

1 **SEXUAL SELECTION AND POPULATION DIVERGENCE II.**
2 **DIVERGENCE IN DIFFERENT SEXUAL TRAITS AND**
3 **SIGNAL MODALITIES IN FIELD CRICKETS**
4 **(*TELEOGRYLLUS OCEANICUS*)**

5
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20 **Running Title:** Divergence in Multiple Sexual Trait Modalities

21

22 **Data Archive Location:** Microsatellite, cuticular hydrocarbon and calling song data are
23 archived on the Dryad Digital Repository at doi:10.5061/dryad.tb552. Additional
24 morphometric data presented here will be archived upon acceptance.

25 *Abstract*

26 Sexual selection can target many different types of traits. However, the relative influence of
27 different sexually-selected traits during evolutionary divergence is poorly understood. We
28 used the field cricket *Teleogryllus oceanicus* to quantify and compare how five traits from
29 each of three sexual signal modalities and components diverge among allopatric
30 populations: male advertisement song, cuticular hydrocarbon (CHC) profiles and forewing
31 morphology. Population divergence was unexpectedly consistent: we estimated the among-
32 population (genetic) variance-covariance matrix, \mathbf{D} , for all 15 traits, and \mathbf{D}_{\max} explained
33 nearly two-thirds of its variation. CHC and wing traits were most tightly integrated, whereas
34 song varied more independently. We modelled the dependence of among-population trait
35 divergence on genetic distance estimated from neutral markers to test for signatures of
36 selection vs. neutral divergence. For all three sexual trait types, phenotypic variation among
37 populations was largely explained by a neutral model of divergence. Our findings illustrate
38 how phenotypic integration across different types of sexual traits might impose constraints
39 on the evolution of mating isolation and divergence via sexual selection.

40

41 **KEY WORDS:** acoustic communication, cuticular hydrocarbons, eigendecomposition,
42 geometric morphometrics, multimodal signalling, sexual selection

43 *Introduction*

44

45 The role of sexual selection in evolutionary diversification has been the subject of research
46 scrutiny, because it is predicted to increase the evolutionary rate of traits that cause
47 reproductive isolation such as sexual signals and mating preferences (Lande 1981; West-
48 Eberhard 1983; Ritchie 2007; Kraaijeveld et al. 2011). If sexual selection causes rapid
49 evolution of such traits in isolated populations, mismatches in sexual communication arising
50 from genetic drift, ecological selection, or other processes will become amplified, and may
51 ultimately decrease the likelihood of gene flow upon secondary contact. Such patterns can
52 then be exacerbated by reinforcement, when genetic incompatibilities between lineages in
53 secondary contact reinforce existing patterns of selection on mate recognition. Sexual
54 selection therefore has the potential to play a two-part role in evolutionary diversification:
55 first, by accelerating the elaboration of sexual signals, and second, by being the causal
56 mechanism by which signal mismatches create mating barriers between taxa. Two critical
57 parameters for empirically testing these ideas are therefore the amount of sexual trait
58 divergence among populations, and the rate at which it evolves relative to other traits
59 (Rodríguez et al. 2013, Wilkins et al. 2016).

60

61 Studies examining the relationship between sexual selection and divergence frequently test
62 how strongly genetic divergence correlates with divergence in male sexual trait values, or,
63 less commonly, female preferences (e.g. Gage et al. 2002; Masta and Maddison 2002;
64 Huang and Rabosky 2014; Hudson and Price 2014). Although drift can independently
65 influence both genetic structure and phenotypic divergence, the rationale of such

66 approaches is that divergence in sexual traits should correlate with reproductive isolation
67 among populations or higher taxonomic groupings (e.g. Mendelson and Shaw, 2005). This
68 implies a possible role for sexual selection to elaborate sexual trait divergence above and
69 beyond what is expected by neutral processes (Ritchie 2007); a prediction that follows is
70 that phenotypic divergence is expected to be greater for sexual traits with a greater
71 influence on reproductive isolation (Rodríguez et al. 2013). Secondly, if sexual traits evolve
72 more rapidly due to coevolutionary feedback dynamics of sexual selection (Lande 1981),
73 these phenotypes should show greater divergence than those not subject to such selection
74 (Funk et al. 2009). However, few studies evaluate patterns of divergence among different
75 traits that might be targets of sexual selection, despite ample evidence that sexual selection
76 acts on traits in more than one modality within a species, for example olfactory, acoustic,
77 visual or tactile signals (Møller and Pomiankowski 1993, Hebets and Papaj 2005, Uetz et al.
78 2009, Girard et al. 2011). In addition, sexual selection can act upon different components of
79 complex or multicomponent signalling traits, for example morphologies and behaviours
80 which together generate a conspicuous acoustic or visual signal (Pomiankowski and Iwasa
81 1993; Rowe 1999). Given the potential multivariate, complex nature of sexual traits,
82 evaluating which are most likely to be targeted by sexual selection during evolutionary
83 elaboration or divergence remains challenging.

84

85 Testing for signatures of selection and drift in more than one sexual trait simultaneously can
86 illuminate constraints on the evolution of reproductive isolation via signal divergence. Here
87 we address this in a field cricket system (*Teleogryllus oceanicus*) by testing the
88 correspondence among patterns of phenotypic divergence in different male sexual traits—
89 acoustic advertisement signals, cuticular hydrocarbons, and morphology of sound-producing

90 wing structures—among allopatric populations, and by using this data with estimates of
91 putatively neutral genetic divergence to subsequently test for signals of selection vs. neutral
92 processes. Our key interest is the correspondence, or not, of population divergence among
93 different sexual traits: Is population divergence of a similar magnitude across trait types,
94 and do selection or other neutral processes similarly exaggerate different trait types? Do
95 individual traits tend to be more integrated within each modality or component than they
96 are between them, or are processes affecting divergence in one modality or component
97 likely to constrain evolutionary responses in another?

98

99 *T. oceanicus* is found in northern and eastern Australia and Oceania (Otte and Alexander
100 1983). As with most grylline crickets, males produce conspicuous acoustic signals which
101 function in mate recognition, mate location, close-range courtship, and aggression (Figure
102 1a) (Alexander 1967). The genus *Teleogryllus* has been a popular system for examining
103 sexual selection on male song traits and the role of song in establishing reproductive
104 barriers (e.g. Hoy et al. 1973, Simmons et al. 2001, Brooks et al. 2005). However, field
105 crickets also express cuticular hydrocarbons (CHCs). CHCs are common in arthropods, and
106 consist of long-chain waxy molecules thought to have evolved under selection for
107 desiccation resistance (Figure 1b). Crickets can discriminate subtle variations in CHCs, the
108 sexes express different CHC profiles, and there is evidence that both males and females
109 discriminate among potential mates and thereby exert sexual selection on the composition
110 of CHC blends (Tregenza and Wedell 1997, Thomas and Simmons 2009, 2010, Steiger et al.
111 2013, Capodeanu-Nägler et al. 2014, Simmons et al. 2014). Finally, acoustical properties of
112 cricket songs are determined not only by variation in behaviours that produce temporal
113 patterns of chirps such as wing closure rate, but also by structural features of the forewing

114 resonators that produce acoustic signals (Figure 1c) (Alexander 1962, Simmons and Ritchie
115 1996, Bennet-Clark 2003, Bailey et al. 2007, Moradian and Walker 2008). The male
116 forewings of *T. oceanicus* contain derived sound-producing structures, including two
117 oscillating membranes bounded by thickened, modified wing veins (Ragge 1955). These
118 morphological structures are also expected to be targets of sexual selection, although the
119 shape and intensity of that selection may differ from that on song, owing to the additional
120 behavioural motor patterns that combine to produce song phenotypes (Klingenberg et al.
121 2010).

122

123 This study combines previously-reported (Pascoal et al. 2016) and new data from allopatric
124 populations of *T. oceanicus* to examine male calling song traits, CHC profiles, and forewing
125 morphometrics measured in common garden laboratory conditions. Patterns of phenotypic
126 divergence were then compared with population genetic divergence. Our analyses tested
127 several hierarchical predictions. First, we predicted, and confirmed, that phenotypic trait
128 values vary across populations. The second prediction was that the three trait types show
129 corresponding patterns of phenotypic divergence among populations. The third was that
130 comparing this divergence to expectations under a neutral processes model derived from
131 neutral genetic markers would reveal a role for sexual selection in promoting variation
132 among populations in all three trait types. We report ample evidence for population
133 divergence within each modality and trait component, and unexpected phenotypic
134 integration (i.e. phenotypic correlation) across all three. However, phenotypic divergence
135 was largely consistent with expectations under neutral processes, and patterns of genetic
136 variation were less consistent with a stepping-stone model of island colonisation than they
137 were with simple isolation-by-distance. We discuss the evolutionary implications of

138 phenotypic integration and patterns of divergence across these three sexual traits.

139

140 *Methods*

141 **CRICKET SAMPLING AND MAINTENANCE**

142 Previously-published data analysed here include microsatellite-based population genetic
143 data, male calling song recordings, and CHC profiles (Pascoal et al. 2016). These are archived
144 on the Dryad Digital Repository (doi:10.5061/dryad.tb552). The calling song parameters
145 from Daintree and Townsville, Australia, that we analyse here were additionally reported in
146 Bailey and Macleod (2014). Detailed methodological descriptions for microsatellite, calling
147 song and CHC analyses are provided in Pascoal et al. (2016), so we briefly summarise the
148 procedures below. To these data we have added a morphometric analysis of male forewing
149 resonating structures.

150

151 We sampled seven *T. oceanicus* populations distributed across eastern Australia and the
152 Pacific. Stock populations were maintained in the lab at approximately 25 °C on a 12:12
153 light:dark cycle in a temperature-controlled chamber. Crickets were kept in 16 L plastic
154 containers and fed Excel Junior and Dwarf rabbit pellets, provisioned with cardboard egg
155 cartons for shelter and moistened cotton wool. Maintenance was carried out twice weekly.
156 When experiments required crickets to be isolated, they were placed into small 118 mL
157 plastic cups provisioned and maintained as above.

