Niche divergence in a brown lemur (*Eulemur* spp.) hybrid zone: Using ecological niche models to test models of stability

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1   **ABSTRACT**

2   Endogenous selection is often implicated in the maintenance of stability of natural hybrid zones.

3   Environmental conditions often vary across these zones, suggesting that local adaptation to

4   ecological conditions could also play a role in this process. We used niche modeling to

5   investigate these alternatives in a hybrid zone between two species of brown lemur (*Eulemur*

6   *rufifrons* and *E. cinereiceps*) in southeastern Madagascar. We produced ecological niche models

7   (ENMs) for parental and hybrid populations and compared values of niche overlap to null

8   expectations using identity and background tests. All three taxonomic groups had non-equivalent

9   ENMs with limited spatial overlap, supporting a role for niche divergence and local adaptation in

10   the maintenance of this zone. However, values of niche overlap between ENMs were not greater

11   than null expectations controlling for background environmental differences. These results could

12   suggest that taxa in this hybrid zone inhabit portions of their environments that are more similar

13   to their backgrounds (i.e., niche conservatism). Nevertheless, we did find evidence of niche

14   divergence when using background tests that examined environmental variables separately.

15   While we could not rule out models indicating selection against hybrids, most lines of evidence

16   were consistent with predictions for the bounded superiority model of hybrid zone stability. This

17   study thus provides support that exogenous, environmental selection may be responsible for

18   maintaining the hybrid zone, and may be implicated in the evolutionary divergence of these taxa.

19   Key words: hybrid zone; ecological niche models; tension zone; geographical selection-

20   gradient; bounded superiority

INTRODUCTION

The potential role of ecological adaptations in speciation – with natural selection driving divergence between populations occupying different habitats – has garnered increased attention (Schluter 2001; Wiens 2004). Hybrid zones, where distinct lineages overlap and reproductive isolation is incomplete, offer a unique perspective on such dynamic evolutionary processes (Arnold 1992). Many hybrid zones are stable over long periods, with hybridizing taxa exchanging genes but remaining distinct (Barton and Hewitt 1985; Hewitt 1988). As environmental conditions may vary across zones of overlap and into allopatric portions of the parental species' ranges, adaptation to local conditions may reinforce premating barriers and ultimately the evolutionary separation among lineages (Schluter 2001).

Several models have been proposed to explain hybrid zone stability (e.g., Barton and Hewitt 1985; Moore 1977). Two models – the tension zone and geographical selection-gradient models – argue that selection acts against hybrids but that continual dispersal of parental forms into hybrid zones maintains them over time (Barton and Hewitt 1985; Bigelow 1965; Endler 1977; Key 1968; Moore and Price 1993). The primary difference between these models is that in tension zones, selection against hybrids is endogenous (heterozygote breakdown), while under the geographical selection-gradient model, selection is exogenous (i.e., potentially driven by niche divergence in the parental species). The bounded superiority model was proposed in opposition to these models, arguing that selection could actually favor hybrids within transitional habitats (Anderson 1949; Moore 1977). Similar to the geographical selection-gradient model, selection is exogenous under the bounded superiority model but in this case favors hybrids. Empirical work has provided support for the tension zone and geographical selection-gradient

43   models, while far less evidence for the bounded superiority model has been documented (e.g.,

44   Alexandrino *et al.* 2005; Barton and Hewitt 1985; Gligor *et al.* 2009; Kawakami *et al.* 2008).

45   Niche modeling provides a valuable framework for investigating questions of ecological

46   adaptation across landscapes (Kozak *et al.* 2008; Swenson 2008), which can be used to

47   corroborate findings from genetic, morphological, and behavioral research. These methods use

48   environmental data from known localities to predict the potential geographic distribution of

49   taxonomic groups (Franklin 2009; Peterson *et al.* 2011). These models are termed ecological

50   niche models (ENMs), and comparisons with known taxonomic boundaries can be used to make

51   inferences regarding ecological separation in hybrids and parental species; in the context of

52   ENMs, niche divergence refers to non-identical or significantly different predicted spatial

53   distributions based on environmental (often climate) predictors. For example, Swenson (2006)

54   modeled the ecological niches of four avian hybrid zones, each consisting of eastern and western

55   species in North America. The ENMs of eastern species matched their known taxonomic

56   boundaries, suggesting that exogenous selection limits these species. By contrast, the ENMs of

57   western species expanded well into the east, suggesting that biotic factors (e.g., competition)

58   may be limiting their ranges.

59   We employ ENMs here to test models of stability in a hybrid zone between two species

60   of brown lemur, red-fronted (*Eulemur rufifrons*) and gray-headed lemurs (*E. cinereiceps*). These

61   species are part of the brown lemur species complex, which includes seven species found

62   throughout Madagascar (Johnson 2007; Markolf and Kappeler 2013; Mittermeier *et al.* 2008).

63   Most species within this complex are arboreal and frugivorous (Johnson 2007), and several

64   confirmed or suspected cases of hybridization have been documented (Lehman and Wright 2000;

65   Mittermeier *et al.* 2006; Pastorini *et al.* 2009; Wyner *et al.* 2002).

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66   *Eulemur rufifrons* and *E. cinereiceps* hybridize in the Andringitra region of southeastern

67   Madagascar. Data collected from this hybrid zone suggest that it is stable: populations at the

68   center of the zone are at Hardy-Weinberg equilibrium (Wyner *et al.* 2002), private sites specific

69   to the hybrid population have been identified (Wyner *et al.* 2002), and the zone is likely too

70   narrow to be explained purely by neutral diffusion (Delmore *et al.* 2013). Further evidence

71   suggests that it may conform to the bounded superiority model: hybrids are apparently equally as

72   fit as parental forms (Delmore *et al.* 2011), clines constructed using phenotypic and genetic data

73   are variable in shape and non-coincident in position, and there is no elevation of linkage

74   disequilibrium at the center of the hybrid zone (Delmore *et al.* 2013). A preliminary analysis of

75   environmental data further suggests that hybrids occupy a transitional or novel habitat

76   characterized by greater seasonality in precipitation, as well as lower seasonality in temperature

77   (Delmore *et al.* 2013). Furthermore, divergence in the feeding ecology of hybrid and parental

78   forms has been reported, including marked differences in diet during seasons with low food

79   abundance (Johnson 2002, 2007).

80   We constructed ENMs for hybrid and parental forms in the Andringitra brown lemur

81   hybrid zone and quantified niche overlap using both identity and background tests (McCormack

82   *et al.* 2010; Warren *et al.* 2008). We predicted that if this zone conforms to the tension zone

83   model, ENMs should not coincide closely with observed geographic boundaries across parental

84   species and the hybrids because environmental differences are not expected to play a role in

85   species divergence. Furthermore, niche divergence should not be observed (although tension

86   zones can settle in density troughs, which may themselves be associated with environmental

87   shifts; Hewitt 1988); thus, either niche conservatism or null models would be supported under

88   the tension zone model. If the Andringitra hybrid zone conforms to the geographical selection-

89   gradient model, we predicted that there would be overlap between the ENMs of parental forms at

90   the known location of the hybrid zone. Moreover, the ENM of hybrids would be located in this

91   region but overlap with ENMs of parental forms. We further predicted that we should observe

92   niche divergence between parental forms – but comparisons between hybrids and each parental

93   form should demonstrate niche conservatism, as hybrids do not occupy separate ecological

94   niches under this model. Instead, they may possess characteristics from both parental forms that

95   are maladaptive when combined in hybrids. Finally, if this zone conformed to the bounded

96   superiority model, we predicted that the ENM of hybrids should line up with the known location

97   of the hybrid zone and show limited overlap with ENMs of parental forms. Niche divergence

98   should also be documented between hybrids and both parental forms.

99   **METHODS**

100   **Study system**

101   Occurrence locations for *Eulemur cinereiceps*, *E. rufifrons*, and hybrids were recorded

102   during previous research in the study region (e.g., Delmore *et al.* 2013; Ingraldi 2010; Johnson

103   2002) (Table 1). For simplicity, we refer hereafter to all three populations (including hybrids) as

104   'taxa'. The distribution of *E. rufifrons* includes a portion of western Madagascar south of the

105   Tsiribihina River, as well as a long expanse of eastern forests from the Mangoro and Onive

106   Rivers in the north to the Andringitra Massif in the south (Mittermeier *et al.* 2008). We only

107   include the eastern portion of this species' range in our analyses, as marked climatic differences

108   between the now isolated eastern and western distributions would introduce a high degree of

109   environmental heterogeneity in our analyses; western dry forest habitats are not present in the

110   eastern populations of *E. rufifrons* that border and contribute to the hybrid zone and inclusion of

111   climatic variables from the more arid western range may bias results. *Eulemur cinereiceps*

112   occupies a narrow range in eastern Madagascar, from the Andringitra Massif to the Mananara

113   River (Johnson *et al.* 2008; Mittermeier *et al.* 2008), though with an isolated population

114   described south of this river (H. Andriamaharoa, pers. comm.). The hybrids are distributed in the

115   mountainous Andringitra region, which serves as a biogeographical crossroads between eastern

116   and central Madagascar (Goodman and Lewis 1996). Based on analysis of morphological,

117   microsatellite and mtDNA markers, the hybrid zone extends up to 70 km in width, from

118   Ambondro in the north to Ankorabe in the south (Delmore *et al.* 2011, 2013; Fig. 1).

119   **Ecological Niche Model (ENM) construction**

120   Presence locations for ecological niche models (ENMs) were drawn from the entirety of

121   the range of *Eulemur cinereiceps*, the hybrid zone, and the eastern range of *E. rufifrons*. The

122   final set of presence locations was refined in order to avoid spatial autocorrelation and to match

123   the spatial resolution of environmental variables (see below). Systemic sampling was conducted

124   by creating a 1-km grid encompassing all of the presence locations, and randomly sampling one

125   occurrence per species per grid cell (Fourcade *et al.* 2014). This resulted in a dataset of 83

126   presence locations (21 *E. cinereiceps,* 25 hybrid, and 37 *E. rufifrons*; Table 1).

