

1                    Molecular evolutionary consequences of island colonisation

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27 **Abstract**

28

29 Island endemics are likely to experience population bottlenecks; they also have  
30 restricted ranges. Therefore we expect island species to have small effective  
31 population sizes ( $N_e$ ) and reduced genetic diversity compared to their mainland  
32 counterparts. As a consequence, island species may have inefficient selection and  
33 reduced adaptive potential. We used both polymorphisms and substitutions to  
34 address these predictions, improving on the approach of recent studies that only  
35 used substitution data. This allowed us to directly test the assumption that island  
36 species have small values of  $N_e$ . We found that island species had significantly  
37 less genetic diversity than mainland species; however, this pattern could be  
38 attributed to a subset of island species that had undergone a recent population  
39 bottleneck. When these species were excluded from the analysis, island and  
40 mainland species had similar levels of genetic diversity, despite island species  
41 occupying considerably smaller areas than their mainland counterparts. We also  
42 found no overall difference between island and mainland species in terms of  
43 effectiveness of selection or mutation rate. Our evidence suggests that island  
44 colonisation has no lasting impact on molecular evolution. This surprising result  
45 highlights gaps in our knowledge of the relationship between census and  
46 effective population size.

47 Word count: 196

48

49 *Key words:* effective population size, genetic diversity, bottlenecks,  
50 polymorphism, substitution.

51

## 52 **Introduction**

53

54 Island species have long been considered to be under greater threat of extinction  
55 than their mainland counterparts (Mckinney 1997; Frankham 1997; Johnson &  
56 Stattersfield 1990; Jones et al. 2003; Purvis et al. 2000). Although extinction itself  
57 is caused by a number of stochastic factors, not least human activity (Burgess et  
58 al. 2013; Pimm et al. 1988), the susceptibility of island populations may also be a  
59 consequence of population genetics. Island species are likely to have experienced  
60 population bottlenecks at some point in their evolutionary history due to  
61 founder events during the initial island colonisation. As only a fraction of  
62 individuals from the original population found an island population, only a  
63 fraction of the original genetic diversity of the population will be maintained, and  
64 effective population sizes ( $N_e$ ) will be small (Nei et al. 1975). In addition, island  
65 species are restricted to relatively small areas, which could impose long-term  
66 restrictions on census population sizes, and in turn on long-term  $N_e$ . Therefore it  
67 may be that island species are genetically vulnerable.

68

69 Low diversity/low  $N_e$  could theoretically reduce the adaptive potential of a  
70 species, as standing levels of genetic variation determine the alleles that are  
71 immediately available for evolution to act upon (Messer & Petrov 2013; Barrett  
72 & Schluter 2007; Hermisson & Pennings 2005). In addition, populations founded  
73 by a small number of individuals will experience increased inbreeding.  
74 Inbreeding results in an increasingly homozygous population, and therefore  
75 there is a greater risk that deleterious recessive alleles will be exposed  
76 (Charlesworth & Charlesworth 1987), which could have significant fitness costs.

77 There is some evidence that bottlenecked species do experience a loss of fitness:  
78 for example, Frankham et al. (Frankham et al., 1999) demonstrated that  
79 laboratory populations of *Drosophila* showed reduced evolvability (in terms of  
80 ability to tolerate increasing concentrations of an environmental pollutant) after  
81 a bottleneck; while Briskie and Mackintosh (Briskie & Mackintosh 2003)  
82 uncovered a link between the severity of population bottlenecks and loss of  
83 fitness in birds.

84

85 In addition, species with low effective population sizes are expected to have  
86 inefficient selection, resulting in high levels of deleterious mutations segregating  
87 and a tendency to fix deleterious mutations. However, past studies investigating  
88 the differences in the efficiency of selection between island and mainland species  
89 have provided only limited support for this prediction. Johnson and Seger  
90 (Johnson & Seger 2001) found some evidence that island species had less  
91 efficient selection, but this was for a small and taxonomically restricted dataset.  
92 Woolfit and Bromham (Woolfit & Bromham 2005) used a much larger and more  
93 varied dataset, however, they reported a difference between island and mainland  
94 species that was only significant at the one-tailed level, while Wright et  
95 al. (Wright et al. 2009) found no significant difference between island and  
96 mainland species. This may be because previous studies have considered rates of  
97 substitution, usually the ratio of the rate of non-synonymous substitution to the  
98 rate of synonymous substitution ( $\omega$ ). The problem with considering substitution  
99 data is that a reduction in  $N_e$  is expected to increase the rate at which slightly  
100 deleterious mutations are fixed, but reduce the rate at which advantageous  
101 mutations are fixed, particularly if the rate of adaptation is limited by the supply

102 of mutations. We therefore cannot make a clear prediction about the effect of  $N_e$   
103 on substitutions. This issue can be addressed by using polymorphism data  
104 instead of substitution data, using the number of nonsynonymous  
105 polymorphisms divided by synonymous polymorphisms, because advantageous  
106 mutations are not expected to significantly contribute to polymorphism (Kimura  
107 1984; Kryazhimskiy & Plotkin 2008; Ho et al. 2011).

