How important is sexual isolation to speciation?

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Abstract

A central role for sexual isolation in the formation of new species and establishment of species boundaries has been noticed since Darwin and is frequently emphasized in the modern literature on speciation. However, an objective evaluation of when and how sexual isolation plays a role in speciation has been carried out in few taxa. We discuss three approaches for assessing the importance of sexual isolation relative to other reproductive barriers, including the relative evolutionary rate of sexual trait differentiation, the relative strength of sexual isolation in sympatry, and the role of sexual isolation in long-term persistence of diverging forms. First, we evaluate evidence as to whether sexual isolation evolves faster than other reproductive barriers

32   during the early stages of divergence. Second, we discuss available evidence as to whether

33   sexual isolation is as strong or stronger than other barriers between closely related sympatric

34   species. Finally, we consider the effect of sexual isolation on long term species persistence,

35   relative to other reproductive barriers. We highlight challenges to our knowledge of and

36   opportunities to improve upon our understanding of sexual isolation from different phases of the

37   speciation process.

38

39   **Introduction**

40

41   How do new species arise? A simple scenario captures the basic idea about which most

42   scientists agree. A progenitor species is comprised of multiple populations across space that are

43   connected genetically. Evolutionary forces act in those populations causing changes among

44   them, and over time, some of those changes become biological barriers to gene flow. Whether

45   similar or distinct processes occur in allopatry, parapatry with gene flow, or sympatry, does not

46   affect this general scenario though the consensus is that divergence is more likely when gene

47   flow is low (such as in allopatry) or when differential selection is strong (such as during adaptive

48   radiation). While Darwin's great works (*On the Origin of Species*; *The Descent of Man and*

49   *Selection in Relation to Sex*) laid the foundations of evolutionary biology, Mayr and Dobzhansky

50   and other architects of the Modern Synthesis ushered in the study of speciation as a distinct

51   discipline. In so doing, Mayr (1942) focused attention on what many regard today as the key

52   question in speciation research: "What are these biological barriers and how do they originate?"

53   Ostensibly, barriers to gene flow that sum to total *reproductive isolation* between two species

54   include all the genetically based biological features that prevent gene flow between different

55   species or populations. During speciation, the evolution of such barriers produces distinct

56   groups of organisms each with the potential to persist as separate, demographically and

57   genetically integrated entities. Here we discuss the evidence that sexual barriers make key

58   contributions to the evolution of reproductive isolation during speciation.

59   Barriers to gene flow will usually evolve independently within each diverging lineage,

60   and while convergent evolution may occur, biological barriers will often be as unique as the taxa

61   that express them. Nevertheless, past researchers have suggested ways to conceptually organize

62   the diversity of barriers to gene flow. Most influentially, Dobzhansky (1937), listed

63 "mechanisms" of isolation (replaced in recent years with the term "barrier", to avoid the

64 assumption that isolation is their function). Beyond geographic isolation, these include so-called

65 "genotypically conditioned" barriers and are thus biological in nature, e.g. ecology and habitat

66 preference, seasonal or temporal differences in the timing of breeding, sexual incompatibilities in

67 the realm of mating behavior, reproductive morphology, gametic interactions, and postzygotic

68 incompatibilities affecting survival, fertility or mating/fertilization success of hybrids. It is also

69 now common to organize the appearance or expression of barriers along the trajectory of the life

70 cycle (e.g. Ramsey et al. 2003; Dopman et al. 2010), beginning with premating barriers, moving

71 to the postmating, prezygotic barrier phase, and culminating in postzygotic barriers, although this

72 too, can be traced to Mayr (1948, 1963).

73   Despite the diversity of barriers that might evolve between newly emerging species,

74 Mayr (1963) repeatedly argued that ethological differences (by which he explicitly means sexual

75 behaviors) are the most important factors preventing mating between closely related forms.

76 Sexual isolation, which would include such factors, is defined here as a barrier to gene flow

77 arising from divergence in prezygotic phenotypes that change reproductive interactions between

78 the sexes. This can happen due to differences in mating rituals, courtship behaviors, sensory

79 detection mechanisms and mate preferences, physical characteristics that prevent copulation and

80 the successful transfer of gametes and other mechanisms of syngamy including gamete

81 recognition phenotypes. For example, if two species of birds have different mating calls or

82 displays, they may not respond to one another as mates and therefore will not attempt to

83 reproduce. Similarly, if two species of insects express different sex pheromones or genital

84 structures, barriers to mating and insemination may result. When two populations are sexually

85 isolated, mating partners from each fail to successfully fertilize gametes, and ultimately produce

86 fewer hybrids, leading to depressed gene flow between the populations. This promotes

87 independent evolution in each population, resulting in the accumulation of additional genetic and

88 phenotypic differences. Through such mating incompatibilities, sexual isolation maintains the

89 genetic integrity of differentiated forms and contributes to the separate evolutionary trajectories

90 of different species or populations.

91   Ideas on the primacy of sexual isolation in speciation have deep roots. Indeed, Mayr

92 (1982) marshals evidence that Darwin's early position on the importance of ethological barriers

93 was similar (based on Darwin's notebooks from the 1830's), despite being more agnostic in the

Origin: "My definition of species has nothing to do with hybridity, is simply an instinctive impulse to keep separate…". In the *Descent of Man*, Darwin states that secondary sexual traits often make up the most obvious differences between closely related species. Extensive research in the ensuing decades has substantiated this claim with respect to a wide variety of sexual phenotypes, from genitalic structures to nuptial coloration to courtship signals such as pheromones or songs. Despite this, several decades of doubt about the agency of female mate choice in sexual selection and evolution followed (Richards 2017) before Fisher (1930) made an important link between the interaction of female preferences and exaggerated evolution of male traits. Although Fisher said little about its consequences for sexual isolation, Lande (1981, 1982) later explicitly modelled the Fisherian runaway process, and clearly emphasized how this could generate assortative mating between nascent species. At a conceptual level, a role for sexual selection in speciation was cemented with the seminal paper by West-Eberhard (1983). Simultaneously, other models during and since the Modern Synthesis have recruited sexual traits into the process of completing speciation (e.g. reinforcement; reviewed in Servedio and Noor 2003). Thus, a strong role for sexual isolation at multiple stages of the speciation process has developed on theoretical grounds.

