

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

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12 **Abstract**

13 Divergent ecological selection may diversify conspecific populations evolving into different
14 niches, and may lead to speciation if gene flow is ceased, e.g, due to reinforcement leading to
15 character displacement. Adaptation to the same niche is expected to be parallel. Whether
16 selection against maladaptive hybridization in secondary sympatry also results in parallel
17 divergence in traits that are not directly related to the ecological niches remains an empirical
18 challenge, although such character displacement can be decisive for if ecotypes develop into
19 new species. Here, we use a host shift in the phytophagous peacock fly *Tephritis conura*, with
20 both host races represented in two geographically separate areas East and West of the Baltic
21 Sea to investigate convergence in morphological adaptations. We asked i) if there are
22 consistent morphological adaptations to a host plant shift and ii) if the response to secondary
23 sympatry with the alternate host race is parallel across contact zones. We found surprisingly
24 low and variable, albeit significant, divergence between host races. Only one trait, the length
25 of the female ovipositor, which serves an important function in the interaction with the hosts,
26 was consistently different. Instead, co-existence with the other host race significantly affected
27 the degree of morphological divergence, but the divergence was largely driven by different
28 traits in different contact zones. Thus, local stochastic fixation or reinforcement could
29 generate trait divergence, and additional evidence is needed to conclude whether divergence
30 is locally adaptive.

31 **Introduction**

32 Adaptation to novel niches through ecological selection has been identified as a major driver
33 of speciation (Schluter 2008; Schluter 2009; Nosil 2012). Classic adaptive radiations
34 including the Darwin Finches (Weiner 1994; Loo et al 2019), the Hawaiian tarweeds and
35 silverswords (Baldwin et al 2003) and the recurrent evolution of limnetic-benthic stickleback
36 pairs (Schluter 1993; Bay et al 2017) are driven by adaptations to novel diets and habitats.
37 Speciation driven by expansion of novel dietary niches has potential to be rapid, like in
38 *Rhagoletis* flies, where populations of hawthorn-feeding flies have adapted to use the
39 introduced apple (*Malus domestica*) as a host during the last 150 years (Bush 1969; Feder et
40 al 1988; Linn et al 2004; Meyers et al 2020). It remains an empirical challenge to resolve to
41 which extent morphological adaptations are parallel and predictable under parallel selection
42 regimes (Bolnick et al 2018). Few studies have explicitly compared parallelism in multiple
43 traits in such scenarios, but a recent study of Bahamas mosquitofish (*Gambusia hubbsi*)
44 suggests that only a few traits are highly predictable across parallel predation environments
45 (Langerhans 2018).

46
47 Parallelism in response to co-existence with congeners provide a further empirical challenge,
48 as evolutionary divergence may be affected by the local presence or absence of the other
49 incipient species (Amarasekare 2003; Calabrese and Pfennig 2020). Reducing gene flow to
50 populations adapted to other niches is crucial for ecological specialization to result in
51 diversification, as ecological divergence does not necessarily lead to speciation (Taylor et al
52 2006; Bolnick 2011; Lackey and Boughman 2017). Divergence and specialization may be
53 reversed in the absence of reproductive isolation (Taylor et al 2006; Lackey and Boughman
54 2017). Hence, if reproductive isolation between the incipient species is incomplete, secondary
55 contact may break up important ecological adaptations. This could be particularly evident

56 when different populations of the diverging species are inhabiting discrete niches, like in the
57 case of insect host races. Close congeners inhabiting different niches may be poorly adapted
58 to the alternative environment (Rundle and Nosil 2005; Nilsson et al 2017; Cronemberger et
59 al 2020; Martin et al 2020), which may lead to reinforcement (Servedio and Noor 2003) and
60 character displacement with stronger differences in traits involved in mate choice (Hinojosa et
61 al 2020). While parallel divergence is expected for ecologically selected traits (Fig. 1a), either
62 parallel- or nonparallel character displacement (Fig. 1b,c) or reduced divergence due to
63 introgression (Fig. 1d) could be expected for traits that are not strongly coupled with host
64 plant use.

65

66 To understand whether selection to reduce maladaptive mating in the presence of conspecifics
67 results in parallel morphological divergence to the same extent as ecological adaptations to a
68 novel niche, it is necessary to compare patterns of divergence in allopatry and sympatry
69 across geographic regions. Such studies would test the hypothesis that morphological
70 responses to sympatry can be more arbitrary than adaptations of ecological traits, which are
71 expected to diverge in parallel across populations of similar niches (Fig. 1). In particular,
72 studies asking how co-existence with congeners affects trait distributions, e.g. through
73 parallel- or non-parallel character displacement (Fig. 1), may provide insight into the nature
74 of the selection pressures exerted by co-existence of close congeners. Here, we address how
75 morphological traits evolve and diversify in allopatric and sympatric areas using two host
76 races of the fly *Tephritis conura*. This species has recently undergone a host shift resulting in
77 host races specializing on utilizing different *Cirsium* thistle species as larval food plants
78 (Diegisser et al 2006a; Diegisser et al 2006b; Diegisser et al 2007; Diegisser et al 2008).

79

80 Macroevolutionary analyses reveal that host plant driven speciation, as in the case of *T.*
81 *conura*, is one of the main factors explaining the tremendous diversity of phytophagous
82 insects as many speciation events can be attributed to host plant shifts (Berlocher and Feder
83 2002; Dres and Mallet 2002; Nylin et al 2014). Moreover, diversification rates are elevated in
84 herbivorous insects compared to their non-herbivorous relatives (Mitter et al 1988; Farrell
85 1998; Wiens et al 2015). For an insect specialist like *T. conura*, the host plant provides a
86 discrete environment imposing a multidimensional selection pressure. Hence, insect host plant
87 adaptations are predicted to affect multiple traits involved in e.g. phenological matching,
88 female host preference and larval performance (Matsubayashi et al 2010). The putatively
89 strong selection pressures involved in host plant adaptation provide a strong prediction of
90 parallelism in host-use related traits of phytophagous insects at early stages of the speciation
91 process (Nosil et al 2002; Meyers et al 2020). However, the prediction is less straightforward
92 for responses to sympatry, because the traits involved in putative reinforcement are not
93 necessarily associated with host use.

