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1 Detecting the true extent of introgression during anthropogenic hybridization

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3

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8

## 9 **Abstract**

10 Hybridization among naturally separate taxa is increasing due to human impact,  
11 and can result in taxon loss. Previous classification of anthropogenic  
12 hybridization has largely ignored the case of bimodal hybrid zones, in which  
13 hybrids commonly mate with parental species resulting in many backcrossed  
14 individuals with a small proportion of introgressed genome. Genetic markers can  
15 be used to detect such hybrids, but until recently too few markers have been  
16 used to detect the true extent of introgression. Recent studies of wolves and  
17 trout have used thousands of markers to reveal previously undetectable  
18 backcrosses. This improved resolution will lead to increased detection of late  
19 generation backcrosses, shed light on the consequences of anthropogenic  
20 hybridization, and pose new management issues for conservation scientists.

21

## 22 **Anthropogenic hybridization**

23 **Anthropogenic hybridization** (see Glossary), in which human disturbance  
24 leads to range overlap and **hybridization** of previously reproductively isolated  
25 populations or species is a growing conservation concern [1-3]. With increased  
26 human-generated movement of species into new ranges, there is an increasing  
27 number of cases of hybridization between species that were historically  
28 **allopatric** [4]. Disturbance of habitats can also result in a breakdown of  
29 reproductive isolation between previously isolated, **sympatric** species [1].  
30 **Introgression** is usually hard to detect from phenotypes and there is growing  
31 evidence that backcrossing has often proceeded further than is detectable by low  
32 density genetic marker panels. In this article we make the case that genomic  
33 approaches are essential and increasingly available to disentangle late  
34 generation backcrosses from parental populations after introgression has  
35 occurred.

36

## 37 **The benefits of anthropogenic hybridization**

38 There are possible benefits of anthropogenic hybridization. Policy makers can  
39 use hybridization as a management tool to help endangered populations. In  
40 'genetic rescue' programs (i.e. breeding programs designed to release small  
41 populations from inbreeding depression), individuals from a closely related  
42 population or subspecies are introduced to an inbred population to manage  
43 inbreeding depression. For example, when Florida panthers (*Puma concolor*  
44 *coryi*) were threatened due to inbreeding depression, eight Texas panthers (*P.*  
45 *concolor cougaur*) were introduced. The **hybrid** kittens survived better, and the  
46 population is now recovering [5]. Approximately 90% of such genetic rescue  
47 attempts have been successful, showing that anthropogenic hybridization is a  
48 viable conservation method [6]. Adaptive introgression ('evolutionary rescue') in  
49 which beneficial alleles from an introduced population are selected for in hybrid  
50 individuals is another possible benefit of anthropogenic hybridization. For  
51 example, a segment of chromosome 15 that has naturally introgressed from  
52 *Populus balsamifera* into *P. trichocarpa* appears to allow *P. trichocarpa* to live in  
53 colder, drier areas than *P. trichocarpa* individuals without this haplotype [7].  
54 This suggests that there is potential for adaptive introgression to facilitate

55 evolutionary rescue of populations at risk of extinction due to climate change [8],  
56 although such genomic management of at risk populations much enabling  
57 research, and should be approached with caution [9, 10].

58

### 59 **The problems with anthropogenic hybridization**

60 Anthropogenic hybridization can cause problems for native species. When no  
61 offspring or sterile offspring are produced, reproductive effort is wasted [11].  
62 When fertile F1s are formed, introgression between the two previously diverged  
63 species is possible. There are two reasons why even low levels of introgression  
64 of non-native alleles are of concern from a conservation perspective. First, if all  
65 individuals of a species are hybrids then the species as it was is extinct. This has  
66 been termed 'extinction by hybridization' [11-15]. Note, however, that there may  
67 still be many copies of the native alleles represented in the population, so long as  
68 the population itself is large enough, and from a 'gene view point' we may be  
69 content with this mode of conservation [16].

70

71 The second problem with hybridization is that introgression and recombination  
72 break up linked gene complexes, and non-native alleles that are favoured (or no  
73 longer in linkage with deleterious alleles) can be swept to fixation [17]. While  
74 this leads to an initial increase in biodiversity (because alleles from both the  
75 native and non-native populations are present) as non-native alleles sweep to  
76 fixation, native alleles are lost. If we again take a gene view point of biodiversity,  
77 any alleles lost from the native population are a loss in biodiversity from the  
78 system. For example, non-native alleles at three out of 68 genetic markers have  
79 gone to fixation in some populations of California Tiger Salamanders  
80 (*Ambystoma californiense*) after hybridization with Barred Tiger Salamanders (*A.*  
81 *mavortium*) [18]. This has occurred in California Tiger Salamander populations  
82 that are nearly 100km from the original Barred Tiger Salamander introduction  
83 site, suggesting that these alleles have higher fitness than the native, California  
84 Tiger Salamander alleles that they have replaced [18].

85

### 86 **Goals of studies of anthropogenic hybridization**

87 Studies of anthropogenic hybridization have different goals. A researcher might  
88 be interested to know if hybridization has occurred at all in a population to  
89 determine whether it should provide the breeding stock for new populations,  
90 and or whether it should be quarantined because of hybridization. Relatively few  
91 informative markers are needed to detect individuals of hybrid origin in any  
92 particular population, as the detection of any non-native allele is a clear  
93 indication of hybridization [19].

94  
95 However, if a researcher wishes to understand more about the underlying  
96 process of hybridization and introgression, then many more markers are  
97 required. Specific goals might include: to select individuals for breeding  
98 programs; to understand the relationship between genotype and phenotype; to  
99 understand the type of hybrid system involved (see next section); and to  
100 investigate mating patterns and fitness. For any of these goals, it is ideal to  
101 quantify individual **admixture** accurately, and to do this this hundreds or  
102 thousands of informative markers may be required (see below).

103

#### 104 **Classifying hybridization**

105 To assist researchers and policy makers in addressing anthropogenic  
106 hybridization, Allendorf and colleagues [11] categorized hybridization outcomes.  
107 Types 1-3 applied to naturally-occurring hybridization while Types 4-6 applied  
108 to anthropogenic hybridization. Type 4 results in few or sterile F1 hybrids, and is  
109 characterized by wasted reproductive effort. Type 5 results in a **hybrid swarm**  
110 with widespread introgression into particular populations, but some populations  
111 do not experience hybridization at all. Finally, Type 6 results in a complete  
112 hybrid swarm following break down of reproductive isolation between species  
113 across all populations [11].