158

159 **POPULATION GENETICS**

160 Twenty-four wild-caught individuals from each population were screened using a panel of

161 10 polymorphic microsatellite loci (Beveridge and Simmons 2005, Pascoal et al. 2016). DNA
162 extraction details, primer sequences and PCR conditions are provided in Pascoal et al.
163 (2016), and samples were run on an ABI 3730 sequencer at Edinburgh Genomics. We
164 calculated estimates of F_{ST} and F'_{ST} (Peakall and Smouse 2012) and constructed population-
165 pairwise genetic distance matrices for subsequent analyses using GenePop v.4.0.10
166 (Raymond and Rousset 1995; Rousset 2008), FSTAT v.1.2 (Goudet 1995) and the Microsoft
167 Excel add-in GenAlEx v.6.5 (Peakall and Smouse 2012; Verity and Nichols 2014).

168

169 **TRAIT QUANTIFICATION**

170 *Calling Song*

171 We previously reared crickets in a common garden environment in the lab and recorded the
172 calling songs of between 18-21 adult males per population (Bailey and Macleod 2014;
173 Pascoal et al. 2016). Stock populations experienced at least two generations of lab rearing,
174 thereby reducing the potential for maternal effects arising from field conditions. Recordings
175 were made using a Sennheiser ME66 microphone under red light between 23 – 27 °C during
176 the crickets' dark cycle, and we only analysed males from which we could obtain ten
177 complete song phrases. We used Sony Sound Forge 7.0a to quantify 15 song traits.

178

179 *Cuticular Hydrocarbons*

180 We previously analysed the CHC profiles of 768 adult male crickets between the ages of 7 –
181 10 days post-eclosion (Pascoal et al. 2016). Frozen crickets were thawed and immersed in 4
182 mL of HPLC-grade hexane (Fisher Scientific) for five minutes. 2 μ L samples of a 100 μ L
183 aliquot reconstituted in hexane with a 10ppm pentadecane standard were processed in an
184 Agilent 7890 gas chromatographer and an Agilent 5975B mass spectrometer (GC-MS) on a

185 30 m x 0.25 mm internal diameter DB-WAX column with helium as a carrier gas. GC-MS
186 conditions are described fully in Pascoal et al. (2016). We estimated the relative abundance
187 of 26 CHC peaks using MSD CHEMSTATION v.E.02.00.493 (Agilent). Ion 57 was the target
188 and we corrected peak abundances by dividing each by the abundance of the pentadecane
189 standard. Log₁₀ transformed relative peak abundances were used in subsequent statistical
190 analyses.

191

192 *Forewing Morphometrics*

193 Shape and relative placement of sound-producing structures on male forewings were
194 measured using landmark-based geometric morphometrics (Webster and Sheets 2010). We
195 removed the right forewings from crickets that were used for the CHC analyses above
196 (Pascoal et al. 2016) and mounted them between two microscope slides (n = 13 exclusions
197 for torn or mislabelled wings). Wings were photographed using a Leica DFC295 digital
198 camera attached to a Leica M60 dissecting microscope, and a 1 mm grid scale was included
199 in photographs to facilitate later measurement. Using the program tpsDIG v.2.16 (Rohlf
200 2005), 11 landmarks were placed at prominent vein junctions defining the harp, scraper and
201 mirror of the male forewing (Ragge 1955). Figure 1 illustrates the landmarks, which are
202 modelled after those used in a morphometric study of a closely-related cricket, *Gryllus*
203 *firmus* (Klingenberg et al. 2010). Several programs from the Integrated Morphometrics
204 Package were used to superimpose landmark data from all samples and quantify shape
205 variation using Procrustes distances (Zelditch 2012). Landmark data was combined from all
206 individuals into a common dataset, and the program CoordGen6f (Zelditch 2012) was used
207 to produce Procrustes distances. From this, we calculated principal components and scores
208 describing the shape of resonating structures for each individual using PCAgen6l (Rohlf and

209 Slice 1990, Zelditch 2012).

210

211 Harp and mirror surface areas were calculated by measuring the area of the polygon
212 enclosing each wing structure (Figure 1). This technique was adopted for convenience, and
213 we validated it in a randomly-chosen subset of 50 wings for which the exact outlines of the
214 harp and mirror were drawn manually and the surface areas calculated. The validation
215 showed a strong positive correlation between the two measurement techniques (see
216 Supplemental Figure S1), so analysis proceeded using the original polygon-based
217 measurements. A further validation was performed on the same set of 50 wings, in which
218 we placed landmarks on the original photos a second time, and re-calculated harp and
219 mirror surface area. The results of this validation (see Supplemental Figure S1) similarly
220 indicated confidence in the precision of our protocol. Landmark placement and
221 measurement for the validation were performed blind to sample identity.

222

223 **ANALYSES**

224 *Population Variation in Sexual Traits*

225 We focused on a subset of five key sub-traits from each modality and component to
226 facilitate statistical modelling of divergence across populations, and to test how such
227 patterns of divergence did or did not correspond among the three types of traits. Wing (n =
228 755) and CHC (n = 768) traits were quantified from the same individuals in the previously
229 described experiment, which examined social environment effects, while calling song traits
230 were quantified from a different set of individuals (n = 137) (Pascoal et al. 2016). The five
231 calling song traits were: number of long chirps, number of short chirps, carrier frequency,
232 long chirp-short chirp interval, and inter-song interval. We chose these traits because they

233 were found to be the main targets of selection in a multivariate selection analysis of calling
234 song in the closely-related sister species *T. commodus* (Brooks et al. 2005). The five CHC
235 traits comprised the first 5 PCs based on the same extraction implemented in Pascoal et al.
236 (2016), which cumulatively explained 71.9% of variation in CHC profiles (PC1 = 38.4%, PC2 =
237 16.5%, PC3 = 7.3%, PC4 = 5.1%, PC5 = 4.6%). Landmark-based morphometric data captured
238 information about the shape and relative placement of key wing vein junctions independent
239 of the absolute size of the surrounding features. However, harp and mirror surface area also
240 have an important influence on male carrier frequency (Alexander 1962, Simmons and
241 Ritchie 1996, Bennet-Clark 2003, Bailey et al. 2007, Moradian and Walker 2008), so our five
242 wing morphology traits included absolute measures of both harps and mirrors, plus the first
243 three relative warps which cumulatively explained just over 50% of the variation in forewing
244 shape, independent of size (variance explained by relative warps for wing landmarks: RW1 =
245 25.1%, RW2 = 15.0%, RW3 = 10.2%).

246

247 The experiment described in Pascoal et al. (2016) examined the effects of a social
248 environment manipulation on CHC expression. However, this effect was not of direct
249 interest here and sample sizes were balanced across treatments in the experiment, so for
250 the CHC and wing morphometric data we did not model the social environment (or
251 incubator, for which we found no significant effect in the previous study (Pascoal et al.
252 (2016)). Each trait was divided by its standard deviation (across all populations), giving a
253 standard unit variance, to ensure that they all entered models scale-independent.

254

255 We used canonical variates analyses (CVA) implemented in SPSS v.21 to visualise patterns of
256 population variation in song, CHC, and wing traits. This was only done for purposes of

257 illustrating overall patterns of phenotypic differentiation among populations, as the five
258 individual traits selected for each trait type included existing latent variables extracted from
259 PC analyses. CVA maximises variation among pre-defined groups and it is a useful tool for
260 visualising differences among such groups. We therefore modelled “population” as a factor,
261 and plotted scores from the first two canonical variates axes for each trait type. In addition,
262 we used CVAgen v.6l to visualise the main sources of variation in wing landmark data across
263 populations. The latter analysis used all relative warps from the landmark-based
264 morphometric approach described above, and wing landmark variation was regressed on
265 the first significant canonical variate axis to produce a Procrustes deformation grid and
266 vectors describing the relative magnitude and direction of landmark displacement among
267 populations. The scaling factor was set to 0.2.

268

269 *Comparison of Phenotypic Divergence in Different Traits*

270 We used REML linear models to formally evaluate among population differences within
271 each trait type, and facilitate subsequent comparison against population divergence in
272 individual traits. We first fit three multivariate linear models using REML, one for each
273 modality (song, CHC, wing morphology). In each case, the five observed traits (in standard
274 deviation units) were treated as response variables with population as a predictor (i.e.
275 analogous to a classical MANOVA analysis). Given evidence of population effects on each
276 modality (see Results), univariate REML models were used to test the significance of
277 population effects on individual traits.

278

279 We then estimated the among-population (genetic) variance covariance matrix (**D**) for the
280 complete set of 15 traits. Although **D** is defined as the among–trait covariance matrix of

281 population specific means, we chose to re-estimate these parameters using MCMC rather
282 than REML to better carry statistical uncertainty forward to subsequent analytical steps.
283 Thus, we re-estimated population specific trait means using a multivariate (15 trait) linear
284 model fitted in MCMCglmm, with a single (fixed) factor of Population specified for each
285 trait. The model was run with default priors for 20,000 iterations with a burn-in of 5,000
286 iterations and a thinning interval of 10. Model convergence was checked visually and by
287 comparison of posterior means for each parameter to the REML estimates (which were very
288 similar in all cases). **D** was then determined as the among-trait covariance matrix of the trait
289 means. We defined credible intervals (CIs) as the 95% highest posterior density interval of
290 the posterior for each element of **D**, and consider off-diagonal elements (i.e. covariances) to
291 be significant at $P < 0.05$ if the CI did not span zero. We note that CIs for diagonal elements
292 (i.e. variances) are constrained to positive space so cannot be used for inference, but
293 among-population variance was already tested in the REML analysis. To better interpret the
294 covariance structure of **D** matrix, we subjected it to eigendecomposition and also rescaled
295 to the correlation matrix **D**_{cor}. We also calculated the traces (with CI) of the 5x5 submatrices
296 of **D** corresponding to each trait type to test whether among-population divergence was
297 different between the three trait types.

