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1 # Independent introductions and sequential founder events shape genetic differentiation and diversity of the invasive green anole (*Anolis* *carolinensis*) on Pacific Islands
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4 Running title: Sequential founder events in *A. carolinensis*
5

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23 ## ABSTRACT

24 **Aim** Natural range expansions and human-mediated colonizations usually involve a small
25 number of individuals that establish new populations in novel habitats. In both cases, founders
26 carry only a fraction of the total genetic variation of the source populations. Here we used native
27 and non-native populations of the green anole, *Anolis carolinensis*, to compare the current
28 distribution of genetic variation in populations shaped by natural range expansion and human-
29 mediated colonization.

30 **Location** North America, Hawaiian Islands, Western Pacific Islands

31 **Methods** We analyzed 401 mtDNA haplotypes to infer the colonization history of *A. carolinensis*
32 on nine islands in the Pacific Ocean. We then genotyped 576 individuals at seven microsatellite
33 loci to assess the levels of genetic diversity and population genetic differentiation for both the
34 native and non-native ranges.

35 **Results** Our findings support two separate introductions to the Hawaiian Islands and several
36 western Pacific islands, with subsequent colonizations within each region following a stepping-
37 stone model. Genetic diversity at neutral markers was significantly lower in the non-native
38 range because of founder effects, which also contributed to the increased population genetic
39 differentiation among the non-native regions. In contrast, a steady reduction in genetic diversity
40 with increasing distance from the ancestral population was observed in the native range
41 following range expansion.

42 **Main conclusions** Range expansions cause serial founder events that are the spatial analogue
43 of genetic drift, producing a pattern of isolation-by-distance in the native range of the species. In
44 human-mediated colonizations, after an initial loss of genetic diversity, founder effects appear

to persist, resulting in overall high genetic differentiation among non-native regions but an absence of isolation-by-distance. Contrasting the processes influencing the amount and structuring of genetic variability during natural range expansion and human-mediated biological invasions can shed new light on the fate of natural populations exposed to novel and changing environments.

Keywords *Anolis* lizards, founder effects, human-mediated colonizations, islands, microsatellites, mtDNA, range expansion

INTRODUCTION

The current range of a species reflects a combination of its dispersal ability and climatic tolerances as well as the influence of interspecific interactions (Gaston, 1996; Sexton, McIntyre, Angert & Rice, 2009). Range expansions and colonization events occur throughout evolutionary history and over long time periods, for example, during the Pleistocene in post-glacial Europe (Hewitt, 1999). However, rapid range expansions (and shifts) have been documented in parallel with climate change in many taxonomic groups and geographical locations during the 20th century (Parmesan, 2006; Pecl, Araujo, Bell, Blanchard, Bonebrake et al., 2017), and via human-mediated introductions of organisms into new locations (Suarez & Tsutsui, 2008). In all instances, modifications of a species' distribution, population size and connectivity should be reflected in the amount and structuring of genetic diversity within and genetic differentiation among contemporary populations, largely because of the effects of natural selection, genetic drift and gene flow. Understanding how these mechanisms drive population differentiation as well as generate diversity is a major aim in evolutionary biology. A key question is whether recent human-mediated changes in species ranges (i.e., non-native species introductions) result in different patterns of genetic diversity and differentiation in the non-native compared to the native ranges of a species.

72 Historical range expansions, through a series of colonization events, have resulted in a
73 steady reduction of heterozygosity and increased between-population genetic
74 differentiation with increasing geographic distance from the ancestral population
75 (Slatkin & Excoffier, 2012). However, gene flow from nearby subpopulations can reduce
76 the effects of genetic drift and potentially erode genetic differentiation among
77 established populations. On the contemporary (and faster) side of the spectrum of
78 species movements, human-mediated introductions often lead to a loss of genetic
79 diversity because of founder effects (Uller & Leimu, 2011). Subsequent introductions
80 from established populations in the non-native range should further reduce genetic
81 diversity and increase genetic differentiation (i.e., serial founder scenario, Clegg,
82 Degnan, Kikkawa, Moritz, Estoup et al., 2002). However, species introductions often
83 involve complex routes with multiple introductions and admixture that counteract the
84 severity of founder events (Kolbe, Glor, Rodríguez Schettino, Lara, Larson et al., 2004;
85 Kolbe, Larson, Losos & de Queiroz, 2008; Michaelides, While, Zajac, Aubret, Calsbeek et
86 al., 2016). In both natural range expansions and human-mediated invasions, the specific
87 details of the colonization process will determine the evolutionary trajectory of
88 populations. Consequently, evaluating the mechanisms and processes influencing the
89 amount and structure of genetic diversity in human-mediated colonizations and
90 contrasting this with the patterns associated with natural range expansions could assist
91 in planning better conservation practices (e.g. species translocations), predicting the
92 evolutionary potential of organisms under climate change and preventing further
93 spread of invasive species.

94

95 The green anole, *Anolis carolinensis*, provides an excellent opportunity to investigate
96 whether natural range expansions and human-mediated colonizations unfold in a

97 similar way. The species is the only anole native to North America and is a natural
98 colonizer (arrived in Florida from Cuba: Glor, Losos & Larson, 2005) widely distributed
99 in the south-eastern United States. The phylogeographic structure of the species across
100 its native range has received considerable attention regarding the initial colonization of
101 the continent and subsequent range expansion. These studies support an origin of the
102 species in southern Florida with northward range expansion accompanied by a
103 latitudinal gradient in genetic diversity and niche expansion leading to increased
104 genetic isolation between populations in different versus similar thermal environments
105 (Glor et al., 2005; Campbell-Staton, Goodman, Backstrom, Edwards, Losos et al., 2012;
106 Tollis, Ausubel, Ghimire & Boissinot, 2012; Tollis & Boissinot, 2014; Campbell-Staton,
107 Edwards & Losos, 2016; Manthey, Tollis, Lemmon, Moriarty Lemmon & Boissinot,
108 2016). The species is also a successful invader, having been introduced to Europe
109 (Spain), Caribbean islands (Anguilla, Bahamas) and many islands in the Pacific since the
110 1940s (Lever, 2003; Kraus, 2009). Historical records and observational data associate
111 the occurrence of the green anole (and other non-native reptiles) in the Pacific region to
112 shipment-cargo movements and military activities during and after World War II (Fritts
113 & Rodda, 1998; Crombie & Pregill, 1999; Chapple, Miller, Kraus & Thompson, 2013).
114 The current distribution of the species in these regions probably conforms to a pattern
115 of stepping-stone colonization, from one island to another, within and among
116 archipelagos. However, testing and confirming these hypotheses requires a combination
117 of molecular markers and analytical tools to unravel a potentially complex introduction
118 history (Estoup & Guillemaud, 2010).

119

120 In this study, we (1) inferred the colonization history of *A. carolinensis* in the Hawaiian
121 Islands (Oahu, Hawaii, Maui and Lanai) and on other islands in the Western Pacific

(Guam, Palau, Saipan, Yap and Rota) and (2) assessed the population-genetic structure and levels of genetic diversity between and within the native and non-native ranges. To complement our sampling of the native range in the U.S. and non-native range on Pacific islands, we also used published microsatellite data for introduced *A. carolinensis* on three Japanese Islands (Chichijima, Hahajima and Anijima; Sugawara, Takahashi & Hayashi, 2015) for our comparative analyses. We hypothesize that the genetic characteristics of the native-range sampling locations will vary spatially in accordance with a historical range expansion model, whereas in the non-native range, these characteristics will be influenced by the specific details of recent colonizations. We predict that in the native range (1) genetic diversity (i.e., heterozygosity and allelic richness) will show a steady reduction and (2) increased population differentiation (F_{ST} values) with increasing distance from the ancestral population(s) in southern Florida. In the non-native range, we predict (1) lower overall levels of genetic diversity in relation to the native range, (2) reduced genetic diversity in stepping-stone colonizations, (3) increased population differentiation from native-range source(s) due to sequential founder events and (4) stronger population differentiation between rather than within archipelagos (i.e., isolation-by-colonization). We evaluate these predictions and discuss our findings in relation to historical and observational data and the mechanisms generating population genetic structure in the native and non-native range.

METHODS

Sampling, sequencing and genotyping

We used 590 lizards, 492 previously sampled by Campbell-Staton et al. (2012) and 98 new (including 59 museum specimens), from 27 locations (18 in the native range and nine in the non-native range). Tail tip or liver tissue preserved in 70-90% ethanol was

147 used to extract genomic DNA using Bioline DNA Isolate Kits (Bioline, USA). For the
148 phylogenetic analysis, we amplified an approximately 1200 base-pair (bp) region of the
149 mtDNA including the genes encoding ND2, $tRNA^{Trp}$, and $tRNA^{Ala}$ from 98 individuals
150 from the non-native range with primer pair H5730 (5'- AGCGAATRGAAGCCCGCTGG-
151 3')(Glor, Gifford, Larson, Losos, Schettino et al., 2004) and L4437a (5'-
152 AAGCTTTCGGGCCCATACC-3')(Macey, Larson, Ananjeva & Papenfuss, 1997).
153 Amplifications were carried out in a total volume of 30 µl consisting of 15 µl of MyTaq
154 HS Mix (Bioline), 1.2 µl (0.4 mM) of each primer, 10.6 µl PCR grade H_2O and 2 µl
155 template DNA (20 ng). PCR conditions were as follows: an initial denaturation step at 95
156 ^{o}C for 1 min, followed by 30 cycles at 95 ^{o}C for 1 min, 53 ^{o}C for 35 sec and 72 ^{o}C for 80
157 sec and a final extension step at 72 ^{o}C for 5 min. PCR products were purified using the
158 QIAquick PCR Purification Kit (Qiagen) and sequencing reactions were performed on
159 the ABI 3130xl genetic analyzer at URI Genomics and Sequencing Centre.
160

