



# Rapid evolution of a native species following invasion by a congener

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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 4<sup>th</sup> 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

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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#](http://www.sciencemag.org/content/###/###/###/suppl/XX#)

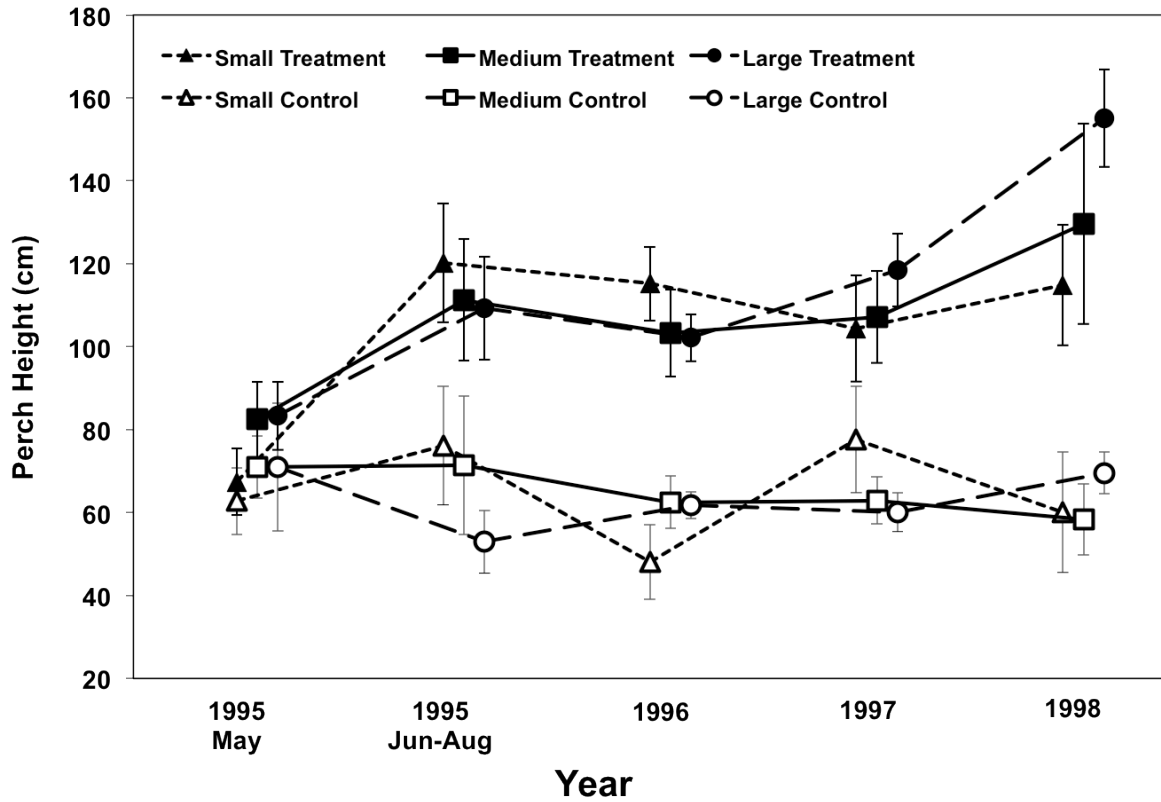
261 Materials and Methods

262 Figs. S1 to S2

263 Tables S1 to S7

264 References (29-45)

265

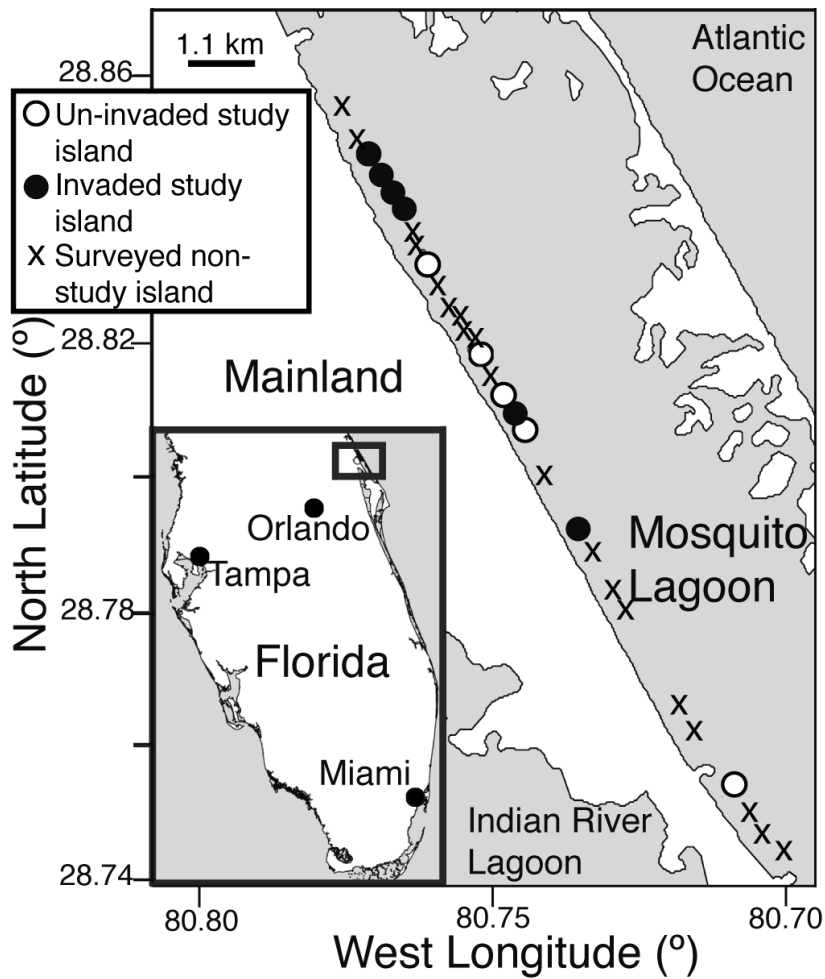


267

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 ( $\pm 1$ 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



275

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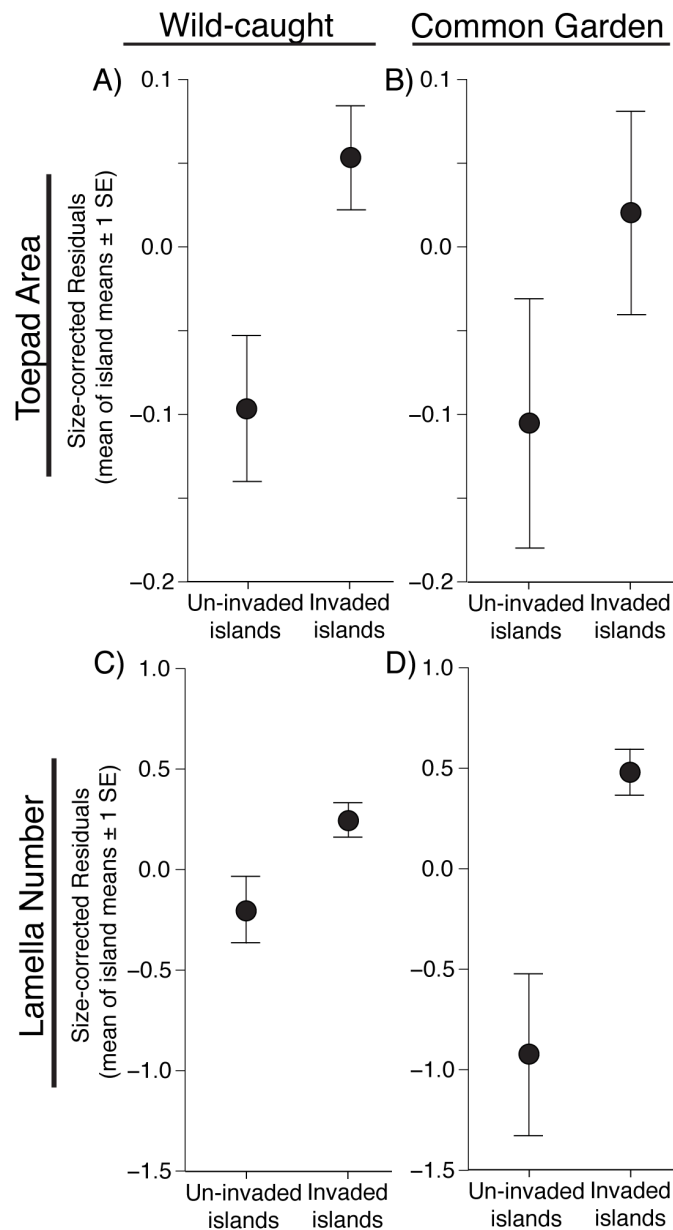
277