298

299 *Selection Versus Neutral Divergence of Phenotypes*

300 To determine whether patterns of among-population divergence in song, CHC and wing
301 traits were consistent with a neutral model we used several complementary approaches.
302 First, using the point estimates of the multivariate phenotypic mean (from the MCMC model
303 described above), we calculated the phenotypic distance matrix (as the Euclidean distance
304 in 15 dimensional trait space) among populations and tested whether this was correlated

305 with the microsatellite-based F_{ST} and F'_{ST} distance matrices (where F'_{ST} scales from 0 to 1).
306 Second, we used Mantel tests to check for correlation of the phenotypic distance matrix
307 (and the microsatellite distance matrices) with geographic distance. Geographic distances
308 among all population pairs were calculated using the Great Circle Mapper
309 (www.gcmap.com), under two putative models of cricket dispersal and colonisation. The
310 first calculated point-to-point distances between population pairs assuming direct,
311 unimpeded movement from one location to the other, whereas the second calculated
312 pairwise distances assuming an island-hopping model in which crickets migrated from
313 coastal/mainland populations in Australia across successive Pacific islands. Patterns of allelic
314 diversity in this species are consistent with serial bottlenecks experienced by founding
315 propagules of crickets that dispersed from west to east across Oceania (Tinghitella et al.
316 2011). The second geographic distance model accounted for the different geographic
317 structure expected under such a scenario by assuming free movement of crickets among the
318 three mainland Australian populations, while constraining distance calculations involving
319 island populations to the following sequence: mainland → Fiji → Mangaia → Tahiti →
320 Hawaii. Such a sequence might be expected if crickets accompanied humans during early
321 migrations across Oceania, or where range expansion occurred in a stepping-stone fashion.
322
323 Finally, we followed the mixed-model approach described in Pascoal et al. (2016) to test
324 whether there was more among-population variance than expected under a neutral model.
325 For each trait, we fitted a mixed model using REML in which the phenotype was predicted
326 by a single fixed effect of the mean and a random effect of population. We assumed
327 populations have diverged neutrally (i.e., under neutral processes alone), such that levels of
328 the random effects are drawn from a normal distribution with mean 0 and variance, to be

329 estimated, of $V_{POP(neutral)}$. Provided the microsatellite data provide an unbiased expectation
330 of neutral divergence, then the expected covariance between a pair of observations, one on
331 an individual in population i and one on an individual in population j , is equal to $(1-$
332 $F'_{STij}) * V_{POP(neutral)}$. For each trait this model was then compared to one in which a second
333 random effect of population was added to account for additional among-population
334 variance above that expected under neutrality ($V_{POP(sel)}$). We assumed that twice the
335 difference in model log-likelihoods (LnL) is distributed as a 50:50 mix of χ^2_1 and χ^2_0 (following
336 Visscher 2006), with a significant improvement in fit being indicative of selection
337 contributing to total among-population variance. As also noted in Pascoal et al (2016), we
338 stress that the asymptotic approximation of the test statistic to a χ^2 distribution may not
339 give reliable results with only seven levels (i.e. populations) for each random effect. Thus,
340 while P values are provided they should be interpreted cautiously.

341

342 *Results*

343 **POPULATION VARIATION IN SEXUAL TRAITS**

344 Table 1 shows the results of multivariate fixed effect models and the univariate fixed effect
345 models for each of the 15 traits. The multivariate model showed a clear difference in song
346 traits across populations and the univariate models confirm that all traits contribute
347 significantly to this overall multivariate effect (Table 1). There were also significant
348 differences in the CHC profiles of males across populations in the multivariate model, and
349 each of the five vectors describing CHC expression contributed to this overall multivariate
350 effect (Table 1). Similarly, multivariate analysis showed that wing morphology varied
351 significantly across populations (Table 1). Univariate analyses confirmed that the geometric

352 shape of the wings (Rw1-3), as well as mirror and harp area, significantly contributed to this
353 overall multivariate effect (Table 1). Supplemental Table S1 reports details of the canonical
354 variates analyses implemented to visualise population variation in each trait.

355

356 **POPULATION DIVERGENCE IN DIFFERENT TRAIT TYPES**

357 Table 2 presents the among-population variance-covariance matrix, **D**, for the five traits
358 contributing to each modality. The among-population variances in each modality are
359 provided along the diagonal of this matrix and the sum of these estimates within each
360 modality (the trace) provides an estimate of the total amount of divergence of traits in each
361 modality. The estimated amount of divergence was greatest in wing morphology (1.311,
362 95% CIs: 1.187, 1.501), followed by song traits (1.281, 95% CIs: 1.203, 1.950) and then CHC
363 traits (1.139, 95% CIs: 1.029, 1.316). However, overlapping credible intervals indicate there
364 were no significant differences in the amount of divergence between the three trait types.
365 The mean magnitude of correlations calculated using point estimates from Table 2 was
366 0.477 within types, and 0.507 between types. However, these were statistically
367 indistinguishable using an anti-conservative *t*-test (2-tailed *t*-test: $t = -0.528$, $P = 0.599$). The
368 magnitudes of within-type trait correlations were also similar when disaggregated by trait
369 type: they were 0.369 for song traits, 0.590 for CHCs and 0.472 for wings, and again
370 indistinguishable in an anti-conservative test (one-way ANOVA: $F_{2,27} = 1.264$, $P = 0.299$).

371

372 Table 3 presents the eigendecomposition of **D**. We retained the first six vectors from this
373 decomposition for interpretation, which collectively explained >99.9% of the variation in **D**.
374 The dominant vector (**D**_{max}) explained 63.5% of this variance and was significantly loaded to
375 all CHC traits and four out of five wing morphology traits. In contrast, for song traits only the

376 number of long chirps and the number of short chirps were significantly loaded to D_{\max}
377 (Table 3).

378

379 **TESTING FOR A SIGNAL OF SEXUAL SELECTION**

380 Using Mantel tests, we compared the multivariate divergence in trait means across types to
381 geographic distance matrices to determine if mean phenotypic divergence could be
382 explained by the degree of geographical isolation. We used two different geographic
383 distance matrices: the first was based on the shortest physical distance between population
384 pairs, while the second was based on the hypothetical west-east island hopping colonization
385 route proposed by Tinghitella *et al.* (2011). In both cases, mean trait divergence was
386 significantly correlated with geographic distance (physical distance: $r = 0.738$, $P = 0.010$;
387 island hopping: $r = 0.554$, $P = 0.010$), although the correlation was weaker in the latter
388 scenario.

389

390 Univariate mixed models comparing the among population divergence expected under
391 neutral divergence (based on the F'_{ST} matrix across populations) to a model that allows
392 additional among population divergence (i.e. implicating a role for selection) are presented
393 in Supplemental Table S2. Significance of these models could be taken as evidence that
394 neutral processes alone are insufficient to explain divergence between populations for a
395 given trait. However, for all traits, the neutral model adequately explained population
396 divergence. Collectively, these analyses suggest that drift coupled to restricted gene flow is
397 the likeliest explanation for most divergence in traits across populations. In support of this
398 argument, a comparison of the multivariate divergence in trait means to the F'_{ST} matrix
399 showed that these matrices were significantly positively correlated ($r = 0.764$, $P = 0.010$).

400

401 *Discussion*

402 Causally linking the process of sexual selection with patterns of phenotypic differentiation is
403 a fundamental challenge in evolutionary and behavioural research. Key to this is
404 understanding the form and features of total sexual selection; that is, the combined effects
405 of episodes of sexual selection arising from discrete mechanisms such as male-male
406 competition and female choice, or episodes of sexual selection occurring at different
407 timescales or through different sexual traits (Hunt et al. 2009). On a trait-by-trait basis, the
408 shape of sexual selection might be expected to differ among modalities and among trait
409 components, owing to variable constraints imposed by other sources of selection and
410 genetic architectures, and thus provoke disjointed evolutionary responses (Greig et al.
411 2015). Our results clearly indicate that *T. oceanicus* populations show phenotypic
412 divergence in sexually-selected traits. In addition, the three trait types—male calling song,
413 CHCs and wing morphology—show evidence of phenotypic divergence at roughly equal
414 levels. Populations diverge in a fully multivariate way, with the major axis of overall
415 differentiation in **D** loading on all three trait types.

416

417 The fact that a signal of selection was undetectable for all three sexual traits was
418 unexpected, particularly in view of the finding that female preferences for male calling song
419 vary across other populations of the same species (Simmons et al. 2001). Numerous studies
420 have documented mate choice for all three types of traits in field crickets; their use as
421 exemplars in sexual selection research is well-established. A potential explanation may lie in
422 the fact that most studies infer the action of sexual selection (a) within populations (b) using

423 mate choice experiments and (c) while keeping constant other potential sources of selection
424 such as fecundity or ecological selection. Studies that demonstrate causal links between
425 sexual selection, an evolutionary response to that selection, and patterns of phenotypic
426 diversification are surprisingly uncommon, given theoretical expectations about the rapid
427 rate of evolution by sexual selection (Svensson and Gosden 2007). Thus, while there is an
428 abundance of evidence that sexual selection operates on a wide variety of traits in a
429 multitude of organisms, extending that insight to demonstrate its causal role in promoting
430 diversification is a challenge that has largely remained unmet. A recent meta-analysis
431 highlights the importance of this conceptual distinction, finding that absolute phenotypic
432 divergence in female preferences for male secondary sexual traits best predicts patterns of
433 diversification of those traits, rather than the intensity of selection operating on the traits
434 (Rodríguez et al. 2013).

435

436 Research on multimodal and multicomponent sexual selection is still relatively
437 underdeveloped (Coleman 2009, Prokop and Drobniak 2016), but several recent studies
438 have examined the form and intensity of sexual selection on different types of signalling
439 traits within a single population or species. For instance, a population of the lark bunting
440 *Calamospiza melanocorys* experienced highly variable sexual selection pressures on multiple
441 size and plumage colouration traits across different years (Chaine and Lyon 2008). Other
442 studies have examined different targets of sexual selection in more than one population. For
443 example, closely-related forms of the flycatcher *Monarcha castaneiventris* in the Solomon
444 Islands behaviourally discriminate male plumage and song characters, and both contribute
445 to premating isolation (Uy et al. 2008). In a similar study, Veltsos et al. (2011)
446 simultaneously estimated sexual selection on male calling song and olfactory profiles in the

447 fruit fly *Drosophila montana*. Both traits were targets of sexual selection, but the form of
448 selection differed between them, and also between two populations (Veltos et al. 2011). A
449 recent study tested the relationship between acoustic signals in a sister species of field
450 cricket, *Teleogryllus commodus*, and morphological features of male forewings that
451 contribute to their resonant properties (Pitchers et al. 2014). Pitchers et al. (2014) found
452 that wing morphology and acoustic signal properties covaried with differing strength in
453 different populations of this species, but that overall covariance was minimal and appeared
454 unrelated to patterns of population divergence. Such a pattern may be influenced by a
455 greater degree of lability in behavioural traits compared to morphological traits which are
456 fixed during development (Pitchers et al. 2014, Ower et al. 2016).

