Non-parallel morphological divergence following colonization of a new host plant

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Keywords: Parallelism, host plant adaptation, secondary sympatry, adaptation to co-existence

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Abstract

Divergent ecological selection may diversify populations of the same species evolving in different niches. However, for adaptation to result in speciation, the ecologically divergent populations have to experience at least some degree of reproductive isolation. While ecological selection pressures in similar environments are expected to result in convergent adaptation, sexually selected traits may diverge in different directions in different locations. Here, we use a host shift in the phytophagous peacock fly *Tephritis conura*, with both host races represented in two geographically separate areas, East and West of the Baltic Sea, to investigate convergence in morphological adaptations. We asked i) if there are consistent morphological adaptations to a host plant shift and ii) if the adaptations to secondary sympatry with the alternate host race are consistent across contact zones. We found low, albeit significant, divergence between host races, but only a few traits, including the female ovipositor, were consistently different. Interestingly, co-existence with the other host race significantly increased the degree of morphological divergence, but the patterns of divergence were not consistent across the two sympatric contact zones. Thus, local stochastic fixation or reinforcement could generate trait divergence, and evidence from additional sources is hence needed to conclude whether divergence is adaptive.

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Introduction

Adaptation to novel niches through ecological selection has been identified as a major driver of speciation (Schluter, 2008, Schluter, 2009, Nosil, 2012). Classic adaptive radiations including the Darwin Finches (Weiner, 1994, Loo et al., 2019), the Hawaiian tarweeds and silverswords (Baldwin et al., 2003) and the recurrent development of limnetic-benthic stickleback pairs (Schluter, 1993, Bay et al., 2017) are driven by adaptations to novel diets and habitats. Speciation driven by expansion of novel dietary niches has potential to be rapid, like in *Rhagoletis* flies, where populations of hawthorn-feeding flies have adapted to use the introduced apple (*Malus domesticus*) as a host during the last 150 years (Bush, 1969, Feder et al., 1988, Linn et al., 2004, Meyers et al., 2020). Ecological divergence does not necessarily lead to speciation, however, as divergence and specialization may be reversed in the absence of reproductive isolation (Taylor et al., 2006, Lackey & Boughman, 2017).

While there are numerous examples of strong ecological divergence between young species pairs, the early stages of speciation may be reversible if the drivers of reproductive isolation are behavioral, plastic or depend on environmental context (Taylor et al., 2006, Lackey & Boughman, 2017). The coupling of traits involved in ecological adaptation to traits causing reproductive isolation is crucial for speciation to occur, and is pivotal in models of speciation (Kirkpatrick & Ravigne, 2002). For this reason, speciation is most readily achieved under some degree of allopatry (Mayr, 1947, Coyne & Orr, 2004, Rundle & Nosil, 2005) or if genetic incompatibilities arise, causing postzygotic barriers (Hollinger & Hermisson, 2017). At early stages of divergence postzygotic isolation is generally weak, and behavioral barriers to maladaptive mating may be absent in allopatric populations (Nosil, 2012). Prezygotic barriers, arising more readily during the early stages of diversification (Mendelson, 2003, Sanchez-Guillen et al., 2014) may be needed for specialization to persist. These may be

environment dependent (Funk et al., 2002, Nosil et al., 2002, Lamont et al., 2003). Models suggest that traits under natural selection may be co-opted as mating cues (Thibert-Plante & Gavrilets, 2013). However, sexually selected traits that are not co-opted ecological traits are instead predicted to conform to a mutation order scenario (Mendelson et al., 2014) where incompatible alleles are fixed in different populations and may drift together with mating preferences along a line of equilibrium (Uyeda et al., 2009).

It remains an empirical challenge to resolve whether ecological adaptations and sexually selected/mate recognition traits diverge to the same extent under parallel selection regimes. Few studies have explicitly compared parallelism in multiple traits under different selection regimes. A recent study of Bahamas mosquitofish (*Gambusia hubbsi*) suggests that only a few traits are highly predictable across parallel predation environments (Langerhans, 2018). In a series of studies of Skyros wall lizards (*Podarcis gaigeae*) traits under natural selection were found to evolve in parallel (Runemark et al., 2014, Runemark et al., 2015), whereas traits under sexual selection diverged stochastically (Runemark et al., 2011). Parallelism in responses to co-existence with congeners is less well understood, although the evolutionary divergence of both ecological and sexual traits may be affected also by the local presence or absence of the other incipient species (Amarasekare, 2003, Calabrese & Pfennig, 2020). If the reproductive isolation between the incipient species is incomplete, such secondary contact may break up important ecological adaptations, which may be particularly evident in e.g. insect host races inhabiting different discrete plant niches. Close congeners inhabiting different niches may be poorly adapted to the alternative environment (Rundle & Nosil, 2005, Nilsson et al., 2017, Cronemberger et al., 2020, Martin et al., 2020), which may lead to reinforcement (Servedio & Noor, 2003) and character displacement with stronger differences in traits involved in mate choice (Hinojosa et al., 2020). While parallel divergence is expected

