Peer Community In Evolutionary Biology

Demographic effects may affect adaptation to islands

Emma Berdan *based on peer reviews by* ***Steven Fiddaman*** *and 3 anonymous reviewers*

Mathilde BARTHE, Claire DOUTRELANT, Rita COVAS, Martim MELO, Juan Carlos ILLERA, Marie-Ka TILAK, Constance COLOMBIER, Thibault LEROY , Claire LOISEAU , Benoit NABHOLZ (2022) Evolution of immune genes in island birds: reduction in population sizes can explain island syndrome. Missing preprint_server, ver. 4, peer-reviewed and recommended by Peer Community in Evolutionary Biology.
https://doi.org/10.1101/2021.11.21.469450

Submitted: 29 November 2021, Recommended: 02 November 2022

Cite this recommendation as:
Berdan, E. (2022) Demographic effects may affect adaptation to islands. *Peer Community in Evolutionary Biology*, 100153. https://doi.org/10.24072/pci.evolbiol.100153

Published: 02 November 2022

The unique challenges associated with living on an island often result in organisms displaying a specific suite of traits commonly referred to as “island syndrome” (Adler and Levins, 1994; Burns, 2019; Baeckens and Van Damme, 2020). Large phenotypic shifts such as changes in size (e.g. shifts to gigantism or dwarfism, Lomolino, 2005) or coloration (Doutrelant et al., 2016) abound in the literature. However, less obvious phenotypes may also play a key role in adaptation to islands.

One such trait, reduced immune function, has important implications for the future of island populations in the face of anthropogenic-induced changes. Due to lower parasite pressure caused by a less diverse and less virulent parasite population, island hosts may show a decrease in immune defenses (Beadell et al., 2006; Pérez-Rodríguez et al., 2013). However, this hypothesis has been challenged, as many studies have found ambiguous or conflicting results (Matson, 2006; Illera et al., 2015).

While most previous work has examined various immunological parameters (e.g., antibody concentrations), here, Barthe et al. (2022) take the novel approach of examining molecular signatures of immune genes. Using comparative genomic data from 34 different species of birds the authors examine the ratio of synonymous substitutions (i.e., not changing an amino acid) to non-synonymous substitutions (i.e., changing an amino acid) in innate and acquired immune genes (Pn/Ps ratio). Because population sizes on islands are lower which will affect molecular evolution, they compare these results to data from 97 control genes. Assuming relaxed selection on islands predicts that the difference between the Pn/Ps ratio of immune genes and of control genes (ΔPn/Ps) is greater in island species compared to mainland ones.

As with previous work the authors found that the results differ depending on the category of immune genes. Both forms of innate defense: beta-defensins and Toll-like receptors did not show higher ΔPn/Ps for island populations. As these genes still have a higher Pn/Ps than control genes, the authors argue these results are in line with these genes being under purifying selection but lacking an "island effect". Instead, the authors argue that demographic effects (i.e., reductions in *Ne*) may lead to the decreased immunity documented in other studies. In contrast, there was a reduction in Pn/Ps in MHC II genes, known to be under balancing selection. This reduction was stronger in island species and thus the authors argue that this is the only class of genes where a role for relaxed selection can be invoked.

Together these results demonstrate that the changes in immunity experienced by island species are complex and that different categories of immune genes can experience different selective pressures. By including control genes in their study, they particularly highlight the importance of accounting for shifts in *Ne* when examining patterns of island species evolution. Hopefully, this kind of framework will be applied to other taxa to determine if these results are widespread or more specific to birds.

References:

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Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2021.11.21.469450
Version of the preprint: 2

Authors' reply, 27 September 2022

Dear Dr Berdan,

Please find attached the revised manuscript with the changes highlighted in blue. In this revised manuscript, we took all your corrections and those of the reviewers into account. The revised manuscript has been submitted to biorxiv (https://doi.org/10.1101/2021.11.21.46945). Thank you for handling our manuscript.

Best regards,

Mathilde Barthe on the behalf of all co-authors

Download tracked changes file

Decision by Emma Berdan, posted 09 September 2022, validated 12 September 2022

Minor revision

I want to apologize again for the delay, I had to find a chunk of time to make extensive comments on the paper. Overall, I find that the authors did a great job of responding to the reviewer comments and improving the technical aspects of the paper. However, I still think that the readability needs to be improved and agree with the suggestions of reviewer 3. In addition, I attach an edited and noted copy that I have worked on with several questions as well as edits. In particular, the tense issue needs to be addressed as the manuscript switches between past and present tense. These are all superficial issue and can be easily addressed. I think having the manuscript read by a native speaker again before submission could be helpful as well. I am excited to see the next (and final) version of this manuscript!

