

Peer Review Information

Journal: Nature Ecology & Evolution

Manuscript Title: Meta-analytic evidence that animals rarely avoid inbreeding

Corresponding author name(s): Raïssa A. de Boer, Regina Vega-Trejo

Editorial Notes:

Reviewer Comments & Decisions:

Decision Letter, initial version:
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15th December 2020

*Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors.

Dear Dr de Boer,

Your manuscript entitled "Meta-analytic evidence that animals rarely avoid inbreeding" has now been seen by 3 reviewers, whose comments are attached. The reviewers have raised a number of concerns which will need to be addressed before we can offer publication in Nature Ecology & Evolution.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

* Include a "Response to reviewers" document detailing, point-by-point, how you addressed each reviewer comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the reviewers along with the revised manuscript.

* If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions at <http://www.nature.com/natecolevol/info/final-submission>. Refer also to

any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

Please use the link below to submit your revised manuscript and related files:

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Ecology & Evolution or published elsewhere.

Nature Ecology & Evolution is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit www.springernature.com/orcid.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

[REDACTED]

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

This manuscript describes a major meta-analysis designed to summarise the overall evidence for inbreeding avoidance in experimental studies (i.e. mate choice trials) in animals. I agree with the authors that the assumption that inbreeding avoidance is typical still prevails in biology, even though there are both theoretical and empirical suggestions that this should not necessarily be the case. A thorough meta-analysis of the substantial literature on experimental studies is therefore timely and is likely to be of wide interest. Indeed, the authors have done a commendable and careful job of assembling a big literature. Accordingly, I just have some suggestions for sharpening and clarifying

parts of the manuscript, and queries about the interpretation of the meta-analysis.

I am not an expert in the technicalities of meta-analyses, but it appears that everything was done sensibly and carefully. I was, however, struck by the apparent evidence for publication bias (towards inbreeding avoidance). I could imagine that there might possibly be publication bias against studies that find neither inbreeding avoidance nor preference (i.e. against studies that find 'inbreeding tolerance'). However I find it harder to see why there should be publication bias against studies that find inbreeding preference, as the authors suggest (lines 94-96). Why should studies that show 'significant' and noteworthy results that go against common expectation be less likely to be published? Indeed, there are examples of studies that show apparent inbreeding preference being published as relatively high-profile papers. Hence, I need a little more convincing that the data really show evidence of publication bias, as opposed to a skewed distribution of effect sizes that is in fact correct. Such a skewed distribution might potentially arise if both inbreeding avoidance and tolerance are common, but inbreeding preference is genuinely rare. This is plausible, and broadly in line with theory. Perhaps the authors' interpretation is informed also by study precision, and so then it would be helpful if the funnel plot could be presented for readers to see. Perhaps this information is in figure 3, but here I cannot really see the point sizes (the precision), and I did not understand what is on the Y-axis (what is the scale?). I suspect this key information could be more clearly presented. These points are important because if in fact the degree of publication bias is less than currently inferred, for example because the distribution of effects is genuinely skewed, then the authors may be rejecting evidence for overall inbreeding avoidance that is in fact there.

Also, it would be helpful if the authors could do slightly more to help readers interpret the effect sizes presented. For example, what does an overall Hedges g of 0.11 actually mean, for example in the context of studies which record whether or not focal individuals associated with kin vs non-kin?

The authors have done a good job of thinking through some reasonable hypotheses about what factors ('moderators') might explain variation, and testing them as far as feasible with the available data. However it is also the case that there are some factors listed in Box 1 that are not tested but which might be of major importance, for example population size, dispersal and magnitude of inbreeding depression. It may be that the authors cannot do much about this quantitatively in the current manuscript. But perhaps some of these points could be developed a bit more in the Discussion. Would it, in principle, be possible to pull out estimates of the magnitude of inbreeding depression across systems? There must be published estimates for at least some systems. Would it be possible to comment more on whether the species that are typically used for lab experiments are actually the best ones to choose if the aim is to test for evolved inbreeding avoidance? My suspicion is that they are often not – for example I suspect that, over evolutionary time, selection for inbreeding avoidance might often have been weak in things like *Drosophila* and mice and *Physa*, which have large and perhaps relatively mobile (at some life stage) populations. Yet these things are the focus of many published studies. Consequently it would be worth some comment on the degree to which the currently available experimental literature is in any way representative of inbreeding avoidance or preference that might occur in nature.

Finally, I think there are lots of ways in which the writing can be tightened up to make sure it is exactly technically correct and clear. Some of these are perhaps slightly pedantic – but on the other hand I think it is important that a major meta-analysis is tightly rather than loosely written. Here are some examples from the first few paragraphs. Similar things apply elsewhere in the manuscript, implying that a careful edit of everything would be helpful.

L12-13: 'incestuous mating generates inbreeding depression' – not really true. Inbreeding depression exists due to the underlying load, which incestuous mating might even purge. I think what is (correctly) meant here is something like 'incestuous mating generates inbreeding, leading to expression of inbreeding depression' – so maybe edit along those lines.

L13-14: 'theoretical models suggest that inclusive fitness benefits associated with inbreeding can lead to tolerance'. Kind of true, but only part of the story, and a part that seems more relevant to inbreeding preference rather than tolerance? It's hard to see what could lead to evolution of inbreeding preference apart from inclusive fitness benefits, but there are plenty of situations that could lead to inbreeding tolerance (i.e. random mating) without invoking inclusive fitness – most simply if relatives are rarely encountered as potential mates, so then there is no selective force to drive evolution of inbreeding avoidance.

L16: what do you mean by 'importance'? I think you mean something like 'frequency of occurrence'? This links to line 49-50 where you also mention 'evolutionary significance'. This implies to me that you're also going to consider the magnitude of inbreeding depression (since if there is no depression then there is quite little 'evolutionary significance of inbreeding avoidance' I would say). But in fact you don't look at the magnitude of inbreeding depression. Perhaps in the Introduction it would be better to keep focussed on setting up the expectation that you examine the prevalence of inbreeding avoidance/tolerance/preference, and nothing more to do with the fitness consequences.

L40: Could be more precisely worded in context of theory – the expectation is a reduction in mean fitness.

L47-48: I tripped over the phrasing here – are studies really performed with this assumption? I'm pretty sure some of them were done to test whether or not there is evidence of inbreeding avoidance, so then there is no 'assumption' of inbreeding avoidance. The phrasing in L67 is better.

L55: Again loosely worded – in fact recurrent close inbreeding might be expected to reduce inbreeding depression.

Finally, some parts of the manuscript structure seem a bit repetitive, and perhaps this could be streamlined. For example, the point about internal versus external fertilisation is partly dealt with on lines 128-135 and then again from line 180. The points about familiar vs unfamiliar kin, and $r = 0.5$ or not, also seem to come round twice.

Reviewer #2 (Remarks to the Author):

This study represents the most comprehensive and authoritative review of the empirical evidence for inbreeding avoidance via mate choice to date. Investigating data across a wide range of animal taxa using a meticulous pipeline and phylogenetically controlled meta-analytical approach, the study makes a commendable attempt to investigate ecological factors underpinning broad patterns in inbreeding avoidance. Inbreeding avoidance through different mechanisms of mate choice is an important area of research, at the interface of sexual selection, sexual conflict and population genetics and ecology, with ramifications for the management of animal populations, e.g. endangered or fragmented species. As such, the study will attract a broad readership and is highly suited for Nature Ecology & Evolution. The results are clearcut and paint a consistent picture which challenges the notion that inbreeding avoidance should necessarily evolve. The article is written clearly and is accessible to the non-specialist. I don't have significant concerns, the comments below are meant to encourage the authors to consider additional factors and to increase the clarity of the study.

