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Supplementary Materials for

Rapid evolution of a native species following invasion by a congener

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Materials and Methods, and Supplementary Text

Terminology

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

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

Dredge Spoil Island Natural History

The Mosquito Lagoon dredge spoil islands used in these studies were created by the US Army Corps of Engineers (*17*) as a byproduct of the digging of the Intracoastal Waterway (ICW). An old, obsolete section of ICW channel built prior to the 1950s exists along the eastern edge of the lagoon. The new, active channel of the ICW was dredged along the western edge of the lagoon in the 1950s. Spoil islands exist along both the old and the active channel.

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

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

(1) Introduction Experiment (1995-1998)

A pilot introduction of *A. sagrei* to Six-Palm and Coon Islands indicated that *A. sagrei* populations would expand rapidly following introduction (*30*). To assess the speed and magnitude of the effects of *A. sagrei* invasion on *A. carolinensis* demography and habitat use, we conducted an introduction experiment on six spoil islands in Mosquito Lagoon from 1995 to 1998. We chose matched pairs of small (ca. 0.1 ha), medium (ca. 0.2 ha), and large (ca. 1.0 ha) islands and flipped a coin to determine which island in each pair would be subjected to a purposeful introduction of *A. sagrei* (**Table S1**) in a random-blocked design. Throughout May 1995, before initiating the *A. sagrei* introductions, we sampled *A. carolinensis* on all six islands using Rand surveys (*31*), whereby we walked through the habitat slowly until we observed an undisturbed adult lizard. We then measured its perch height to the nearest 1 cm using a tape measure. We marked all lizards with unique numbers (with permanent markers and by toe-clipping) to prevent double-counting; thus, all lizards in the perch height analyses were unique individuals. On May 27 and 28, 1995, we captured 120 *A. sagrei* from urban sites on the surrounding mainland near New Smyrna Beach and marked and released 40 of these *A. sagrei* (20M:20F) on each of the three treatment islands. We only observed four *A. sagrei* on the large treatment island in the few weeks subsequent to their release, so we increased propagule pressure by adding 40 more *A. sagrei* to this island in early June 1995 to encourage the establishment on this much larger island. From June through August 1995, and throughout the summers (May to August) of 1996, 1997, and 1998, we used the same methods to collect perch height data for *A. carolinensis* and the introduced *A. sagrei* populations.

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

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

For the 1995-1998 introduction experiment, we used linear mixed models to analyze *A. carolinensis* perch height data because such models incorporate within-island variation by nesting islands as a random effect within the fixed treatment effect (*i.e.,* the introduction of *A. sagrei*) (*32*). We square-root transformed the perch data to improve normality in the model residuals. We termed the variable representing the five time points during which perch heights were measured "event"; "event" included 1995 pre-introduction (May), 1995-post introduction (June – August), 1996, 1997, and 1998. We conducted our analyses using the *lme()* function in the R package *nlme* (*33*) and built the following full model that includes treatment, event, and sex as explanatory variables: lme(sqrt(perch height) ~ treatment + event + sex + treatment*event + treatment*sex, random = ~sex | island). The treatment*sex interaction was not significant so we built the following reduced model: lme(sqrt(perch height) ~ treatment + event + sex + treatment*event, random = ~sex | island). Residuals from this model were normally distributed and model output is reported in **Table S2**. The treatment*event interaction was significant, as would be expected if *A. sagrei* drives a perch height increase in *A. carolinensis*. At each time point post introduction of *A. sagrei*, *A. carolinensis* perches significantly higher on treatment islands compared to controls (**Table S2**; ($\beta_{treatment}$ ranges from 2.09 to 3.47, t_{1627} ranges from 3.3 to 5.0; all one-tailed $p < 0.001$). Male lizards perch significantly higher than females ($\beta_{male} = 1.85$, $t_{1627} = 10.1$, one-tailed $p < 0.001$). Treatment itself was not significant in this model ($p > 0.36$; **Table S2**) because *A. carolinensis* perch heights were measured on treatment islands before *A. sagrei* introduction in early 1995 (**Fig. 1**). To investigate the effects of treatment further, we built the same model but for a dataset pruned to include only perch height data collected post-introduction. This model found that sex remained a significant predictor of *A. carolinensis* perch height ($\beta_{male} = 1.95$, $t_{1384} = 10.0$, one-tailed $p < 0.001$). The treatment effect was significant in this model ($\beta_{treatment} = 2.98$, $t_4 = 5.4$, one-tailed $p < 0.003$; **Table S2**), but the treatment*event interaction was no longer significant (all $p > 0.39$; **Table S2**). This is consistent with **Fig. 1**: most perch height shift occurred in 1995 just after introduction, and perch height remained mostly level 1996-1998.