127   We developed ENMs using Maxent software (Maxent v3.3.3k; Phillips *et. al.* 2006).

128   Maxent relies on presence-only records to estimate the probability of occurrence for a species. It

129   finds the probability distribution that is most spread out, or closest to uniform (i.e., maximum

130   entropy) and then constrains that distribution by the values of environmental variables at

131   locations where the species is known to occur (Phillips *et al.* 2006). Using the mean of

132   environmental values to constrain the distribution minimizes overfitting. Maxent performs well

133   in comparison to other approaches (Elith *et al.* 2006) and with small numbers of presence

134   locations (Pearson *et al.* 2007). With the exception of cross-validation (see below), we relied on

135   the default settings for all model parameters.

136   We developed Maxent models using five-fold cross-validation and model performance

137   was assessed on the held-out (i.e., test) folds (Elith *et al.* 2011). We constructed receiver-

138   operating characteristic curves (ROC) for each fold and used the area under the curve (AUC) to

139   compare model performance (Fielding and Bell 1997). We calculated the test omission rate for

140   each fold through binary predictions using equal sensitivity and specificity; we used a one-tail

141   binomial test to investigate whether the observed omission rate was better than expected

142   compared to a random prediction (Anderson *et al.* 2002). The AUC is a threshold-independent

143   test statistic that measures the ability of a model to discriminate between sites where a species is

144   present and those where it is absent, which indicates the efficacy of the model for prioritizing

145   areas in terms of their relative importance as habitat for a species. However, Maxent is a

146   presence-only algorithm; therefore we used the approach suggested by Phillips *et al.* (2006),

147   applying randomly selected pseudo-absences instead of observed absences to AUC; we drew

148   background selections from within the known ranges of the three taxa within in eastern

149   Madagascar (Fig. 1). The AUC ranges from 0 to 1, where 1 indicates perfect discrimination and

150   0.5 suggests predictive discrimination is no better than random and values below 0.5 implies

151   performance worse than random. We adopted the interpretation offered by Hosmer and

152   Lemeshow (2000) whereby an AUC value of 0.7–0.8 is considered an acceptable prediction;

153   0.8–0.9 is excellent and >0.9 is outstanding. We ran the models with increasing regularization

154   multiplier settings (2 and 3) but the default multiplier (1) showed the highest model performance

155   (highest AUC) and the lowest variability; furthermore, increasing regularization did not improve

156 model overfitting in two of the three taxa (results not shown). We did not use spatially

157 independent calibration data, which may have inflated performance estimates (Radosavljevic and

158 Anderson 2014); nonetheless, our background tests using Maxent generated ENMs (see below)

159 do not suggest systematic biases leading to overfitting (see Results).

160   We constructed the ENMs using six environmental variables – four climate variables, a

161 measure of forest cover, and elevation. The climate data were downloaded from the WorldClim

162 database (Hijmans *et al.* 2005). We reduced the number of WorldClim climate variables to four

163 (from nineteen), based on a combination of expert knowledge about the physiological and life

164 history requirements of lemurs, as well as correlation analysis. For the latter, we removed

165 collinear variables with Pearson correlation >0.90 (Syfert *et al.* 2013). One of the four retained

166 climate variables represented temperature trends (mean annual temperature); another represented

167 temperature seasonality (standard deviation of temperature), while the others represented

168 precipitation trends (mean annual precipitation) and drought incidence (precipitation of driest

169 month). We derived elevation from a digital elevation model (DEM; USGS 2012) and obtained

170 forest cover from layers classified by Conservation International using 2005 satellite imagery

171 (Harper *et al.* 2007). We upscaled the forest layer to match the resolution of the WorldClim data

172 as it was based on Landsat imagery (30 m resolution). The upscaling process also involved

173 converting the categorical forest variable (forest/non-forest) to a gradient representation (percent

174 forest cover). This was accomplished by the use of a 3 x 3 moving window averaging all of the

175 forest pixels that fell within in each final 30 arc-second pixel. We clipped all variables using the

176 forest layer as the mask so as to only include areas that had greater than zero percent forest cover.

177 We completed all analyses using a Mercator projection in ArcGIS v10.1 (ESRI 2012).

178 **Quantitative analyses of niche divergence**

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179   We quantified potential niche divergence using three methods. First, we used the identity

180   test implemented in ENMtools (Warren *et al.* 2008). This test uses a randomization procedure to

181   evaluate whether the ENMs of taxa are more different than expected by chance. We generated

182   pseudoreplicate datasets by pooling occurrence points, randomizing species identities and

183   creating two new samples of the same sizes as the original samples. We created ENMs for these

184   replicates and estimated niche divergence using two measures of niche overlap: Schoener's *D*

185   and Hellinger's *I* (Warren *et al.* 2008). These measures compare estimates of habitat suitability

186   for each taxonomic group in the full study area; values range from 0 (no overlap) to 1 (complete

187   overlap). *D* assumes that habitat suitability scores are proportional to species abundance while *I*

188   treats scores as probability distributions. We repeated this randomization procedure 100 times to

189   generate a null distribution and used a one-tailed test to compare observed values of niche

190   overlap to the null distribution.

191   Second, we used the background test implemented in ENMtools (Warren *et al.* 2008).

192   Similar to the identity test, this test uses ENMs and a randomization procedure; it aims to

193   determine if the ENM of one taxon predicts that of a second better than expected by chance.

194   Briefly, we compared the ENM of one taxon (A) to another ENM created by choosing a random

195   set of points from the background of the opposing taxon (B). We created background

196   environments by selecting the centroid of every pixel within the ranges of the three taxa within

197   eastern Madagascar (Fig. 1). Where ranges overlapped in the hybrid zone, we created boundaries

198   between *Eulemur rufifrons* and the hybrid background and *E. cinereiceps* and the hybrid

199   background with a horizontal line (along the corresponding parallel of latitude) dividing them at

200   the midpoint between the two most proximal presence points (i.e., between *E. rufifrons* and

201   hybrids at the northern edge of the hybrid zone, and between *E. cinereiceps* and hybrids at the

202   southern limit of the zone; Fig. 1). The number of random points drawn from taxon B's

203   background is equivalent to number of occurrence points for taxon B. We compared these ENMs

204   using Schoener's D and Hellingers I. We repeated this procedure 100 times and in both

205   directions to generate two null distributions. We used two-tailed tests to compare observed

206   values of niche overlap between taxon A and B. We considered the niches of taxa diverged if the

207   observed value fell below the 95% CI of the null distribution (i.e., measures of niche overlap are

208   lower). Following Blair *et al.* (2013), we used a conservative approach when interpreting these

209   results; if the test was significant in one direction but not the other, we failed to reject the null.

210   We took the same approach when results were in opposite directions for the two distributions.

211   Finally, we used the background test described by McCormack *et al.* (2010) using custom

212   scripts in R 3.0.3 (R_Development_Core_Team 2014) available in Supplemental Materials. This

213   is a multivariate method that does not rely on ENMs. Instead, we extracted environmental

214   variables from occurrence points and a random set of background points. We calculated

215   differences for each variable between taxonomic groups and compared them to a null distribution

216   (generated by calculating the difference between background points using a bootstrapping

217   approach and 1000 resamples). We used a two-tailed test, the same environmental variables used

218   to construct ENMs and the same background used in the previous test to assess niche overlap

219   among taxa. We considered the niches of taxa diverged if the observed value fell above the 95%

220   CI of the null distribution (i.e., differences between background points were less than differences

221   between the occurrence points). The advantage of this background test (vs. Warren *et al.* (2008))

222   is that environmental data are not summarized into a single value, allowing us to examine each

223   variable separately. For simplicity, we term these two analyses the 'Warren background test'

224   and 'McCormack background test'. We note that comparisons among non-sister taxa (i.e.,

225   *Eulemur cinereiceps* and *E. rufifrons*) may be problematic on theoretical grounds, as we cannot

226   deduce whether niche divergence might have occurred between these species and a possibly

227   large number of more closely related, now extinct lineages since these species originally

228   diverged evolutionarily (Losos 2008; Losos and Glor 2003). However, the parental species are

229   very closely related (e.g., Markolf and Kappeler 2013), and there is no evidence for niche

230   divergence in more recent splits in the brown lemur complex (Blair *et al.* 2013). These

231   comparisons nonetheless should be interpreted with caution.