General comments.

1) While the study considers a wide range of factors that might modulate inbreeding avoidance, some conspicuous mechanisms remain neglected. Inbreeding avoidance through mate choice is not expected to evolve when inbreeding is rare, e.g. in large panmictic populations or in species where at least one sex disperses before mating (here inbreeding avoidance is taken care by dispersal). Similarly, inbreeding avoidance will depend on the magnitude of inbreeding depression, which can be highly variable. For example, low inbreeding depression may occur when populations undergo prolonged periods of inbreeding which purges deleterious recessives. It is possible that some of these effects may be captured by the phylogenetic control, however these factors tend to vary at the level of the population/strain. It would be important for the study to more explicitly explore the role of mechanisms that modulate risk of inbreeding and magnitude of inbreeding depression.

2) Inbreeding avoidance during and after mating can be confounded by antagonistic interactions with the opposite sex, when sexes 'disagree' over inbreeding. For example, female attempts to terminate mating with-, or neutralise the sperm of a related male may be counteracted by male strategies to promote inbreeding. The study should acknowledge and discuss this issue.

3) Inbreeding tolerance vs preference. These terms ought to be defined and clarified early in the manuscript. Tolerance is typically used to refer to situations in which the consequences of a certain event (e.g. infection or inbreeding in this case) are no longer costly. However, I think that in the ms inbreeding tolerance means that individuals of a particular species show no bias with respect to the relatedness of a partner (i.e. same probability of mating with kin and non-kin). This is not the same as saying that inbreeding is selectively neutral. Similarly, it is not clear what is meant by preference: an active bias to mate with kin?

4) The methods of the main document should clarify the range in relatedness of the non-kin category (line 441 states $r = \sim 0$ but it is not clear whether this is based on empirical data or an assumption). If non-kin r is considerably variable, it would be helpful to explore whether such variation can contribute to explain variation in inbreeding avoidance patterns (see point 1 above).

Specific points.

Abstract

a) Line 13- Yet, theoretical models routinely suggest that the inclusive fitness benefits associated with inbreeding can lead to tolerance, or even preference, of inbreeding.
>replace 'routinely' with 'consistently', and add 'under some conditions'.

Intro

b) Line 43- as incestuous mating simultaneously increases the reproductive success of two related individuals

>This is common misconception of inclusive fitness, fitness payoffs cannot be simultaneously assigned to multiple individuals. Rephrase by simply saying that mating between related individuals can lead to indirect fitness gains which might outweigh direct fitness costs.

c) Line 44- Mate choice favouring relatives can therefore be adaptive and even expected, or at least tolerated,

>This is confusing (see point 3 above). The sentence is also awkward because mate choice is

'expected' by biologists but is 'tolerated' by a population of organisms.

d) Line 58- Such moderators alter the potential payoffs and costs associated with inbreeding, which means that different scenarios may call for different adaptive strategies

>Some of these moderators are constraints, which are expected to limit inbreeding avoidance rather than alter pay-offs.

Results

e) Line 203- Since inbreeding avoidance is predicted to be strongest when the costs of inbreeding are highest 11,12

>Actually, some literature proposes an optimal level of inbreeding (see Bateson's work in Japanese quails).

f) Line 262- However, mating status is not expected to influence inbreeding avoidance strategies among males

>Clarify that you mean 'male mating status' not the 'mating status of a male's partner'. I think that it is safer to state that mating status should have a more limited influence on inbreeding avoidance strategies in males than in females.

g) Line 268 V. Mate discrimination

>This is not a particularly helpful description of this section as any mate choice represents some form of discrimination (see also Box 1).

h) Line 287- Effect sizes are influenced weakly by sensory modalities

> Different modalities are expected to drive kin recognition in different taxa. This section of the results should be particularly clear about how the analysis controlled for phylogeny and potential evidence for phylogenetic inertia.

i) Line 306- Inbreeding avoidance strategies are also hypothesized to be influenced by cognitive constraints. Specifically, animals are expected to be better able to discern between kin and non-kin and avoid inbreeding when stimuli are presented simultaneously.

>True, but inbreeding avoidance may also change across simultaneous choice, sequential choice and no choice scenarios for reasons that have nothing to do with cognition. These three scenarios have starkly different fitness consequences determined by the probability of encountering another partner in the near future, the probability surviving to the next mating opportunity, the probability that the next partner will be kin or not kin. This section should be presented in a broader context that considers these mechanisms (see also box 1).

Discussion

j) Line 368- Under natural condition in wild populations however, it is not clear how often individuals would have prior familiarity with their kin,

>Prior familiarity with kin is likely universal in the vast majority of species where sibs grow/develop together for a period of time. If an organism does not encounter kin early in life, it is unclear why they would meet kin later in life (i.e. when searching for mates).

k) Fig 2.

>I think that human references should be 93-98 not 92-98.

Reviewer #3 (Remarks to the Author):

De Boer et al: Meta-analytic evidence that animals rarely avoid inbreeding

There is a widely held assumption that animals should avoid inbreeding to avoid the exposure of deleterious alleles due to increased homozygosity. In their manuscript, De Boer et al conduct a meta-analysis to examine if animals do, in fact, avoid inbreeding. This meta-analysis covers a wide range of species as well as traits/behaviours used to assess mate choice. Interestingly, the authors found little evidence that animals avoid inbreeding.

The authors also conducted a series of meta-regression analyses to test for the effects of potentially important moderators in explaining some of the high heterogeneity. Surprisingly, many of the moderators did not account for much of the total variation, including taxonomic group, pre- vs post-copulatory sexual selection, focal sex, mating status etc. However, they found that inbreeding avoidance does increase with degree of relatedness (although only weakly), as well differences between internal and external fertilisers, familiarity with kin, and the sensory signals used.

This is a very interesting manuscript that provides quantitative evidence challenging the assumption that individuals should avoid inbreeding. It is well written, transparent, and the analysis is mostly sound and thorough. I have a few comments regarding some of the analyses/statistics presented and suggest some expansion of the discussion (see below), but these should all be relatively easy to address. Pending these changes/clarifications, I believe that this manuscript would make a nice addition to Nature Ecology & Evolution.

Comments

1) My main comments regarding the analysis are to do with the random effects in the models. My recommendation is that species (not accounting for phylo relatedness) as well as the corr matrix should be included as random effects in the model. This is because only including phylogeny in the model can greatly overestimate the phylogenetic signal (see <https://ecoevorxiv.org/su4zv/>). Table 1 should therefore present I2 non-phylo species (i.e., due to ecological differences) and I2 (or Pagel's Lambda or deltaAIC) for phylogeny.

Additionally, based on Fig. 2, the number of studies included is approaching the number of species which could result in them becoming non-identifiable, making the variance components unreliable. For a sensitivity analysis, animal group ID rather than study ID could be included as a random effect - assuming that some studies measured different responses from different groups of animals with the same study (making animal group ID > species).

2) Total heterogeneity needs to be reported early on in the results. It is easy to infer from Table 1, but it is important for the reader to be able to clearly understand how variable the data is and why it is important to look at moderator variables (especially those that may explain the large between study variation). This high total heterogeneity should also be mentioned in the first paragraph of the

discussion.

3) The use of the term 'moderators' in Box 1 is misleading as it implies everything listed will be included as a moderator in the meta-regression. It needs to be made clear which factors are actually moderators in the model vs what are other potentially important factors. This shouldn't take much to clarify – perhaps just refrain from using the term moderator unless it is actually included in the analysis. An asterisk or something could be an easy way of identifying which factors were actually included in the analysis. It also needs to be made clear how some of the moderators were categorised/coded (i.e., for taxonomy, the way it's written implies the moderator is at the species level, but it was actually animal class that was included in the analysis). The inclusion of a meta-data file with the supplementary material would be helpful to show how the moderators were coded.