114

115 Three axes of variation determine the outcome of anthropogenic hybridization:  
116 differences in hybrid fitness, time since **secondary contact**, and mating patterns  
117 of hybrids. Time since secondary contact and mating patterns of hybrids were  
118 not explicitly considered in Allendorf et al's original categorization. Type 4  
119 differs from Types 5 and 6 along an axis of hybrid fitness, where intrinsic post

120 zygotic isolation affects hybrids in Type 4, but not in Types 5 or 6. This results in  
121 little to no backcrossing in Type 4 hybrid zones, as hybrids are extremely unfit  
122 compared to parental species. This decrease in hybrid fitness must be extreme,  
123 as even with a 90% decrease in fitness, the proportion of hybrids in a hybridizing  
124 population is expected to increase [20].

125

126 We suggest that the only difference between Allendorf et al's [11] Type 5 and  
127 Type 6 is time since secondary contact. When an F1 reproduces, all of its  
128 offspring and descendants are admixed to some extent [20]. If Type 5  
129 characterizes a system where only one or few populations have introgression,  
130 Type 6 is the logical outcome of this same system, assuming random mating and  
131 sufficient time for migration between populations. Thus, we consider Type 5 and  
132 Type 6 to be the same, both hybrid swarms with a breakdown of assortative  
133 mating, in which hybrids have the same mating success as either of the parental  
134 species individuals, and common enough that hybrid x hybrid matings occur.

135

136 When there is a preference among hybrids for parental species phenotypes, or  
137 hybrids are very rare, we expect a different pattern of introgression.

138 Backcrossing into the parental species leads to an increasingly large number of  
139 individuals with a small proportion (<10%) of their genome that is from the  
140 opposite species. As backcrossing continues, morphological differences between  
141 parental species and backcrossed individuals lessen, making it more and more  
142 difficult to detect a backcross using only phenotypic traits. This results in many  
143 hybrid individuals with very small proportions of another genome, although  
144 with a maintained bi-modal distribution of trait values between the two parental  
145 species (Figure 1). From a conservation perspective we consider this to be a  
146 worst-case scenario as these introgressed individuals are very difficult to detect.  
147 This can be contrasted with a general lack of assortative mating, in which hybrid  
148 individuals are as likely to breed with other hybrid individuals as with parental  
149 species (leading to a hybrid swarm), or, in the unlikely event of true assortative  
150 mating, where hybrid individuals preferentially breed with each other, which  
151 would lead to the eventual formation of a hybrid species e.g. [21]. The contrast  
152 between hybrid zones with unimodal distributions of traits and admixture

153 scores and those with bimodal distributions has previously been described in the  
154 context of naturally occurring hybrid zones [22], but does not yet seem to inform  
155 studies of anthropogenic hybridization.

156

157 The distribution of hybrid scores in a system at equilibrium varies depending on  
158 ecological factors that can affect hybrid fitness, and hybrid encounter rate.

159 Extrinsic post zygotic isolation can vary according to ecological factors, affecting  
160 the ability of hybrids to successfully mate and reproduce [23]. Further, stochastic  
161 factors, particularly when hybrids are rare, or management might alter the  
162 reproductive success of hybrid individuals in wild systems. However, if hybrids  
163 are fertile, the proportion of hybrid individuals in all populations should increase  
164 [20], leading to the extreme end points of majority hybrid populations which  
165 either follow a hybrid swarm or **bimodal hybrid zone** distribution.

166

167

## 168 **Key considerations for genetic analyses of anthropogenic** 169 **hybridization**

170 Published studies of anthropogenic hybridization generally follow a similar  
171 protocol. Researchers use codominant marker genotypes to estimate divergence  
172 between the two species [24] and then use a clustering approach such as  
173 STRUCTURE [25-28], or ADMIXTURE [29, 30] to partition individuals into  
174 different genetic groups ( $K$ ). Those individuals with an admixture score ( $Q$ )  
175 intermediate to the extreme admixture scores associated with parental species  
176 individuals are designated hybrids. Many studies then use HYBRIDLAB [31, 32]  
177 or similar methods to simulate hybrid genotypes from the sampled genotypes to  
178 assess the **efficiency** (i.e. type II error rate, rate of assigning hybrid individuals  
179 as parental species), and **accuracy** (i.e. type I error rate, rate of erroneously  
180 assigning parental species individuals as hybrids; [33]). The 'overall  
181 performance' of an analysis is the product of efficiency and accuracy and this  
182 performance can be used to assess the reliability of the study itself [33]. Here we  
183 outline some best practices and points to consider in order to avoid  
184 underestimation of the extent of hybridization.

185

186

### 187 **Divergence between parental species**

188 It is highly relevant to have an estimate of divergence between the focal species  
189 in the absence of hybridization.  $F_{st}$  is often reported in studies of anthropogenic  
190 hybridization, but is rarely used to motivate the marker density deployed for  
191 estimates of individual admixture, typically because the same markers are used  
192 to determine both  $F_{st}$  and individual  $Q$  estimates. Simulations have clearly shown  
193 that species (or subspecies) with lower divergence will require more markers to  
194 accurately estimate admixture, because of shared polymorphisms between them,  
195 leading to fewer **diagnostic markers** [33]. While it might not be practical to use  
196 markers to estimate  $F_{st}$  and then determine how many markers are needed to  
197 estimate individual admixture scores, an initial assessment of  $F_{st}$  will hint at how  
198 much power a system has to detect advanced backcrosses.