108

109 It seems likely that adaptive evolution might occur for at least some island  
110 species, despite their predicted low effective population sizes, due to the fact that  
111 the species is encountering a novel habitat. Although populations with large  
112 effective population sizes may have more efficient selection, we might also  
113 expect positive selection to play a significant role after a colonisation event, as  
114 species adapt to new environmental requirements and ecological niches.  
115 However, in making predictions regarding adaptive evolution it is important to  
116 consider the direction of colonisation. Although island species most commonly  
117 colonise an island from a nearby mainland, occasionally lineages that originated  
118 on islands re-colonise a mainland, providing an interesting contrast in terms of  
119 molecular evolution. Species colonising mainlands from islands are likely to  
120 experience population size increases, and therefore increases in  $N_e$ . This could  
121 result in a spate of rapid molecular evolution in the new mainland population as  
122 advantageous mutations that were previously effectively neutral become fixed  
123 (Takano-Shimizu 1999; Charlesworth & Eyre-Walker 2007).

124

125 However, predictions about the molecular evolution of island species are  
126 predicated on the crucial assumption that island species do in fact have lower  $N_e$

127 and levels of genetic diversity than mainland species. Whether this is in fact the  
128 case is not certain, because census population size can sometimes be a poor  
129 indicator of genetic diversity (Lewontin 1974; Bazin et al. 2006; Leffler et al.  
130 2012; Romiguier et al. 2014). Although some studies uncover a link between the  
131 two (for overview, see (Frankham 2012)), other authors have not found a  
132 relationship; for example, Nabholz et al. (Nabholz, Mauffrey, et al. 2008) did not  
133 find a strong relationship between mammalian mitochondrial diversity and life-  
134 history traits associated with  $N_e$  (such as body mass), or between diversity and  
135 IUCN category, an index partly based on assessments of census population size.  
136 More generally, there is surprisingly little variation in levels of diversity between  
137 species; one recent paper reported a range of nucleotide diversities of only 800-  
138 fold across a range of taxa, many orders of magnitude smaller than their  
139 estimated census population size differences (Leffler et al. 2012). The  
140 determinants of genetic diversity remain poorly understood.

141

142 One possible complicating factor is the mutation rate. Both Nabholz et al.  
143 (Nabholz, Glémin, et al. 2008) and Romiguier et al. (Romiguier et al. 2014) found  
144 evidence suggesting that there are lineage-specific differences in the mutation  
145 rate, in mitochondrial and nuclear data respectively. How the mutation rate  
146 evolves is contentious: if selection is responsible for determining the mutation  
147 rate, populations with high effective population sizes should have the lowest  
148 mutation rates, because selection will be more effective at reducing the rate: this  
149 is because whether a mutation can be selected depends on the strength of  
150 selection being over  $1/N_e$ . However, support for this prediction remains mixed.  
151 For example, in previous studies of island-mainland systems (all of which

152 controlled for phylogenetic non-independence), two found no difference in  
153 substitution rate between island and mainland lineages (Woolfit & Bromham  
154 2005; Johnson & Seger 2001), while another found that it was mainland species  
155 that had higher rates of substitution (Wright et al. 2009), the opposite of what  
156 we might expect if the mutation rate depends on the population size. Another  
157 factor that may contribute to unexpected patterns of diversity is selection at  
158 linked sites: this reduces genetic diversity, particularly in genomic regions with  
159 low rates of recombination (Frankham 2012; Gillespie 2000; Maynard Smith &  
160 Haigh 1974). Linked selection may occur more frequently in populations with  
161 high values of  $N_e$ , reducing diversity more rapidly than in populations with a low  
162  $N_e$ . On the other hand, it could be that selective sweeps occur more commonly in  
163 species adapting to a new environment e.g. (Montgomery et al. 2010).

164

165 In summary, we expect island species to have low effective population sizes and  
166 because of this we expect them to have low genetic diversities. We also expect  
167 selection to be less efficient in island species, leading to higher ratios of  
168 nonsynonymous to synonymous polymorphism, and potentially to increases in  
169 the mutation rate (the mutation rate might increase to such an extent that island  
170 and mainland species have similar diversities). Whether we expect island species  
171 to have higher ratios of nonsynonymous to synonymous substitution depends on  
172 how much adaptive evolution there is, and how this is affected by effective  
173 population size and the act of colonisation. If there is no adaptive evolution then  
174 island species are expected to have higher values for  $\omega$ ; however, adaptive  
175 evolution could potentially be either reduced in island species because of their  
176 low  $N_e$  or increased because of adaptation to a new environment, given that in

177 most cases the island is the new environment that is colonised. Here we perform  
178 the first analysis of polymorphism data from a dataset of phylogenetically  
179 independent pairs of island and mainland species, and combine this with  
180 substitution data. The paired study design is crucial: there are a large number of  
181 life history traits that are known to influence molecular evolution (e.g. body size,  
182 fecundity, generation times) and could therefore act as confounding factors  
183 (Bromham 2011; Lanfear et al. 2013). Closely related island and mainland  
184 species have similar life-history traits, and even if there is variation it is not  
185 expected to be systematic, and so should not bias our results. Therefore, island  
186 colonisation itself will be the primary reason for any differences in molecular  
187 evolution between island and mainland species (Johnson & Seger 2001; Woolfit  
188 & Bromham 2005).