457

458 In this context, we would have predicted that behaviour associated with the production of
459 calling song in *T. oceanicus*, i.e. the temporal dynamics of wing opening and closure, could
460 play a more important role in responses to sexual selection than the structural wing
461 features determining carrier frequency of male song. Although the overall magnitude of
462 population divergence in each sexual trait was similar, the observation that song traits
463 showed the lowest level of phenotypic integration, i.e. did not load as strongly or
464 significantly onto D_{\max} as wing or CHC traits, supports this idea. A potential explanation is
465 that the development of male wing structures may be less susceptible to the influence of
466 environmental noise compared to motor neurons, central pattern generators and sensory
467 apparatus involved in the behavioural production of song, and for CHCs, the direction of
468 evolutionary change might be more heavily influenced by stabilising natural selection on
469 CHC composition, which plays an important role in desiccation resistance (Foley and Telonis-
470 Scott 2011). Apart from these differences, male *T. oceanicus* traits generally covaried within

471 and between modalities in a consistent manner in our study, suggesting that unconstrained
472 axes of variation capable of independently responding to selection might be relatively
473 minor.

474

475 *Conclusion*

476 Despite progress documenting the action of sexual selection in multimodal and
477 multicomponent signals modalities across taxa (Candolin 2003), it remains challenging to
478 test whether different sexually selected traits diverge among populations in a uniform
479 versus inconsistent manner. Such data can provide an important step towards establishing
480 the relative contributions of different sexual traits to evolutionary diversification in species
481 where selection potentially targets more than one sexual signal. Our results suggest that
482 phenotypic integration across multiple sexual traits can act as a significant evolutionary
483 constraint. Traits least constrained by genetic correlation and countervailing natural
484 selection might be behaviours that can be flexibly adjusted, such as wing movements
485 associated with acoustic signals in *T. oceanicus*, but we did not find evidence that selection
486 acting on these has contributed to patterns of phenotypic divergence among allopatric
487 populations. Instead, neutral processes such as drift appear to have played a dominant role
488 in generating population differences in the phenotypic values of all three sexual traits.

489

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502

503 LITERATURE CITED

504

505 Alexander, R. D. 1962. Evolutionary change in cricket acoustical communication. *Evolution*
506 16:443-467.

507

508 Alexander, R. D. 1967. Acoustical communication in arthropods. *Annual Reviews in*
509 *Entomology* 12:495-526.

510

511 Bailey, N. W., D. T. Gwynne, W. V. Bailey, and M. G. Ritchie. 2007. Multiple differences in
512 calling songs and other traits between solitary and gregarious Mormon crickets from
513 allopatric mtDNA clades. *BMC Evolutionary Biology* 7:5.

514

515 Bailey, N. W., and E. Macleod. 2014. Socially flexible female choice and premating isolation
516 in field crickets (*Teleogryllus* spp.). *Journal of Evolutionary Biology* 27:170-180.

517

518 Bennet-Clark, H. C. 2003. Wing resonances in the Australian field cricket *Teleogryllus*

519 *oceanicus*. The Journal of Experimental Biology 206:1479-1496.

520

521 Beveridge, M., and L. W. Simmons. 2005. Microsatellite loci for the Australian field cricket

522 *Teleogryllus oceanicus* and their cross-utility in *Teleogryllus commodus*. Molecular

523 Ecology Notes 5:733-735.

524

525 Brooks, R., J. Hunt, M. W. Blows, M. J. Smith, L. F. Bussière, and M. D. Jennions. 2005.

526 Experimental evidence for multivariate stabilizing sexual selection. Evolution 59:871-

527 880.

528

529 Candolin, U. 2003. The use of multiple cues in mate choice. Biological Reviews 78:575-595.

530

531 Capodeanu-Nägler, A., J. Rapkin, S. K. Sakaluk, J. Hunt, and S. Steiger. 2014. Self-recognition

532 in crickets via on-line processing. Current Biology 24:R1117-R1118.

533

534 Chaine, A. S., and B. E. Lyon. 2008. Adaptive plasticity in female mate choice dampens sexual

535 selection on male ornaments in the lark bunting. Science 319:459-462.

536

537 Coleman, S. W. 2009. Taxonomic and sensory biases in the mate-choice literature: there are

538 far too few studies of chemical and multimodal communication. Acta Ethologica

539 12:45-48.

540

541 Foley, B. R., and M. Telonis-Scott. 2011. Quantitative genetic analysis suggests causal

542 association between cuticular hydrocarbon composition and desiccation survival in

543 *Drosophila melanogaster*. Heredity 106:68-77.

544

545 Funk, W. C., D. C. Cannatella, and M. J. Ryan. 2009. Genetic divergence is more tightly
546 related to call variation than landscape features in the Amazonian frogs *Physalaemus*
547 *petersi* and *P. freibergi*. Journal of Evolutionary Biology 22:1839-1853.

548

549 Gage, M. J. G., G. A. Parker, S. Nylin, and C. Wiklund. 2002. Sexual selection in speciation in
550 mammals, butterflies and spiders. Proceedings of the Royal Society of London, Series
551 B 269:2309-2316.

552

553 Girard, M. B., M. M. Kasumovic, and D. O. Elias. 2011. Multi-modal courtship in the peacock
554 spider, *Maratus Volans* (O.P.-Cambridge, 1874). PLoS ONE e25390.

555

556 Goudet, J. 1995. FSTAT (version 1.2) A computer program to calculate F-statistics. Journal of
557 Heredity 86:385-386.

558

559 Greig, E. I., D. T. Baldassare, and M. S. Webster. 2015. Differential rates of phenotypic
560 introgression are associated with male behavioral responses to multiple signals.
561 Evolution 69:2602-2612.

562

563 Hebets, E. A., and D. R. Papaj. 2005. Complex signal function: developing a framework of
564 testable hypotheses. Behavioral Ecology and Sociobiology 57:197-214.

565

566 Hoy, R. R., J. Hahn, and R. C. Paul. 1973. Hybrid cricket auditory behavior: evidence for

567 genetic coupling in animal communication. *Science* 195:82-84.

568

569 Huang, H., and D. L. Rabosky. 2014. Sexual selection and diversification: re-examining the
570 correlation between dichromatism and speciation rate in birds. *American Naturalist*
571 184:E101-E114.

572

573 Hudson, E. J., and T. D. Price. 2014. Pervasive reinforcement and the role of sexual selection
574 in biological speciation. *Journal of Heredity* 105:821-833.

575

576 Hunt, J., C. J. Breuker, J. A. Sadowski, and A. J. Moore. 2009. Male-male competition, female
577 mate choice and their interaction: determining total sexual selection. *Journal of*
578 *Evolutionary Biology* 22:13-26.

579

580 Klingenberg, C. P., V. Debat, D. A. Roff. 2010. Quantitative genetics of shape in cricket wings:
581 developmental integration in a functional structure. *Evolution* 64:2935-2951.

582

583 Kraaijeveld, K., F. J. L. Kraaijeveld-Smit, and M. E. Maan. 2011. Sexual selection and
584 speciation: the comparative evidence revisited. *Biological Reviews* 86:367-377.

585

586 Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proceedings of*
587 *the National Academy of Sciences, USA* 78:3721-3725.

588

589 Masta, S. E., and W. P. Maddison. 2002. Sexual selection driving diversification in jumping
590 spiders. *Proceedings of the National Academy of Sciences, USA* 99:4442-4447.

591

592 Mendelson, T. C., and K. L. Shaw. 2005. Rapid speciation in an arthropod. *Nature* 433:375-

593 376.

594

595 Møller, A. P., and A. Pomiankowski. 1993. Why have birds got multiple sexual ornaments?

596 *Behavioral Ecology and Sociobiology* 32:167-176.

597

598 Moradian, N. R., and S. E. Walker. 2008. Relationships between body size and sound-

599 producing structures in crickets: do large males have large harps? *Invertebrate*

600 *Biology* 127:444-451.

601

602 Otte, D., and R. D. Alexander. 1983. *The Australian crickets (Orthoptera: Gryllidae)*. Academy

603 of Natural Sciences of Philadelphia. Philadelphia, PA.

604

605 Ower, G. D., Hunt, J., and S. K. Sakaluk. 2016. Multivariate sexual selection on male tegmina

606 in wild populations of sagebrush crickets, *Cyphoderris strepitans* (Orthoptera:

607 Haglidae). *Journal of Evolutionary Biology* 30:338-351.

608

609 Pascoal, S., Mendrok, M., Mitchell, C., Wilson, A.J., Hunt, J., and N. W. Bailey. 2016. Sexual

610 selection and population divergence I. The influence of socially flexible cuticular

611 hydrocarbon expression in male field crickets (*Teleogryllus oceanicus*). *Evolution*

612 70:82-97.

613

614 Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. *Population*

615 genetic software for teaching and research—an update. *Bioinformatics* 28:2537-
616 2539.

617

618 Pitchers, W. R., C. P. Klingenberg, T. Tregenza, J. Hunt, and I. Dworkin. 2014. The potential
619 influence of morphology on the evolutionary divergence of an acoustic signal.
620 *Journal of Evolutionary Biology* 27:2163-2176.

621

622 Pomiankowski, A., and Y. Iwasa. 1993. Evolution of multiple sexual preferences by Fisher's
623 runaway process of sexual selection. *Proceedings of the Royal Society of London, B*
624 253:173-181.

625

626 Prokop, Z. M., and S. M. Drobniak. 2016. Genetic variation in male attractiveness: It is time
627 to see the forest for the trees. *Evolution* 70:913-921.

628

629 Ragge, D. R. 1955. *The wing-venation of the Orthoptera Saltatoria*. Adlard and Son, Ltd.
630 Surrey, UK.

631

632 Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software
633 for exact tests and ecumenicism. *Journal of Heredity* 86:248-249.