Just a suggestion, but it might be logical to group Box 1 by 'included moderators' and 'other potentially important moderators'.

4) I found the discussion a bit lacking. Most of the discussion of the moderators seem to repeat what was already said in the results and Box 1. Perhaps summarising the important and unimportant moderators in a more succinct way would allow more space to unpack some of the theoretical literature mentioned in the introduction and discuss how this study is consistent/inconsistent with such theories. Further discussion of the clear publication bias and why this may arise would also be beneficial. Additionally, the last paragraph is particularly anticlimactic. These are exciting and interesting results and the novelty should be emphasised!

Minor comments

Line 57: It's not clear what 'motivational or intrinsic state' means

Line 58: potential to potentially

Lines 77-78: This sentence isn't really needed and takes away the excitement of reading the results

Line 108: Add CI in text following Hedges g (same throughout when mean ES is reported in text)

Line 109: This sentence isn't really needed

Line 149: It's not really 'accounting' for variation as it's a sub-analysis rather than included as a moderator

Line 173: taxonomical to taxonomic

Line 182: relate to relative (I think)

Line 262: What about role-reversed species?

Line 287: 'influenced weakly' to 'weakly influenced'

Lines 359 - 360: This isn't clear

Lines 512-513: It's not intuitive which traits/behaviours will fit into the subcategories under 'before mating' and 'during mating'

Lines 569-579: This belongs in the results

Lines 591-592: How can the data be binary or continuous? Are you referring to Hedges g?

Table 2: 'human animals' and 'nonhuman animals' sounds a bit strange. Maybe just call it humans vs animals (even though we are animals...)

Fig. 1: Reiterate how the searches differed in the legend

Fig. 1: The 'excluded' arrows seem misplaced. For example, '4931 excluded' is coming off '3677' which looks like 4931 studies were excluded from 3677

Fig. 2: Need to mention what the colours mean in the legend

Fig. 3 and 4: It's not possible to discern the size of each point. Perhaps change the alpha level or have

a black/dark outline to make it clearer

Box 1: Is 'time constraints' essentially mating rate? There are no predictions mentioned here compared to the other moderators

*****END*****

Author Rebuttal to Initial comments

Reviewer #1 (Remarks to the Author):

This manuscript describes a major meta-analysis designed to summarise the overall evidence for inbreeding avoidance in experimental studies (i.e. mate choice trials) in animals. I agree with the authors that the assumption that inbreeding avoidance is typical still prevails in biology, even though there are both theoretical and empirical suggestions that this should not necessarily be the case. A thorough meta-analysis of the substantial literature on experimental studies is therefore timely and is likely to be of wide interest. Indeed, the authors have done a commendable and careful job of assembling a big literature. Accordingly, I just have some suggestions for sharpening and clarifying parts of the manuscript, and queries about the interpretation of the meta-analysis.

- We thank the reviewer for their kind words. We appreciate the useful comments they provided and we thank them for their time and providing us with a thorough review of our work.

Comment 1

I am not an expert in the technicalities of meta-analyses, but it appears that everything was done sensibly and carefully. I was, however, struck by the apparent evidence for publication bias (towards inbreeding avoidance). I could imagine that there might possibly be publication bias against studies that find neither inbreeding avoidance nor preference (i.e. against studies that find 'inbreeding tolerance'). However I find it harder to see why there should be publication bias against studies that find inbreeding preference, as the authors suggest (lines 94-96). Why should studies that show 'significant' and noteworthy results that go against common expectation be less likely to be published? Indeed, there are examples of studies that show apparent inbreeding preference being published as relatively high-profile papers. Hence, I need a little more convincing that the data really show evidence of publication bias, as opposed to a skewed distribution of effect sizes that is in fact correct. Such a skewed distribution might potentially arise if both inbreeding avoidance and tolerance are common, but inbreeding preference is genuinely rare. This is plausible, and broadly in line with theory. Perhaps the authors' interpretation is informed also by study precision, and so then it would be helpful if the funnel plot could be presented for readers to see. Perhaps this information is in figure 3, but here I cannot really see the point sizes (the precision), and I did not understand what is on the Y-axis (what is the scale?). I suspect this key information could be more clearly presented. These points are important because if in fact the degree of publication bias is less than currently inferred, for example because the distribution of effects is genuinely skewed, then the authors may be rejecting evidence for overall inbreeding avoidance that is in fact there.

- We should have been more careful in our phrasing and we thank the reviewer for pointing this out. We agree with the reviewer that publication bias is unlikely to be the case for asymmetrical funnel plots if the asymmetry is mostly found in large, precise studies with significant results. If a researcher finds evidence of inbreeding preference, they may think that this result goes against the conventional wisdom so much that they don't trust it, but this is

less likely to keep the researcher from publishing this work if it was a large study with significant findings. However, what we find is that there are small, imprecise studies with statistically insignificant results showing inbreeding preference missing in the publication record and this is likely to be due to publication bias. We now address this more elaborately in the text and provide three lines of evidence, including 1) we now include a funnel plot in the main manuscript (this was previously only presented in the Supplemental Material) with contours indicating areas of statistical significance, showing that studies with low standard errors (i.e. sample size) and statistically insignificant studies showing inbreeding preference are missing, 2) we confirm asymmetry in the funnel plot biased towards studies showing inbreeding avoidance using Egger's regressions on meta-analytic residuals, and 3) we demonstrate that the overall meta-analytical effect size estimates switches signs to becomes negative in two of the three analytical trim-and-fill methods we applied to examine the impact of publication bias in our dataset.

- We agree that our figures could have been clearer. We have now redesigned all of our figures. Specifically, we have increased the font size, made each part of a multi-panel figure larger, and reorganized the figures so there are fewer parts to each multi-panel figure. We also added an expanded explanation of the x-axis and y-axis in each figure legend.

Comment 2

Also, it would be helpful if the authors could do slightly more to help readers interpret the effect sizes presented. For example, what does an overall Hedges g of 0.11 actually mean, for example in the context of studies which record whether or not focal individuals associated with kin vs non-kin?

- Great point. We should have made this crystal clear in our original submission. We have now corrected this oversight in the revised manuscript. Effect size estimates are generally considered to be small if they are ~ 0.2 , medium if they are ~ 0.5 and large if they are 0.8 . In our study, effect size estimates from every meta-analysis we perform are in the 'small' range. We now point this out in the text. In addition, a good way for interpreting the magnitude of the overall effect size is to compare it to previous meta-analyses within the same research topic (Durlak, 2009). Accordingly, we now add in our revised version of the manuscript that previous meta-analyses within the same topic have also found small effects. (Lines 174-177)

Durlak, J.A., 2009. How to select, calculate, and interpret effect sizes. *Journal of pediatric psychology*, 34(9), pp.917-928.

Comment 3

The authors have done a good job of thinking through some reasonable hypotheses about what factors ('moderators') might explain variation, and testing them as far as feasible with the available data.

- The reviewer raises a lot great points in this paragraph. We respond to each point in turn rather than responding in a block text.

However it is also the case that there are some factors listed in Box 1 that are not tested but which might be of major importance, for example population size, dispersal and magnitude of inbreeding depression. It may be that the authors cannot do much about this quantitatively in the current manuscript. But perhaps some of these points could be developed a bit more in the Discussion.