94
95 *Tephritis conura* provides an excellent system for studying parallelism in divergence in
96 response to ecological adaptation and co-existence with congeners, as it enables comparisons
97 of trait variation across replicated sympatric and allopatric settings. The two recently
98 established host races inhabit two geographically isolated sympatric zones with adjacent
99 allopatric populations, one East and one West of the Baltic Sea (Fig. 2a). *Tephritis conura*
100 females of the two host races are ovipositing into buds of either *C. heterophyllum* (the
101 ancestral host; Fig. 2b) or *C. oleraceum* (the novel host; Fig. 2c), and larvae show host
102 specific performance and survival (Diegisser et al 2008). Hence, the system enables
103 investigating response to sympatry across two geographically separated contact zones.
104 Specifically, we test the hypotheses (i) that there are consistent morphological responses to a

105 host plant shift across the Eastern and Western areas and (ii) that responses to sympatry are
106 parallel in the two contact zones. Given the consistent genetic differentiation between host
107 races both in allopatry and sympatry (Diegisser et al 2006a; Ortega et al *in prep*) we predict
108 the host races to be morphologically divergent, and this divergence to be consistent across
109 geographical settings. As larval survival is strongly reduced in the alternate host (Diegisser et
110 al 2008), we predict hybridization to be maladaptive, potentially resulting in reinforcement
111 and character displacement between the host specialists in sympatry.

112

113 **Methods**

114 **Study species and sampling**

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

130 while wing traits are more likely shaped by sexual selection. For simplicity, we refer to flies
131 infecting *C. heterophyllum* as CH-flies, while the flies infecting *C. oleraceum* will be denoted
132 CO-flies.

133

134 We used a parallel sampling design to examine phenotypic adaptation to a novel host plant
135 and effects of co-existence between host races, by sampling each host race both in sympatry
136 and allopatry on each side of the Baltic (Fig. 2a). We collected thistle buds infested by *T.*
137 *conura* larvae/pupae and allowed the adults to eclose in a common environment. CO-fly
138 larvae were sampled in Germany and Lithuania (allopatric areas), and both host races were
139 collected in sympatric areas in southern Sweden and Estonia. Allopatric CH-fly larvae were
140 sampled in central-Sweden and Finland (Fig. 2a, Table S1). All sampling took place during
141 June and July 2018. The sampling scheme enables examining to what extent patterns of
142 phenotypic divergence are explained by host plant adaptations, by co-existence with the other
143 host race, and if these patterns differ between the two transects. Typically, *C. heterophyllum*
144 and *C. oleraceum* do not grow in the same microhabitat. Thus, the sympatric and allopatric
145 definitions here refer to the presence of one or both thistle species in a region (Fig. 2a).

146

147 **Morphological measurements**

148 *Tephritis conura* adults eclosed from field-collected thistle buds in a common lab
149 environment, (see Supplementary material S1). One male and one female per bud were
150 euthanized by freezing a few days after emergence and subsequently included in the
151 morphological analysis. For each individual, we took magnified photographs using a
152 Celestron 44308 USB microscope. We photographed a lateral image of the fly body after
153 removal of the wings and a dorsal image of the right wing on a transparent background to
154 allow better visibility of the wing veins. Body length and ovipositor length were measured

155 digitally from lateral photographs (Fig. S1). We placed 14 landmarks, adapted from Pieterse
156 et al. (2017), digitally on the dorsal wings (Fig. 2d,S2) for geometric morphometrics (Zelditch
157 2004). We added a landmark; number 15, to reflect the high variance in the proximal area on
158 the wing. Digitization was performed in TPSDig2 v2.31(Rohlf 2017) and we used TPSUtil
159 v1.76 (Rohlf 2018) for file handling. Wing melanisation was measured with an automated
160 script without any user queries developed in MATLAB (Matlab 2017). As the wings do not
161 display any chromaticity, analysis is based on the red spectral band only. The script extracted
162 the intensity of the red spectral band for each pixel, and performed white calibration by
163 division with the statistical mode corresponding to the white background of the images.
164 Subsequently, images were inverted to represent absorbance rather than reflectance, so the
165 melanised wing would extend from the white background (set to zero). The script identified
166 the wing and separated melanised areas from non-melanised areas, and the size of these areas
167 were divided to estimate the fraction of the wing that was melanised.

168

169 **Statistical analysis**

170 We used PAST3 v3.20 (Hammer et al 2001) to apply a Procrustes fit to the landmark data to
171 align and scale the wings (Fig. 2d). To produce relative warps (i.e. principal components of
172 shape) to compare shape between groups, a wing-shape principal component analysis (PCA)
173 was performed with the Procrustes fitted data using PAST3 v3.20 (Hammer et al 2001) (Fig.
174 S3). Based on the variance explained by the eigenvalues (Fig. S4) and the broken stick
175 criterion (Jackson 1993), six relative warps, principal components of shape, which jointly
176 explain 68% of the wing shape variance were identified (Fig. S5). These relative warps were
177 included in subsequent analyses of phenotypic divergence to represent wing shapes. All
178 subsequent statistical analysis were performed in the statistical software R (R Core Team
179 2019).

180

181 We quantified five morphological traits (body length, ovipositor length, wing length, wing
182 width and melanisation ratio) in addition to wing shape (represented by relative warps
183 produced from landmark analysis) for 583 flies. As an exploratory analysis to investigate
184 whether host race was the major factor explaining variation in fly morphology, we performed
185 a full PCA on the variables fly body length (mm), wing length (mm), wing width (mm), wing
186 melanisation ratio (%) and relative warps 1-6 reflecting wing shape, excluding ovipositor
187 length to be able to include both sexes. We identified four significant dimensions of variation
188 from the full PCA analysis using the broken stick criterion (Jackson 1993) (Fig. S6).