189

## 190 **Results**

191

### 192 *Dataset overview*

193 To investigate the consequences of island colonisation on molecular evolution  
194 we compiled data for 120 island-mainland comparisons. Some comparisons  
195 comprised a single island and single mainland species, while some consisted of  
196 multiple island and multiple mainland species. In the majority of cases, the  
197 inferred direction of colonisation is from mainland-to-island. The data is  
198 dominated by mitochondrial sequences from birds (Table 1a) but we have a  
199 reasonable number of mitochondrial sequence comparisons available for  
200 invertebrates and reptiles, and a moderate number of nuclear sequence  
201 comparisons. For 70 of our comparisons, multiple sequences from the same

202 species were available, allowing us to conduct polymorphism analyses. Again,  
203 this dataset is dominated by mitochondrial data from birds (Table 1b). For a full  
204 list of species used in this analysis, and for complete details of results, please see  
205 the archived data at: <http://dx.doi.org/10.6084/m9.figshare.1296151>.

206

### 207 *Geography*

208 Island species are studied from a molecular evolutionary perspective because  
209 they are expected to have smaller populations than mainland species due to their  
210 small ranges. However, this assumption is rarely tested. In this study, the ranges  
211 of the species used were confirmed where possible using the IUCN database  
212 (IUCN 2014). The mean range of island species was 5,780 km<sup>2</sup>, while for  
213 mainland species this mean range was over 4,080,000 km<sup>2</sup>. The ratio of island to  
214 mainland range sizes did not exceed 0.25 for any of the comparisons used, and in  
215 the majority of cases island species had ranges which were less than 1% of the  
216 area of those of their mainland counterparts (Figure 1). Therefore we have  
217 evidence that the island species used in this study inhabit substantially smaller  
218 geographic regions than their mainland relatives, although we have no  
219 information on population density.

220

### 221 *Synonymous diversity*

222 We might expect island species to have lower diversity than their mainland  
223 counterparts for two reasons. Firstly, island species inhabit substantially smaller  
224 areas than their mainland relatives; resulting in a smaller census population size  
225 and hence potentially a smaller long-term  $N_e$ . Secondly, island populations are  
226 likely to be founded by few individuals, which again is expected to result in a

227 small  $N_e$ . As expected, we find that island species have significantly lower  
228 synonymous site nucleotide diversity overall, and when we consider  
229 mitochondrial and nuclear DNA separately (Table 2). Chloroplast sequences  
230 show the opposite pattern, but as there are only 4 comparisons this is likely to be  
231 due to sampling error. When different taxonomic groups were considered  
232 separately, island birds had significantly lower levels of diversity than mainland  
233 birds, while for both reptiles and invertebrates there was no significant pattern  
234 (Table 2) (for other groups we do not have enough data to make a valid  
235 comparison). However, despite being statistically significant, the differences  
236 between mainland and island species are relatively modest. Mainland species  
237 have on average 40% more diversity than island species, and in about one third  
238 of cases, island species have higher diversity than their mainland relatives.

239  
240 It is potentially possible to differentiate between the two possible causes of the  
241 lower diversity in island species by considering the ratio of island to mainland  
242 nucleotide diversity as a function of the divergence between the island and  
243 mainland species. In this analysis we use the total number of island and  
244 mainland synonymous substitutions ( $dS$ ) as an estimator of species divergence,  
245 however it should be noted that this is a crude estimator as  $dS$  is dependant on  
246 both generation time and mutation rate. If most of the reduction in diversity is  
247 due to a bottleneck during colonisation, then we expect the ratio of island to  
248 mainland diversity to be greatest when the evolutionary divergence is longest. In  
249 contrast, if diversity is largely determined by population sizes after colonisation  
250 then we might expect the ratio of island to mainland diversity to decline with  
251 evolutionary divergence. Consistent with the bottleneck hypothesis, we find that

252  $\pi_s(\text{island})/(\pi_s(\text{island})+\pi_s(\text{mainland}))$ , the normalised level of neutral island  
253 diversity, is positively correlated to the total number of synonymous  
254 substitutions between island and mainland species (Pearsons correlation  $r=$   
255  $0.318$ ,  $p= 0.012$ ) (Figure 2). The correlation increases in strength if we restrict  
256 the analysis to mainland-to-island colonisation events ( $r = 0.384$ ,  $p=0.004$ ), and  
257 is negative, though non-significant, if we consider colonisations that occurred in  
258 the opposite direction ( $r = -0.129$ ,  $p = 0.74$ ). This positive correlation appears to  
259 be driven by a group of island species/clades that are recent colonists and have  
260 no synonymous diversity (Figure 2), because the positive correlation disappears  
261 when these species are removed ( $r = 0.214$ ,  $p = 0.150$ ). Although the low levels of  
262 diversity we have recorded could be a result of low levels of mutation and/or  
263 short sequences, this explanation is unlikely because we would expect equal  
264 numbers of island and mainland species to have low diversity (i.e. in Fig 2 we  
265 would expect an equal number of points clustering at 1 on the y-axis as at 0),  
266 which is not what we observe.

267

268 Reptiles are disproportionately represented amongst the species with no genetic  
269 diversity in the island species/clades (6 out of 14 reptiles compared to 9 out of  
270 36 birds and 0 out 9 invertebrates). If each phylogenetic group is considered  
271 individually we find a significant positive correlation between  
272  $\pi_s(\text{island})/(\pi_s(\text{island})+\pi_s(\text{mainland}))$  and  $dS$  for invertebrates ( $r = 0.752$ ,  $p =$   
273  $0.012$ ) and positive but non-significant correlations for birds and reptiles (figure  
274 2) (we do not have enough data to study the other groups individually). As a  
275 group, birds appear to retain the highest levels of diversity, with some species  
276 seemingly not undergoing a population bottleneck during the colonisation event,

277 perhaps because there are more individuals initially founding the island  
278 population and/or because there is continued migration from the mainland. This  
279 is compatible with the greater dispersal ability of birds compared to other  
280 animal groups. Reptiles on the other hand appear to experience a quite severe  
281 loss of diversity during founder events.

