Review of Increased birth rank of homosexual males: disentangling the older brother effect and sexual antagonism hypothesis (doi: https://doi.org/10.1101/2022.02.22.481477)

Confidential Comments to Editor

My overall impression of the manuscript is somewhat positive, although you will notice that I nonetheless have some larger concerns about the work. The topic is certainly interesting, and the authors are weighing in on an important discussion within this literature—whether the female relatives of homosexual males genuinely reproduce more than is to be expected, or if these (scattered) past findings are simply artefacts of the more well-established fraternal birth order effect (FBOE) for male same-sex attraction. The authors conclude the latter, although as my full review makes clear I find their route to this conclusion to be rather opaque. I also mention in the main review that the statistical equations and proofs should be evaluated by someone with the requisite knowledge to do so adequately. I simply don't have the statistical sophistication to adjudicate the accuracy of the equations in the text and supplementary material. In short, I think the work has merit, but if the authors found more order and clarity for the manuscript this would be appreciated by readers and likely lead to wider adoption (and comprehension) of their proposed methods.

Comments for Authors

In the present manuscript, the authors seek to examine two important questions for understanding the evolution of male same-sex attraction. First, some work has shown that the female relatives of homosexually attracted males have higher reproductive output than the female relatives of heterosexually attracted males. This has been interpreted as suggesting one of several potential genetic effects that could account for the existence of male homosexuality, given that genes associated with this attraction pattern in males might lead to elevated reproduction when carried by females. Alternatively, the fraternal birth order effect (FBOE), or as the authors prefer the Older Brother Effect (OBE), is the finding that each older brother a male has increases the likelihood that he will have exclusive same-sex attractions. Few attempts have been made to disentangle whether the elevated reproduction of female relatives due to some separate genetic effect or simply a result of highly fecund women being more likely to produce homosexual sons. The work is timely, interesting, and a solution to this empirical problem would be welcomed by many. Although I find much to praise in the manuscript, and think the authors' desire to bring innovative solutions to this challenging problem is laudable, I cannot recommend the manuscript in its present form. My largest concern, as detailed below, revolves around ease of understanding the proposed analytic models. Many analytic models are difficult to understand and this should not be avoided if they nonetheless improve accuracy and understanding of phenomena. However, all analytic frameworks must eventually be implemented, and I think more researchers in this area would be likely to implement the proposed frameworks if they were easier to understand. More specific comments are found below, and I hope they are of use to the authors. I similarly hope that my focus on areas of concern does not overshadow my largely positive impression of the manuscript—these comments are offered in the hope that the manuscript can be refined and hence made more useful to the scientific community who will pay attention to such scholarship.