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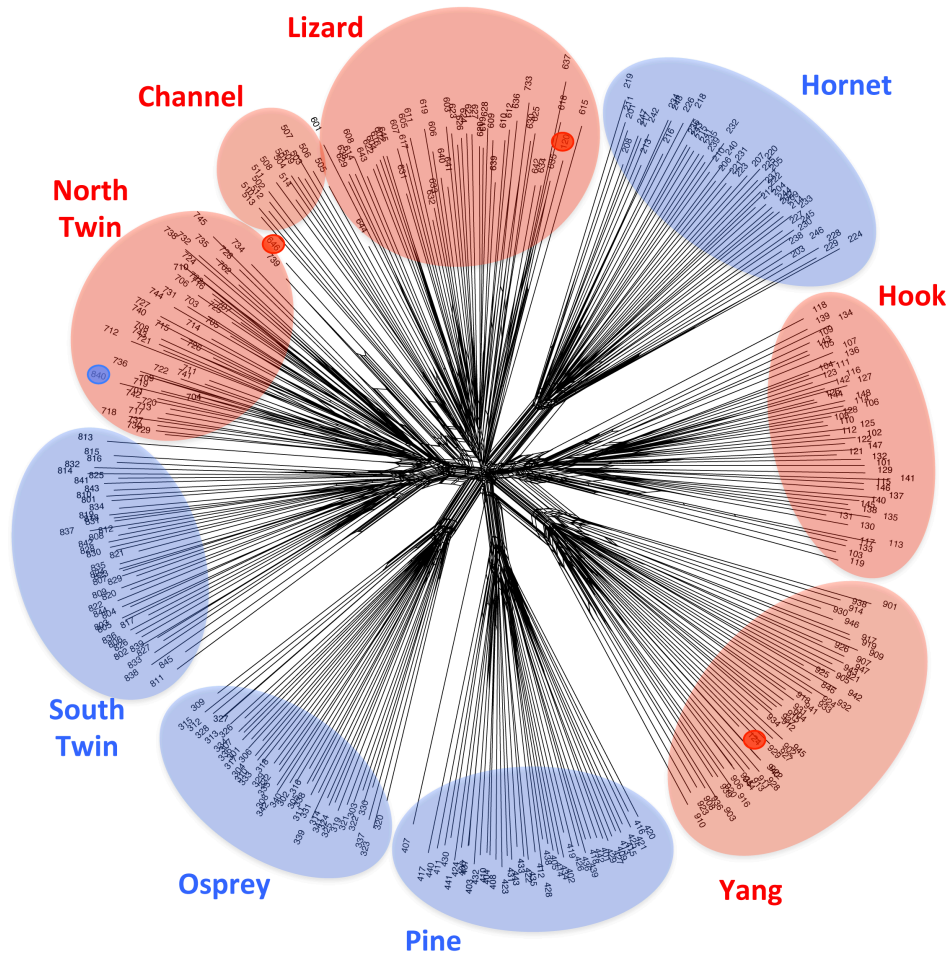
280

**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 ( $\pm 1$ 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:  $\text{lme}(\text{sqrt}(\text{perch height}) \sim \text{treatment} + \text{event} + \text{sex} + \text{treatment}*\text{event}$   
412  $+ \text{treatment}*\text{sex}, \text{random} = \sim \text{sex} \mid \text{island})$ . The  $\text{treatment}*\text{sex}$  interaction was not significant so  
413 we built the following reduced model:  $\text{lme}(\text{sqrt}(\text{perch height}) \sim \text{treatment} + \text{event} + \text{sex} +$   
414  $\text{treatment}*\text{event}, \text{random} = \sim \text{sex} \mid \text{island})$ . 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**).