282

283 Although our results are consistent with the idea that the genetic diversity of  
284 island species is able to recover over time, either through continued immigration  
285 or the accumulation of new genetic diversity *in situ*, an alternative interpretation  
286 is that island species that are not diverse simply go extinct. This may be why only  
287 young species have low levels of diversity (out of 62 comparisons, only the  
288 chameleon *Archaius tigris* was moderately divergent without any synonymous  
289 diversity at all). These explanations are not necessarily mutually exclusive.  
290 Nevertheless it is surprising that aside from those species with no synonymous  
291 diversity, in most cases island species have similar and in some cases more  
292 genetic diversity than their mainland counterparts. If we remove the  
293 comparisons in which island diversity is zero and re-analyse the data we find  
294 that the remaining island species do not have lower synonymous diversity than  
295 mainland species (Wilcoxon signed-ranks test,  $n = 48$ ,  $p = 0.258$ ). This suggests  
296 that island species/clades only have lower levels of diversity if they have  
297 recently (in terms of generations) undergone a population bottleneck.

298

### 299 *Effective population sizes*

300 The fact that the genetic diversity of island species is generally not lower than  
301 that of mainland species suggests that they do not have lower effective

302 population sizes. To investigate this, we estimated effective population size by  
303 dividing synonymous diversity by synonymous divergence (using synonymous  
304 divergence to approximate mutation rate) and compared island species to their  
305 mainland counterparts. Note that these effective population size estimates can  
306 only be compared against each other, since we are dividing the diversity by the  
307 product of the mutation rate per generation and the number of generations since  
308 the mainland and island species diverged. Mainland species had significantly  
309 greater effective population sizes than island species overall (Wilcoxon signed-  
310 ranks test,  $n = 66$ ,  $p = 0.030$ ); however, if we exclude those comparisons in which  
311 the island species had no synonymous diversity, the difference between island  
312 species and mainland species is no longer significant ( $n = 45$ ,  $p = 0.281$ ).

313

#### 314 *Efficiency of selection*

315 Selection is expected to be less efficient in species with small  $N_e$ . However, we  
316 have found little evidence to suggest that island species have lower long-term  
317 effective population sizes than mainland species. It is therefore perhaps not  
318 surprising that we find little evidence for selection being less efficient in island  
319 species. Using polymorphism data we compared  $\pi_n/(\pi_n+\pi_s)$  between island and  
320 mainland species and found no significant differences between island and  
321 mainland species/clades (Wilcoxon signed-ranks test,  $n = 51$ ,  $p = 0.389$ ); we also  
322 found no difference when considering different DNA types separately, although  
323 when splitting by taxonomic group the difference between island and mainland  
324 bird species is just significant (Table 3). It should be noted however that most of  
325 the island species that have no synonymous polymorphisms also have no non-

326 synonymous polymorphisms and hence are excluded from the analysis because

327  $\pi_n / (\pi_n + \pi_s)$  is undefined.

328

329 We also find no significant differences between island and mainland species for

330  $\omega$  (nonsynonymous divided by synonymous divergence) overall, or if we split the

331 data by phylogenetic group or genome type (Table 4). However, there is an

332 expectation that  $\omega$  will increase during a population size expansion

333 (Charlesworth & Eyre-Walker 2007) and so we might expect island-to-mainland

334 colonisations to show different patterns to mainland-to-island colonisations. If

335 we restrict our analysis to mainland-to-island colonisations we still do not

336 observe a significant difference between island and mainland  $\omega$  overall, or for

337 each genome, although if we split by phylogenetic group the result for birds is

338 significant (Table 4). We also do not observe any significant difference in

339  $\omega(\text{mainland}) / \omega(\text{island})$  between species that have colonised the island from the

340 mainland, and the mainland from the island (independent samples t-test,  $p =$

341 0.315), contrary to the results of Charlesworth and Eyre-Walker (Charlesworth

342 & Eyre-Walker 2007).

343

344 *Adaptive evolution*

345 Colonisation of an island might be expected to lead to a burst of adaptive

346 evolution, since the colonisers are experiencing a new environment that might

347 have empty niches into which the species can adaptively evolve. To investigate

348 whether colonisation leads to higher rates of adaptive evolution we estimated

349 the rate of adaptive amino acid substitution along the island and mainland

350 lineages using two approaches. First we calculated the direction of selection

351 (DoS) statistic for each lineage. We find that on average the DoS is negative in  
352 both island and mainland species (Table 5), indicating that slightly deleterious  
353 mutations are prevalent in our data. We find no significant difference in values of  
354 DoS between island and mainland species, either when considering the dataset  
355 as a whole, or when the results are analysed separately depending on the  
356 direction of colonisation. However, DoS is sensitive to slightly deleterious  
357 mutations segregating in the population, and therefore any changes in the  
358 relative frequencies of deleterious mutations between island and mainland  
359 species will influence DoS, potentially masking a signal of adaptive evolution  
360 (Nielson 2005). Unfortunately, we did not have sufficient polymorphism data to  
361 correct for slightly deleterious mutations by removing low frequency  
362 polymorphisms (Fay et al. 2001; Charlesworth & Eyre-Walker 2008).