199

### 200 **Historical admixture**

201 Many systems have a history of repeated secondary contact and hybridization.  
202 Documenting historical admixture using genomic resources can determine  
203 whether the introgression found is due to recent, anthropogenic forces, or to  
204 natural causes, which will change the conservation status of the situation [34,  
205 35]. There are techniques for detecting historical admixture. For example, the  
206 ABBA-BABA test can be used to determine if there has been historical  
207 introgression from a third species or population into each of two closely related  
208 sister taxa, to explain variation that is not well explained by a null assumption of  
209 bifurcating phylogeny [36]. This technique can be applied to either sequences of  
210 single individuals from each population, or to multiple individuals from each  
211 population [37], and can be used to indicate historical (hundreds to thousands of  
212 generations before present) admixture. Similarly,  $\delta a \delta i$  analyses can be used to  
213 determine how well different demographic models fit the pattern of variation in  
214 the data, where demographic models can include admixture at different time  
215 points [38]. For example demographic modeling was used to demonstrate that  
216 hybridization between golden-winged (*Vermivora chrysoptera*) and blue winged  
217 warblers (*V. cyanoptera*) has probably been occurring since the original species  
218 split, and is not solely due to anthropogenic forces [39]. Finally, researchers can



219 examine the length of haplotype blocks that are identical by descent, as linkage  
220 disequilibrium decays over time due to recombination [40, 41]. The distribution  
221 of haplotype block lengths should follow a Poisson distribution [41] and  
222 deviation from this distribution can be used to infer population admixture over  
223 both short (tens of generations) [42] and long time spans [41]. These and other  
224 techniques for disentangling historical and contemporary admixture are  
225 reviewed in [43].

226

### 227 **Generations since secondary contact and recombination rates**

228 It is important to estimate the number of generations since secondary contact to  
229 estimate the potential number of backcross generations in a system. This  
230 estimate might have substantial uncertainty, but in many cases of anthropogenic  
231 hybridization there are historical records that suggest when a non-native species  
232 was first introduced or sighted that can be combined with typical generation  
233 times for the taxa involved. The expected proportion of invasive genome in a  
234 backcrossed individual halves with each successive generation of backcrossing  
235 [44].

236

237 Recombination each generation leads to less linkage disequilibrium between  
238 non-native loci, which means that genotype at a species-specific marker in one  
239 position is less informative about surrounding, un-sampled loci. For example,  
240 genomic regions with high recombination rates were found to be associated with  
241 more introgression of the non-native genome in replicate swordtail (*Xiphophorus*  
242 *birchmanni* and *X. malinche*) hybrid zones [17]. Due to obligatory crossing over,  
243 which is expected to occur once per chromosome arm [45], at least twice as  
244 many markers as there are chromosome arms are needed to cover each  
245 independent section of the genome. In some cases, there is a species-specific  
246 estimate of recombination (e.g. [46]), or one can refer to taxon-specific patterns.  
247 For example, there is as much as 10 times more recombination in avian genomes  
248 than in mammalian genomes [47]. Additionally, information on recombination  
249 rate can be combined with genomic methods examining haplotype block lengths  
250 to date introgression events (as discussed above). We discuss how many  
251 markers are needed further in Box 1.

252

### 253 **Assessing the power of markers**

254 Many studies of anthropogenic hybridization assess the power of genetic  
255 markers used by simulating hybrid genotypes and then determining the power  
256 the markers have to detect these hybrid genotypes [48]. When assessing the  
257 power of markers in this way, it is important to ensure that the biology of the  
258 system is reflected in the simulation. In particular, if the two species of interest  
259 have been in contact for many generations and F1s are thought to be fertile  
260 (Figure 1), then simulations should account for the possibility of many  
261 generations of backcrossing. This is rarely done in conservation genetic studies -  
262 many studies simulate backcrosses to assess the power of their markers, and  
263 find low power to detect even first generation backcrosses, for example finding  
264 less than 80% of first generation backcrosses are properly assigned [49, 50].  
265 Further information obtained from laboratory or field studies, such as  
266 asymmetry in hybrid fertility (e.g. between sexes, Haldane's Rule [51] or  
267 according to the species of the mother of the F1, Darwin's Corollary [52]), should  
268 also be included in simulations. For example, if previous laboratory work has  
269 established that backcrossing is largely unidirectional because of decreased  
270 fitness of hybrid individuals in the opposite direction (as expected by Darwin's  
271 Corollary) or due to the relative abundance of the parental species, then  
272 mitochondrial markers should be integrated into future analyses to add power to  
273 detect hybrids.

274

### 275 **Defining hybrid individuals**

276 To be defined as a hybrid, a focal individual must be genetically differentiated  
277 from both parental species. Parental species are assumed to have an admixture  
278 (Q) score of 0 or 1, although because of error (e.g. non-diagnostic markers,  
279 genotyping errors), very few individuals will have an estimated score of exactly 0  
280 or 1. Any score in between indicates a hybrid [25]. It is typical for a researcher to  
281 set a Q score as a cut-off for hybrid individuals, so any individual above (or  
282 below) this score is considered parental. Thresholds are determined either by  
283 power, specifically, at what level can the markers differentiate between hybrids  
284 and parental species, or by the number of acceptably mis-matched markers, e.g.

285 one allele indicative of the other species might be an error, but two markers  
286 suggest hybridization [53]. These thresholds can range widely between studies,  
287 from 0.8 [54] to 0.999 [30] in relation to a parental species score of 1.0.  
288 Determination of the threshold is a balancing act between Type I and Type II  
289 errors, in which the researcher must decide whether it is better to mistakenly  
290 assign a parental species individual as a hybrid (Type I; too low 'accuracy'; [33])  
291 or assign hybrid individuals as parental types (Type II; too low 'efficiency'; [33]).  
292 If the researcher accepts a higher level of Type II errors, they consider advanced  
293 backcrosses as parental species. For example, an admixture score threshold of  
294 0.8 would include most second-generation backcrosses (87% of the genome is  
295 species A, 13% of the genome is species B on average) as parental species.  
296 Similarly, with a Q score of 0.9, third-generation backcrosses (average of 93%  
297 species A) would be included as parental species individuals.

298

299 There are two ways to ameliorate error introduced in species assignment using  
300 thresholds. One obvious way is to employ more markers (Box 1), which  
301 increases the power of a study and allows the setting of thresholds approaching  
302 0 and 1. Studies that have used thousands of markers use the most stringent  
303 thresholds e.g. [30]. A second solution to the threshold problem is to do away  
304 with them entirely. Rather than assigning individuals to species classes based on  
305 point estimates, it is more appropriate to use **credible** or **confidence intervals**  
306 around point estimates which capture uncertainty in the marker system  
307 appropriately (Box 2). In this scenario any individual with a credible interval  
308 overlapping 0 or 1 is considered a parental species and all others are considered  
309 hybrids.

