depends on

544   habitat. Furthermore, in agamid lizards, density can drive the directionality of SSD between

545   species (female-biased to male-biased) while ecological generalism predicts the extent of

546   male-biased SSD between species. These results highlight that that the interaction between

547   natural and sexual selection in the evolution of SSD can result in different patterns at

548   differing evolutionary levels. By combining macro evolutionary and fine-scale evolutionary

549   patterns to better understand patterns of SSD, this study serves as a template for future

550   comparative analyses across other taxa. In order to extend between-population and within-

551   population level analyses, it would be insightful to compare this study with patterns found in

552   female-biased size dimorphic agamid species. Moreover, our results suggest that human-

553   engineered environments can lead to the increased frequency of alternative male reproductive

554   tactics when population density exceeds a particular threshold, thus reversing the direction of

555   the effect of sexual selection on male-biased SSD. This suggests that city landscapes, in

556   addition to altering genetic and morphological patterns (Littleford‐Colquhoun et al. 2017),

557   can also influence the relationship between natural and sexual selection.

571   **Data accessibility**

572   Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.jm8r78r

573   (Littleford-Colquhoun et al. 2019).

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Supporting Information

771 **Supporting Information**

772 Table S1. Mean (μ) and standard deviation (s) of seven morphological traits for 43 agamid

773 species.

774 Table S2. Sample sizes used at the between-species level analyses.

775 Table S3. Population sample sizes, mean and standard deviation (stdev) of SSD PC scores

776 used for between-population level analyses.

777 Table S4. Loadings from the first PC of a phylogenetic PCA at the between-species level and

778 a standard PCA at the between-population and within-population levels.

779 Table S5. Loadings from the first PC of a morphological PCA.

780 Fig. S1. Boxplots showing variation in morphology at the between-population level analyses.

781 Fig. S2. Boxplots showing variation in morphology at the within-population level analyses.

782 Fig. S3. Map showing locations of all four city park populations.

783 Fig. S4. Satellite images showing land use and vegetative cover of isolated native habitat

784 populations and city park populations.

785 Fig. S5. Map of CP3 study site.

786 Fig. S6. Graph showing high correlation between two different calculations of sexual

787 dimorphism index.

788 Fig. S7. Between-species measures of SSD PC scores plotted next to the agamid phylogeny.

789 Fig. S8. Scatterplot of species size against SSD PC scores.

790

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795   **Figure legend**

796   **Fig. 1.** Predictors of sexual size dimorphism (SSD) at the a) between-species, b) between-

797   population and c) within-population level. (ai) shows between-species SSD PC scores

798   mapped onto the agamid phylogeny, (aii) boxplot showing the PC scores of smaller than

799   average male-biased and female-biased SSD species (red) and larger than average (blue)

800   male-biased SSD species for each number of climate zones inhabited (Table 1a-c). The boxes

801   show the median and first and third quartiles, and whiskers represent the 95% CI. (aiii)

802   scatterplot of logDensity against SSD PC scores for smaller than average male-biased and

803   female-biased SSD species (red) and larger than average (blue) male-biased SSD species

804   (Table 2a-c). (bi) shows the average SSD PC scores for each eastern water dragon population

805   mapped onto the phylogeny (phylogeny is based on ND5 subunits of CP1-4 and INH1-3,

806   however INH4 and INH5 have been added to the phylogeny arbitrarily, therefore this

807   phylogeny is an approximation), (bii) scatterplot showing the interaction between logDensity

808   and every population pairwise SSD PC score depending on habitat (city park; blue and native

809   habitat; green; Table 2). (ci) representation of the SSD PC scores between a focal male

810   eastern water dragon (blue dragon) and the females (red dragons) present in his 95% home

811   range (phylogeny is a graphical representation only), (bii) scatterplot showing the relationship

812   between SSD PC scores and the frequency of agonistic displays (Table 4).

814 **Table 1.** Table outlining models used in sexual size dimorphism (SSD) analyses.

Evolutionary scale	Testing	No. species	No. Populations	No. microhabitats	No. males	No. females	Calculation of SDI and SSD	No. SSD measurements	Calculation of body size	% variance PC1 explained	Type of model used	Dependent variable	Predictor variables	Random effects	Model weighted
Between-species	SSD	43	NA	NA	502	499	For each species, SDI measurements were calculated for each male to every female. Then for each species, the mean SDI measurement for each morphological trait was used to generate overall species SSD	43	Morphological measurements for all individuals from all 43 species were used in a PCA. First PC used to calculate species size as [(mean male PC1 + mean female PC1) / 2] for each species	89%	Phylogenetic Generalised Least Squares (PGLS)	SSD	logDensity + Number climate zones + Species size	NA	NA
Between-populations	SSD	1	9	NA	124	121	SDI for each morphological measurement calculated for every male to every female within each population. All SDI measures used in PCA to generate overall SSD measurement for every male-female pairwise comparison within each population	1913	NA	55%	REML based linear-mixed models (LME)	SSD	logDensity * Habitat	Male ID Female ID Population	NA
	Coefficient of variation (CV) of body size	1	9	NA	124	121	NA	NA	Morphological measurements for all individuals used in PCA. First PC (PC1) defined as body size. PC scores then split by sex. Absolute difference between PC scores calculated for every male-male and female-female pair within each population. Coefficient of variation (CV) then calculated for each sex in each habitat type	86%	Linear models (LM)	CV of body size	logDensity * Habitat	NA	NA