189 Collectively, these full PCA-axes explained 78% of the morphological variance in the dataset.

190

191 To formally test if the two host races were significantly differentiated, we applied a
192 multivariate analysis of variance (MANOVA), with all variables measured included as
193 response variables (body length, ovipositor length, wing width, wing length, wing
194 melanisation and PC1-6 of wing shapes). To further test if the patterns of morphological
195 adaptation were parallel in the Eastern and the Western transects, and explicitly address if co-
196 existence affected host plant races in the same way in these replicates, we performed a full
197 MANOVA with host race, co-existence and geographical setting and their 3-way and 2-way
198 interactions as factors. The MANOVAs were performed separately on females and males as
199 this enabled including the biologically important trait ovipositor length (Diegisser et al 2007)
200 in analyses of females. This division of sexes reduced the multicollinearity of explanatory
201 factors to below recommended values (Hair, 2010).

202

203 To further investigate parallelism in differentiation of the host race pairs, we applied separate
204 linear discriminant function analyses (LDAs) on the data from the Eastern and Western

205 transects. We used host races and co-existence with the other host race as factors in the
206 models. This analysis was performed on males and females separately. To test if the patterns
207 of divergence differed significantly between transects, we performed each LDA 10,000 times
208 using the bootstrap R package ‘boot’ (Canty and Ripley 2020) and used the confidence
209 intervals to assess if the loadings differed between analyses. In addition, we assessed the
210 proportions of divergence that is shared among host races and unique among the populations,
211 respectively, in a nested MANOVA using size and shape variables against host race and
212 transect following Langerhans & DeWitt (2004).

213

214 **Results**

215 We found no strong evidence for separation between host races along the two major axes of
216 divergence when including both sexes (Fig. 2e). CO-flies had, on average, lower values of
217 PC1 compared to CH-flies, reflecting smaller body and wing size, as predominantly size
218 variables load positively on PC1 (GLM: $F_{1, 581} = 5.189$, $p = 0.023$; Fig. 2e; Table S2). The
219 significant host race differences also hold true in a more complex model, including co-
220 existence and geographic origin (see below; Table 1). However, the difference between host
221 races was small compared with the variation among populations within host races. There was
222 no separation between host races along the second PC axis, with higher values representing
223 higher wing melanisation and lower representation of wings that are larger than average in the
224 distal and proximal areas of the wing, while being smaller in the posterior area, see relative
225 warp 5 in Fig. S5.

226

227 Intriguingly, host plant and co-existence affected female morphology differently East and
228 West of the Baltic Sea, as illustrated by a significant 3-way interaction (Pillai’s trace = 0.117,
229 $F_{11, 277} = 3.22$, $p < 0.001$; Table 1). All main effects and 2-way interactions were also

230 significant in the full model (Table 1). These patterns hold true also for males (Pillai's trace =
231 0.092, $F_{10, 271} = 2.74$, $p = 0.003$; Table 1). Hence, depending on geography, host race and co-
232 existence affected fly morphology differently. Co-existence with the other host race decreased
233 morphological divergence in both females and males (Pillai's trace = 0.295, $F_{11, 277} = 10.15$, p
234 < 0.001 and Pillai's trace = 0.194, $F_{10, 271} = 6.54$, $p < 0.001$ respectively), and flies from the
235 two transects differed significantly in morphology for both sexes (Pillai's trace = 0.374, $F_{10,$
236 $271 = 14.52$, $p < 0.001$ for females and Pillai's trace = 0.393, $F_{10, 271} = 17.51$, $p < 0.001$ for
237 males).

238

239 Interestingly, host race differences in females depend on co-existence, with CO-fly females
240 becoming more similar in sympatry compared to CH-fly females (Pillai's trace = 0.218, $F_{11,$
241 $277 = 6.79$, $p < 0.001$; Tables 1 and S3; Fig. 3 and S7). In contrast to females, co-existence
242 affected CO-fly males more strongly, with CO-fly males becoming more similar to CH-fly
243 males in sympatry whereas CH-fly males were more similar across all populations (Pillai's
244 trace = 0.179, $F_{10, 271} = 5.92$, $p < 0.001$; Tables 1 and S3; Fig. S8-S11). The differences
245 between host races varied between transects in females, with a stronger host race divergence
246 in the Western than the Eastern transect (Pillai's trace = 0.18, $F_{11, 277} = 6.79$, $p < 0.001$; Tables
247 1 and S3; Fig. 3 and S7; Tables 1 and S3; Fig. 3 and S7). This pattern holds true also for
248 males (Pillai's trace = 0.16, $F_{10, 271} = 5.12$, $p < 0.001$; Tables 1 and S3; Fig. S8-S11). Finally,
249 the differences between allopatric and sympatric populations are stronger in Western flies
250 compared to Eastern both in females (Pillai's trace = 0.163, $F_{11, 277} = 4.74$, $p < 0.001$; Tables 1
251 and S3; Fig. 3 and S7) and males (Pillai's trace = 0.131, $F_{10, 271} = 4.1$, $p < 0.001$; Tables 1 and
252 S3; Fig. S8-S11).

253

254 To assess how much of the female divergence was unique and shared between host races we
255 also estimated Wilk's partial η^2 . While a high share of the partial variance was explained by
256 shared divergence between host races ($F_{11,273} = 4.96$, 30.6%) and transect specific patterns of
257 host race divergence ($F_{11,273} = 3.81$, 25.3%), divergence between transects explained the
258 highest percentage of partial variance ($F_{11,273} = 6.85$, 37.8% (Table S4).