Today, the assertion that sexual isolation evolves early and rapidly during lineage divergence (e.g. Brand et al. 2021; Mendelson and Shaw 2005; Turbek et al. 2021), and is necessary for species coexistence in sympatry (Gröning & Hochkirch 2008; Irwin and Schluter 2022), is not uncommon in the empirical literature on species formation. Several phylogenetic comparative studies have shown that secondary sexual traits or sexually selected mating systems are associated with elevated speciation rates (e.g. Kraaijeveld et al. 2011; Ellis and Oakley 2016), though not all such studies support the relationship, or even find a negative one (e.g. Morrow et al. 2003). Direct measures of the strength of sexual selection are correlated with species richness (Janicke et al. 2018), but overall consideration of alternative forms of reproductive isolation is often overlooked (but see Cally et al. 2021). Decisive quantitative comparative analyses of multiple reproductive barriers are still needed.

Here, we ask whether and how sexual isolation is "important" to speciation. In other words, considering recent decades of modern speciation research, is there evidence that sexual isolation has a vital role in speciation? A primary hurdle in answering such a question is to decide upon the evolutionary context in which an answer would make sense. For example, given

125 that speciation can be a long and complex process, sexual isolation may vary in its importance at

126 different points along the speciation continuum. With the complexities acknowledged (Lowry

127 2012), we therefore sought an answer to the question of whether sexual isolation is important

128 from three perspectives (Figure 1).

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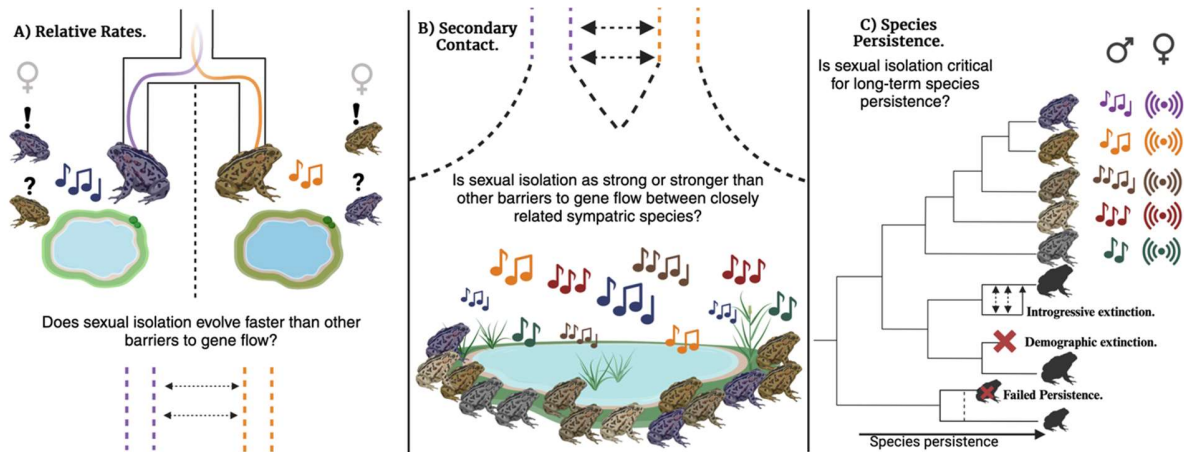
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133 Figure 1. How important is sexual isolation to speciation: three expectations. A) Rapid divergence of
134 sexual traits contributes to the early evolution of sexual isolation during species formation in a pair of
135 toads. Divergence of acoustic signals in males and female preferences for them forms a common sexual
136 barrier contributing to sexual isolation. B) Sexual isolation, along with other barriers, is directly
137 challenged in areas of species overlap; if sexual isolation is important in speciation, it should be relatively
138 strong in sympatry. C) Is sexual isolation vital to the long-term persistence of species? Long-term
139 persistence can fail for three reasons: (i) merging due to gene flow between within-species lineages
140 (termed introgressive extinction), (ii) the extinction of lineages before speciation (i.e. before reproductive
141 isolation is complete (termed demographic extinction), (iii) the extinction of lineages shortly after
142 speciation (i.e. after reproductive isolation is complete; termed failed persistence), such that species-level
143 lineages do not contribute to long term diversity patterns or have the opportunity to speciate themselves.
144 Panel C) illustrated after Dynesius and Jansson (2014).

145

146   First, we ask whether sexual isolation evolves faster than other reproductive barriers, a

147 pattern sometimes seen during the early stages of divergence. Evidence suggests that sexual

148 selection (one likely causal force underlying the evolution of sexual isolation) is strong and

149 persistent in nature (Kingsolver et al. 2001). As a result, rapid differentiation in sexual traits

150 might characterize early stages of lineage divergence, with the prediction that sexual isolation

151 evolves relatively rapidly. Secondary contact might also lead to rapid sexual trait differentiation,

152 as it can create conditions for reproductive interference (Gröning and Hochkirch 2008; Pfennig

153 and Pfennig 2009; Shuker and Burdfield-Steel 2017) or reinforcing selection (though the latter

154 works in conjunction with the existence of hybrid unfitness).

155   Second, we ask whether sexual isolation is as strong or stronger than other barriers

156 between closely related sympatric species. The efficacy of sexual isolation as a barrier to gene

157 flow in nature will be most evident in areas of overlap between very closely related species, at a

158 stage prior to any long-term persistence which may still depend on a variety of additional factors.

159 If sexual isolation is important to the coexistence of newly emerged species, its contribution to

160 overall reproductive isolation between such species should be relatively strong.