95 for ecologically selected traits (Figure 1A), either parallel- or nonparallel character
96 displacement (Figure 1B-C) or reduced divergence due to introgression (Figure 1D) could be
97 expected for sexually selected traits that are not strongly coupled with host plant use.
98
99 To understand if ecological selection and selection to reduce maladaptive mating result in
100 different patterns of divergence, there is a need for joint investigations of how traits mediating
101 ecological specialization and sexually selected traits develop during adaptation to novel
102 niches. Such studies should test the hypothesis that the direction of change in sexually
103 selected traits, during the early stages of speciation, can be more arbitrary than the ecological
104 traits, which are expected to diverge in parallel across populations of similar niches (Figure
105 1). In particular, studies asking how co-existence with congeners affects the sexually selected
106 trait distribution, e.g. through parallel- or non-parallel character displacement (Figure 1),
107 compared to the distribution of ecologically important traits, may provide insight into the
108 nature of the selection pressures acting on those different trait types. Here, we address how
109 ecologically and putatively sexually selected traits evolve and diversify in allopatric and
110 sympatric areas using two host races of the fly *Tephritis conura.* This species has recently
111 undergone a host shift resulting in host races specializing on utilizing different *Cirsium* thistle
112 species as larval food plants (Diegisser et al., 2006a, Diegisser et al., 2006b, Diegisser et al.,
113 2007, Diegisser et al., 2008). Macroevolutionary analyses reveal that such host plant driven
114 speciation is one of the main factors explaining the tremendous diversity of phytophagous
115 insects, because many speciation events can be attributed to host plant shifts (Berlocher &
116 Feder, 2002, Dres & Mallet, 2002, Nylin et al., 2014), and diversification rates are elevated in
117 herbivorous insects compared to their non-herbivorous relatives (Mitter et al., 1988, Farrell,
118 1998, Wiens et al., 2015). For an insect specialist like *T. conura,* the host plant provides a
119 discrete environment imposing a multidimensional selection pressure. Hence, insect host plant

120 adaptations are predicted to affect multiple traits involved in e.g. phenological matching,
121 female host preference and larval performance (Matsubayashi et al., 2010).The putatively
122 strong selection pressures involved in host plant adaptation provides a strong prediction of
123 parallelism in host-use related traits of phytophagous insects at early stages of the speciation
124 process (Nosil et al., 2002, Meyers et al., 2020). However, the prediction is less
125 straightforward for sexually selected traits, because these are not necessarily associated with
126 host use.
127
128 *Tephritis conura* provides an excellent system for studying patterns of divergence in
129 ecological and sexually selected traits, as it enables comparisons of trait variation across
130 replicated sympatric and allopatric settings. The two recently established host races inhabit
131 two geographically isolated sympatric zones with adjacent allopatric populations, one East
132 and one West of the Baltic Sea (Figure 2A). *Tephritis conura* females of the two host races
133 are ovipositing into buds of either *Cirsium heterophyllum* (the ancestral host; Figure 2B) or *C.*
134 *oleraceum* (the novel host; Figure 2C), and larvae show host specific performance and
135 survival (Diegisser et al., 2008). During courtship, *Tephritid* flies performs a mating ritual
136 that includes elaborate wing movements from both sexes (Sivinski, 2000, but see Briceno &
137 Eberhard, 2017). Hence, *T. conura* offers an opportunity to compare the diversification of
138 traits that are likely to be under ecological (e.g. body size, ovipositor length) or sexual (wing
139 size, wing morphology) across different zones of secondary contact. Specifically, we test the
140 hypotheses (i) that there are morphological effects of a host plant shift in ecologically and
141 sexually selected characters, (ii) that host race divergence in ecologically selected traits is
142 parallel in the two geographic areas and (iii) that traits putatively under sexual selection also
143 diverge in parallel in the two replicas. Given the consistent genetic differentiation between
144 host races both in allopatry and sympatry (Diegisser et al., 2006a, Ortega et al., [*in prep.]*) we

predict the host races to be morphologically divergent, and this divergence to be consistent across geographical settings. As larval survival is strongly reduced in the alternate host (Diegisser et al., 2008), we predict hybridization to be maladaptive, potentially resulting in reinforcement and character displacement between the host specialists in sympatry.

Methods

Sampling

The dipteran *T. conura* infest several species of the thistle genus *Cirsium* (Asteraceae) (Romstock-Volkl, 1997). Adult *T. conura* oviposit into thistle buds during early summer, wherein the adolescent flies remain during larval development and pupation. *Tephritis conura* infesting *Cirsium heterophyllum*, the melancholy thistle, have recently colonized and adapted to *C. oleraceum*, the cabbage thistle. Haplotype analyses suggest that a peripatric host shift took place during the last ice age in the Alps (Diegisser et al., 2006b). The two host races are largely reproductively isolated, but there is evidence of some small amounts of gene flow (Diegisser et al., 2006b). The flies infesting *C. oleraceum* have adapted ecologically to the smaller bud sizes (Diegisser et al., 2007), and have a significantly shorter ovipositor to body size ratio. Another variable character is wing pigmentation. Wing patterns in Tephritid flies have been suggested to be under sexual selection, as males perform dances with their wings to attract females (Sivinski & Pereira, 2005). Tephritid males attempting to initiate copulation situate themselves in front of female flies and posture with their wings (Sivinski, 2000, but see Briceno & Eberhard, 2017). We therefore have reason to assume that ecological selection pressures are more important for size traits in general, and for ovipositor length in particular, while wing traits are more likely shaped by sexual selection. For simplicity, we refer to flies infecting *C. heterophyllum* as CH-flies, while the flies infecting *C. oleraceum* will be denoted CO-flies.

170

171 We used a parallel sampling design to examine phenotypic adaptation to a novel host plant
172 and effects of co-existence between host races, by sampling each host race both in sympatry
173 and allopatry on each side of the Baltic (Figure 2A). We collected thistle buds infested by *T.*
174 *conura* larvae/pupae and allowed the adults to eclose in a common environment. CO-fly
175 larvae were sampled in Germany and Lithuania (allopatric areas), both host races were
176 collected in the sympatric areas in southern Sweden and Estonia, and allopatric CH-fly larvae
177 were sampled in mid-Sweden and Finland (Figure 2A, Table S1). All sampling took place
178 during June and July 2018. The sampling scheme enables examining to what extent patterns
179 of phenotypic divergence are explained by host plant adaptations, by co-existence with the
180 other host race, and if these patterns differ between the two transects. Typically, *C.*
181 *heterophyllum* and *C. oleraceum* do not grow in the same microhabitat. Thus, the sympatric
182 and allopatric definitions here refer to the presence of one or both thistle species in the general
183 area (Figure 2A).