(2) Character Displacement in Toepads (2010)

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

Study Island History and Choice, and Accounting for Environmental Heterogeneity

In 1994, along the western edge of Mosquito Lagoon following the main channel of the ICW, Campbell surveyed for *A. carolinensis* and *A. sagrei* on 23 spoil islands. Of these 23 islands, all but two had populations of *A. carolinensis*. Of the 21 islands with *A. carolinensis* on them, by 1994, two islands were already invaded by *A. sagrei*. Four more of these 21 islands had *A. sagrei* purposefully introduced to them in 1994 and 1995: LT and MT from the introduction experiment described above, and islands Six-Palm and Coon as part of a separate pilot study described in (*30*). By the end of the introduction experiment, island LC had been colonized naturally by *A. sagrei,* bringing the total invaded to seven of the 21. We surveyed these 23 islands again in 2009 and 2010 and found that *A. sagrei* had also invaded 12 more islands through natural colonization (including MC from the introduction experiment), leaving just two islands of the original 23 with just *A. carolinensis* (recall that two islands were empty in 1994 and remained so in 2010). We surveyed 7 more islands along the western edge of the lagoon, revealing three additional islands with only *A. carolinensis*, making for a total of 5 un-invaded islands with just *A. carolinensis* out of 30 islands surveyed. Thus, we chose these 5 islands as our "controls" and complemented them with six "treatment" islands from the original 23 that were similar to the controls in size, shape, and vegetation structure but were invaded by *A. sagrei* sometime between 1995 and 2010 (**Table S4**). The five un-invaded islands are interspersed between invaded islands (**Fig. 2**). Two of the six invaded islands (LC and LT) were part of the introduction experiment described above.

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

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

To test for differences in available tree heights and vegetation species richness, we conducted point-quarter habitat surveys of island vegetation. Islands have two distinct habitat types: a forested edge and an open center. Within the forested edge, we used Google Earth to haphazardly choose survey points along an outer circle close to the forest/water edge and an inner circle near the forest/center edge. For the open center, we surveyed three to four points along three to four regularly placed north-south transects, the number of points and transects per island depending on island size. At each point, we recorded the species identity for the four closest trees (one in each quarter) and then measured their heights. We also recorded the species identities of the four closest shrubs (one in each quarter). As above, we used logistic regression with invaded/un-invaded status as the response variable and available tree heights and two species richness metrics used as the predictor variables. Species richness was calculated using both the Shannon and Simpson diversity indices using the *diversity()* function in the R (version 2.14.1, R Development Core Team) package *vegan* (*34*). Results are shown in **Table S7**.

Perch Height

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

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

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

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

Toepad Evolution

We captured lizards with noose poles and returned captured lizards to our field laboratory. For every adult lizard caught, we measured toepad area and lamella number from flatbed digital scans (2400 dpi) of the fourth toe of each hind foot. This toe is commonly used in studies of *Anolis* toepad functional morphology, so we measured it in our study to maximize the comparability of our data to that obtained in other research; however, we also note that lamellae measures from different toes are significantly correlated in *A. carolinensis* (*18*). Specifically, Glossip and Losos (*18*) counted lamellae on toes 2-5 on the fore- and hindfeet of 42 male and 24 female *A. carolinensis*. They found that males have more lamellae on each toe than females (mean difference = 1.2; *t*-test > 2.74, $p < 0.01$ in all cases), which is consistent with the sex effect in our data (see below). Glossip and Losos also found that for males, 25 of 28 pairwise comparisons showed significant correlations between lamella number on different toes (hindfoot toe 2 vs. hindfoot toe 4 and hindfoot toe 5 versus hindfeet toes 3 and 4 being the exceptions). Fifteen of 28 pairwise comparisons for females showed significant correlations for lamella number among toes; specific non-significant comparisons for females were not reported but the authors noted "no pattern of which comparisons are significant and which are not" (*18*).