- Great point. We now discuss these other important factors that we were not able to address in our analyses directly in more detail in the Discussion (specifically in the 6th and 7th paragraph in the Discussion).

Would it, in principle, be possible to pull out estimates of the magnitude of inbreeding depression across systems? There must be published estimates for at least some systems.

- Another great point. Yes, it is possible in principle to do this. In fact, we are currently working on doing exactly this. The trouble with incorporating this kind of analysis in the current manuscript is that there is limited overlap with our measures of inbreeding avoidance and estimates of inbreeding depression (data is available for both metrics for ~20 species). So any analysis we run will come with a large caveat that we are assessing less than ¼ of species in the full dataset. Second, there are some real challenges with disentangling the variance in inbreeding avoidance measures with variance in inbreeding depression measures. As we report in the present manuscript, effect size estimates of inbreeding avoidance sometimes vary depending on what is being considered. Similarly, different traits show different degrees of inbreeding depression. All of this is super interesting, but it makes it challenging to develop a way to address this question. This is something we are still working on. Therefore, given where things currently stand we have elected to not directly include data on inbreeding depression in the current manuscript. Instead, we discuss this point in more detail in the discussion and describe the range of inbreeding depression values that are reported for some of the species assessed in our meta-analysis (Lines 487-509).

Would it be possible to comment more on whether the species that are typically used for lab experiments are actually the best ones to choose if the aim is to test for evolved inbreeding avoidance? My suspicion is that they are often not – for example I suspect that, over evolutionary time, selection for inbreeding avoidance might often have been weak in things like *Drosophila* and mice and *Physa*, which have large and perhaps relatively mobile (at some life stage) populations. Yet these things are the focus of many published studies. Consequently it would be worth some comment on the degree to which the currently available experimental literature is in any way representative of inbreeding avoidance or preference that might occur in nature.

- Thanks. Another very interesting point. We now describe in the Results that roughly half of the effect sizes are derived from only 11 species. The reviewer correctly points out that some of the most well studied systems are those like

Drosophila, mice, and *Physa* (along with crickets, guppies, gerbils, voles, humans, chimpanzees, rats, and zebra finches). We now directly discuss some of the limitation in drawing general conclusions from our data based on the history of selection for inbreeding avoidance that may have been present in these species. However, we also note that it is really challenging to say that species X is not a great model for studying inbreeding avoidance, because the attributes that make for a good model of inbreeding avoidance can differ among populations within a species (something Reviewer 2 points out). Instead, we suggest that researchers should take a more holistic approach when studying inbreeding avoidance, starting by demonstrating inbreeding depression and validating the ecological and life-history context for inbreeding avoidance strategies to evolve in the first place. (Lines 510-538)

Comment 4

Finally, I think there are lots of ways in which the writing can be tightened up to make sure it is exactly technically correct and clear. Some of these are perhaps slightly pedantic – but on the other hand I think it is important that a major meta-analysis is tightly rather than loosely written. Here are some examples from the first few paragraphs. Similar things apply elsewhere in the manuscript, implying that a careful edit of everything would be helpful.

- We thank the reviewer for their helpful writing suggestions!

L12-13: 'incestuous mating generates inbreeding depression' – not really true. Inbreeding depression exists due to the underlying load, which incestuous mating might even purge. I think what is (correctly) meant here is something like 'incestuous mating generates inbreeding, leading to expression of inbreeding depression' – so maybe edit along those lines.

- Changed according to reviewer's suggestion. It now reads "*incestuous mating can lead to the expression of inbreeding depression*". (Lines 12-13)

L13-14: 'theoretical models suggest that inclusive fitness benefits associated with inbreeding can lead to tolerance'. Kind of true, but only part of the story, and a part that seems more relevant to inbreeding preference rather than tolerance? It's hard to see what could lead to evolution of inbreeding preference apart from inclusive fitness benefits, but there are plenty of situations that could lead to inbreeding tolerance (i.e. random mating) without invoking inclusive fitness – most simply if relatives are rarely encountered as potential mates, so then there is no selective force to drive evolution of inbreeding avoidance.

- We clarified this sentence (and others in the text) according to the reviewer's suggestion: "*...and that under some conditions the inclusive fitness benefits associated with inbreeding can even lead to a preference for mating with kin*"
Lines 15-16

L16: what do you mean by 'importance'? I think you mean something like 'frequency of occurrence'? This links to line 49-50 where you also mention 'evolutionary significance'. This implies to me that you're also going to consider the magnitude of

inbreeding depression (since if there is no depression then there is quite little 'evolutionary significance of inbreeding avoidance' I would say). But in fact you don't look at the magnitude of inbreeding depression. Perhaps in the Introduction it would be better to keep focussed on setting up the expectation that you examine the prevalence of inbreeding avoidance/tolerance/preference, and nothing more to do with the fitness consequences.

- We changed "importance" to "prevalence" in the abstract (Line 17) and focus on the prevalence of inbreeding avoidance in the introduction, according to the reviewer's suggestion.

L40: Could be more precisely worded in context of theory – the expectation is a reduction in mean fitness.

- Changed to "mean fitness" according to reviewer's suggestion. (Line 42)

L47-48: I tripped over the phrasing here – are studies really performed with this assumption? I'm pretty sure some of them were done to test whether or not there is evidence of inbreeding avoidance, so then there is no 'assumption' of inbreeding avoidance. The phrasing in L67 is better.

- We rephrased this sentence according to the reviewer's suggestion: "*Despite these longstanding theoretical predictions^{1,8,9}, the assumption that inbreeding avoidance is widespread in animals remains, possibly due to human moral judgment against incest*". (Lines 52-54)

L55: Again loosely worded – in fact recurrent close inbreeding might be expected to reduce inbreeding depression.

- We rephrased this sentence to say that inbreeding depression, at least initially, increases with relatedness. (Line 64-65)

Finally, some parts of the manuscript structure seem a bit repetitive, and perhaps this could be streamlined. For example, the point about internal versus external fertilisation is partly dealt with on lines 128-135 and then again from line 180. The points about familiar vs unfamiliar kin, and $r = 0.5$ or not, also seem to come round twice.

- We thank the reviewer for pointing this out. Because of how the data was subsetting and analysed, fertilisation mode was highlighted when considering an overall mean and then again when using fertilisation and reproductive mode as moderators. We have now shorted these sections to avoid repetition.

Reviewer #2 (Remarks to the Author):

This study represents the most comprehensive and authoritative review of the empirical evidence for inbreeding avoidance via mate choice to date. Investigating data across a wide range of animal taxa using a meticulous pipeline and phylogenetically controlled meta-analytical approach, the study makes a commendable attempt to investigate ecological factors underpinning broad patterns in inbreeding avoidance. Inbreeding avoidance through different mechanisms of mate choice is an important area of research, at the interface of sexual selection, sexual conflict and population genetics and ecology, with ramifications for the management of animal populations, e.g. endangered or fragmented species. As such, the study will attract a broad readership and is highly suited for Nature Ecology & Evolution. The results are clearcut and paint a consistent picture which challenges the notion that inbreeding avoidance should necessarily evolve. The article is written clearly and is accessible to the non-specialist. I don't have significant concerns, the comments below are meant to encourage the authors to consider additional factors and to increase the clarity of the study.

- We thank the reviewer for their time and their appreciation of our study. We are happy they think our work is suited for the broad readership of Nature Ecology & Evolution. We have addressed the useful comments suggested by the reviewer in the manuscript and provided a response for each of their comments below.

Comment 5

General comments.

