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3

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24 **Abstract**

25 Hybridization is among the evolutionary mechanisms most frequently hypothesized to drive the
26 success of invasive species, in part because hybrids are common in invasive populations. One
27 explanation for this pattern is that biological invasions coincide with a change in selection
28 pressures that limit hybridization in the native range. To investigate this possibility, we studied
29 the introduction of the brown anole (*Anolis sagrei*) in the southeastern United States. We find
30 that native populations are highly genetically structured. In contrast, all invasive populations
31 show evidence of hybridization among native-range lineages. Temporal sampling in the invasive
32 range spanning 15 years showed that invasive genetic structure has stabilized, indicating that
33 large-scale contemporary gene flow is limited among invasive populations and that hybrid
34 ancestry is maintained. Additionally, our results are consistent with hybrid persistence in
35 invasive populations resulting from changes in natural selection that occurred during invasion.
36 Specifically, we identify a large-effect X chromosome locus associated with variation in limb
37 length, a well-known adaptive trait in anoles, and show that this locus is often under selection in
38 the native range, but rarely so in the invasive range. Moreover, we find that the effect size of
39 alleles at this locus on limb length is much reduced in hybrids among divergent lineages,
40 consistent with epistatic interactions. Thus, in the native range, epistasis manifested in hybrids
41 can strengthen extrinsic post-mating isolation. Together, our findings show how a change in
42 natural selection can contribute to an increase in hybridization in invasive populations.

43 **Significance**

44 Hybridization is common in invasive species and can be important for their success. The
45 connection between hybridization and bioinvasions could result in part because of a disruption in
46 the selection pressures that limit hybridization in the native range. We demonstrate that, in the
47 lizard *Anolis sagrei*, hybridization is rare in native populations, which show frequent evidence of
48 natural selection at a large effect X chromosome locus. Conversely, little selection at this locus
49 was detected in invasive populations, which do not experience large-scale contemporary gene
50 flow, but instead maintain a mosaic of hybrid ancestries formed during invasive range
51 colonization. Ecological changes during biological invasions can affect hybridization frequency
52 and stability, which can in turn drive the success of invasive taxa.

53 **Introduction**

54 Evolutionary change can affect the success of invasive species. This possibility has been
55 considered by biologists for more than half a century (1). Recent decades, however, have seen a
56 remarkable growth in research on this topic (2-5), simultaneous with the greater emphasis on
57 contemporary evolution driving ecological phenomena (6, 7). It is now widely accepted that
58 substantial evolution can occur over a few generations, well within the timeframe in which the
59 establishment and spread stages of biological invasions play out (6, 7).

60

61 Among evolutionary factors that can facilitate invasions, hybridization is noteworthy for several
62 reasons (8-10). For one, hybridization between species or among lineages within species has
63 repeatedly been observed in genetic surveys of invasive taxa. As a result, invasive populations
64 often show levels of genetic diversity equal to or larger than those in native populations (11-14).
65 Also, meta-analyses have shown that invasive hybrids are frequently larger and more fecund than
66 their parents (15). Moreover, genetically, hybridization can contribute to invasive spread through
67 hybrid vigor or adaptive introgression (12, 13, 16).

68

69 Nonetheless, studies directly connecting hybridization to invasive spread are still in the minority.
70 As a result, we do not know how often hybridization is a true driver of invasion success, or a
71 consequence of propagule pressure and repeated introductions of allopatric lineages (10).

72 Further, why hybridization is less common in the native range than in the invasive range of some
73 species is unclear, particularly when opportunities for human-mediated inter-population dispersal
74 and contemporary physical barriers to natural dispersal do not clearly differ between ranges (e.g.,
75 refs. 14 and 17).

76

77 Changes in natural selection that occur during biological invasions may provide part of the
78 answer for why hybrids are more common in invasive populations, especially when biological
79 invasions occur in disturbed habitats or in habitats that are ecologically novel from the
80 perspective of the invader. In a landmark paper on hybridization and invasiveness, Ellstrand &
81 Schierenbeck (9) discussed 28 species for which hybridization preceded invasive spread. All of
82 these were found to occur in habitats characterized by disturbance, indicating that the change of
83 selection pressures experienced by parental lineages or the opening of new niches to which
84 hybrids are better adapted might facilitate hybridization in invasive species (9). Originally
85 proposed by Anderson (18), the possibility that unstable, rapidly changing or novel habitats
86 promote hybridization is now well documented in non-invasive species (19). Particularly
87 compelling examples come from long-term field studies that show a lack of hybridization before
88 the environment changes (20) or a return to non-hybrid status once the ancestral environment is
89 restored (21). In a similar way, hybrids may more easily form or persist in invasive populations
90 when biological invasions coincide with a temporary or permanent change in patterns of
91 adaptation that evolved in the native range (22, 23).

92

93 To investigate whether changes in selection may facilitate hybridization during biological
94 invasions, we studied the Cuban brown anole (*Anolis sagrei*), an excellent organism for such
95 research for multiple reasons. First, it is one of the best-known examples of hybridization
96 occurring during biological invasion. Previous studies indicated that most invasive populations in
97 Florida, in the southeastern United States, derive from admixture between divergent native-range
98 lineages (11, 24). Second, *A. sagrei* is a highly successful invader. Since the mid- to late-1800's,

99 when the first populations were established in Florida, the species has colonized the entire
100 peninsula and expanded to the north and west (11). From there, it has since seeded secondary
101 invasions globally (11). Third, niche structure, natural selection and local adaptation are
102 exceptionally well-studied in anoles, thanks to decades of observational and experimental work
103 (25). A number of these studies focused on natural and experimental populations of *A. sagrei* in
104 its native range in the Caribbean, predominantly on islands in the Bahamas (e.g., refs. 26-33). As
105 such, an important baseline regarding phenotypic and environmental components of native range
106 local adaptation is available in this system.