232   **Ethical note**

233   We obtained location points for study taxa in previous research and re-analyzed them

234   here (see above; Table 1). Original sources provide details regarding animal handling procedures.

235   US Fish and Wildlife Services, Institutional Animal Care and Use Committee (Omaha's Henry

236   Doorly Zoo and Aquarium) and Animal Care Committee (University of Calgary) approved all

237   procedures. The research adhered to the legal requirements of the government of Madagascar.

238   **RESULTS**

239   The Maxent models showed strong discrimination on held out folds, with a mean cross-

240   validated AUC of 0.989 (SD = 0.01) for *Eulemur cinereiceps*, 0.967 (SD = 0.01) for hybrids and

241   0.836 (SD = 0.02) for *E. rufifrons* (Table 1). These AUC values suggest excellent to outstanding

242   discrimination (Hosmer and Lemeshow 2000; Table 1) and were further supported by significant

243   binomial tests (omission error, $0 - 0.36$; all folds significant at $P < 0.0001$, except fold 3 for *E.*

244   *rufifrons* with $P = 0.002$; Table 1).

245   Visual inspection of ENMs indicated that areas with a high probability of occurrence in

246   models for each taxon closely aligned with their known distributions (Figs. 1-2). Observed

247   values of niche overlap (Schoener's *D* and Hellingers *I*) are provided in Table 2 for each

248   comparison. Congruent with visual inspection of the ENMs, we rejected the null hypothesis of

249   niche equivalency in all cases; values of niche overlap were lower than expected by chance

250   (Table 2; Fig. 3).

251   Results were less clear when incorporating background divergence into the null

252   hypothesis. Using ENMs and randomization tests in the Warren background test, we found that

253   the niches of *Eulemur rufifrons* and *E. cinereiceps* were more similar than expected by chance

254   (i.e., the observed value of niche overlap fell above the 95% CI, Table 2; Fig. 4ad). We were

255   unable to reject the null hypothesis consistently (and in the same direction) in the remaining two

256   comparisons (Fig. 4be,cf); thus the most conservative interpretation is that the observed

257   divergence in niches viewed in ENMs (Fig. 2) and non-identical niches shown in the identity test

258   (Fig. 3) may be explained primarily by differences in the environmental background of taxa.

259   However, results for the comparison between *E. rufifrons* and hybrids may indicate more

260   nuanced patterns: niche conservatism for hybrids and niche divergence for *E. rufifrons* (although

261   there was only a trend for divergence when quantifying niche overlap using Hellinger's *I*, Table

262   2; Fig. 4be). This result suggests that hybrids occupy regions within their environment more

263   similar to the background of *E. rufifrons*, while *E. rufifrons* inhabits regions within its

264   environment that are more different from the background of hybrids than would be expected by

265   chance (Table 2; Fig. 4be).

266   Using the multivariate McCormack background test, we documented significant

267   differences from null in all six of the environmental variables compared between *Eulemur*

268   *rufifrons* and *E. cinereiceps*. We found niche divergence for five of the six environmental

269   variables (i.e., the observed difference was greater than the 95% CI); the remaining variable

270   exhibited niche conservatism (i.e., the observed difference was less than the 95% CI; Table 2).

271   When considering *E. rufifrons* and hybrids, all six environmental variables showed significant

272   differences; we recorded niche divergence for two of these variables, and niche conservatism in

273   the remaining four variables (Table 2). Four of six environmental variables exhibited significant

274   differences between hybrids and *E. cinereiceps*. Three of these variables showed niche

275   divergence and one showed conservatism (Table 2). All three comparisons exhibited niche

276   conservatism in forest cover, but niche divergence in the standard deviation (SD) of temperature

277   (Table 2).

278   **DISCUSSION**

279   **Testing models for the maintenance of the Andringitra hybrid zone**

280   Our results indicated variable support for each of the three models for hybrid zone

281   stability: the tension zone, the geographical selection-gradient, and the bounded superiority

282   models (Table 3). The tension zone and the geographical selection-gradient models both invoke

283   selection against hybrids, with the former indicating endogenous selection, such as reproductive

284   impairment, and the latter exogenous (environmental) selection (Barton and Hewitt 1985; Endler

285   1977; Moore and Price 1993); the bounded superiority model also involves exogenous selection,

286   but in this case favoring hybrids within particular ecotones (Good *et al.* 2000; Moore 1977).

287   Under the tension zone model, we predicted substantial overlap among ENMs across parentals

288   and hybrids (or niche conservatism), because adaptation to local environmental conditions was

289   not expected to delimit boundaries across populations (Barton and Hewitt 1985). However,

290   visual inspection of the ENMs indicated well-demarcated ranges across these brown lemur

291   populations, at least in terms of the areas with the highest predicted suitability (Fig. 2).

292   Furthermore, identity tests, which compare the overlap of ENMs through randomization

293   procedures (Warren *et al.* 2008), indicated that all three taxa were significantly diverged in their

294   niches (i.e., had non-identical niches). However, under the Warren background tests, we found

295   convergence between the two parental species, while the hybrid-parental comparisons showed no

296   difference from null. Using a conservative interpretation wherein a lack of clear divergence

297   suggests niche conservatism (Blair *et al.* 2013), these results support the tension zone model. On

298   the other hand, the McCormack background tests (McCormack *et al.* 2010) suggested niche

299   conservatism for forest cover, divergence for mean annual temperature, and conflicting patterns

300   across the remaining variables (Table 2). In all, our results offer only mixed support for the

301   tension zone model, the most commonly cited for hybrid zone stability across many animal

302   species (e.g., Alexandrino *et al.* 2005; Bronson *et al.* 2003; Kawakami *et al.* 2008).

303   Under the geographical selection-gradient model, we predicted that the ENMs of parental

304   forms would meet at the hybrid zone and overlap substantially with the ENM of hybrids (Table

305   3). According to visual inspection of the modeled niches, this was not the case: the areas of high

306   suitability in the ENMs of parental forms did not meet, and there was limited overlap between

307   the ENMs of hybrids and both parental forms (Fig. 2). Indeed, the transitions between parental

308   forms and hybrids coincided well with boundaries of the hybrid zone documented through

309   genetic and morphological evidence (Delmore *et al.* 2013). We also predicted that niche

310   divergence would be documented between parental forms under this model, but not between

311   hybrid and parental forms. Identity test results support the prediction of non-identical niches

312   between parentals, but they did not support the prediction that hybrids and parentals would be

313   identical. Meanwhile, the Warren background tests showed no support for the former prediction,

314   but support for the latter (Tables 2-3). Finally, with the McCormack background tests, the

315   finding of divergence between parentals and convergence (or no difference from null) between

316   hybrids and each parental in elevation does fit the predictions of the geographical selection-

317   gradient model. Nonetheless, the weight of evidence does not provide clear support for this

318   model.

319   Instead, we found stronger support for the bounded superiority model, which predicts

320   niche separation across both parental species and hybrids (Flockhart and Wiebe 2009; Good *et al.*

321   2000; Moore 1977). Both visual inspection of high-suitability areas (Fig. 2) and identity tests

322   (Table 2) indicate divergence (i.e., non-identical niches) in the ENMs of hybrids and parentals.

323   While the Warren background test suggested niche conservatism, we did document significant

324   divergence for mean standard deviation of temperature using McCormack background tests

325   (Table 2). As each environmental variable used to construct ENMs was examined separately in

326   this set of analyses, it is possible that only temperature seasonality is relevant to niche divergence

327   and the maintenance of reproductive isolation in this system; the inclusion of additional variables

328   which do not distinguish among parentals and hybrids in the ENMs may have swamped out any

329   signal of divergence along relevant niche axes in the Warren background tests.

330   We also note that while the overall results from the Warren background tests suggest

331   niche conservatism, we did document some trends that may support niche divergence.

332   Specifically, there was a trend towards niche divergence in the comparisons between hybrids and

333   each parental species (Fig. 3). For example, it appears that while hybrids are occupying a portion

334   of their environment that is similar to the background environment of *E. rufifrons*, *E. rufifrons* is

335   occupying a portion of its environment that is different than the hybrid background (Fig. 3be).

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336 This could be considered niche divergence and would support both the geographical selection-

337 gradient and bounded superiority models.

338   We thus view our analyses as cautious support for the bounded superiority model, but we

339 cannot rule out the tension zone or geographical selection-gradient models across all lines of

340 evidence. This assessment is largely congruent with findings from previous research in the

341 Andringitra brown lemur hybrid zone (Table 3). These investigations have shown no indication

342 of strong selection against hybrid forms, as suggested by body condition and the shape and non-

343 coincidence of genetic and morphological clines across the zone (Delmore *et al.* 2011, 2013).

344 Furthermore, the parental species and hybrids appear to vary in ecological adaptations, such as

345 overall and scarce-season diets (Johnson 2007). We caution however that such ecological

346 differences do not provide definitive evidence of niche divergence. As suggested by the Warren

347 background tests, brown lemurs regardless of ancestry might selectively use similar habitats (and

348 perform similar ecological behaviors) when they are available; it could instead be differences in

349 available environmental types that cause these taxa to appear distinct in their modeled

350 distributions (Godsoe 2010; Warren *et al.* 2008). A recent study comparing ENMs across

351 *Eulemur* also found niche conservatism in all brown lemur sister species pairs (Blair *et al.* 2013).