161 Mitochondrial DNA sequences in both directions were corrected by eye and aligned to
162 obtain a consensus sequence. Accepted sequences were then aligned using MAFFT
163 (Katoh, Misawa, Kuma & Miyata, 2002) implemented in GENEIOUS 8 (Kearse, Moir,
164 Wilson, Stones-Havas, Cheung et al., 2012) and trimmed to a uniform length of 1172 bp.
165 For 29 sequences, we amplified a length of 705 bp because of poor DNA quality. We
166 translated the sequenced *ND2* regions to amino acid sequences to verify that no
167 premature stop codons disrupted the reading frame. Unique sequences were submitted
168 to GenBank (accession numbers MG252703 - MG252730). We also genotyped 576
169 individuals from 23 locations (four locations in the non-native range, Lanai, Maui, Rota
170 and Yap were not genotyped because of limited sample size, N<4) at seven polymorphic
171 microsatellite loci (Table S1). Single locus and multiplexed PCRs were carried out in a

172 total volume of 10 μl consisting of 5 μl of MyTaq HS Mix (Bioline), 0.25 μl (0.25 mM) of
173 forward-labelled primer, 0.75 μl (0.75 mM) of forward primer and 1 μl (1 mM) of
174 reverse primer, 1 μl PCR grade H2O and 2 μl template DNA (20 ng). PCR conditions
175 were as follows: an initial denaturation step at 95 ^{o}C for 3 min, followed by 30 cycles at
176 95 ^{o}C for 45 sec, Ta (58-62 ^{o}C anneal temperature modified for each locus) for 45 sec
177 and 72 ^{o}C for 45 sec and a final extension step at 72 ^{o}C for 5 min. PCR products were co-
178 plexed and run on a 3730xl 96-Capillary Genetic Analyzer at the Yale DNA Analysis
179 Facility.

180

181 **Phylogenetic analyses**

182 We used the phylogenetic analysis to reconstruct relationships among haplotypes and
183 to assign genetic origin of the introduced haplotypes. We combined our sequences with
184 371 sequences (of varying lengths) obtained from GenBank from across the native
185 range of the species (see Table S2, Glor et al., 2005; Kolbe, Glor, Schettino, Lara, Larson
186 et al., 2007; Campbell-Staton et al., 2012; Tollis et al., 2012). We also included two
187 sequences from the non-native populations in Ogasawara (Bonin) Islands, Japan
188 (Hayashi, Shima & Suzuki, 2009). Three sequences from *Anolis altitudinalis* (AY654023,
189 Glor et al., 2004), *Anolis isolepis* (AY654022, Glor et al., 2004), *Anolis porcatus*
190 (AY654025, Glor et al., 2004) were used as outgroup in the phylogenetic analysis using
191 Bayesian inference (BI). We implemented BI analyses using the MRBAYES 3.2.6
192 (Huelsenbeck & Ronquist, 2001) plugin in GENEIOUS 8 (Kearse et al., 2012), under the
193 HKY+G nucleotide substitution model as selected by the best-fit model applying the
194 Akaike Information criterion (AICc) in MEGA 7 (Tamura, Peterson, Peterson, Stecher, Nei
195 et al., 2011). The BI analysis was run with four chains of 2,000,000 generations and
196 sampling every 1,000 trees, with default priors (unconstrained branch lengths). We

197 discarded (burn-in-length) the first 10% of trees after checking for convergence of the
198 chains and the posterior probability branch support was estimated from the 50%
199 majority-rule consensus tree.
200
201 To investigate the source location of the introduced haplotypes further, we calculated
202 pairwise nucleotide distance (average number of nucleotide substitutions per site
203 between populations, Dxy) between native and non-native populations in DNASP
204 (Librado & Rozas, 2009) and constructed a neighbour-joining (NJ) phylogenetic tree in
205 MEGA 7 (Tamura et al., 2011) with default parameters. We also constructed
206 parsimonious phylogenetic networks using a median–joining algorithm in Network
207 v.4.6.12 (Bandelt, Forster & Röhl, 1999). The method uses median vectors as a
208 hypothetical ancestral sequence required to connect existing sequences in the network
209 with maximum parsimony. For this analysis, we first used a subset of 286 sequences
210 from the Gulf/Atlantic native-range clade (see Results), which included all haplotypes
211 from non-native populations. Haplotypes were trimmed to a uniform length of 705 bp.
212 We also constructed a median-joining network with all the non-native range haplotypes
213 and 27 haplotypes from possible source populations identified in the NJ phylogenetic
214 tree analysis (see Results).
215

216 **Population-genetic analyses**

217 We calculated basic genetic diversity indices, observed and unbiased-expected
218 heterozygosities (H_O, H_E) and private alleles (P_A) with GENALEX 6.5 (Peakall & Smouse,
219 2012), allelic richness (A_R) with FSTAT v.2.9.3 (Goudet, 2001) and nucleotide diversity
220 (Pi) with DNASP v.5.10 (Librado & Rozas 2009). We compared H_E and A_R in native versus
221 introduced populations with a Welch Two Sample t-test in R (R Development Core

222 Team, 2011). Populations for these comparisons and additional population-level
223 analyses were defined as those locations with ten or more sampled individuals. We then
224 inferred and contrasted the population genetic consequences of range expansion (in the
225 native range) and human-mediated colonization (in the non-native range). First, we
226 calculated the geographic distance of native populations from a reference population
227 (since the diversification in *A. carolinensis* occurred northward from southern Florida,
228 we considered the southern sampling location, SEFL, as the reference, Manthey et al.,
229 2016) and then regressed levels of genetic diversity (H_E, A_R) and differentiation
230 (linearized F_{ST}). We contrasted patterns in the non-native range by ordering
231 populations from east (Hawaiian Islands) to west (Japanese Islands) and by age (oldest
232 introduction) within each archipelago. For this comparison, we also used multilocus
233 microsatellite genotypes (five loci) from five populations on three Japanese Islands
234 (Chichijima, Hahajima and Anijima: Sugawara et al., 2015) and calculated A_R and H_E. We
235 also tested whether genetic distance (linearized F_{ST}) was related to geographic distance,
236 separately for non-native and native sampling locations, using Mantel tests
237 implemented in the 'vegan' package in R (Oksanen, Blanchet, Friendly, Kindt, Legendre
238 et al., 2016).
239
240 To evaluate the magnitude of founder events on population differentiation, we first
241 calculated all pairwise F_{ST} values in Arlequin v3.5.1.2 (Excoffier & Lischer 2010). Then,
242 using native populations belonging to the Gulf-Atlantic clade (see Results), we averaged
243 their pairwise F_{ST} values with non-native populations on Oahu, Hawaii, Guam, Palau and
244 Saipan. We then plotted these values along with pairwise F_{ST} values for comparisons
245 among and within non-native regions (Hawaiian Islands, Western Pacific Islands and
246 Japanese Islands). Also, since under a sequential colonization model, F_{ST} values should

247 increase with the number of founder events from the source, we calculated the number
248 of founder events from a particular source (based on colonization scenario, see below)
249 and the F_{ST} values within each event. We also included averaged F_{ST} values from the
250 three Japanese islands (combined sampling locations within each island).
251
252 We used two approaches to detect whether populations in both the native and non-
253 native ranges had undergone genetic bottlenecks after range expansion and
254 colonization, respectively. First, we calculated the degree of heterozygosity excess,
255 which occurs because of the loss of rare alleles shortly after bottlenecks using
256 BOTTLENECK (Piry, Luikart & Cornuet, 1999). We used a two-phase mutation model, with
257 95% stepwise and 5% non-stepwise mutations. The significance of heterozygosity
258 excess was then calculated using Wilcoxon tests. Second, we calculated Garza and
259 Williamson's index (M), by dividing the number of alleles in a population (k) by the
260 range in allele size (r) (Garza & Williamson, 2001) in Arlequin (Excoffier & Lischer,
261 2010). This statistic is sensitive to population bottlenecks because the number of alleles
262 is usually reduced more than the range of alleles by a recent reduction in population
263 size, such that the distribution of allele length will show "vacant positions" (Excoffier &
264 Lischer 2010). Consequently, the M-index should be very small in populations that have
265 been through a bottleneck and close to unity in stationary populations. We expected
266 significantly lower values in non-native populations, because they are recent
267 introductions, compared to populations in the native range, and tested this prediction
268 with a Welch Two Sample t-test.
269
270 We used two approaches to infer population structure in our sampling locations. First,
271 we implemented a Bayesian clustering analysis in STRUCTURE v.2.3.4 (Pritchard,

272 Stephens & Donnelly, 2000), using the admixture model (Falush, Stephens & Pritchard,
273 2003) with correlated allele frequencies. We ran simulations with a burn-in of 100,000
274 iterations and a run length of 10^6 iterations from *K* = 2 through 15. Runs for each *K* were
275 replicated four times and the best supported *K* was determined according to the method
276 described by Evanno et al., (2005) in the online software STRUCTURE HARVESTER v.0.6.93
277 (Earl & vonHoldt, 2011). Multiple runs were combined with CLUMPP (Jakobsson &
278 Rosenberg, 2007). We also ran simulations for each range separately. Second, we used a
279 discriminant analysis of principal components (DAPC) implemented in the R package
280 'adegenet' (Jombart, Devillard & Balloux, 2010; Jombart & Ahmed, 2011). This approach,
281 as opposed to STRUCTURE, uses coefficients of the alleles in linear combinations and
282 seeks to maximize between-group variance and minimize within-group variance
283 without the assumption of Hardy-Weinberg equilibrium (Jombart et al., 2010).
284 Preliminary analysis revealed that locations in central to southern Florida and North
285 Carolina differ considerably from the rest of the native range, which shows little
286 population structure from the Atlantic coast to Texas. We therefore re-ran the analysis
287 excluding sampling locations in North Carolina and southern Florida to increase
288 resolution.