310

311 An additional problem in separating hybrid individuals from parental species is  
312 that some hybrids, particularly later generation of backcrosses, will be  
313 homozygous for all sampled diagnostic loci by chance. This is due to increased  
314 variation around the proportion of genome inherited from each parental species  
315 with each generation of backcrossing ([44]; Box 1). The hybrid nature of these  
316 individuals will be undetectable, and they will be classified as parental species,  
317 even though unmarked genome regions may be introgressed. Increasing the

318 number of markers increases the probability of sampling a hybrid individual at  
319 loci that are heterozygous or homozygous for alleles representative of both  
320 parental species (Box 1).

321

### 322 **Higher density markers to identify bimodal hybrid zones**

323 When researchers apply higher density marker panels to examples of  
324 anthropogenic hybridization, they generally uncover more backcrossed  
325 individuals compared to studies using low-density panels, and can draw more  
326 accurate conclusions about the system. These newly-detected backcrosses are  
327 often genetically very similar to the parental species, with less than 10%  
328 introgression, indicative of a bimodal hybrid zone. For example, in a study of  
329 Italian wolves that hybridize with domestic dogs, use of 170,000 SNPs found that  
330 hybridization had occurred 3 -5 generations prior to sampling [30]. This multi-  
331 generation backcrossing was not detectable in the population when 18  
332 microsatellite markers were used [49]. Further, while very few individuals were  
333 found to have Q scores between 0.25 and 0.75, as would be expected in a hybrid  
334 swarm with a complete breakdown of reproductive isolation, 62% of sampled  
335 Eurasian wolves had a small proportion (<5%) of admixture with domestic dogs  
336 [55]. The Eurasian wolf – domestic dog system has the distribution of admixture  
337 scores and phenotypes that characterizes a bimodal hybrid zone with some  
338 degree of mating preference for parental phenotypes, or rare intermediate  
339 hybrids. In this system, most individuals are either phenotypically dog-like with  
340 extreme Q scores at one end of the distribution, or phenotypically wolf-like with  
341 Q scores at the other end of the distribution. There are few individuals with  
342 intermediate Q scores and phenotypes. This can be contrasted with the  
343 westslope cutthroat (*Oncorhynchus clarki lewisi*) – rainbow trout (*O. mykiss*)  
344 system, which has also recently been genotyped using 3180 diagnostic SNPs  
345 [56]. While the increase in number of markers did lead to increased detection of  
346 advanced backcrosses, there were also many individuals with intermediate Q  
347 scores and phenotypes [56, 57]. This suggests that the westslope cutthroat-  
348 rainbow trout system is a hybrid swarm that has little assortative mating.

349

### 350 **Designing an ideal study of an anthropogenic hybrid zone**

351 When embarking on a study of anthropogenic hybridization, there are many  
352 considerations in deciding on the genetic resources to be used (Box 1). As whole  
353 genome sequencing (WGS) becomes cheaper [58], conservation biologists should  
354 consider whether WGS is the best way forward. Firstly, WGS data allows for  
355 detection of heterogeneity of introgression across the genome. If conservation  
356 biologists truly adopt a 'gene view point' of hybridization [16] then individuals  
357 ought to be classified based on whether they carry specific alleles at identified  
358 loci, rather than by overall Q scores (but see [10] for a discussion of the difficulty  
359 of implementing this approach). Secondly, WGS enables the researcher to  
360 distinguish between historical and contemporary introgression. Finally, we  
361 anticipate that the use of WGS will result in more diagnostic or **ancestry**  
362 **informative markers** being detected, and thus make studies more powerful.  
363 Researchers will be more confident in their estimates of individual admixture,  
364 and will report the power and confidence associated with their analyses (Box 2).  
365 While the bioinformatics skills required to assemble a genome and call SNPs may  
366 seem intimidating, we believe that 1) these are skills are now routinely taught in  
367 universities and 2) WGS presents an additional opportunity for conservation  
368 biologists to collaborate with speciation geneticists (Box 3). Another  
369 consideration is that high quality DNA is needed for the most accurate  
370 assemblies, although progress is being made towards high quality sequences  
371 from poor quality samples (e.g. [59]). While the use of WGS is more expensive  
372 than microsatellite marker studies, when the cost of microsatellite markers,  
373 including the cost of labour, was compared to the use of SNP markers in  
374 European wolves, SNPs were less expensive if at least 24 samples were  
375 genotyped [60]. This suggests that the use of thousands of variable genome wide  
376 markers (e.g. from ddRAD [61]) may represent a practical middle ground for  
377 conservation biologists, depending on the history and biology of the system.  
378 Taken together, we believe that the best way forward to accurately detect  
379 backcrossing in studies of anthropogenic hybrid zones is to routinely use higher  
380 density markers, including WGS when possible.

381

## 382 **Concluding Remarks**

383 Advanced backcrosses are unlikely to have been detected with many of the  
384 methods that biologists studying anthropogenic hybridization have used to date.  
385 Most studies of anthropogenic hybridization have used fewer than 20 markers  
386 [13], too few to reliably detect individuals that are more than two generations  
387 backcrossed [33], unless markers are perfectly species diagnostic [44]. For this  
388 reason, it is rare for studies to consider backcrossed individuals past the second  
389 generation of backcrossing, regardless of the number of generations that have  
390 passed since secondary contact. Here, we suggest that studies should attempt to  
391 go much further. By accounting for the number of generations since secondary  
392 contact and increasing the density of genetic markers accordingly, many more  
393 backcrossed individuals will become distinguishable from the parental  
394 populations. We echo the call for more genetic markers to be used in these  
395 studies to allow for higher accuracy and efficiency [1, 3, 13, 33, 62], particularly  
396 since we have now entered the genomics era, making tens or hundreds of  
397 thousands of markers obtainable even in non-model systems [58]. It seems likely  
398 that anthropogenic hybridization will only increase in frequency and result in  
399 increased gene flow between previously isolated species [1]. The increase in  
400 number of markers and associated power will also open up the opportunity to  
401 ask new questions in these systems, parallel to those speciation biologists  
402 explore in natural hybrid zones (Box 3). There are new challenges with  
403 increased marker density, but a genomic approach to studying these systems will  
404 help researchers to detect backcrosses and make the best policy  
405 recommendations.