352 **Issues of scale, resolution, and variables for ecological niche models**

353   There are inherent difficulties when attempting to infer small-scale processes – such as

354 difference in how animals interact with their local environments – from large-scale analyses as

355 presented here. Indeed there are real concerns that ENMs are at best indirect, coarse predictors of

356 the ecological niches and spatial distribution of organisms, and debate continues about which

357 niche dimensions are predicted from these models (Warren 2012). One concern for ENMs is the

358   potential for high levels of environmental heterogeneity to influence model results. Specifically,

359   a high degree of variation in the background range can bias results toward niche divergence

360   (Blair *et al.* 2013; Godsoe 2010). Given the considerably larger range of *Eulemur rufifrons*, it is

361   likely that the distribution of this species maintains greater environmental variation (which

362   would have been even greater had we included the arid west). Nonetheless, we found no

363   evidence of niche divergence in the Warren background tests, suggesting our results are

364   sufficiently conservative despite potential environmental heterogeneity in background ranges.

365   While background tests are designed to assess the differential use of habitats within

366   larger ranges (McCormack *et al.* 2010; Warren *et al.* 2008, 2010), it may be difficult to

367   determine potential niche divergence at small spatial scales. While we were able to detect niche

368   divergence according to mean annual temperature in the McCormack background test, other

369   climate variables showed conflicting patterns across comparisons, no difference from null, or

370   niche conservatism (Table 2). This suggests that *Eulemur cinereiceps, E. rufifrons,* and their

371   hybrids would be similar in niche requirements and behavior in identical environments, despite

372   recorded differences in dietary ecology in allopatry (e.g., Johnson 2007; Overdorff 1993). Two

373   species of mouse lemurs (*Microcebus murinus* and *M. griseorufus*) and their hybrids demonstrate

374   a somewhat contrasting but instructive pattern in southeastern Madagascar. Unlike the brown

375   lemurs, the parentals are broadly similar in allopatry (Rakotondranary and Ganzhorn 2011).

376   However, where they overlap in Andohahela National Park, they occupy different microhabitats

377   according to tree size and differ in temporal occupancy via extended torpor in *M. murinus*;

378   meanwhile hybrids in these environments are more generalist, overlapping with the niches of

379   both parentals (Rakotondranary and Ganzhorn 2011). In general, differential adaptation to

380   environmental types and gradients may play a role in the extent and direction of hybridization in

381   this system (Hapke *et al.* 2011). Interestingly, a recent study by Kamilar *et al.* (in press)

382   indicated higher niche overlap using identity tests between these mouse lemur species ($I = 0.779$,

383    $D = 0.476$) than recorded in our study taxa. It is possible that some of the evidence pointing to

384   niche conservatism in our regional-scale analysis might obscure similar patterns of divergence

385   among brown lemurs in shared habitats, where reinforcement of population differences is most

386   critical. Indeed, researchers have long recognized subtle niche partitioning in sympatric *Eulemur*

387   species (Tattersall and Sussman 1998).

388   In addition to appropriate spatial scales, the selection of relevant variables is an important

389   step in building ENMs (Elith and Leathwick 2009; Warren 2012). Given the ongoing reduction

390   and fragmentation of Madagascar's forest ecosystems (Allnutt *et al.* 2008; Harper *et al.* 2007)

391   with likely substantial impacts on lemur populations (e.g., Schwitzer *et al.* 2014), it may be

392   prudent to consider human factors when attempting to predict lemur distributions and niches.

393   Kamilar and Tecot (2015; this volume) found that including anthropogenic disturbance variables

394   (e.g., distance to human settlements) in *Eulemur* ENMs improved their performance over models

395   using climate variables alone. Such predictors may have altered the ENMs of the Andringitra

396   hybrids and parental species in our analyses, including the degree of niche overlap. We note

397   however that we included an implicit measure of ecosystem integrity – percentage of forest cover

398   – which did not indicate niche divergence in these taxa (Table 2).

399   **Implications for evolutionary processes and conservation**

400   In summary, aspects of our results are consistent with all three models of hybrid zone

401   stability, with the bounded superiority model receiving support from the most lines of evidence.

402   This model underscores the potential importance of ecological selection in the maintenance of

403   reproductive isolation and speciation (Nosil 2012; Schluter 2009), and highlights the creative

404   role hybridization could play in these evolutionary processes. Under the bounded superiority

405   model, hybrids occupy transitional or novel habitats and exist relatively independent of parental

406   forms – which could represent the early stages of hybrid speciation (Mallet 2007). Given the lack

407   of empirical support for the bounded superiority model in other systems, future work to

408   disentangle models of hybrid zone stability in this zone will be important. The collection of

409   behavioral data from individuals of distinct ancestry in shared environments could inform this

410   question by identifying differences in the ecological strategies of hybrid and parental forms.

411   Such research could provide insight into the mechanisms behind the patterns observed in the

412   present study, at the appropriate local spatial scales.

413   If climatic niche divergence among hybrids and parentals maintains the stability of the

414   zone over time, then encroachment into a neighboring taxon's range should be limited. Thus, an

415   expanding hybrid zone does not likely pose a risk to 'pure' populations of *Eulemur cinereiceps*, a

416   Critically Endangered species (IUCN 2015). However, while estimates of historic land cover

417   change vary, deforestation remains a persistent threat to Madagascar's ecosystems (Agarwal *et al.*

418   2005; Grinand *et al.* 2013; Harper *et al.* 2007; Ingram and Dawson 2005; McConnell and Kull

419   2014). Habitat loss could have the secondary effect of disrupting hybrid zone stability,

420   potentially increasing hybridization and posing particular risks for species with small ranges and

421   populations (Detwiler *et al.* 2005) such as *E. cinereiceps* (Brenneman *et al.* 2012; Irwin *et al.*

422   2005). Therefore, preserving the integrity of the forests surrounding the Andringitra Massif may

423   be crucial for conserving this species, as well as the evolutionary and ecological processes that

424   maintain the unique brown lemur hybrid zone.

425   **ACKNOWLEDGEMENTS**

19

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646

FIGURE CAPTIONS

648 **Figure 1.** Locations of background environments for *Eulemur rufifrons*, hybrids and *E.*

649 *cinereiceps.* Points representing the centroid of each pixel (30 arc-seconds) within these ranges

650 were selected for background tests. Limits of the ranges correspond to known river barriers,

651 forest within the eastern escarpment and lowland fragments where these taxa have been recorded,

652 and midpoints between the most proximate parental and hybrid sampling localities.

653 **Figure 2.** Ecological niche models (ENMs) showing the probability of species presence (logistic

654 output) ranging from 0 – 1 for (A) *Eulemur cinereiceps*, (B) *E. rufifrons* and (C) Hybrids in

655 Madagascar. Darker colors represent high probability of species presence. Each distribution is

656 based on models developed using five-fold cross-validation.

657 **Figure 3**. Results from identity test evaluating whether ENMs of *Eulemur cinereiceps*, *E.*

658 *rufifrons* and their hybrids in southeastern Madagascar are more different than expected by

659 chance. Observed niche overlap values are shown with red arrows and null distributions

660 generated using a randomization procedure are shown in black. Table 2 includes raw values for

661 niche overlap and p-values.

662 **Figure 4**. Results from background test to determine if the ENM of one taxon predicts that of a

663 second better than expected by chance. Taxa include *Eulemur cinereiceps*, *E. rufifrons* and their

664 hybrids in southeastern Madagascar. Schoener's *D* is indicated in the top panels and Hellinger's *I*

665 in the bottom panels. Observed niche overlap values are shown with black arrows; null

666 distributions generated using a randomization procedure are shown. Colors correspond to the

667 focal species in each comparison (e.g., panels a and d show comparison of *E. rufifrons*

668   distributions (blue) in the *E. cinereiceps* background (red)). C (conservatism) and D (divergence)

669   denote significant differences from null; see Table 2 for raw values and p-values).

1 **Table 1.** Performance of Maxent models for *Eulemur cinereiceps*, *E. rufifrons* and their hybrids in southeastern Madagascar, number
2 of presence locations used to build ecological niche models (ENMs) and data sources for presence locations. AUC (Area under the
3 receiver-operating characteristic (ROC) curve) values show average and standard deviation (SD) of five-fold cross-validation and the
4 test omission rate and P-value of the one tail binomial test for each fold.

