

Female mating status has been overlooked in mate choice research: a comment on Richardson and Zuk

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The study of mate choice and sex roles has come a long way from the assumption that only males are promiscuous and females are coy. However, fragments of this mindset might still influence our research today. In their review, Richardson and Zuk (2022) bring forward the important issue that many mate choice experiments use only virgin females for measuring female preferences, despite the fact that in systems with multiply mating females, most of the mating events should occur with non-virgin females. As full monandry has been found to be much rarer than polyandry in most animal species (Taylor et al. 2014), this is an issue that most mate choice studies should consider carefully.

We find Richardson and Zuk's review both informative and eye-opening. This is so, despite the fact that their meta-analysis did not reveal large biases in mating preferences between virgin and non-virgin females in previously published literature. Their review still shows how using virgin females is the standard of most mate choice studies, and that only a few papers explicitly state that mated females were used. We strongly agree that more studies should be aware of the potential shortcomings that arise from this discrepancy between experimental animals and wild animal populations.

This problem has gone largely unnoticed until now, as even in the otherwise comprehensive book on mate choice by Rosenthal (2017), virgin females are only mentioned when discussing potential male preferences. While the meta-analysis by Richardson and Zuk did not find differences in choosiness between virgin and mated females, this might be due partly to the low number of publications with non-virgin females and thus warrants further investigating. More studies comparing experimentally these differences within single systems would be especially helpful to evaluate if this is a large concern for mate choice research.

There are several reasons why using virgin females might be justified in mate choice studies. Virgin females are often seen as a "clean slate" unbiased by previous mating experience. A study on reproductive isolation might therefore be more interested in investigating innate female preferences, and using virgin females may yield more reliable results through this approach. However, when estimating the overall reproductive isolation between populations or species, the role of behavioral isolation in restricting gene flow might be seriously underestimated if learned mate recognition is not taken into account (Magurran and Ramnarine 2004). We therefore agree with the Richardson and Zuk in that future studies should consider whether the mating status of their females fully represents the natural conditions.

Richardson and Zuk choose to limit their focus to studies of three categories: reproductive isolation, inbreeding avoidance and sexually transmitted disease. We are impressed with the thoroughness of the meta-analysis in this framework, but also recognize that it leaves out some large categories of mate choice research. For example, we still lack corresponding knowledge of studies that measure sexual selection acting on male phenotypic traits. If the use of virgins is widespread in this field as well, we could expect potential underestimation of female choosiness. The authors also do not consider the effects of different mating systems in their analysis, but it might

warrant further study. In systems where females are significantly mate-limited, virgin females may have a stronger reason to be less choosy than mated females. The differences in choosiness between virgin and mated females might therefore be the largest in species where females also compete for access to males (Hare and Simmons 2019).

The authors caution future researchers to be aware of the unknown mating status of wild-caught females. However, we would argue that a sufficiently large sample of wild-caught females should be the best representation of the types of potential mates the males would encounter in the wild. Using only wild-caught females would therefore avoid most of the concerns presented by this review, at least when it comes to estimating realistic female preferences in wild populations.

In summary, Richardson and Zuk raise an important concern for mate choice studies. Future research needs to take this issue into careful consideration. In addition, we think that the extent to which mating status might affect choosiness deserves to be delved into in its own right, and in both sexes, to estimate behavioral differences between virgin and non-virgin individuals.

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The complex interplay between male and female mating history: a comment on Richardson and Zuk

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Choosing a mate is a complex and multifaceted process (Rosenthal and Ryan 2022). Female mating status, in particular, is expected to influence their choice decisions, as reproduction is generally considered more costly and limiting for females compared to males. Therefore, previous encounters with potential suitors, as well as female mating history, should fundamentally affect both the strength and direction of female choice. In their recent meta-analysis, Richardson and Zuk (2022) explored the role of female mating status on mate choice. They found that empirical studies of female choice under a range of ecologically important scenarios are very often tested using only virgin females, resulting in an

incomplete—and potentially even misleading—understanding of how female choice decisions play out in the real world. In addition, Richardson and Zuk's (2022) review touched on the potential role of male mating behaviors in mediating the response of virgin and non-virgin females. Here, we extend on this discussion by delving more deeply into the importance of male mating status and male reproductive strategies, and how these, in turn, might interact with female mating status to affect female choice outcomes.