107

108 **Results and Discussion**

109 **Genetic structure and genetic diversity in native and invasive *A. sagrei***

110 We first aimed to understand how genetic variation is partitioned among native and invasive
111 populations. For the native range, we included 10 populations, nine of which are representative
112 of lineages known to have seeded the Florida invasion (11). For the invasive range, we included
113 34 populations predominantly from Florida (SI Appendix, Fig. S1 and Dataset S1). We obtained
114 genome-wide SNPs for all lizards ($N = 824$) *via* double-digest RAD-seq reduced representation
115 sequencing (SI Appendix, Methods). Principal component analyses (PCA), phylogenetics, and
116 Bayesian clustering all pointed towards strong genetic structure in the native range producing six
117 main lineages, with limited evidence of among-lineage genetic exchange (Fig. 1A, B; SI
118 Appendix, Figs. S2-S4). Specifically, only one of the 134 genotypes was clearly a hybrid (Fig.
119 1A). Further genetic structure was apparent within clades, albeit to a lesser extent (Fig. 1A,
120 inset).

121

122 Previous studies have indicated that geographical distance is an important determinant of genetic
123 structure for *A. sagrei* in Cuba (34, 35). Our results reinforce these findings by providing
124 evidence of strong isolation by distance (IBD; Mantel's $r = 0.88$, $P = 5 \times 10^{-4}$; SI Appendix, Fig.
125 S6A). However, contrary to expectations under true IBD, genetic distances did not increase
126 uniformly with geographic distance. Rather, moderate within-clade genetic structure was
127 complemented by much stronger among-clade genetic subdivisions (SI Appendix, Fig. S6A).
128 Similar hierarchical genetic structure has been shown to lead to significant IBD (36). Strong
129 among-clade genetic fragmentation has been found in the native range of other anole species
130 that, like *A. sagrei*, have a broad and continuous distribution (discussed in ref. 25). The fact that
131 some of the genetic breaks, including those described in Cuban *A. sagrei*, overlap in different
132 species while also corresponding to inferred past geological barriers (35) suggests that they
133 resulted from divergence in allopatry, likely during periods of partial island submergence (35,
134 37).

135

136 The mechanisms that allow these genetic subdivisions to be maintained in the absence of
137 contemporary geographical barriers are, however, unknown. This genetic differentiation is
138 particularly striking given that *A. sagrei* clades have geographically abutting distributions in
139 Cuba (35). One possibility is that local adaptation and resulting ecological selection against
140 immigrants and hybrids is involved. Such ecological structuring of genetic variation across the
141 landscape is suggested by results from previous studies that indicated ecology has an important
142 role in shaping spatial genetic divergence in anoles, albeit to a lesser extent as compared to
143 geographical distance (34). We revisit the contribution of local adaptation below.

144

145 Compared to the native range, population structure in the invasive range was markedly different.
146 There was no clear grouping of genotypes in PCA space (Fig. 1A), and all populations showed
147 evidence of admixture, deriving ancestry from more than one native Cuban lineage (Fig. 1B; SI
148 Appendix, Figs. S3 and S5). Also, within-population estimates of relatedness were more similar
149 to among-population estimates of relatedness in the invasive range than in the native range,
150 indicating that ancestral population structure has collapsed during invasion (SI Appendix, Fig.
151 S7). Lastly, the strength of IBD was much reduced relative to the native range (Mantel's $r =$
152 0.28 , $P = 0.004$; SI Appendix, Fig. S6B), as expected given that dispersal of invasive *A. sagrei*
153 occurred with the contribution of human-mediated long-distance transport.

154

155 Shifts in genetic structure between the native and invasive ranges were accompanied by
156 important changes in genetic variation. Genome-wide heterozygosity, which we used as a proxy
157 for neutral genetic variation, was almost completely non-overlapping between the two ranges,
158 with invasive populations significantly more diverse (two-sided Wilcoxon rank-sum test, $P =$
159 4.719×10^{-9} ; Fig. 1C; SI Appendix, Fig. S8A). This pattern was reversed for SNPs predicted to
160 be detrimental – those that produce premature stop codons, frameshift mutations, or the loss of
161 start codons (SI Appendix, Methods). Invasive populations showed a lower proportion of these
162 putatively deleterious mutations as compared to native populations (two-sided Wilcoxon rank-
163 sum test, $P = 0.015$; Fig. 1C; SI Appendix, Fig. S8B), indicating that the purging of genetic load
164 occurred more readily in the invasive range. This could have happened if invasion was
165 accompanied by a change in the fitness landscape, such that weakly deleterious alleles in native
166 populations are more readily visible to selection in the new environment (38). As well, if
167 recessive, even strongly deleterious alleles would have persisted at low frequency in native

168 populations. These rare alleles should have been preferentially lost during genetic bottlenecks
169 that occurred when invasive populations were seeded.

170

171 **Invasive genetic structure has stabilized to a mosaic of hybrid ancestries**

172 The observation of widespread hybridization in invasive *A. sagrei* is notable given that hybrids
173 among divergent lineages are rare in the native range. To investigate whether this is due to the
174 more recent history of *A. sagrei* in Florida, such that removal of hybrids in these populations is
175 ongoing, we resampled a subset of invasive populations fifteen years after they were first
176 sampled. We included SNPs from six Florida populations ($N = 172$) that we surveyed in both
177 2003 and 2018 (SI Appendix, Fig. S9).

