



Rapid evolution of a native species following invasion by a congener

Citation

Stuart, Y. E., T. S. Campbell, P. A. Hohenlohe, R. G. Reynolds, L. J. Revell, and J. B. Losos. 2014. "Rapid Evolution of a Native Species Following Invasion by a Congener." *Science* 346 (6208) [October 23]: 463–466. doi:10.1126/science.1257008.

Published Version

10.1126/science.1257008

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1 **Title: Rapid evolution of a native species following invasion by a congener**

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14

15 **Abstract:** In recent years, biologists have increasingly recognized that evolutionary change can
16 occur rapidly when natural selection is strong; thus, real time studies of evolution can be used to
17 test classic evolutionary hypotheses directly. One such hypothesis, that negative interactions
18 between closely related species can drive phenotypic divergence, is thought to be ubiquitous
19 though well-documented cases are surprisingly rare. On small islands in Florida, we found that
20 the lizard *Anolis carolinensis* moved to higher perches following invasion by *Anolis sagrei* and,
21 in response, adaptively evolved larger toepads after only 20 generations. These results illustrate
22 that interspecific interactions can drive evolutionary change on observable time scales.

23

24 **One Sentence Summary:** Island populations of the lizard *Anolis carolinensis* have rapidly
25 undergone morphological change in response to shifts in habitat use driven by competitive
26 interactions with an invading, closely related lizard.

27

28 **Main Text:**

29 In their classic paper, Brown and Wilson (1) proposed that mutually negative interactions
30 between closely-related species could lead to evolutionary divergence when those species co-
31 occurred. In the six decades since, this idea has been debated vigorously, with support that has
32 vascillates based on the latest set of theoretical treatments and comparative studies (reviewed in
33 [(2-5)]). However, tests of interaction-driven evolutionary divergence have been slow to
34 capitalize on the growing recognition that evolutionary change can occur rapidly in response to
35 strong divergent natural selection (but see [(6-9)]); thus, evolutionary hypotheses about
36 phenomena once thought to transpire on time scales too long for direct observation can be tested
37 in real time while using replicated statistical designs.

38 An opportunity to study real-time divergence between negatively interacting species has
39 been provided by the recent invasion of the Cuban brown anole lizard, *Anolis sagrei*, into the
40 southeastern United States, where *Anolis carolinensis* was the sole native anole. These species
41 have potential to interact strongly (e.g., [(10)]), being very similar in habitat use and ecology
42 (11). We investigated the eco-evolutionary consequences of this interaction on islands in Florida
43 (12) using an *A. sagrei* introduction experiment, well-documented natural invasions by *A. sagrei*,
44 genomic analyses of population structure, and a common garden experiment. This multifaceted
45 approach can rule against several of the most difficult alternative hypotheses (e.g., plasticity,
46 ecological sorting, environmental gradients [(2, 5)]) while directly testing two predictions for
47 how *A. carolinensis* responds to its congeneric competitor.

48 Typical of solitary anoles (13), *A. carolinensis* habitat-use spans ground to tree crown
49 (14). However, where *A. carolinensis* and *A. sagrei* (or their close relatives) co-occur elsewhere,
50 *A. carolinensis* perches higher than *A. sagrei* (13-16). Thus, we used an introduction experiment
51 to test Collette's prediction (14) that competitive interactions with *A. sagrei* should drive an
52 increase in *A. carolinensis* perch height. In early May 1995, we chose six islands that contained
53 resident populations of *A. carolinensis* and collected pre-introduction perch height data from
54 undisturbed lizards (12). Later that month, we introduced small populations of *A. sagrei* to three
55 treatment islands, leaving three control islands containing only *A. carolinensis* (12). From May-
56 August 1995-1998, we measured perch heights for both species. The *A. sagrei* populations grew
57 rapidly (**Table S1**; [(17)]), and by August 1995, *A. carolinensis* on treatment islands already

58 showed a significant perch height increase relative to controls, which was maintained through the
59 study (**Fig. 1; Fig. S1; Table S2; [(12)]**).

60 We next predicted, following (14), that this arboreal shift by *A. carolinensis* would drive
61 the evolution of larger toepads with more lamellae (adhesive, setae-laden, subdigital scales).
62 Toepad area and lamella number (body-size corrected) correlate positively with perch height
63 among anole species (14, 18-20). Larger and better developed toepads improve clinging ability
64 (20), permitting anoles to better grasp unstable, narrow, and smooth arboreal perches. We tested
65 the prediction in 2010 on a set of islands partially overlapping those used in 1995-1998 (12). We
66 surveyed 30 islands and found that *A. sagrei* had colonized all but five (12). We compared *A.*
67 *carolinensis* populations on these five islands without the invader (hereafter “un-invaded”) to *A.*
68 *carolinensis* populations on six islands that, based on 1994 surveys, were colonized by *A. sagrei*
69 sometime between 1995 and 2010 (hereafter “invaded”) (**Fig. 2; [(12)]**).

70 From May-August 2010, we measured perch height for undisturbed lizards and found
71 that, as in the 1995 introduction experiment, *A. carolinensis* perch height was significantly
72 higher on invaded islands (**Fig. S2; Table S3; [(12)]**). We then tested whether the perch height
73 shift had driven toepad evolution by measuring toepad area and lamella number of the 4th toe of
74 each hindleg for every *A. carolinensis* captured (12). We found that *A. carolinensis* on invaded
75 islands indeed had larger toepads and more lamellae (traits corrected for body size; **Fig. 3; Table**
76 **S3; [(12)]**).

77 This morphological change occurred quickly. Assuming conservatively that *A. sagrei*
78 reached all six invaded islands in 1995, *A. carolinensis* populations on invaded and un-invaded
79 islands have diverged at mean rates of 0.091 (toepad area) and 0.077 (lamellae) standard
80 deviations per generation (*haldanes* [(21)]; rates > zero, each one-tailed $p < 0.02$; [(12)]),
81 comparable to other examples of rapid evolution (21) such as soapberry bug beak length (22) or
82 guppy life history (23).

83 We tested several alternative processes that could have generated the observed
84 divergence. First, we used a common garden experiment to investigate possible post-hatching,
85 developmental responses to physical challenges imposed by arboreality during growth (*i.e.*,
86 phenotypic plasticity). We took gravid *A. carolinensis* females from four invaded and four un-
87 invaded islands in July 2011, collected their eggs in the lab, and raised the offspring in identical
88 conditions (12). The effect of *A. sagrei* invasion on *A. carolinensis* toepad characteristics

89 persisted in the common garden (**Fig. 3; Table S4; [(12)]**), suggesting genetically based
90 divergence in nature (though we cannot rule out trans-generational plasticity).

91 Second, observed divergence in *A. carolinensis* could have arisen through non-random
92 migration of individuals with large toepads among invaded islands, instead of independently on
93 each island. Thus, we tested whether relatedness among *A. carolinensis* populations is
94 independent of *A. sagrei* invasion. In 379 *A. carolinensis* individuals from 4 un-invaded and 5
95 invaded islands, we genotyped 121,973 single nucleotide polymorphisms across the genome
96 (**Table S5, [(12)]**). Individuals from the same island were closely related, and islands were
97 largely genetically independent (pairwise- F_{ST} 0.09-0.16; **Table S6**). We found no evidence that
98 population relatedness in *A. carolinensis* was correlated with whether an island had been
99 colonized by *A. sagrei* (**Fig. 4; [(12)]**) or with distance between islands (Mantel test; $p > 0.25$),
100 suggesting that gene flow is relatively limited among islands and that island populations were
101 independently founded from the mainland.

102 Third, toepad changes could have been generated by adaptation to environmental
103 differences among islands that are confounded with the presence of *A. sagrei* [*e.g.*, (24)].
104 Invaded and un-invaded islands, however, do not differ in characteristics important to perching
105 or arboreal locomotion (*e.g.*, vegetated area, plant species richness, or available tree heights;
106 **Table S7; [(12)]**). Fourth, toepad changes could have arisen through ecological sorting, wherein
107 *A. sagrei* was only able to colonize those islands on which the existing *A. carolinensis*
108 population was already sufficiently different. However, *A. sagrei* seems capable of successfully
109 colonizing every island it reaches, regardless of resident *A. carolinensis* ecology/morphology: all
110 ten *A. sagrei* populations introduced in 1994-1995 are still extant (12), and *A. sagrei* inhabits
111 nearly every other island surveyed in the lagoon (**Fig. 2**). Finally, toepad changes observed in
112 2010 could be unrelated to interactions with *A. sagrei* if the latter's invasion merely missed the
113 five islands with the lowest *A. carolinensis* perch heights (**Fig. S2**) by chance; however, this
114 would occur only one time in 462. In sum, alternative hypotheses of phenotypic plasticity,
115 environmental heterogeneity, ecological sorting, non-random migration, and chance are not
116 supported; our data suggest strongly that interactions with *A. sagrei* have led to evolution of
117 adaptive toepad divergence in *A. carolinensis*.