289

290 **Approximate Bayesian computation (ABC) analyses**

291 We estimated the relative likelihood of alternative scenarios to explain the colonization
292 route(s) of *A. carolinensis* to islands in the Pacific using approximate Bayesian
293 computation (ABC, Beaumont, Zhang & Balding, 2002) in the program DIYABC v.2.0.4
294 (Cornuet, Pudlo, Veyssier, Dehne-Garcia, Gautier et al., 2014). We first pooled native
295 populations of the Gulf-Atlantic mtDNA clade to create a native (N) pool of genotypes
296 that could be evaluated against the genotypes of introduced populations. Considering

297 the population on Oahu (oldest introduction) as an independent colonization, we tested
298 plausible scenarios of colonization for the other four islands (HI-Hawaii, WP-Guam, WP-
299 Palau and WP-Saipan). We followed a sequential-event approach, based on historical
300 records of introduction dates (or first observed-documented), to create a pool of
301 introduced genotypes (I_A) from populations preceding the next non-native population
302 (I_B). Then, we tested for an introduction event directly from the native range (primary
303 colonization, scenario I), an introduction from an already established non-native
304 population (I_A; stepping-stone colonization, scenario II) and an introduction from an
305 unsampled location (unsampled source, scenario III) (Figure 2). For populations for
306 which stepping-stone colonization was supported, we further tested scenarios to clarify
307 their source (Figure S1a and b). We also used the microsatellite dataset from three
308 Japanese islands (Chichijima, Hahajima and Anijima) to infer their colonization patterns.
309 To simplify the analyses and because more specific historical records were limited, we
310 pooled individuals from all three locations on Chichijima and considered them as one
311 independent colonization (oldest documented in the archipelago). We then tested
312 whether populations on Hahajima (pooled into one population) and Anijima were
313 established from Chichijima or from an unknown source (see Figure S1c for a graphical
314 representation). The parameters defining each scenario (i.e., effective population sizes
315 (N_E), effective number of founders (N_F), time of introduction (t), and duration of
316 bottlenecks (db)) were considered random variables drawn from prior distributions
317 (see Table S3 in supplementary material). The mutation model for microsatellite loci
318 was assumed to be a generalized stepwise-mutation (GSM) model (Estoup, Jarne &
319 Cornuet, 2002) and default values were used (Cornuet et al., 2014). The coalescent-
320 based algorithm simulates data sets for a number of predefined scenarios and compares
321 the summary statistics of these with the summary statistics of the observed data.

322 Summary statistics used in ABC were one-sample summary statistics including mean
323 genetic diversity and mean number of alleles, two-sample summary statistics including
324 mean genetic diversity, mean number of alleles and pairwise F_{st} values. We first
325 performed pre-evaluation of scenarios and prior distributions (option implemented in
326 DIYABC v.2.04) to check that at least one combination of scenarios and priors can
327 produce simulated data sets that are close enough to the observed data set. We then
328 simulated 10^6 data sets for each competing scenario and estimated posterior
329 probabilities using the direct approach, the 500 simulated data sets closest to the
330 observed, and a polychotomous logistic regression on 1% of simulated data sets closest
331 to the observed data set. For this analysis, summary statistics were transformed by
332 linear discriminant analysis (LDA) (Estoup, Lombaert, Marin, Guillemaud, Pudlo et al.,
333 2012). We also performed model checking following standard procedures in DIYABC.
334

335 **RESULTS**

336 **Phylogenetic origin**

337 We found 30 unique haplotypes in the non-native range, six from locations in the
338 Hawaiian Islands (HI), 22 from locations in the Western Pacific Islands (WP) and two
339 (previously published) from the Japanese Islands (JP); all were nested within the Gulf-
340 Atlantic native-range clade (Bayesian inference tree, supplementary Figure S2). One
341 haplotype from Hawaii (HI-H4) was identical to a haplotype from Brownsville, Texas
342 (TX-H24) and one haplotype from Guam (WP-H17) was identical to a haplotype from
343 the Japanese Islands (JP-H1). There was further haplotype sharing among islands within
344 each non-native region (Table 2). Also, after sequences were trimmed to a uniform
345 length of 705 bp (for the network analyses, see below), more haplotypes in the Western

346 Pacific islands became identical as did some haplotypes in the native range (e.g. LA-H18
347 identical to LA-H21 and TX-H13, see also Figure 3b).
348
349 The network analysis, conducted using sequences from the Gulf-Atlantic clade, grouped
350 haplotypes roughly into two geographic regions (one along the Gulf coast and one
351 farther north, Figure S3). The location of haplotypes from non-native populations in the
352 network indicates that the source region(s) is probably somewhere in Louisiana and
353 Texas (see also Table S4 in supplementary material). The NJ tree based on nucleotide
354 distance also grouped all non-native populations with four southern Louisiana locations
355 and the Brownsville, Texas population (Figure 3a) suggesting separate source regions
356 for the Hawaiian Islands (Oahu, Hawaii) and the other Pacific populations (Guam, Palau,
357 Saipan, Yap and the Japanese islands). Constructing the median-joining network
358 including only introduced-range haplotypes and 27 haplotypes from these five potential
359 sources (Figure 3b) also suggests two possible source regions based on the number of
360 mutation steps between haplotypes (1-9 steps).
361
362 **Population genetic diversity, differentiation and structure**
363 Expected heterozygosity was 0.57-0.82 in the native range and 0.62-0.71 in the non-
364 native range, and allelic richness was 5.01-7.44 in the native and 4.12-4.87 in the non-
365 native ranges (Table 1). Genetic diversity (H_E and A_R) was significantly lower in the non-
366 native range (H_E; $t = -5.67$, $df = 7.68$, $p < 0.001$, A_R; $t = -9.48$, $df = 18.19$, $p < 0.001$) and
367 the loss of genetic diversity was greater after secondary colonizations (with the
368 exception of the population on Saipan, see Figure 4a). Private allele analyses showed
369 few low frequency alleles in non-native populations. There were two private alleles on
370 Guam not sampled in the native range (Table S7). Population genetic differentiation in

371 the native range was significantly related to geographic distance (Mantel test, $R = 0.48$, P
372 < 0.001) suggesting a pattern of isolation-by-distance (IBD), but no pattern was
373 detected in the non-native range (Figure S6). Allelic richness and heterozygosity were
374 both reduced significantly in the native range compared to the ancestral population in
375 southern Florida (H_E; $R^2 = 0.34$, $p < 0.05$, A_R; $R^2 = 0.49$, $p < 0.001$, Figure 4a), whereas
376 genetic differentiation increased over the same distance (linearized F_{ST}; $R^2 = 0.40$, $p <$
377 0.05, Figure 4b). The bottleneck index (M) was significantly lower in the non-native
378 range (M; $t = -8.15$, $df = 14.74$, $p < 0.001$, Table 1, Figure 4b) and there was a bottleneck
379 signal (i.e., heterozygosity excess test) in Saipan and Chichijima ($p < 0.05$, Table 1).
380 Population differentiation, measured as F_{ST}, was low to moderate (0.02-0.10) between
381 populations within the three introduced regions (HI-Islands, WP-Islands and JP-
382 Islands), moderate to high (0.06-0.12) between native and introduced populations, and
383 high (0.18-0.20) between populations when comparing across the two regions in the
384 non-native range (Figure 4a, see also Table S6). Between successive founder events, F_{ST}
385 values increase (with the exception of the colonization on Saipan, Figure 5b).
386
387 The Bayesian clustering analysis in Structure including all sampling locations revealed
388 *K*=3 as the most likely number of genetic clusters (Figure 1a). One cluster included
389 populations in central and southern Florida, the second cluster included all non-native
390 populations and native populations from the Gulf coast and the third cluster included
391 mainly the northern native range populations. When looking at the plot for *K*=4 (second
392 highest Delta K, Figure S4a), non-native populations from the two regions (Hawaiian
393 and Western Pacific Islands) form two separate clusters, which was also supported in
394 the best fit model when running the analysis including only non-native locations (Figure
395 S4c). Three clusters (*K*=3) was the most likely number of genetic clusters for native

396 range locations (Figure S4b). Similarly, the discriminant analysis of principal
397 components (DAPC) placed non-native populations with locations along the Gulf coast
398 (Figure S5) and indicated different source(s) for the two regions (Hawaiian and
399 Western Pacific Islands) in the non-native range.
400
401 **Approximate Bayesian computation (ABC) analyses**
402 Pre-evaluation of scenarios and prior distributions showed that the summary statistics
403 from the observed data produced eigenvectors that were within the margins of the sets
404 of simulated data (data not shown). Considering the population on Oahu (oldest
405 introduction) as an independent colonization from the native range, the analyses
406 supported a second independent introduction to Guam (posterior probabilities of P =
407 0.42 and P = 0.51 based on logistic regression and direct approach, respectively) and a
408 stepping-stone colonization scenario for Palau (from Guam), Saipan (from Palau) and
409 Hawaii (from Oahu) (Table S5). Secondary colonizations were also supported for
410 populations in the Japanese archipelago with introductions from Chichijima to Hahajima
411 and Anijima (Table S5).
412
413 **DISCUSSION**
414 We established the phylogenetic origin of introduced populations of *Anolis carolinensis*
415 on islands in the Pacific and assessed the level of genetic diversity and population
416 genetic differentiation in relation to populations in the native range of the south-eastern
417 U.S. Our findings support at least two introduction events, one on Oahu in the Hawaiian
418 Islands and another on Guam in the Western Pacific Islands, and further colonizations
419 within each of these non-native regions following a stepping-stone model. Based on the
420 phylogenetic analysis and haplotype similarity, the likely geographic origin of these