178

179 We found that hybrid ancestry is not eliminated in Florida, and that genetic differences among
180 populations resulting from independent introduction and hybridization events are maintained
181 over at least 15 years. First, genetic structure inferred using PCA did not change between 2003
182 and 2018 (SI Appendix, Fig. S10A). Second, while populations differed in the proportion of
183 Western Cuba ancestry ($F_{5,165} = 217.4$; $P < 2 \times 10^{-16}$; SI Appendix, Fig. S10B), reflecting the
184 mosaic of hybrid origins that occur across Florida (Fig. 1B; see also ref. 11), each population
185 maintained similar proportions over the 15-year period ($F_{1,165} = 0.207$; $P = 0.649$; SI Appendix,
186 Fig. S10B). Third, there was no effect of sampling time on the index of admixture ($F_{1,165} =$
187 0.972 ; $P = 0.325$; SI Appendix, Fig. S10C), which takes into account the contribution of each
188 native-range lineage to the ancestry of hybrids (12).

189

190 The only metric that did change was heterozygosity, which decreased significantly over the 15
191 years across all populations ($F_{1,165} = 50.72$; $P = 3.12 \times 10^{-11}$; SI Appendix, Fig. S11A). Two lines
192 of evidence suggest that this drop in heterozygosity is not a result of ongoing purging of common
193 intrinsic incompatibilities, but is instead driven by the loss of rare alleles, likely due to genetic
194 drift. First, heterozygosity decreased irrespective of whether hybrids originated from closely
195 related or distantly related lineages (SI Appendix, Fig. S11B). This is inconsistent with selection
196 against intrinsic incompatibilities, which should be more common in hybrids among divergent
197 lineages (39). Second, polymorphism lost in the 2018 samples involved alleles that were already
198 rare in 2003 (SI Appendix, Fig. S11C), consistent with genetic drift. We note, however, that
199 these results do not exclude the possibility that any intrinsic incompatibilities have been purged
200 by selection prior to 2003, the first timepoint represented in our sampling.

201

202 The finding that genetic differences established among Florida populations by 2003 remain
203 unchanged by 2018 argues against large-scale ongoing gene flow in the invasive range. Such
204 gene flow would have been expected to shift population genetic structure over the span of 15
205 years. Rather, a more plausible interpretation for hybrid ancestry in Florida populations is that
206 distinct hybridization events took place during the evolutionary history of invasive *A. sagrei*.
207 These hybridization events would have occurred when divergent lineages met after repeated
208 introductions from Cuba and following progressive range expansion from these points of initial
209 introduction.

210

211 An equally important finding is that hybrid ancestry is not only common, but it is also stable in
212 the invasive range. This contrasts with observations in the native range where hybrids are much

213 rarer, particularly among lineages that are representative of major phylogenetic splits in *A.*
214 *sagrei*, and even when these lineages have geographically abutting distributions (e.g., the Central
215 Cuba and East-Central Cuba lineages; SI Appendix, Fig. S2). Limited genetic exchange among
216 these divergent lineages in the native range is supported by our data as well as a previous study
217 that surveyed more Cuban populations (35). Differences in hybridization frequency between the
218 native and invasive ranges suggest that at least some gene flow barriers in the native range are
219 conditional on environment (i.e., they are extrinsic). If barriers to gene flow were predominantly
220 unconditional (i.e., represented by intrinsic genetic incompatibilities), we would have expected
221 to find that hybridization is equally rare in both ranges or that hybrid ancestry present in invasive
222 populations is progressively eliminated.

223

224 **Genome scans are consistent with changes in natural selection**

225 To understand differences in hybridization frequency between ranges, we focused on local
226 adaptation, which can limit gene flow between populations that inhabit different environments
227 (40, 41). To this end, we contrasted the genomic signature of selection for the native and
228 invasive ranges. In the native range, population differentiation was strongest on the X
229 chromosome, where fixed differences ($F_{ST} = 1$) occurred even among closely related
230 populations, for which median genome-wide F_{ST} was over 26 times lower than on the X
231 chromosome ($F_{ST} = 0.038$; Fig. 2A; SI Appendix, Fig. S12). High X chromosome F_{ST} windows
232 were concentrated in a region of 18 Mbp in size (chromosome 7 coordinates 70Mbp – 88Mbp;
233 Fig. 2B). While representing 16% of the X chromosome length, this locus accounted for 47% of
234 all X chromosome outliers and 62% of extreme X-chromosome outliers.

235

236 Compared to the native range, genome-wide F_{ST} in the invasive range was only a third as high
237 (Fig. 2A) and there were no fixed differences among sampling sites (all windowed $F_{ST} < 1$; SI
238 Appendix, Fig. S13). Even so, the genomic landscape was similar to the one we observed in the
239 native range, with the X chromosome contributing more to population differentiation (Fig. 2A;
240 SI Appendix, Fig. S13). Compared to the native range, however, the largest X chromosome F_{ST}
241 peaks resulted from comparisons among divergent lineages (SI Appendix, Fig. S13), which we
242 could infer for invasive hybrids at this chromosomal region due to reduced recombination (SI
243 Appendix, Methods). This indicates that introduction history, which can create sharp breaks in
244 allele frequencies among populations (42), likely has an important role in elevating invasive
245 range F_{ST} at this genomic region. Specifically, because this locus is frequently differentiated
246 among native range populations, any two invasive populations with X chromosome haplotypes
247 of different origins will also show high X chromosome F_{ST} . Lastly, outliers were less
248 concentrated in the divergent X chromosome region identified from native populations (Fig. 2B).
249 In invasive populations, this locus contained 35% of all X chromosome outliers and 43% of
250 extreme X chromosome outliers. After accounting for introduction history by excluding
251 comparisons made among divergent X chromosome lineages, these estimates dropped to 19%
252 and 25%, respectively.