184

185 **Morphological measurements**

186 *Tephritis conura* adults eclosed from field-collected thistle buds in a common lab
187 environment, (see Supplementary material S1). One male and one female per bud were
188 euthanized by freezing a few days after emergence and subsequently included in the
189 morphological analysis. For each individual, we took magnified photographs using a
190 Celestron 44308 USB microscope. We photographed a lateral image of the fly body after
191 removal of the wings and a dorsal image of the right wing on a transparent background to
192 allow better visibility of the wing veins. Body length and ovipositor length were measured
193 digitally from lateral photographs (Figure S1). We placed 14 landmarks, adapted from
194 Pieterse et al. (2017), digitally on the dorsal wings (Figures 2D and S2) for geometric

195 morphometrics (Zelditch, 2004). We added a landmark; number 15, to reflect the high
196 variance in the proximal area on the wing. Digitization was performed in TPSDig2
197 v2.31(Rohlf, 2017) and we used TPSUtil v1.76 (Rohlf, 2018) for file handling.
198
199 **Statistical analysis**
200 We used PAST3 v3.20 (Hammer et al., 2001) to apply a Procrustes fit to the landmark data to
201 align and scale the wings (Figure 2D). To produce relative warps (i.e. principal components
202 of shape) to compare shape between groups, a principal component analysis (PCA) was
203 performed with the Procrustes fitted data using PAST3 v3.20 (Hammer et al., 2001) (Figure
204 S3). Based on the variance explained by the eigenvalues (Figure S4) and the broken stick
205 criterion (Jackson, 1993), six principal components which jointly explain 67.6% of the
206 variance were used in further analyses of wing shape. All subsequent statistical analysis were
207 performed in R (R Core Team, 2019).
208
209 To investigate whether host race was the major factor explaining variation in fly morphology,
210 we performed a PCA on the variables fly body length (mm), wing length (mm), wing width
211 (mm), wing melanisation ratio (%) and relative warps 1-6 reflecting wing shape. We
212 quantified five morphological traits and wing shape for 583 flies. We found six significant
213 dimensions of wing shape variation between flies (Figure S5), that were included in
214 subsequent analyses of phenotypic divergence. We identified four significant dimensions of
215 variation from a PCA-analysis using the broken stick criterion (Jackson, 1993) (Figure S6).
216 Collectively, these PCA-axes explained 78.0% of the morphological variance in the dataset.
217
218 To formally test if the two host races were significantly differentiated, we applied a
219 multivariate analysis of variance (MANOVA), with all of these variables included as response

220 variables (body length, ovipositor length, wing width, wind length, wing melanisation and
221 PC1-6 of wing shapes). To investigate both whether host races differed, and if co-existence
222 with the other host race affected morphology, we ran a generalized linear model (GLM)
223 including host origin, co-existence, and their interaction as explanatory factors. To further test
224 if the patterns of morphological adaptation were parallel in the Eastern and the Western
225 transects, and explicitly address if co-existence affected host plant races in the same way in
226 these replicates, we performed a final MANOVA with host race, co-existence and
227 geographical setting and their 3-way and 2-way interactions as factors. The MANOVAs were
228 performed separately on females and males as this enabled including the biologically
229 important ovipositor (Diegisser et al., 2007) in analyses of females. This also reduced the
230 multicollinearity of explanatory factors to below recommended values (Hair, 2010). To find
231 which combination of factors that best explained the data, we estimated and compared AIC-
232 scores (Akaike, 1974) for all models.
233
234 To further investigate parallel and non-parallel differentiation of the host race pairs, we
235 applied separate linear discriminant function analyses (LDAs) on the data from the Eastern
236 and Western transects. We used host races and co-existence with the other host race as factors
237 in the models. This analysis was performed on males and females separately. To test if the
238 patterns of divergence differed significantly between the transects, we performed each LDA
239 10,000 times using the bootstrap R package 'boot' (Canty & Ripley, 2020) and used the
240 confidence intervals to assess if the loadings differed between analyses. In addition, we
241 assessed the proportion of divergence is shared among host races and unique among the
242 populations in a nested MANOVA using size and shape variables against host race and
243 transect following Langerhans & DeWitt (2004). We applied this test to sympatric

244 populations separately from allopatric populations, to avoid confounding effects of co-
245 existence.
246
247 **Results**
248 We found weak evidence for separation between host races along the two first major axes of
249 divergence (Figure 2E), with CO-flies, on average had lower values of PC1 compared to CH-
250 flies, reflecting predominantly size variables (GLM: $F_1 = 5.189$, $p = 0.023$; Figure 2E). This
251 host race difference was small compared with the variation among populations.
252
253 We found multivariate divergence between host races (Pillai's trace = 0.237, $F_{11, 271} = 7.66$,
254 $p<0.001$), as well as an effect of co-existence with the other host race (Pillai's trace = 0.257,
255 $F_{11, 271} = 8.51$, $p <0.001$), and a significant interaction between host race and co-existence
256 (Table 1; Pillai's trace = 0.155, $F_{11, 271} = 4.52$, $p <0.001$) in females. For males, co-existence
257 (Pillai's trace = 0.165, $F_{10, 275} = 5.45$, $p <0.001$) and the interaction between host race and co-
258 existence (Pillai's trace = 0.141, $F_{10, 275} = 4.5$, $p <0.001$) affected morphology, while host race
259 alone did not significantly affect morphology (Table 1). The differences between males and
260 females could potentially be explained by the strong divergence in ovipositor length, included
261 only in the analyses of females.
262
263 Females of the two host plant races differed in ovipositor length and in loadings of relative
264 warp 5, reflecting wing shape (Tukeys post hoc tests; Table 2). Co-existence influenced a
265 suite of characters, including ovipositor length, wing length, wing width, melanisation ratio,
266 and wing shape. Moreover, body length, wing width, and several wing shape components
267 were significantly affected by the interaction between host race and co-existence (Table 2;
268 figures 3 and S7). Ovipositor length diverged as expected under ecological selection,