363

#### 364 *Mutation rate*

365 We also investigated potential differences in the mutation rates of island and  
366 mainland species. In this study we inferred the mutation rate from dS, the  
367 number of synonymous substitutions, along the lineages leading to the mainland  
368 and island species (and where there were multiple island and mainland species,  
369 from their averages).  $N_e$  is predicted to influence mutation rate, and as we found  
370 no consistent differences in  $N_e$  between island and mainland species we do not  
371 expect mutation rate to differ between the two groups. This is in fact the case:  
372 comparing dS values between island and mainland species revealed no  
373 significant trend (Table 6)( $n = 112$ ,  $p = 0.251$ ). However, when different  
374 genomes were considered separately, there was one statistically significant  
375 difference between island and mainland species for nuclear DNA ( $n = 32$ ,  $p =$

376 0.004). The trend in this instance was for mainland species to have higher values  
377 of dS than island species.

378

### 379 **Discussion**

380

381 It is generally assumed that island species will have smaller effective population  
382 sizes than mainland species. Island species are expected to have low effective  
383 population sizes initially because they are likely to be founded by a small number  
384 of individuals (one pregnant female is sufficient) and hence experience a  
385 bottleneck. We find some evidence for this: some island species, which are very  
386 closely related to their mainland counterparts, have little or no diversity,  
387 consistent with these species experiencing extreme bottlenecks during  
388 colonisation. However, besides these species, island species have similar levels of  
389 diversity to mainland species. There is no evidence to suggest that island species  
390 have low long-term effective populations sizes, despite the fact that island  
391 species occupy considerably smaller ranges than mainland species; in this  
392 analysis, island species had ranges of on average 0.14% of the area of their  
393 mainland counterparts. Consistent with island and mainland species having  
394 similar effective population sizes, we find no evidence that natural selection is  
395 less efficient in island species.

396

397 Our results are perhaps not surprising. It is well established that the relationship  
398 between population size and genetic diversity is not straightforward, with levels  
399 of genetic diversity remaining remarkably constant across groups of organisms  
400 which are incredibly disparate in terms of population size (Leffler et al. 2012;

401 Gillespie & Ohta 1996). What is unique about the current data is that only closely  
402 related species are compared to each other- many of the island and mainland  
403 species pairs are in the same genus. They therefore share life history traits, many  
404 of which influence molecular evolution. In addition, our paired study design  
405 allows us to correct for phylogenetic effects (Lanfear et al. 2010). This is crucial,  
406 as it has been well demonstrated that molecular evolution is influenced by  
407 taxonomy. For example, Romiguier et al. (Romiguier et al. 2014) demonstrated  
408 that levels of diversity differ between families but are similar within a family.  
409 Correcting for phylogenetic effects has allowed us to study the effects of island  
410 colonisation on molecular evolution across a wide range of taxa.

411

412 There are a number of possible reasons why island species might not have lower  
413 effective population sizes than their mainland counterparts. First, it is possible  
414 that many island species are founded by multiple individuals, and gene flow is  
415 maintained as they speciate, thereby allowing island species to inherit much of  
416 the variation of the mainland species. We have evidence that this is true of some  
417 species: birds in particular appear to experience relatively few bottlenecks as a  
418 taxonomic group, which is probably a due to their increased dispersal ability  
419 relative to other animals. However, after the initial colonisation event, we might  
420 then expect the genetic diversity of island species to decline due to their  
421 restricted range. We see no evidence of this: even if we exclude those young  
422 island species with no diversity, the correlation between synonymous nucleotide  
423 diversity and synonymous divergence remains positive ( $r = 0.214$ ,  $p = 0.150$ ).

424

425 Second, it has been suggested that levels of diversity are relatively constant  
426 across species because of an inverse relationship between population size and  
427 the mutation rate per generation (Lynch 2007; Piganeau & Eyre-Walker 2009), a  
428 relationship for which we have some evidence (Lynch 2010; Sung et al. 2012).  
429 This is hypothesised to occur because populations with large effective  
430 population sizes can more effectively select for modifiers of the mutation rate.  
431 Therefore, selection to reduce the mutation rate will be more effective in larger  
432 populations, resulting in lower mutation rates and hence levels of genetic  
433 diversity similar to those found in small populations. There is no evidence that  
434 this is the case in this analysis. When we analysed the levels of synonymous  
435 divergence, an indicator of the neutral mutation rate, we did not find a difference  
436 between island and mainland species, indicating that island species do not have  
437 higher mutation rates. In addition, there is no evidence, from considering the  
438 efficiency of selection, that island species have lower effective population sizes.  
439 Finally, upon excluding those species with no diversity we do not find that  
440 diversity increases with divergence, which we might expect if higher mutation  
441 rates evolve over time in island species.  
442  
443 Third, it is also possible that there is selection on synonymous mutations, which  
444 could obscure a relationship between genetic diversity and effective population  
445 size. If selection acts on synonymous codons to optimise the accuracy of  
446 translation, we expect there to be a distribution of fitness effects of synonymous  
447 mutations (Akashi 1994; Stoletzki & Eyre-Walker 2007). We therefore might find  
448 that as  $N_e$  increases, the proportion of effectively neutral mutations would  
449 decrease as selection becomes more efficient. This process could allow the levels

450 of genetic diversity to remain constant as effective population sizes increase, but  
451 only if the distribution of fitness effects of synonymous mutations is exponential  
452 (Welch et al. 2008). There is no evidence to suggest that this is the case, and  
453 therefore this is an unlikely explanation of our results.