118 Brown and Wilson called evolutionary divergence between closely related, sympatric
119 species 'character displacement' (1), and our data constitute a clear example. Resource

120 competition has been the interaction suggested most often as the source of divergent selection
121 during character displacement (sometimes specifically called ‘ecological character displacement’
122 [(1-3)]). For *A. carolinensis* and *A. sagrei*, resource competition for space likely is important:
123 allopatric *A. carolinensis* and *A. sagrei* overlap in their use of the habitat (12-14, 16); moreover,
124 when they co-occur, the two species interact agonistically (10), and our experimental data show a
125 rapid spatial shift by *A. carolinensis* following *A. sagrei* introduction. The two species also
126 overlap in diet and thus may compete for food (17). Competition for food is strong among co-
127 occurring *Anolis* and has been shown to be mitigated by differences in perch height (11).
128 Evolutionary divergence may also arise, however, from selection to reduce interspecific
129 hybridization, yet such ‘reproductive character displacement’ (4) seems an unlikely explanation
130 for our results as *A. carolinensis* and *A. sagrei* already differ markedly in species-recognition
131 characteristics, males of both species nearly exclusively ignore heterospecifics in staged
132 encounters (25), and the species have never been reported to successfully produce hybrids. We
133 note, finally, that other mutually negative interactions like apparent competition (26) and
134 intraguild predation (27) could also produce divergence among overlapping species. These
135 remain to be explored in this system, though some evidence exists for at least the latter (17).

136 Here, we have provided evidence from a replicated, natural system to support the long-
137 held idea (4) that interspecific interactions between closely related species are an important force
138 for evolutionary diversification (2). Moreover, we show that evolutionary hypotheses like
139 character displacement can be rigorously tested in real time following human-caused
140 environmental change. Our results also demonstrate that native species may be able to respond
141 evolutionarily to strong selective forces wrought by invaders. The extent to which the costs of
142 invasions can be mitigated by evolutionary response remains to be determined (28), but studies
143 such as this demonstrate the ongoing relevance of evolutionary biology to contemporary
144 environmental issues.

145

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248

249 We thank A. Kamath, C. Gilman, A. Algar, J. Allen, E. Boates, A. Echternacht, A. Harrison, H.
250 Lyons-Galante, T. Max, J. McCrae, J. Newman, J. Rifkin, M. Stimola, P. VanMiddlesworth, K.

251 Winchell, C. Wiench, K. Wollenberg, and three reviewers; M. Legare and J. Lyon (Merritt Island
252 National Wildlife Refuge), J. Stiner and C. Carter (Canaveral National Seashore); Harvard
253 University, Museum of Comparative Zoology, University of Massachusetts, University of
254 Tennessee, University of Tampa, NSF (DEB-1110521) and NIH (P30GM103324) for funding.
255 Y.E.S., T.S.C., and J.B.L. designed the study; Y.E.S., T.S.C., P.A.H., L.J.R, and R.G.R.
256 collected the data; Y.E.S., T.S.C., and P.A.H. analyzed the data; all authors contributed to the
257 manuscript. Data are accessioned on datadryad.org:xxxxxxx.

258

259 **Supplementary Materials:**

260 www.sciencemag.org/content/###/###/###/suppl/XX#

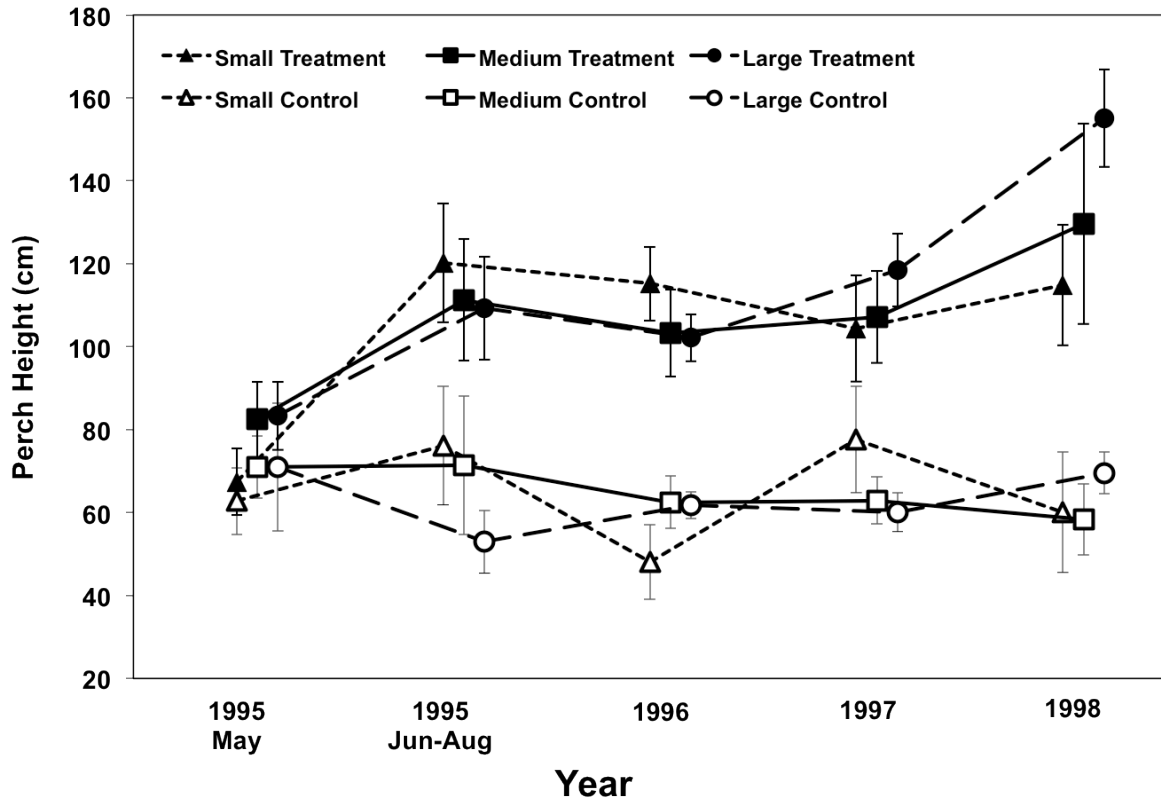
261 Materials and Methods

262 Figs. S1 to S2

263 Tables S1 to S7

264 References (29-45)

265

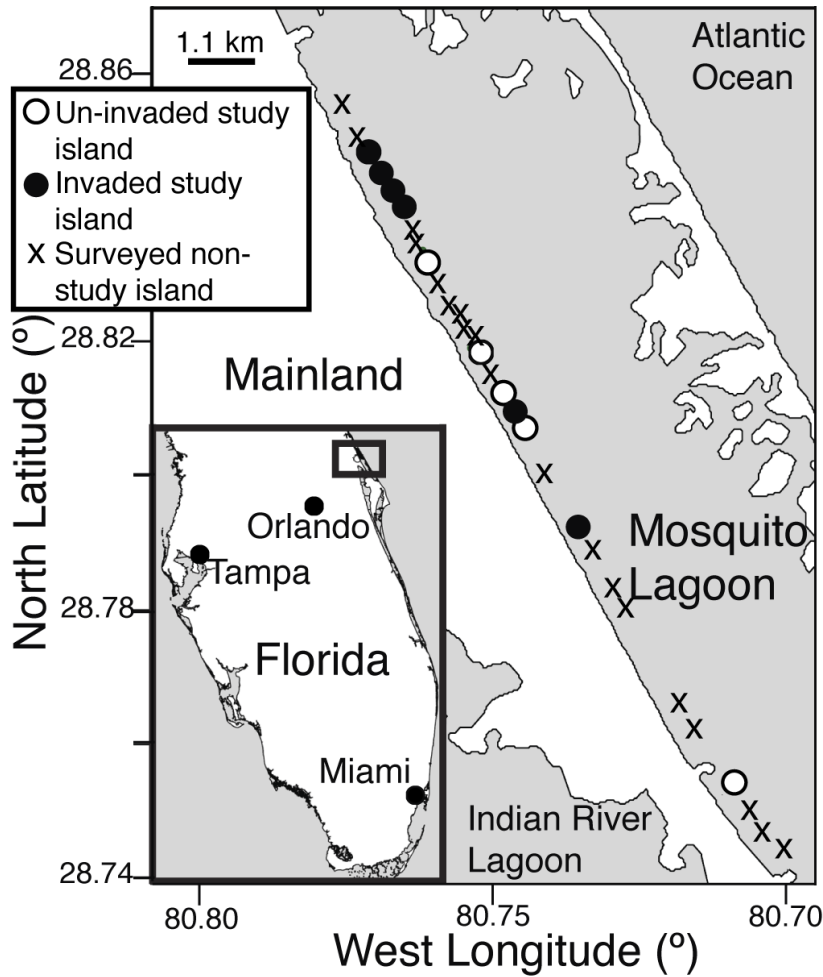


267

268 **Fig. 1. Perch height shift by *A. carolinensis* after the experimental introduction of *A. sagrei*.**

269 We introduced *A. sagrei* to one small, one medium, and one large island (treatment; closed
 270 shapes) in 1995, keeping three similarly-sized control islands (open shapes). Island means
 271 (± 1 s.e.) are shown for perch height. *Anolis sagrei* introduction corresponds with a significant
 272 perch height increase by *A. carolinensis* (Linear Mixed Models: treatment x time interactions, all
 273 $p < 0.001$; [(12)]; **Table S1**; **Table S2**).