253

254 We next asked whether the same outliers have repeatedly contributed to differentiation among
255 independent population pairs from within either the native or the invasive ranges. Non-parallel
256 F_{ST} windows accounted for the majority of outliers (85.4%-89.2%; SI Appendix, Table S1).
257 While representing a smaller fraction of the genome, repeated outliers were more common than
258 expected by chance, for both ranges ($P < 0.001$ all permutation tests; SI Appendix, Fig. S14).

259 Also, relative to null expectations, there were more repeated outliers in the invasive range than in
260 the native range (SI Appendix, Fig. S14). Repeated F_{ST} differentiation in both ranges is
261 consistent with divergence under natural selection. In the invasive range, higher repeatability can
262 be the result of the increased linkage disequilibrium that characterizes these populations (SI
263 Appendix, Fig. S15), thereby enhancing non-independence of adjacent genomic windows. While
264 the genome-wide proportion of repeated outliers was larger in invasive populations, this pattern
265 was reversed at the X chromosome locus, which contributed proportionately more to parallel
266 differentiation in the native range (SI Appendix, Fig. S16). This further underscores that the
267 genetic basis of population differentiation is different between the two ranges, with the X
268 chromosome locus having a larger contribution in the native range.

269

270 In support of F_{ST} results, we found that Tajima's D is often reduced at the same X chromosome
271 locus in native populations, but rarely so in invasive populations (Fig. 2C; SI Appendix, Figs
272 S17-S18). Reduced Tajima's D indicates an excess of low-frequency variants, as expected after
273 positive or negative selection (43). Among these possibilities, positive selection was likely
274 involved, as indicated by Fay and Wu's H test (Fig. 2D). This metric quantifies the excess of
275 derived (i.e., non-ancestral) alleles, which is expected under positive selection. We found that H
276 was lower in the native range than in the invasive range at the X chromosome divergent locus
277 (one-sided Wilcoxon rank-sum test, $P = 1.26 \times 10^{-6}$). Also, H estimates at this genomic region
278 were lower than background neutral values only for native populations (Fig. 2D), consistent with
279 positive selection in the native range only. Background neutral H values were similar between
280 the native and invasive ranges (one-sided Wilcoxon rank-sum test, $P = 1$), indicating that
281 admixture or demography, which should impact genetic variation more broadly rather than a

282 single locus, are unlikely to be a major source of bias in our results. Moreover, positive selection
283 should have been easy to identify using Fay and Wu's H in invasive populations, because this
284 metric has more power in admixed than in non-admixed populations (44). Lastly, we note that
285 while analyses presented above focused on the most recent (i.e., 2018) samples from the invasive
286 range, we also contrasted H between the 2003 and 2018 timepoints, for the six invasive
287 populations with temporal data. These analyses provided no evidence of change in H values in
288 the invasive range, at least over the span of the 15 years covered in our sampling (Wilcoxon
289 rank-sum tests, all $P > 0.05$).

290

291 Compared to metrics discussed above, the signature of selection persists in nucleotide diversity
292 (π) and absolute differentiation (D_{xy}) for more generations (45, 46). Therefore, π and D_{xy} can be
293 informative with regards to whether selection also occurred in the common ancestor of
294 populations under investigation. Consistent with a “selective sweep before selective population
295 differentiation” model (47, 48), we found that both metrics tend to be reduced at the same X-
296 linked locus relative to the rest of the chromosome in both ranges (Fig. 2E; SI Appendix, Figs.
297 S19-S22). These findings highlighting an important role of this locus to local adaptation in the
298 native range, and at multiple points throughout the evolutionary history of *A. sagrei*.

299

300 In summary, we find that the genomic signature of selection is different between the two ranges.

301 In the native range, an X chromosome locus frequently retains a signature consistent with
302 positive selection. By contrast, the same genomic region is evolving neutrally in most invasive
303 populations. Two non-mutually exclusive explanations can account for these results. First, if
304 evolution in both ranges is driven by similar selective forces, changes in the genomic target of

305 selection could have occurred in invasive populations, following hybridization among divergent
306 lineages and introgression of adaptive alleles at other loci in the genome. We consider this less
307 likely, given that the X chromosome locus is part of the genetic architecture of local adaptation
308 in most native-range lineages that contributed ancestry to invasive populations. Also, relative to
309 native populations, most invasive populations showed limited evidence of selection at the X
310 chromosome locus, despite varying in ancestry and extent of hybridization. The second
311 explanation is that temporary or permanent changes in selection pressure occurred during
312 biological invasion in *A. sagrei*.

313

314 Changes in selection pressure can contribute to differences in hybridization frequency that we
315 observe between ranges. For example, to the extent that dispersal in the native range occurs
316 between populations that are adapted to different environments, natural selection is expected to
317 limit within or among clade gene flow *via* both pre-mating and post-mating mechanisms (40,
318 41), thereby enhancing population genetic structure. Pre-mating isolation occurs when selection
319 removes maladapted immigrant genotypes before these can produce hybrid offspring (40). Post-
320 mating isolation occurs because hybrids are maladapted to the new environment as a result of
321 additive genetic effects when hybrids are intermediate relative to parents, dominant genetic
322 effects when hybrids are mismatched for different parental phenotypes (49), or epistatic genetic
323 effects when interactions among loci create departures from additivity (50).