634

635 Ritchie, M. G. 2007. Sexual selection and speciation. *Annual Reviews in Ecology and*
636 *Systematics* 38:79-102.

637

638 Rodríguez, R. L., J. W. Boughman, D. A. Gray, E. A. Hebets, G. Höbel, and L. B. Symes. 2013.

639 Diversification under sexual selection: the relative roles of mate preference strength
640 and the degree of divergence in mate preferences. *Ecology Letters* 16:964-974.
641

642 Rohlf, F. J. 2005. tpsDig, digitize landmarks and outlines, version 2.05. Department of
643 Ecology and Evolution, State University of New York at Stony Brook.
644

645 Rohlf, F.J., and D. Slice. 1990. Extensions of the Procrustes method for the optimal
646 superimposition of landmarks. *Systematic Zoology* 39:40-59.
647

648 Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for
649 Windows and Linux. *Molecular Ecology Resources* 8:103-106.
650

651 Rowe, C. 1999. Receiver psychology and the evolution of multicomponent signals. *Animal*
652 *Behaviour* 58:921-931.
653

654 Simmons, L. W., M. G. Ritchie. 1996. Symmetry in the songs of crickets. *Proceedings of the*
655 *Royal Society of London, Series B* 263:1305-1311.
656

657 Simmons, L. W., M. L. Thomas, B. Gray, M. Zuk. 2014. Replicated evolutionary divergence in
658 the cuticular hydrocarbon profile of male crickets associated with the loss of song in
659 the Hawaiian archipelago. *Journal of Evolutionary Biology* 27:2249-2257.
660

661 Simmons, L. W., M. Zuk, J. T. Rotenberry. 2001. Geographic variation in female preference
662 functions and male songs of the field cricket *Teleogryllus oceanicus*. *Evolution*

663 55:1386-1394.

664

665 Steiger, S., G. D. Ower, J. Stökl, C. Mitchell, J. Hunt, and S. K. Sakaluk. 2013. Sexual selection
666 on cuticular hydrocarbons of male sagebrush crickets in the wild. Proceedings of the
667 Royal Society of London, Series B 280:1773.

668

669 Svensson, E. I., and T. P. Gosden. 2007. Contemporary evolution of secondary sexual traits in
670 the wild. Functional Ecology 21:422-433.

671

672 Thomas, M. L., and L. W. Simmons. 2009. Sexual selection on cuticular hydrocarbons in the
673 Australian field cricket, *Teleogryllus oceanicus*. BMC Evolutionary Biology 9:162.

674

675 Thomas, M. L., and L. W. Simmons. 2010. Cuticular hydrocarbons influence female
676 attractiveness to males in the Australian field cricket, *Teleogryllus oceanicus*. Journal
677 of Evolutionary Biology 23:707-714.

678

679 Tinghitella, R. M., M. Zuk, M. Beveridge, and L. W. Simmons. 2011. Island hopping
680 introduces Polynesian field crickets to novel environments, genetic bottlenecks and
681 rapid evolution. Journal of Evolutionary Biology 24:1199-1211.

682

683 Tregenza, T., and N. Wedell. 1997. Definitive evidence for cuticular pheromones in a cricket.
684 Animal Behaviour 54:979-984.

685

686 Uetz, G. W., J. A. Roberts, and P. W. Taylor. 2009. Multimodal communication and mate

687 choice in wolf spiders: female response to multimodal versus unimodal signals.
688 Animal Behaviour 78:299-305.
689
690 Uy, J. A. C., R. G. Moyle, and C. E. Filardi. 2008. Plumage and song differences mediate
691 species recognition between incipient flycatcher species of the Solomon Islands.
692 Evolution 63:153-164.
693
694 Veltsos, P., C. Wicker-Thomas, R. K. Butlin, A. Hoikkala, and M. G. Ritchie. 2011. Sexual
695 selection on song and cuticular hydrocarbons in two distinct population of
696 *Drosophila montana*. Ecology and Evolution 2:80-94.
697
698 Verity, R., and R. A. Nichols. 2014. What is genetic differentiation, and how should we
699 measure it— G_{ST} , D , neither, or both? Molecular Ecology 23:4216-4225.
700
701 Visscher, P. M. 2006. A note on the asymptotic distribution of likelihood ratio tests to test
702 variance components. Twin Research and Human Genetics 9:490-495.
703
704 Webster, M., and H. D. Sheets. 2010. A practical introduction to landmark-based geometric
705 morphometrics. In: Quantitative Methods in Paleobiology, pp. 163-188. The
706 Paleontological Society Papers, volume 16, John Alroy and Gene Hunt (eds.)
707
708 West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. The
709 Quarterly Review of Biology 58:155-183.

710

711 Zelditch, M. L. 2012. <http://www.canisius.edu/~sheets/morphsoft.html>

712 **TABLES**

713

714 **Table 1.** Analysis of divergence in songs, CHCs and wing morphology across populations in *T.*
 715 *oceanicus*. We started the analysis of each trait type by running a multivariate linear model
 716 including each of the 5 sub-traits per type (described in the main text) as the response
 717 variables. Each multivariate model was then followed by separate univariate linear models
 718 for each sub-trait to determine how these individual traits contribute to the overall
 719 multivariate difference between populations.

720

	Trait	df ¹	F	P
calling song	Multivariate	30,321.5	7.07	<0.0001
	Univariate			
	LONG CHIRPS	6,130	5.73	<0.0001
	SHORT CHIRPS	6,130	19.20	<0.0001
	FREQUENCY	6,129	3.50	<0.0001
	LC-SC INTERVAL	6,130	6.40	<0.0001
	INTER-SONG INTERVAL	6,130	3.56	<0.0001
cuticular hydrocarbons	Multivariate	30,2004.4	58.53	<0.0001
	Univariate			
	CHC1	6,761	36.08	<0.0001
	CHC2	6,761	25.47	<0.0001
	CHC3	6,761	68.33	<0.0001
	CHC4	6,761	18.37	<0.0001
	CHC5	6,761	13.72	<0.0001
wing morphology	Multivariate	30,1969.8	33.30	<0.0001
	Univariate			
	RWA1	6,748	11.85	<0.0001
	RWA2	6,748	67.63	<0.0001
	RWA3	6,748	24.34	0.0030
	MIRROR	6,748	55.87	<0.0001
	HARP	6,748	35.23	0.0027

721 ¹ (numerator,denominator)

722

723

724 Table 2: The among-population variance-covariance matrix (**D**) among trait means for song, CHC and wing morphology traits showing among-
 725 population variances (shaded diagonal) and covariances (above diagonal), as well as corresponding correlations (below diagonal). 95% CIs are
 726 provided in brackets and bold font denotes statistically significant parameters (based on 95% CIs not overlapping zero).