274



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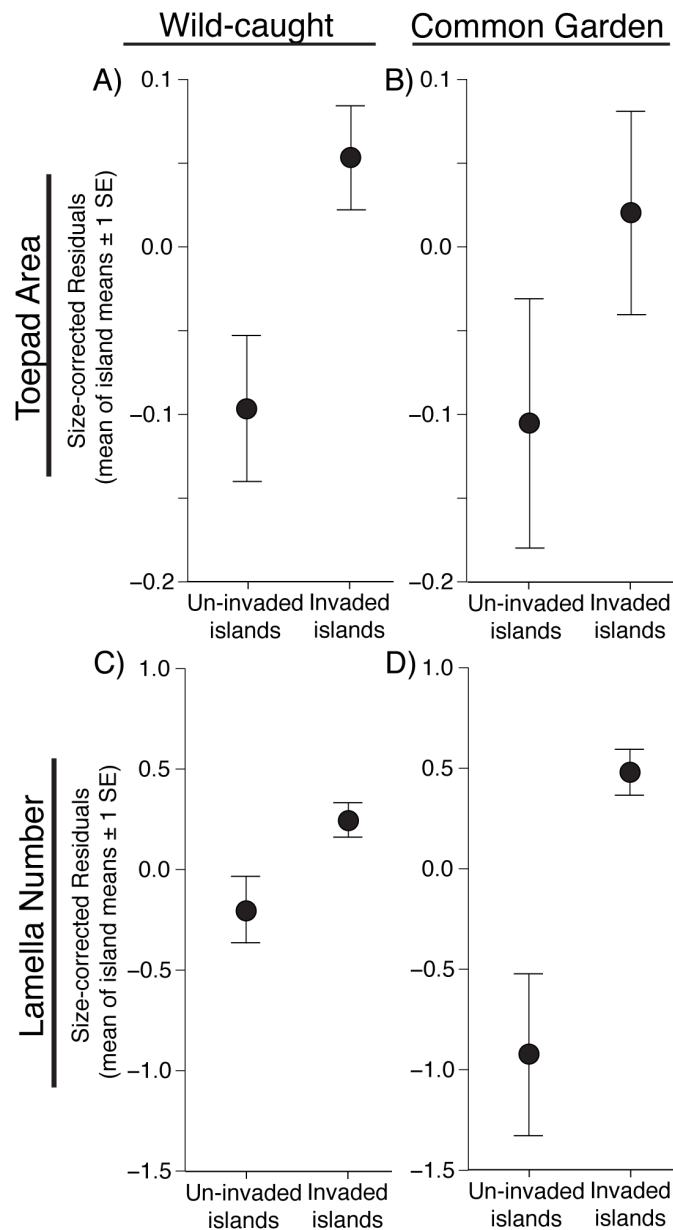
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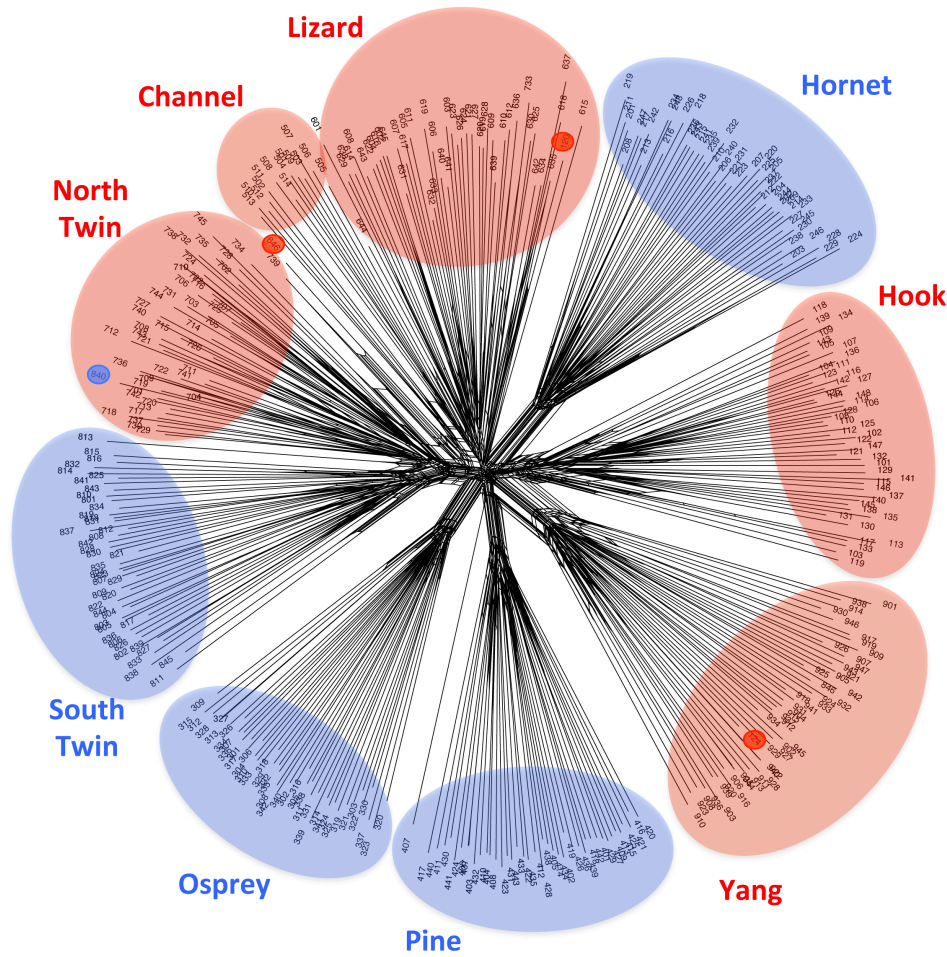
280

Fig. 2. 2010 study islands along the Intracoastal Waterway. *Anolis carolinensis* inhabits all study islands. Six study islands were invaded by *A. sagrei* sometime between 1995 and 2010 (closed circles) and five study islands remain un-invaded today (open circles). 19 additional non-study islands were surveyed ('x'; [(12)]); 17 were invaded by *A. sagrei* and two were empty of both species.



281

282 **Fig. 3. Divergence in wild-caught (A, C) and common garden *A. carolinensis* (B, D).** Mean-
 283 of-island-means, size-corrected residuals (± 1 s.e.) are shown. The invasion of *A. sagrei*
 284 corresponds to a significant increase in both traits for wild-caught lizards (A, C) in 2010 (5
 285 islands un-invaded, 6 invaded; Linear Mixed Models [LMM]; **Panel A:** Toepad Area, $\beta_{invaded}$
 286 $=0.15$, $t_9=2.7$, $p=0.012$; **Panel C:** Lamella Number, $\beta_{invaded}=0.54$, $t_9=3.1$, $p=0.009$). Common
 287 garden offspring from invaded islands had significantly larger toepad characteristics (4 un-
 288 invaded islands; 4 invaded; LMM; **Panel B:** Toepad Area, $\beta_{invaded}=0.14$, $t_6=2.1$, $p=0.043$; **Panel**
 289 **D:** Lamella Number, $\beta_{invaded}=1.45$, $t_6=3.6$, $p=0.006$). All p -values one-tailed.



291

292 **Figure 4. Neighbor-net analysis of genetic distance for *A. carolinensis* individuals from**
 293 **invaded (red) and un-invaded (blue) islands (12).** Small shaded areas enclose individuals that
 294 do not cluster with their own island; the color of these areas represents invasion status of their
 295 home islands.

296

297 **Supplementary Materials:**

298 Materials and Methods

299 Tables S1-S7

300 Figures S1-S2

301 References (31-44)

302 Full Acknowledgments

303

304 **Materials and Methods:**

305 Terminology

306 The terms native, invasive, invaded, natural, and introduced have accrued multiple
307 connotations across the invasive species literature. Therefore, we define our use of these terms
308 here. We treat *A. carolinensis* as a native species because it has existed on the mainland United
309 States for ca. 2 million years (29). *Anolis carolinensis* is ubiquitous in the Mosquito Lagoon
310 region and its colonization of the spoil islands does not constitute a range expansion; therefore,
311 we consider it a native species on the spoil islands even though colonization of those man-made
312 islands is recent. By contrast, *A. sagrei* is native to Cuba and the Bahamas. It colonized southern
313 Florida in the 1940s (14) and spread into the rest of Florida as well as Georgia and Louisiana.
314 Hence, we refer to *A. sagrei* as an invasive species, and we term the spoil islands on which it has
315 established populations as invaded. Furthermore, we wish to make a distinction between
316 colonization by *A. sagrei* that is the result of natural processes versus those that are purposefully
317 manipulated by researchers. We term those instances where we purposefully colonized islands
318 with *A. sagrei* as introductions; thus, the 1995-1998 study is an introduction experiment.