			Omission error[*]				
Species	**N[**]**	**AUC (SD)[***]**	**Fold 1**	**Fold 2**	**Fold 3**	**Fold 4**	**Fold 5**
E. cinereiceps[1]	21	0.990 (0.01)	0.07, P < 0.0001	0, P < 0.0001	0.07, P < 0.0001	0, P < 0.0001	0.07, P < 0.0001
E. rufifrons[2]	37	0.842 (0.02)	0.02, P < 0.0001	0.27, P < 0.0001	0.34, P = 0.002	0.36, P = 0.0003	0.21, P < 0.0001
Hybrid[3]	25	0.967 (0.01)	0, P < 0.0001	0.18, P < 0.0001	0.09, P < 0.0001	0.27, P < 0.0001	0, P < 0.0001

5 [1]Delmore *et al.* 2011, 2013; Ingraldi 2010; Johnson 2002

6 [2]Delmore *et al.* 2011, 2013

7 [3]Delmore *et al.* 2011, 2013; Johnson 2002

8 [*]Optimum threshold determined where sensitivity equals specificity

9 [**]Number of presence locations used to build models

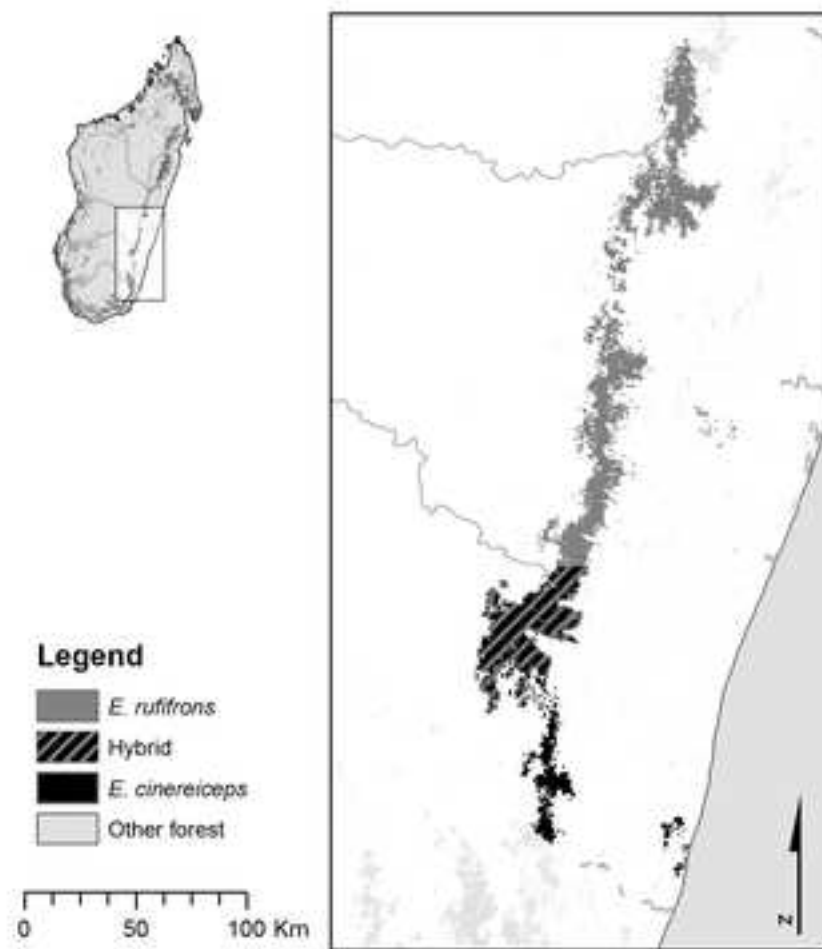10 [***]Area under the receiver-operating characteristic (ROC) curve

11

12 **Table 2.** Observed values of niche overlap and results from identity and background tests for *Eulemur cinereiceps*, *E. rufifrons* and
13 hybrids in southeastern Madagascar. 'Identical' vs. 'non-identical' may be more appropriate terms for the inferences from identity
14 tests; however, we use 'convergence' and 'divergence' here for simplicity of comparison to other tests. Statistics for the Warren
15 background tests are provided for each pairwise comparison (i.e., the ENM of Taxon A in the background of Taxon B and vice versa).
16 Values of niche overlap (Hellinger's *I* and Schoener's *D*) are used in these tests, and inferences are provided below statistics. Note that