324

325 **Epistatic interactions occur in hybrids among divergent lineages**

326 The availability of different hybrid genomic backgrounds and detailed trait information in
327 Florida *A. sagrei* allowed us to test if epistasis increases post-mating isolation among divergent

328 clades in the native range. Under this scenario, long-term isolation of *A. sagrei* clades led to the
329 random accumulation of divergent alleles genome-wide that interact with alleles at the adaptive
330 X chromosome locus. Thus, two native populations that are adapted to the same environment,
331 but are members of different clades, would still experience limited genetic exchange because
332 hybrids will be maladapted as a result of epistatic interactions between divergent alleles. In the
333 invasive range however, due to changes in selection pressure, hybrids are common and can
334 persist. To test for epistasis, we used genome-wide association (GWA) and included 13 traits that
335 describe the size and shape of lizards (SI Appendix, Methods and Dataset S2). Previous studies
336 have indicated that variation in all these traits might have an adaptive basis in anoles (51),
337 although most support so far has been obtained for limb length (discussed in ref. 25).

338

339 The linear-mixed model implemented in GEMMA (52) indicated that SNPs suggestively
340 associated with the length of the distal portion of hindlimb, including metatarsals and phalanges,
341 map to the same candidate adaptive locus that we identified on the X chromosome (Fig. 3A, B;
342 SI Appendix, Fig. S23). A stronger and genome-wide significant signal was observed for the
343 same trait at this genomic region using the asaMap association model (53), which allows effect
344 sizes to vary depending on ancestry ($P = 3.40 \times 10^{-7}$; Fig. 3A; SI Appendix, Fig. S23). As well,
345 asaMap analyses indicated that the same locus affects variation in several other components of
346 limb length (Fig. 3A; SI Appendix, Fig. S23). That the same locus is involved in the control of
347 multiple limb components is expected, given the strong positive correlation among these traits
348 across samples, after removing the effect of body size (SI Appendix, Fig. S24). Identifying the
349 genes that control variation in limb length in *A. sagrei* is outside the scope of this study.

350 Nonetheless, we note that among the 267 genes that span the 18 Mbp region on the X

351 chromosome, there are several candidate genes known to be involved in limb development.
352 These include *Cut Like Homeobox 2* (*Cux2*; ref. 54), *Growth Differentiation Factor 11* (*GDF11*;
353 ref. 55), *Noggin* (*Nog*; ref. 56), *T-Box Transcription Factor 1* (*Tbx1*; ref. 57), and
354 *Xylosyltransferase 1* (*Xylt1*; ref. 58). In sum, GWA findings indicate that the X chromosome
355 locus singled out by genome scan analyses affects limb length, variation of which is known to be
356 adaptive in anoles (25). These results reinforce our conclusion that positive selection (rather than
357 background selection) is acting at the X chromosome locus and implicate limb length as an
358 important component of adaptive divergence in the native range.

359

360 The asaMap analyses further indicated that the strength of association between alleles at the X
361 chromosome locus and limb length phenotypes vary among the lineages that are hybridizing in
362 Florida. Specifically, for three of the four traits for which the X chromosome locus was the top
363 genome-wide association, an effect was inferred for the Western Cuba ancestry component of
364 invasive hybrids (SI Appendix, Table S2), but not for the Eastern Cuba ancestry component.
365 These results are consistent with epistatic interactions between alleles at the X chromosome limb
366 length locus and alleles of Eastern Cuba origin that are located elsewhere in the genome. While
367 epistasis has traditionally been considered in relation to intrinsic (i.e., unconditional) isolation
368 under the Bateson–Dobzhansky–Muller model (59), evidence has been accumulating for a
369 contribution of such interactions to extrinsic isolation as well (e.g., ref. 50).

370

371 To further investigate these results, we stratified the Florida samples based on ancestry into two
372 groups. The first of these consisted of samples with predominantly Western Cuba ancestry and
373 low heterozygosity (SI Appendix, Methods and Fig. S25). Therefore, we refer to this group as

374 the “hybridization limited” group. The second group consisted of samples with ancestry from all
375 parental lineages and high heterozygosity (SI Appendix, Fig. S25). Therefore, we refer to these
376 samples as the “hybridization common” group. We then tested for an effect of genotype at the
377 limb length locus in each of these sample groups. In line with results presented above, when
378 considering samples with limited hybrid ancestry, we detected a large and significant effect of
379 genotype on all limb length traits but one. Effect sizes in this case ranged from moderate (PVE
380 5.7% - 9.3%) to large (PVE 10.03% - 13.08%; Fig. 3C, D). By contrast, no such effect was
381 observed in the sample group for which hybridization is common (Fig. 3C, D).

382

383 Similar patterns could arise if hybrid and non-hybrid samples differ with respect to linkage
384 between alleles at the genotyped SNP and the causal limb length SNP. This may occur in our
385 dataset, given that we used reduced representation sequencing, and therefore are likely not
386 genotyping causal variants. To evaluate this possibility, we repeated these analyses using only
387 samples with Western Cuba ancestry at the X chromosome locus (SI Appendix, Methods),
388 reasoning that linkage relationships are more likely to be similar among closely related
389 haplotypes. Results were equivalent to those for the complete dataset, as expected if epistatic
390 interactions rather than linkage disequilibrium underpin differences in effect sizes between
391 hybrid categories (SI Appendix, Fig. S26). A limitation of epistasis analyses presented above is
392 that we could not compare the effect of genotype at the limb length locus in hybrids relative to
393 decidedly non-hybrid *A. sagrei*. This limitation is because pure parental genotypes are rare in
394 Florida. Nonetheless, because of residual hybridization in the “hybridization limited” group, the
395 effect sizes that we estimate for these samples may well be conservative.