319

320 We first discuss the natural history of the dredge spoil islands and then describe the two
321 studies reported in the main text: (1) the 1995-1998 introduction experiment, and (2) the 2010
322 study of character displacement in toepad characteristics.

323

324 Dredge Spoil Island Natural History

325 The Mosquito Lagoon dredge spoil islands used in these studies were created by the US
326 Army Corps of Engineers (17) as a byproduct of the digging of the Intracoastal Waterway
327 (ICW). An old, obsolete section of ICW channel built prior to the 1950s exists along the eastern

328 edge of the lagoon. The new, active channel of the ICW was dredged along the western edge of
329 the lagoon in the 1950s. Spoil islands exist along both the old and the active channel.

330 Along with other flora and fauna from the nearby mainland, *A. carolinensis* colonized the
331 islands in the decades following island creation (17). We observed *A. carolinensis* in
332 (presumably) marginal mangrove and salt marsh environments on every island visited in 2010.
333 This suggests that *A. carolinensis* populations could have reached the islands through natural
334 colonization shortly after the creation of the islands without requiring the late-successional,
335 present-day plant community dominated by broad-stemmed woody species (e.g., *Juniperus*
336 *virginiana* and *Sabal palmetto*). *Anolis sagrei* arrived to the mainland surrounding the lagoon in
337 the late 1980s (30).

338 But for the occasional nocturnal gecko (*Hemidactylus sp.*), we observed no other lizards
339 on the islands during research from 2009-2011. The bird faunas on these islands are depauperate
340 and mostly feature waterfowl; we observed red-winged blackbirds (*Agelaius phoeniceus*) and
341 common nighthawks (*Chordeiles minor*) infrequently, and other insectivorous birds were
342 observed even more rarely, suggesting little competition for insects with the *Anolis* species from
343 birds. Several spider species inhabited the islands at noticeable frequency (*Nephila clavipes*,
344 *Gasteracantha cancriformis*, *Argiope aurantia*, *Eriophora ravilla*, *Phidippus spp.*), but their
345 competitive relationship with the lizards on these islands remains to be studied (see [(11)] for
346 discussion of anole-spider interactions). The most commonly observed lizard predators on these
347 islands were black racers (*Coluber constrictor*) and raccoons (*Procyon lotor*). Racers were seen
348 only occasionally and not often enough to compare invaded and un-invaded islands. We did not
349 collect quantitative data on raccoons but they were observed on nearly every island and likely
350 only prey on lizards opportunistically. Very little is known about parasites in *A. carolinensis* and
351 *A. sagrei* (see [(11)]). Occasionally, we observed unidentified insect larvae that were living
352 subcutaneously emerge through the skin of adult *A. carolinensis*.

353

354 (1) Introduction Experiment (1995-1998)

355 A pilot introduction of *A. sagrei* to Six-Palm and Coon Islands indicated that *A. sagrei*
356 populations would expand rapidly following introduction (30). To assess the speed and
357 magnitude of the effects of *A. sagrei* invasion on *A. carolinensis* demography and habitat use, we
358 conducted an introduction experiment on six spoil islands in Mosquito Lagoon from 1995 to

359 1998. We chose matched pairs of small (ca. 0.1 ha), medium (ca. 0.2 ha), and large (ca. 1.0 ha)
360 islands and flipped a coin to determine which island in each pair would be subjected to a
361 purposeful introduction of *A. sagrei* (**Table S1**) in a random-blocked design. Throughout May
362 1995, before initiating the *A. sagrei* introductions, we sampled *A. carolinensis* on all six islands
363 using Rand surveys (31), whereby we walked through the habitat slowly until we observed an
364 undisturbed adult lizard. We then measured its perch height to the nearest 1 cm using a tape
365 measure. We marked all lizards with unique numbers (with permanent markers and by toe-
366 clipping) to prevent double-counting; thus, all lizards in the perch height analyses were unique
367 individuals. On May 27 and 28, 1995, we captured 120 *A. sagrei* from urban sites on the
368 surrounding mainland near New Smyrna Beach and marked and released 40 of these *A. sagrei*
369 (20M:20F) on each of the three treatment islands. We only observed four *A. sagrei* on the large
370 treatment island in the few weeks subsequent to their release, so we increased propagule pressure
371 by adding 40 more *A. sagrei* to this island in early June 1995 to encourage the establishment on
372 this much larger island. From June through August 1995, and throughout the summers (May to
373 August) of 1996, 1997, and 1998, we used the same methods to collect perch height data for *A.*
374 *carolinensis* and the introduced *A. sagrei* populations.

375 The small treatment (ST) and small control (SC) islands are located on the eastern edge
376 of Mosquito Lagoon in the old channel of the ICW near Eldora, FL (28.91, -80.82; [(17)]). Island
377 ST, 0.5 km north of Eldora, is 0.16 ha in total area, with a central forested area of 0.04 ha
378 (dominant species: *Juniperus virginiana*, *Schinus terebinthifoliusis*, *Sabal palmetto*) flanked on
379 the north, east, and south by extensive regularly inundated salt marsh (*Spartina alterniflora* and
380 *Batis* sp.). Island SC, 0.2 km south of Eldora, is 0.12 ha in total area, with a central forested area
381 of 0.02 ha (same dominant species) flanked on the east and south by a narrow strip of regularly
382 inundated salt marsh (*Spartina alterniflora* and *Batis* sp.). The medium treatment (MT) is located
383 in the island chain along the western edge of Mosquito Lagoon (where the 2010 toepad study
384 was conducted) and is 0.17 ha, with vegetation the same as ST and SC, but the forested area
385 (0.10 ha) comprises a larger percentage of this island, and the salt marsh only occurs on the north
386 and west edges. The medium control (MC; 0.15 ha) is also located along the western edge of
387 Mosquito Lagoon near the south end of the island chain. It is very similar to Island MT in
388 forested area (0.08 ha) and marsh area, which only flanks the south and east edges of the island.

389 Finally, the small and medium islands are similar to the large islands in that they represent
390 smaller versions of the forested area on the large islands and support similar vegetation (17).

391 The two large treatment and control islands (LT and LC, respectively) are also located on
392 the western edge of Mosquito Lagoon along the new, active channel of the ICW. Both are large
393 sand piles with open, desert-like central areas rimmed by forested ‘hedges’ and relatively small,
394 triangular, marsh ‘tails’ extending westward towards the mainland. LT (0.89 ha) has 0.21 ha
395 forested area composed of *Juniperus virginiana*, *Schinus terebinthifoliusis*, and *Sabal palmetto*.
396 LC (0.94 ha) is physically very similar to Island LT, with 0.16 ha forested area. LC, a National
397 Park Service backcountry campsite is frequently used by boaters, and thus was naturally invaded
398 by *A. sagrei* at the end of the introduction experiment in 1998. We removed a few *A. sagrei* in
399 early May of 1998 to maintain its integrity as a control island for the introduction experiment
400 throughout that summer. By 2010, this LC population of *A. sagrei* was fully established; both LT
401 and LC were used as invaded islands for the 2010 toepad study, described next (**Table S1**). (MC
402 and SC were also invaded naturally by *A. sagrei* between 1998 and 2010).

403 For the 1995-1998 introduction experiment, we used linear mixed models to analyze *A.*
404 *carolinensis* perch height data because such models incorporate within-island variation by
405 nesting islands as a random effect within the fixed treatment effect (*i.e.*, the introduction of *A.*
406 *sagrei*) (32). We square-root transformed the perch data to improve normality in the model
407 residuals. We termed the variable representing the five time points during which perch heights
408 were measured “event”; “event” included 1995 pre-introduction (May), 1995-post introduction
409 (June – August), 1996, 1997, and 1998. We conducted our analyses using the *lme()* function in
410 the R package *nlme* (33) and built the following full model that includes treatment, event, and
411 sex as explanatory variables: $\text{lme}(\sqrt{\text{perch height}}) \sim \text{treatment} + \text{event} + \text{sex} + \text{treatment}*\text{event}$
412 $+ \text{treatment}*\text{sex}$, random = $\sim \text{sex} \mid \text{island}$). The $\text{treatment}*\text{sex}$ interaction was not significant so
413 we built the following reduced model: $\text{lme}(\sqrt{\text{perch height}}) \sim \text{treatment} + \text{event} + \text{sex} +$
414 $\text{treatment}*\text{event}$, random = $\sim \text{sex} \mid \text{island}$). Residuals from this model were normally distributed
415 and model output is reported in **Table S2**. The $\text{treatment}*\text{event}$ interaction was significant, as
416 would be expected if *A. sagrei* drives a perch height increase in *A. carolinensis*. At each time
417 point post introduction of *A. sagrei*, *A. carolinensis* perches significantly higher on treatment
418 islands compared to controls (**Table S2**; ($\beta_{\text{treatment}}$ ranges from 2.09 to 3.47, t_{1627} ranges from 3.3
419 to 5.0; all one-tailed $p < 0.001$). Male lizards perch significantly higher than females ($\beta_{\text{male}} =$

420 1.85, $t_{1627} = 10.1$, one-tailed $p < 0.001$). Treatment itself was not significant in this model ($p >$
421 0.36; **Table S2**) because *A. carolinensis* perch heights were measured on treatment islands
422 before *A. sagrei* introduction in early 1995 (**Fig. 1**). To investigate the effects of treatment
423 further, we built the same model but for a dataset pruned to include only perch height data
424 collected post-introduction. This model found that sex remained a significant predictor of *A.*
425 *carolinensis* perch height ($\beta_{\text{male}} = 1.95$, $t_{1384} = 10.0$, one-tailed $p < 0.001$). The treatment effect
426 was significant in this model ($\beta_{\text{treatment}} = 2.98$, $t_4 = 5.4$, one-tailed $p < 0.003$; **Table S2**), but the
427 treatment*event interaction was no longer significant (all $p > 0.39$; **Table S2**). This is consistent
428 with **Fig. 1**: most perch height shift occurred in 1995 just after introduction, and perch height
429 remained mostly level 1996-1998.