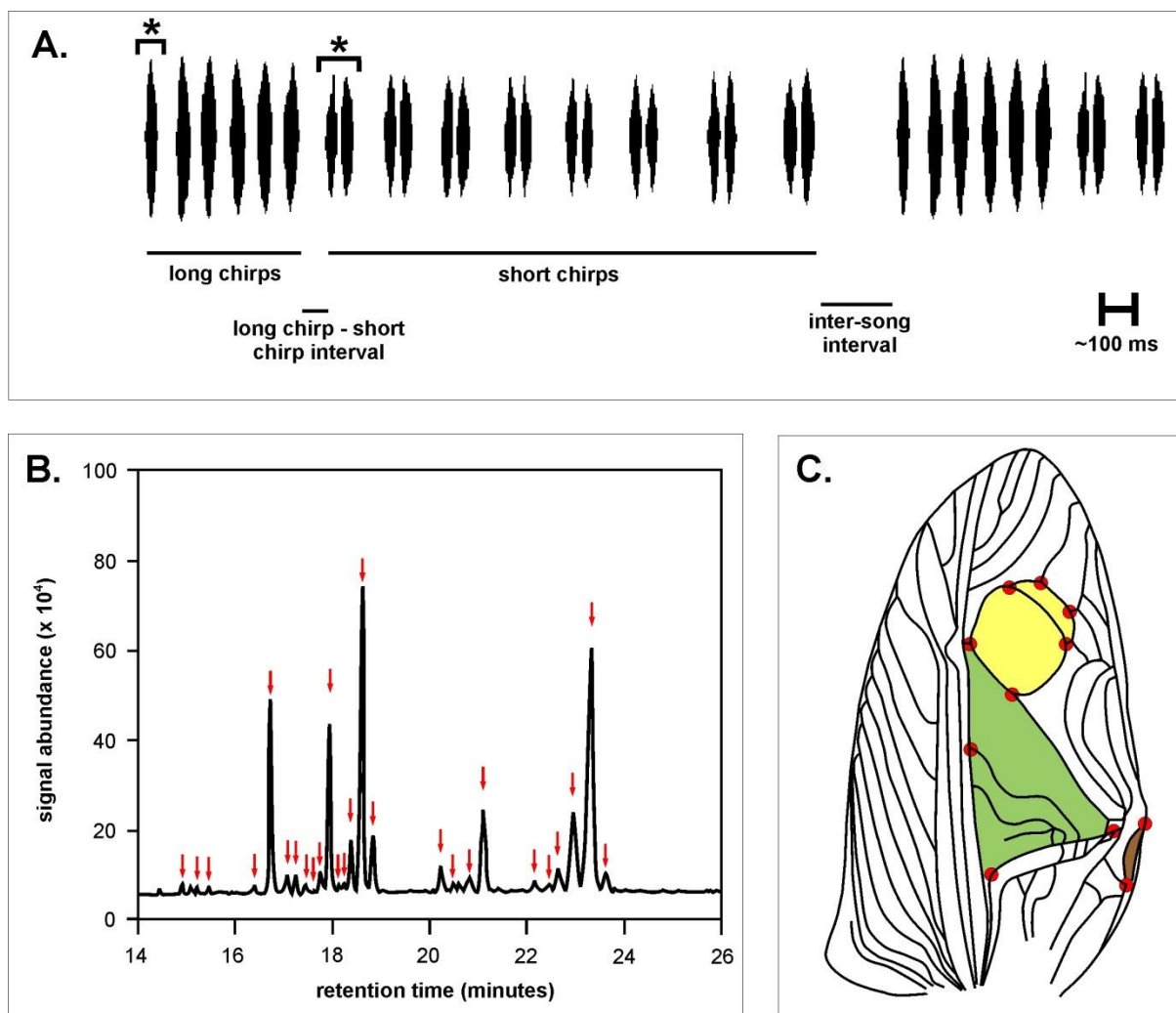
		calling song					cuticular hydrocarbons					wing morphology				
		LONG CHIRPS	SHORT CHIRPS	FREQUENCY	LC-SC INTERVAL	INTER-SONG INTERVAL	CHC1	CHC2	CHC3	CHC4	CHC5	RWA1	RWA2	RWA3	MIRROR	HARP
calling song	LONG CHIRPS	0.224 (0.118,0.445)	-0.306 (-0.461,-0.182)	-0.074 (-0.184,0.033)	0.056 (-0.062,0.169)	0.046 (-0.044,0.183)	-0.118 (-0.226,-0.05)	0.106 (0.045,0.206)	-0.186 (-0.295,-0.074)	-0.037 (-0.111,0.038)	0.066 (0.012,0.153)	-0.062 (-0.143,-0.009)	-0.228 (-0.345,-0.133)	0.089 (0.018,0.18)	-0.171 (-0.285,-0.083)	-0.19 (-0.276,-0.102)
	SHORT CHIRPS	-0.896 (-0.967,-0.607)	0.521 (0.368,0.777)	0.088 (-0.067,0.208)	-0.017 (-0.153,0.129)	-0.037 (-0.21,0.074)	0.248 (0.171,0.339)	-0.188 (-0.259,-0.102)	0.404 (0.318,0.515)	0.171 (0.097,0.248)	-0.173 (-0.264,-0.118)	0.136 (0.063,0.205)	0.429 (0.342,0.538)	-0.01 (-0.081,0.078)	0.362 (0.288,0.476)	0.322 (0.238,0.409)
	FREQUENCY	-0.411 (-0.737,0.137)	0.321 (-0.179,0.629)	0.145 (0.057,0.354)	-0.015 (-0.13,0.097)	-0.058 (-0.16,0.043)	0.125 (0.036,0.22)	-0.103 (-0.188,-0.018)	0.001 (-0.122,0.108)	-0.023 (-0.097,0.053)	-0.04 (-0.111,0.024)	0.047 (-0.013,0.116)	0.109 (-0.012,0.218)	-0.081 (-0.16,-0.001)	0.102 (-0.011,0.205)	0.11 (0.009,0.187)
	LC-SC INTERVAL	0.236 (-0.22,0.557)	-0.047 (-0.378,0.299)	-0.08 (-0.48,0.407)	0.252 (0.132,0.478)	0.17 (0.069,0.299)	-0.033 (-0.133,0.05)	0.054 (-0.027,0.14)	0.046 (-0.055,0.159)	0.095 (0.043,0.192)	-0.088 (-0.165,-0.031)	0.109 (0.033,0.16)	-0.048 (-0.147,0.075)	0.082 (0.025,0.178)	-0.026 (-0.117,0.099)	-0.009 (-0.102,0.079)
	INTER-SONG INTERVAL	0.259 (-0.185,0.712)	-0.135 (-0.566,0.25)	-0.406 (-0.72,0.197)	0.898 (0.468,0.977)	0.143 (0.078,0.368)	-0.087 (-0.186,-0.007)	0.092 (0.022,0.188)	0.023 (-0.114,0.115)	0.059 (-0.022,0.124)	-0.036 (-0.095,0.035)	0.048 (-0.026,0.106)	-0.082 (-0.229,-0.004)	0.058 (-0.02,0.142)	-0.071 (-0.204,0.008)	-0.05 (-0.158,0.022)
cuticular hydrocarbons	CHC1	-0.503 (-0.736,-0.183)	0.693 (0.491,0.806)	0.659 (0.224,0.87)	-0.133 (-0.46,0.177)	-0.464 (-0.811,-0.112)	0.246 (0.199,0.335)	-0.17 (-0.221,-0.126)	0.197 (0.151,0.26)	0.093 (0.041,0.135)	-0.116 (-0.161,-0.073)	0.076 (0.031,0.118)	0.283 (0.243,0.353)	0.024 (-0.028,0.063)	0.279 (0.234,0.335)	0.217 (0.171,0.266)
	CHC2	0.492 (0.221,0.78)	-0.569 (-0.721,-0.34)	-0.592 (-0.801,-0.11)	0.236 (-0.107,0.543)	0.535 (0.159,0.824)	-0.748 (-0.883,-0.614)	0.208 (0.142,0.268)	-0.085 (-0.145,-0.034)	-0.028 (-0.072,0.012)	0.055 (0.013,0.099)	-0.084 (-0.123,-0.04)	-0.203 (-0.267,-0.157)	0.042 (-0.008,0.078)	-0.167 (-0.23,-0.122)	-0.13 (-0.175,-0.079)
	CHC3	-0.613 (-0.794,-0.253)	0.873 (0.767,0.952)	0.003 (-0.47,0.331)	0.143 (-0.145,0.463)	0.094 (-0.355,0.45)	0.617 (0.477,0.725)	-0.289 (-0.466,-0.106)	0.412 (0.339,0.509)	0.223 (0.161,0.27)	-0.177 (-0.237,-0.135)	0.102 (0.044,0.146)	0.344 (0.297,0.411)	0.117 (0.057,0.163)	0.323 (0.271,0.384)	0.252 (0.202,0.309)
	CHC4	-0.194 (-0.507,0.176)	0.591 (0.359,0.773)	-0.153 (-0.568,0.273)	0.472 (0.214,0.792)	0.391 (-0.111,0.66)	0.469 (0.229,0.62)	-0.155 (-0.392,0.059)	0.866 (0.731,0.942)	0.161 (0.1,0.215)	-0.115 (-0.152,-0.078)	0.074 (0.029,0.104)	0.155 (0.098,0.209)	0.126 (0.081,0.163)	0.162 (0.104,0.209)	0.099 (0.05,0.142)
	CHC5	0.419 (0.068,0.736)	-0.718 (-0.885,-0.524)	-0.317 (-0.641,0.18)	-0.523 (-0.782,-0.205)	-0.288 (-0.605,0.22)	-0.704 (-0.83,-0.477)	0.36 (0.1,0.587)	-0.829 (-0.932,-0.684)	-0.861 (-0.953,-0.686)	0.111 (0.074,0.173)	-0.086 (-0.12,-0.05)	-0.158 (-0.221,-0.111)	-0.06 (-0.105,-0.024)	-0.16 (-0.207,-0.11)	-0.13 (-0.174,-0.087)
wing morphology	RWA1	-0.387 (-0.741,-0.103)	0.559 (0.292,0.775)	0.366 (-0.092,0.679)	0.645 (0.197,0.791)	0.379 (-0.178,0.678)	0.457 (0.218,0.675)	-0.544 (-0.739,-0.313)	0.472 (0.247,0.679)	0.546 (0.222,0.703)	-0.768 (-0.906,-0.501)	0.114 (0.068,0.172)	0.107 (0.052,0.156)	0.01 (-0.038,0.04)	0.091 (0.042,0.139)	0.09 (0.042,0.13)
	RWA2	-0.757 (-0.914,-0.483)	0.931 (0.817,0.971)	0.451 (-0.024,0.729)	-0.149 (-0.406,0.2)	-0.339 (-0.719,-0.02)	0.894 (0.804,0.957)	-0.698 (-0.821,-0.565)	0.841 (0.766,0.908)	0.607 (0.396,0.731)	-0.746 (-0.868,-0.57)	0.498 (0.284,0.699)	0.406 (0.346,0.511)	0.024 (-0.029,0.082)	0.369 (0.328,0.444)	0.296 (0.247,0.36)
	RWA3	0.445 (0.084,0.709)	-0.034 (-0.256,0.243)	-0.504 (-0.764,-0.023)	0.387 (0.13,0.725)	0.366 (-0.042,0.708)	0.116 (-0.13,0.271)	0.216 (-0.033,0.409)	0.432 (0.219,0.571)	0.747 (0.553,0.871)	-0.429 (-0.636,-0.161)	0.071 (-0.256,0.289)	0.089 (-0.113,0.275)	0.178 (0.127,0.246)	0.065 (0.005,0.105)	-0.008 (-0.055,0.036)
	MIRROR	-0.605 (-0.833,-0.315)	0.839 (0.713,0.937)	0.445 (-0.018,0.728)	-0.085 (-0.372,0.252)	-0.313 (-0.751,-0.015)	0.939 (0.849,0.969)	-0.613 (-0.768,-0.463)	0.842 (0.763,0.909)	0.673 (0.478,0.803)	-0.802 (-0.899,-0.607)	0.449 (0.187,0.627)	0.968 (0.923,0.992)	0.257 (0.022,0.394)	0.358 (0.297,0.451)	0.275 (0.224,0.332)
	HARP	-0.793 (-0.947,-0.513)	0.882 (0.749,0.963)	0.572 (0.072,0.762)	-0.037 (-0.37,0.277)	-0.262 (-0.665,0.107)	0.866 (0.76,0.937)	-0.564 (-0.731,-0.403)	0.776 (0.68,0.88)	0.489 (0.278,0.666)	-0.769 (-0.892,-0.583)	0.525 (0.307,0.737)	0.919 (0.834,0.963)	-0.039 (-0.246,0.164)	0.909 (0.84,0.969)	0.256 (0.181,0.319)

727

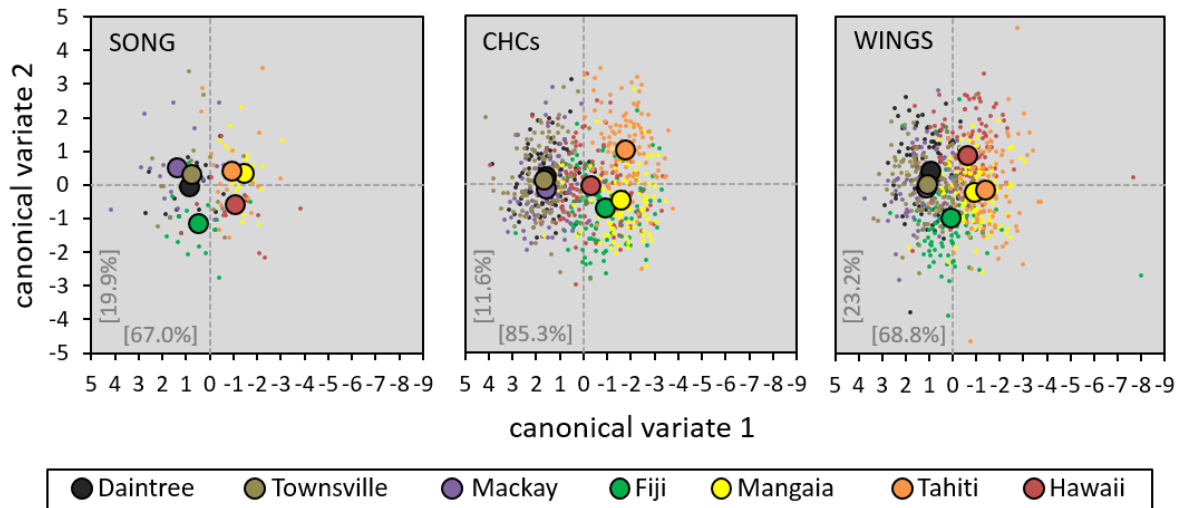
728 **Table 3.** Eigendecomposition of the **D** matrix. Only the first six vectors are retained for
 729 interpretation as they collectively explain >99.9% of the observed among-population
 730 (co)variance in song, CHC and wing morphology traits. 95% CIs are provided in brackets.
 731 Estimates of trait loadings are considered statistically significant (bold font) if 95% CIs do not
 732 overlap zero (note this is necessarily true for the eigenvalues themselves).
 733