1) Abstract: I'm not sure I know what the authors mean by "evolutionary determinants." This leaves quite a bit of ambiguity as to whether the considerations are proximate or ultimate in nature.
2) Abstract: It's unclear why an antagonistic effect (AE) would lead to a discernible birth rank effect rather than simply a greater number of siblings in general.
3) The first sentence of the introduction is rather lengthy, and can be a difficult compound sentence to parse. I might recommend splitting this into two sentences to reduce reader burden.
4) When discussing the origin of male homosexuality, it would be helpful if the readers distinguished between proximate origins (e.g., certain genes, maternal immune factors, etc.) as opposed to ultimate explanations for the origins of the trait (balancing selection, spandrel, etc.). These explanations are of course enmeshed, but clarity would be appreciated.
5) The Fraternal Birth Order Effect (FBOE) is typically referred to with the accompanying acronym. Would the authors be open to modifying the recurring acronym OBE (older brother effect) for the FBOE to maintain slightly more consistency in this literature?
6) Page 4 & 5. The authors point out that the FBOE has been examined in a huge diversity of samples. This point is well taken, but I would hasten to point out that each of these samples share key developmental features and findings—the FBOE is found among males who are androphilic, and this pattern emerges irrespective of other factors such as gender identity or age-preferences. Although other developmental factors likely play into the variations discussed, these groups all share a remarkably similar FBOE as it pertains to the development of male androphilia. A more relevant caveat in this literature are stopping rules (e.g., Blanchard 2022; https://doi.org/10.1080/00224499.2021.1984379)
7) Page 5, discussion of the OSE. Just a note to say that I thought this paragraph was well articulated. Sometimes as reviewers we get mired in finding areas for potential improvement, but some praise also seems warranted.
8) Page 6. The authors state: "Indeed, a higher fertility of mother of homosexual men implies that, when sampling homosexuals from a population, the mean birth order of homosexuals is higher than the mean birth order of heterosexuals." I'm not sure if I agree with the logic here. The high fertility doesn't speak to birth order *per se*, but simply family size. The AE would imply that family size of homosexual males will be large irrespective of actual birth order. The OBE, as stated in the next sentence, would imply that mothers with especially large reproductive output would have more (later born) homosexual sons.
9) Page 6. From my understanding of Khovanova (2019, as published in Archives of Sexual Behavior) is that one proposed approach (Method 1) examines restrictive cases of families with either 1 or 2 children. A more relaxed formulation (Method 2), also detailed in that paper, is to ignore sisters and consider families with either 1 or 2 males (which from a data analytic perspective amounts to participants with either no brothers, or one brother). The authors are correct, however, to note that these restrictions can substantially reduce sample sizes.
10) Page 7. I'm not sure what the authors mean when they say "index ratios." Price and Hare (1969) as cited certainly use the term "ratio," but "index ratio" does not appear in that manuscript. The authors' point about limitations are well taken, but there is a certain "hollowness" in this criticism given that one couldn't reasonably expect to have

"matched controls" in the same way as many medical studies and such that employ Odds ratios. Groups of homosexual individuals are already (statistically) unusual, and so removing potential "confounds" that differentiate them from heterosexual individuals may also remove important causative factors. The authors continue by saying, "Also, using an index ratio implicitly assumes that the expression of OBE is independent of the level of fertility, which remains to be shown." I'm not sure I understand the meaning here. If the MIH is correct, and the FBOE is genuine, then higher fertility will invariably lead to more (later born) sons who are same-sex attracted. This fact, and this fact alone, may explain related findings that the mothers of homosexual males sometimes show elevated reproduction—they end up in these "samples" precisely because they had high fertility to begin with.

11) I hope the authors don't take my concerns detailed above as an indication that I do not see merit in their arguments. They have conveyed a key concern in this literature about both theoretical considerations, and the data typically collected, and argued that we need more refined methodological tools to disentangle AE and OBE.
12) Page 9. I don't think I follow (or agree with) the logic outlined for the twin comparison. The authors state: "under the AE hypothesis, there should thus be more homosexual men among the dizygotic twins than among the monozygotic twins." There is little (if anything) in the introduction that would assist readers in seeing the rationale for this prediction without devoting their own further thinking to the matter. The authors could help lead readers down this path more clearly. I understand that the authors are making a simple "prevalence rate" prediction (i.e., not a concordance rate between twins), but I'm not sure why the presence of an AE would imply this prediction. Tong and Short (1998) discuss the ebb and flow of dizygotic twinning rates across time, which seem to be marked by initial decreases in developed countries, and have increased since the 1980s because of exogenous fertility treatments. Indeed, the abstract of Tong and Short (1998) states: "The rise in the dizygotic twinning rates which occurred from the 1980s onwards in developed countries is almost certainly due to increasing use of ovulation-inducing agents, but this rise may have masked a continuing decline in dizygotic twinning." The authors have outlined an evolutionary rationale, and numerous potential confounds in the extant literature. It is unclear to me why examining the prevalence of homosexuality among dizygotic and monozygotic twins represents a step forward when the elevation in dizygotic twinning observed in recent decades is attributable to exogenous fertility treatments that were unavailable to ancestral women. Indeed, women seeking fertility treatments are arguably more likely to be compensating for suppressed natural fertility. This seems to answer a confound with an even larger confound. Furthermore, greater fertility must be distinguished from actual reproductive output—dizygotic twins may be an indicator of "fertility" as defined by ease of conception, but this does not mean that these women invariably have higher overall reproductive output.
13) The authors note many exclusions regarding data in published literature, and I think that many of them are justifiable (or at least understandable). However, the authors explain in the introduction that individuals with gender-dysphoria would be excluded. Given this exclusion, it is curious to me that samples from both Samoa and Southern Mexico (i.e., Paul Vasey's research group) are included. These samples are not comprised of heterosexual and homosexual "men," but instead heterosexual men and third-gender individuals known as either fa'afafine (Samoa) or muxes (Mexico). Although not