259

260 As host race affected morphology differently depending on geography, we further tested if the
261 major axis of divergence separated host races in both transects, and if the same traits
262 separated groups using a Linear Discriminant Analysis (LDA). This analytical approach
263 revealed that the importance of host race for population separation differed between transects.
264 In the Western LDA, host races separated along the first discriminant axis whereas the
265 sympatric and allopatric populations separated along the second discriminant axis (Fig. 4). In
266 the Eastern LDA, the first discriminant instead divided the two CO-fly populations and the
267 second discriminant axis divided the two CH-fly host race populations (Fig. 4). These
268 analyses also revealed that the host races from transects East and West of the Baltic had
269 diverged in different sets of characters as bootstrap loadings from the two LDAs show that
270 different characters loaded on the first discriminant axes (Table S5). For the Eastern transect,
271 body length and wing warp 6 had positive loadings, and wing size, ovipositor length and wing
272 warp 1 had negative loadings on LD1, whereas for the Western transect, LD1 had positive
273 loadings for wing width and shape. In the Eastern transect, wing shape, body length and wing
274 width had negative loadings, but wing length positive loadings on LD2, while for the Western
275 transect body length, ovipositor length and wing lengths loaded positively on LD2. Wing
276 warp 5 loaded positively on LD1 for both transects, but the loading was substantially stronger
277 for Western populations.

278

279 **Discussion**

280 Contrary to previous studies that found clear differences between the *T. conura* host races
281 (Diegisser et al 2007) we found low and variable morphological differentiation. The
282 multivariate differentiation between host races was driven by subtle differences across many
283 traits, and the traits that were divergent between host races differed in the two transects. Host
284 races separated slightly but significantly along the main axis of variation, reflecting mainly
285 ovipositor length differences, with CH-fly ovipositors being slightly longer than CO-fly
286 ovipositors, but with unexpected substantial variation among populations. Previous work on
287 the *T. conura* host races has reported consistent divergence for loci inferred to be involved in
288 host plant adaptation (*Peptidase D* and *Hexokinase*; see (Diegisser et al 2006b), and recently
289 obtained whole genome data (Ortega et al *in prep*) support the presence of discrete genetic
290 clusters for each host race. Moreover, the flies also have poor performance on the alternate
291 host plant (Diegisser et al 2008). In light of this, the utterly moderate and variable host race
292 divergence in morphology was unexpected. However, our findings are in line with the
293 observation that parallelism in fitness is typically higher than parallelism in both phenotypic
294 divergence and genetic divergence (Bolnick et al 2018). Patterns of parallel divergence only
295 in traits under very strong ecological selection is consistent with the findings of parallelism in
296 response to different predation regimes in Bahamas Mosquitofish (Langerhans 2018), where
297 only a few traits show highly predictable patterns of diversification. Hence, at early stages of
298 diversification driven by ecological adaptation, parallelism may be high only for traits that are
299 strongly coupled to the ecological factor that adaptively diversify the incipient species.

300

301 We find that two out of five female traits, ovipositor length and body length, match a parallel
302 divergence scenario, and body length only in the Western transect (Fig. 1a). Hence, the degree
303 of parallelism among host races in morphological divergence was lower than expected.

304 Ovipositor length was significantly diverged between host races, but the strength of
305 divergence differed between sympatric and allopatric populations as well as between the
306 Eastern and Western populations. In Western populations, the sympatric CH-fly population
307 has intermediate ovipositor lengths, whereas CO-flies and Eastern CH-flies show parallel
308 ecological divergence between sympatry and allopatry, corresponding to the expectations
309 under parallel ecological selection (Fig. 1a). The intermediate measurements for traits found
310 in the Western CH-flies could suggest selection for shorter ovipositors in sympatry, or reflect
311 introgression. We find host race divergence in body size, with clear host race differences in
312 body length in the Western transect (corresponding to Fig. 1a), while Eastern flies have a
313 shuffled distribution of body sizes. Size typically varies with temperature in insects (Atkinson
314 1994; but see Shelomi 2012). While the parallel sampling design would partly correct for
315 temperature effects, temperature could still differ between the Eastern and Western transects.
316
317 When we jointly investigated effects of co-existence, geographic origin and their interactions
318 on host race divergence, we found that both co-existence with the other host race and whether
319 the population originate from East or West of the Baltic Sea affected morphology (Fig. 3).
320 Hence, the low host race divergence may, to a high extent, be explained by interacting effects
321 of co-existence with the other host race, with non-parallel patterns of divergence in the two
322 transects, which could indicate a mutation order scenario (Fig. 1c) (Mendelson et al 2014). If
323 reinforcement due to maladaptive hybridization would have been a prominent force acting on
324 the *T. conura* host races, we would expect traits to be more divergent between host races in
325 sympatry compared to allopatry, i.e. for character displacement to arise (Comeault et al 2016;
326 Calabrese and Pfennig 2020; Kyogoku and Wheatcroft 2020; Fig. 1b,c). This was generally
327 not the case. Instead, the most common pattern was that traits in sympatric populations are
328 more similar between host races than traits in allopatry. Contrary to our expectations, based

329 on the fact that *Tephritid* flies perform a mating ritual that includes elaborate wing
330 movements from both sexes during courtship (Sivinski 2000; but see Briceno and Eberhard
331 2017), this pattern was strongest for wing morphology traits. This is the opposite of what
332 would be predicted under a reinforcement scenario but consistent with introgression (Fig. 1d),
333 as found by Ortega et al. [*in prep.*], or effects of a shared environment.