430

431 (2) Character Displacement in Toepads (2010)

432 We wanted to determine whether a perch height shift by *A. carolinensis* in response to the
433 invasion of *A. sagrei* drove toepad evolution in the former species. From presence absence
434 surveys in 2009 and 2010, we found five islands un-invaded by *A. sagrei* with only *A.*
435 *carolinensis* present. We compared perch heights and toepads of *A. carolinensis* populations on
436 these islands to *A. carolinensis* on six islands where *A. sagrei* had invaded. The six invaded
437 islands were chosen because they were similar in size, shape, and vegetation to the un-invaded
438 islands (see below).

439

440 *Study Island History and Choice, and Accounting for Environmental Heterogeneity*

441 In 1994, along the western edge of Mosquito Lagoon following the main channel of the
442 ICW, Campbell surveyed for *A. carolinensis* and *A. sagrei* on 23 spoil islands. Of these 23
443 islands, all but two had populations of *A. carolinensis*. Of the 21 islands with *A. carolinensis* on
444 them, by 1994, two islands were already invaded by *A. sagrei*. Four more of these 21 islands had
445 *A. sagrei* purposefully introduced to them in 1994 and 1995: LT and MT from the introduction
446 experiment described above, and islands Six-Palm and Coon as part of a separate pilot study
447 described in (30). By the end of the introduction experiment, island LC had been colonized
448 naturally by *A. sagrei*, bringing the total invaded to seven of the 21. We surveyed these 23
449 islands again in 2009 and 2010 and found that *A. sagrei* had also invaded 12 more islands
450 through natural colonization (including MC from the introduction experiment), leaving just two

451 islands of the original 23 with just *A. carolinensis* (recall that two islands were empty in 1994
452 and remained so in 2010). We surveyed 7 more islands along the western edge of the lagoon,
453 revealing three additional islands with only *A. carolinensis*, making for a total of 5 un-invaded
454 islands with just *A. carolinensis* out of 30 islands surveyed. Thus, we chose these 5 islands as our
455 “controls” and complemented them with six “treatment” islands from the original 23 that were
456 similar to the controls in size, shape, and vegetation structure but were invaded by *A. sagrei*
457 sometime between 1995 and 2010 (**Table S3**). The five un-invaded islands are interspersed
458 between invaded islands (**Fig. 2**). Two of the six invaded islands (LC and LT) were part of the
459 introduction experiment described above.

460 We did not use MT, MC, ST, or SC because they were much smaller than required,
461 compared to the five un-invaded “control” islands. Beyond LT, MT, and ST, seven more
462 purposeful introductions of *A. sagrei* were made by Campbell: two on the western edge of the
463 lagoon along the new, active ICW channel in 1994 (Six-Palm and Coon described above; [(30)]),
464 and five in 1995 on the eastern edge of the lagoon along the old ICW channel. Similarly, these
465 five introduced old-channel islands were not used because they were not comparable to the five
466 controls in size or age. However, that 10 of 10 purposeful introductions of *A. sagrei* were
467 successful on islands that varied in size and age suggests that *A. sagrei* can colonize any spoil
468 island and that ecological sorting is not responsible for the patterns observed in 2010 (see main
469 text).

470 We tested for environmental heterogeneity between invaded and un-invaded islands in
471 the 2010 study. To estimate distance to the mainland, island area, and vegetated area for each
472 island in the study, we used Google Earth. We used logistic regression to test whether these
473 variables are associated with the presence or absence of *A. sagrei* (**Table S7**).

474 To test for differences in available tree heights and vegetation species richness, we
475 conducted point-quarter habitat surveys of island vegetation. Islands have two distinct habitat
476 types: a forested edge and an open center. Within the forested edge, we used Google Earth to
477 haphazardly choose survey points along an outer circle close to the forest/water edge and an
478 inner circle near the forest/center edge. For the open center, we surveyed three to four points
479 along three to four regularly placed north-south transects, the number of points and transects per
480 island depending on island size. At each point, we recorded the species identity for the four
481 closest trees (one in each quarter) and then measured their heights. We also recorded the species

482 identities of the four closest shrubs (one in each quarter). As above, we used logistic regression
483 with invaded/un-invaded status as the response variable and available tree heights and two
484 species richness metrics were used as the predictor variables. Species richness was calculated
485 using both the Shannon and Simpson diversity indices using the *diversity()* function in the R
486 (version 2.14.1, R Development Core Team) package *vegan* (34). Results are shown in **Table**
487 **S7**.

488

489 *Perch Height*

490 First, to establish that individual *A. carolinensis* were still perching higher in the presence
491 of *A. sagrei*, as found in the introduction experiment, we visited each island on average 8.3 times
492 from May-August 2010, usually visiting sometime between 7am and 2pm. We collected lizard
493 perch height data using the Rand survey method (31), whereby we walked through the habitat
494 slowly until we observed an undisturbed adult lizard. We noted the perch at which the lizard was
495 first observed and measured the height of the perch to the nearest cm with a tape measure.
496 Sample sizes are in **Table S3**.

497 We again used linear mixed models to analyze perch height data (32). We square-root
498 transformed the perch data to improve normality in the model residuals. We conducted our
499 analyses using the *lme()* function in the R package *nlme* (33) and built a full model that includes
500 sex as an explanatory variable as follows: $\text{lme}(\text{sqrt}(\text{lizard perch height}) \sim \text{sagrei presence} + \text{sex} +$
501 $\text{sagrei presence} * \text{sex}, \text{random} = \sim \text{sex} | \text{island})$. The interaction term in the full model was not
502 significant so we built the following reduced model: $\text{lme}(\text{sqrt}(\text{lizard perch height}) \sim \text{sagrei}$
503 $\text{presence} + \text{sex}, \text{random} = \sim \text{sex} | \text{island})$. Residuals from this model were normally distributed.
504 The presence of *A. sagrei* significantly predicts perch height in *A. carolinensis* populations (see
505 main text for statistics), even after significant perch differences by sex are taken into account
506 ($\beta_{\text{male}} = 1.94, t_{807} = 3.7$, one-tailed $p < 0.001$).

507 Previous studies of *Anolis* have found that limb length correlates positively with lizard
508 perch diameter (reviewed in [(11)]), so we also measured diameter of lizard perches to the
509 nearest 0.1cm. We found no difference in perch diameter use by *A. carolinensis* on invaded and
510 un-invaded islands (Linear Mixed Model, log-transformed data, no interaction: $\beta_{\text{invaded island}} =$
511 $0.17, t_9 = 1.49, p = 0.17$; $\beta_{\text{male}} = -0.02, t_{768} = -0.27, p = 0.29$; island sample sizes 52-108), so there
512 was no functional basis to predict limb length evolution. Thus, we focused solely on the

513 prediction that *A. sagrei* should drive the evolution of enhanced toepads in sympatric *A.*
514 *carolinensis*.

515 The focus of both the 1995-1998 introduction experiment and the 2010 study has been
516 the influence of the invader *A. sagrei* on habitat use and morphology in *A. carolinensis*. We
517 weren't able to ask the converse, whether *A. carolinensis* influences *A. sagrei* perch use (and
518 subsequently toepad morphology), because of a dearth of comparable islands with just *A. sagrei*
519 present. However, comparisons among populations throughout the Caribbean suggest that *A.*
520 *carolinensis* does indeed influence *A. sagrei* ecomorphology. Compared to populations where *A.*
521 *sagrei* is the lone anole, *A. sagrei* sympatric with *A. carolinensis* perch lower (13, 35) and have
522 fewer lamellae (36). This suggests that the negative interactions between the two species are
523 indeed mutual although perhaps not always symmetric. On the spoil islands, we should expect
524 the response to be asymmetrical. *Anolis sagrei* have invaded Florida from Cuba, where close
525 relatives of *A. carolinensis* exhibit a similar ecomorphology to *A. carolinensis* (15). Spoil island
526 *A. carolinensis*, on the other hand, are being exposed to *A. sagrei* for the first time, and therefore
527 have the potential to be affected more strongly, as they have not already evolved to interact with
528 *A. sagrei*.