269 especially in the Eastern transect (Figure 1A). In contrast, wing length showed tendencies to
270 diverge as expected by character displacement in the Eastern transect (Figure 1B; Figure 3C).
271 Male host races differed in fewer variables than females, with only relative warp 5 differing
272 significantly between the host races (Table 2, figures S8-S11). Co-existence reduced male
273 wing length with both host races having shorter wings in sympatric regions. Male wing width,
274 melanisation ratio and wing shape also differed between populations that co-exist with the
275 other host race and those that do not.
276
277 Host race and co-existence affected female morphology differently East and West of the
278 Baltic Sea, as illustrated by a significant 3-way interaction (Pillai's trace = 0.117, $F_{11, 277}$ =
279 3.22, p <0.001; Table S2). All 2-way interactions and main effects were also significant in the
280 full model (Table 1). This pattern holds true also for males (Pillai's trace = 0.092, $F_{10, 271}$ =
281 2.74, p = 0.0031; Table S3 and S4). Hence, depending on geography, host race and co-
282 existence affects fly morphology differently.
283
284 To assess how much of the female divergence that was unique to a transect, shared between
285 the host races and differentially affected by host race in the two transects, we estimated
286 Wilk's partial η^2. We find that while a high share of the partial variance was explained by
287 shared divergence between host races ($F_{11.273}$ = 4.96, 30.6%) and transect specific patterns of
288 host race divergence ($F_{11.273}$ = 3.81, 25.3%), divergence between transects explained the
289 highest percentage of partial variance ($F_{11.273}$ = 6.85, 37.8% (Table S5).
290
291 As host race affected morphology differently depending on geography, we further tested if the
292 major axis of divergence separated host races in both transects, and if the same traits
293 separated groups using a Linear Discriminant Analysis (LDA). We find that the importance of

host race differed between transects. In the Western LDA, host races separated along the first discriminant axis whereas the sympatric and allopatric populations separated along the second discriminant axis (Figure 4). In the Eastern LDA, the first discriminant instead divided the two CO populations and the second discriminant axis divided the two CH populations (Figure 4). Moreover, different sets of characters separated populations in the two transects as bootstrap loadings show that different characters loaded on the first discriminant axes in the LDAs (Fig. 4C; Table S6).

Discussion

Contrary to previous studies that found clear differences between the host races (Diegisser et al., 2007) we found low and variable morphological differentiation between host races of *T. conura*. Multivariate differentiation between host races was driven by subtle differences across many traits. Host race separation reflected mainly ovipositor length differences with CH-fly ovipositors being longer than CO-fly ovipositors, although this varied among populations. Previous work report consistent divergence for two loci (Diegisser et al., 2006b), and whole genome data (Ortega et al., [*in prep.*]) support the presence of discrete genetic host races. Moreover, the flies also have poor performance on the alternate host plant (Diegisser et al., 2008). In light of this, the moderate host race divergence in morphology was unexpected, but this result is in line with the observation that parallelism in fitness is typically higher than for both phenotypic divergence and genetic divergence (Bolnick et al., 2018). Moreover, parallel divergence only in traits under strong ecological selection is consistent with the findings in response to different predation regimes in Bahamas Mosquitofish (Langerhans, 2018), where only a few traits show highly predictable patterns of diversification. Hence, at early stages of diversification driven by ecological adaptation, parallelism may be high only for traits that are strongly coupled to the ecological factor the species is adapting to.

319

320 Two out of five female traits match a parallel divergence scenario, ovipositor length, and
321 body length in the Western transect. Hence, the degree of parallelism among host races in
322 morphological divergence was lower than expected. Intermediate ovipositor lengths in
323 Western CH-flies could suggest selection for shorter ovipositors in sympatry, potentially due
324 to introgression or to be able to utilize also *C. oleraceum* buds to a higher degree. Many of the
325 traits measured are highly correlated with body size, a factor that typically varies with
326 temperature in insects (Atkinson, 1994, but see Shelomi, 2012). While the parallel sampling
327 design with allopatric regions both South and North of both sympatric zones would partly
328 correct for such temperature effects, temperature could still differ between the Eastern and
329 Western transects, potentially contributing to non-parallel patterns of divergence.

330

331 When we jointly investigated effects of co-existence, geographic origin and their interactions
332 on host race divergence, we found that both co-existence with the other host race and transect
333 affected morphology. Hence, the low host race divergence may be explained by interacting
334 effects of co-existence with the other host race, with non-parallel patterns of divergence in the
335 two transects. This pattern would be consistent a mutation order scenario (Figure 1C). If
336 reinforcement due to maladaptive hybridization would have been a prominent force in
337 sympatric populations, we would expect traits to be more divergent between the host races in
338 sympatry compared to allopatry, i.e. for character displacement to arise (Comeault et al.,
339 2016, Calabrese & Pfennig, 2020, Kyogoku & Wheatcroft, 2020; Figure 1B-C). This was
340 generally not the case. While the degree of host race divergence differed between sympatry
341 and allopatry, the most common pattern was that the traits in sympatric populations are more
342 similar between host races than traits in allopatry, most prominent in wing morphology traits.
343 This could be indicative by introgression (Figure 1D), consistent with findings by Ortega et

344 al. [*in prep*.], or effects of a shared environment. Co-existence most strongly affects on wing
345 size, with wing width being shorter in sympatry than in allopatry in the Western transect. This
346 could be consistent with selection for shorter dispersal distances (Claramunt et al., 2012),
347 potentially to avoid dispersal to the other host plant, reducing survival. We also found patterns
348 consistent with character displacement in in wing length in the Eastern transect. Hence,
349 contrary to reports of parallelism also in sexually selected traits in ecotypes in e.g.
350 sticklebacks (Boughman et al., 2005), we find mixed evidence for parallelism in wing traits
351 that putatively are under sexual selection. This could reflect a mutation order scenario, where
352 the difference may be important but the specific trait or direction of the difference is arbitrary
353 (Mendelson et al., 2014). Some degree of sexual selection may further differentiate
354 populations under a mutation order scenario (Rundle & Rowe, 2018), underlining the need to
355 further investigate if there is sexual selection at work in this system and identifying the traits
356 under selection.
357
358 The degree of divergence differ between transects. While host races are separated by the main
359 discriminant axis in the Western transect, host race was a less important predictor of
360 divergence in the Eastern transect. The traits that differ between host races also differs
361 between our two geographic replicates. A mutation order scenario (Mendelson et al., 2014)
362 could explain the seemingly arbitrary divergence between the transects (Figure 1C). However,
363 the differences in patterns between the transects East and West of the Baltic Sea could have
364 several additional explanations related to demographic history, population size and the extent
365 to which the host plant races co-exist locally. Possibly, one contact zone may be older than
366 the other and populations in older sympatry would have had more time for character
367 displacement to develop. Alternatively, if the Eastern transect has a higher proportion suitable
368 thistle habitat, this could have increased both within- and between host race connectivity and