161   Third, we consider whether sexual isolation is critical for long-term species persistence.

162 Sexual differentiation and ensuing sexual isolation may occur rapidly, but other barriers alone or

163 in combination may be necessary for the persistence of new species. For example, sexually

164 isolated groups may arise frequently but not persist if they have no accompanying ecological

165 differences that enable a defensible place in a given community. The reverse might also be true,

166 i.e. sexual differences may be necessary for ecological variants to persist. Whether the evolution

167 of multiple and/or interacting barrier traits are required to establish sympatry and coexistence of

168 new species is an active area of study (Dopman et al. Ch. XX). How these factors play out may

169 depend on the demographic landscape of reproductive and/or ecological competition in the taxa

170 considered (M'Gonigle et al. 2012). Recognizing that more research needs to be done, our

171 discussion evaluates the "importance" of sexual isolation as a key form of reproductive barrier in

172 relation to other forms of isolation from these three perspectives. We highlight areas in need of

173 further research.

174

175 **I. Relative rates of evolution of different reproductive barriers**

176

177   The idea that sexual isolation evolves relatively quickly arises from both theoretical

178 predictions and empirical observations, presumably as a consequence of the rapid evolution of

179 courtship (pre-mating), mechanical (e.g. genitalic morphologies) and gamete-associated (post-

180 mating, pre-zygotic or PMPZ) traits (Garlovsky et al. Ch. XX; Matute and Cooper 2021;

181 Servedio and Boughmann 2017; Simmons and Fitzpatrick 2019). The rapid evolution of

182 reproductive traits and genes is well-documented in a variety of taxa (e.g. Arnegard et al. 2010;

183 Cooney et al. 2019; Dapper and Wade 2020; Masta and Maddison 2002; Mendelson and Shaw

184   2005; Ritchie 2007; Simmons and Fitzpatrick 2019). Moreover, a demonstration that the rate of

185   evolution of reproductive traits outpaces eco-morphological traits is growing increasingly

186   common (e.g. Arnegard et al. 2010; Friis and Milá 2020; Hersch and Moore, 2023). Notably, the

187   two animal groups with the highest recorded speciation rates (African cichlid fishes (McGee et

188   al. 2020) and Hawaiian *Laupala* crickets (Mendelson and Shaw 2005)) are both characterized by

189   rapid divergence in premating intersexual signaling.

190   In the context of relative rates of evolution of reproductive barriers, sexual isolation is

191   often estimated in mate choice trials by contrasting the frequency of conspecific versus

192   heterospecific matings; such has been the approach for many decades of research in *Drosophila*

193   speciation (Sobel and Chen 2014; Westram et al. 2022). In their seminal comparative analyses,

194   Coyne and Orr (1989, 1997) showed that sexual isolation evolved faster than postzygotic barriers

195   in *Drosophila* species pairs, and this difference was primarily driven by young, sympatric

196   species pairs (we further discuss geographic considerations below). In other animal systems,

197   Mendelson (2003) compared rates of sexual isolation and hybrid inviability in allopatric

198   populations of darter fish (*Etheostoma*), finding that sexual isolation evolved significantly faster

199   than hybrid inviability, and implicating sexual selection in driving initial divergence. A detailed

200   comparison of reproductive barriers between stickleback species pairs with variable divergence

201   times also finds evidence for faster evolving premating isolation (Lackey and Boughman 2017).

202   However, this study also demonstrates that habitat isolation and sexual isolation contribute

203   almost equally to the result of faster premating isolation, while postmating barriers contributed to

204   reproductive isolation later in the process, as revealed between older stickleback pairs. In

205   contrast, sexual isolation has been shown to evolve more slowly than postmating isolation in

206   some animal taxa. For example, in stalk-eyed flies hybrid male sterility evolves twice as fast as

207   sexual isolation (Christianson et al. 2005), while, freshwater and saltwater populations of

208   killifish (*Lucania parva*) show emergence of postzygotic isolation before any form of sexual

209   isolation (Kozak et al. 2012). Taken together, these results suggest an important, but non-

210   uniform, role for rapid sexual isolation in the early divergence and speciation of animals.

211   In plants, sexual isolation might arise from differences in 1) flowering phenology, 2)

212   pollinator specialization, and 3) PMPZ barriers (e.g. pollen-stigma interactions and pollen

213   competition; Baack et al. 2015; Garlovsky et al. Ch. XX; Haghighatnia et al. 2023). The relative

214   rate (as opposed to strength, see below) of floral-related divergence during speciation compared

215 to other sources of reproductive isolation can be difficult to disentangle, especially when there

216 may be interacting effects, e.g. pollination-related phenotypes and ecogeographic components.

217 Nevertheless, evidence suggests that floral evolution can occur rapidly upon shifts in pollinators

218 (e.g Mackin et al. 2021). Harder and Johnson (2009) argue that such traits, along with others

219 (e.g. floral timing), experience episodes of selection during diversification. Thus, we might

220 reasonably hypothesize that such divergence will have consequences for gene flow and may act

221 early in the process of divergence.

222   Two comprehensive reviews of reproductive isolation in flowering plants have compared

223 the presence and strength of pre- versus post-zygotic isolation (Baack et al. 2015; Christie et al.

224 2022). Both conclude that prezygotic isolation is approximately twice as strong as postzygotic

225 isolation, with pollinator isolation ranking highest among other different contributions to the

226 prezygotic component (more on this in the next section). A prime example of this comes from a

227 study by Ramsey et al. (2003). They showed that pollinator-mediated reproductive isolation

228 between *Mimulus* species was one of two significant contributors to total reproductive isolation

229 (the second being an ecogeographic contribution). Regarding the relative appearance (and by

230 proxy, rate) of sexual isolation, Christie et al. (2022) found that nearly three times as many taxon

231 pairs were strongly isolated *only* by prezygotic barriers, compared to postzygotic barriers alone.