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

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

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

Rates of Divergence

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

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

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

Common Garden Experiment

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

We checked plant pots for eggs three times per week from August-November 2011. We collected, incubated, and hatched all laid eggs. We raised the offspring in individual cages and shuffled cages regularly to randomize any within room environmental variation. Offspring were fed and misted by the same regimen as adults, except that smaller cricket sizes were used as appropriate to the size of the lizard.

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

For toepad area and lamella number, individually, we built a full model that included lizard svl as a random effect: lme(trait ~ sagrei presence*svl, random = ~svl | island). The interaction term was not significant so we chose the following reduced model: lme(trait ~ sagrei presence + svl, random = ~svl | island).

Population genetics

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

We obtained just over 404 million sequence reads. We de-multiplexed raw reads and filtered for the presence of a correct barcode and restriction site using Stacks (*39*), leaving 314.8 million reads. We then aligned raw reads against the *A. carolinensis* reference genome (version 2.0.75) using Bowtie2 (*40*), discarding reads that aligned to more than one location in the reference. We called diploid genotypes using a maximum likelihood model (as described by [(*39*, *41*)], implemented using code available at http://webpages.uidaho.edu/hohenlohe/software.html, with a Phred quality score minimum of 10 and prior bounds on the nucleotide error rate of 0.001 and 0.1. Genotypes were called at 161,038 RAD tag loci. From these genotypes we identified single-nucleotide polymorphisms (SNPs) across the complete set of individuals. We removed 5 individuals for low numbers of called genotypes (*i.e.,* low coverage), and we removed any putative SNPs genotyped in fewer than 150 individuals, with minor allele frequency less than 0.05 across the combined sample set, or with more than two alleles. This analysis and filtering produced a final dataset of 121,973 biallelic SNPs genotyped across 379 individuals.

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

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

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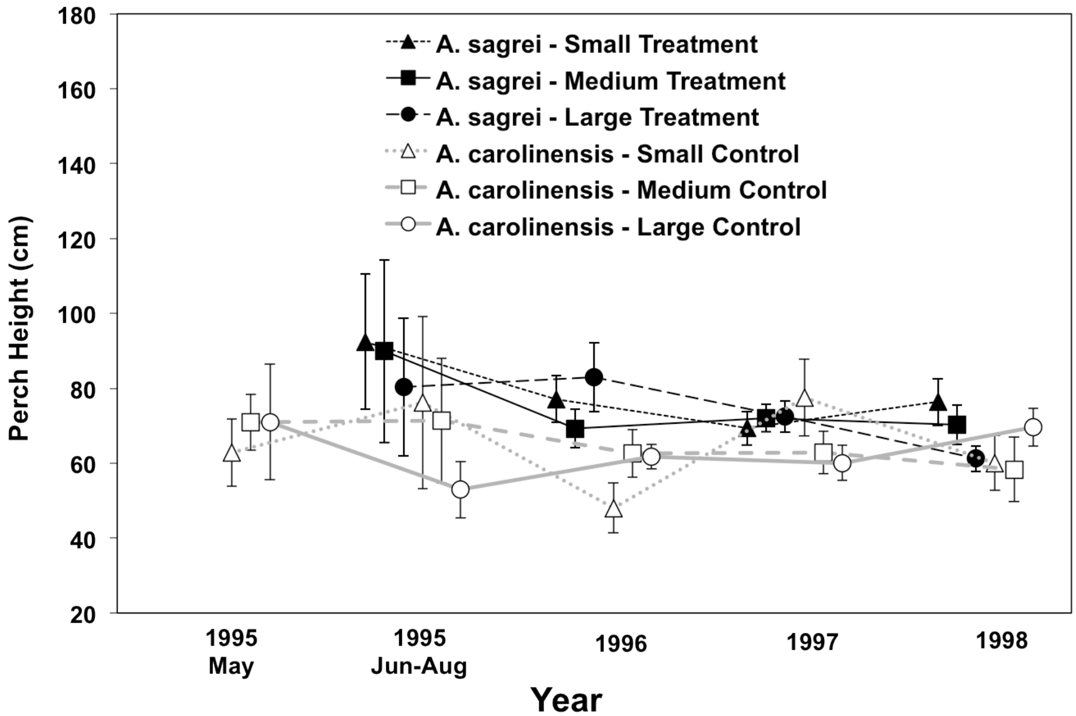

Fig. S1.