406

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411 **Additional Elements**

412

413 **Glossary**

- 414 • **Anthropogenic hybridization:** the breakdown of reproductive isolation  
415 between two species due to human action, including but not limited to,  
416 species introduction, habitat disturbance or escape of domestic species.
- 417 • **Accuracy:** the proportion of identified hybrids that are actually of hybrid  
418 ancestry [33]. A low accuracy suggests a high rate of type I errors, in  
419 which parental species individuals are erroneously assigned as hybrids.
- 420 • **Admixture:** the mixing of genomes from structured or diverged  
421 populations
- 422 • **Allopatry:** species in non-overlapping ranges
- 423 • **Ancestry informative markers:** genetic markers with substantial allele  
424 frequency differences across populations, which can be used to assign  
425 individuals to each population [63]
- 426 • **Bimodal hybrid zone:** a hybridizing population in which preference for  
427 parental phenotypes, or scarcity of hybrids with which to mate, results in  
428 a population that includes few F1 hybrids, and many backcrossed  
429 individuals with a low level of introgression that often resemble the  
430 parental species in phenotype. Can be unimodal (if backcrossing is into  
431 just one parental species) or bimodal (backcrossing into both parental  
432 species) [22]
- 433 • **Credible interval:** the range of possible values surrounding a point  
434 estimate, representing the uncertainty in the estimate
- 435 • **Diagnostic markers:** markers with fixed allele differences across  
436 populations
- 437 • **D<sub>xy</sub>:** an absolute measure of genetic differentiation, calculated as the  
438 proportion of nucleotides that differ between two homologous sequences  
439 within the same or different population.
- 440 • **Efficiency:** Proportion of correctly identified individuals in each group  
441 [33]. If the null hypothesis is that an individual is from the parental  
442 species rather than a hybrid individual, then low efficiency suggests a

- 443 high rate of type II errors, in which hybrid individuals are incorrectly  
444 assigned as parental species.
- 445 • **F<sub>ST</sub>** –A measure of genetic differentiation between populations based on  
446 the difference in allele frequencies within and between populations [64]
  - 447 • **Hybridization:** mating of individuals from diverged populations
  - 448 • **Hybrid:** an individual that has an intermediate genotype between two  
449 diverged, parental populations, as the result of interbreeding between  
450 these populations
  - 451 • **Hybrid swarm:** a hybridizing population that includes F1 hybrids and  
452 various backcrosses, due to a total breakdown of assortative mating. Also  
453 known as a unimodal hybrid zone [22].
  - 454 • **Introgression:** the movement of alleles between genetically  
455 differentiated forms (including populations, species, etc), mediated by  
456 backcrossing [65]
  - 457 • **Secondary contact:** Occurs when two (or more) species that have been in  
458 allopatry come back into sympatry
  - 459 • **Sympatry:** species in overlapping ranges
- 460



461 Figure 1: Anthropogenic hybridization falls into three main categories. These are  
462 1) systems with inviable or infertile hybrids, 2) bimodal hybrid zones in which  
463 there is either mating preference for parental species phenotypes or the relative  
464 abundance of parental species means most matings are backcrosses and 3)  
465 hybrid swarms in which there is random mating and many hybrid individuals. In  
466 this schematic figure we illustrate for each type of anthropogenic hybridization  
467 system how many individuals of each admixture (Q) score might be found and  
468 typical distributions of mating success across Q scores according to whether  
469 there is a high likelihood of hybrid individuals mating with the parental species  
470 phenotypes present. While we represent hybrid swarms and bimodal hybrid  
471 zones as categorically different, these are probably ends of a continuum and  
472 some systems may be intermediate between them. Note that we have  
473 represented (2) as a bimodal hybrid zone due to backcrossing into both parental  
474 species. Alternatively there can be a single (i.e. unimodal) hybrid zone due to  
475 unidirectional backcrossing.

476 **Box 1 – How many markers do I need to discover backcrossed individuals**  
477 **in my system?**

478

479 Substantial power is needed to detect individuals that are the result of repeated  
480 generations of backcrossing. General rules have been suggested, including that  
481 for each additional generation twice as many markers are needed [44], and that  
482 at least 48 markers would be needed to consistently detect first generation  
483 backcrossing in hybrids with parental species that have an  $F_{st} = 0.21$  [33].

484 However, we are now in the age of genomics, when the cost of increasing marker  
485 density is dramatically decreasing [58], and thus marker numbers should be less  
486 of a barrier than previously. So, how many markers does a study need to reliably  
487 detect backcrossed individuals?

488

489 To maximize detection of backcrossed individuals, researchers can increase their  
490 power in three ways; through increased divergence, the use of diagnostic  
491 markers, or with increased numbers of markers. Studies with high divergence  
492 between hybridizing species have high power [33]. However, as many  
493 conservation biologists choose their study system based on conservation  
494 concerns and not to maximize power, this advice is not helpful. Diagnostic  
495 markers have fixed allelic differences between parental species and are the most  
496 powerful for backcross detection [25]. Ancestry informative markers, those with  
497 strong allele frequency divergence between species, are also very powerful [63].  
498 Loci with weak allele frequency divergence between species are least useful.

499 Diagnostic and ancestry informative markers can be determined based on  
500 genotyping and contrasting known parental species individuals, although this is  
501 not always feasible (e.g. [55]). Additionally, the diagnostic properties of markers  
502 are a function of the populations and individuals that have been sampled; more  
503 extensive sampling sometimes demonstrates that selected markers are not  
504 diagnostic for all populations [66]. Generally speaking, the more markers used,  
505 the higher the chance of detection of admixture in an individual [33, 44].

506

507 Assuming diagnostic markers, it is ideal to know the number of elapsed  
508 generations since the initial hybridization, as, for every further generation of

509 backcrossing, the proportion of introgressed genome halves [44]. The number of  
510 generations since hybridization should be interpreted with an eye to policy.  
511 After some number of generations of uni-directional backcrossing, policy will  
512 dictate that we consider an individual to be parental species (again) [67]. It's  
513 best to make this decision prior to marker selection, as it is impossible to apply  
514 policy decisions regarding the acceptability of backcrossed individuals without  
515 sufficient detection power.