Vector	1	2	3	4	5	6	
Eigenvalue	2.372 (2.184, 2.789)	0.680 (0.558, 0.987)	0.297 (0.266, 0.522)	0.269 (0.172, 0.360)	0.101 (0.066, 0.183)	0.016 (0.013, 0.080)	
Proportion of variance	0.635 (0.556, 0.659)	0.182 (0.148, 0.239)	0.080 (0.066, 0.123)	0.072 (0.045, 0.089)	0.027 (0.017, 0.044)	0.004 (0.002, 0.019)	
Trait load							
calling song	LONG CHIRPS	0.236 (0.142, 0.345)	-0.184 (-0.417, 0.054)	-0.188 (-0.647, 0.442)	0.461 (-0.163, 0.631)	0.036 (-0.369, 0.316)	-0.083 (-0.430, 0.530)
	SHORT CHIRPS	-0.446 (-0.518, -0.364)	0.015 (-0.175, 0.169)	0.088 (-0.438, 0.470)	-0.402 (-0.557, 0.051)	0.174 (-0.347, 0.353)	-0.211 (-0.550, 0.304)
	FREQUENCY	-0.107 (-0.217, 0.027)	0.235 (-0.065, 0.475)	0.365 (-0.328, 0.725)	0.295 (-0.359, 0.645)	-0.373 (-0.669, 0.031)	-0.466 (-0.604, 0.179)
	LC-SC INTERVAL	0.008 (-0.117, 0.126)	-0.503 (-0.704, -0.259)	0.501 (-0.129, 0.655)	0.136 (-0.497, 0.568)	-0.068 (-0.358, 0.332)	0.090 (-0.446, 0.375)
	INTER-SONG INTERVAL	0.054 (-0.032, 0.206)	-0.399 (-0.582, -0.171)	0.259 (-0.243, 0.494)	-0.156 (-0.472, 0.348)	-0.036 (-0.477, 0.301)	-0.229 (-0.483, 0.506)
cuticular hydrocarbons	CHC1	-0.280 (-0.327, -0.235)	0.116 (-0.022, 0.235)	-0.031 (-0.420, 0.428)	0.424 (-0.026, 0.500)	-0.147 (-0.406, 0.202)	0.099 (-0.255, 0.446)
	CHC2	0.191 (0.133, 0.237)	-0.240 (-0.332, -0.086)	-0.169 (-0.416, 0.325)	-0.309 (-0.571, 0.169)	-0.692 (-0.772, -0.285)	-0.097 (-0.412, 0.479)
	CHC3	-0.367 (-0.412, -0.314)	-0.290 (-0.378, -0.135)	-0.250 (-0.421, 0.149)	-0.242 (-0.449, 0.214)	-0.028 (-0.283, 0.229)	0.113 (-0.267, 0.435)
	CHC4	-0.172 (-0.214, -0.107)	-0.345 (-0.400, -0.209)	-0.137 (-0.279, 0.131)	0.065 (-0.175, 0.271)	0.126 (-0.184, 0.300)	-0.316 (-0.493, 0.214)
	CHC5	0.177 (0.123, 0.224)	0.200 (0.096, 0.281)	-0.117 (-0.256, 0.129)	-0.122 (-0.295, 0.192)	0.124 (0.201, 0.297)	0.029 (-0.380, 0.383)
wing morphology	RWA1	-0.127 (-0.174, -0.072)	-0.146 (-0.249, 0.004)	0.402 (-0.071, 0.459)	0.102 (-0.486, 0.500)	0.306 (-0.052, 0.600)	0.263 (-0.202, 0.590)
	RWA2	-0.409 (-0.452, -0.366)	0.079 (-0.042, 0.159)	-0.089 (-0.214, 0.091)	0.047 (-0.137, 0.217)	0.081 (-0.125, 0.285)	-0.240 (-0.450, 0.193)
	RWA3	-0.031 (-0.079, 0.034)	-0.391 (-0.497, -0.205)	-0.409 (-0.573, 0.236)	0.277 (-0.381, 0.567)	0.081 (-0.225, 0.452)	0.122 (-0.399, 0.385)
	MIRROR	-0.373 (-0.413, -0.327)	0.002 (-0.112, 0.118)	-0.180 (-0.337, 0.212)	0.233 (-0.171, 0.351)	-0.174 (-0.361, 0.100)	-0.123 (-0.378, 0.283)
	HARP	-0.310 (-0.348, -0.257)	0.064 (-0.049, 0.152)	0.102 (-0.104, 0.218)	0.002 (-0.232, 0.197)	-0.392 (-0.561, 0.049)	0.617 (0.011, 0.723)

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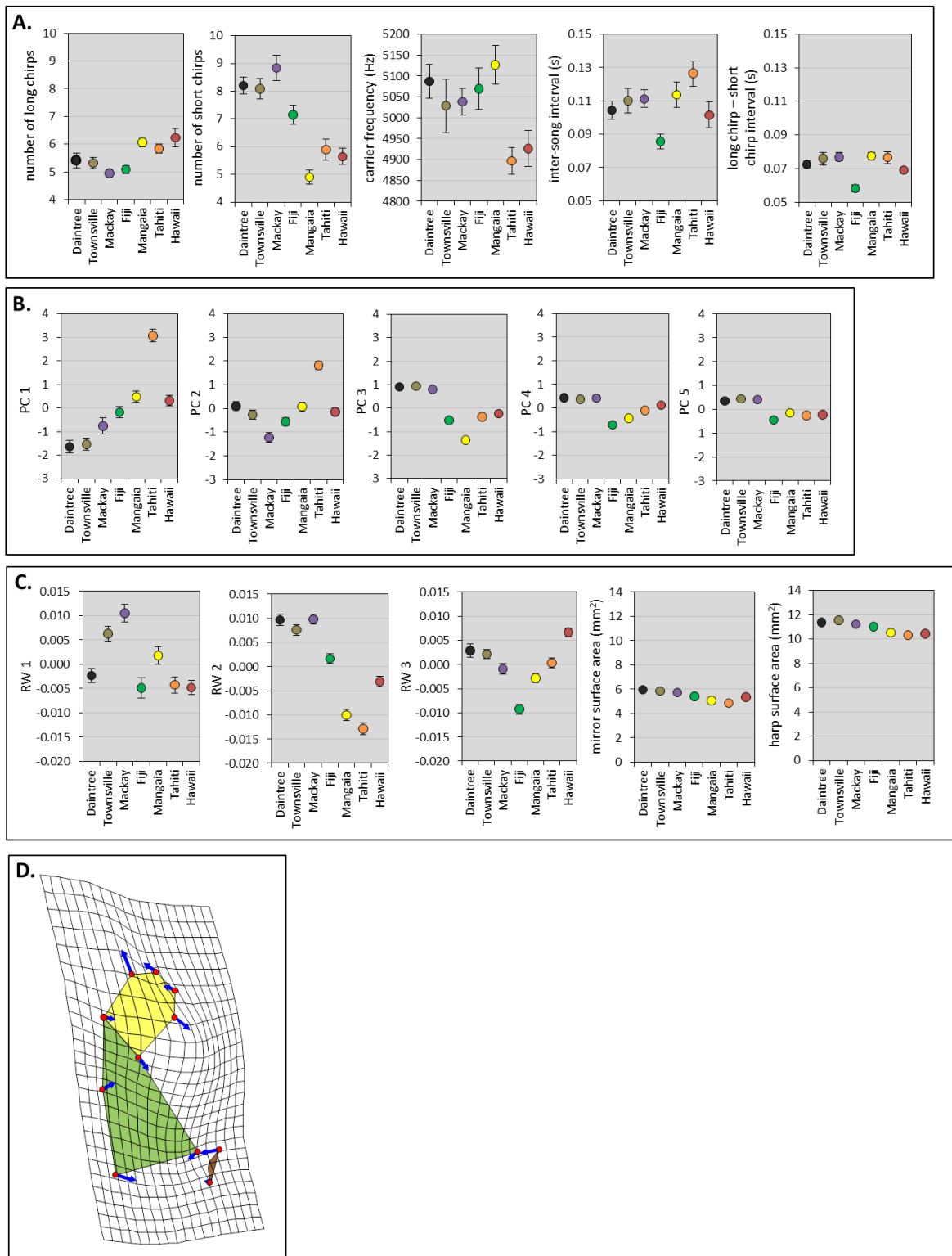


736
 737 **Figure 1.** Male *T. oceanicus* traits subject to sexual selection. (A) Oscillogram of a typical
 738 male calling song, indicating the temporal parameters measured in the present study
 739 (modified from Bailey and Macleod (2014)). The brackets indicated with asterisks highlight a
 740 single long chirp (one pulse) and a single short chirp (typically paired pulses). (B)
 741 Diagrammatic illustration of a gas chromatograph of a male cuticular hydrocarbon profile.
 742 Peaks analysed in the present study are indicated with red arrows. (C) Principal sound-
 743 producing structures on the male forewing, adapted from Pascoal et al. (2014). Red circles
 744 indicate the 11 landmarks used in this study, which define the harp (green shading), mirror
 745 (yellow shading) and scraper (brown shading).
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Figure 2. Population divergence in three sexually-selected male traits. Canonical variate analyses (CVAs) were used to visualise overall patterns of population divergence for calling song (n = 137), CHC profiles (n = 768), and forewing morphology (n = 755). All five individual traits for each sexual trait type were used in the respective CVAs. Data from the first two canonical variates components are plotted, and the proportion of variance explained by each axis is indicated by the grey text in brackets (see Table S1 for additional statistical details). Centroids for each population are depicted with larger dots. Colour-coding is indicated in the key. Some X-axes are reversed to maintain consistency with other figures.