421 introductions was Louisiana or Texas in the native range. Genetic diversity at neutral
422 markers was significantly lower in the non-native range because of founder effects,
423 which also contributed to increased population genetic differentiation among the
424 introduced regions. In contrast to an isolation-by-colonization pattern observed in the
425 non-native range, a steady reduction in genetic diversity and increased population
426 differentiation with increasing distance from the ancestral population in southern
427 Florida suggests a pattern of isolation-by-distance following natural range expansion in
428 the native range.
429
430 Despite some earlier confusion concerning the identity of the species occurring in the
431 Pacific (some authors have claimed that it is Cuban in origin, *Anolis porcatus* or *A. c.*
432 *porcatus*; Crombie & Pregill, 1999; Lever, 2003; Kraus, 2009), we confirmed with
433 genetic analyses that populations on Pacific islands are typical of *A. carolinensis* of the
434 south-eastern United States. It is difficult to pinpoint the exact geographic origin(s)
435 within the native range because of the relatively weak phylogeographic structure in the
436 source region. However, mitochondrial DNA haplotypes from the non-native range
437 cluster within the Gulf-Atlantic clade and are closely related to sequences from locations
438 in Louisiana and Texas, with small differences (1-3 bp out of 1172 bp haplotypes).
439 Louisiana has a major shipping port (New Orleans) and data from the mid-1990s
440 suggest that nearly a million *A. carolinensis* a year are collected and sold commercially
441 as pets (at least one major supplier is located in Louisiana, Losos, 2009). Certain
442 populations in eastern Tennessee may have been established from escapees and/or
443 released animals from Louisiana (Wade, Echternacht & McCracken, 1983). At least two
444 introductions via the pet trade have also been documented in Texas as well as seven
445 other introductions (Kraus, 2009). Our mtDNA analyses showed that one haplotype

446 from Brownsville, Texas was 99.9% similar (at 1172 bp) to two haplotypes from
447 Louisiana, suggesting an affinity between these locations. It is thus plausible that the
448 geographic origin of all introduced populations in the Pacific was several locations
449 around southern Louisiana.

450

451 Historical records and museum collections first documented the occurrence of green
452 anoles on the Hawaiian Island of Oahu in 1950, the records believed to be based on the
453 offspring of released or escaped pets (reviewed in Kraus, 2009). The species was
454 subsequently introduced to Maui (1964), Hawaii (1978) and Kauai (1987). Shared
455 haplotypes among islands in this region support a scenario of introductions from a
456 common source. Specifically, for the population on Hawaii, our ABC analysis supported
457 a stepping-stone colonization scenario from the previously established population on
458 Oahu. Levels of genetic diversity were lower on Oahu than in the native range and the
459 secondary introduction to Hawaii has resulted in further loss, a characteristic of serial
460 founder events (Clegg et al., 2002).

461

462 The situation in the Western Pacific appears more complicated. Our analyses indicate a
463 separate introduction to this region with no evidence of haplotypes shared with
464 Hawaiian Island populations and high genetic differentiation between the two non-
465 native regions. The introduction on Guam (1953) was probably an independent event
466 from a source(s) in the native range. Our analysis showed that the population in Palau
467 (1960) was established from Guam and this colonization scenario is supported with
468 historical records (Lever, 2003). The population on Saipan (1979) has probably
469 established through stepping-stone colonization from Palau. Levels of genetic diversity
470 on Saipan were higher than in Palau, which might indicate multiple introduction events

471 (or a large propagule size). Indeed, a unique haplotype on Saipan, not found in other
472 non-native populations, might indicate an undocumented, independent introduction.
473 *Anolis carolinensis* was also introduced to the Ogasawara Islands in the late 1960s
474 where it has expanded its range substantially (but see Suzuki-Ohno, Morita, Nagata,
475 Mori, Abe et al., 2017) and caused negative impacts on native species and the ecosystem
476 (e.g. competing with and preying upon an endemic lizard, *Cryptoblepharus boutonii*
477 *nigropunctatus*; Abe, Makino & Okochi, 2010; Toda, Takahashi, Nakagawa & Sukigara,
478 2010; Sugawara et al., 2015; Suzuki-Ohno et al., 2017). One mtDNA haplotype from
479 Chichijima (Hayashi et al., 2009) was identical to one from Guam, indicating a common
480 source (it has been suggested that islanders or American soldiers brought in several
481 green anoles from Guam; Hasegawa, Kusano & Miyashita, 1988). Two additional mtDNA
482 haplotypes were found in Okinawa (Suzuki-Ohno et al., 2017), one of which was
483 identical to Texas and Louisiana haplotypes. The colonization pattern within the
484 Japanese archipelago also followed a stepping-stone model with decreasing levels of
485 genetic diversity from island to island (Sugawara et al., 2015) mirroring the patterns
486 observed in other Pacific populations. These introductions in the region were associated
487 with intentional and/or unintentional release of captive animals via the pet trade as
488 well as post-World War II shipment-cargo movements. Green anoles may have been
489 introduced as pets for American military personnel or came with supplies and
490 construction material during the rebuilding of cities after the end of WWII (Fritts &
491 Rodda, 1998; Crombie & Pregill, 1999; Lever, 2003; Kraus, 2009).
492
493 Over the last 100 years, Pacific islands have been the recipient of numerous non-native
494 species, causing significant ecological impacts in many cases (e.g. Harper & Bunbury,
495 2015). Colonization varied from single events and a reduction in genetic diversity (e.g

496 brown tree boa, *Boiga irregularis*, Richmond, Wood, Stanford & Fisher, 2015), to
497 multiple waves of introductions (e.g. brown skink, *Carlia ailanpalai*; Austin, Rittmeyer,
498 Oliver, Andermann, Zug et al., 2011) and genetic admixture in source population(s)
499 leading to increased genetic diversity (e.g. house gecko, *Hemidactylus frenatus*; Tonione,
500 Reeder & Moritz, 2011). The stepping-stone colonizations in the non-native range of *A.*
501 *carolinensis* are consistent with a process of isolation-by-colonization, whereby
502 independent introductions have resulted in overall high genetic differentiation between
503 the two non-native regions (Western Pacific and Hawaiian archipelagos). This results in
504 no relationship between the pattern of neutral genetic differentiation and geographic
505 distance. Within each archipelago, there is a small reduction in genetic variation but
506 levels of population differentiation remain relatively low (with the exception of the
507 population on Saipan). In recently introduced populations and experimental studies, it
508 is unclear how long such founder effects will persist (Kolbe, Leal, Schoener, Spiller &
509 Losos, 2012). In populations that have been separated for longer periods of time, other
510 forces (i.e., natural selection) may contribute additional differences to the apparent
511 patterns of population genetic structure.
512
513 Differences in the amount and structuring of genetic variability during range expansions
514 and biological invasions have implications for the fate of natural populations exposed to
515 novel and changing environments. Human-mediated introductions of *A. carolinensis* in
516 the Pacific have resulted in strong erosion of genetic diversity, which appeared lower
517 than that in populations at the expansion front in the native range. Climate matching is
518 considered the most important predictor of global establishment of non-native reptiles
519 (Mahoney, Beard, Durso, Tallian, Long et al., 2015; Tingley, Thompson, Hartley &
520 Chapple, 2016); however, low levels of genetic diversity, small population sizes and

521 isolation could restrict the survival and persistence of non-native populations. It
522 remains to be tested whether these independent evolutionary units will have the
523 capacity for rapid adaptation if faced with unfavourable conditions. On the other hand,
524 our analyses support an isolation-by-distance pattern in the native range of *A.*
525 *carolinensis* (but see Campbell-Staton et al., 2016) where natural range expansion has
526 resulted in a steady reduction of genetic diversity at the leading edge of the expansion
527 front. However, these larger and more connected populations may be better able to
528 respond to novel conditions compared to the low diversity, isolated introduced
529 populations in the Pacific. Indeed, green anole populations near their southern range
530 limit in Texas showed an adaptive response to an extreme cold weather event by
531 decreasing their low-temperature tolerance, and shifting gene expression and allele
532 frequencies to be more similar to cold-adapted northern populations (Campbell-Staton,
533 Cheviron, Rochette, Catchen, Losos et al., 2017). Whether lower diversity, more isolated
534 introduced populations are capable of similar adaptive responses is unknown. Also,
535 when rapid climate change is accompanied by habitat loss and fragmentation, a species'
536 ability to respond to the combined effect could be significantly limited (see Henle,
537 Andres, Bernhard, Grimm, Stoev et al., 2016). We will need to incorporate an eco-
538 evolutionary framework to understand the complex effect of species range expansions
539 and climate change on genetic diversity and adaptive potential (Bailey, Genung, Ware,
540 Gorman, Van Nuland et al., 2014; Fronhofer & Altermatt, 2015).