334

335 Wing size was most strongly affected by co-existence. Interestingly, wing length was overall
336 significantly shorter in sympatry than in allopatry, consistent on both sides of the Baltic Sea.
337 Disproportionally shorter wing length in relation to body size could be consistent with
338 selection for shorter dispersal distances (Claramunt et al 2012), potentially to avoid dispersal
339 to the alternate host plant which could lead to maladaptive introgression. We also found
340 patterns consistent with character displacement, as sympatric flies have similarly narrower
341 wings than their respective allopatric counterparts in the Western transect. In contrast, Eastern
342 sympatric CH-flies have broader and sympatric CO-flies narrower wings. These observations
343 are consistent with a mutation order scenario, where the difference may be important but the
344 specific trait or direction of the difference is arbitrary (Mendelson et al 2014). Some degree of
345 sexual selection may further differentiate populations under a mutation order scenario
346 (Rundle and Rowe 2018), underlining the need to investigate if there is sexual selection at
347 work in this system and disentangling how it would operate.

348

349 Contrary to studies of parallelism in sexually selected traits in ecotypes in e.g. sticklebacks
350 (Boughman et al 2005), we find mixed evidence for parallelism. While shared host race
351 divergence constituted the main share of variation, and host races are separated by the main
352 discriminant axis in the Western transect, host race was a less important predictor of
353 divergence in the Eastern transect where the two CO-fly populations instead separate along

354 the first discriminant axis. The traits that differ between host races also differs between our
355 two geographic replicates. The differences in patterns between the transects East and West of
356 the Baltic Sea could have several additional or alternative explanations related to
357 demographic history, population size and the extent to which the host plant races co-exist
358 locally. Possibly, one contact zone may be older than the other and populations in older
359 sympatry would have had more time for character displacement to develop. Alternatively, if
360 the Eastern transect has a higher proportion suitable thistle habitat, this could have increased
361 both within- and between host race connectivity and potentially gene flow (Servedio and
362 Noor 2003). Genetic data and detailed analyses of introgression should be used to resolve
363 whether selection against hybridization could be expected.

364

365 Morphological differentiation does not always strongly reflect even crucial ecological
366 adaptations. For instance, cultural evolution contributes to reproductive isolation in *Cassia*
367 crossbills, *Loxia sinesciuris* (Porter and Benkman 2019) and *Rhagoletis pomonella* have
368 adapted their phenologies to host fruits ripening at different times of the year (Filchak et al
369 2000). Another potential explanation to the low consistency of host race divergence may be
370 the traits included in this analysis. Females were more diverged than males likely as
371 ovipositor length was differentiated, consistent with previous findings (Diegisser et al 2007).
372 These findings are similar to those of Jourdan et al. (2016) where divergence in female
373 mosquitofishes (*Gambusia*) divergence was more parallel than male divergence. Potentially,
374 the other traits measured are not important enough for host plant adaptation to result in
375 strongly parallel divergence.

376

377 Traits that have been shown to differ strongly between host races include female ovipositor
378 length (Diegisser et al 2007) and the larval ability to survive on the different host plant

379 species (Diegisser et al 2008). A performance experiment showed low viability of *T. conura*
380 larvae adapted to *C. heterophyllum* when reared on *C. oleraceum* (Diegisser et al 2008). Thus,
381 the larval ability to process plant tissue is likely under strong selection, and expected to show
382 similar patterns across populations. Furthermore, host plant preference may have a potential
383 to act as a magic trait (Gavrilets 2004; Thibert-Plante and Gavrilets 2013) in *T. conura*,
384 separating the habitats of the populations and simultaneously providing reproductive isolation
385 as these flies mate on their host plant (Diegisser et al 2007). Finally, we cannot rule out that
386 other, unmeasured traits are important for sexual selection, as pheromones have been
387 suggested to play a role in tephritid mate choice (Roriz et al 2019), as well as overexpression
388 of antioxidants, which have been shown to increase male performance under certain
389 conditions (Teets et al 2019). Regardless of the exact selection pressures acting on our set of
390 study traits, our findings are important, because they show a context dependence for host race
391 adaptation. Parallelism was found only in ecologically strongly selected traits, whereas we
392 found non-parallel changes, some consistent with character displacement for other traits.
393 These insights should guide the design and interpretation when studying ecologically driven
394 divergence.

395

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

403

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412

413 **Declarations**

414

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418 **Conflicts of interest**

419 The authors declare no conflicts of interest.

420 **Ethics approval**

421 Not applicable

422 **Consent to participate**

423 All co-authors consent to participation.

424 **Consent for publication**

425 All co-authors consent to publication.

426 **Availability if data and material**

427 All data will be deposited in dryad (upon acceptance of the MS).

428 **Code availability**

429 The code used to analyse data is available at github.com/kalnil/tephritis.

430 **Author contribution**

431 A.R conceived and designed the study. KJ.N., J.O. and A.R. performed the field work. KJ.N.

432 reared the flies, quantified the morphological traits and performed the statistical analyses,

433 with advice from A.R. and M.F. KJ.N. wrote a draft of the manuscript. A.R. and M.F. helped

434 writing the manuscript and all co-authors commented on and approved the final version of the

435 manuscript.