529

530 *Toepad Evolution*

531 We captured lizards with noose poles and returned captured lizards to our field
532 laboratory. For every adult lizard caught, we measured toepad area and lamella number from
533 flatbed digital scans (2400 dpi) of the fourth toe of each hind foot. This toe is commonly used in
534 studies of *Anolis* toepad functional morphology, so we measured it in our study to maximize the
535 comparability of our data to that obtained in other research; however, we also note that lamellae
536 measures from different toes are significantly correlated in *A. carolinensis* (18). Specifically,
537 Glossip and Losos (18) counted lamellae on toes 2-5 on the fore- and hindfeet of 42 male and 24
538 female *A. carolinensis*. They found that males have more lamellae on each toe than females
539 (mean difference = 1.2; t -test > 2.74 , $p < 0.01$ in all cases), which is consistent with the sex effect
540 in our data (see below). Glossip and Losos also found that for males, 25 of 28 pairwise
541 comparisons showed significant correlations between lamella number on different toes (hindfoot
542 toe 2 vs. hindfoot toe 4 and hindfoot toe 5 versus hindfeet toes 3 and 4 being the exceptions).
543 Fifteen of 28 pairwise comparisons for females showed significant correlations for lamella

544 number among toes; specific non-significant comparisons for females were not reported but the
545 authors noted “no pattern of which comparisons are significant and which are not” (18).

546 We measured lamella number by counting all lamellae on the third and fourth phalanges
547 of the toe and traced the area encompassed by those lamellae to measure toepad area. We
548 measured both traits for right and left toes and averaged sides for each trait for analysis. We also
549 measured snout-to-vent length (svl) using calipers, as a proxy for body-size used for correction
550 during analysis. Captured lizards were released at site of capture following measurement. To
551 prevent repeated measures of the same individual, lizards were marked with temporary ink and
552 permanent subcutaneous VI Alpha Tags (Northwest Marine Technologies) prior to release.
553 Sample sizes are in **Table S3**.

554 As above, we used linear mixed models to nest island random effects within our *A.*
555 *sagrei*-presence fixed effect. For toepad area and lamella number, separately, we built full
556 models that included lizard sex and svl as random effects: $\text{lme}(\text{trait} \sim \text{sagrei presence} * \text{sex} * \text{svl},$
557 $\text{random} = \sim \text{sex} + \text{svl} \mid \text{island})$, where trait is either toepad area or lamella number. Neither the
558 three-way interaction term nor any of the two way interaction terms were significant so we chose
559 a reduced model that did not include interaction terms: $\text{lme}(\text{trait} \sim \text{sagrei presence} + \text{sex} + \text{svl},$
560 $\text{random} = \sim \text{sex} + \text{svl} \mid \text{island})$. Residuals from this model were normally distributed for both
561 traits.

562 The presence of *A. sagrei* was a significant predictor for both toepad area and lamella
563 number (see main text for statistics). Toepad area was also significantly predicted by sex ($\beta_{\text{male}} =$
564 $0.46, t_{551} = 4.4, \text{one-tailed } p < 0.001$) and svl ($\beta_{\text{svl}} = 0.12, t_{551} = 12.8, \text{one-tailed } p < 0.001$), as
565 was lamella number ($\beta_{\text{male}} = 0.88, t_{551} = 4.5, \text{one-tailed } p < 0.001$) and svl ($\beta_{\text{svl}} = 0.04, t_{551} = 2.4,$
566 $\text{one-tailed } p = 0.008$). Some evidence suggests that scale number in lizards might be fixed at
567 hatching (37), suggesting that size correction for lamella number is unnecessary. We built a
568 model, as above, but without including svl as a main effect. Results were qualitatively
569 unchanged. The presence of *A. sagrei* remained a significant predictor for lamella number
570 ($\beta_{\text{invaded island}} = 0.53, t_9 = 3.0, \text{one-tailed } p = 0.002$) as did sex ($\beta_{\text{male}} = 1.27, t_{547} = 13.4, \text{one-tailed}$
571 $p < 0.001$).

572

573 *Rates of Divergence*

574 We calculated the mean rate of divergence for toepad area and lamella number using the
575 *haldane* (h), a measure of the proportional change per generation in standard deviation units (21).
576 This method assumes that the two populations (or sets of populations) are diverging from a
577 similar ancestral state. We used the equation

$$578 \quad h = \left((x_s / s_p) - (x_a / s_p) \right) / g .$$

579 x is the mean of island trait-means for either size-corrected toepad area or size-corrected lamella
580 number. Subscript s represents islands where *A. carolinensis* is sympatric with *A. sagrei* (*i.e.*,
581 invaded islands) while subscript a represents islands where *A. carolinensis* is allopatric to *A.*
582 *sagrei* (*i.e.*, un-invaded islands). g is the number of generations since divergence began, which
583 we conservatively take to be 20 generations as *A. carolinensis* likely has slightly more than one
584 generation per year and *A. sagrei* began colonizing the islands during or after 1995. s_p is the
585 pooled standard deviation of the island means across a and s islands; this value was calculated as
586 the square root of the within mean-squared error taken from a linear regression of size-corrected
587 trait mean against *A. sagrei* presence or absence. p -values were calculated using a randomization
588 test, whereby a and s were assigned to island means in every possible permutation and h was
589 recalculated in each case to provide a distribution of h values. We compared our observed h
590 values to this distribution. R scripts are available from the authors.

591

592 *Common Garden Experiment*

593 In late July 2011, we collected gravid *A. carolinensis* females from four invaded and four
594 un-invaded islands. We returned these gravid females to common cage conditions in an
595 environmentally controlled room within the University of Massachusetts Boston animal care
596 facility. Females were housed individually in Critter Keepers with bamboo dowels, cage carpet,
597 and a potted plant for laying eggs. Cages were illuminated with full-spectrum lighting. Lizards
598 were misted twice daily and fed 2-3 times per week with crickets that had been fed Flukers
599 Orange Cubes and Flukers High Calcium Cricket Diet. Directly before feeding to lizards,
600 crickets were also dusted with vitamin and calcium powders.

601 We checked plant pots for eggs three times per week from August-November 2011. We
602 collected, incubated, and hatched all laid eggs. We raised the offspring in individual cages and
603 shuffled cages regularly to randomize any within room environmental variation. Offspring were

604 fed and misted by the same regimen as adults, except that smaller cricket sizes were used as
605 appropriate to the size of the lizard.

606 We raised the offspring for six months and then measured toepad area and lamella
607 number, as described above. Because of low sample sizes (**Table S4**), we did not differentiate by
608 sex in our models as our field data demonstrate significant effects of the presence of *A. sagrei*
609 regardless of whether sex is included in the model. We did not include an indicator for each
610 hatchling's dam, as there were no differences among dams from invaded and un-invaded islands
611 in svl, mass, or body condition (mass/svl) (Linear Mixed Models. svl: $\beta_{\text{sagrei present}} = -0.13$, $t_6 = -$
612 0.19 , $p = 0.86$; mass: $\beta_{\text{sagrei present}} = 0.11$, $t_6 = 1.07$, $p = 0.33$; body condition: $\beta_{\text{sagrei present}} = 0.002$,
613 $t_6 = 1.34$, $p = 0.23$).

614 For toepad area and lamella number, individually, we built a full model that included
615 lizard svl as a random effect: $\text{lme}(\text{trait} \sim \text{sagrei presence} * \text{svl}, \text{random} = \sim \text{svl} | \text{island})$. The
616 interaction term was not significant so we chose the following reduced model: $\text{lme}(\text{trait} \sim \text{sagrei}$
617 $\text{presence} + \text{svl}, \text{random} = \sim \text{svl} | \text{island})$. Juvenile svl was not a significant predictor of lamella
618 number in this model ($\beta_{\text{svl}} = 0.07$, $t_{41} = 1.4$, one-tailed $p = 0.09$).

619

620 *Population genetics*

621 To test the hypothesis that the observed evolutionary changes in multiple invaded islands
622 are independent, we assessed genetic relationships among the study populations of *A.*
623 *carolinensis* with genomic data. We used restriction-site associated DNA sequencing (RADseq)
624 to discover and genotype a large number of single-nucleotide polymorphism (SNP) loci across
625 individuals from nine study islands (**Table S5**). Following established protocols (38), we created
626 libraries for sequencing from 384 individuals. We used unique 6bp barcodes to multiplex 192
627 samples in each of two lanes of 100bp single-end sequencing on an Illumina HiSeq machine (U.
628 Oregon).