541 **REFERENCES**

542 Abe, T., Makino, S.i. & Okochi, I. (2010) Why have endemic pollinators declined on the Ogasawara
543 Islands? *Restoring the Oceanic Island Ecosystem: Impact and Management of Invasive Alien*
544 *Species in the Bonin Islands* (ed. by K. Kawakami and I. Okochi), pp. 75-83. Springer Japan,
545 Tokyo.
546 Austin, C.C., Rittmeyer, E.N., Oliver, L.A., Andermann, J.O., Zug, G.R., Rodda, G.H. & Jackson, N.D.
547 (2011) The bioinvasion of Guam: inferring geographic origin, pace, pattern and process of an
548 invasive lizard (*Carlia*) in the Pacific using multi-locus genomic data. *Biological Invasions*, **13**,
549 1951-1967.

550 Bailey, J.K., Genung, M.A., Ware, I., Gorman, C., Van Nuland, M.E., Long, H., . . . Fox, C. (2014)
551 Indirect genetic effects: an evolutionary mechanism linking feedbacks, genotypic diversity
552 and coadaptation in a climate change context. *Functional Ecology*, **28**, 87-95.
553 Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific
554 phylogenies. *Molecular Biology and Evolution*, **16**, 37-48.
555 Beaumont, M.A., Zhang, W.Y. & Balding, D.J. (2002) Approximate Bayesian computation in
556 population genetics. *Genetics*, **162**, 2025-2035.
557 Campbell-Staton, S.C., Edwards, S.V. & Losos, J.B. (2016) Climate-mediated adaptation after
558 mainland colonization of an ancestrally subtropical island lizard, *Anolis carolinensis*. *Journal*
559 *of Evolutionary Biology*, **29**, 2168-2180.
560 Campbell-Staton, S.C., Goodman, R.M., Backstrom, N., Edwards, S.V., Losos, J.B. & Kolbe, J.J. (2012)
561 Out of Florida: mtDNA reveals patterns of migration and Pleistocene range expansion of the
562 Green Anole lizard (*Anolis carolinensis*). *Ecology and Evolution*, **2**, 2274-84.
563 Campbell-Staton, S.C., Cheviron, Z.A., Rochette, N., Catchen, J., Losos, J.B. & Edwards, S.V. (2017)
564 Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole
565 lizard. *Science*, **357**, 495-498.
566 Chapple, D.G., Miller, K.A., Kraus, F. & Thompson, M.B. (2013) Divergent introduction histories
567 among invasive populations of the delicate skink (Lampropholis delicata): has the
568 importance of genetic admixture in the success of biological invasions been
569 overemphasized? *Diversity and Distributions*, **19**, 134-146.
570 Clegg, S.M., Degnan, S.M., Kikkawa, J., Moritz, C., Estoup, A. & Owens, I.P. (2002) Genetic
571 consequences of sequential founder events by an island-colonizing bird. *Proceedings of the*
572 *National Academy of Sciences of the United States of America*, **99**, 8127-32.
573 Cornuet, J.M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., . . . Estoup, A. (2014)
574 DIYABC v2.0: a software to make approximate Bayesian computation inferences about
575 population history using single nucleotide polymorphism, DNA sequence and microsatellite
576 data. *Bioinformatics*, **30**, 1187-1189.
577 Crombie, R.I. & Pregill, G.K. (1999) A Checklist of the Herpetofauna of the Palau Islands (Republic of
578 Belau), Oceania. *Herpetological Monographs*, **13**, 29.
579 Earl, D.a. & vonHoldt, B.M. (2011) STRUCTURE HARVESTER: a website and program for visualizing
580 STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*,
581 **4**, 359-361.
582 Estoup, A. & Guillemaud, T. (2010) Reconstructing routes of invasion using genetic data: why, how
583 and so what? *Molecular Ecology*, **19**, 4113-4130.
584 Estoup, A., Jarne, P. & Cornuet, J.M. (2002) Homoplasy and mutation model at microsatellite loci and
585 their consequences for population genetics analysis. *Molecular Ecology* **11**, 1591-604.
586 Estoup, A., Lombaert, E., Marin, J.M., Guillemaud, T., Pudlo, P., Robert, C.P. & Cornuet, J.M. (2012)
587 Estimation of demo-genetic model probabilities with Approximate Bayesian Computation
588 using linear discriminant analysis on summary statistics. *Molecular Ecology Resources*, **12**,
589 846-55.
590 Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the
591 software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611-20.
592 Excoffier, L. & Lischer, H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform
593 population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**,
594 564-567.
595 Falush, D., Stephens, M. & Pritchard, J.K. (2003) Inference of population structure using multilocus
596 genotype data: Linked loci and correlated allele frequencies. *Genetics*, **164**, 1567-1587.
597 Fritts, T.H. & Rodda, G.H. (1998) The role of introduced species in the degradation of island
598 ecosystems: A case history of Guam. *Annual Review of Ecology and Systematics*, **29**, 113-140.
599 Fronhofer, E.A. & Altermatt, F. (2015) Eco-evolutionary feedbacks during experimental range
600 expansions. *Nat Commun*, **6**, 6844.

601 Garza, J.C. & Williamson, E.G. (2001) Detection of reduction in population size using data from
602 microsatellite loci. *Molecular Ecology*, **10**, 305-318.
603 Gaston, K.J. (1996) Species-range-size distributions: patterns, mechanisms and implications. *Trends*
604 *in Ecology & Evolution*, **11**, 197-201.
605 Glor, R.E., Losos, J.B. & Larson, A. (2005) Out of Cuba: overwater dispersal and speciation among
606 lizards in the Anolis carolinensis subgroup. *Molecular Ecology*, **14**, 2419-32.
607 Glor, R.E., Gifford, M.E., Larson, A., Losos, J.B., Schettino, L.R., Chamizo Lara, A.R. & Jackman, T.R.
608 (2004) Partial island submergence and speciation in an adaptive radiation: a multilocus
609 analysis of the Cuban green anoles. *Proceedings of the Royal Society B*, **271**, 2257-65.
610 Goudet, J. (2001) *FSTAT, a program to estimate and test gene diversities and fixation indices (version*
611 *2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html*.
612 Harper, G.A. & Bunbury, N. (2015) Invasive rats on tropical islands: Their population biology and
613 impacts on native species. *Global Ecology and Conservation*, **3**, 607-627.
614 Hasegawa, M., Kusano, T. & Miyashita, K. (1988) Range expansion of *Anolis c. carolinensis* on Chichi-
615 Jima, the Bonin Islands, Japan. *Journal of Herpetology*, **12**, 115-118.
616 Hayashi, F., Shima, A. & Suzuki, T. (2009) Origin and genetic diversity of the lizard populations, *Anolis*
617 *carolinensis,* introduced to the Ogasawara Islands, Japan. *Biogeography* **11**, 119-124.
618 Henle, K., Andres, C., Bernhard, D., Grimm, A., Stoev, P., Tzankov, N. & Schlegel, M. (2016) Are
619 species genetically more sensitive to habitat fragmentation on the periphery of their range
620 compared to the core? A case study on the sand lizard (*Lacerta agilis*). *Landscape Ecology*,
621 **32**, 131-145.
622 Hewitt, G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean*
623 *Society*, **68**, 87-112.
624 Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian Inference of Phylogenetic Trees. *BMC*
625 *Bioinformatics*, **17**, 754-755.
626 Jakobsson, M. & Rosenberg, N.A. (2007) CLUMPP: a cluster matching and permutation program for
627 dealing with label switching and multimodality in analysis of population structure.
628 *Bioinformatics*, **23**, 1801-6.
629 Jombart, T. & Ahmed, I. (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data.
630 *Bioinformatics*, **27**, 3070-1.
631 Jombart, T., Devillard, S. & Balloux, F. (2010) Discriminant analysis of principal components: a new
632 method for the analysis of genetically structured populations. *BMC Genetics*, **11**, 94.
633 Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple
634 sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059-3066.
635 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., . . . Drummond, A. (2012)
636 Geneious Basic: an integrated and extendable desktop software platform for the
637 organization and analysis of sequence data. *Bioinformatics*, **28**, 1647-9.
638 Kolbe, J.J., Larson, A., Losos, J.B. & de Queiroz, K. (2008) Admixture determines genetic diversity and
639 population differentiation in the biological invasion of a lizard species. *Biology Letters*, **4**,
640 434-7.
641 Kolbe, J.J., Leal, M., Schoener, T.W., Spiller, D.A. & Losos, J.B. (2012) Founder effects persist despite
642 adaptive differentiation: a field experiment with lizards. *Science*, **335**, 1086-9.
643 Kolbe, J.J., Glor, R.E., Rodríguez Schettino, L., Lara, A.C., Larson, A. & Losos, J.B. (2004) Genetic
644 variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177-81.
645 Kolbe, J.J., Glor, R.E., Schettino, L.R., Lara, A.C., Larson, A. & Losos, J.B. (2007) Multiple sources,
646 admixture, and genetic variation in introduced anolis lizard populations. *Conservation*
647 *Biology*, **21**, 1612-25.
648 Kraus, F. (2009) *Alien reptiles and amphibians: a scientific compendium and analysis*. Springer
649 Science and Business Media B.V, Dordrecht, Netherlands.
650 Lever, C. (2003) *Naturalized Reptiles and Amphibians of the World*. Oxford University Press, Oxford.