precisely fitting the characterization of gender dysphoria, these third-gender individuals are not necessarily the same as Western gay men (although they do share in common the fact that they are male, and attracted to men/masculinity, among many other correlates such as the FBOE). Additionally, I am not sure how convinced I am that including bisexual individuals in the French, Greek, and Indonesian samples is appropriate. Male bisexuality in the West is relatively rare, and the determinants are not well understood (e.g., Bailey et al., 2016; Jabbour et al., 2020; www.pnas.org/cgi/doi/10.1073/pnas.2003631117). It is not theoretically parsimonious to lump bisexual and homosexual men together, and this is not typically done in this literature (and when it does occur, it likely should not). Furthermore, the sample described in Nila et al. (2019), which must actually be traced back to Nila et al. (2018; https://link.springer.com/article/10.1007/s10508-018-1202-y) for full sample information, also includes a substantial number (n = 34) of bisexual men. This aspect of the Nila data is not discussed presently, and is difficult to ascertain for readers unfamiliar with this literature. Beyond this concern, the "homosexual men" group in Nila et al. (2018) contains data from 11 gender-variant waria (from my understanding, these individuals are broadly comparable to the Samoan fa'afafine or the Mexican muxes). This is an even more confusing inclusion give that the authors are at great pains to exclude samples documenting the FBOE among androphilic males who are not cisgender. In short, readers shouldn't have to dig this much for sample information, and the samples considered should also remove the rather easy critique/confound of lumping together males who report bisexuality with those who report exclusive homosexuality.

14) Page 11. The authors compute mean birth order as OB/N + 1. This is not a metric that I have seen previously in this literature. I presume that the capital N indicates that this metric is for the entire sample, although it is arguably best applied within sexual orientation category to compensate for sample size differences across groups that are common in this literature. I have perhaps misunderstood the metric, but if I have this only serves to illustrate that the authors could briefly contextualize on the use of this parameterization and how readers should think about it.
15) Page 12. Including a standard deviation to accompany age would be helpful. The authors have presumably used a Wilcoxon test for this group difference due to the skewed nature of the data, but a brief note to this effect would ensure readers do not have to engage in any guess-work.
16) Regarding the statistical models, the Editor (or for this preprint service, the "recommender") will likely need to have this evaluated by a statistician who can appropriately adjudicate the models. That said, the authors have done little to explicate their approach and explain why it represents a useful innovation over previous approaches. Doing so is vitally important, as most readers curious about the FBOE will not have the requisite mathematical sophistication to evaluate the meaning or utility of the models presented. If the authors believe their approaches are a step forward, it is imperative that the rationale be conveyed to researchers in a clear and understandable manner. (Ablaza et al., 2022 have done exactly this, giving researchers a clear road-map to mimic their analytic framework for both data analysis and interpretation.) To be frank, after several readings of the mathematical portion of the manuscript, I am not sure what the models represent, how to interpret the values, or how this helps push our understanding forward. This is not helped by the fact that model specifics are found in the