Download recommender's annotations

Reviewed by Steven Fiddaman, 22 July 2022

In my opinion, the authors have satisfactorarily responds to the points made and I have no further comments.

Reviewed by anonymous reviewer 1, 29 August 2022

Dear Editors,

I have reviewed this new version of the manuscript and am happy to say that it has fully accounted for the reviewer's comments. I attach some very minor corrections directly on the pdf. In conclusion, I think that the manuscript can be published.

Download the review

Reviewed by anonymous reviewer 3, 16 August 2022

The authors have made a thorough job replying to all of my comments and revised the ms accordingly. I still have some additional comments.

It is quite difficult to follow all the model testing and selection processes, I understand they are difficult to explain, but it would be good to make those parts more clear. I recommend reading all the newly added paragraphs and checking once more for correct spelling and grammar.

A comment on the new analysis based on high nucleotide diversity and low FIS: it is true that this pattern is expected when paralog genes are accidentally analysed as one gene, but it is also expected with very strong and long-term balancing selection. It can be difficult to tell these apart, so I agree that leaving the suspiciously low FIS and high Pi genes out is at least a clear solution to the potential paralog problem.

11: immune fonctions
 immune functions

101: (Santonastaso et al., 2017) demonstrated
 Remove the parentheses.

276-286: Check that the singular and plural forms are in correct places. (e.g. HO is the average number of heterozygous individualS).

358-361: Five linear mixed models were defined i) null model, ii) model with only the origin parameter, iii) model with only the gene category parameter, iv) model using both origin and gene category parameters, and finally v) model including those two parameters and the interaction effect.
 Use the same numbering (1-5) for the models in the text and in the tables.

Figure 3 is not referred to in the text.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2021.11.21.469450
Version of the preprint: 1

Authors' reply, 03 July 2022

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Decision by Emma Berdan, posted 07 March 2022, validated 08 March 2022

I want to apologize for the delay with this decision; it was very difficult to find reviewers. The preprint has now been read by four reviewers and myself. All of us found merit in the study but there are several issues that should be addressed before publication.

By far the biggest issue raised is that the authors used different species for the island vs. mainland. This means that species itself is confounded with island vs. mainland and it seems that the phylogenetic distribution is skewed as well. This issue needs to be addressed up front.

Furthermore, several methodological questions need to be clarified such as sampling strategy and choice of "control genes". Finally, the reviewers had a number of minor comments will improve readability that should be addressed as well. In addition, I had some comments that follow below. I look forward to reading a revised version of this manuscript.

Best,
Emma Berdan

Comments:

Species names should always be in italics.

Lines 128-150 - This section is somewhat convoluted and it is hard to understand what your predictions are.

Figure 2 - The same color (purple) should not refer to different groupings in the two figures (A and B). This is confusing for the reader.

I found table 3 very difficult to read. Please re-do the formatting.

Reviewed by anonymous reviewer 3, 14 February 2022

Preprint "Reduction in population size and not a shift in parasite community affect evolution of immune genes in island birds" by Barthe et al. challenges the hypothesis of relaxed selection pressure on immune genes due to reduced diversity of pathogens. Instead, they come to the conclusion that the apparent lack of genomic signatures of selection is, in other than MHC class II genes, due to the decrease in population size and thus stronger genetic drift. The authors study 20 island and 14 mainland bird species. They compare the synonymous and non-synonymous diversity of various innate and acquired immune genes and use a set of non-immune function genes as a control group.

In general, the preprint is well and clearly written. The analyses and conclusions are justified and well explained. The introduction goes through relevant theory and introduces the hypotheses. A minor detail: both immune gene and immunity gene are used in the text, I suggest using only "immune gene" throughout the paper.

In the preprint, the immune genes are handled in three different classes instead of individual examination. I wonder if one would expect all the immune genes to respond in a similar way under relaxed pressure? Would you expect some genes to be more sensitive to selection? Is it possible that some interesting aspects are lost when only the means of the three classes of genes are included in the analyses? Did the authors look at the variation in Pn/Ps among the genes within a species? It is a challenging task to compare mainland and island populations that have differences both in population size, demographic history, time of speciation event and parasite load. Nevertheless, the authors have done a thorough job trying to control for different sources of variation.