629 We obtained just over 404 million sequence reads. We de-multiplexed raw reads and
630 filtered for the presence of a correct barcode and restriction site using Stacks (39), leaving 314.8
631 million reads. We then aligned raw reads against the *A. carolinensis* reference genome (version
632 2.0.75) using Bowtie2 (40), discarding reads that aligned to more than one location in the
633 reference. We called diploid genotypes using a maximum likelihood model (as described by
634 [(39, 41)], implemented using code available at

635 <http://webpages.uidaho.edu/hohenlohe/software.html>, with a Phred quality score minimum of 10
636 and prior bounds on the nucleotide error rate of 0.001 and 0.1. Genotypes were called at 161,038
637 RAD tag loci. From these genotypes we identified single-nucleotide polymorphisms (SNPs)
638 across the complete set of individuals. We removed 5 individuals for low numbers of called
639 genotypes (*i.e.*, low coverage), and we removed any putative SNPs genotyped in fewer than 150
640 individuals, with minor allele frequency less than 0.05 across the combined sample set, or with
641 more than two alleles. This analysis and filtering produced a final dataset of 121,973 biallelic
642 SNPs genotyped across 379 individuals.

643 We assessed genetic clustering of individuals based on this set of SNPs with a neighbor-
644 joining phylogenetic network using SplitsTree4 version 4.13.1 (42), by using custom scripts to
645 convert genotypes at the 121,973 SNPs to nexus format. We used default settings for
646 SplitsTree4, which estimates uncorrected Hamming distance between individuals based on
647 diploid genotypes and generates a phylogenetic network with the NeighborNet algorithm (43).
648 We found island populations to be well-defined. There is no indication of clustering of islands by
649 invasion status, and the few individuals that do not cluster with their home island population
650 show no sign of preferential migration among islands of similar invasion status (**Figure 4**).

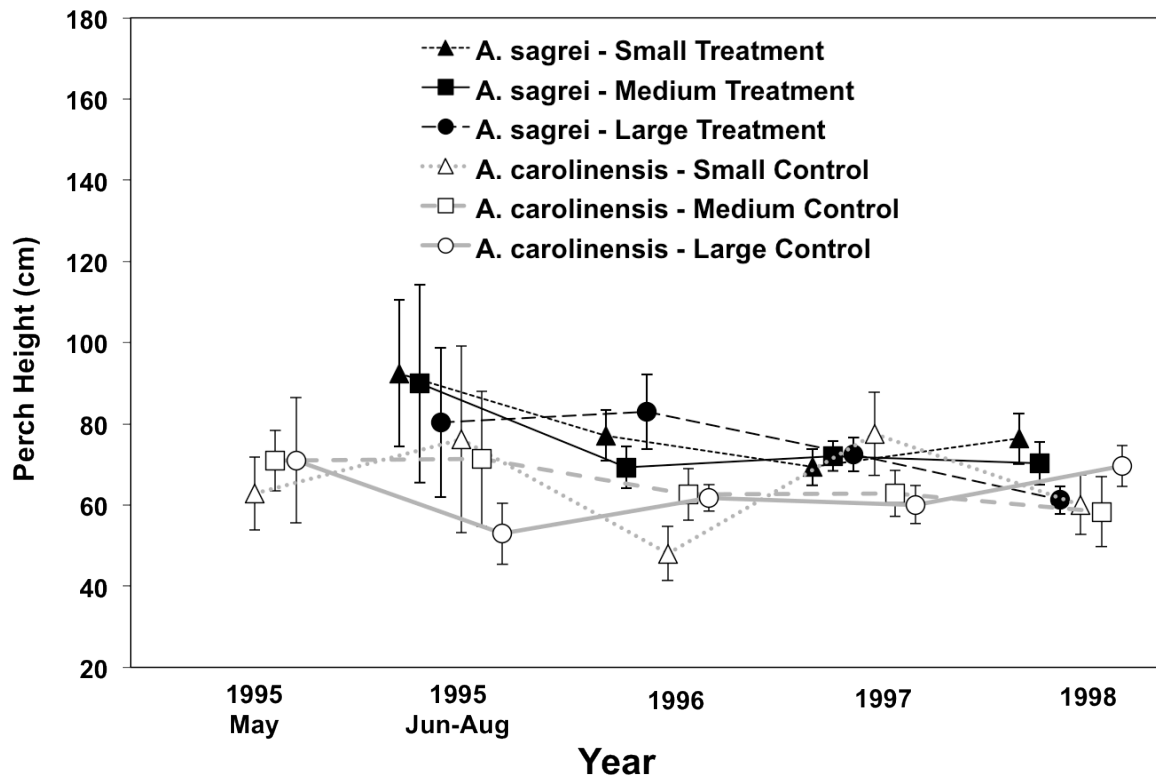
651 We also calculated the genome-wide average pairwise F_{ST} using the variance
652 decomposition method of (44) among all islands from the set of 121,973 SNPs (code available at
653 <http://webpages.uidaho.edu/hohenlohe/software.html>). We assessed grouping of islands based on
654 the pairwise F_{ST} matrix (**Table S6**) with several approaches: principal coordinates analysis using
655 the R function *cmdscale()* with varying levels of the number of dimensions k ; neighbor-joining
656 trees using the R package *APE* (45); and the NeighborNet algorithm in SplitsTree4. None of
657 these suggested any relationship between invasion status and genetic grouping of populations.
658 We also tested for a difference in mean F_{ST} depending on similarity or difference in invasion
659 status with a 2-sample t-test using the R function *t.test()*, which was not significant ($p > 0.5$). We
660 tested for isolation by distance using a Mantel test [R function *mantel.test()*] to compare matrices
661 of pairwise F_{ST} and geographic distance (**Table S6**) and found no relationship ($p > 0.25$).

662

663 **Full Acknowledgments:**

664 We thank A. Kamath, C. Gilman, A. Algar, J. Allen, J. Archer, E. Boates, A. Echternacht, F.
665 Gregg, A. Harrison, J. Kolbe, H. Lyons-Galante, J. McCrae, J. Newman, R. Pringle, J. Rifkin, M.

666 Stimola, P. VanMiddlesworth, K. Winchell, and K. Wollenberg for assistance; A. Algar and A.
667 Kamath for photographs; T. Max and C. Wiench for preparing RADseq libraries; three
668 anonymous reviewers for helpful comments and improvements; M. Legare and J. Lyon from
669 Merritt Island National Wildlife Refuge and J. Stiner and C. Carter from Canaveral National
670 Seashore for permission to conduct this research; Harvard University, Museum of Comparative
671 Zoology, University of Massachusetts Boston, University of Tennessee Knoxville, University of
672 Tampa, NSF (DEB-1110521) and NIH (P30GM103324) for funding.
673



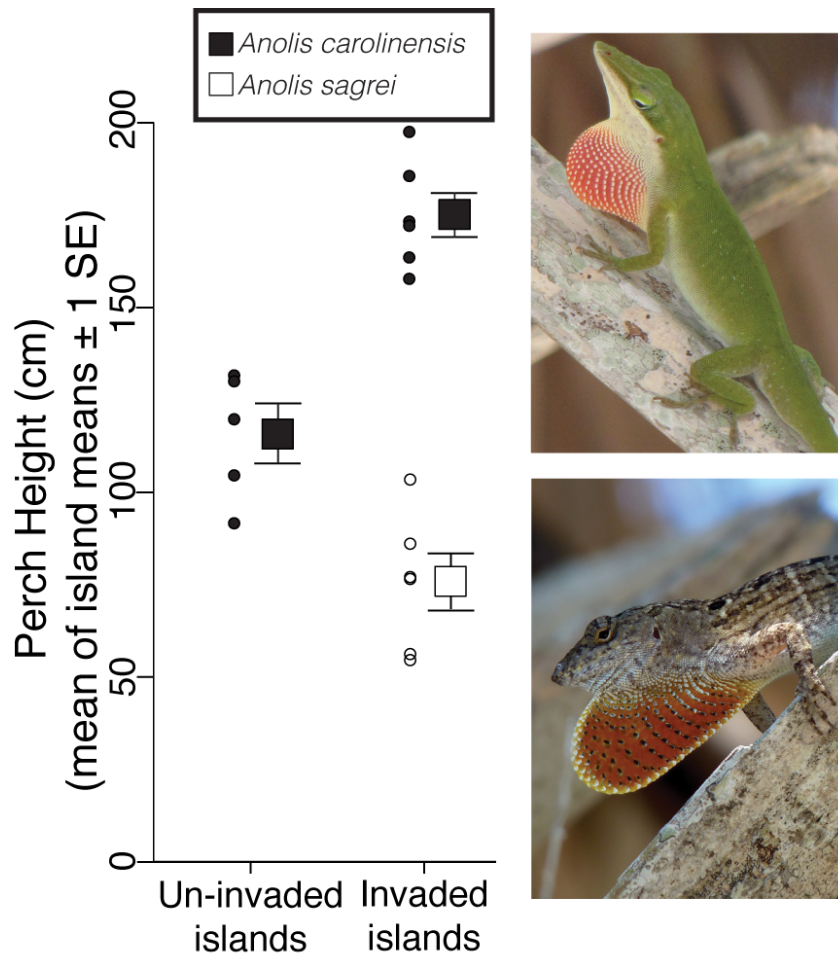
674

675 **Fig. S1** Perch height through time during the 1995-1998 introduction experiment for *A. sagrei*

676 (filled shapes) on treatment islands and allopatric *A. carolinensis* (open shapes) on control

677 islands. Island means (± 1 s.e.) are shown for each island.