17 a convergence or divergence inference would only be indicated if a) both comparisons are significantly different from null and b) both
18 comparisons indicate either convergence or divergence (i.e. the same direction). Each environmental variable is examined separately
19 in the McCormack background test (MA = mean annual). Variables showing significant divergence (D) or conservatism (C) are
20 shown in bold. Confidence intervals (95%) for the null distribution of background divergence are shown in parentheses.
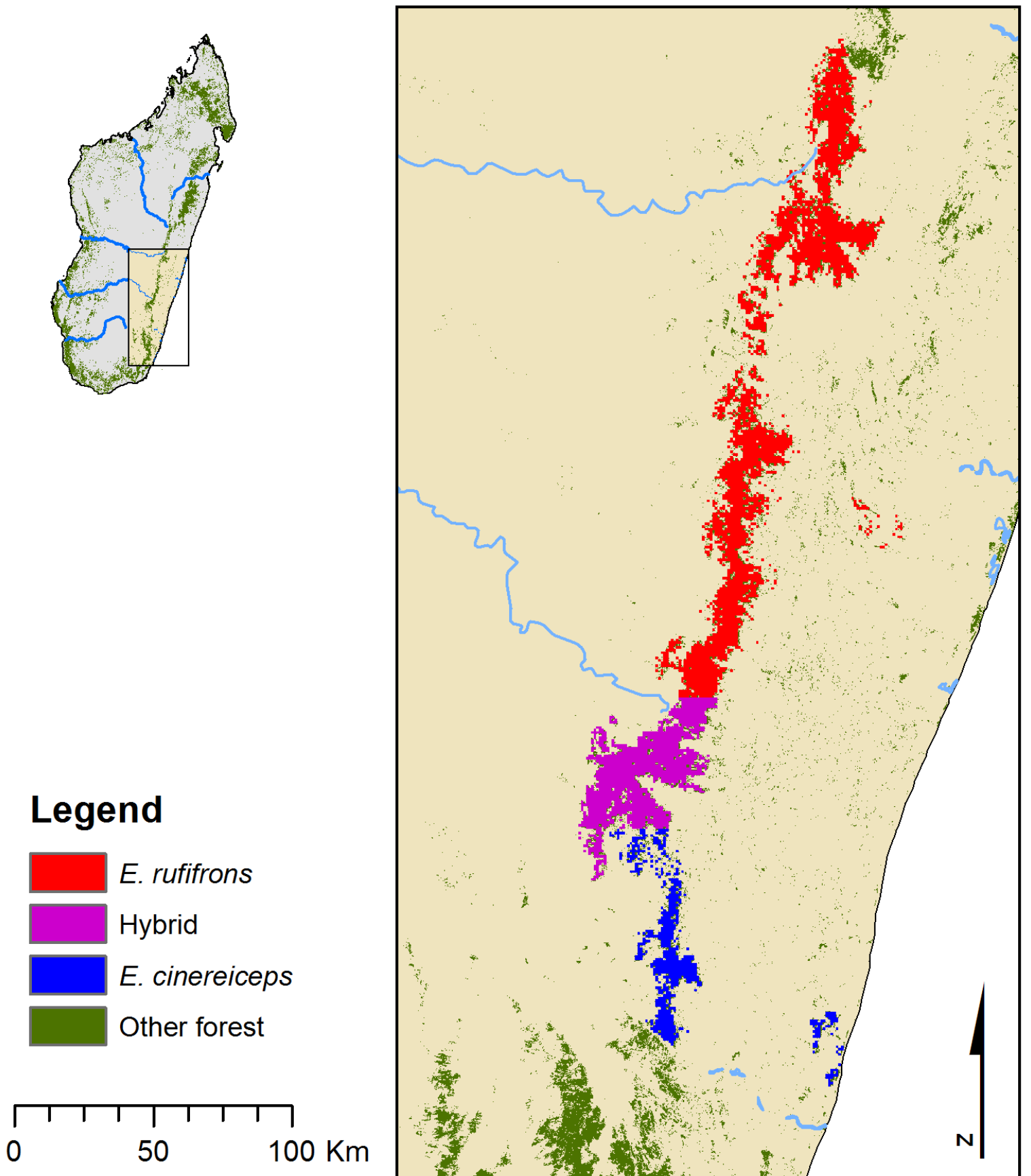
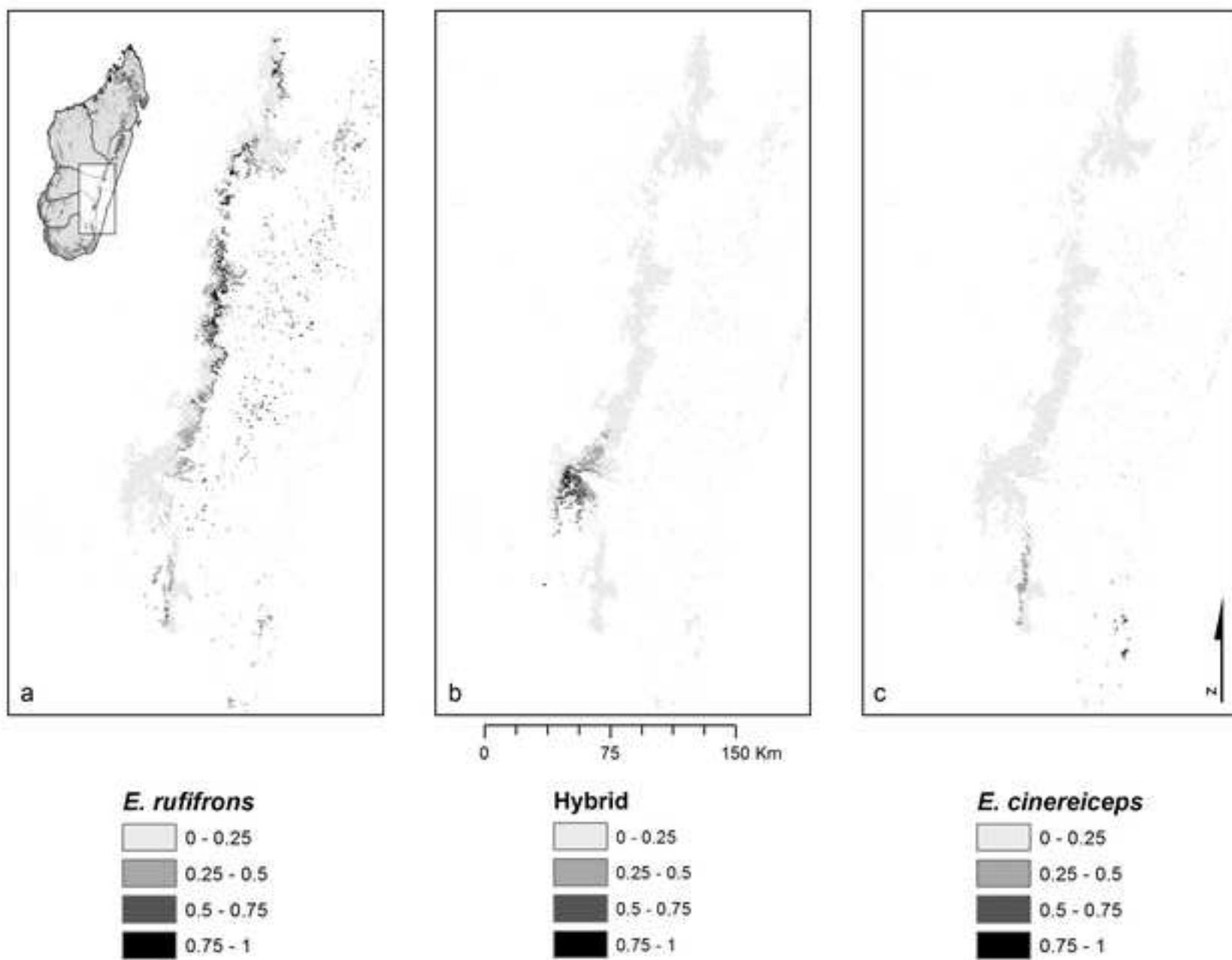|		*E. rufifrons* vs. *E. cinereiceps*	*E. rufifrons* vs. Hybrid	Hybrid vs. *E. cinereiceps*
Observed niche overlap	*I*	0.62	0.58	0.39
	D	0.3	0.29	0.15
Identity	*I*	*P* < 0.01	*P* < 0.01	*P* < 0.01
	D	*P* < 0.01	*P* < 0.01	*P* < 0.01
		Divergence	**Divergence**	**Divergence**
Warren background	*I*	*P* < 0.01, *P* < 0.01	*P* = 0.08, *P* = 0.01	*P* = 0.38, *P* = 0.34
	D	*P* < 0.01, *P* < 0.01	*P* = 0.04, *P* = 0.01	*P* = 0.26, *P* = 0.24
		Convergence	**Null**	**Null**
McCormack background	Forest cover	**4.03 C** (10.52, 22.74)	**13.7 C** (25.02, 36.11)	**9.76 C** (40.05, 53.48)
	MA rainfall	**363 D** (147.5, 238.8)	**427 C** (485.2, 542.4)	**790 D** (668.7, 752.0)
	MA temperature	**3.4 D** (2.39, 2.78)	**0.66 D** (0.012, 0.33)	2.74 (2.60, 2.94)
	SD temperature	**1.2 D** (0.78, 1.02)	**0.23 D** (0.032, 0.15)	**0.97 D** (0.70, 0.90)
	Driest month	**2.87 D** (1.20, 1.92)	**2.08 C** (2.83, 3.23)	**4.95 D** (4.31, 4.89)
	Elevation	**692.5 D** (459.5, 553.6)	**45.5 C** (100.1, 168.3)	647 (602.9, 679.2)