516

517 If we are interested in all generations of backcrossing, then we can extend the  
518 deterministic model developed by Boecklen and Howard ([44]; Equation 2) for  
519 the genomics era. We made the same assumptions, specifically that backcrossing  
520 is unidirectional, loci are independent and Mendelian, all markers are diagnostic,  
521 all backcrossing is between the previous generation of backcrosses and parental  
522 species, and all genotypes are equally fecund [44]. We asked what proportion of  
523 backcrossed individuals are undetectable because they are homozygous for all  
524 diagnostic markers. We modeled 10 generations of backcrossing, and each of 10,  
525 100 and 1000 diagnostic markers (Figure I). When using 10 diagnostic markers,  
526 52% of 4<sup>th</sup> generation backcrosses are homozygous for one parental species at  
527 all loci, and thus undetectable as backcrosses. In contrast, 1000 diagnostic  
528 markers allow for powerful (85%) detection of 9<sup>th</sup> generation backcrosses.

529

530

531

532 Figure I: An extension of the deterministic model presented by Boecklen and  
533 Howard [44]. The proportion of hybrid individuals that are homozygous at all  
534 the (diagnostic) markers, and are hence indistinguishable from the parental  
535 species that is being introgressed, increases with each generation of  
536 backcrossing, but decreases with increased marker density. This demonstrates  
537 that more markers than are typically used in studies of anthropogenic  
538 hybridization are needed to detect advanced backcrosses.

539

540 **Box 2 – Reporting Error**

541 Credible (or confidence) intervals (CIs) are a powerful, intuitive way to assess  
542 confidence in the estimates being presented [68, 69]. Measures of uncertainty  
543 are not always presented in estimates of anthropogenic hybridization (although  
544 see [53, 70-73] for exceptions), perhaps because the uncertainty is so high where  
545 estimated. Credible intervals can be calculated using STRUCTURE [25] and  
546 standard errors can be calculated using ADMIXTURE [29], so reporting of error  
547 estimates is easily implemented in a routine workflow.

548

549 There are practical implications of the reporting of credible intervals,  
550 particularly for individuals with very low or very high admixture values (Q). Cut-  
551 off thresholds have been used to determine if individuals are members of the  
552 parental populations or are admixed, but these thresholds are usually based on  
553 the detection power of a study (see main text). Since these are hard cut-offs,  
554 individuals with very similar levels of admixture can be assigned to very  
555 different populations. For example, with a Q cut-off of 0.80, if individual 1 is  
556 assessed as  $Q=0.79$ , it is determined to be admixed and, depending on the  
557 management of the system, may be culled. In contrast, if individual 2 is estimated  
558 to have  $Q=0.81$ , it would be considered a parental species individual and be  
559 retained for breeding. There may be no substantive difference between these  
560 individuals, although this is impossible to tell using only point estimates.

561

562 We recommend that credible intervals should also be included in visual  
563 depictions of admixture. Typically, the key figure from a paper on anthropogenic  
564 hybridization is the characteristic “STRUCTURE Bar Plot” [25], that uses stacked  
565 colours to denote genetic contributions from different source populations. These  
566 plots show the point estimates for each individual, and allow the author to  
567 determine thresholds for inclusion in each group. While such figures are  
568 compelling and easily interpreted, they do not convey the uncertainty around  
569 individual point estimates.

570

571 Allendorf and colleagues [11] noted that it is very difficult to make policy  
572 decisions when comparing different low point estimates of admixture. We

573 recommend that researchers should focus on the uncertainty around Q estimates  
574 when making decisions about the genetic group each individual belongs to. It has  
575 been pointed out that the use of credible intervals demonstrates the high levels  
576 of uncertainty researchers are facing [70]. As they should! This problem will of  
577 course be substantially alleviated by using more markers (see Box 1).  
578

579 **Box 3: Lessons from Natural Systems**

580

581 Naturally occurring hybrid zones have long been used as ‘natural laboratories’ to  
582 study the speciation process [74]. The field of speciation genomics works to  
583 understand how genomic differences build up to cause eventual reproductive  
584 isolation [75-78]. Recently, population geneticists have used genome wide  
585 markers to ask questions regarding the genomic architecture of reproductive  
586 isolation and speciation, and how the genomes of diverged populations change in  
587 the face of on-going gene flow [43, 78, 79]. Further, many studies of natural  
588 hybrid zones have focused on isolating signals from historical vs. contemporary  
589 hybridization (main text 2.1.1, [78]). These questions that speciation biologists  
590 ask using hybrid zones could equally be asked in anthropogenic hybrid zones,  
591 particularly in studies that used whole genome sequence data. Indeed, studies of  
592 anthropogenic hybrid zones may even have more power than those with  
593 naturally occurring secondary contact as in some cases of introduced or escaped  
594 heterospecifics, phenotypic divergence is more extreme, meaning that fewer  
595 individuals would need to be sampled for, for example, admixture mapping [78].

596

597 Use of genomic data allows speciation geneticists to examine heterogeneity in  
598 divergence across the genome. Indeed, the questions we noted above are most  
599 interesting when heterogeneity is found. Genome scans look for regions of high  
600 divergence between species ( $F_{st}$  or  $d_{xy}$ ) which may indicate regions that resist  
601 introgression, also known as ‘speciation islands’ [80], or ‘islands of  
602 differentiation’ [79]. While such signals are not without controversy [81], and in  
603 some cases may represent phylogenetically derived regions of low  
604 recombination, rather than reproductive isolation [82], they represent  
605 interesting candidate regions for fixed differences between hybridizing species,  
606 and thus could be used diagnostically by conservation biologists. For example,  
607 golden-winged (*Vermivora chrysoptera*) and blue-winged warblers (*V.*  
608 *cyanoptera*), which hybridize in eastern North America are phenotypically  
609 distinct but undistinguishable when using low density, microsatellite marker  
610 panels [83]. Only with the use of whole genome sequencing were six small  
611 divergent regions of the genome discovered, four of which are associated with

612 either pigmentation or feather development genes and explain more than 90% of  
613 the variation in plumage [39]. This demonstrates that a focus on the use of high  
614 density markers to explore heterogeneity across the genome allows for higher  
615 power to both distinguish between closely related, hybridizing species  
616 genetically, and to associate genomic regions with diverged phenotypes, two  
617 possible goals of conservation biologists working on anthropogenic hybrid  
618 zones. We echo the call of [1] that conservation biologists can take a cue from  
619 speciation biologists that have, in many cases, developed methods that use  
620 genomics to ask interesting questions of hybrid zones.  
621