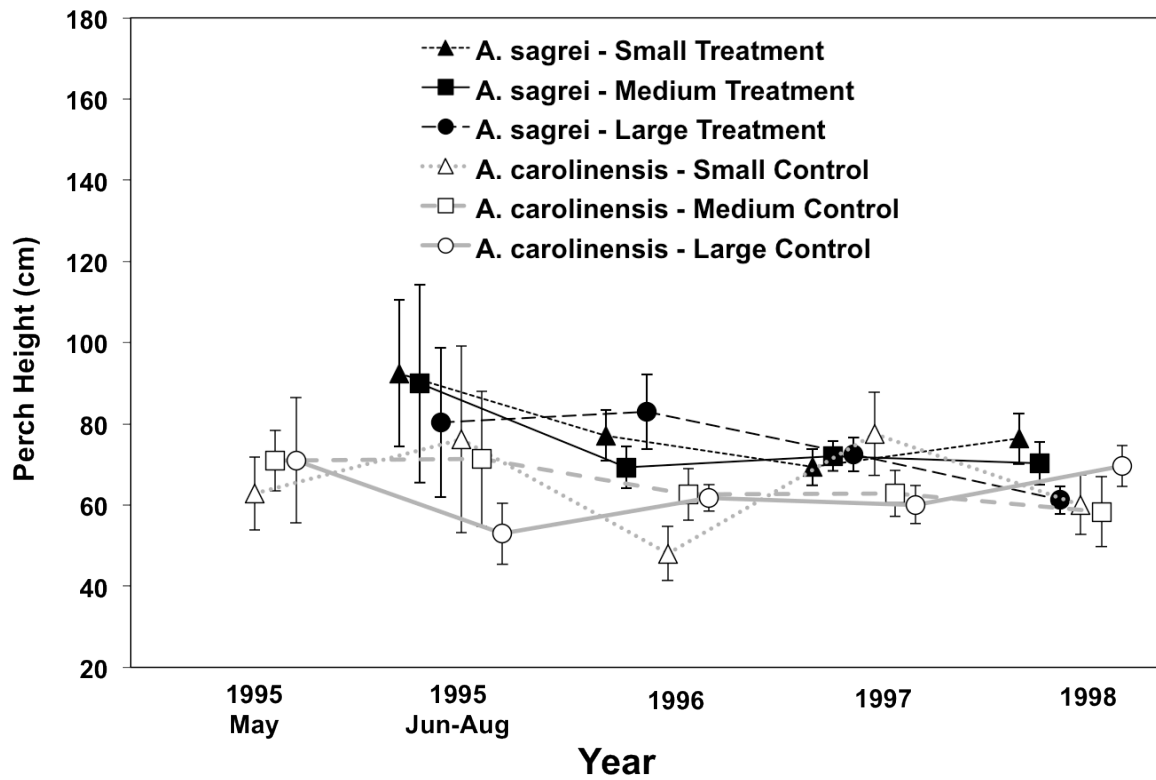
651 We also calculated the genome-wide average pairwise  $F_{ST}$  using the variance  
652 decomposition method of (44) among all islands from the set of 121,973 SNPs (code available at  
653 <http://webpages.uidaho.edu/hohenlohe/software.html>). We assessed grouping of islands based on  
654 the pairwise  $F_{ST}$  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