396

397 **Phenotype-environment correlations are consistent with changes in natural**
398 **selection during biological invasion**

399 Previous studies of local adaptation in anoles have relied, among other methods, on phenotype-
400 environment correlations. In the native range, a positive relationship exists both interspecifically
401 and among conspecific populations between the diameter of the perches that anoles use and limb
402 length (reviewed in ref. 25). Biomechanical studies reveal the underlying basis for this
403 relationship, specifically that the optimal limb length for lizard sprint speed and agility is a
404 function of surface diameter (60, 61). Although this relationship has been found repeatedly in
405 natural and experimental *A. sagrei* populations (25, 28, 29), it was not found in a comparison of
406 invasive *A. sagrei* populations in Florida (62).

407
408 To investigate whether this lack of a relationship is still the case, we used the 30 populations
409 from Florida and Southern Georgia described above for genomic and trait analyses, for which we
410 additionally obtained 1028 observations of habitat use (SI Appendix, Dataset S3). These
411 populations were chosen to avoid heavily disturbed or urban sites, such that nearly all habitat
412 measurements originated from natural vegetation, similar to Kolbe *et al.* (62). We found the
413 situation to be the same as in the Kolbe *et al.* (62) study, with no relationship between population
414 average values of relative limb length and perch diameter ($R^2 = 4.61 \times 10^{-5}$; $P = 0.97$; SI
415 Appendix Fig. S27). Thus, results from phenotype-environment correlation analyses are
416 consistent with genomic results above, indicating that changes in natural selection occurred
417 during biological invasion in *A. sagrei*. We note, however, that a caveat of these analyses is that
418 native populations used in previous studies are predominantly from islands in the Bahamas (25,

419 28, 29). Comparable data from Cuban populations that sourced the Florida invasion are currently
420 not available.

421

422 **Conclusions**

423 Our results indicate that changes in natural selection as inferred from genomic variation at a
424 large-effect adaptive X chromosome locus contribute to differences in hybridization frequency
425 among native and invasive populations of *A. sagrei*. In the native range, evidence of frequent
426 selective sweeps suggests that the X chromosome locus, which affects variation in limb length, is
427 an important component in the adaptive response of *A. sagrei* populations to the environment. To
428 the extent that migration occurs among contrasting environments in the native range, adaptive
429 divergence could limit gene flow among populations within or between clades. Ancestry-specific
430 association analyses, which we could perform thanks to the availability of invasive hybrids,
431 additionally showed that the same X chromosome locus is involved in epistatic interactions when
432 hybridization occurs among divergent lineages. This result indicates that native range extrinsic
433 isolation may be stronger between populations from different clades and provides an example on
434 the value of studying invasive populations for understanding evolution in the native range.

435

436 Although *A. sagrei* has been reasonably well sampled across its range in Cuba, more detailed
437 study of the contact zones between clades is needed. Combining genomic data with trait and
438 habitat data will provide in-depth information on native range gene flow and environmental
439 drivers of local adaptation. In the invasive range, natural selection as it is manifested in the
440 native range appears to have been disrupted. Here, hybrid ancestry occurs in all populations and
441 has stabilized irrespective of whether gene flow occurred within or among divergent lineages.

442

443 Whether hybridization had a major role in the success of the *A. sagrei* invasion remains to be
444 definitively established. Nonetheless, available evidence points towards hybridization-mediated
445 biological invasion. Specifically, hybrid ancestry occurs in all invasive populations. Were
446 hybridization merely a consequence of repeated introductions, we would have expected to find a
447 mosaic of hybrid and non-hybrid ancestry across the invasive range. Aside from being
448 widespread, hybrid ancestry has stabilized: samples collected 15 years apart, while representing a
449 narrow snapshot in the history of invasive *A. sagrei*, did not reveal changes in ancestry. Thus,
450 even if currently neutral, stability of new ancestry combinations should increase the chance that
451 adaptive allele combinations are available when invasive populations are exposed to novel
452 selective pressures.

453 **Methods**

454 **Sequencing and Variant Calling**

455 To obtain genome-wide SNP data, we used reduced representation sequencing (RADseq; SI
456 Appendix, Methods). We aligned quality-filtered reads to the *A. sagrei* reference genome v2.0
457 (63) in the dDocent v2.2.20 pipeline (64). We then performed joint genotyping using Freebayes
458 v. 1.3.2 (65) including data from the 897 *A. sagrei* libraries (885 samples and 12 replicates),
459 along with 128 other *A. sagrei* libraries that were part of a related project. To decrease SNP
460 calling runtime and following Freebayes manual recommendations, we only called the six best
461 alleles. We next applied stringent variant filtering and estimated post-filtering genotyping errors
462 (SI Appendix, Methods).

463

464 **Spatial population genetic structure across the range of *A. sagrei***

465 To summarize population structure, we used PCA in “adeget” (v. 2.1.1; ref. 66). Fine-scale
466 population structure in Western Cuba was explored using a separate PCA. For each analysis, we
467 identified markers genotyped in at least 99% of samples with a minor allele frequency > 1%.
468 From this set, to decrease computational time, we selected 10,000 random SNPs using the
469 “vcfrandomsample” tool from *vcflib* (<https://github.com/vcflib/vcflib>). These SNPs were located
470 on chromosomes 1-5 of the v2.0 reference genome, which are equivalent to chromosomes 1-6 of
471 the v2.1 reference genome. For consistency, we will refer only to genome coordinates v2.1
472 throughout. We complemented the PCA with estimates of the *A. sagrei* phylogeny, Bayesian
473 clustering, identity-by-state, and isolation-by-distance (SI Appendix, Methods).