Another general note: How did the authors take into account gene families? E.g. MHC genes often have multiple repeats in an individual genome and it may be very demanding to pick only one of those genes while alleles are likely to be also recombining into different loci and the copy number changes even between individuals. How many MHC genes were found per species and were there any problems in selecting the correct counterparts from different species?

Detailed comments:

126-127: In this context, the ratio Pn/Ps is typically interpreted as the result of a change in natural selection.

 Why would Pn/Ps indicate change in natural selection, and not a long-lasting evolutionary state of natural selection?

129-132: However, the fixation probability depends on the product Ne*s, and a variation in Ne is also expected to impact the efficacy of selection and thus the ratio Pn/Ps across the entire transcriptome, particularly in the presence of slightly deleterious mutations (Ohta 1992; Charlesworth and Eyre-Walker 2008; Loire et al. 2013; Leroy et al. 2021).

 When talking about immune gene diversity, advantageous mutations are also important and likely lost due to drift.

133: Therefore, we predict a significant effect of drift on island species leading to a genome-wide reduction in genetic diversity and efficacy of selection, as reported by previous studies. In addition, due to their lower population sizes, island birds compared to continental species exhibit a genome-wide reduction in genetic diversity and efficacy of selection (Kutschera et al. 2020; Leroy et al. 2021).

 Here the authors seem to state the same point twice: the effect of drift and population size. Population size is directly linked to the strength of genetic drift, so I suggest either combining these sentences or modifying to make more clear what is the difference that the authors mean here.

141-143: we randomly selected protein-coding genes (i.e., control genes) implied in various biological functions (Fijarczyk et al. 2016; Leroy et al. 2021).

 Is "implied" the correct word here?

145-147: More specifically, for genes under purifying selection, non-synonymous weekly deleterious mutations, normally eliminated under strong selection, would be maintained, leading to an increase of genetic diversity.

 I wonder if it is possible to draw such a direct conclusion about the fate of the alleles of genes under purifying genes. Anyhow, I assume that for many immune genes, the negative selection pressure also rises from autoimmunity reactivity, so the parasite pressure is not the only driving force.

Figure 2: This figure is good for visualizing the main hypotheses of the manuscript. However, wouldn't we expect genes evolving under purifying selection to follow a similar low ratio of Pn/Ps as random protein-coding genes that often also evolve under purifying selection?

Table 1: It is not clear which data is from which reference. Also the settings of the table could be more clear, especially for the two last columns.

Table 1: Correct "Ploceus cuculatus" to "Ploceus cucullatus".

268: The island effective population size of 110,000 sounds very large assuming that the census size is much larger. Do you have estimates of the census sizes of the study populations and gene flow between the different populations? I assume gene flow between different island populations and/or between island and mainland populations can have a large effect on both the selection pressures and amount of drift/effective population size.

283-285: Unlike for all other species (e.g. Fringilla coelebs, Figure S3), synonymous polymorphism level was very dependent on the number missing data tolerated in P. trochilus alignments (Figure S3).

 Is there something missing from this sentence?

298-301: A linear mixed model was performed, using the Pn/Ps ratio as dependant variable and, as explanatory variables, the mainland or insular origin of species as well as the category of genes (packages lme4 and

lmerTest (Bates et al. 2012; Kuznetsova et al. 2017))

 In general, the regression models in the preprint seem to be well performed and justified. However, the Pn/Ps ratio does not immediately seem like a normally distributed variable. How well does the linear model fit your data with Ps/Pn bound to be positive (and relatively close to zero)? Did you try to fit other error structures and check for the distribution of residuals in the linear model?

337: an average of 3.3 millions paired-ends reads per individual was generated

 "millions" "million"

Table 2 & Table 4: The p-values of ANOVA test between simpler models are not reported if a more complex model is significant.

 I suggest changing this sentence into: "The p-values of ANOVA test between simpler models are not reported if a more complex model explains a larger proportion of the variance".

 In addition, please clarify in the tables what are models 1-4 in the ANOVA part of the table.

486-487: "MHC class I genes are primarily involved in the recognition intracellular pathogens (Kappes and Strominger 1988)"

 Add "of" to: "in the recognition of intracellular pathogens"

Supplementary material:

Figure S2: Correlation between Pn/Ps (a) and Ps (b) calculated on the control genes in this study's dataset and those calculated by (Leroy et al. 2021) Leroy et al. (2021).