232 From this we can conclude that overall, prezygotic isolation arises faster than postzygotic

233 isolation, but like animal taxa, there is also heterogeneity among plants. For example, Moyle et

234 al. (2004) analyzed patterns of post-pollination prezygotic isolation and postzygotic isolation,

235 and demonstrated that pre- and postzygotic barriers evolved at similar rates in *Glycine* and

236 *Silene*, although notably, pollinator isolation was not examined. In the nightshade genus *Nolana*,

237 PMPZ barriers evolved considerably more slowly than postzygotic isolation (Jewell et al. 2012);

238 although again, pollinator isolation was not examined. In addition, species pairs of food-

239 deceptive orchids show no evidence of increasing premating and PMPZ isolation, but do show

240 some evidence of increasing postzygotic isolation with time (Scopece et al. 2007; Sobel and

241 Randle 2009). Careful dissection of factors contributing to both premating and postzygotic

242 barriers in the California jewelflower *Streptanthus* indicated ecological differentiation may be

243 driving faster evolution of premating barriers at shallow genetic distances, rather than sexual trait

244 differentiation (Christie and Strauss 2018). In summary, sexual isolation, especially due to

245 pollinator isolation, has emerged as an important form of isolation in early stages of plant

246 divergence, with the acknowledged caveat that taxon sampling remains sparse and variation in

247 overall patterns will undoubtedly continue to emerge among taxa.

248

249 Sexual isolation, reproductive interference and reinforcement

250   During the Modern Synthesis, sexual isolation was thought to arise as a consequence of

251 the process of reinforcement. Under reinforcement, indirect selection increases sexual isolation

252 between partially differentiated forms in response to direct selection against unfit hybrid

253 offspring. That is, natural selection is said to act indirectly on incipient sexual barriers (including

254 premating barriers such as flowering time). Critically, analyses of rates of sexual isolation under

255 contrasting geographic distributions have been a productive source of evidence for

256 reinforcement. By intersecting range overlap estimates with divergence and the magnitude of

257 premating isolation in *Drosophila* species pairs, Coyne and Orr (1989, 1997) showed that

258 sympatric species pairs evolved premating isolation faster (at lower genetic divergence) than

259 allopatric pairs. In a follow-up to this study, Noor (1997) tested for reinforcement by comparing

260 the magnitude of sexual isolation in *Drosophila* species that were known to be either sympatric

261 or allopatric with an outgroup species. Sympatric species were expected to be more sexually

262 isolated with the outgroup relative to the allopatric species, as a consequence of reinforcing

263 (indirect) selection during secondary contact. Consistent with this expectation, Noor (1997)

264 found that over 21% of sympatric comparisons show higher levels of sexual isolation than

265 allopatric comparisons. Similarly, Yukilevich (2012) tested the rarer female effect prediction of

266 reinforcement (that reinforcing selection will be stronger where hybridization is more common)

267 in *Drosophila* by utilizing range overlap data and estimates of reproductive isolation. This work

268 showed that in sympatry more costly heterospecific matings are associated with greater

269 premating isolation; similar patterns were not observed in allopatry. Likewise, Yukilevich (2014)

270 showed that almost all cases of weak premating isolation occur between allopatric pairs, while

271 strong premating isolation occurs between sympatric pairs.

272   The interpretations offered in the *Drosophila* works above focus on reinforcing selection.

273 Direct selection via reproductive interference could also result in a faster evolution of sexual

274 isolation in sympatry. Reproductive interference is manifest as negative reproductive

275 interactions between different species (e.g. competition for signaling locations or signaling

276 channels, and any consequential interactions; Gröning and Hochkirch 2008; Pfennig and Pfennig

277   2009; Shuker and Burdfield-Steel 2017), but could arguably also occur between incipient species

278   upon secondary contact. Like reinforcement, reproductive interference also results in

279   reproductive character displacement and thus sexual isolation, though it is often thought a less

280   likely explanation in *Drosophila* due to the typically low genetic divergence and incomplete

281   postmating isolation between focal pairs (Coyne and Orr; 1989, 1997) Alongside reproductive

282   interference, resource-based competition and other ecological processes might also explain faster

283   evolution of sexual isolation in sympatry or parapatry compared to allopatry, for example if

284   magic traits are involved (Kondrashov and Kondrashov 1999, Funk et al 2006). Magic traits are

285   traits that experience divergent selection and as a consequence, give rise to non-random mating.

286   Rapid evolution of sexual isolation arising from divergence in body size, a magic trait, in an

287   experimental evolution study of feather lice shows such potential (Villa et al. 2020). However, a

288   role for ecology and/or magic traits was undermined by a re-analysis of Coyne and Orr's (1989,

289   1997) study, which found no clear link between ecological differentiation, premating isolation

290   and sympatry (Turelli et al. 2014).

291   Other rate tests involving changes in geographical status of incipient species have

292   explored the importance of sexual isolation over time. For example, a meta-analysis by

293   Gourbière and Mallet (2010) showed that sympatric *Drosophila* and *Leptasterias* pairs fit a

294   'slowdown' model, compared to a 'snowball' or linear model, of reproductive isolation, possibly

295   due to contributions of sexual isolation. 'Snowball' models of the evolution of incompatibilities

296   are expected to show an accelerating accumulation between species, if mutations have many

297   epistatic interactions. However, model fitting suggested that the rate of increase of prezygotic

298   incompatibilities declined in these groups. Gourbière and Mallet (2010) offer the interpretation

299   that once strong assortative mating evolves during initial divergence, there is little 'need' to

300   further strengthen sexual barriers, resulting in a slowdown of sexual isolation with an increasing

301   emphasis on postmating barriers to complete species divergence. Such patterns and

302   interpretations are consistent nonetheless with the early and rapid evolution of sexual isolation in

303   secondary contact, involving a strong selective advantage to assortative mating in zones of

304   sympatry in very early stages of overlap.