474 **Temporal changes in the ancestry of invasive hybrids**

475 To investigate whether the ancestry of invasive hybrids has stabilized or is changing, we
476 revisited in 2018 six populations that we sampled in 2003 ($N = 172$; SI Appendix, Fig. S9). We
477 targeted the same sites, or sites located as close as possible to the original 2003 sampling. We
478 first used PCA, as described above. Additionally, we modeled temporal changes at three metrics
479 of hybrid status, as follows. First, we identified a set of ancestry informative markers (AIMs) that
480 are diagnostic of the Western Cuba lineage. To be classified as AIMs, SNPs needed to be scored
481 in 70% or more of Western Cuba and non-Western Cuba samples, and show an allele frequency
482 of 20% or lower in one group, and 80% or higher in the other. In all, 711 SNPs fit these criteria,
483 of which 469 were scored at high quality in the 2003 and 2018 invasive samples. To summarize
484 Western Cuba ancestry, we then averaged AIM allele frequency for each invasive genotype.

485
486 Second, we calculated an index of admixture (H_A), following Keller & Taylor (12). As input, we
487 used the STRUCTURE results from the $K=6$ analysis with prior population information (Fig.
488 1B). Third, we calculated heterozygosity using 155,905 filtered SNPs from chromosomes 1-6
489 (for details on SNP filtering, see identity-by-state analyses section, SI Appendix, Methods). To
490 test whether AIM allele frequency, H_A , or heterozygosity changed over 15 years, we used three
491 linear models in R v. 3.6.1 (67). These had each of the three metrics as the response variable, and
492 population IDs and time (2003 or 2018) as the predictor variable.

493

494 **The genomic signature of natural selection in *A. sagrei***

495 We combined information from relative differentiation (F_{ST}), Tajima's D , Fay and Wu's H test,
496 nucleotide diversity (π), and absolute differentiation (D_{xy}). For the invasive range, we used the

497 30 populations sampled in 2018 from Florida and Southern Georgia ($N = 560$; SI Appendix,
498 Dataset S1). For the native range, we used all 10 populations ($N = 134$; SI Appendix, Dataset
499 S1). Prior to performing analyses along the genome, we updated genome coordinates from v2.0,
500 which was used to align reads and call SNPs to the most recent version (v2.1), which includes
501 changes to sequence coordinates but does not differ in sequence content. We then removed
502 gametolog SNPs (i.e. SNPs resulting from Y-chromosome reads that align to the X chromosome;
503 SI Appendix, Methods), and imputed any missing data at the remaining 123,882 SNPs in
504 BEAGLE v5.0 (68).

505

506 For F_{ST} analyses, we used population-pairwise comparisons and calculated F_{ST} in non-
507 overlapping windows of 50 kb in VCFtools (v. 0.1.16; ref. 69). To avoid pseudoreplication, we
508 used only unique population pairs (see SI Appendix, Methods for details on population pairing).
509 We then classified windows as “outliers” if weighted F_{ST} was in the top 5% of observations (i.e.,
510 we sorted windowed F_{ST} for each population pair, and obtained windows in the top 5%).
511 Similarly, “extreme outliers” were windows in the top 1%. Aside from evaluating how F_{ST} varies
512 along the genome, this approach additionally allowed us to investigate the repeatability of F_{ST}
513 differentiation, using a permutation approach implemented from Rennison *et al.* (ref. 70; SI
514 Appendix, Methods).

515

516 Tajima’s D was calculated in non-overlapping windows of 50kb in VCFtools. To estimate Fay
517 and Wu’s H , we used “PopGenome” (v. 2.7.5; ref. 71). We first incorporated an outgroup from
518 publicly available sequence data (SI Appendix, Methods), retaining 139 SNPs scored at the
519 divergent X chromosome locus for all samples and for the outgroup. To get an estimate of

520 background neutral H values at the X chromosome, we obtained another set of 139 SNPs with
521 outgroup data. Similar to SNPs from the divergent X chromosome locus, these were located in
522 the male-hemizygous region, between PAR1 and PAR2. To minimize effects of genetic
523 hitchhiking which would extend the signature of selection in the vicinity of an adaptive locus,
524 candidate neutral SNPs were from the opposite end of the X chromosome (i.e., adjacent to
525 PAR1). We then compared H values obtained for each range at the divergent X chromosome
526 locus to neutral values, and for each locus category between ranges, using Wilcoxon rank-sum
527 tests in R, adjusting P values for multiple comparisons using the Bonferroni method. Lastly,
528 while analyses of selection focused on the 2018 invasive range samples, we also contrasted H
529 values between the 2003 and 2018 samples, for the six invasive populations with temporal data.
530 To do this, we followed the same approach as described above for the full dataset.

531
532 To calculate nucleotide diversity (π), and absolute differentiation (D_{xy}), we repeated the SNP
533 calling step for the X chromosome, retaining monomorphic sites as well. We filtered the output
534 keeping genotypes supported by at least four reads, and sites with data in at least 70% of samples
535 used for the genome scan analyses. Also, we removed gametolog SNPs using the same approach
536 as for the 123,882 SNP set described above, used in the rest of the genome scan analyses (see
537 also SI Appendix, Methods). We then used the python script “popgenWindows.py”
538 (https://github.com/simonhmartin/genomics_general; ref. 72) to estimate π and D_{xy} in non-
539 overlapping windows of 50kb along the X chromosome, based on windows with at least 50 sites
540 with data. For D_{xy} , we used the same pairs of populations as in the F_{ST} analyses. We compared
541 average per-population π from within the divergent X chromosome locus (107 windows) to a

542 candidate neutral locus of the same size (107 windows) using the same approach as for Fay and
543 Wu's *H* test above.