Perch height through time during the 1995-1998 introduction experiment for *A. sagrei* (filled shapes) on treatment islands and allopatric *A. carolinensis* (open shapes) on control islands. Island means (± 1 s.e.) are shown for each island.

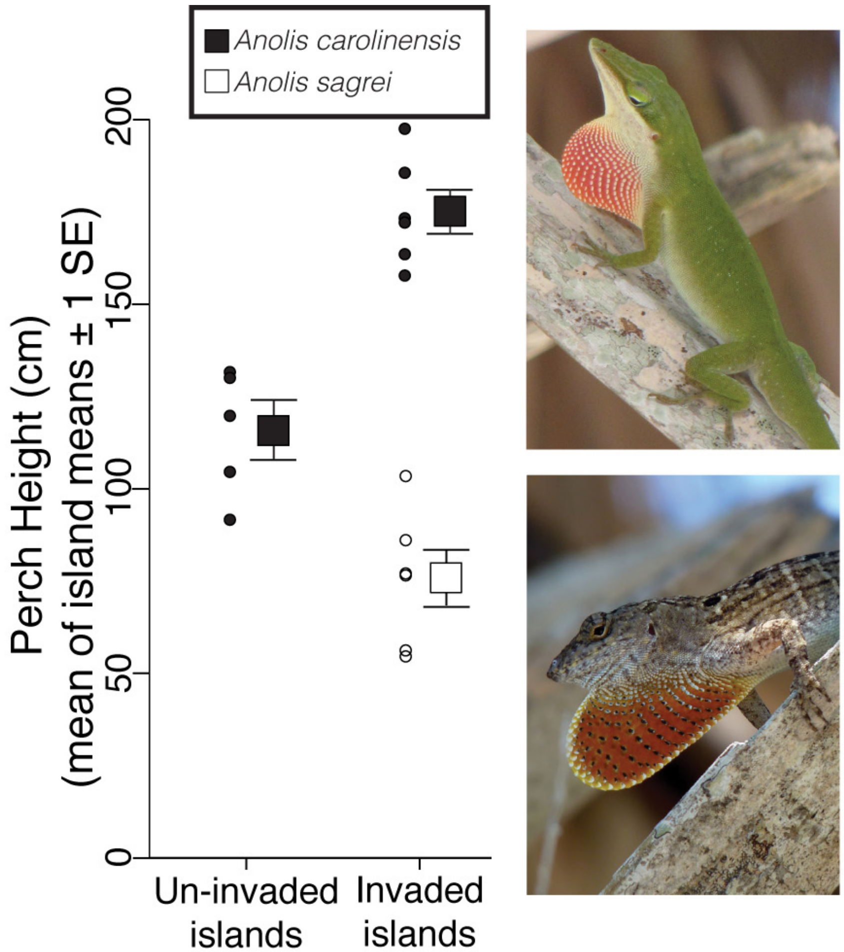

Fig. S2

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

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

Island	Size	Type	1995 Pre-Introduction	1995 Post-Introduction	1996	1997	1998
			Anolis carolinensis				
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
			Anolis sagrei				
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

[a] The number of first-captures of introduced individuals
[b] Yin (LT) and Lizard (LC) were included as “invaded” islands in the 2010 toepad study.

Table S2.

Perch height analysis for the1995-1998 *A. sagrei* introduction experiment. Mixed model output is shown for a datasets (A) including and (B) excluding pre-introduction perch height data (*12*).

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

	β Coefficient	Standard Error	Degrees of Freedom	*t*-value	2-sided *p*-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 perch height data from treatment and control islands.

	β Coefficient	Standard Error	Degrees of Freedom	*t*-value	2-sided *p*-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

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

[b] Treatment represents the effect of introduction on perch height, compared to controls.

[c] 1995 June-August, post-introduction.

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

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

Table S3.

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

Island	*A. sagrei* 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

[a] The large control (LC) island in the 1995-1998 study.

[b] The large treatment (LT) island in the 1995-1998 study.

Table S4.

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

Island	*A. sagrei* 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

Table S5.

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

Island	*A. sagrei* invasion	*n*	# 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

Table S6.

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

Table S7.

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

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

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