305   Available evidence then, does support the idea that sympatry impacts the rate of

306   evolution of sexual isolation. As there are open questions about what causes this change in rate,

307   the impact of changing geography of emerging species remains a necessary area of future

308 investigation. While comparative data fit with the idea that reinforcement is needed to 'complete'
309 sexual (or more generally, prezygotic) isolation, plausible alternatives exist. Sexual isolation
310 arising as a by-product of intense sexual selection diversifying mating systems is one such
311 mechanism, one which should be strong in any geographic mode. Likewise, sexual isolation may
312 increase as a by-product of ecological character displacement, or other consequences of
313 sympatry in the absence of hybridization (Pfennig and Pfennig 2009; Mendelson and Safran
314 2021). Regarding evidence involving geographic range comparisons, we must be cautious of
315 historical inference. For example, the 'differential fusion' argument has been proposed
316 (Templeton 1981), that species can only become sympatric where sexual isolation has previously
317 evolved, creating a bias in evolutionary outcomes toward elevated instances of sexual isolation in
318 sympatry. However, the observation that similar levels of sexual isolation are rarely if ever seen
319 between allopatric species (e.g. Coyne & Orr 1989, 1997) at comparably low levels of
320 divergence counters this idea. In addition, whether range overlap estimates accurately capture
321 speciation histories remains an open question (Losos and Glor 2003; Fitzpatrick et al. 2009;
322 Butlin et al. 2008). Genomic investigations lead Peñalba et al. (2019) to question the relationship
323 between contemporary biogeography and historical biogeography; additional comparative
324 studies incorporating genomic analyses will be helpful to further test this assumption. It is likely
325 that simple, discrete categories of allopatry and sympatry do not capture complex, dynamic
326 processes in speciation.
327
328 **II. The importance of sexual isolation in sympatric species: is it the strongest barrier to**
329 **gene flow?**
330
331   Another way to assess the "importance" of sexual isolation is to evaluate whether sexual
332 isolation is the strongest barrier to gene flow between sympatric species. Studies that measure
333 and directly compare the strengths of multiple reproductive barriers in one or more pairs of
334 sympatric species are well-suited to address this question. In such studies, the strength of
335 reproductive barriers is typically measured on a standardized scale (for example from 0 - 1) (e.g.,
336 Sobel & Chen 2014; Ramsey et al. 2003), such that their magnitudes are directly comparable. An
337 open question is whether such a scale represents comparable effects on gene flow for different
338 reproductive barriers. Sobel & Chen (2014) suggest that the relationship between barrier strength

339 and the consequences for gene flow varies by reproductive barrier, and is not necessarily linear.

340 However, given a growing number of studies that use these metrics and draw conclusions about

341 the importance of reproductive barriers in sympatry, it is instructive to consider the results.

342   At least three substantive reviews compile data from multiple studies to compare the

343 strength of different reproductive barriers, in fungi (Le Gac & Giraud 2008), seed plants

344 (Christie et al. 2022), and *Drosophila* (Yukilevich 2012). Based on these analyses, sexual

345 isolation is, on average, the strongest barrier to gene flow for sympatric species in most groups.

346 The exception is the fungal group Ascomycota, in which the overall trend suggests that

347 postzygotic barriers are stronger than prezygotic barriers for sympatric species, though both are

348 generally weak. Authors suggest this may be due to their particular lifestyle, characterized by

349 gametes with low dispersal abilities and thus little physical contact among heterospecifics.

350 Within the larger group of fungi examined by Gac & Giraud (2008) (the Homobasidiomycota),

351 prezygotic barriers--measured as evidence of "clamp connections" (fungal mating)--were the

352 only barriers reported. However, since they were typically complete (RI = 1), and stronger for

353 sympatric compared to allopatric species, it may be safe to conclude that sexual isolation is the

354 strongest barrier to gene flow for sympatric species in this group.

355   For seed plants, authors compiled data for mostly sympatric species pairs from 31

356 families and reported the strength of a dozen unique reproductive barriers (Christie et al. 2022).

357 Data suggest that floral isolation (mean RI = 0.61), specifically via pollen deposition (mean RI =

358 0.72) is the strongest barrier to gene flow. The barrier with the next greatest strength was

359 ecogeographic isolation (mean RI = 0.59), while the strength of postzygotic barriers was

360 significantly lower (mean RI = 0.068 - 0.333).

361   For *Drosophila*, Yukilevich (2012) reported the strength of sexual isolation, measured as

362 copulation success, and postzygotic isolation, measured as a composite of hybrid sterility and

363 viability, for 73 sympatric species pairs. The strength of sexual isolation was, on average, clearly

364 larger (mean RI = 0.82) than that of postzygotic isolation (mean RI = 0.47).

365   These comprehensive reviews thus lend support to the idea that sexual isolation plays an

366 important role in reducing gene flow between currently sympatric species. This conclusion is not

367 without caveats. For both *Drosophila* and the fungal group Homobasidiomycota, no data were

368 presented to assess the strength of ecological barriers. For the seed plants, a comprehensively

369 wide range of reproductive barriers were measured, but the data set was dominated by plants

370 with showy flowers. Additionally, ecological and sexual barriers were markedly similar in

371 strength, which is to say, sexual isolation is not the only strong barrier. Thus, whether ecological

372 differentiation is necessary in addition to sexual isolation to substantially limit gene flow

373 between sympatric species, requires additional data from additional taxa.

374   In the absence of further broad-scale taxonomic comparisons, some case studies are

375 potentially informative about the relative strengths of different types of barriers. For example,

376 the effects of ecological barriers, in addition to sexual and post-zygotic isolation, have been

377 quantified for several species-pairs of *Heliconius* butterflies that are sympatric over broad

378 geographical areas (Garzón-Orduña et al. 2018, Mérot et al. 2017, Rosser et al. 2019).