369 potentially gene flow (Servedio & Noor, 2003). Genetic data and detailed analyses of
370 introgression should be used to resolve whether selection against hybridization in allopatric
371 regions could be expected.
372
373 Morphological differentiation does not always strongly reflect even crucial ecological
374 adaptations. For instance, cultural evolution contributes to reproductive isolation in Cassia
375 crossbills (*Loxia* sinesciuris) (Porter & Benkman, 2019) and *Rhagoletis pomonella* have
376 adapted their phenologies to host fruits ripening at different times of the year (Filchak et al.,
377 2000). A potential explanation to the low consistency of host race divergence may lie with the
378 traits included in this analysis. Females were more diverged than males. The strong
379 divergence in females likely reflects the inclusion of ovipositor length in the analyses, as
380 ovipositor length was one of the traits that differed significantly between the host races,
381 consistent with previous findings (Diegisser et al., 2007). These findings are similar to those
382 of Jourdan et al. (2016) where divergence in female mosquitofishes (*Gambusia*) divergence
383 was more parallel than male divergence. Potentially, the other traits measured are not
384 important enough for host plant adaptation to result in strongly parallel divergence. Traits that
385 have been shown to differ strongly between host races include female ovipositor length
386 (Diegisser et al., 2007) and the larval ability to survive on the different host plant species
387 (Diegisser et al., 2008), and these traits are more likely to show parallel divergence.
388 Moreover, host plant preference may have a potential to act as a magic trait (Gavrilets, 2004,
389 Thibert-Plante & Gavrilets, 2013) for *T. conura*, separating the habitats of the populations and
390 simultaneously providing reproductive isolation as these flies mate on their host plant
391 (Diegisser et al., 2007). Finally, we cannot rule out that other, unmeasured traits are important
392 for sexual selection, as pheromones have been suggested previously to play a role in tephritid
393 mate choice (Roriz et al., 2019), as well as overexpression of antioxidants, which have been

shown to increase male performance under certain conditions (Teets et al., 2019). Regardless of the exact selection pressures acting on our set of study traits, our findings are important, because they show a context dependence for host race adaptation, with parallelism only in ecologically strongly selected traits, whereas there are non-parallel changes, some consistent with character displacement for other traits. These insights should guide the design and interpretation when studying ecologically driven divergence.

In conclusion, our work suggests that morphological responses to niche shifts can be highly context dependent. Co-existence with closely related congeners and demographic origin may affect easily measured morphological characters, potentially masking underlying parallelism in traits important for adaptation to specific niches. Moreover, we find intriguing patterns of non-parallel divergence in putatively sexually selected traits and even character displacement in opposite directions in the two geographic replicas, suggesting that mutation order dependent divergence may lead to different solutions to avoid introgression in independent contact zones.

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Author contribution

A.R conceived and designed the study. KJ.N., J.O. and A.R. performed the field work. KJ.N. reared the flies, quantified the morphological traits, performed the statistical analyses, with advice from A.R. and wrote a draft of the manuscript. A.R. and M.F. helped writing the manuscript and all co-authors commented on and approved the final version of the manuscript.

Conflict of interest statement

The authors have no conflict of interest to declare.

Data availability statement

All data sets will be uploaded to the Dryad Digital Repository (www.datadryad.org) upon acceptance.

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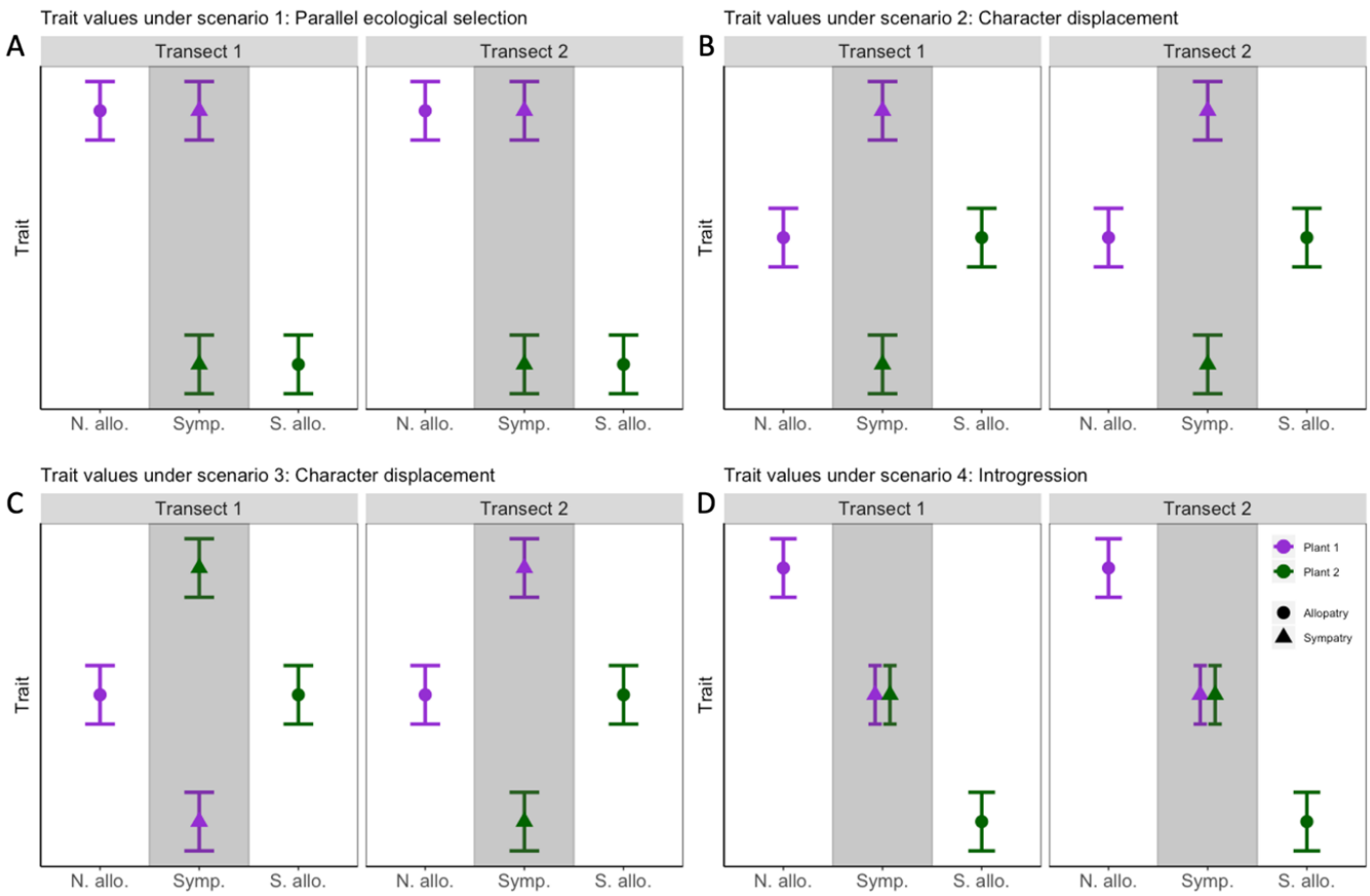