454

455 Fourth, it has been suggested that levels of genetic diversity might not be  
456 correlated to population size due to selection at linked sites (Gillespie 2000;  
457 Maynard Smith & Haigh 1974). Gillespie has argued that if the rate of adaptive  
458 evolution is mutation limited then as population sizes increase so does the rate  
459 of adaptive evolution and hence the level of genetic hitch-hiking – a phenomenon  
460 that he has termed genetic draft. Some authors have found evidence to suggest  
461 that draft has an important role in reducing genetic diversity. However, studies  
462 generally report that draft has relatively weak effects, which may not be  
463 powerful enough to reduce genetic diversity to observed levels (Gossmann et al.  
464 2011; Weissman & Barton 2012; Andolfatto 2007). Furthermore, there is no  
465 evidence in our data that draft is important. Firstly, if genetic draft was prevalent  
466 in our dataset we might expect different patterns for the organellar genomes,  
467 which have little or no recombination, and the nuclear genome (Campos et al.  
468 2014). However, they behave qualitatively in a similar fashion. Secondly, we do  
469 not find a significant difference between island and mainland species in terms of  
470 their DoS. If selective sweeps were responsible for the low diversity of mainland  
471 species, we might expect mainland species to have greater values of DoS than  
472 their island counterparts. In addition, our results indicate that it is deleterious  
473 mutations that are dominating evolutionary dynamics, rather than advantageous  
474 mutations. However, it is worth noting that the signal of adaptive evolution could

475 be obscured by a shift in the distribution of fitness effects for island species.  
476 Correcting for this with the current dataset is difficult due to a lack of sufficient  
477 polymorphism data (we have very few datasets which contain more than 4  
478 alleles), although the results from our limited sample indicate that it is island  
479 species that undergo a greater degree of adaptive evolution, rather than species  
480 with large population sizes.

481

482 Romiguier et al. (Romiguier et al. 2014) recently showed that geographic factors  
483 likely to influence population size are poor correlates of genetic diversity when  
484 diversity is considered across the full breadth of the animal kingdom.  
485 Surprisingly, they find that propagule size is the single best predictor of diversity.  
486 Those species with few large propagules had low genetic diversity, and those  
487 with a large number of small propagules had high genetic diversity, and were  
488 termed K and r strategists respectively. However, the authors do not present a  
489 clear hypothesis as to why these strategies should affect genetic diversity. One  
490 possibility is that propagule size is related to population density, and that the  
491 variance in population density is far greater than the variance in population  
492 range size, so that the degree to which species differ in effective and census  
493 population sizes is largely determined by density and not range size. However,  
494 our results would tend to suggest that population density is not the missing  
495 factor, because there is no reason to believe that densities differ systematically  
496 between island and mainland species.

497

498 Alternatively, it may be that the mutation rate itself is an important determinant  
499 of diversity, particularly in organellar genomes (Lynch et al. 2006; Nabholz,

500 Mauffrey, et al. 2008; Bazin et al. 2006). Although the issue is controversial,  
501 Nabholz et al. showed that mutation rate is a major determinant of  
502 mitochondrial diversity, and as our dataset is dominated by mitochondrial  
503 sequences this could explain why we did not find a difference between island  
504 and mainland species, considering that we also did not find a difference in  
505 mutation rate between them. We found a strong positive correlation between the  
506 mutation rate, as measured by the rate of synonymous divergence, and levels of  
507 synonymous diversity, both for our entire dataset ( $n = 138$ ,  $r = 0.337$ ,  $p < 0.000$ ),  
508 and considering mitochondrial sequences separately ( $n = 112$ ,  $r = 0.269$ ,  $p =$   
509  $0.004$ ), which lends some support to this theory, however, we are unable to  
510 recover this correlation if we correct for phylogenetic independence by  
511 comparing island and mainland species (i.e.  
512  $\pi_s(\text{island}) / (\pi_s(\text{island}) + \pi_s(\text{mainland}))$  is not significantly correlated to  $dS(\text{island})$   
513  $/ (dS(\text{island}) + dS(\text{mainland}))$ ).

514

515 In conclusion, our analysis demonstrates that island colonisation typically has  
516 little impact on a species' molecular evolution. For some species the initial  
517 colonisation event results in a period of low diversity, but this effect appears to  
518 be short-lived with no discernible lasting effects. Our results confirm that census  
519 population size is a poor correlate of effective population size.

520

## 521 **Methods**

522

523 *Dataset*

524 The dataset was created by combining all of the independent island-mainland  
525 species comparisons used in two previous studies: the 48 comparisons of island  
526 and mainland bird species used in (Wright et al. 2009), and the 44 comparisons  
527 used in (Woolfit & Bromham 2005), which cover a wide range of taxa. This  
528 dataset was then expanded using a keyword search ('endemic') of the Arkive  
529 species database (<http://www.arkive.org/>). One or more mainland relatives and  
530 outgroup species were then identified for each island species. This added 56  
531 species comparisons to the dataset. Some comparisons contained a single island  
532 and mainland species, while some consisted of multiple island and/or mainland  
533 species. All phylogenies were checked for agreement with the literature, and  
534 apparent direction of colonisation was noted. In addition, the recorded range  
535 area of the species used was calculated from IUCN records (IUCN 2014) using  
536 ArcGIS. Protein coding sequences were collected from NCBI  
537 ([www.ncbi.nlm.nih.gov/genbank/](http://www.ncbi.nlm.nih.gov/genbank/)). Sequences were collected if there was an  
538 orthologous gene available for each of the island, mainland, and outgroup species  
539 in a comparison, or if there were multiple sequences of the same loci available  
540 for both the island and the mainland species in a comparison. A note was made  
541 of whether the sequences were nuclear, mitochondrial or chloroplast. All  
542 alignment files and further details of this analysis are available at:  
543 <http://dx.doi.org/10.6084/m9.figshare.1296151>.