651 Librado, P. & Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA
652 polymorphism data. . *Bioinformatics* **25**, 1451-1452.
653 Losos, J.B. (2009) *Lizards in an Evolutionary Tree: ecology and adaptive radiation of anoles*.
654 University of California Press, Berkeley and Los Angeles, California.
655 Macey, J.R., Larson, A., Ananjeva, N.B. & Papenfuss, T.J. (1997) Evolutionary shifts in three major
656 structural features of the mitochondrial genome among iguanian lizards. *Journal of*
657 *Molecular Evolution*, **44**, 660-74.
658 Mahoney, P.J., Beard, K.H., Durso, A.M., Tallian, A.G., Long, A.L., Kindermann, R.J., . . . Richardson, D.
659 (2015) Introduction effort, climate matching and species traits as predictors of global
660 establishment success in non-native reptiles. *Diversity and Distributions*, **21**, 64-74.
661 Manthey, J.D., Tollis, M., Lemmon, A.R., Moriarty Lemmon, E. & Boissinot, S. (2016) Diversification in
662 wild populations of the model organism *Anolis carolinensis*: A genome-wide phylogeographic
663 investigation. *Ecology and Evolution*, **6**, 8115-8125.
664 Michaelides, S.N., While, G.M., Zajac, N., Aubret, F., Calsbeek, B., Sacchi, R., . . . Uller, T. (2016) Loss
665 of genetic diversity and increased embryonic mortality in non-native lizard populations.
666 *Molecular Ecology*, **25**, 4113-25.
667 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., . . . Wagner, H. (2016)
668 *vegan: Community Ecology*.
669 Parmesan, C. (2006) Ecological and Evolutionary Responses to Recent Climate Change. *Annual*
670 *Review of Ecology, Evolution, and Systematics*, **37**, 637-669.
671 Peakall, R. & Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software
672 for teaching and research--an update. *Bioinformatics (Oxford, England)*, **28**, 2537-9.
673 Pecl, G.T., Araujo, M.B., Bell, J.D., Blanchard, J., Bonebrake, T.C., Chen, I.C., . . . Williams, S.E. (2017)
674 Biodiversity redistribution under climate change: Impacts on ecosystems and human well-
675 being. *Science*, **355**
676 Piry, S., Luikart, G. & Cornuet, J.M. (1999) BOTTLENECK: A computer program for detecting recent
677 reductions in the effective population size using allele frequency data. *Journal of Heredity*,
678 **90**, 502-503.
679 Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus
680 genotype data. *Genetics*, **155**, 945-959.
681 R Development Core Team (2011) *R: A language and environment for statistical computing*. R
682 Foundation for Statistical Computing.
683 Richmond, J.Q., Wood, D.A., Stanford, J.W. & Fisher, R.N. (2015) Testing for multiple invasion routes
684 and source populations for the invasive brown treesnake (*Boiga irregularis*) on Guam:
685 implications for pest management. *Biological Invasions*, **17**, 337-349.
686 Sexton, J.P., McIntyre, P.J., Angert, A.L. & Rice, K.J. (2009) Evolution and Ecology of Species Range
687 Limits. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 415-436.
688 Slatkin, M. & Excoffier, L. (2012) Serial founder effects during range expansion: a spatial analog of
689 genetic drift. *Genetics*, **191**, 171-81.
690 Suarez, A.V. & Tsutsui, N.D. (2008) The evolutionary consequences of biological invasions. *Molecular*
691 *Ecology*, **17**, 351-60.
692 Sugawara, H., Takahashi, H. & Hayashi, F. (2015) Microsatellite Analysis of the Population Genetic
693 Structure of *Anolis carolinensis* Introduced to the Ogasawara Islands. *Zoological Science*, **32**,
694 47-52.
695 Suzuki-Ohno, Y., Morita, K., Nagata, N., Mori, H., Abe, S., Makino, T. & Kawata, M. (2017) Factors
696 restricting the range expansion of the invasive green anole *Anolis carolinensis* on Okinawa
697 Island, Japan. *Ecology and Evolution*, **7**, 4357-4366.
698 Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular
699 Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and
700 Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731-2739.

701 Tingley, R., Thompson, M.B., Hartley, S. & Chapple, D.G. (2016) Patterns of niche filling and
702 expansion across the invaded ranges of an Australian lizard. *Ecography*, **39**, 270-280.
703 Toda, M., Takahashi, H., Nakagawa, N. & Sukigara, N. (2010) Ecology and control of the green anole
704 (*Anolis carolinensis*), an invasive alien species on the Ogasawara Islands. *Restoring the*
705 *Oceanic Island, Ecosystem: Impact and Management of Invasive Alien Species in the Bonin*
706 *Islands* (ed. by K. Kawakami and I. Okochi), pp. 145–152. Springer, Tokyo.
707 Tollis, M. & Boissinot, S. (2014) Genetic variation in the green anole lizard (*Anolis carolinensis*)
708 reveals island refugia and a fragmented Florida during the quaternary. *Genetica*, **142**, 59-72.
709 Tollis, M., Ausubel, G., Ghimire, D. & Boissinot, S. (2012) Multi-locus phylogeographic and population
710 genetic analysis of *Anolis carolinensis*: historical demography of a genomic model species.
711 *PLoS One*, **7**, e38474.
712 Tonione, M.A., Reeder, N. & Moritz, C.C. (2011) High genetic diversity despite the potential for
713 stepping-stone colonizations in an invasive species of gecko on Moorea, French Polynesia.
714 *PLoS One*, **6**, e26874.
715 Uller, T. & Leimu, R. (2011) Founder events predict changes in genetic diversity during human-
716 mediated range expansions. *Global Change Biology*, **17**, 3478-3485.
717 Wade, J.K., Echternacht, A.C. & McCracken, G.F. (1983) Genetic variation and similarity in *Anolis*
718 *carolinensis* (Sauria: Iguanidae). *Copeia*, **2**, 529-530.

719

720 **Data Accessibility Statement**

721 Unique haplotypes generated in this study are deposited on GenBank (accession numbers
722 MG252703 - MG252730). A list of published sequences used in the phylogenetic analyses can be
723 found in the supplementary information (Table S1).

724 **Author Contributions**

725 J.J.K conceived the idea; S.N.M. generated and analysed the data; S.N.M. and J.J.K led the writing.
726 R.M.G. collected tissue samples and generated data; R.I.C. collected tissue samples from Pacific
727 Islands. All authors commented on the manuscript and approved the final version.

728

729 **Biosketches**

730 **Sozos N. Michaelides** is a postdoctoral researcher in the Department of Biological Sciences at
731 the University of Rhode Island. He is a molecular ecologist interested in the causes and
732 consequences of genetic variation in natural populations.

733 **Jason J. Kolbe** is an Associate Professor in the Department of Biological Sciences at the
734 University of Rhode Island. His research focuses on the ecological and evolutionary responses of
735 natural populations to recent, human-mediated global change, such as biological invasions,
736 climate change, and urbanization.

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Tables and Figures:

Table 1. Summary statistics from population-level genetic analyses, with populations defined as those sampling locations with ten or more sampled individuals (see Methods). Number of genotyped individuals (N), observed (H_O) and expected heterozygosity (H_E), allelic richness (A_R), number of haplotypes (H), nucleotide diversity (Pi), Garza-Williamson's index (M) and Wilcoxon tests for heterozygosity excess (P_{HZe}). Values highlighted in bold are those indicative of a bottleneck ($P \leq 0.05$ for the Wilcoxon test).

Range	State/Region	PopID	N	H_O	H_E	A_R	H	Pi (%)	M	P_{HZe}
Native	Alabama	AL	33	0.65	0.73	6.01	3[#]	0.21	0.17	0.34
	Arkansas	AR	30	0.57	0.68	5.01	3[#]	0.18	0.14	0.53
	Florida	NFL	32	0.71	0.77	6.06	4[#]	0.55	0.20	0.77
	Florida	MFL	28	0.58	0.78	6.39	5[#]	3.71	0.20	0.99
	Florida	SEFL	32	0.67	0.82	7.44	5[#]	1.2	0.24	0.95
	Florida	SWFL	16	0.68	0.78	6.61	4[#]	1.5	0.17	0.41
	Florida	NWFL	32	0.72	0.75	5.78	3[#]	2.1	0.17	0.71
	Georgia	GA	26	0.75	0.81	6.69	3[#]	0.29	0.18	0.15
	Louisiana	SLA	20	0.76	0.78	6.16	3[#]	0.47	0.17	0.29
	Louisiana	NLA	21	0.69	0.74	6.05	4[#]	0.98	0.16	0.34
	Mississippi	MS	31	0.69	0.72	5.77	3[#]	0.21	0.16	0.71
	North Carolina	NC	33	0.74	0.77	6.34	4[#]	0.26	0.19	0.41
	South Carolina	SC	28	0.76	0.80	6.57	4[#]	0.30	0.20	0.85
	Tennessee	ETN	25	0.66	0.77	6.34	3[#]	0.55	0.19	0.97
	Tennessee	WTN	33	0.52	0.72	6.01	4[#]	0.60	0.17	0.81
	Texas	TYTX	18	0.61	0.71	5.64	4[#]	0.18	0.15	0.66
	Texas	CCTX	21	0.59	0.76	5.56	3[#]	0.3	0.16	0.53
	Texas	ORTX	32	0.69	0.70	5.53	3[#]	0.29	0.18	0.99
Non-native	Hawaiian Islands	HI-Oahu	12	0.65	0.66	4.87	5	0.42	0.11	1.00
	Hawaiian Islands	HI-Hawaii	17	0.55	0.62	4.47	2	0.3	0.13	0.96
	Western Pacific Islands	WP-Palau	19	0.6	0.66	4.41	10	0.58	0.11	0.34
	Western Pacific Islands	WP-Saipan	17	0.75	0.71	4.80	7	0.47	0.11	**0.01**
	Western Pacific Islands	WP-Guam	20	0.62	0.66	4.76	7	0.57	0.13	0.95
	Ogasawara Islands (Japan)	JP-Chi1*	25	0.64	0.59	4.48	2	0.88	0.35	0.41
	Ogasawara Islands (Japan)	JP-Chi2*	24	0.68	0.62	4.53	2	0.88	0.34	**0.03**
	Ogasawara Islands (Japan)	JP-Chi3*	22	0.76	0.65	5.00	2	0.88	0.36	**0.03**
	Ogasawara Islands (Japan)	JP-Haha1*	27	0.60	0.55	4.31	1	-	0.33	0.31
	Ogasawara Islands (Japan)	JP-Haha2*	25	0.50	0.55	4.50	1	-	0.35	0.92
	Ogasawara Islands (Japan)	JP-Ani1*	59	0.74	0.62	4.73	1	-	0.36	0.31