1) While the study considers a wide range of factors that might modulate inbreeding avoidance, some conspicuous mechanisms remain neglected. Inbreeding avoidance through mate choice is not expected to evolve when inbreeding is rare, e.g. in large panmictic populations or in species where at least one sex disperses before mating (here inbreeding avoidance is taken care by dispersal). Similarly, inbreeding avoidance will depend on the magnitude of inbreeding depression, which can be highly variable. For example, low inbreeding depression may occur when populations undergo prolonged periods of inbreeding which purges deleterious recessives. It is possible that some of these effects may be captured by the phylogenetic control, however these factors tend to vary at the level of the population/strain. It would be important for the study to more explicitly explore the role of mechanisms that modulate risk of inbreeding and magnitude of inbreeding depression.

- This is a really helpful suggestion. We rewrote large parts of the discussion and included the issues suggested by the reviewer in the new version of the manuscript.

Comment 6

2) Inbreeding avoidance during and after mating can be confounded by antagonistic interactions with the opposite sex, when sexes 'disagree' over inbreeding. For example, female attempts to terminate mating with-, or neutralise the sperm of a

related male may be counteracted by male strategies to promote inbreeding. The study should acknowledge and discuss this issue.

- This is a good point and one that we considered generally in our analyses (although we didn't highlight it sufficiently) by assessing sex-specific effects at different episodes of sexual selection. We found no evidence that this was the case and we highlight this in the Results. We also acknowledge that our dataset may not be ideal for testing the idea of sexually antagonistic interactions and therefore have now addressed sexual conflict in more detail in the discussion (Lines 446-461).

Comment 7

3) Inbreeding tolerance vs preference. These terms ought to be defined and clarified early in the manuscript. Tolerance is typically used to refer to situations in which the consequences of a certain event (e.g. infection or inbreeding in this case) are no longer costly. However, I think that in the ms inbreeding tolerance means that individuals of a particular species show no bias with respect to the relatedness of a partner (i.e. same probability of mating with kin and non-kin). This is not the same as saying that inbreeding is selectively neutral. Similarly, it is not clear what is meant by preference: an active bias to mate with kin?

- This is a good point of the reviewer that we hadn't considered. We agree that using the term "*inbreeding tolerance*" is at odds with the existing literature and doesn't get the point that we are trying to make across. We now use different terms throughout the manuscript and define them specifically. We describe "*kin avoidance*" as cases where non-kin are preferred over kin (i.e. significant values with positive effect sizes), "*no kin bias*" as cases where mating preference are not based on relatedness, and "*kin preference*" as cases where kin are preferred over non-kin (i.e. significant values with negative effect sizes). We define these terms in the introduction (Lines 83-84) and then use them consistently throughout the manuscript.

Comment 8

4) The methods of the main document should clarify the range in relatedness of the non-kin category (line 441 states $r = \sim 0$ but it is not clear whether this is based on empirical data or an assumption). If non-kin r is considerably variable, it would be helpful to explore whether such variation can contribute to explain variation in inbreeding avoidance patterns (see point 1 above).

- The reviewer makes a very good point. It was sometimes noted that unrelated individuals were at least less related than a certain level (e.g. relatedness < 0.0625), but in the majority of studies (~90%) it was not specified what the relatedness level of "unrelated" individuals were. We were therefore not able to account for varying levels of unrelatedness. We now state this explicitly in the methods (Lines 592-596). If there is a large variation in how unrelated individuals are within inbreeding avoidance studies, this would pose a problem. We now bring this issue up in the discussion (Lines 539-554).

Comment 9

Specific points.

Abstract

a) Line 13- Yet, theoretical models routinely suggest that the inclusive fitness benefits associated with inbreeding can lead to tolerance, or even preference, of inbreeding.

>replace 'routinely' with 'consistently', and add 'under some conditions'.

- Changed according to this reviewer's and Reviewer 1's suggestion: "*Yet, theoretical models consistently predict that unbiased mating with regards to kinship should be common, and that under some conditions the inclusive fitness benefits associated with inbreeding can even lead to a preference for mating with kin.*". (Lines 13-16)

Intro

b) Line 43- as incestuous mating simultaneously increases the reproductive success of two related individuals

>This is common misconception of inclusive fitness, fitness payoffs cannot be simultaneously assigned to multiple individuals. Rephrase by simply saying that mating between related individuals can lead to indirect fitness gains which might outweigh direct fitness costs.

- We rephrased this sentence according to the reviewer's suggestion: "*Moreover, mating between related individuals can lead to indirect fitness benefits that could outweigh the direct fitness costs associated with inbreeding, and thus inbreeding can increase inclusive fitness under some conditions.*". (Lines 48-51)

c) Line 44- Mate choice favouring relatives can therefore be adaptive and even expected, or at least tolerated,

>This is confusing (see point 3 above). The sentence is also awkward because mate choice is 'expected' by biologists but is 'tolerated' by a population of organisms.

- We rephrased this sentence, and are now avoiding these confusing terms "*Mate choice in favour of relatives can therefore be adaptive, provided the costs of inbreeding depression aren't excessive.*" (Lines 51-52)

d) Line 58- Such moderators alter the potential payoffs and costs associated with inbreeding, which means that different scenarios may call for different adaptive strategies

>Some of these moderators are constraints, which are expected to limit inbreeding avoidance rather than alter pay-offs.

- We added that moderators may also constrain the opportunity for inbreeding avoidance in addition to altering costs and pay-offs (Lines 68-69)

Results

e) Line 203- Since inbreeding avoidance is predicted to be strongest when the costs

of inbreeding are highest 11,12

>Actually, some literature proposes an optimal level of inbreeding (see Bateson's work in Japanese quails).

- We rephrased this sentence to put more emphasis on that the costs generally increase with increasing relatedness, and that therefore inbreeding avoidance is more likely to occur at higher relatedness levels. In addition, we address in the discussion that there may be a non-linear relationship between relatedness and inbreeding depression depending on the costs and benefits of inbreeding at different relatedness levels (Lines 544-547).

f) Line 262- However, mating status is not expected to influence inbreeding avoidance strategies among males

>Clarify that you mean 'male mating status' not the 'mating status of a male's partner'. I think that it is safer to state that mating status should have a more limited influence on inbreeding avoidance strategies in males than in females.

- We rephrased this sentence according to the reviewer's suggestions (Lines 396-398)

g) Line 268 V. Mate discrimination

>This is not a particularly helpful description of this section as any mate choice represents some form of discrimination (see also Box 1).

- We agree with the reviewer in that mate discrimination could be confusing given other subsections also include some sort of mate choice. We have now renamed this subheading as "*mating conditions*" to capture that these analyses reflect the various conditions that animals have been tested under (i.e. manipulation of their experience with kin, sensory modalities, choice type and mating status).

h) Line 287- Effect sizes are influenced weakly by sensory modalities

> Different modalities are expected to drive kin recognition in different taxa. This section of the results should be particularly clear about how the analysis controlled for phylogeny and potential evidence for phylogenetic inertia.

- We now include a random effect for species in addition to the phylogenetic matrix as suggested by Reviewer #3. We reiterate that both species (due to ecological differences) and phylogeny are included in this section in the revised version. We also emphasize that there is limited scope for interpreting the difference in sensory modalities that we report since this effect stems from a limited number of effect sizes and species examined (Lines 368-373).

i) Line 306- Inbreeding avoidance strategies are also hypothesized to be influenced by cognitive constraints. Specifically, animals are expected to be better able to discern between kin and non-kin and avoid inbreeding when stimuli are presented simultaneously.