544

#### 545 *Statistical tests*

546 This study has a paired design, in that each island species/clade is compared to a  
547 closely related mainland species/clade, with each comparison occurring only  
548 once in each analysis. If a choice had to be made between comparisons (for

549 example, if statistics from both the mitochondrial and nuclear genomes were  
550 available for a single comparison) the statistics that corresponded to the longest  
551 sequence alignment were used. This decision should reduce sampling error,  
552 because longer sequences are more representative than short sequences.  
553 Island and mainland species were compared using Wilcoxon signed-ranks tests.  
554 This is a paired, non-parametric test that takes into account the direction of the  
555 difference between pairs, and gives greater weight to those pairs that are the  
556 most different, making it more powerful than a sign test (Sokal & Rohlf 1995).

557

#### 558 *Polymorphism data*

559 Polymorphism data was calculated by aligning sequences of the same loci from  
560 the same species using a Geneious translation alignment, which was then  
561 analysed using our own scripts. A number of statistics were recorded, including  
562 nucleotide diversity and number of polymorphisms. If a comparison included  
563 multiple island and/or multiple mainland species, average values of each statistic  
564 were taken across the species. Similarly, if multiple sequences from the same  
565 genome were available for a particular island/mainland comparison, the average  
566 value of the sequences was used. Therefore, each comparison is represented by a  
567 single island, mainland, and outgroup value of each polymorphism statistic for a  
568 particular genome.

569 The data was used to calculate  $\pi_n/(\pi_n+\pi_s)$ , where  $\pi_n$  is nonsynonymous diversity  
570 and  $\pi_s$  is synonymous diversity. This ratio is used because, unlike polymorphism  
571 counts, nucleotide diversity is unaffected by the number of chromosomes  
572 sampled. In addition, using total diversity as the denominator reduces the

573 number of undefined ratios. Any comparisons with undefined values were  
574 excluded from the analysis.

575

#### 576 *Substitution data*

577 Substitution data was calculated by aligning orthologs of island, mainland and  
578 outgroup species. If multiple sequences at different loci were available for all of  
579 the species in a comparison, sequences were concatenated prior to alignment;  
580 however, sequences from different genomes of the same organism were treated  
581 separately. The alignments were pruned so that they included equal numbers of  
582 island and mainland species to control for the node-density effect (Hugall & Lee  
583 2007), and then used to generate phylogenetic trees with RaxML (Stamatakis  
584 2014), in combination with PartitionFinder (Lanfear et al. 2012). The trees were  
585 subsequently used to run the codeml programme of PAML version 4.7 (Yang  
586 2007), which calculated  $\omega$  (dN/dS) for island, mainland, and outgroup branches  
587 of each tree, as well as separate dN and dS values for each branch.

588

#### 589 *Adaptive evolution tests*

590 Polymorphism and substitution data was combined to test for differences in  
591 levels of adaptive evolution between island and mainland species. The direction  
592 of selection (DoS) statistic was used, calculated as:  $DoS = dN / (dN + dS) -$   
593  $pN / (pN + pS)$  This statistic has the advantage over using the neutrality index in  
594 that it is defined for all datasets in which there is at least one substitution and  
595 one polymorphism, so fewer species comparisons had to be excluded; it is also  
596 expected to be unbiased (Stoletzki & Eyre-Walker 2011). Positive values indicate

597 that the dynamics of evolution are dominated by positive selection and negative  
598 values that slightly deleterious mutations predominate.

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600

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737 **Tables**

<b>Divergence</b>	<b>Mitochondrial</b>	<b>Nuclear</b>	<b>Chloroplast</b>	<b>Combined dataset</b>
Amphibian	1	2	-	2
Bird	60	9	-	60
Invertebrate	15	3	-	15
Mammal	2	2	-	2
Plant	-	2	10	12
Reptile	18	14	-	21
Total	96	32	10	112

738 Table 1a

<b>Polymorphism</b>	<b>Mitochondrial</b>	<b>Nuclear</b>	<b>Chloroplast</b>	<b>Combined dataset</b>
Amphibians	-	1	-	1
Bird	37	2	-	37
Invertebrate	11	1	-	11
Mammal	1	-	-	1
Plant	-	1	4	4
Reptile	11	9	-	16
Total	60	14	4	70

739 Table 1b

740 Table 1a and b

741 An overview of the sequences gathered in this analysis, split by DNA type and  
742 taxonomic group. For analyses that combined data across DNA types, each  
743 species comparison appeared only once: the numbers of sequences available in  
744 these cases are given in the 'combined dataset' column. When choosing between  
745 sequences from different genomes for a particular comparison, we always used  
746 the longest sequence.