Figure 1: **Predictions to adaptation.** Four possible scenarios populations may follow when faced by ecological or sexual selection under a secondary contact of host races. **A:** Scenario 1**,** parallel ecological selection between host races. This scenario would be expected for ecologically important traits where natural selection imposed by the environment provided by the host differ. **B**: Scenario 2, character displacement in a shared direction across transects. Should a sexual character be reinforced by maladaptive hybridization consistently across transects, this scenario would be expected. **C**: Scenario 3, character displacement in different directions across transects. This would be expected in a sexual character which is reinforced by maladaptive hybridization, similarly to Scenario 2, however in this scenario the direction of adaptation is arbitrary, which would be expected under a mutation order regime. **D:** Scenario 4, hybridization in sympatric areas is causing introgression resulting in intermediary traits.

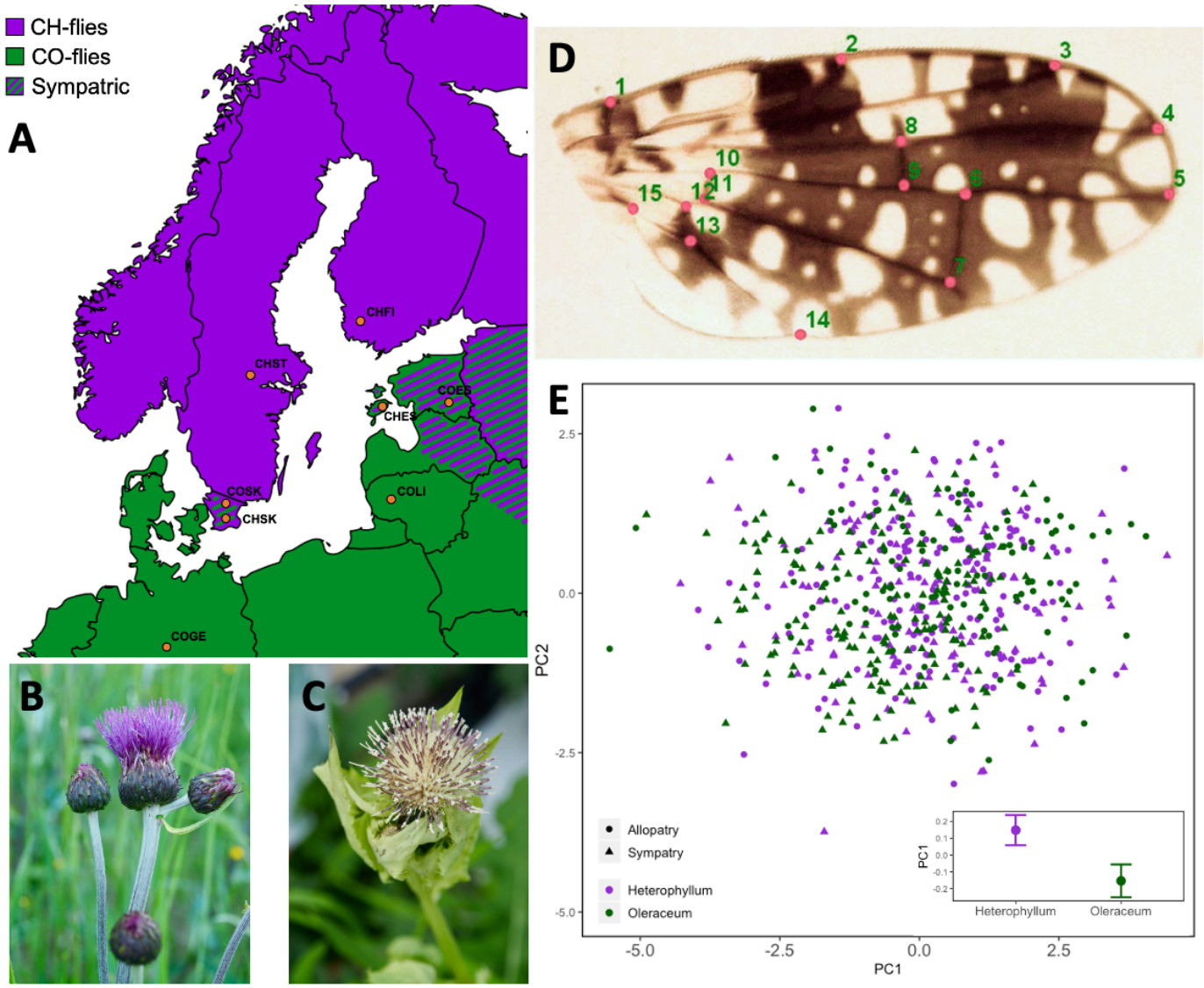

Figure 2: **Methods. A**: Map showing distribution of *T. conura* host races and our sampling locations. **B**: *Cirsium heterophyllum*. **C**: *Cirsium oleraceum*. **D**: Dorsal photograph of a *T. conura* wing annotated with 15 landmarks used to analyze wing shape. **E**: Principal component analysis of *T. conura* individuals measured. The first two principal components explain 37.84% of all variation. Insert shows the mean and standard error of the mean of PC1 in the host races.

645 Table 1: **Results from MANOVA analysis.** On factors explaining fly host race, whether the

646 flies were allopatric or sympatric and the interaction of factors.

MANOVA	df	Pillai	Approx. F	num df	den df	P	
Host plant	1	0.237	7.66	11	271	< 0.001	***
Co-existence	1	0.257	8.51	11	271	< 0.001	***
Host plant x Co-existence	1	0.154	4.42	11	271	< 0.001	***
Residuals	281						

647

648

Table 2: **Post-Hoc ANOVA analyses.** Showing only traits which significantly affected differences between groups in the above MANOVA.

ANOVA	Independent	Dependent	F	num df	den df	P	
Females	Host plant	Ovipositor length	43.25	1	281	< 0.001	***
		Wing shape, warp 5	8.87	1	281	0.003	**
	Co-existence	Ovipositor length	12.28	1	281	< 0.001	***
		Wing length	9.05	1	281	0.003	**
		Wing width	17.45	1	281	< 0.001	***
		Melanisation ratio	9.26	1	281	0.003	**
		Wing shape, warp 2	10.84	1	281	0.001	**
		Wing shape, warp 3	6.50	1	281	0.011	*
		Wing shape, warp 5	12.09	1	281	< 0.001	***
	Interaction	Body length	6.37	1	281	0.012	*
		Wing width	5.61	1	281	0.018	*
		Wing shape, warp 1	7.42	1	281	0.007	**
		Wing shape, warp 3	6.80	1	281	0.01	**
		Wing shape, warp 5	6.92	1	281	0.009	**
Males	Host plant	Wing shape, warp 5	6.93	1	284	0.009	**
	Co-existence	Wing length	13.79	1	284	< 0.001	***
		Wing width	14.35	1	284	< 0.001	***
		Melanisation ratio	7.14	1	284	0.008	**
		Wing shape, warp 2	12.69	1	284	< 0.001	***
		Wing shape, warp 3	8.75	1	284	0.003	**
		Wing shape, warp 5	5.97	1	284	0.015	*
	Interaction	Body length	11.47	1	284	< 0.001	***
		Wing shape, warp 1	5.48	1	284	0.02	*
		Wing shape, warp 3	9.17	1	284	0.003	**
		Wing shape, warp 6	7.32	1	284	0.007	**

