

1 Molecular evolutionary consequences of island colonisation

2

3 Jennifer E. James¹, Robert Lanfear^{2,3}, Adam Eyre-Walker¹

4

5 ¹Centre for the Study of Evolution, School of Life Sciences, University of Sussex,
6 Brighton, United Kingdom, BN1 9QG

7 ²Department of Biological Sciences, Macquarie University, Sydney, New South
8 Wales 2109, Australia

9 ³ Division of Evolution Ecology and Genetics, Research School of Biology, The
10 Australian National University, Canberra, Australian Capital Territory 0200,
11 Australia

12

13 Jennifer James: E-mail: j.e.james@sussex.ac.uk, telephone number: 07743312606

14 Robert Lanfear: E-mail: rob.lanfear@gmail.com

15 Adam Eyre-Walker (Corresponding author): E-mail: [a.c.eyre-](mailto:a.c.eyre-walker@sussex.ac.uk)
16 walker@sussex.ac.uk.

17

18 Data archived on figshare at: <http://dx.doi.org/10.6084/m9.figshare.1296151>

19 Word count: 5,766

20 Table count: 8, (1a, 1b, 2, 3, 4a, 4b, 5 and 6)

21 Figure count: 2

22

23

24

25

26

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 (ω). 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

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

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

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

Results

Dataset overview

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

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

Geography

Island species are studied from a molecular evolutionary perspective because they are expected to have smaller populations than mainland species due to their small ranges. However, this assumption is rarely tested. In this study, the ranges of the species used were confirmed where possible using the IUCN database (IUCN 2014). The mean range of island species was 5,780 km², while for mainland species this mean range was over 4,080,000 km². The ratio of island to mainland range sizes did not exceed 0.25 for any of the comparisons used, and in the majority of cases island species had ranges which were less than 1% of the area of those of their mainland counterparts (Figure 1). Therefore we have evidence that the island species used in this study inhabit substantially smaller geographic regions than their mainland relatives, although we have no information on population density.

Synonymous diversity

We might expect island species to have lower diversity than their mainland counterparts for two reasons. Firstly, island species inhabit substantially smaller areas than their mainland relatives; resulting in a smaller census population size and hence potentially a smaller long-term N_e . Secondly, island populations are 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,

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

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

Effective population sizes

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

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

Efficiency of selection

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

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

363

364 *Mutation rate*

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

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

Discussion

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

Our results are perhaps not surprising. It is well established that the relationship between population size and genetic diversity is not straightforward, with levels of genetic diversity remaining remarkably constant across groups of organisms 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/). 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

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

Polymorphism data

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

The data was used to calculate $\pi_n/(\pi_n+\pi_s)$, where π_n is nonsynonymous diversity and π_s is synonymous diversity. This ratio is used because, unlike polymorphism counts, nucleotide diversity is unaffected by the number of chromosomes 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 ω (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: $\text{DoS} = \text{dN}/(\text{dN} + \text{dS}) -$
593 $\text{pN}/(\text{pN} + \text{pS})$ 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.

599

600

601 **Literature Cited**

602

603 Akashi, H., 1994. Synonymous codon usage in *Drosophila melanogaster*: Natural
604 selection and translational accuracy. *Genetics*, 136, pp.927–935.

605 Andolfatto, P., 2007. Hitchhiking effects of recurrent beneficial amino acid
606 substitutions in the *Drosophila melanogaster* genome. *Genome Research*, 17,
607 pp.1755–1762.

608 Barrett, R.D.H. & Schluter, D., 2007. Adaptation from standing genetic variation.
609 *Trends in ecology & evolution*, 23, pp.38–44.

610 Bazin, E., Glémin, S. & Galtier, N., 2006. Population size does not influence
611 mitochondrial genetic diversity in animals. *Science*, 312, pp.570–2.

612 Briskie, J. V & Mackintosh, M., 2003. Hatching failure increases with severity of
613 population bottlenecks in birds. *PNAS*, 101, pp.558–561.

614 Bromham, L., 2011. The genome as a life-history character: why rate of
615 molecular evolution varies between mammal species. *Phil. Trans. R. Soc. B*, 366,
616 pp.2503–13.

617 Burgess, S.C., Waples, R.S. & Baskett, M.L., 2013. Local adaptation when
618 competition depends on phenotypic similarity. *Evolution*, 67, pp.3012–22.

619 Campos, J.L. et al., 2014. The relation between recombination rate and patterns
620 of molecular evolution and variation in drosophila melanogaster. *Molecular*
621 *Biology and Evolution*, 31, pp.1010–1028.

622 Charlesworth, B. & Charlesworth, D., 1987. Inbreeding Depression and its
623 Evolutionary Consequences. *Ann. Rev. Ecol. Syst*, 18, pp.237–268.