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673



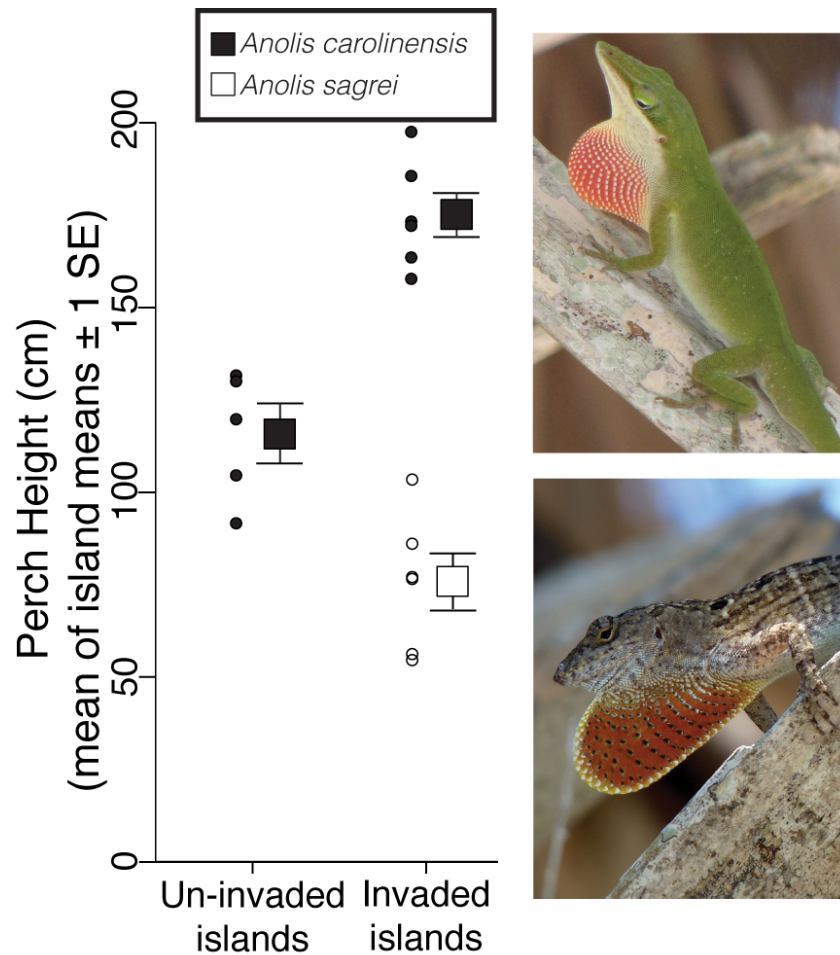
674

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 ( $\pm 1$  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 <sup>b</sup>	Large	Treatment	56	30	89	68	54
Fellers	Small	Control	22	9	34	27	32
Tarp	Medium	Control	45	23	84	78	41
Lizard <sup>b</sup>	Large	Control	18	45	213	146	121
<i>Anolis sagrei</i>							
Zero	Small	Treatment	n/a	23 <sup>a</sup>	89	157	140
Ant	Medium	Treatment	n/a	10 <sup>a</sup>	97	289	144
Yin	Large	Treatment	n/a	4 <sup>a</sup>	41	218	291

690 <sup>a</sup> The number of first-captures of introduced individuals

691 <sup>b</sup> 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.

	$\beta$ Coefficient	Standard Error	Degrees of Freedom	<i>t</i> -value	2-sided <i>p</i> - value
Intercept <sup>a</sup>	6.28	0.41	1627	17.18	0.000
Treatment <sup>b</sup>	0.50	0.49	4	1.02	0.365
1995 <sup>c</sup>	-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 <sup>d</sup>	1.85	0.18	1627	10.12	0.000
Treatment*1995 <sup>e</sup>	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.

	$\beta$ Coefficient	Standard Error	Degrees of Freedom	<i>t</i> -value	2-sided <i>p</i> - value
Intercept <sup>a</sup>	5.76	0.43	1384	13.54	0.000
Treatment <sup>b</sup>	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 <sup>d</sup>	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 <sup>a</sup> The intercept represents control islands at first collection (A: May 1995; B: June-August 1995).

697 <sup>b</sup> Treatment represents the effect of introduction on perch height, compared to controls.

698 <sup>c</sup> 1995 June-August, post-introduction.

699 <sup>d</sup> The sex coefficient represent the effect of being male on perch heights, compared to females.

700 <sup>e</sup> 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 <sup>a</sup>	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 <sup>b</sup>	Yes	48 / 12	27 / 16

708 <sup>a</sup> The large control (LC) island in the 1995-1998 study.

709 <sup>b</sup> 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	$\beta$	Standard Error	Z-value	p-value (two-sided)
Distance to Shore (m)	0.006	0.007	0.770	0.44
Island Area (m <sup>2</sup> )	0.0002	0.0002	0.995	0.34
Vegetated Area (m <sup>2</sup> )	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