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759

760 **Figure 3.** Population variation among the 5 individual traits measured for each modality in
 761 male *T. oceanicus*. Means and standard errors are indicated, and colour coding follows
 762 Figure 2. Where standard error bars are not visible, it is because they were obscured by the
 763 data points. (A) Calling song. The five traits examined in this study; data from Bailey and
 764 Macleod (2014) and Pascoal et al. (2016) are shown, and terminology follows Figure 1. (B)
 765 Cuticular hydrocarbons. The first five principal components describing relative abundances
 766 of 26 CHC peaks; data from Pascoal et al. (2016) are shown. (C) Wing venation. Population

767 variation in the first 3 relative warps describing variation in landmark placement on male
768 wings are depicted, as well as mean harp and mirror surface area in each population. (D)
769 Male forewing landmark deformation across all populations. The deformation grid
770 illustrates the main sources of variation in the shape of sound-producing structures among
771 populations, and the blue arrows are vectors showing the magnitude and direction of
772 landmark displacement. Highlighted structures are as in Figure 1C and demonstrate how
773 landmarks were joined to calculate mirror and harp surface area. Vectors were scaled using
774 a Procrustes deformation scaling factor of 0.2.

775 *Online Supporting Information For:*

776

777 **SEXUAL SELECTION AND POPULATION DIVERGENCE II.**
778 **DIVERGENCE AMONG DIFFERENT SEXUAL TRAITS AND**
779 **SIGNAL MODALITIES IN FIELD CRICKETS**
780 **(*TELEOGRYLLUS OCEANICUS*)**

781

782 **Sonia Pascoal¹, Magdalena Mendrok², Alastair J. Wilson³, John Hunt^{3,4}, Nathan W. Bailey^{5,6}**

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801 *Contents*

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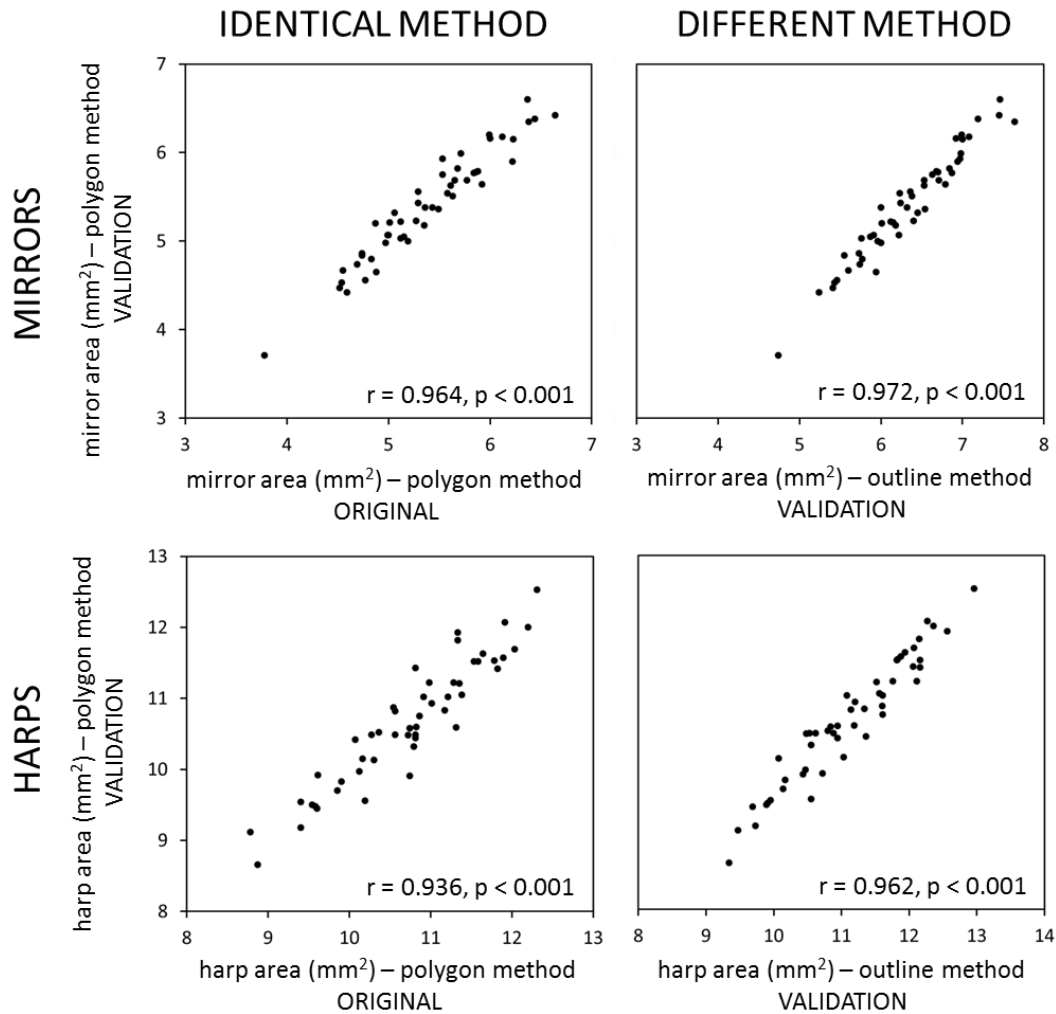
803 p. 1 **Figure S1.** Validations of wing morphometric measurements.

804

805 p. 2 **Table S1.** Details of canonical variates axes for each sexual trait type.

806

807 p. 3 **Table S2.** Models evaluating neutral vs. non-neutral divergence.



808
 809 **Figure S1:** Graphs illustrating methodological validations of wing morphometrics. Blind
 810 validations were carried out on a randomly-chosen subset of 50 individual male wings.
 811 Technical replicability was assessed by recalculating mirror (top row) and harp (bottom row)
 812 surface areas. Graphs on the left show the correlation between original and blind validation
 813 measurements, in which surface area was measured by enclosing boundary landmarks
 814 within a convex polygon and calculating its area. Graphs on the right show the correlation
 815 between two methods of calculating surface area: the polygon method, and manually
 816 outlining the exact structure in question followed by calculation of the enclosed area. Both
 817 sets of comparisons utilise the same validation data for the polygon method indicated by
 818 the y-axes. Statistics were calculated using Pearson product-moment correlations, and data
 819 were checked for normality and homogeneity of variances (all $P > 0.505$).

820 **Table S1.** Canonical variate axes for each sexual trait type (song, CHCs and wings),
 821 derived from analyses in which “population” is the classification variable.

Trait	Function	Eigenvalue	% Variance	Wilks' λ^a	Chi-square	df	P
calling song	1	1.142	67.0	0.283	164.192	30	<0.001
	2	0.340	19.9	0.606	65.176	20	<0.001
	3	0.160	9.4	0.812	27.146	12	0.007
	4	0.056	3.3	0.942	7.817	6	0.252
	5	0.006	0.3	0.994	0.764	2	0.682
cuticular hydrocarbons	1	2.037	85.3	0.40	1086.266	30	<0.001
	2	0.277	11.6	0.729	240.841	20	<0.001
	3	0.034	1.4	0.930	55.052	12	<0.001
	4	0.030	1.3	0.961	29.901	6	<0.001
	5	0.009	0.4	0.991	7.138	2	0.028
wing morphology	1	0.925	68.8	0.356	771.769	30	<0.001
	2	0.312	23.2	0.686	282.049	20	<0.001
	3	0.068	5.1	0.900	78.768	12	<0.001
	4	0.027	2.0	0.961	29.570	6	<0.001
	5	0.013	1.0	0.987	9.529	2	0.009

822 ^a The null hypothesis is that the canonical correlation of the given function, plus all
 823 functions following it, are not significantly different from zero.

824

825 **Table S2.** Univariate mixed model results showing estimated among-population variance
826 partitioned into components attributable to neutral processes ($V_{POP(neutral)}$) and putative
827 selection ($V_{POP(sel)}$) as well as residual (within-population, V_R) for each trait. Also shown are
828 likelihood ratio tests comparing model fit to a reduced model in which all among-population
829 variance is attributable to neutral processes. Standard errors are shown in parentheses
830 (note – denotes a SE that was non-estimable due to the variance component being bound to
831 zero in the REML solution).
832

	Trait	$V_{POP(neutral)}$	$V_{POP(sel)}$	V_R	$X^2_{0,1}$	P
calling song	LONG CHIRPS	0.379 (0.413)	0.016 (0.075)	0.827 (0.103)	0.069	0.397
	SHORT CHIRPS	0.697 (0.485)	0.000 (-)	0.553 (0.068)	0.000	0.500
	FREQUENCY	0.000 (-)	0.115 (0.093)	0.900 (0.112)	1.991	0.079
	LC-SC INTERVAL	0.000 (-)	0.225 (0.154)	0.808 (0.100)	0.000	0.500
	INTER-SONG INTERVAL	0.449 (0.386)	0.000 (-)	0.895 (0.111)	0.000	0.500
cuticular hydrocarbons	CHC1	0.334 (0.405)	0.081 (0.093)	0.785 (0.040)	1.879	0.085
	CHC2	0.194 (0.414)	0.119 (0.128)	0.840 (0.043)	0.729	0.197
	CHC3	0.954 (0.577)	0.000 (-)	0.660 (0.034)	0.000	0.500
	CHC4	0.514 (0.328)	0.000 (-)	0.879 (0.045)	0.000	0.500
	CHC5	0.232 (0.239)	0.012 (0.036)	0.909 (0.047)	0.172	0.339
wing morphology	RWA1	0.303 (0.205)	0.000 (-)	0.920 (0.048)	0.000	0.500
	RWA2	0.447 (0.275)	0.000 (-)	0.653 (0.034)	0.000	0.500
	RWA3	0.000 (-)	0.172 (0.104)	0.843 (0.044)	1.848	0.087
	MIRROR	0.536 (0.448)	0.021 (0.052)	0.696 (0.036)	0.304	0.291
	HARP	0.321 (0.276)	0.022 (0.039)	0.786 (0.041)	0.808	0.184

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