379 Comparing barriers in four sympatric species-pairs spanning the melpomene-cydno clade, pre-

380 mating sexual isolation was reported to be the strongest and nearly complete (average RI= 0.93)

381 followed by ecological isolation (i.e., habitat preference, average RI = 0.69). Post-zygotic

382 barriers were generally weaker, with average RI ranging from 0 to 0.44 (Garzón-Orduña et al.

383 2018, Mérot et al. 2017). In another study, sexual isolation via mate choice between sympatric

384 *Heliconius elevatus* and *H. pardalinus butleri* was complete (RI = 1), while ecological isolation

385 via host plant preference and measured post-mating barriers were weak (RI = -0.04 – 0.06)

386 (Rosser et al. 2019). However, an assessment of micro-habitat differences between species,

387 characterized by annual mean temperature and rainfall, showed *H. p. butleri* inhabiting a much

388 narrower climatic niche than *H. elevatus* (Rosser et al. 2019), suggesting potential for isolation

389 via micro-habitat preference in this species-pair. With the potential for heterogeneous patterns,

390 clearly more data and larger sample sizes are needed across taxa.

391   Importantly, our discussion above draws attention to data on the absolute contribution of

392 sexual isolation as a barrier to gene flow. However, the importance of sexual isolation may be

393 accentuated due to its position in the sequence in which reproductive barriers act. From the

394 initial point of contact between interacting species, sexual barriers act first, reducing or

395 neutralizing the impact of later-acting postzygotic barriers (Ramsey et al. 2003; Sobel et al.

396 2010). For example, in the species pair *Heliconius erato chestertonii* and *H.e. venus*, the strength

397 of sexual isolation due to color pattern preference differences, measured individually on a scale

398 of zero to one, is estimated at 0.57 and the strength of hybrid viability at 0.56. However, the

399 "absolute contribution" of sexual isolation (taking sequential filtering effects into account) is

400 0.87 and for hybrid viability is 0.07 (Muñoz et al. 2010).

401   We note that case studies of other kinds may be useful in teasing apart ecological versus

402 sexual isolation factors. For example, two species of reed frog in São Tomé differ in habitat

403 preferences, body size, coloration, and advertisement call, yet only hybridize where their

404 preferred habitats intersect, suggesting the importance of ecogeographic factors in maintaining

405 species boundaries (Bell & Irian 2019). Two species of land snail on the island of Anijima in

406 Japan differ in habitat preferences, genitalia, and shell morphology; they too hybridize in the

407 transition zones between their preferred habitats (Chiba 1998). In these cases, the strength of

408 reproductive barriers was not quantified, but such observations suggest that habitat

409 differentiation prevents species from coming into contact. When habitat distinctions break down,

410 sexual differences are insufficient to prevent hybridization and gene flow. In summary, different

411 approaches and case studies are informative, but a careful answer to the question of whether

412 sexual isolation generally forms an important barrier to gene flow between sympatric species

413 will require estimates of the strength of more reproductive barriers from more species pairs with

414 broader taxonomic representation.

415

416 **III. What is the role of sexual isolation relative to other reproductive barriers in long term**

417 **persistence?**

418

419   Viewed from a macroevolutionary perspective, speciation involves the splitting of one

420 lineage into two or more independent lineages, followed by their persistence over evolutionary

421 time (Fig. 1). To generate a comprehensive understanding of the origin of new species in a

422 macroevolutionary sense requires study of different factors that promote persistence and build-up

423 of species diversity. In this section, we discuss the importance of sexual isolation relative to

424 other reproductive barriers in promoting the long-term persistence of incipient and newly formed

425 species, and in relation to other factors (e.g. ecological differentiation, demographic factors). We

426 note that while some potential consequences of sexual selection - a major cause of sexual

427 isolation - may influence species persistence (Kokko and Brooks 2003), we concentrate on

428 sexual isolation *per se* and its importance to lineage persistence.

429

430 Persistence as an important constraint on speciation rates

431   Long-term persistence can be defined simply as the maintenance of populations through

432   time (Harvey et al. 2019). In the context of speciation, divergent within-species populations

433   ('incipient species') can fail to persist either because reproductive isolation is incomplete and

434   gene flow causes them to merge back into a single population, or because all members of a

435   population die out (Fig. 1), i.e., 'introgressive extinction' and 'demographic extinction',

436   respectively (Rudman and Schluter 2016). The failure of incipient species to persist due to either

437   of these mechanisms represents a potentially important constraint on speciation rates and species

438   diversity (Dynesius and Jansson 2014; Harvey et al. 2019; Tobias et al. 2020).

439   In addition to the persistence of incipient species, failed persistence of species-level

440   lineages shortly after the completion of speciation (Fig. 1) can also place constraints on

441   speciation rates and species diversity. The idea that new species frequently form but almost

442   never persist can be traced back to Mayr (1963) and is now referred to as 'ephemeral speciation'

443   (Rosenblum et al. 2012). As with the survival of divergent populations ('population

444   persistence'), the ability of newly formed species to persist over long time frames ('species

445   persistence') may place a strong limit on effective speciation rates. Indeed, a high rate of

446   extinction of newly formed species potentially explains large discrepancies between speciation

447   rates measured at microevolutionary and macroevolutionary scales (Rosenblum et al. 2012;

448   Rabosky 2016).

449

450   The role of sexual isolation in population persistence

451   Sexual isolation theoretically can play an important role in several processes linked to

452   lineage survival and persistence. On the one hand, by effectively reducing the probability of

453   mating and gene flow, and thus the risk of genetic introgression, sexual isolation may promote

454   the persistence of diverging populations in the early stages of speciation (Dynesius and Jansson

455   2014; Tobias et al. 2020) (Fig. 1). Similarly, by reducing the probability that individuals from

456   related populations engage in costly reproductive interactions, either before or after the evolution

457   of complete reproductive isolation (Fig. 1), sexual isolation may reduce the probability of

458   demographic extinction and promote the maintenance of viable populations (Gröning and

459   Hochkirch 2008). On the other hand, in some cases sexual isolation may *promote* population

460   extinction (i.e. *reduce* persistence) if, for example, sexual isolation limits effective population

461 size and/or prevents potentially adaptive gene flow into small populations (Dynesius and Jansson
462 2014). Thus, we might expect the role of sexual isolation in long-term population persistence to
463 reflect a balance between the costs and benefits of reduced reproductive interactions with other
464 closely related species.