Table 3. Predictions for models of hybrid zone stability from previously published work (a – d) and the present study (e) for *Eulemur cinereiceps*, *E. rufifrons* (parentals) and their hybrids in southeastern Madagascar. When evidence supports a single prediction, **bold** text is used. Conflicting results for the same prediction are indicated in *italics* (see text).
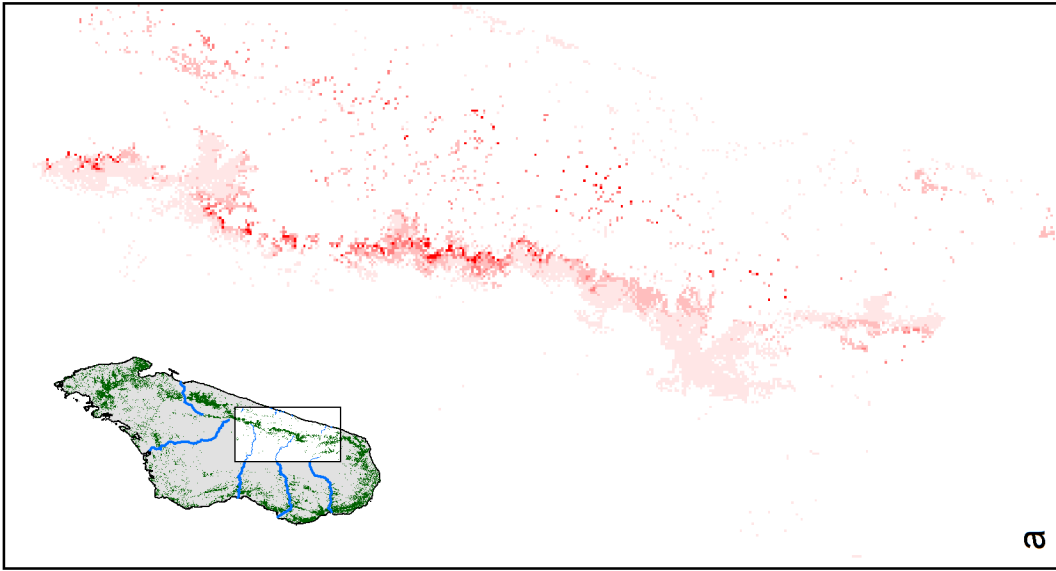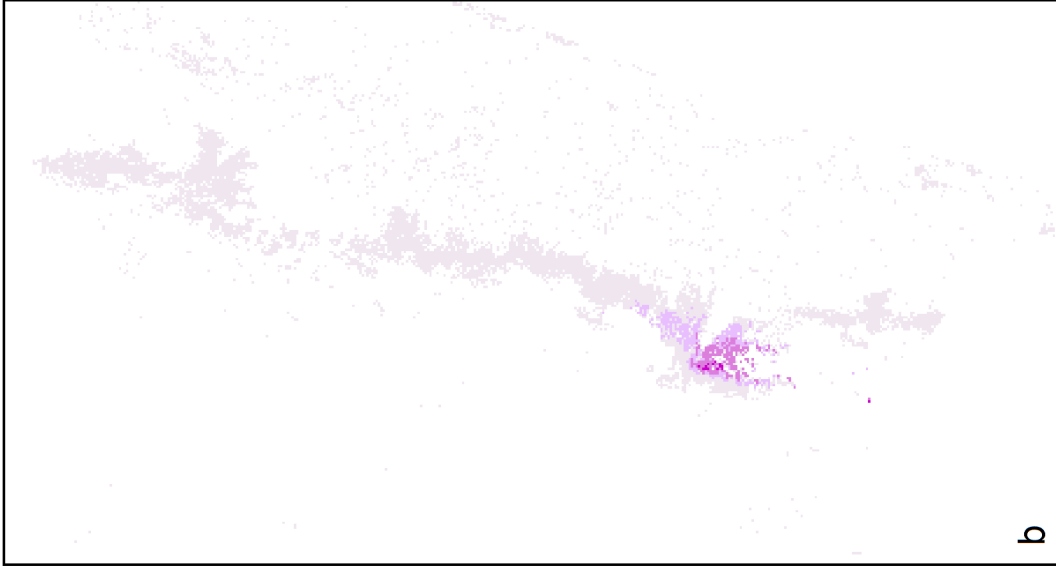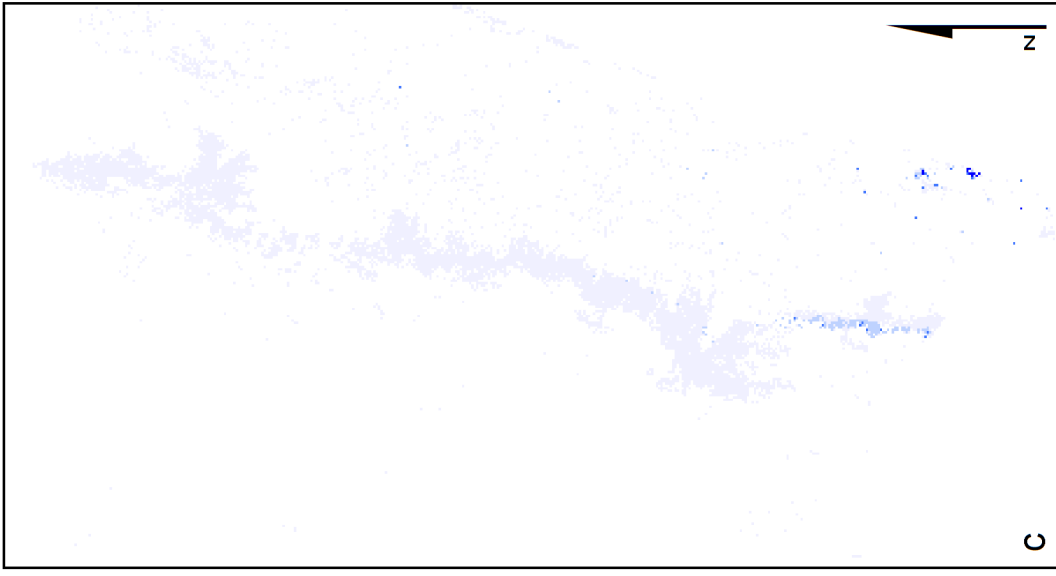
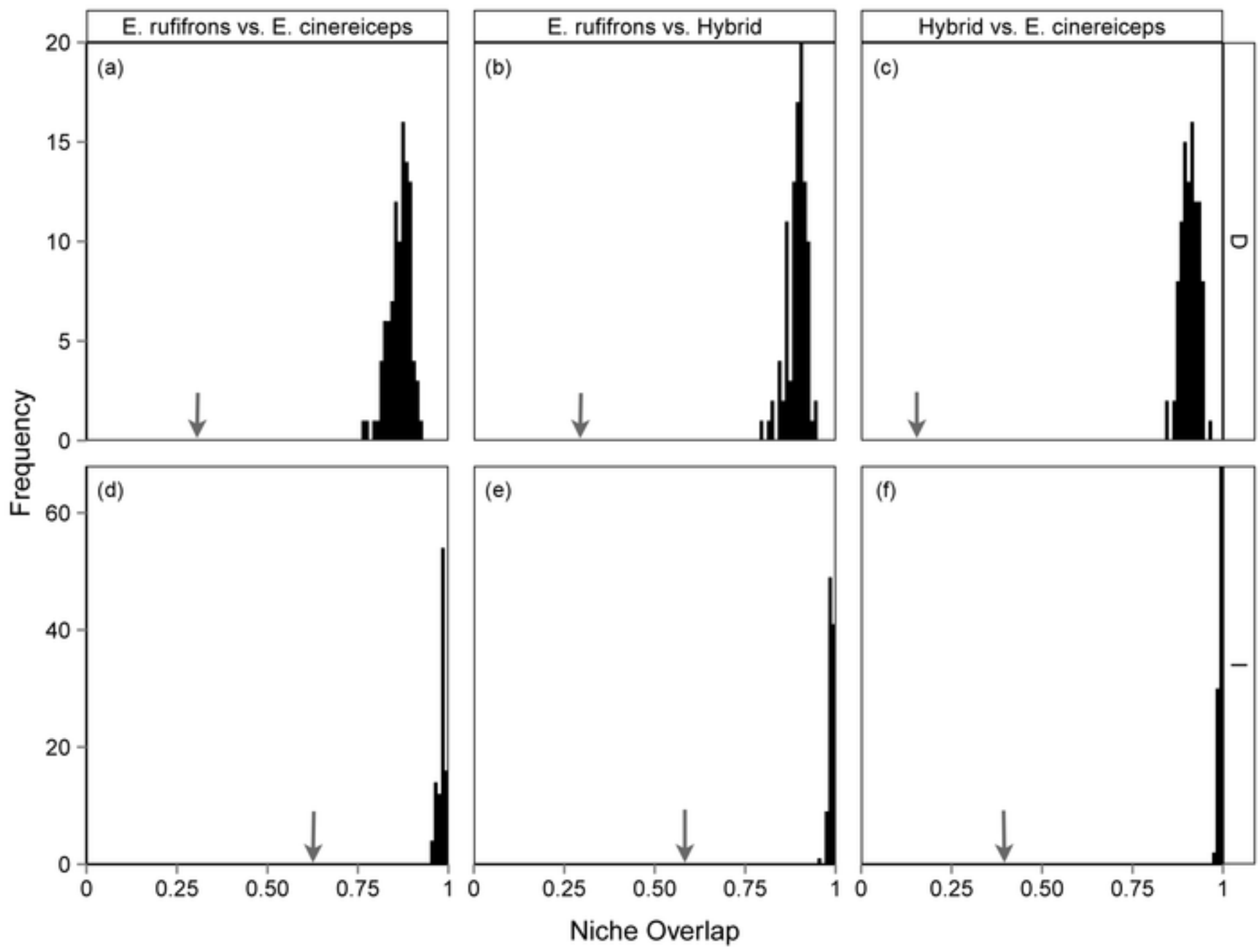|	Tension zone	Geographical selection-gradient	Bounded superiority	Source
Population density in hybrid zone	Low	Low	**≥ Density in parental range**	a
Hybrid zone width	Narrow relative to dispersal	Narrow relative to dispersal	**Width of ecological correlate**	bc
Hybrid zone composition	Parentals and F_1 hybrids	Parentals and F_1 hybrids	**Later generation hybrids**	bcd
Hybrid fitness	Low	Low	**≥ Parental fitness**	b
Cline shape and coincidence	Sigmoid, coincident	Sigmoid, coincident	**Variable, non-coincident**	c
ENM vs. geographic range	No correspondence	ENMs of parentals should overlap at hybrid zone	**Correspondence**	e
Niche conservatism vs. divergence	*Niche conservatism*	*Niche divergence between parentals; niche conservatism between hybrids and parentals*	*Niche divergence*	e*