MALE EXPERIENCE MATTERS

Like female experience, male mating history can also affect female choice. In general, females might prefer non-virgin males because of the potential advantages of mating with a sexually experienced partner that can provide females with better resources. For example, in the nereidid polychaete *Neanthes acuminata*, females prefer males with a more extensive mating history as experienced males were better fathers (Fletcher et al. 2009). Also, while virgin males might be more invested in their mating effort, experienced males are often more successful in mating (Aich et al. 2021). In contrast, males with a higher mating history might become sperm-limited, and mating with them could lower female fertilization success. In such cases, females should benefit by discriminating against non-virgin males, as demonstrated, for example, in the European grapevine moth *Lobesia botrana* (Muller et al. 2016). Therefore, in the context of female mating history, virgin and non-virgin females could differ in their mating preferences based on male mating history. Thus, we recommend that future studies, ideally, should focus on teasing apart both male and female mating history, either experimentally or statistically, to determine how they might interact to influence female mate choice (see Aich et al. 2020).

AND MALE BEHAVIORS TOO

The effects of female mating status on her mate choice decisions are also expected to be affected by male mating strategies. As mentioned in Richardson and Zuk's (2022) review, males could benefit from mating with virgin females if virgins are more receptive, and, in pursuing such females, males end up achieving higher fertilization success. Thus, if virgin females are preferred by males, then this could lead to more mating effort being directed by males toward virgin females. For instance, in the terrestrial isopod *Armadillidium vulgare*, males prefer virgin over mated females, even if the latter are infested with parasites (Fortin et al. 2018). Indeed, emerging evidence suggests that males do adjust their mating behavior based on female mating status. For example, in species with alternative reproductive tactics, such as guppies *Poecilia reticulata*, males engage in more courtship behaviors toward virgin females, but direct more coercive, sneaky copulations toward non-virgins (Guevara-Fiore et al. 2009). Such biases in male mating behavior toward females differing in mating status could potentially result in variation in female mate perception, preference, and, ultimately, mating opportunities. Unfortunately, only a handful of studies have looked at the effect of female mating status on male mating investment, especially in vertebrates. Here, there is scope for future studies to test the effects of female mating experience on male mating strategies in a broader range of taxa. Results from such studies will help us to better understand the substantial variation in mating preferences in both virgin and non-virgin females.

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Assumptions, models and data: a comment on Richardson and Zuk

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To test theoretical models (e.g. Kokko and Mappes 2005) and allied verbal arguments, Richardson and Zuk (2022) (RZ) conducted a meta-analysis to see if virgins are less choosy than mated females when presented with potential mates. Spoiler alert: they found no statistically significant difference. This is reassuring as most experimental mate choice studies either use only one type of female or ignore female mating status. Nonetheless, RZ raise questions about how to test theory using meta-analysis.

First, testing for within-species differences using across-species comparisons is risky. Researchers might be more likely to use mated females in species where they are known, or assumed, to be choosy (e.g. due to cumulative material benefits to mate choice). More generally, confounding inherent differences among species could obscure the true effect of mating on female choosiness. Ideally we need within-species, or even within-study, effect sizes from females randomly assigned as virgin or mated. Experimental studies that compare the choosiness of such females exist (e.g. Aich et al. 2020), but are rare (RZ, Figure 3).

Second, can we generalize from RZ's datasets? Female choosiness was based on avoiding: 1) hybrid mating (e.g. heterospecifics males); 2) close relatives; 3) males with STDs. However, hybridization often