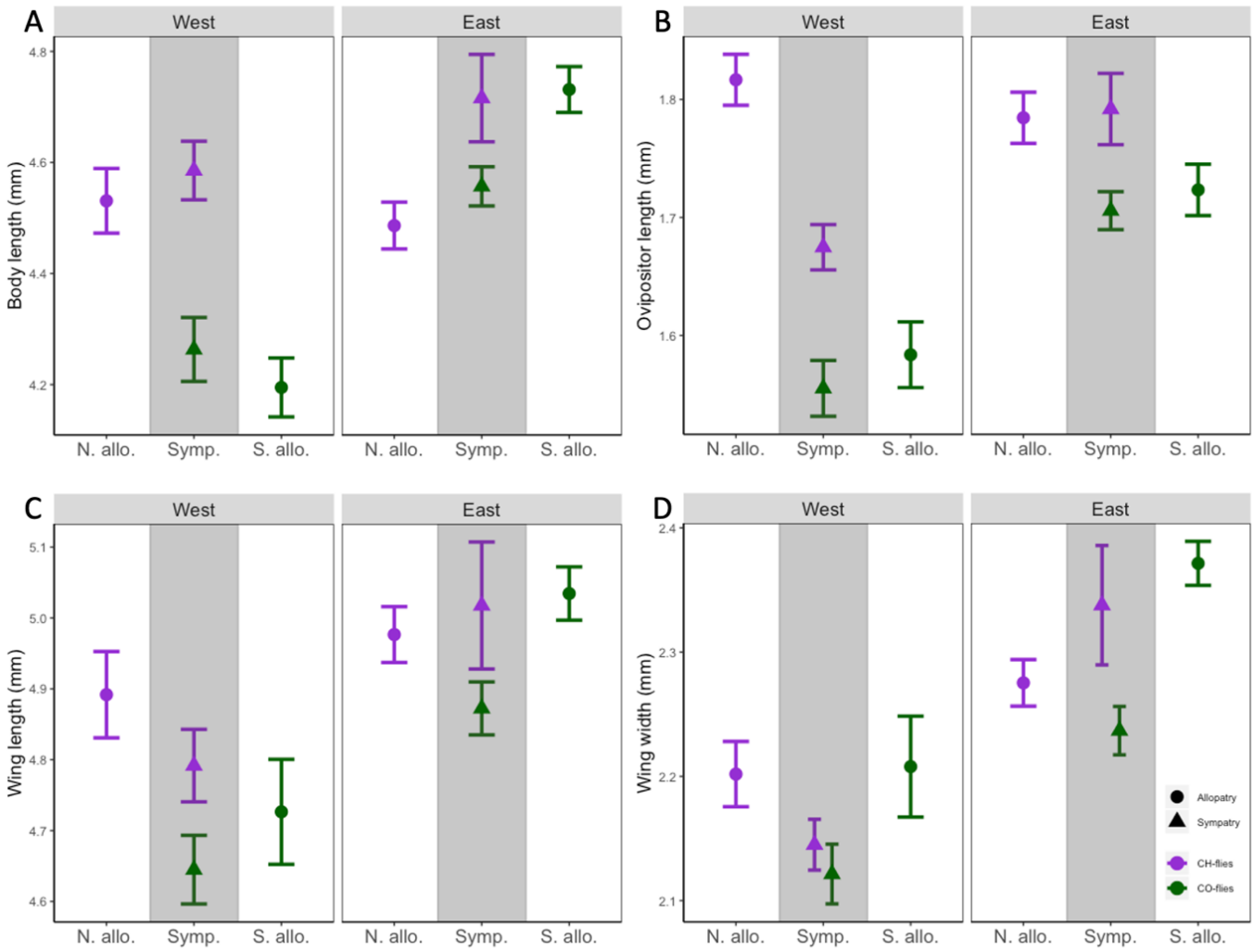

Figure 3: **Trait measurements:** All plots portray mean trait values with SE bars of the mean. Only female *T. conura* are included. 'West' and 'East' represents from which side of the Baltic Sea the population is sampled. X axis labels represent which state of co-existence the fly population is in. 'N.allo.' stands for Northern allopatric, 'Symp.' stands for sympatric and 'S. allo.' stands for Southern allopatric. **A**: Mean values of *T. conura* body length separated by population. **B:** Mean values of *T. conura* ovipositor length separated by population. **C:** Mean