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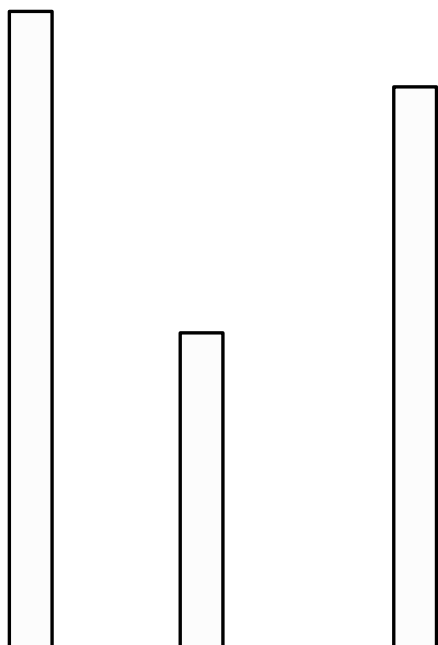
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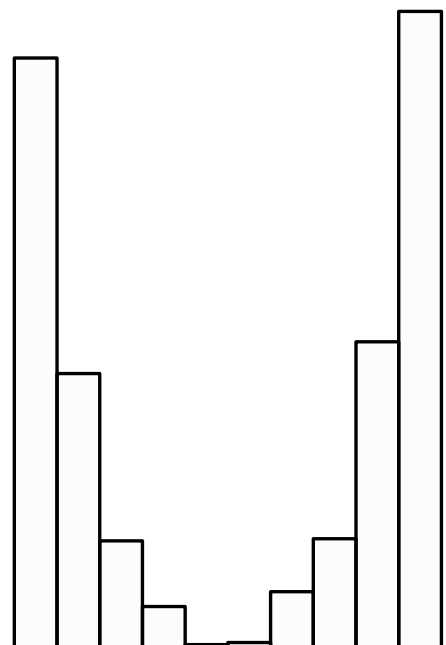
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Figure Inviably or Sterile F1s

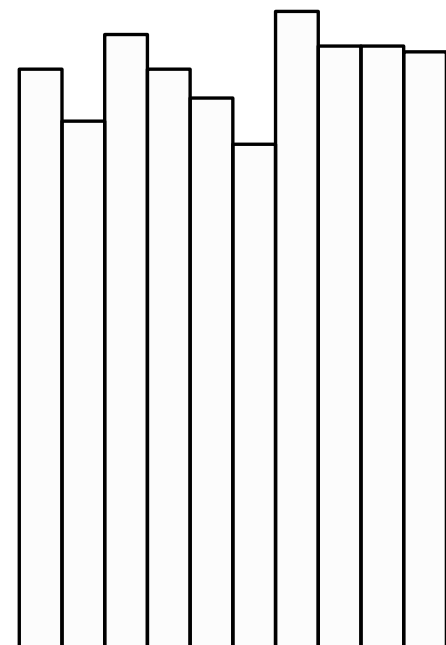
Number of Individuals



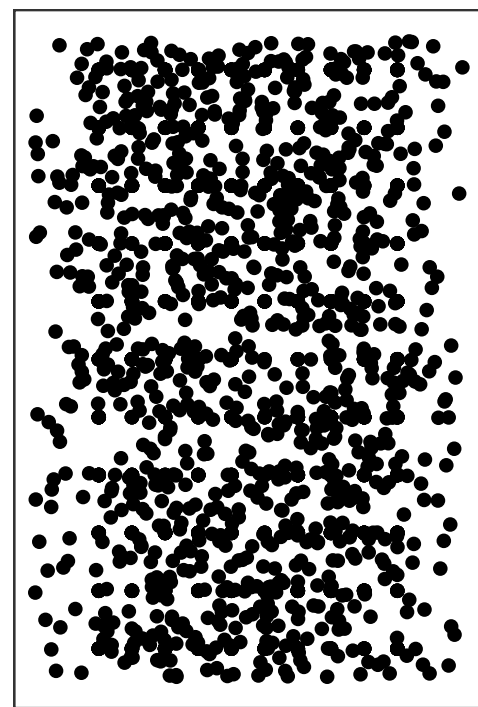
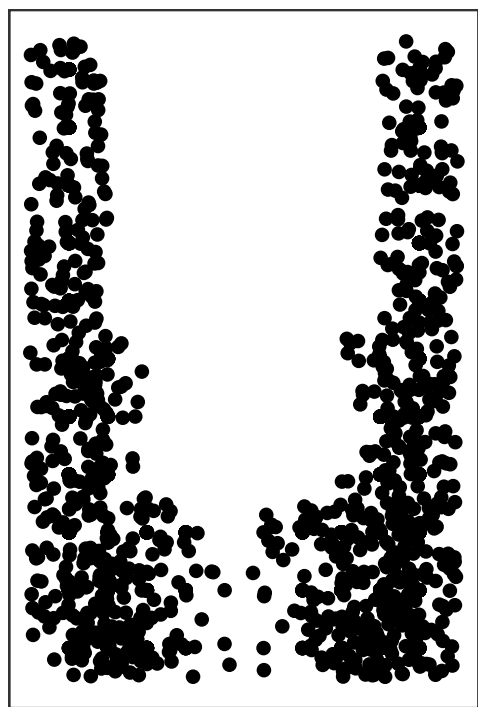
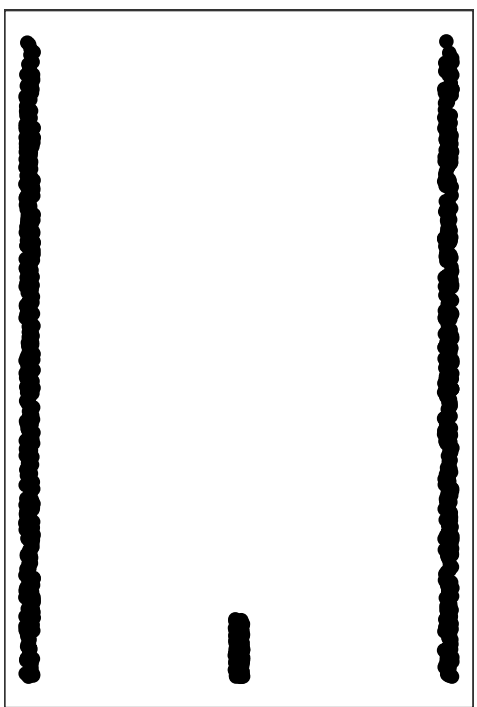
Bimodal Hybrid Zone