624 Charlesworth, J. & Eyre-Walker, A., 2008. The McDonald-Kreitman test and
625 slightly deleterious mutations. *Molecular biology and evolution*, 25(6), pp.1007–
626 15.

627 Charlesworth, J. & Eyre-Walker, A., 2007. The other side of the nearly neutral
628 theory, evidence of slightly advantageous back-mutations. *PNAS*, 104, pp.16992–
629 7.

630 Fay, J.C., Wyckoff, G.J. & Wu, C., 2001. Positive and Negative Selection on the
631 Human Genome. *Genetics*, 158, pp.1227–1234.

632 Frankham, R., 1997. Do island populations have less genetic variation than
633 mainland populations? *Heredity*, 78, pp.311–27.

634 Frankham, R. et al., 1999. Do population size bottlenecks reduce evolutionary
635 potential? *Animal Conservation*, 2, pp.255–260.

636 Frankham, R., 2012. How closely does genetic diversity in finite populations
637 conform to predictions of neutral theory? Large deficits in regions of low
638 recombination. *Heredity*, 108, pp.167–78.

639 Gillespie, J.H., 2000. Genetic Drift in an Infinite Population: The
640 Pseudohitchhiking Model. *Genetics*, 155, pp.909–919.

641 Gillespie, J.H. & Ohta, T., 1996. Development of neutral and nearly neutral
642 theories. *Theor. Pop. Biol.*, 49, pp.128–148.

643 Gossmann, T.I., Woolfit, M. & Eyre-Walker, A., 2011. Quantifying the variation in
644 the effective population size within a genome. *Genetics*, 189, pp.1389–402.

645 Hermisson, J. & Pennings, P.S., 2005. Soft sweeps: molecular population genetics
646 of adaptation from standing genetic variation. *Genetics*, 169, pp.2335–52.

647 Ho, S.Y.W. et al., 2011. Time-dependent rates of molecular evolution. *Molecular*
648 *ecology*, 20, pp.3087–101.

649 Hugall, A.F. & Lee, M.S.Y., 2007. The likelihood node density effect and
650 consequences for evolutionary studies of molecular rates. *Evolution*, 61,
651 pp.2293–307.

652 IUCN, 2014. IUCN Red List of Threatened Species. Available at:
653 <www.iucnredlist.org>.

654 Johnson, K.P. & Seger, J., 2001. Elevated Rates of Nonsynonymous Substitution in
655 Island Birds. *Molecular biology and evolution*, 18, pp.874–881.

656 Johnson, T.H. & Stattersfield, A.J., 1990. A global review of island endemic birds.
657 *Ibis*, 132, pp.167–180.

658 Jones, K.E. et al., 2003. Biological Correlates of Extinction Risk in Bats. *The*
659 *American Naturalist*, 161, pp.601–614.

660 Kimura, M., 1984. *The neutral theory of molecular evolution*, Cambridge
661 University Press.

662 Kryazhimskiy, S. & Plotkin, J.B., 2008. The population genetics of dN/dS. *PLoS*
663 *genetics*, 4.

664 Lanfear, R. et al., 2012. Partitionfinder: combined selection of partitioning
665 schemes and substitution models for phylogenetic analyses. *Molecular biology*
666 *and evolution*, 29, pp.1695–701.

667 Lanfear, R. et al., 2013. Taller plants have lower rates of molecular evolution.
668 *Nature Communications*, 4, p.1879.

669 Lanfear, R., Welch, J.J. & Bromham, L., 2010. Watching the clock: studying
670 variation in rates of molecular evolution between species. *Trends in ecology &*
671 *evolution*, 25, pp.495–503.

672 Leffler, E.M. et al., 2012. Revisiting an old riddle: what determines genetic
673 diversity levels within species? *PLoS biology*, 10.

674 Lewontin, R., 1974. *The genetic basis of evolutionary change*, New York: Columbia
675 University Press.

676 Lynch, M., 2010. Evolution of the mutation rate. *Trends in genetics*, 26, pp.345–
677 52.

678 Lynch, M., 2007. *The Origins of Genome Architecture*, Sunderland: Sinauer
679 Associates Inc.

680 Lynch, M., Koskella, B. & Schaack, S., 2006. Mutation pressure and the evolution
681 of organelle genomic architecture. *Science*, 311, pp.1727–30.

682 Maynard Smith, J. & Haigh, J., 1974. The hitch-hiking effect of a favourable gene.
683 *Genetical Research*, 23, pp.23–35.

684 Mckinney, M.L., 1997. Extinction Vulnerability and Selectivity: Combining
685 Ecological and Paleontological Views. *Annu. Rev. Ecol. Syst.*, 28, pp.495–516.