>True, but inbreeding avoidance may also change across simultaneous choice, sequential choice and no choice scenarios for reasons that have nothing to do with

Reviewer #3 (Remarks to the Author):

De Boer et al: Meta-analytic evidence that animals rarely avoid inbreeding

There is a widely held assumption that animals should avoid inbreeding to avoid the exposure of deleterious alleles due to increased homozygosity. In their manuscript, De Boer et al conduct a meta-analysis to examine if animals do, in fact, avoid inbreeding. This meta-analysis covers a wide range of species as well as traits/behaviours used to assess mate choice. Interestingly, the authors found little evidence that animals avoid inbreeding.

The authors also conducted a series of meta-regression analyses to test for the effects of potentially important moderators in explaining some of the high heterogeneity. Surprisingly, many of the moderators did not account for much of the total variation, including taxonomic group, pre- vs post-copulatory sexual selection, focal sex, mating status etc. However, they found that inbreeding avoidance does increase with degree of relatedness (although only weakly), as well differences between internal and external fertilisers, familiarity with kin, and the sensory signals used.

This is a very interesting manuscript that provides quantitative evidence challenging the assumption that individuals should avoid inbreeding. It is well written, transparent, and the analysis is mostly sound and thorough. I have a few comments regarding some of the analyses/statistics presented and suggest some expansion of the discussion (see below), but these should all be relatively easy to address. Pending these changes/clarifications, I believe that this manuscript would make a nice addition to Nature Ecology & Evolution.

We thank the reviewer for their time and comprehensive review of our work. We are glad the reviewer considers our study suitable for Nature Ecology & Evolution. The reviewer provided valuable improvements to our analyses, which we were happy to address. We provide more detailed responses to the reviewer's suggestions below.

Comments

Comment 10

1) My main comments regarding the analysis are to do with the random effects in the models. My recommendation is that species (not accounting for phylo relatedness) as well as the corr matrix should be included as random effects in the model. This is because only including phylogeny in the model can greatly overestimate the phylogenetic signal (see <https://ecoevorxiv.org/su4zv/>). Table 1 should therefore present I2 non-phylo species (i.e., due to ecological differences) and I2 (or Pagel's Lambda or deltaAIC) for phylogeny.

- As suggested by the reviewer, we have now included a random effect for species without phylogenetic correlation in our analyses. We describe this in the methods. Accordingly, we note I² for species in Table 1. Species explained (much) more variation than the phylogenetic correlation matrix, so we are grateful that the reviewer noted this. We must note that some of our

results changed by adding an additional random effect. Most notably, the estimate for the full dataset in the meta-analysis became significantly different from zero, although again this effect was diminished by publication bias as confirmed with trim and fill methods. In addition, the significant difference between internal and external fertilizing animals became a trend. We have adjusted the Results section accordingly. Importantly, despite these few changes in our results, the broad take home message of our manuscript remains unchanged from the original submission.

Additionally, based on Fig. 2, the number of studies included is approaching the number of species which could result in them becoming non-identifiable, making the variance components unreliable. For a sensitivity analysis, animal group ID rather than study ID could be included as a random effect - assuming that some studies measured different responses from different groups of animals with the same study (making animal group ID > species).

- Good point. We ran a sensitivity analysis where we replaced study ID with animal group ID, which is now added to the supplementary material and explained in the methods section. Replacing study ID with animal group ID did not qualitatively change the results of the meta-analyses. Of all studies, ~53% used one group of animals, ~31% used two groups, and the rest (~17%) three or more groups of animals. The I^2 was a little bit lower when including study ID compared to animal group ID (see Supplementary Material Section 11 for more details), so we opted to retain the original random effect for study ID.

Comment 11

2) Total heterogeneity needs to be reported early on in the results. It is easy to infer from Table 1, but it is important for the reader to be able to clearly understand how variable the data is and why it is important to look at moderator variables (especially those that may explain the large between study variation). This high total heterogeneity should also be mentioned in the first paragraph of the discussion.

- We now added the total heterogeneity in Table 1, as well as species heterogeneity as per the previous comment. We moved the paragraph on heterogeneity from the methods into the results section, and also mention the high levels of heterogeneity in the discussion (Lines 433-441).

Comment 12

3) The use of the term 'moderators' in Box 1 is misleading as it implies everything listed will be included as a moderator in the meta-regression. It needs to be made clear which factors are actually moderators in the model vs what are other potentially important factors. This shouldn't take much to clarify – perhaps just refrain from using the term moderator unless it is actually included in the analysis. An asterisk or something could be an easy way of identifying which factors were actually included in the analysis. It also needs to be made clear how some of the moderators were categorised/coded (i.e., for taxonomy, the way it's written implies the moderator is at the species level, but it was actually animal class that was included in the analysis).

The inclusion of a meta-data file with the supplementary material would be helpful to show how the moderators were coded.

Just a suggestion, but it might be logical to group Box 1 by 'included moderators' and 'other potentially important moderators'.

- We thank the reviewer for these suggestions. We now only use the term 'moderator' for factors affecting inbreeding avoidance that we included in the analyses. We also have clarified which factors were included in the text for Box 1 using asterisks. We changed the text for taxonomy to state clearly that we are looking at differences on the level of animal class. We also adjusted the Box figure to make more clear which factors were included or not in the current analyses by indicating them with an asterisk, but we now also use different colours to make the distinction easier. We changed the text associated with the Box to animal class to clarify we are looking at the animal class level. In addition, we have added a meta-data file to the supplementary material that explains how moderators were coded (see Supplementary Material Section 2), and point to this table in the manuscript (Lines 695-696).

Comment 13

4) I found the discussion a bit lacking. Most of the discussion of the moderators seem to repeat what was already said in the results and Box 1. Perhaps summarising the important and unimportant moderators in a more succinct way would allow more space to unpack some of the theoretical literature mentioned in the introduction and discuss how this study is consistent/inconsistent with such theories. Further discussion of the clear publication bias and why this may arise would also be beneficial. Additionally, the last paragraph is particularly anticlimactic. These are exciting and interesting results and the novelty should be emphasised!

- We rewrote large parts of the discussion in line with the suggestions of this reviewer and the other reviewers. We now address publication bias and rewrote the last paragraph.

Comment 14

Minor comments

Line 57: It's not clear what 'motivational or intrinsic state' means

- We have now rephrased this to "*motivation to mate*" and "*intrinsic differences associated with each species*". (Lines 66-68)

Line 58: potential to potentially

- Changed according to reviewer's suggestion. (Line 67)

Lines 77-78: This sentence isn't really needed and takes away the excitement of reading the results

- We deleted this sentence.

Line 108: Add CI in text following Hedges g (same throughout when mean ES is reported in text)

- We have added confidence intervals throughout the Results section in the revised version of the manuscript

Line 109: This sentence isn't really needed

- We deleted this sentence.

Line 149: It's not really 'accounting' for variation as it's a sub-analysis rather than included as a moderator

- We changed this to "*we considered differences in experimental design*". (Line 217)

Line 173: taxonomical to taxonomic

- Changed according to reviewer's suggestion.

Line 182: relate to relative (I think)

- We rephrased this sentence

Line 262: What about role-reversed species?

- We added "*in species with conventional sex roles*" to this sentence. (Line 395)

Line 287: 'influenced weakly' to 'weakly influenced'

- The placement of "weakly" in the original sentence was grammatically correct. This is an example of a split infinitive, where it is not grammatically correct to place a word (weakly) between the verb (influence) and the infinitive marker of that verb (are). However, we agree it doesn't exactly roll off the tongue. We changed this subheading to "*Effect sizes differ weakly among sensory modalities*" (Line 355).

Lines 359 - 360: This isn't clear

- We decided this sentence didn't add much information so we removed this sentence.