 Remove the extra reference.

Figure S4: Boxplot of Pn/Ps according to population size for simulated sequences under overdomiance with SliM.

 Correct the word "overdominance".

Table S3: This is missing, there was no Table S3 in the supplementary material. And I could not find any list of genes that were studied. This was referred as Table S3 in the results on line 341.

Reviewed by anonymous reviewer 2, 23 January 2022

This manuscript tests the hypothesis that immune genes of birds living on islands experience reduced selective pressure from parasites, compared to the immune genes of birds living on the mainland. The authors analyze 35 species and compare genetic diversity in synonymous (Pn) and nonsynonymous sites (Ps) between species from mainland vs. island in control (randomly selected) genes and genes linked to immune functions. To test the hypothesis authors calculate Pn/Ps (or πN/πS), a statistic meant to detect selection within a population, where Pn, in brief, is influenced by effective population size and selection (and mutation rate), and Ps is influenced by effective population size (and mutation rate). The authors want to distinguish between the effects of population size differences (smaller on the island), and selection, (expected to be also smaller on the island). Although the idea is quite simple and attractive, I found major problems with this approach and its implementation. The major problem is the use of a different set of species for scoring Pn/Ps on the mainland and island. I'm afraid this leads to uninterpretable results. I think it is not possible to distinguish with this system effects of relaxed selection caused by reduced Ne, and reduced selective pressure from parasites because both of these properties can vary significantly between species.

Major comments:

1) The statistical test considers two measures, one of them is delta Pn/Ps which compares the difference in selection between immune and control genes, and another is a difference between Pn/Ps between mainland

and island species. Whereas delta Pn/Ps is calculated for the same species, the difference between mainland and island is calculated between groups of different species. Delta Pn/Ps will correspond to the average difference in selective pressure between control and immune genes, but the difference mainland-island here corresponds not to the average difference in Ne/selection between origins, but the average difference in Ne/selection between several species. In other words, one can't untangle the effects of origin from the effects of species. I think the only potential solution to test this hypothesis would be to use simulations to find parameters of Ne and selection which correspond to genetic diversity observed in immune and control genes, separately in mainland and island populations, and then compare if estimated selection coefficients are the same or lower in island compared to the mainland.

2) Not much is mentioned about populations. Pn/Ps is a statistic that only makes sense in populations, eg population structure can impact nucleotide diversity and therefore Pn/Ps ratio. From the text and tables, it's not clear if sampled individuals come from single populations or are just randomly selected from a species. The authors should clarify this or show some results indicating that all individuals from each species come from the same population.

3) In MHC simulations, apart from the above-mentioned problem with different species, it seems from simulations of balancing selection that the selection coefficient does not have any effect on the difference between mainland and island genes for a set of chosen Ne sizes (Figure S6A). In this case, testing for the origin effect is not supported because there is no power to distinguish between different selection coefficients. Another thing is that simulated mainland Pn/Ps is different from the observed, so naturally, the category will turn out as significant. I think the results of this test are not interpretable unless simulation parameters are chosen such that simulated Pn/Ps in the mainland will match observed.

4) Pn/Ps depends on Ps and this will differ across species. Authors should show the distribution of Ps for each species and group, to make sure that differences in Pn/Ps are not driven by Ps only.

Minor comments:

line 80: natural selection"on" low-effect mutations

line 128: This parapgraph is not clear, it would be help the reader to explain what is expected with lower Ne, what is expected with lower s, in terms of Pn, Ps, and Pn/Ps

line 129: Please explain why lower s would lead to higher Pn/Ps

line 131: Is there a reason why do you specifically use "transcriptome" here? If it's a general statement, "genome" would fit better.

line 133: The two sentences ("Therefore, we predict…" and "In addition, …") refer to the same thing: effect of drift (Ne) on selection. I think authors meant to refer in one to lower parasite pressure and in another one to population size decrease.

line 144: Genetic diversity of immunity genes is used interchangeably with pn/ps but it's not the same thing, I would stick to pn/ps.

line 147: Please use "nonsynonymous genetic variation" or pn/ps instead of " genetic variation" .

line 149: Mutations could be eliminated or fixed.

line 259: This sentence is confusing. Control genes preferentially evolve under purifying selection because this is commonly found across genes in the genome but not because balancing selection is rare.

line 296: Should be nucleotide diversity instead of genetic diversity

line 298: Is there a reason why PGLS model was not used for the first analysis (immune genes)? Please explain why two different tests were applied to these two similar analyses?

line 337: It's not clear what N in parentheses referes to. Please explain.

line 341: couldn't find Table S3 anywhere

line 338: Please add information about the total length of target regions and average coverge per sample.

line 341: It's not clear how many species were sequenced with targeted sequencing and how many with WGS. Please add information.

line 386: Some additional results are shown in supplementary material but not commented at all in the main manuscript, eg variation in selection coefficient. Please mention them in the results.