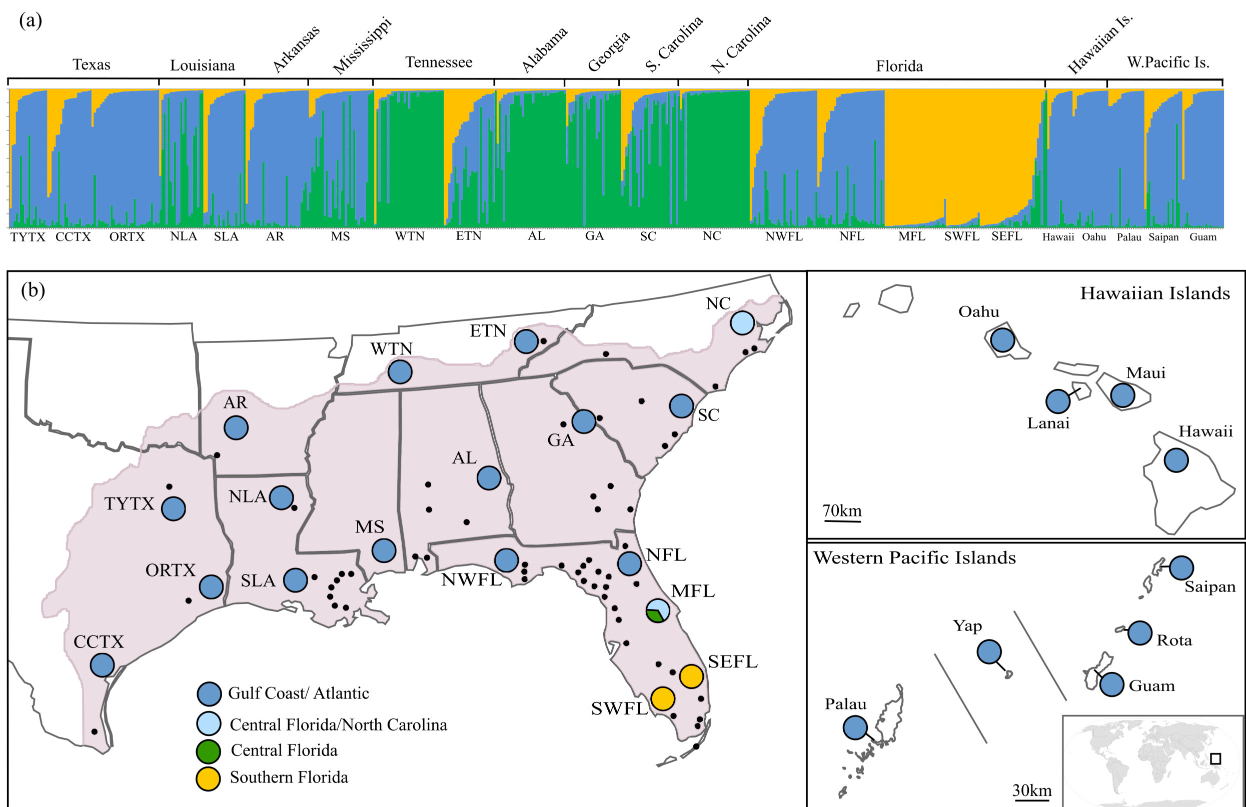
[#] Data from Campbell-Staton et al., 2012.

* Data from Sugawara et al., 2015; three sampling locations on Chichijima (Chi1-Chi3), two sampling locations on Hahajima (Haha1 and Haha2) and one location on Anijima (Ani1).

762 **Table 2.** Date of introduction (or first documented occurrence) and mtDNA haplotype information for
763 the Pacific Islands, including the number of individuals sequenced (N) and number of haplotypes (Nh).

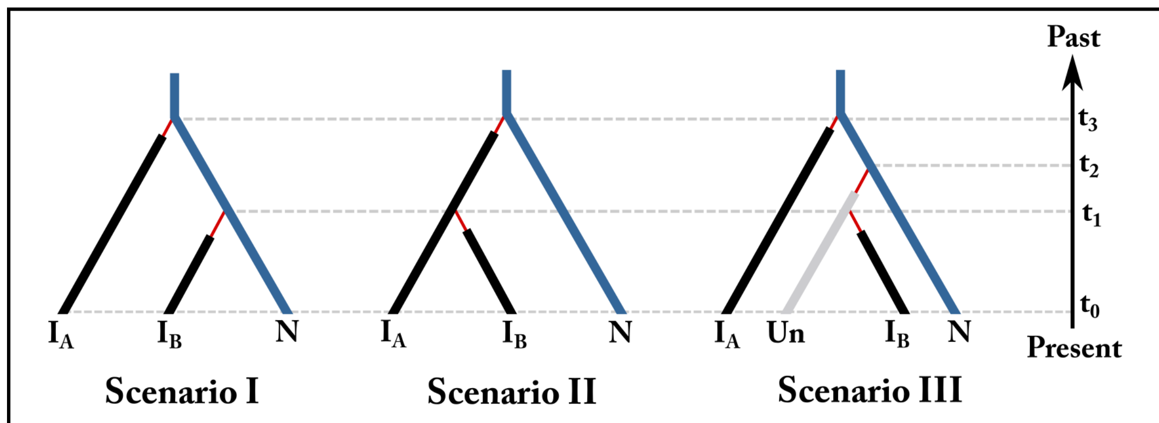
Region	Island	Date	N	Nh	Haplotype (frequency)[1]
Hawaiian Islands (HI)	Oahu	1950	16	5	**HI-H1(2),** HI-H3(2), HI-H4, **HI-H5(10)**, HI-H6
	Maui	1964	2	1	**HI-H1**
	Hawaii	1978	4	2	HI-H2, **HI-H5(3)**
	Lanai	NA	3	1	**HI-H1**
Western Pacific Islands (WP)	Guam	1953	20	7	**WP-H15(4)**, WP-H17(5), WP-H18(5), WP-H19(3),WP-H20, WP-H21, WP-H22
	Palau	1960*	33	10	**WP-H1(9)**, **WP-H3(4)**, WP-H4, WP-H10, WP-H11(2), WP-H12, WP-H13(2), WP-H14(2), **WP-H15(9),** WP-H16(2)
	Yap	1968	3	2	**WP-H2**, WP-H5(2)
	Saipan	1979	16	7	**WP-H1**, **WP-H2**, **WP-H3**, WP-H6, WP-H7, **WP-H8,** WP-H9(10)
	Rota	1988	1	1	**WP-H8**
Japanese Islands (JP)	Chichijima	1960	NA	2	**JP-H1,** JP-H2
	Hahajima	1980	NA	1	**JP-H1**
	Anijima	2013	NA	1	**JP-H1**

764 [1] Haplotypes highlighted in bold are shared between sampling locations.
765 *Approximate date based on Crombie and Pregill (1999).
766