values of *T. conura* wing length separated by population. **D:** Mean values of *T. conura* wing width separated by population.

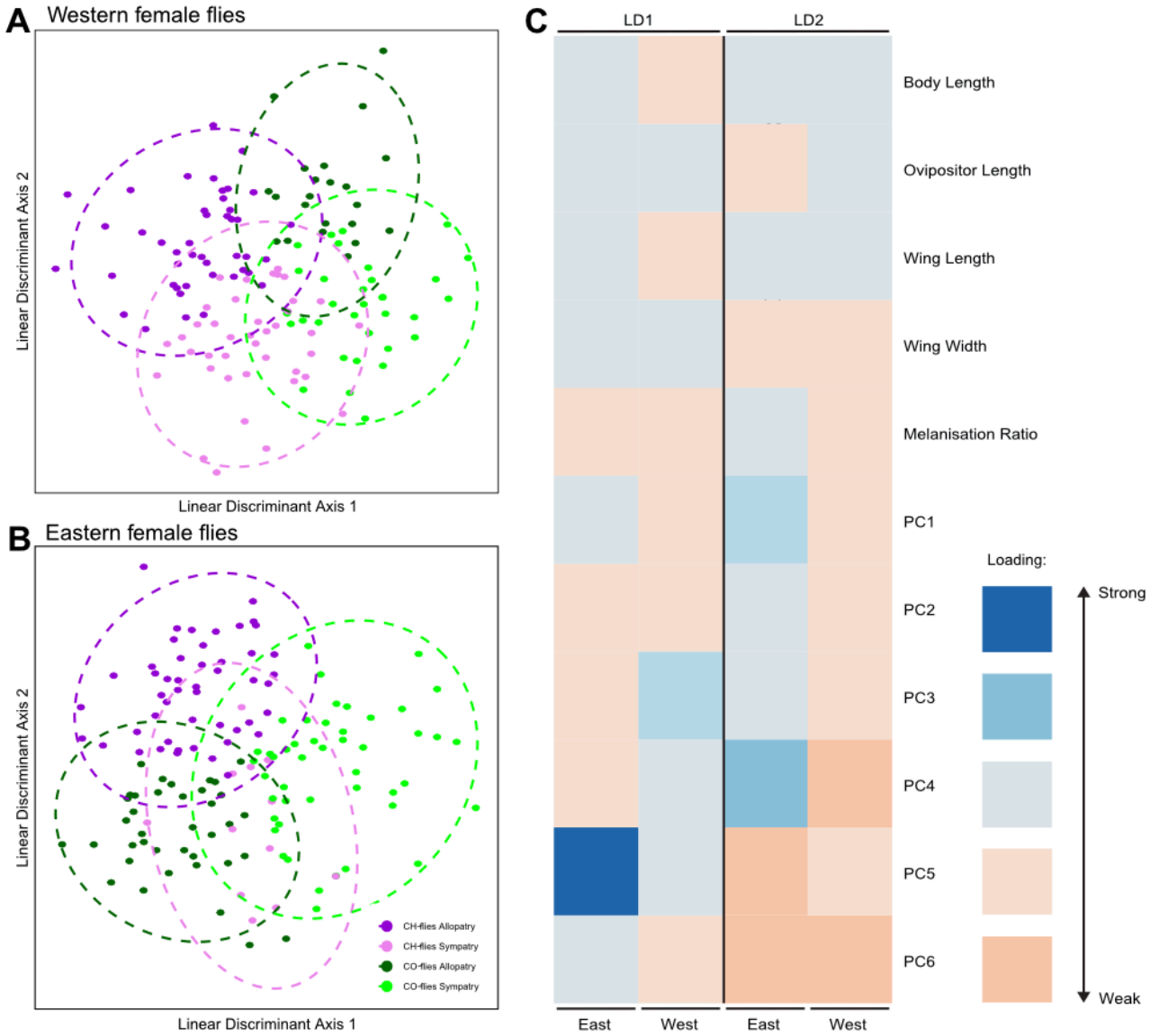

Figure 4: **Linear discriminant function analyses and bootstrapped loadings.** All female *T. conura* included**. A and B**: LDAs showing a different structure when comparing Eastern and Western flies **C:** Bootstrapped loadings colored from blue to red based on how much standard error surpasses zero or not, where loadings with standard errors which never surpassing zero are colored blue. Bootstrap performed with 100 000 replications.