465

466 Evidence for the effects of sexual isolation on population persistence

467   Studying population persistence and extinction directly in natural systems is difficult,
468 given the large temporal and spatial scales of investigation required (Harvey et al. 2019). As a
469 result, theoretical studies comprise the main source of insight into the relative importance of
470 sexual isolation for promoting population persistence in the context of speciation. However,
471 relevant insights from empirical studies are increasing and come from a range of different
472 sources (e.g. experiments, genomic analyses and phylogenetic comparative studies).

473   In terms of theoretical work, a recent simulation study (Irwin and Schluter 2022)
474 investigated persistence outcomes for sympatric species pairs under different scenarios of
475 reproductive isolation and ecological differentiation. Importantly, the authors separately modeled
476 the roles of prezygotic (sexual) isolation and postzygotic isolation in their study. An important
477 finding was that sexual isolation is generally required for ecologically differentiated populations
478 to stably coexist in sympatry. Specifically, when sexual isolation in the form of conspecific mate
479 preference is absent or weak between incipient, sympatric species, they tend to merge
480 (introgression extinction) when hybrid fitness is high, or only one persists (demographic
481 extinction) when hybrid fitness is low. Together, these results imply that postzygotic isolation
482 alone generally does not lead to the persistence of incipient species in sympatry. Rather, the
483 evolution of sexual isolation is likely a crucial step in promoting the persistence of populations
484 and young species in sympatry, particularly by preventing population merging (Irwin and
485 Schluter 2022).

486   In support of this, the absence of complete sexual isolation between lineages has been
487 implicated in several instances of failed lineage persistence in nature. Many examples concern
488 instances of 'speciation collapse' or 'speciation reversal'. In such cases, formerly distinct
489 populations/species (e.g. benthic and limnetic sticklebacks; alternative color morphs of African
490 cichlids; Darwin's finches) have merged via hybridization in sympatry due to the lack of

491 persistent sexual isolation among individuals from different populations (e.g. Seehausen 1997,
492 2006; Kleindorfer et al. 2014; Lackey & Boughman 2017). While introgression can be
493 sometimes be adaptive, the study of invasive species has revealed several cases where genetic
494 introgression and/or costly reproductive interactions in sympatry have seemingly caused severe
495 population declines and local extinctions in native species, primarily as a result of incomplete
496 sexual isolation between native and invasive taxa (Rhymer and Simberloff 1996; Wolf et al.
497 2001). In addition to the negative effects of hybridization and genetic introgression between
498 incompletely isolated taxa, reproductive interference between fully reproductively isolated taxa
499 has also been proposed as an important constraint on persistence and coexistence in some cases
500 (Gröning and Hochkirch 2008). Indeed, there are several empirical examples of reproductive
501 interference between related species causing negative fitness, population declines and apparent
502 sexual exclusion of taxa from certain habitats (e.g. Carrasquilla and Lounibos 2011; Hochkirch
503 et al. 2007; Mikkelsen and Irwin 2021). In both cases (i.e. interactions between incompletely or
504 completely reproductively isolated taxa) negative persistence consequences stem from
505 overlapping preferences/recognition mechanisms for conspecific and heterospecific individuals,
506 highlighting the potentially long-lasting effects of incomplete sexual independence for the
507 persistence of related species in sympatry.

508 While the available evidence suggests that sexual isolation may be a crucial step for the
509 persistence of incipient or even fully reproductively isolated species in sympatry, a broader issue
510 concerns the extent to which sexual isolation (and reproductive isolation) represents the primary
511 control in determining rates of lineage persistence. For example, courtship traits may evolve
512 rapidly in many taxa (e.g. birds:Turbek et al. 2021; fish: Seehausen et al. 2008; insects:
513 Mendelson and Shaw 2005; see above), leading to a rapid evolution of sexual isolation, but in
514 such cases it is possible that factors other than reproductive isolation between lineages represent
515 the 'rate-limiting step' determining persistence rates in young taxa. For example, ecological
516 differentiation and demographic resilience have both been suggested as primary controls on
517 persistence rates of populations during speciation (Rabosky 2016; Harvey et al. 2019; Tobias et
518 al. 2020). These factors can be disentangled from alternatives particularly if differentiating
519 populations never meet (i.e. remain allopatric) and therefore never face the possible
520 consequences of interacting reproductively.

521   Generally speaking, most theoretical investigations (e.g. Chesson 2000; Mittelbach and

522   McGill 2019; Germain et al. 2021), find that some degree of ecological differentiation is

523   necessary for species to stably coexist (but see M'Gonigle et al. 2012). This is also the case in

524   Irwin and Schluter's (2022) analysis, where the significance of sexual isolation for promoting

525   population persistence was contingent on the extent of ecological differentiation between

526   populations. Similarly, other studies of population survival in the context of speciation have

527   emphasized the importance of demographic factors in promoting population persistence (Harvey

528   et al. 2019). For example, simulation work has demonstrated the importance of intrinsic

529   population growth rate (r) in influencing the outcome of contact between two sexually

530   interacting populations (Liou and Price 1994; Irwin and Schluter 2022). Taken together, the

531   available evidence suggests that strong sexual isolation between incipient species is necessary

532   but not sufficient for promoting long term persistence, if and when incipient species occur in

533   sympatry. Sexual isolation, therefore, emerges as a more specific explanation for the general

534   view that reproductive isolation between incipient species is an essential component for the

535   transition to sympatry. Long term persistence likely also depends on additional demographic

536   (M'Gonigle et al. 2012) or ecological factors including niche differentiation (Cooney et al. 2017;