Lines 512-513: It's not intuitive which traits/behaviours will fit into the subcategories under 'before mating' and 'during mating'

- We described our choice for categorizing different traits and behaviours into "pre-mating" and "during mating" elaborately in the Results (section C3.1). We also reiterate this in the methods to clarify the subcategorization of behaviours into these categories (Lines 667-680).

Lines 569-579: This belongs in the results

- We moved the heterogeneity section to the results (Section A2).

Lines 591-592: How can the data be binary or continuous? Are you referring to Hedges g?

- We clarified this sentence. (Lines 749-751)

Table 2: 'human animals' and 'nonhuman animals' sounds a bit strange. Maybe just call it humans vs animals (even though we are animals...)

- We have changed this to humans vs. other animals to reflect the fact that we are also animals but avoid the clunky term of non-human animals.

Fig. 1: Reiterate how the searches differed in the legend

- We now reiterate how the searches differed within the figure legend.

Fig. 1: The 'excluded' arrows seem misplaced. For example, '4931 excluded' is coming off '3677' which looks like 4931 studies were excluded from 3677

- We thank the reviewer for spotting this. We have now edited this figure.

Fig. 2: Need to mention what the colours mean in the legend

- We have included this information in the revised version of the manuscript

Fig. 3 and 4: It's not possible to discern the size of each point. Perhaps change the alpha level or have a black/dark outline to make it clearer

- As also suggested by Reviewer 1, we have edited these plots so that individual points are easier to distinguish

Decision Letter, first revision:

22nd February 2021

Dear Dr. de Boer,

Thank you for submitting your revised manuscript "Meta-analytic evidence that animals rarely avoid inbreeding" (NATECOLEVOL-201112162A). It has now been seen again by the original reviewers and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Ecology & Evolution, pending minor revisions to satisfy the reviewers' final requests and to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Ecology & Evolution. Please do not hesitate to contact me if you have any questions.

[REDACTED]

Reviewer #3 (Remarks to the Author):

I have reread the manuscript by de Boer et al examining the prevalence of inbreeding avoidance through a large and comprehensive meta-analysis.

The authors have made substantial improvements to the manuscript and I believe that it will indeed make a nice contribution of Nature Ecology & Evolution.

I have very few comments to make on the revised version but see below for my final suggestions.

1) In the results section, I'm a little confused by some of the data subsetting vs testing moderators in meta-regression. The authors first do a series of subsetting in an attempt to make inbreeding avoidance more detectable, then go on to do a series of meta-regressions. However, some of these meta-regressions appear to be on the restricted dataset (e.g., C3.1), but it is unclear if others were also done on the restricted dataset (e.g., C1.1). It seems that some of the meta-regressions were done of the full dataset as they are testing for the effects of factors that were used to subset the data previously (e.g., C1.2) – this seems a little bit redundant given that the effect sizes (and presumably heterogeneity?) did not change substantially in the previous section.

2) Page 4, line 80: perhaps say 'many diploid animals' or simply state how many species as 'all diploid animals' instantly implies that somehow all diploid species were included.

3) Page 8, line 193: This sentence is a little bit confusing. It kind of sounds like it is talking about environmental conditions but presumably, it means creating a dataset that should have reduced heterogeneity

4) Page 9, lines 220-223: I'm confused about how this data was subsetting. Was it split by familiar or unfamiliar kin or both? Perhaps present this the same way as the previous sections. What was k for familiar and unfamiliar effect sizes?

5) Page 12, line 310: Does parameters = traits?

6) Page 13, lines 312 – 315: Probably don't need this section. It may just create confusion and doesn't seem to add much.

7) Page 13, lines 323 – 328: This section is quite confusing and seems to say and test the same thing but through different methods that aren't quite clear

8) Page 18, line 452: Is this meant to say 'unrelated female' instead of 'unrelated male'?

9) Page 25, line 669: It's not intuitive how 'mating' and 'reproduction' categories differ within the 'during mating' avoidance mechanisms.

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Author Checklist

Manuscript:	NATECOLEVOL-201112162A
Author:	Raïssa A. de Boer
Article type:	Article

Title:

Meta-analytic evidence that animals rarely avoid inbreeding

Abstract:

Animals are usually expected to avoid mating with relatives (i.e. kin avoidance), as incestuous mating can lead to the expression of inbreeding depression. Yet, theoretical models consistently predict that unbiased mating with regards to kinship should be common, and that under some conditions the inclusive fitness benefits associated with inbreeding can even lead to a preference for mating with kin. This mismatch between empirical and theoretical expectations generates uncertainty as to the prevalence of inbreeding avoidance in animals. Here, we synthesised 677 effect sizes from 139 experimental studies of mate choice for kin vs. non-kin in diploid animals, comprising 40 years of research using a phylogenetically informed meta-analytical approach. Our meta-analysis revealed little support for the widely held view that animals avoid mating with kin, despite clear evidence of publication bias. Instead, unbiased mating with regards to kinship appears widespread across animals and experimental conditions. The significance of a variety of moderators was explored using meta-regressions, and this revealed that the degree of relatedness and prior experience with kin explained some variation in the effect sizes. Yet, we found no difference in effect among males and females, kin avoidance before and after mating, simultaneous, sequential and no choice experiments, mated and virgin animals, or between humans and other animals. Our findings highlight the need to rethink the widely held view that inbreeding avoidance is a given in experimental studies.

Your paper will be accompanied by the editor's summary below. Please let us know if there are any inaccuracies.

Editor's Summary:

A meta-analysis of 139 studies of diploid animals shows that they rarely avoid mating with kin, although the degree of relatedness and prior experience with kin do alter the effect size, and there is evidence of publication bias.

Additional Abstract Requests:



The abstract — which should be roughly 200 words long and contain no references — should serve both as a general introduction to the topic and a non-technical summary of your main results and their implications. It should contain a brief account of the background and rationale of the work, followed by a statement of the main conclusions introduced by the phrase 'Here we show' or some equivalent phrase. Because we hope that researchers in a wide range of disciplines will be interested in your work, the abstract should be as accessible as possible, explaining essential but specialized terms concisely. We encourage you to show your abstract to colleagues outside of your direct field of expertise to uncover any problematic concepts.

Author information

Please review your complete author list to verify that it is complete and accurate. We ask that you consult with your coauthors to ensure that all names, affiliations, and titles are represented correctly. Note that if any authors are added or removed after this point then all authors will be requested to provide approval documentation that could potentially delay the production of your paper.

The following changes must be made to the author information:

☐ Please supply an email address for each corresponding author.

Article structure

We can accommodate up to 6 display items (Figures or Tables) in the main article and up to 10 Extended Data figures, which will be integrated into the full-text HTML version of your paper and will be appended to the online PDF. Each Figure, Table and Extended Data figure must fit easily within an A4 page (260 x 179 mm). Please ensure that the number and size of your Figures, Tables and Extended Data figures fulfil these requirements to avoid any delay in the acceptance of your Article.

To comply with our format and optimise the presentation of data in your Article, we suggest the following changes to the number of Figures, Tables and Extended Data:

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☐ We estimate that the word count of your Article is approximately 6498 words. This is longer than our usual limit of 5000 so please shorten it where possible, making use of the Methods and Supplementary Information sections, which are unlimited.

Please ensure your main manuscript file includes the following sections:

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- ☐ Author list
- ☐ Affiliations
- ☐ Abstract
- ☐ Main text
- ☐ References
- ☐ Acknowledgements
- ☐ Author Contributions Statement
- ☐ Competing Interests Statement
- ☐ Figure Legends/Captions (for main text figures)
- ☐ Tables
- ☐ Methods (including Data Availability, Code Availability and Statistics subsections where relevant)

Main Text

The following changes must be made to the main text:

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Please see the guidelines linked below for detailed instructions about how your figures should be prepared. Following these instructions will reduce the chances of delays should we need to request replacement artwork from you at a later stage.