(equally confusing) supplementary material, yet the main text makes constant reference to specific model numbers in that supplementary material. This creates an incredibly cumbersome reading process that compounds comprehension difficulties. To put my concerns into concrete terms, when I read Ablaza et al. (2022), I am able to see the way they have set up their models, and which exact parameters I should use if I wanted to replicate their models in my own (or publicly available) data. When I examine Table S1, I am completely at a loss for how I would translate this information into parameterization that I could apply to my own data. The authors may attribute this lack of ability to this reviewer's ignorance, and they may be correct to do so, but I will once again say that most researchers in this area do not have the kind of statistical sophistication to decipher and apply these models in their own work. If the authors believe these analytic approaches are a step forward and would like to see them widely implemented on both past and future data, then they will need to translate practical steps for doing so to us mere mortals.

17) Regarding some parameters in the model, the authors use a 1:1 Male:Female ratio, whereas this number typically shows a slight bias to males (i.e., ~105 Males for each 100 females born; https://ourworldindata.org/gender-ratio). I understand that the slight adjustments are unlikely to impact the models much, but it is worth either adjusting things slightly, or pointing out these caveats.
18) The authors employ WAIC, but do not explain what this statistical approach is. Much like the mathematical proofs and models put forward, the authors seem to have assumed a level of mathematical sophistication that will in fact be quite rare among the readers of this kind of manuscript. Similarly, the authors employ RJMCMC without any explanation of the approach, again presuming a huge amount of reader background knowledge and mathematical sophistication.
19) Page 24. The authors state, "First, we filtered out dubious samples, such as samples not corresponding to adult homosexuality, such as pedophiles, or corresponding to non-representative populations, such as sex offenders, transexuals, psychoanalytic or hospital patients (Zietsch, 2018). Thus, our results can be safely associated with standard homosexual men." In defense of Ray Blanchard's (many) contributions to this literature, I hasten to point out once again that these individuals all share in common the relevant factors for the FBOE—older brothers increasing the likelihood of androphilia among males. These other aspects of sexual orientation or presentation notwithstanding (e.g., age preferences, commission of sexual crimes, etc.), that simple fact remains. Indeed, it is interesting that in such diverse samples, the FBOE for male sexual orientation can be found. Indeed, rather than being "dubious" samples, I think they represent a fascinating convergence of evidence. Last, Paul Vasey's group have argued that other expressions of male androphilia have been common throughout most cultures and history (e.g., VanderLaan et al., 2013; DOI 10.1007/s12110-013-9182-z), meaning that "standard homosexual men" may not be an entirely intelligible way to characterize our understanding of the evolution of male androphilia.
20) Page 24. The authors note that low fecundity could be at play in studies that do not find an OBE, but do not similarly acknowledge (here or elsewhere) the way that stopping rules influence these patterns.
21) Page 26. The authors state: "Based on current knowledge, this OBE is only found in humans and not in any other species of mammals, even those closely related. This

suggests that the OBE is not a mere constraint of the gestation in primates, and thus the interference of male birth order with sexual orientation requires an evolutionary explanation." I'm not sure this point is entirely fair given that 1) exclusive same-sex preference is not found among other primates (although bisexual behavior can be widespread), and 2) to my knowledge, no studies of birth order and sexuality have been undertaken among non-human primates. Domesticated rams are the only species to show exclusive same-sex preference among males. Although a birth order study of these rams would be hugely informative, to my knowledge no such study has ever been conducted. The authors go on to speculate that the OBE could be "an adaptive plastic manipulation of the phenotype of male offspring by the mother." This adaptationist logic has a straightforward developmental rebuttal—the OBE is simply a developmental byproduct (i.e., spandrel; Gould & Lewontin, 1979) of actual adaptive processes (i.e., maternal immune system functioning then colliding with the typical neurodevelopment of a male fetus).

22) The authors' conclusion that the OBE is operating, whereas evidence for the AE and OSE is more limited, are well taken. This conclusion represents a step forward in this literature. However, I would strongly encourage the authors to convey the path taken to this conclusion in a more parsimonious and easy to follow manner.