436

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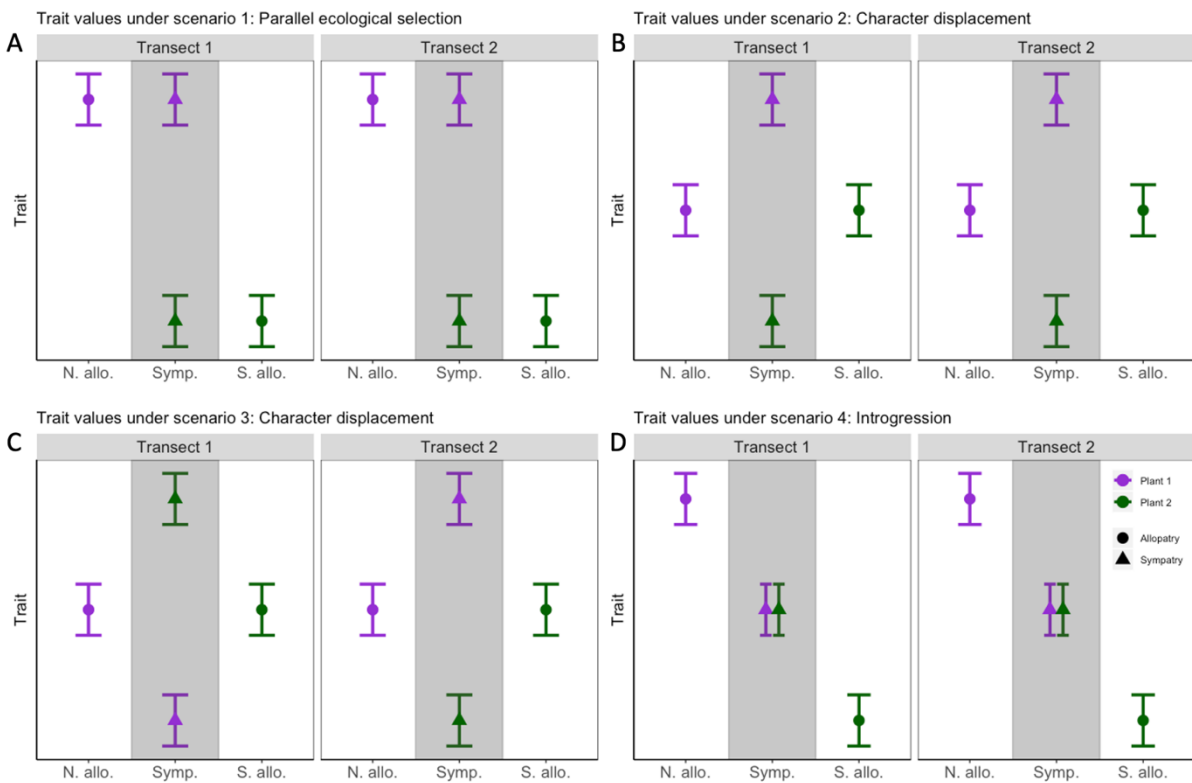
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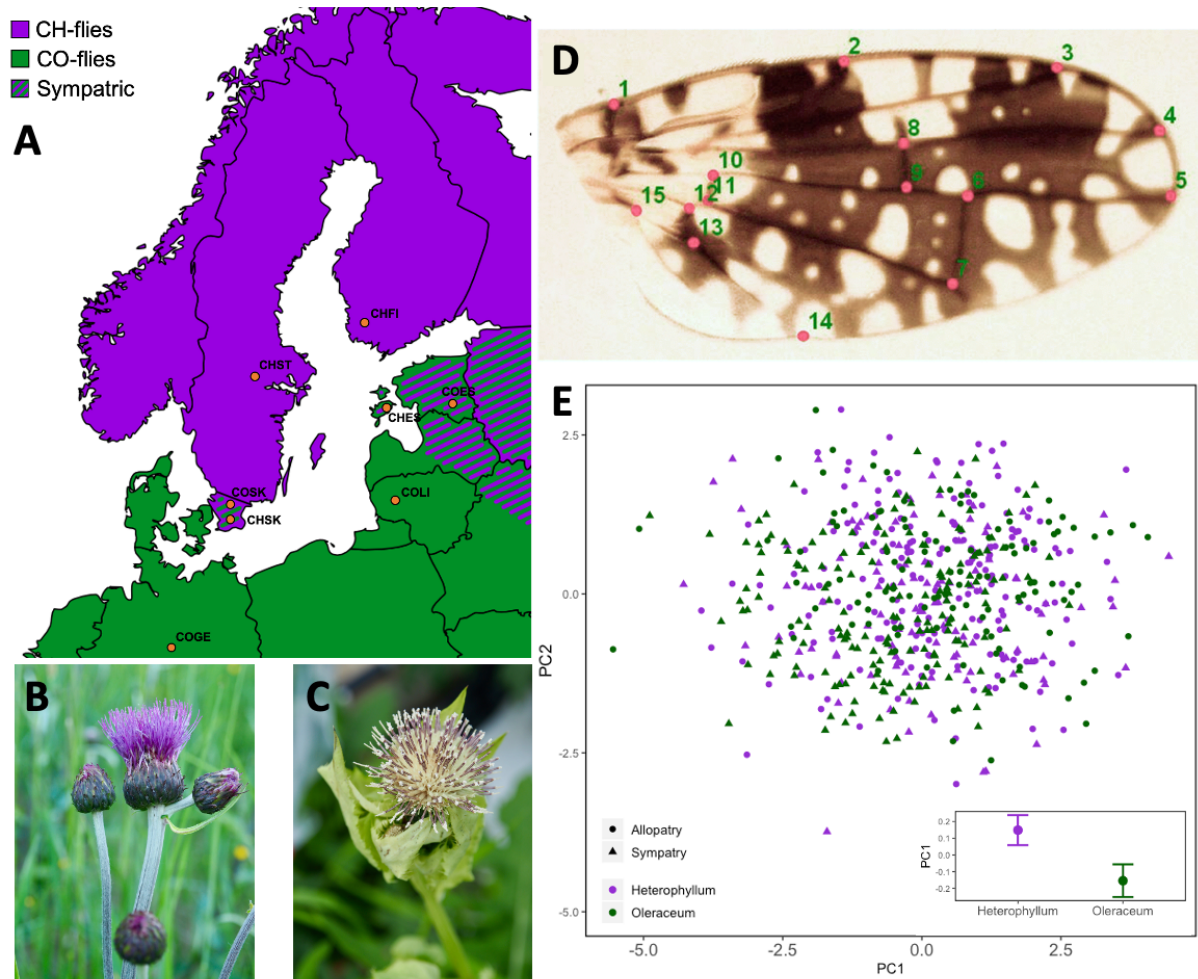