678



679

680 **Fig. S2.** Habitat use shift by *A. carolinensis* in the 2010 toepad study. Mean of island means (\pm 1
681 s.e.) for perch height by *A. carolinensis* (closed squares) on un-invaded (n = 5) and invaded
682 islands (n = 6). The invasion of *A. sagrei* corresponds with a significant increase in perch height
683 by *A. carolinensis* (Linear Mixed Model: $\beta_{invaded\ island} = 2.77$, $t_9 = 6.6$, one-tailed $p < 0.001$; island
684 sample sizes 57-110). Perch height of *A. sagrei* shown for comparison (open square; n = 6).
685 Mean perch heights for each island for *A. carolinensis* (small, closed circles) and *A. sagrei*
686 (small, open circles) are shown also. Top right: *Anolis carolinensis*. Bottom right: *Anolis sagrei*.
687

688 **Table S1.** Sample sizes for *A. carolinensis* and *A. sagrei* perch heights by island in the 1995-
 689 1998 introduction experiment.

Island	Size	Type	1995 Pre- Introduction	1995 Post- Introduction	1996	1997	1998
<i>Anolis carolinensis</i>							
Zero	Small	Treatment	40	45	54	47	17
Ant	Medium	Treatment	64	26	88	15	11
Yin ^b	Large	Treatment	56	30	89	68	54
Fellers	Small	Control	22	9	34	27	32
Tarp	Medium	Control	45	23	84	78	41
Lizard ^b	Large	Control	18	45	213	146	121
<i>Anolis sagrei</i>							
Zero	Small	Treatment	n/a	23 ^a	89	157	140
Ant	Medium	Treatment	n/a	10 ^a	97	289	144
Yin	Large	Treatment	n/a	4 ^a	41	218	291

690 ^a The number of first-captures of introduced individuals

691 ^b Yin (LT) and Lizard (LC) were included as “invaded” islands in the 2010 toepad study.

692

693 **Table S2.** Perch height analysis for the 1995-1998 *A. sagrei* introduction experiment. Mixed
 694 model output is shown for a datasets (A) including and (B) excluding pre-introduction perch
 695 height data (12).

A) Includes pre-introduction (May 1995) perch height data from treatment and control islands.

	β Coefficient	Standard Error	Degrees of Freedom	<i>t</i> -value	2-sided <i>p</i> - value
Intercept ^a	6.28	0.41	1627	17.18	0.000
Treatment ^b	0.50	0.49	4	1.02	0.365
1995 ^c	-0.47	0.58	1627	-0.81	0.418
1996	-0.37	0.45	1627	-0.83	0.405
1997	-0.23	0.46	1627	-0.51	0.607
1998	-0.04	0.47	1627	-0.09	0.925
Sex ^d	1.85	0.18	1627	10.12	0.000
Treatment*1995 ^e	2.48	0.74	1627	3.34	0.001
Treatment*1996	2.09	0.59	1627	3.57	0.000
Treatment*1997	2.34	0.63	1627	3.70	0.000
Treatment*1998	3.48	0.69	1627	5.03	0.000

B) Excludes pre-introduction (May 1995) perch height data from treatment and control islands.

	β Coefficient	Standard Error	Degrees of Freedom	<i>t</i> -value	2-sided <i>p</i> - value
Intercept ^a	5.76	0.43	1384	13.54	0.000
Treatment ^b	2.98	0.55	4	5.45	0.006
1996	0.09	0.46	1384	0.21	0.837
1997	0.23	0.47	1384	0.48	0.628
1998	0.42	0.49	1384	0.86	0.392
Sex ^d	1.95	0.20	1384	9.99	0.000
Treatment*1996	-0.39	0.63	1384	-0.62	0.533
Treatment*1997	-0.13	0.67	1384	-0.19	0.846
Treatment*1999	0.99	0.73	1384	1.36	0.175

696 ^a The intercept represents control islands at first collection (A: May 1995; B: June-August 1995).

697 ^b Treatment represents the effect of introduction on perch height, compared to controls.

698 ^c 1995 June-August, post-introduction.

699 ^d The sex coefficient represent the effect of being male on perch heights, compared to females.

700 ^e This is the interaction between treatment and June-August 1995, post-introduction.

701 **Table S3.** *Anolis sagrei* invasion status, *A. carolinensis* perch height sample size, and *A.*
702 *carolinensis* morphology sample size by island for the 2010 toepad study. For sample sizes,
703 males are listed before the “/” and females after. Yin and Lizard were the LT and LC islands,
704 respectively, in the 1995-1998 introduction experiment. For reference, in Fig. 2, from north to
705 south, the study islands (circles) are Lizard, Hook, Yin, Yang, Hornet, Crescent, Pine, North
706 Twin, South Twin, Channel, and Osprey.
707

Island	<i>A. sagrei</i> invasion	Perch height sample size (M/F)	Morphology sample size (M/F)
Channel	Yes	51 / 15	38 / 15
Crescent	No	50 / 12	38 / 10
Hook	Yes	53 / 22	42 / 16
Hornet	No	60 / 27	44 / 15
Lizard ^a	Yes	70 / 40	41 / 19
North Twin	Yes	49 / 21	33 / 11
Osprey	No	52 / 15	33 / 10
Pine	No	38 / 19	27 / 14
South Twin	No	60 / 38	34 / 24
Yang	Yes	57 / 14	41 / 16
Yin ^b	Yes	48 / 12	27 / 16

708 ^a The large control (LC) island in the 1995-1998 study.

709 ^b The large treatment (LT) island in the 1995-1998 study.

710

711

712 **Table S4.** *Anolis sagrei* invasion status, dam and hatchling sample size by island for the
 713 common garden experiment in the 2010 toepad study. For the column describing hatchlings per
 714 female, the numbers separated by colons denote how many hatchlings were reared to
 715 measurement per female.

716

Island	<i>A. sagrei</i> invasion	Dam sample size	Hatchling sample size	Hatchlings per female
Hornet	No	3	6	1:2:3
Lizard	Yes	6	12	1:1:1:2:3:4
North Twin	Yes	8	10	1:1:1:1:1:1:2:2
Osprey	No	5	8	1:1:1:2:3
Pine	No	1	2	2
South Twin	No	5	7	1:1:1:2:2
Yang	Yes	6	10	1:1:1:2:2:3
Yin	Yes	5	6	1:1:1:1:2

717

718

719 **Table S5.** RADseq summary statistics for the 2010 toepad study. *n* is number of individuals,
720 with the number after filtering for low coverage in parentheses. Number of SNPs is the mean
721 number genotyped per individual within each population, after filtering to a total of 121,973
722 SNPs.

723

Island	<i>A. sagrei</i> invasion	<i>n</i>	# SNPs genotyped
Channel	Yes	14	80,909.5
Hook	Yes	48	71,930.2
Hornet	No	48	96,405.3
Lizard	Yes	48 (46)	40,262.1
North Twin	Yes	46 (45)	15,628.0
Osprey	No	42	81,783.3
Pine	No	43	89,439.1
South Twin	No	47 (46)	94,641.3
Yang	Yes	48 (47)	94,794.1
Total		384 (379)	74,524.4

724

725

726 **Table S6.** Pairwise F_{ST} between islands estimated from 121,973 SNP loci above the diagonal,
 727 and geographic distance between island centers in meters below the diagonal. Invaded islands:
 728 Hook, Channel, Lizard, North Twin, Yang. Un-invaded islands: Hornet, Osprey, Pine, South
 729 Twin.

	Hook	Hornet	Osprey	Pine	Channel	Lizard	North Twin	South Twin	Yang
Hook	-	0.15	0.14	0.14	0.12	0.12	0.13	0.14	0.14
Hornet	1360	-	0.16	0.16	0.15	0.14	0.15	0.15	0.16
Osprey	12085	10726	-	0.16	0.14	0.13	0.15	0.15	0.16
Pine	4102	2742	7984	-	0.14	0.14	0.15	0.15	0.15
Channel	6659	5299	5428	2557	-	0.11	0.13	0.134	0.14
Lizard	499	1858	12584	4600	7157	-	0.11	0.13	0.14
North Twin	4471	3111	7615	370	2188	4969	-	0.09	0.15
South Twin	4758	3399	7328	656	1901	5256	288	-	0.15
Yang	482	879	11604	3620	6177	980	3989	4276	-

730

731

732

733 **Table S7.** Tests for environmental heterogeneity between un-invaded (n=5) and invaded (n=6)
 734 islands in the 2010 toepad study. Invasion status was treated as a binary variable and we used
 735 logistic regression to test whether the environmental variable could predict invasion status.
 736

Variable	β	Standard Error	Z-value	p-value (two-sided)
Distance to Shore (m)	0.006	0.007	0.770	0.44
Island Area (m ²)	0.0002	0.0002	0.995	0.34
Vegetated Area (m ²)	0.00001	0.00001	0.115	0.908
Available Tree Heights (cm)	0.282	1.03	-0.275	0.784
Shannon Diversity Index	4.99	6.61	0.775	0.450
Simpson Diversity Index	18.33	22.29	0.822	0.411

737