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Dataset	n	Mean Island $\pi_s$	Mean Mainland $\pi_s$	Larger ranks-Island:Mainland	p-value
Combined	70	0.0270	0.0388	19:43	0.010
Chloroplast	4	0.00231	0.000575	2:0	0.180
Mitochondrial	60	0.0320	0.0524	19:39	0.014
Nuclear	14	0.00147	0.00686	1:7	0.036
Bird mitochondrial	37	0.0117	0.0283	11:25	0.012
Invertebrate mitochondrial	11	0.0784	0.0582	4:6	0.646
Reptile mitochondrial	11	0.0562	0.116	4:7	0.131

752 Table 2

753 Table 2

754 Differences in synonymous nucleotide diversities ( $\pi_s$ ) between island and  
 755 mainland species. The number of comparisons used in each analysis is given in  
 756 the second column (n), with the significance level of the Wilcoxon signed-ranks  
 757 test given in the last column. Each particular species comparison appears only  
 758 once in each dataset.

759

760

Dataset	n	Mean Island $\pi_n/(\pi_n+\pi_s)$	Mean Mainland $\pi_n/(\pi_n+\pi_s)$	Larger ranks-Island:Mainland	p-value
Combined	51	0.175	0.093	25:23	0.389
Chloroplast	1	0.257	0.223	1:0	-
Mitochondrial	48	0.170	0.092	23:22	0.569
Nuclear	3	0.182	0.126	1:2	-
Bird	30	0.268	0.103	17:11	0.050
Invertebrate	10	0.035	0.055	5:5	0.646
Reptile	20	0.027	0.095	2:5	0.063

761 Table 3

762 Table 3

763 Differences in  $\pi_n/(\pi_n+\pi_s)$  between island and mainland species. The number of  
 764 comparisons used in each analysis is given in the second column (n), with the

765 significance level of the Wilcoxon signed-ranks test given in the last column. Each  
 766 particular species comparison appears only once in each dataset.

767

768

Dataset	n	Mean Island $\omega$	Mean Mainland $\omega$	Larger ranks- Island:Mainland	p-value
Combined	108	0.103	0.087	58:48	0.511
Chloroplast	9	0.341	0.158	6:3	0.173
Mitochondrial	92	0.042	0.051	49:41	0.480
Nuclear	26	0.368	0.238	11:14	0.677
Bird	59	0.083	0.062	36:22	0.092
Invertebrate	14	0.059	0.028	6:7	0.861
Plant	11	0.309	0.167	7:4	0.286
Reptile	20	0.092	0.106	8:12	0.313

769 Table 4a

Dataset	n	Mean Island $\omega$	Mean Mainland $\omega$	Ratio Island:Mainland	p-value
I→M	14	0.162	0.191	4:10	0.363
M→I	94	0.095	0.071	54:38	0.235
M→I	8	0.257	0.151	5:3	0.327
Chloroplast					
M→I	80	0.040	0.035	46:32	0.155
Mitochondrial					
M→I Nuclear	23	0.387	0.232	10:12	0.592
M→I Bird	50	0.088	0.044	34:15	0.019
M→I	14	0.059	0.028	6:7	0.861
Invertebrate					
M→I Plant	10	0.238	0.162	6:4	0.508
M→I Reptile	16	0.069	0.073	7:9	0.569

770 Table 4b

771 Table 4a and 4b

772 Differences in  $\omega$ , (nonsynonymous divided by synonymous divergence) between

773 island and mainland comparisons. The number of comparisons used in each

774 analysis is given in the second column (n), with the significance level of the

775 Wilcoxon signed-ranks test given in the last column. Each particular species

776 comparison appears only once in each dataset. In a), the total dataset is analysed

777 and then divided by DNA type and taxonomic group, while in b), the comparisons

778 are split by colonisation direction; I→M refers to comparisons in which the  
 779 colonisation direction was island-to-mainland, while M→I is mainland-to-island.  
 780 Where the colonisation direction was mainland-to-island, comparisons were  
 781 further divided by genome and taxonomic group

782

783

Dataset	n	Mean Island DoS	Mean Mainland DoS	Larger ranks: Island:Mainland	p-value
Combined	50	-0.180	-0.167	25:25	0.783
I → M	8	-0.144	-0.109	3:5	0.674
M → I	42	-0.187	-0.178	22:20	0.965

784 Table 5

785 Table 5

786 Differences in DoS between island and mainland species, for the combined  
 787 dataset, and for the dataset split by the direction of colonisation. The number of  
 788 comparisons used in each analysis is given in the second column (n), with the  
 789 significance level of the Wilcoxon signed-ranks test given in the last column.

790 I→M refers to comparisons in which the colonisation direction was island-to-  
 791 mainland, while M→I is mainland-to-island

792

793

Dataset	n	Mean Island dS	Mean Mainland dS	Ratio Island:Mainland	p-value
Combined	112	0.351	1.15	52:59	0.251
Chloroplast	10	0.0164	0.0129	6:4	0.646
Mitochondrial	96	0.559	1.42	48:48	0.527
Nuclear	32	0.0582	0.157	6:22	0.004

794 Table 6

795 Table 6

796 Differences in synonymous divergence (dS) between island and mainland  
797 species. The number of comparisons used in each analysis is given in the second  
798 column (n), with the significance level of the Wilcoxon signed-ranks test given in  
799 the last column. Each particular species comparison appears only once in each  
800 dataset.

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819 **Figure legends**

820

821 Fig. 1 The frequency distribution of the ratios of island:mainland species range

822 areas

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824 Fig. 2 The ratio of island diversity to the combined island and mainland diversity,

825  $\pi_s(\text{island}) / (\pi_s(\text{island}) + \pi_s(\text{mainland}))$ , where  $\pi_s$  is synonymous diversity, plotted

826 against total divergence (dS) between island and mainland species.

827