Supplementary methods:

1) Some methods which are discribed in supplements are not metioned in the main paper methods section, eg. test of contaminants. Please add some information in the main methods that is was done.

2) In supplementary methods some analyses are not clear: eg homology detection, please precise at which stage of the data analysis it was performed and what was the goal.

3) Why tests for balancing selection (line 42) were performed only for a single species?

4) Supplementary tables are not sorted, and in different formats. Table S3 is missing. Several tables include sequencing information for species, but it's not clear what the refer to (eg Table S4).

Table 1: It is hard to see for which species reference correpsonds to. Please mark clearly the rows with reference species.

Table 2: it's very unclear what columns 1,2,3 and 4 refer to. Probably you should add model numbers before "Model" column.

Table 3 : Here data is given for one or two best models? It seems like two models, not only the best statistical model.

Reviewed by anonymous reviewer 1, 07 February 2022

This paper is very interesting and adds to the growing evidence that in islands genetic drift is the main force driving the evolution of populations, even in genes that are supposed to be subject to string selection. The paper is generally very well written and easy going. I quite enjoyed reading it. I have only made a few remarks which you will find in the pdf attached.

Download the review

Reviewed by Steven Fiddaman, 14 January 2022

In this study, Barthe and colleagues use genomic and targeted-capture sequencing data from 20 island-dwelling and 14 mainland bird species to test the hypothesis that immune genes experience relaxed selection pressure in island species due to reduced pathogen diversity. They use Toll-like receptors, beta-defensins and MHC classes I and II as genetic proxies for immune function. They identify that neutral processes, such as demographic effects (i.e. bottlenecks), need to be disentangled from the extrinsic effects of a relaxed selection pressure due to changes in pathogen profiles. The authors find no evidence of relaxed selection due to reduced pathogen pressure in the innate immune genes studies, although there is some evidence of this in the MHC class II locus.

Overall, the study has significant merits and makes a good and worthy contribution to the field. It is well written and figures are presented with appropriate clarity. I do, however, have some comments which are detailed below.

Line 77. While there is evidence of decrease in MHC diversity leading to a reduction in immune function, as far as I am aware there is no strong evidence for this for TLRs and BDs, and the authors do not cite any (that I can see). This is quite a big assumption and is important for the validity of using genetic proxies for immune function.

Line 78. I wanted to chase up information in 'Hale & Briskie 2007' but I cannot find this citation in the reference list.

Line 96. "TLRs and BDs are under purifying selection which usually results in the selective removal of deleterious

alleles and stabilizing selection." TLRs, in particular the extracellular domain, have been found many times to evolve under positive selection. This is demonstrated clearly in the Velova et al. 2018 paper cited within this study. It has also been demonstrated over relatively short timescales (at the subspecies level) so it is certainly relevant here; see for instance Levy et al 2020 (doi.org/10.1093/molbev/msaa040). It is not correct to assume that immune genes, especially Toll-like receptors, are exclusively subject to purifying selection in all cases. See also 10.7554/eLife.41815. Different TLRs are subject to different selective pressures – nucleic acid-recognising TLRs are typically under stronger purifying selection than bacterial-recognising TLRs.

Line 146. Typo "weekly".

Line 186. Missing information about ethical approval and/or permits required for blood taking.

Line 191. The MHC locus is notorious for duplications etc (the authors acknowledge this later in the manuscript), which is especially problematic in non-model species. How were loci ensured to be single-copy?

Line 348. 0.12 and 0.2 should be the other way round.

Figure 3. What is the outlier species in the TLR box-plot? A Pn/Ps ratio of >1.5 across the entire coding sequence of all TLRs is implausible – perhaps there is a mistake here, or there is a significant amount of missing data?

Line 409. Presumably meant to be $P < 0.05$?