686 Messer, P.W. & Petrov, D. a, 2013. Population genomics of rapid adaptation by
687 soft selective sweeps. *Trends in ecology & evolution*, 28, pp.659–69.

688 Montgomery, M.E. et al., 2010. Widespread selective sweeps affecting
689 microsatellites in *Drosophila* populations adapting to captivity: Implications for
690 captive breeding programs. *Biological Conservation*, 143, pp.1842–1849.

691 Nabholz, B., Mauffrey, J.-F., et al., 2008. Determination of mitochondrial genetic
692 diversity in mammals. *Genetics*, 178, pp.351–61.

693 Nabholz, B., Glémin, S. & Galtier, N., 2008. Strong variations of mitochondrial
694 mutation rate across mammals- the longevity hypothesis. *Molecular biology and*
695 *evolution*, 25, pp.120–30.

696 Nei, M., Maruyama, T. & Chakraborty, R., 1975. The Bottleneck Effect and Genetic
697 Variability in Populations. *Evolution*, 29, pp.1–10.

698 Nielson, R., 2005. Molecular Signatures of Natural Selection. *Annu. Rev. Genet.*, 39,
699 pp.197–218.

700 Piganeau, G. & Eyre-Walker, A., 2009. Evidence for variation in the effective
701 population size of animal mitochondrial DNA. *PloS one*, 4.

702 Pimm, S.L. et al., 1988. On the Risk of Extinction. *The American Naturalist*, 132,
703 pp.757–785.

704 Purvis, A. et al., 2000. Predicting extinction risk in declining species. *Proc. R. Soc.*,
705 267, pp.1947–52.

706 Romiguier, J. et al., 2014. Comparative population genomics in animals uncovers
707 the determinants of genetic diversity. *Nature*, 515, pp.261–263.

708 Sokal, R. & Rohlf, F.J., 1995. *Biometry*, New York: W. H. Freeman.

709 Stamatakis, A., 2014. RAxML Version 8: A tool for Phylogenetic Analysis and
710 Post-Analysis of Large Phylogenies. *Bioinformatics*.

711 Stoletzki, N. & Eyre-Walker, A., 2011. Estimation of the neutrality index.
712 *Molecular biology and evolution*, 28, pp.63–70.

713 Stoletzki, N. & Eyre-Walker, A., 2007. Synonymous codon usage in *Escherichia*
714 *coli*: Selection for translational accuracy. *Molecular Biology and Evolution*, 24,
715 pp.374–381.

716 Sung, W. et al., 2012. Drift-barrier hypothesis and mutation-rate evolution. *PNAS*,
717 109, pp.18488–18492.

718 Takano-Shimizu, T., 1999. Local Recombination and Mutation Effects on
719 Molecular Evolution in *Drosophila*. *Genetics*, 153, pp.1285–1296.

720 Weissman, D.B. & Barton, N.H., 2012. Limits to the rate of adaptive substitution
721 in sexual populations. *PLoS genetics*, 8.

722 Welch, J.J., Eyre-Walker, A. & Waxman, D., 2008. Divergence and polymorphism
723 under the nearly neutral theory of molecular evolution. *Journal of Molecular*
724 *Evolution*, 67, pp.418–426.

725 Woolfit, M. & Bromham, L., 2005. Population size and molecular evolution on
726 islands. *Proc. R. Soc.*, 272, pp.2277–2282.

727 Wright, S.D. et al., 2009. Slower tempo of microevolution in island birds:
728 implications for conservation biology. *Evolution*, 63, pp.2275–2287.

729 Yang, Z., 2007. PAML 4: a program package for phylogenetic analysis by
730 maximum likelihood. *Molecular biology and evolution*, 24, pp.1586–1591.

731

732

733

734

735

736

737 Tables

Divergence	Mitochondrial	Nuclear	Chloroplast	Combined dataset
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

Polymorphism	Mitochondrial	Nuclear	Chloroplast	Combined dataset
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.

747

748

749

750

751

Dataset	n	Mean Island π_s	Mean Mainland π_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 (π_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

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

Dataset	n	Mean Island ω	Mean Mainland ω	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

Table 4a

Dataset	n	Mean Island ω	Mean Mainland ω	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

Table 4b

Table 4a and 4b

Differences in ω , (nonsynonymous divided by synonymous divergence) between

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

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

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

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

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

are split by colonisation direction; I→M refers to comparisons in which the colonisation direction was island-to-mainland, while M→I is mainland-to-island. Where the colonisation direction was mainland-to-island, comparisons were 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 dataset, and for the dataset split by the direction of colonisation. The number of comparisons used in each analysis is given in the second column (n), with the 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-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.

801

802

803

804

805

806

807

808

809

810

811

812

813

814

815

816

817

818

819 **Figure legends**

820

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

822 areas

823

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 π_s is synonymous diversity, plotted

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

827