595

596 **Figure 1 Predictions for morphological divergence under different scenarios.** Four
597 possible scenarios for population differentiation depending on the ecological and/or sexual
598 selection pressures at play. Bars represent mean values of morphological traits, with one
599 population being represented by each bar. While the white areas of the graph depict allopatric
600 populations, the grey area includes co-existing populations. ‘N. allo.’ stands for northern
601 allopatry, ‘Symp.’ stands for sympatry and ‘S. allo.’ stands for southern allopatry. Two
602 hypothetical transects are depicted side by side. **A:** Scenario 1, parallel ecological selection
603 between host races. This scenario would be expected for ecologically important traits where
604 natural selection imposed by the environment provided by the host differ. **B:** Scenario 2,
605 character displacement in a shared direction across transects. Should a sexual character be
606 reinforced by maladaptive hybridization consistently across transects, this scenario where
607 traits involved in mate recognition diverge further in sympatry due to character displacement
608 would be expected. **C:** Scenario 3, character displacement in different directions across

609 transects. This would be expected in a sexual character which is reinforced by maladaptive
610 hybridization, similarly to Scenario 2, however in this scenario the direction of adaptation is
611 arbitrary, which would be expected under a mutation order regime. **D:** Scenario 4,
612 hybridization in sympatric areas is causing introgression resulting in reduced divergence in
613 these areas.

614



615

616 Figure 2: **Methods.** A: Map showing distribution of the *T. conura* host races, with the

617 distribution of CH-flies infesting *Cirsium heterophyllum* depicted in purple and that of CO-

618 flies infesting *Cirsium oleraceum* depicted in green. Sampling locations are indicated by red

619 circles. B: *Cirsium heterophyllum*. C: *Cirsium oleraceum*. D: Dorsal photograph of a *T.*

620 *conura* wing annotated with 15 landmarks used to analyze wing shape. E: Principal

621 component analysis of *T. conura* morphology based on females. The first two principal

622 components explain 37.84% of the variation. Insert shows the mean and standard error of the

623 mean of PC1 in the two host races.

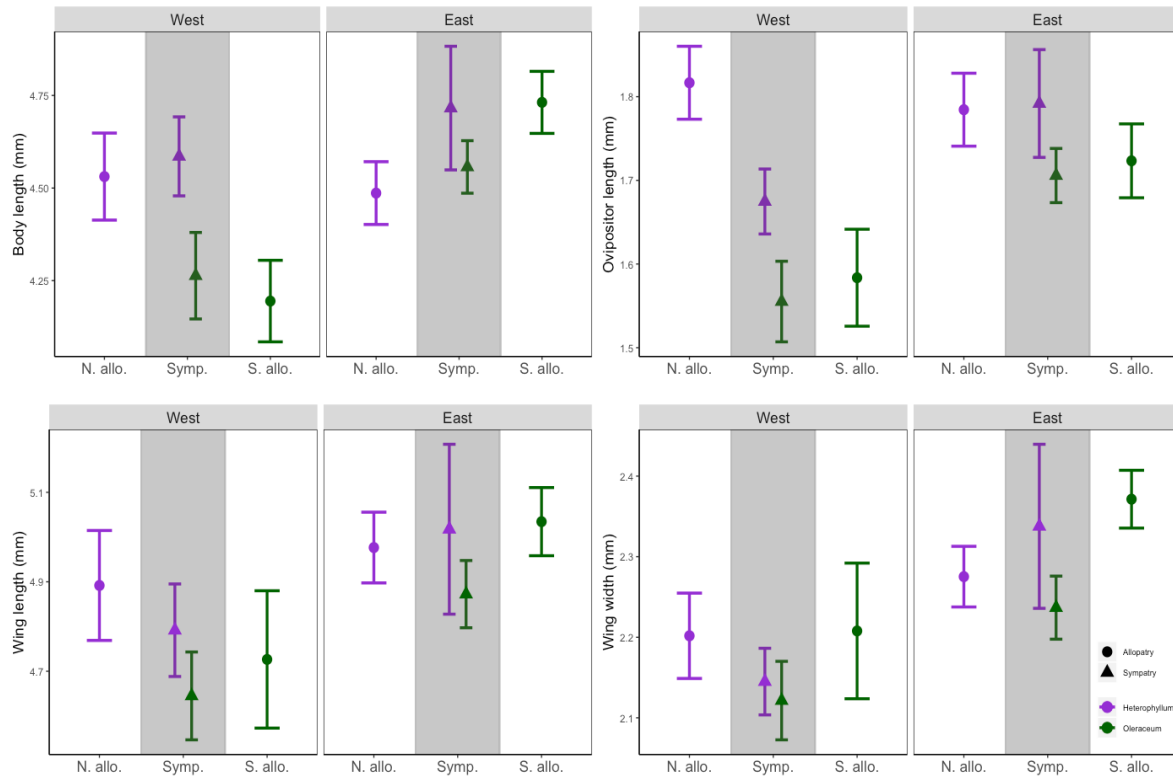
624

625 Table 1: **MANOVA**. Addressing the effect of fly host race, co-existence, transect and the
 626 interaction of these factors on multivariate morphological divergence in five morphological
 627 and six wing shape traits for females and four morphological traits and six wing shapes for
 628 males.