537   Tobias et al. 2020).

538

539   Conclusion and future directions

540

541   The synthesis provided here leads to the enticing conclusion that sexual isolation does

542   play a central role in speciation. Available evidence points to the relatively rapid evolution of

543   sexual isolation, the relatively strong contribution of sexual isolation to reproductive isolation,

544   especially in sympatry, and the importance of sexual isolation in species persistence. These

545   conclusions are not without caveats and sometimes contradictory patterns, and further research is

546   needed to both strengthen this conclusion and identify heterogeneity across different taxonomic

547   groups and demographic histories. To gain further insight into the relative importance of

548   different sexual isolating barriers, we require a great deal more information about the rate and

549   order of appearance of these reproductive barriers, the relative strength of effect of different

550   modes of reproductive isolation, and insight into the long-term persistence of incipient and

551   recently formed species as a function of phenotypic divergence. Such studies will require both

552   comparative analyses and more research on relatively understudied taxa. Future research should

553   therefore address the uneven phylogenetic distribution of information about these three areas,

554   each of which provides a perspective on the importance of sexual isolation to speciation. In

555   addition, critical areas identified below and questions identified in Box 1 will benefit from

556   further research.

557

558   Box 1 here

559

560   First, it is vital to demonstrate when or if there is always a causal connection between

561   rapidly diverging sexual traits and sexual isolation. Such a demonstration would further support

562   a correspondence between rapid divergence in sexual traits and the early appearance of sexual

563   isolation during speciation, as suggested in a few taxa to date (e.g. birds: Campagna et al. 2017;

564   Turbek et al. 2021; fish: Martin and Mendelson 2014a, b; Martin and Mendelson 2016;

565   fruitflies: Higginson et al. 2012; Manier et al. 2013; crickets: Mendelson and Shaw 2005; Oh

566   and Shaw 2013; damselflies: Barnard et al. 2017; Wellenreuther, M. and R. A. Sanchez-Guillen

567   2015; Sánchez-Guillén et al. 2014; Wellenreuther, M. and R. A. Sanchez-Guillen 2015). This

568   connection is not a foregone conclusion. For example, courtship traits in the *willistoni* group of

569   *Drosophila* have evidently diverged even faster than sexual isolation (albeit, considerably faster

570   than postmating isolation; Gleason and Ritchie 1998). Although sexually dimorphic colors

571   diverge quickly in birds and other animals (Cooney et al. 2019; Seddon et al. 2013), comparative

572   analyses leave open multiple potential explanations for this pattern, female preference leading to

573   mate choice being just one potential driver of male color evolution. For example, competition for

574   nesting sites may influence male coloration in tropical birds. Moreover, elaboration in females

575   can contribute to sexual dimorphism as well, for example due to female-female competition in

576   cooperatively breeding birds (Dale et al. 2015), and due to competition among females for male

577   nuptial gifts in some insects and spiders (Murray et al. 2020). Thus, courtship displays and

578   sexual dimorphism can diverge rapidly, but not always due to female choice, or necessarily

579   resulting in sexual isolation (e.g. Watts et al. 2019).

580   Second, by regressing genetic distance against different measures of reproductive

581   isolation, meta-analyses have shown that different isolating barriers show variable rates of

582   evolution with time, and in different geographic contexts. Recent years have thus shown an

583   increasing support for reinforcement by such studies. But as population and phylo-genomic data

584   have increased, so has our appreciation for complex species histories. These large genomic data

585   sets are yielding profound insights into past species ranges and the potential for behavioral,

586   ecological and genomic interactions among incipient species, enabling better inferences about

587   introgression and processes such as reproductive interference, reinforcement and interpretations

588   of reproductive character displacement. New creative tests of reinforcement versus reproductive

589   interference, ecological divergence, differential fusion or other complex speciation histories,

590   contrasting current and historic gene flow in a diversity of taxonomic groups, will be necessary

591   to further strengthen our understanding of when and how sexual barriers are causally involved in

592   speciation.

593   Third, more studies designed to measure the relative strength of reproductive isolation are

594   vital to firm conclusions about sexual isolation. We found that the current slate of studies (not

595   all discussed here) is surprisingly small and taxonomically narrow, which almost certainly

596   speaks to the difficulty in conducting such research. Additional detailed studies from a broader

597   range of taxa will be needed to accommodate heterogeneity in the importance of sexual isolation.

598   The characterization of sexual isolation in relation to other reproductive barriers (e.g. Dell'Aglio

599   et al. Ch. XX; Thompson et al. Ch. XX; Reifová et al. Ch. XX) is particularly important given its

600   apparent involvement in multiple modes and mechanisms of species divergence, for example

601   speciation by sexual selection in allopatry, 'magic trait' speciation, reinforcement (Yukilevich et

602   al. Ch. XX) and other forms of reproductive coupling (Dopman et al. Ch. XX; Aubier et al. Ch.

603   XX; Ritchie and Butlin Ch. XX). Under all of these mechanisms, it will be necessary to

604   understand the connection between measured reproductive barriers and their relative impacts on

605   gene flow (Sobel and Chen 2014).

606   Developing more multidisciplinary approaches will allow us to fairly address the role of

607   sexual isolation (indeed, any form of isolation) during speciation. Dobzhansky (1951) and early

608   proponents of reinforcement theory specifically suggested that sexual isolation is strengthened in

609   secondary contact as an indirect response to selection against hybrids, indicating an important

610   role for sexual isolation in completing speciation, following its initiation via postzygotic

611   incompatibilities. Since that time, as we have highlighted here, sexual isolation has been shown

612   to play a more centrally vital role in speciation, by virtue of the rapid rate of evolution of

613   characters important in sexual isolation, through sexual or natural selection, or both acting in

614 concert. This can occur in any geographical context, but we need more decisive studies of their
615 consequential effects on sexual isolation, and the likelihood of lineage persistence.
616

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