<https://www.nature.com/documents/NRJs-guide-to-preparing-final-artwork.pdf>

Additional requests: ☐ If bold/italic formatting in tables is necessary, please define its meaning in a table footnote

Data & Code

Data Availability

Nature journals strongly support public availability of data and code. Please deposit the data and code used in your paper into a public data repository. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor.

Please note that for some data types, deposition in a public repository is mandatory. Any restrictions on sharing of these data types must be clearly indicated in the statement and discussed with the editor. More information on our data deposition policies and available repositories can be found here:

<https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data>

Data Availability Statements

All published manuscripts reporting original research in Nature Research journals must include a data availability statement, as a separate section before the References and under the heading 'Data Availability'.

The data availability statement must make the conditions of access to the "minimum dataset" that are necessary to interpret, verify and extend the research in the article, transparent to readers.

This minimum dataset may be provided through deposition in public community/discipline-specific repositories, custom proprietary repositories or general repositories like Figshare, Zenodo and Dryad. Providing large datasets in supplementary information is strongly discouraged and the preferred approach is to make data available in repositories. Scientific Data, a Nature Research journal, maintains a list of approved and recommended data repositories to support researchers seeking suitable repositories for their data (<https://www.nature.com/sdata/policies/repositories>).

The Data Availability Statement should also reference any source data published alongside the paper.

If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year).

Reporting Summaries

- ☐ An updated life sciences reporting summary must be completed and uploaded as a supplementary information file with the revised manuscript. All points on the reporting summary must be addressed; if needed, please revise your manuscript in response to these points. This checklist is published alongside your manuscript online. Please note that this form is a dynamic "smart pdf" and must therefore be downloaded and completed in Adobe Reader, instead of opening it in a web browser. <https://www.nature.com/documents/nr-reporting-summary.pdf>

Additional requests related to Data and Code:

- ☐ Please supply a completed PRISMA checklist and ensure the relevant flow diagram is included with the final version of your manuscript

Methods

References

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End Matter

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Author Rebuttal, first revision:

Reviewer #3 (Remarks to the Author):

I have reread the manuscript by de Boer et al examining the prevalence of inbreeding avoidance through a large and comprehensive meta-analysis.

The authors have made substantial improvements to the manuscript and I believe that it will indeed make a nice contribution of Nature Ecology & Evolution.

I have very few comments to make on the revised version but see below for my final suggestions.

- We thank the Reviewer for his/her insightful revision of our manuscript. We have now followed the Reviewer's suggestions to improve our revised version.

1) In the results section, I'm a little confused by some of the data subsetting vs testing moderators in meta-regression. The authors first do a series of subsetting in an attempt to make inbreeding avoidance more detectable, then go on to do a series of meta-regressions. However, some of these meta-regressions appear to be on the restricted dataset (e.g., C3.1), but it is unclear if others were also done on the restricted dataset (e.g., C1.1). It seems that some of the meta-regressions were done of the full dataset as they are testing for the effects of factors that were used to subset the data previously (e.g., C1.2) – this seems a little bit redundant given that the effect sizes (and presumably heterogeneity?) did not change substantially in the previous section.

- We see the Reviewer's point here. To clarify our analytical approach, we have now added a statement to the beginning of Part C which states that the data was subsetted based on the availability of the data in order to test different hypotheses. We also highlight that the effect sizes included in each meta-regression are presented in Table 3. It is a fair point to say that there is a bit of redundancy in this approach. However, given that our study is the first broad synthesis of the data on inbreeding avoidance in animals we aired on the side of redundancy over restricting the dataset in the meta-regressions. This was done because it allowed us to test as many moderators hypothesized to influence inbreeding avoidance in animals using the largest dataset available in each case. We argue that this approach allows us to address as many moderators as possible, with as much statistical power as possible (which is essential given the non-significant results we present and the associated challenges of publishing such findings). Therefore, we feel that this approach allows our findings to be of interest to the widest possible audience of researchers.

2) Page 4, line 80: perhaps say 'many diploid animals' or simply state how many species as 'all diploid animals' instantly implies that somehow all diploid species were included.

- We thank the Reviewer for spotting this. The point here was to make it explicit that only diploid animals were included and so now we state '*diploid animals*'.

3) Page 8, line 193: This sentence is a little bit confusing. It kind of sounds like it is talking about environmental conditions but presumably, it means creating a dataset that should have reduced heterogeneity

- We agree with the Reviewer in that we do not refer to environmental conditions. We had highlighted above that the subsetting of the data was indeed done to reduce heterogeneity, but here in particular we refer to conditions under which inbreeding avoidance would be more likely. We have rephrased this sentence, it now reads: "*If kin avoidance is more likely under these restricted conditions, then subsetting the data under conditions that are more likely to elicit kin avoidance should arguably serve to demonstrate any effects that would otherwise be obscured in the global analysis of the full dataset.*" (Lines 194-197)

4) Page 9, lines 220-223: I'm confused about how this data was subsetting. Was it split by familiar or unfamiliar kin or both? Perhaps present this the same way as the previous sections. What was k for familiar and unfamiliar effect sizes?

- We thank the reviewer for suggesting that we clarify this statement. We now clarify how the data were subsetting by including this statement: "*As such, we only considered effect sizes ($k = 284$) where individuals were presented with kin that were familiar and non-kin that were unfamiliar, as this treatment should facilitate inbreeding avoidance more than any other combination of kin and non-kin stimuli (i.e. the remaining effect size estimates ($k = 142$) that assessed unfamiliar kin and non-kin, unfamiliar kin and familiar non-kin, and familiar kin and non-kin).*" (Lines 222-226)

5) Page 12, line 310: Does parameters = traits?

- We were referring to different metrics (e.g. association time vs. courtship duration) used to assess inbreeding avoidance. This has now been edited to refer to '*metrics*'. (Line 325)

6) Page 13, lines 312 – 315: Probably don't need this section. It may just create confusion and doesn't seem to add much.

- This section was included as response to a Reviewer's comment in the previous section as different traits were included within each episode of sexual selection. We wanted to make explicit that the fact that we included different traits within each episode did not influence our results, and therefore have not made changes to this section.

7) Page 13, lines 323 – 328: This section is quite confusing and seems to say and test the same thing but through different methods that aren't quite clear

- We agree and have now clarified the text. This section refers to two sets of tests: The first examines whether effect sizes estimates differed between the sexes at either precopulatory or postcopulatory episodes of sexual selection. The second examines the interaction between sex and episode of sexual selection. We have now clarified this section by providing more details on how this data was analysed. (Lines 338-343)

8) Page 18, line 452: Is this meant to say 'unrelated female' instead of 'unrelated male'?

- Yes. Thanks for catching this. This has now been corrected.

9) Page 25, line 669: It's not intuitive how 'mating' and 'reproduction' categories differ within the 'during mating' avoidance mechanisms.

- We thank the Reviewer for this suggestion. We have now included more detail: "*mating (mating events or mating duration/latency) and reproduction (pairs of individuals producing offspring)*" Lines (639-640)

Final Decision Letter:

24th March 2021

Dear Dr de Boer,

We are pleased to inform you that your Article entitled "Meta-analytic evidence that animals rarely avoid inbreeding", has now been accepted for publication in Nature Ecology & Evolution.

Before your manuscript is typeset, we will edit the text to ensure it is intelligible to our wide readership and conforms to house style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

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