544

545 **Genetic mapping of candidate adaptive traits in natural hybrid populations**

546 We measured 13 morphological traits that describe body size (SVL), as well as the shape of
547 lizards using image analysis of X-rays (SI Appendix, Methods and Dataset S2). Measurements at
548 skeletal traits were isolated from the effect of SVL by calculating residuals from linear
549 regressions of log-transformed trait values on log-transformed SVL in R. Two GWA approaches
550 were then used: a linear mixed model in GEMMA (52), and ancestry-specific association in
551 asaMap (53). Both analyses were based on the samples obtained in 2018 from Florida and
552 Southern Georgia ($N = 560$). We filtered a VCF containing these 560 samples using the same
553 criteria as above. As well, similar to the genome scan analyses, we removed gametolog SNPs
554 and imputed any missing data that remained after filtering. For the GEMMA analyses, we used a
555 leave-one-chromosome out approach when calculating the relatedness matrix. For the asaMap
556 analyses, to account for population structure, we included as covariates the first 10 PCs from a
557 PCA constructed in “adegenet” for all samples in the analysis. Also, for both GWA approaches,
558 we included transect as an additional covariate (see SI Appendix, Methods for additional details).

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722 **Figure legends**

723 **Figure 1: Spatial genetic structure and diversity of *A. sagrei*.** **A**, PCA of all samples, and a
724 separate PCA of the Western Cuba native range subset (inset). Native genotypes are in color,
725 invasive genotypes are in grey. The arrow points to one genotype identified as a hybrid in the
726 native range. **B**, STRUCTURE membership, with samples grouped by range and population.
727 Each of the 44 populations is separated by white dotted lines. With the exception of one sample
728 that showed evidence of admixture, all native range genotypes are treated as learning samples (SI
729 Appendix, Methods). Cuba populations are arranged from West to East. Invasive populations are
730 arranged in decreasing order of Western Cuba ancestry. **C**, Heterozygosity rate and deleterious
731 SNP proportion, averaged per population. *P* values are from two-sided Wilcoxon rank-sum tests.
732

733 **Figure 2: Genome scans for selection.** **A**, average genome-wide F_{ST} for the native range (top)
734 and the invasive range (bottom) for chromosomes 1-10. **B**, Zoomed-in view of average F_{ST} along
735 the X chromosome (chromosome 7). **C**, Zoomed-in view of Tajima's *D* along the X
736 chromosome, averaged for windows with Tajima's *D* estimates at 50% or more of populations
737 within each range. For both **B** and **C**, empty circles are used for the native range and filled circles
738 are used for the invasive range. Colored lines show a loess smoothing ("span" of 0.2) for the
739 native range (red) and the invasive range (black). The grey shading marks the 18 Mbp X
740 chromosome locus where most native-range F_{ST} outliers are located (SI Appendix, Fig. S12).
741 The dashed vertical lines indicate the boundaries of the pseudo-autosomal regions (PARs). **D**,
742 Fay and Wu's *H*, calculated within the X locus and at a region outside of this locus, used to
743 estimate background neutral *H* levels. Lines connect *H* estimates obtained for the same
744 population. **E**, π values for genomic windows within the divergent X locus and for background
745 neutral values. Grey dots show global average π . For both **D** and **E**, *P* values are from paired
746 Wilcoxon rank-sum tests, using averages estimated per population and locus category (Methods).
747

748 **Figure 3: Genetic architecture of limb length.** **A**, Genome-wide associations for the relative
749 length of the distal portion of hindlimb, inferred using the GEMMA association model (top) and
750 the asaMap ancestry-specific association model (bottom). For the asaMap results, white dots
751 show the smallest P values for three other traits for which the same locus was identified as the
752 top genome-wide association (see SI Appendix, Fig. S23 for GWA results for each trait). The red
753 dashed lines are the Bonferroni-corrected significance thresholds, while the black dashed lines
754 indicate the suggestive significance thresholds (SI Appendix, Methods). **B**, Overlap between
755 GWA for limb length and F_{ST} outliers in the native range. The upper plot indicates, for
756 chromosome 7, the position of the top 1% SNPs identified based on strength of GWA (blue
757 lines) or F_{ST} (grey lines). F_{ST} values are from the Mariel x Guanabo population pair (SI
758 Appendix, Fig. S12). The lower plot shows asaMap association results (blue dots), and Mariel x
759 Guanabo F_{ST} values per SNP (grey dots) along chromosome 7. The black line is a loess
760 smoothing (“span” of 0.2) of association results. The grey shading marks the 18 Mbp X
761 chromosome divergent locus, and the dashed vertical lines indicate the boundaries of the two
762 PARs on the X chromosome. **C**, Relative hindlimb length for samples with small (SM) and large
763 (LG) alleles at the lead SNP identified on chromosome 7 using the asaMap model. Trait values
764 are given separately for the “hybridization limited” and the “hybridization common” sample
765 groups. Points and error bars indicate mean and standard deviation. **D**, Effect sizes of genotypes
766 at the same SNP as in panel C, calculated for all traits, for each of the two sample groups
767 separately. Asterisks indicate significant main effects of genotype after Bonferroni correction.
768 For each sample group ancestry proportions are shown, as estimated using a STRUCTURE
769 analysis at $K=2$ and $K=6$ (see also SI Appendix, Fig. S25).