MANOVA	df	Pillai	Approx. F	num df	den df	P	
Females							
Host plant	1	0.258	8.42	11	267	< 0.001	***
Co-existence	1	0.295	10.15	11	267	< 0.001	***
Baltic Sea	1	0.374	14.52	11	267	< 0.001	***
Host plant x Co-existence	1	0.218	6.79	11	267	< 0.001	***
Host plant x Baltic Sea	1	0.18	5.31	11	267	< 0.001	***
Co-existence x Baltic Sea	1	0.163	4.74	11	267	< 0.001	***
Host plant x Co-existence x Baltic Sea	1	0.117	3.22	11	267	< 0.001	***
Residuals	277						
Males							
Host plant	1	0.078	2.29	10	271	0.014	*
Co-existence	1	0.194	6.54	10	271	< 0.001	***
Baltic Sea	1	0.393	17.51	10	271	< 0.001	***
Host plant x Co-existence	1	0.179	5.92	10	271	< 0.001	***
Host plant x Baltic Sea	1	0.16	5.12	10	271	< 0.001	***
Co-existence x Baltic Sea	1	0.131	4.1	10	271	< 0.001	***
Host plant x Co-existence x Baltic Sea	1	0.092	2.74	10	271	0.003	**
Residuals	280						

629

630



631

632 **Figure 3: Trait measurements of female *T. conura* divided by host race, co-existence and**

633 **geographic setting.** Host race is illustrated by color, with purple corresponding to CH-flies

634 infesting purple melancholy thistle and green to CO-flies infesting white cabbage thistle. The

635 grey shaded area in the center contains the sympatric populations (denoted by triangles). X

636 axis labels represent which state of co-existence the fly population is in. ‘N.allo.’ stands for

637 Northern allopatric, ‘Symp.’ stands for sympatric and ‘S. allo.’ stands for Southern allopatric.

638 West and East headers represent from which side of the Baltic Sea the populations are

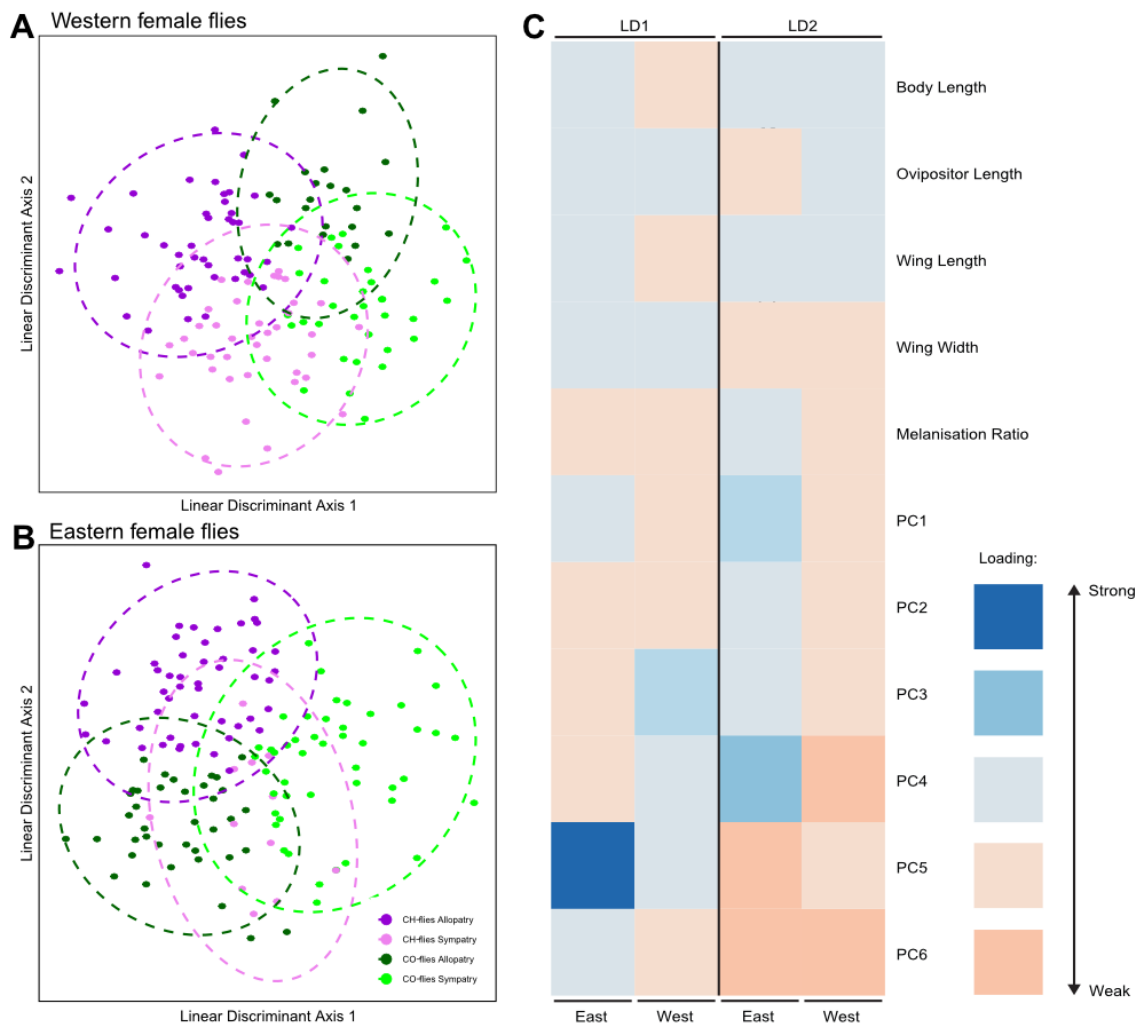
639 sampled. **A:** Mean values of female *T. conura* body length per population. **B:** Mean values of

640 female *T. conura* ovipositor length per population. **C:** Mean values of female *T. conura* wing

641 length per population. **D:** Mean values of female *T. conura* wing width per population. All

642 plots portray mean trait values with 95% confidence interval bars of the mean.

643



644

645 **Figure 4: Linear discriminant function analyses and bootstrapped loadings. A and B:**

646 LDAs illustrating differences in how the host races group along the first two linear

647 discriminant axes in Western (A) and Eastern (B) flies. C: The morphological traits loading

648 on the two first discriminant axes for Eastern and Western flies. Colors illustrate how much

649 the standard error diverges from zero based on 100 000 bootstrap replications. Loading that

650 surpass zero are depicted in red colors whereas loadings significantly lower than zero are

651 colored in blue. Plots and analyses are based on female fly morphology.