a = Irwin *et al.* 2005; b = Delmore *et al.* 2011 (morphological and pelage data); c = Delmore *et al.* 2013 (genetic data); d = Wyner *et al.* 2002 (genetic data); e = current study; * Identity test and McCormack background test support divergence in this category, Warren background test supports conservatism.
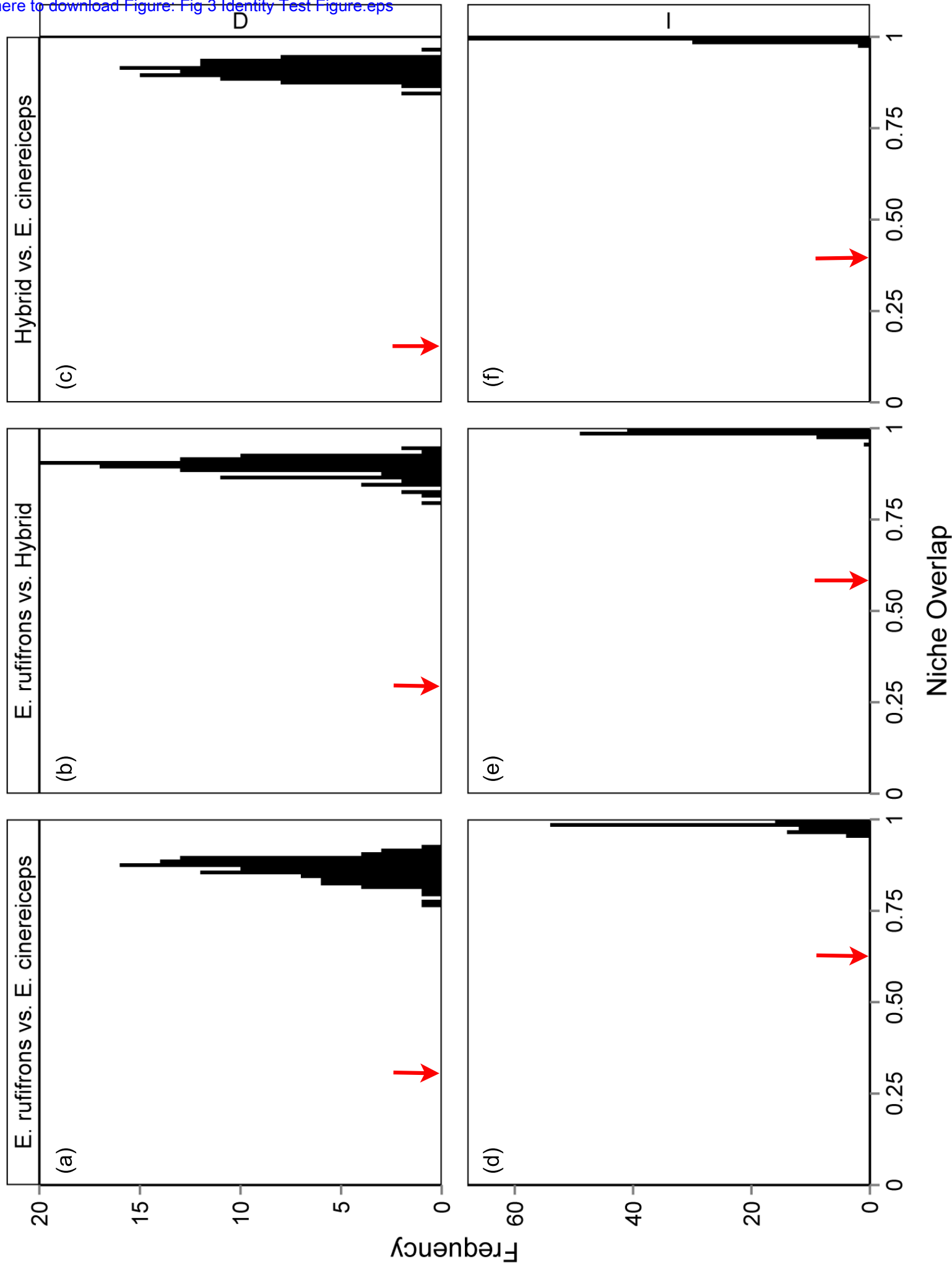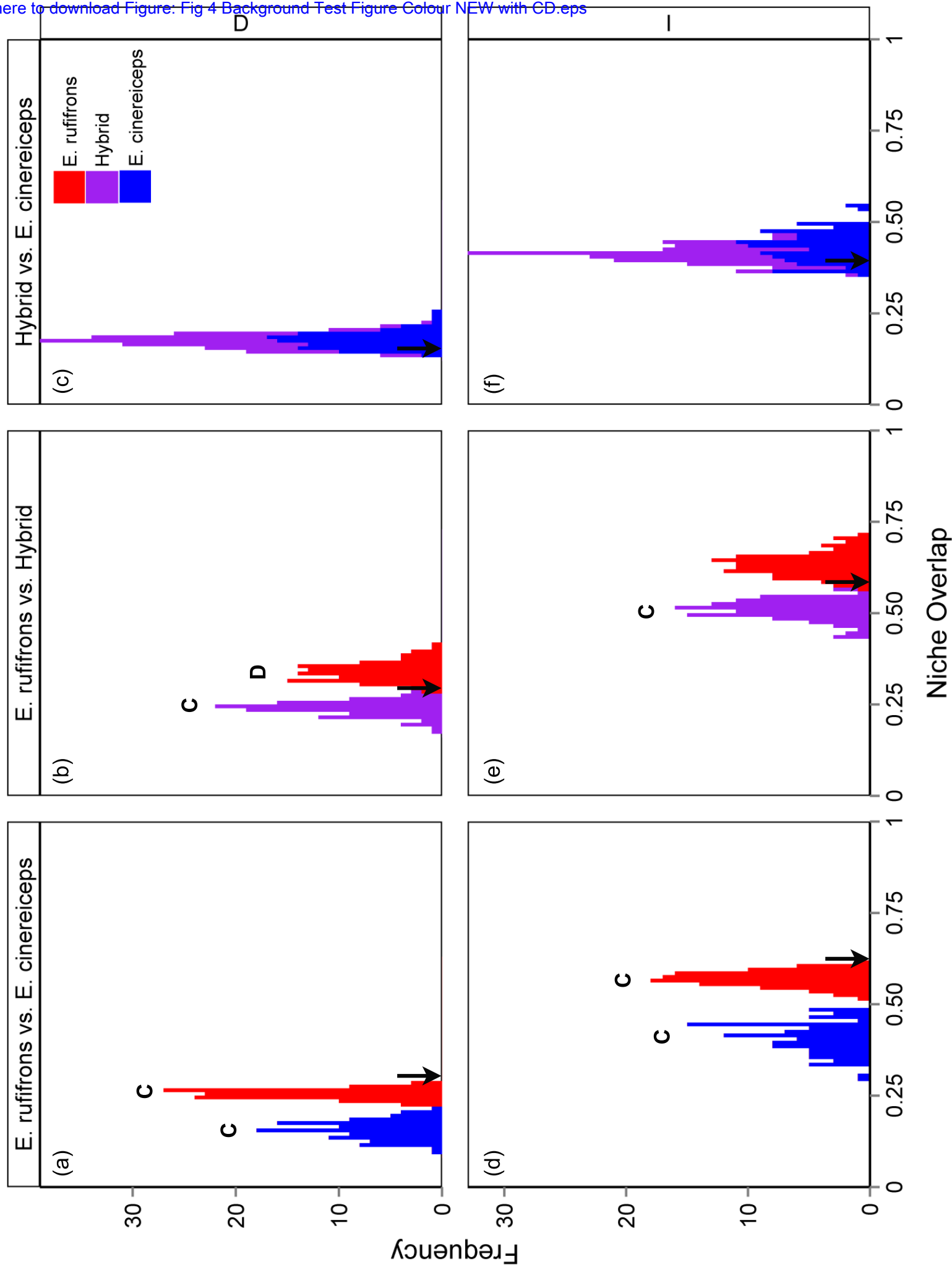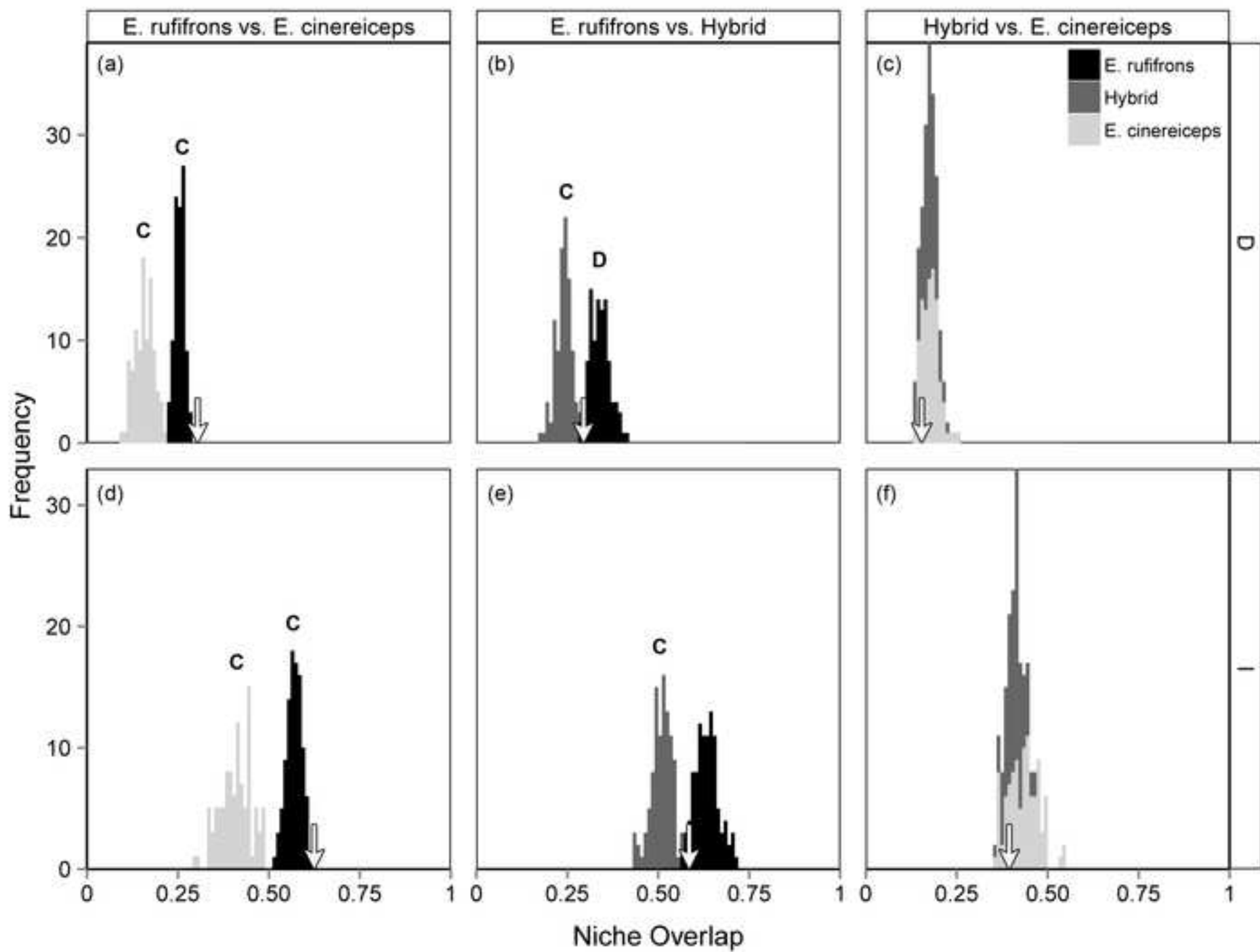
Legend

▨	*E. rufifrons*
▨	Hybrid
■	*E. cinereiceps*
☐	Other forest

0   50   100 Km

Legend

- ■ *E. rufifrons*
- ■ Hybrid
- ■ *E. cinereiceps*
- ■ Other forest

0   50   100 Km

E. rufifrons	Hybrid	E. cinereiceps
0 - 0.25	0 - 0.25	0 - 0.25
0.25 - 0.5	0.25 - 0.5	0.25 - 0.5
0.5 - 0.75	0.5 - 0.75	0.5 - 0.75
0.75 - 1	0.75 - 1	0.75 - 1

0   75   150 Km

Supplemental Materials (R code)

Click here to download Supplemental Video: R code McCormack Background Test.R