Hybrid Swarm

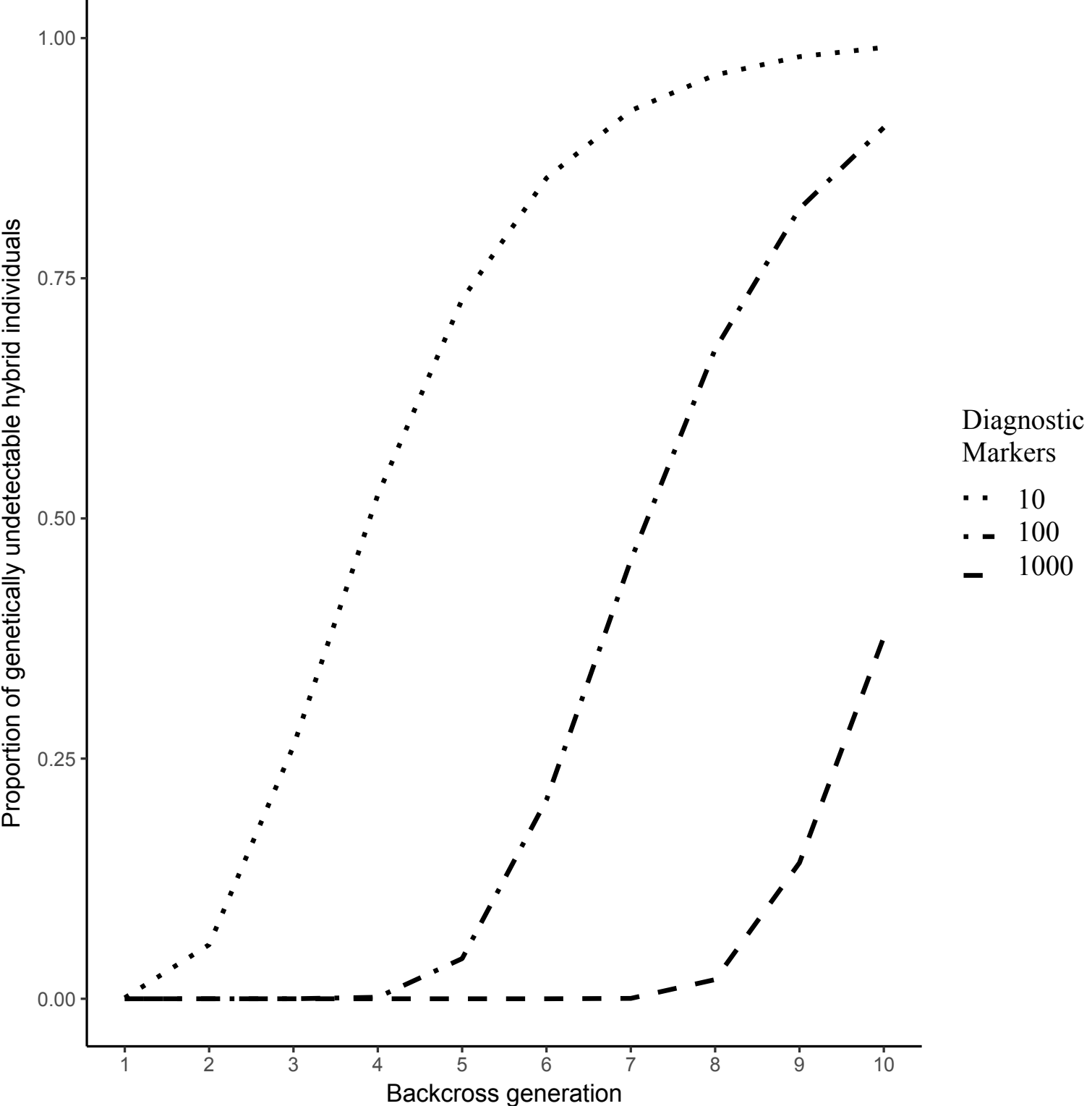


Mating Success



Admixture (Q) scores from 0 - 1

Figure



**Outstanding questions:**

1) Do replicate anthropogenic hybrid zones show similar patterns of introgression?

There are big evolutionary questions that could be answered by the sorts of data that conservation biologists working on anthropogenic hybridization could answer. For example, there are multiple replicate hybrid zones occurring in the wolf/dog, wild cat/domestic cat, red deer/sika deer, westslope cut throat trout/rainbow trout systems. But in many cases, there is limited communication and collaboration between researchers, or different markers are used across studies [60]. Clearly this isn't a problem unique to this field, but it is the case that collaboration between researchers would be made easier with standardized genome wide data aligned to a common genome. Genomic data make cross study comparisons easier, and would allow for easier comparison between studies.

2) Once there has been a breakdown of reproductive isolation characterized as hybridization, how common is maintenance of within parental species assortative mating? Is the strength of assortative mating stronger when species are more diverged, or perhaps between closely related species that have recently evolved reproductive isolation?

3) What is the relative frequency of hybrid swarms vs bimodal hybrid zones? We expect that the prevalence of bimodal hybrid zones has been underestimated because of the difficulty of detecting highly introgressed backcrosses. Increased use of high-density markers will make these cases easier to detect and would enhance our understanding of the systems that are bimodal hybrid zones.

**Highlights:**

Anthropogenic hybridization is increasingly common and likely to result in a breakdown of reproductive isolation between 'good' species.

Backcrossed individuals that have only a small proportion of one parental genome are difficult to differentiate from parental individuals using the most common current technologies.

Bimodal hybrid zones are characterized by introgression and backcrossing. The majority of hybrid individuals in these systems have low levels of introgression. The problems posed by bimodal hybrid zones have been largely overlooked in the literature.

Genome wide sampling of genetic markers at high densities allow for increased precision in the estimate of admixture proportions, which makes it feasible to detect multi-generation backcrosses, and will thus make it easier to differentiate bimodal hybrid zones from hybrid swarms or systems without introgression.