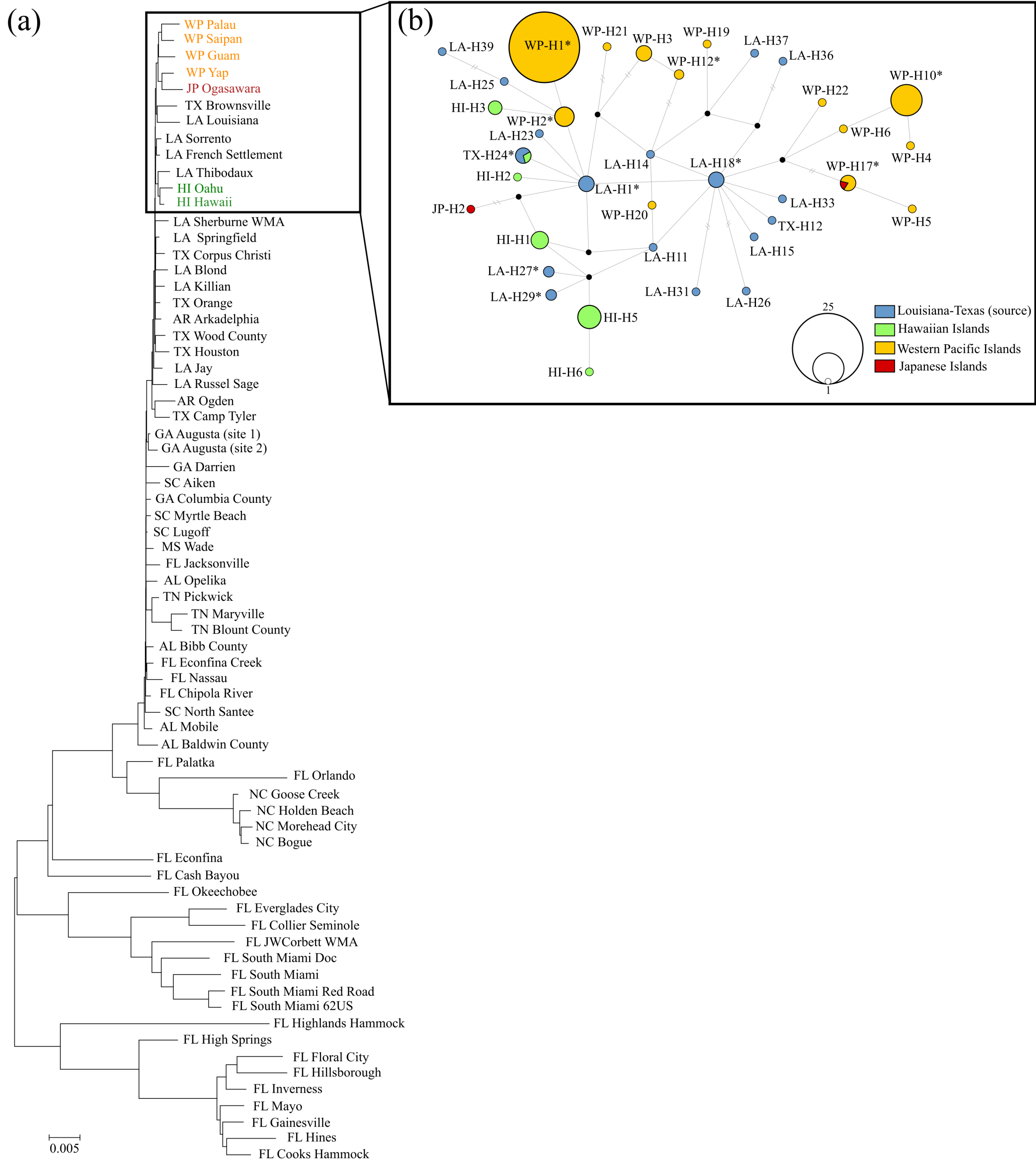


767
768 **Figure 1.** (a) Structure results for all individuals (n=576) from native and non-native locations
769 forming *K = 3* genetic clusters. (b) Distribution of sampling locations in native and non-native
770 ranges with pie charts indicating mtDNA clade assignment (populations on Japanese Islands,
771 not shown here, are assigned to the Gulf coast-Atlantic mtDNA clade, see map in Sugawara et al.,
772 2015). Black dots in the native range indicate sampling locations of mtDNA haplotypes obtained
773 from GenBank and the shaded (light purple) region indicates the approximate native range of *A.*
774 *carolinesis*.

775

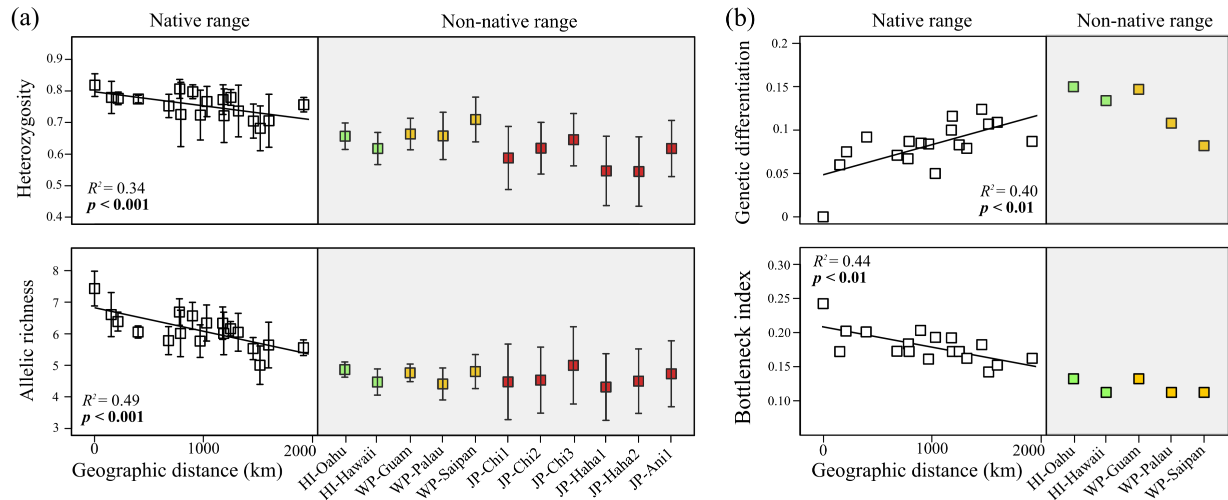


776 **Figure 2.** Graphical representation of possible colonization scenarios of *Anolis carolinensis* in
777 the Pacific as tested using DIY ABC. Scenario I assumes two independent colonization events (I_A
778 and I_B) from a location in the native range (N). Scenario II assumes stepping-stone colonization
779 from an established population in the non-native range (I_A). Scenario III assumes there was an
780 unsampled location (Un) that served as a source for an introduced population. The thin red lines
781 indicate reductions in the effective population size due to bottlenecks following the
782 introductions and t1, t2 and t3 represent time.

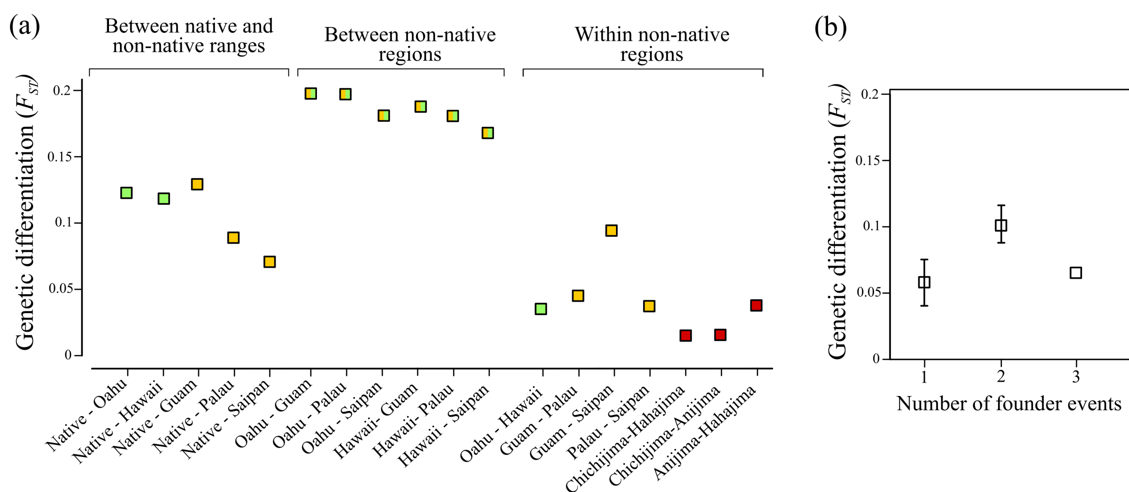


783
784 **Figure 3.** Results from phylogenetic analyses. (a) The evolutionary history of populations was
785 inferred using the Neighbor-Joining method in MEGA7 from pairwise genetic distances (Dxy)
786 calculated in DNAsp. The optimal tree with the sum of branch length = 0.41584892 is shown.
787 The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary
788 distances used to infer the phylogenetic tree. Introduced populations are shown within the box
789 and are colour coded to sampling regions. (b) Median joining network of 125 mtDNA ND2
790 haplotypes (trimmed to 705 bp) from introduced locations on Pacific Islands and five most
791 probable native-range source locations (in the box in panel a). Black dots represent median
792 vectors and connections among haplotypes are single nucleotide mutations unless marked
793 otherwise (slashes). The size of the pie (haplotype) corresponds to the number of individuals
794 sharing the same haplotype. The asterisk denotes a list of identical haplotypes after trimming;
795 WP-H1 (identical to WP-H8, 14, 15, 16), WP-H12 (WP-H13), WP-H2 (WP-H7, WP-H18), WP-H10

796 (WP-H9, H11), WP-H17 (JP-H1), LA-H27 (LA-H28), LA-H29 (LA-H30), LA-H1 (LA-H2, H3, H8),
797 LA-H18 (LA-H21, TX-H13), TX-H24 (TX-H23, HI-H4).



798
799 **Figure 4.** Contrasting genetic trends after range expansion in the native range and human-
800 mediated colonizations in the non-native range. (a) Genetic diversity indices, expected
801 heterozygosity (top) and allelic richness (bottom), in the native range in relation to geographic
802 distance from south Florida (SEFL, as reference population, see text for further explanation) and
803 ordered by island group from east to west (HI; Hawaiian, WP; Western Pacific and JP; Japanese)
804 in the non-native range. (b) Top plot: population differentiation (linearized F_{ST} values)
805 compared to geographic distance within the native range (SEFL as reference population)
806 showing isolation by distance and averaged pairwise F_{ST} values between a non-native
807 population and populations belonging to the Gulf-Atlantic clade; bottom plot: Garza-
808 Williamson's bottleneck index in relation to geographic distance from south Florida.
809 Populations on the three Japanese Islands were genotyped at a different set of microsatellite
810 markers; therefore, comparisons with native and non-native populations should be interpreted
811 with caution.



812
813 **Figure 5.** Founder effects in non-native populations. (a) Genetic differentiation (F_{ST} values)
814 between ranges (native and non-native), between and within non-native regions; (b) the effect
815 of number of founder events (sequential events, based on colonization scenario) on F_{ST} values
816 (averaged pairwise values between a population and its source). Populations on the three

817 Japanese Islands (sampling locations within each island were pooled together) were genotyped
818 at a different set of microsatellite markers; therefore, comparisons with native and non-native
819 populations should be interpreted with caution. Native range populations belonging to the Gulf-
820 Atlantic clade were used for the between ranges analyses.