Within-population														
	SSD	1	1	6	51	51	SDI for each morphological measurement calculated for each focal male to every female within its 95% home range. SDI measures used to generate overall SSD measurements for each male-female pairwise comparison	1423	NA		41%	LME	SSD	logDensity * Microhabitat + Sex ratio + Agonistic frequency	Male ID Female ID	Home range overlap
	Agonistic behaviour	1	1	6	51	51	NA	NA	Morphological measurements for all individuals used in PCA. First PC (PC1) defined as body size.	86%	LME	Agonistic frequency	logDensity + male body size	Microhabitat	NA
	Male and female body size	1	1	6	51	51	NA	NA		84%	LM	Male body size	Mean female size + stdev female size + number of males + number of females	Microhabitat	NA

816   **Table 2.** Results from phylogenetic least squares regressions (PGLS) using Pagel's lambda-
817   transformation for a) overall between-species sexual size dimorphism (SSD) for 43 agamid
818   species, b) 20 agamid species that showed larger than average male-biased SSD PC scores
819   and c) 23 agamid species that showed smaller than average male-biased SSD/female-biased
820   SSD. In all tables, t_{obs} represents the observed t value for each variable in the model.
821   Significant results in bold.

a) Between-species – all species	Parameter	Estimate	SE	t_{obs}	P value	Adjusted R^2	λ
SSD ~ Climate zones + logDensity + Species size						0.31	0
	Intercept	0.29	0.27	1.10	0.280		
	Climate_zones	**0.059**	**0.03**	**2.08**	**0.045**		
	logDensity	**0.16**	**0.08**	**2.18**	**0.035**		
	Species size	**0.05**	**0.02**	**2.23**	**0.032**		

b) Between-species – larger than average SSD	Parameter	Estimate	SE	t_{obs}	P value	Adjusted R^2	λ
SSD ~ Climate zones + logDensity + Species size						0.29	0
	Intercept	0.14	0.25	0.57	0.575		
	Climate_zones	**0.07**	**0.03**	**2.55**	**0.022**		
	logDensity	0.06	0.08	0.77	0.450		
	Species size	0.03	0.02	1.53	0.145		

c) Between-species – smaller than average SSD	Parameter	Estimate	SE	t_{obs}	P value	Adjusted R^2	λ
SSD ~ Climate zones + logDensity + Species size						-0.00	0
	Intercept	0.06	0.22	0.28	0.784		
	Climate_zones	-0.01	0.02	-0.40	0.692		
	logDensity	0.09	0.05	1.69	0.107		
	Species size	-0.01	0.03	-0.29	0.777		

826 **Table 3.** Results of a) REML linear mixed-model testing for associations between predictor variables and between-population sexual size

827 dimorphism (SSD), b) linear model testing for associations between predictor variables and coefficient of variation (CV) in female body size, c)

828 linear model testing for associations between predictor variables and coefficient of variation (CV) in male body size between populations, and d)

829 REML linear mixed-model testing for associations between predictor variables and within-population sexual size dimorphism (SSD). In all

830 tables, t_{obs} represents the observed t value for each variable in the model, 95% t_{rand} represents the 95% confidence intervals of the random t

831 values generated by the randomised null model which shuffled SSD PC scores to assess the significance of observed t values. P values were

832 calculated as the proportion of times the randomised t value was larger than the observed. Significant results in bold.

a) Between-population - overall SSD	Parameter	Estimate	SE	df	t_{obs}	95% t_{rand}	P value	Adjusted R^2
SSD ~ logDensity * Habitat								NA
	Intercept	**11.00**	**3.71**	**234.37**	**2.96**	**-0.02 - 0.10**	**0.002**	
	logDensity	**7.60**	**2.34**	**232.91**	**3.25**	**-0.02 – 0.10**	**0.001**	
	Habitat	**-6.47**	**2.62**	**231.10**	**-2.47**	**-0.11 – 0.01**	**0.010**	
	logDensity:Habitat	**-4.44**	**1.66**	**230.31**	**-2.67**	**-0.11 – 0.01**	**0.007**	

b) Between-population - Female CV of body size	Parameter	Estimate	SE	df	t_{obs}	95% t_{rand}	P value	Adjusted R^2
CV of body size ~ logDensity * Habitat								-0.65
	Intercept	0.78	0.51	NA	1.52	NA	0.203	
	logDensity	0.01	0.32	NA	0.02	NA	0.985	
	Habitat	-0.04	0.40	NA	-0.09	NA	0.930	
	logDensity:Habitat	-0.01	0.25	NA	-0.03	NA	0.976	

c) Between-population - Male CV of body size	Parameter	Estimate	SE	df	t_{obs}	95% t_{rand}	P value	Adjusted R^2
CV of body size ~ logDensity * Habitat								0.90
	Intercept	-0.16	0.33	NA	-0.49	NA	0.648	
	logDensity	-0.38	0.20	NA	-1.85	NA	0.123	
	Habitat	**0.99**	**0.27**	**NA**	**3.74**	**NA**	**0.014**	

	logDensity:Habitat	**0.48**	**0.17**	**NA**	**-2.90**	**NA**	**0.034**	

d) Within-population - overall SSD	**Parameter**	**Estimate**	**SE**	**df**	t_{obs}	**95% t_{rand}**	**P value**	**Adjusted R²**
SSD ~ logDensity * Microhabitat + Sex ratio + Agonistic frequency								NA
	Intercept	-5.82	5.36	50.77	-1.09	-0.04 – 0.12	0.194	
	logDensity	-4.29	3.27	50.66	-1.31	-0.06 – 0.10	0.151	
	Microhabitat	1.52	0.97	50.65	1.57	-0.10 – 0.06	0.089	
	Sex ratio	-4.67	4.27	50.80	-1.09	-0.11 – 0.04	0.180	
	Agonistic frequency	**9.03**	**2.34**	**50.67**	**3.85**	**-0.09 – 0.04**	**< 0.001**	
	logDensity:Microhabitat	0.89	0.60	50.65	1.48	-0.10 – 0.06	0.108	

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