Biological Sciences: Evolution; Ecology; Medical Science

Sex and Power: sexual dimorphism in trait variability and its eco-evolutionary and statistical implications

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ABSTRACT

Biomedical and clinical sciences are experiencing a renewed interest in the fact that males and females differ in many anatomic, physiological, and behavioral traits. Sex differences in trait variability, however, are yet to receive similar recognition. In medical science, mammalian females are assumed to have higher trait variability due to estrus cycles (the 'estrus-mediated variability hypothesis'); historically in biomedical research, females have been excluded for this reason. Contrastingly, evolutionary theory and associated data support the 'greater male variability hypothesis'. Here, we test these competing hypotheses in 218 traits measured in >27,000 mice, using meta-analysis methods. Neither hypothesis could universally explain patterns in trait variability. Sex-bias in variability was trait-dependent. While greater male variability was found in morphological traits, females were much more variable in immunological traits. Sex-specific variability has eco-evolutionary ramifications including sex-dependent responses to climate change, as well as statistical implications including power analysis considering sex difference in variance.

Significance Statement

Males and females differ in many traits. However, we know relatively little about sex differences in trait variability. In many clinical contexts, female subjects have traditionally been excluded, due to assumed higher variability caused by the estrus cycle. Contrastingly, theory from evolutionary biology predicts higher variability in males. Neither explanation universally fits the data, but specific trait groups exhibit strong sex-specific differences. Sex differences in trait variability implies, for example, that the two sexes respond differently to environmental changes, and one sex could fair worse than the other depending on the nature of changes. Also, such sex differences mean that we should regularly include both males and females in biomedical trials, carrying out statistical power calculations separately for both sexes.

Keywords

Sex inequality, gender difference, sexual selection, meta-regression, power analysis

INTRODUCTION

Sex differences arise because selection acts on the two sexes differently, especially on traits associated with mating and reproduction (1). Therefore, sex differences are widespread, a fact which is unsurprising to any evolutionary biologist. However, scientists in (bio-)medical fields have not traditionally regarded sex as a biological factor of intrinsic interest (2–7). Therefore, many (bio-)medical studies have been conducted only with male subjects, or without distinguishing between the sexes. Consequently, our knowledge is biased. For example, we know far more about drug efficacy in male compared to female subjects, contributing to unequal understanding of how the sexes respond to medical intervention (8). Only recently have (bio-)medical scientists started considering sex differences in their research (9–15). The National Institutes of Health (NIH) have implemented new guidelines for vertebrate animal and human research study designs, requiring that sex be included as a biological variable (2, 16, 17). This is an important step, but we can go much further.

[Figure 1 here]

When comparing the sexes, biologists generally focus on mean differences in trait values, placing little or no emphasis on sex differences in trait variability (see Fig. 1 for a diagram explaining differences in means and variances). Despite this, two hypotheses exist that explain why trait variability might be expected to differ between the sexes. Interestingly, these two hypotheses make opposing predictions.

First, the "estrus-mediated variability hypothesis" (Fig. 2), which emerged in the (bio-) medical research field, assumes that the female estrus cycle (see for example 6, 18) causes higher variability across traits in female subjects. This assumption is the major reason for why female research subjects were often excluded from biomedical research trials, especially in the neurosciences, physiology and pharmacology (18). Female exclusion was based on the grounds that including/using females in empirical research led to a loss of statistical power, or that animals must be sampled across the estrus cycle for one to make valid conclusions, requiring more time and resources.

Second, the "greater male variability hypothesis" suggests males exhibit higher trait variability because either: 1) they are subject to stronger sexual selection (19–21) or 2) because they are often the heterogametic sex (22) or both. In mammals, such as mice and humans, we expect males to have higher trait variability under either mechanism. This hypothesis has so far gained some support in the evolutionary and psychological literature (23, 24).

[Figure 2 here]

Here we conduct the first comprehensive test of the greater male variability and estrus-mediated variability hypotheses in mice (Fig. 2; cf. 24–28), examining sex differences in variance across 218 traits in 27,147 animals. To this end, we carry out a series of meta-

analyses in two steps (SI Appendix Fig. S1.1). First, we quantify the natural logarithm of the male to female coefficients of variation, CV (lnCVR) for each cohort (population) of mice, for different traits, along with the variability ratio of male to female standard deviations, SD, on the log scale (lnVR, following 29, see Fig. 1). Then, we analyze these effect sizes to quantify sex bias in variance for each trait using meta-analytic methods. To better understand our results and compare them to previously reported sex differences in trait means (4), we also quantify and analyze the log response ratio (lnRR). Then, we statistically amalgamate the trait-level results to test our hypotheses and to quantify the degree of sex biases in and across nine functional trait groups (for details on the grouping, see below). Our meta-analytic approach allows easy interpretation and comparison with earlier and future studies.

RESULTS

Data characteristics and workflow

We used a dataset compiled by the International Mouse Phenotyping Consortium (31) (IMPC, dataset acquired 6/2018). To gain insight into systematic sex differences, we only included data of wildtype-strain adult mice, between 100 and 500 days of age. We removed cases with missing data, and selected measurements that were closest to 100 days of age (young adult) when multiple measurements of the same trait were available. To obtain robust estimates of sex differences, we only used data on traits that were measured in at least two different institutions (see workflow diagram, SI Appendix Fig. S1.1 A).

Our data set comprised 218 continuous traits (after initial data cleaning and pre-processing; SI Appendix Fig. S1.1 A-D). It contains information from 27,147 mice from 9 wildtype strains that were studied across 11 institutions. We combined mouse strain/institution information to create a biological grouping variable (referred to as “population” in SI Appendix Fig. S1.1 B; see also Table S6.1 for details), and the mean and variance of a trait for each population was quantified. We assigned traits according to related procedures into functionally and/or procedurally related trait groups to enhance interpretability (referred to as “functional groups” hereafter; see also SI Appendix Fig. S1.1 G). Our nine functional trait groups were behaviour, morphology, metabolism, physiology, immunology, hematology, heart, hearing and eye (for the rationale of these functional groups and related details, see Methods and SI Appendix Table S6.3).

[Figure 3 here]

Testing the two hypotheses

We found that some means and variabilities of traits were biased towards males (i.e. ‘male-biased’, hereafter; “turquoise” shaded traits, Fig. 3), but others towards females (i.e. ‘female-biased’, hereafter; “orange” shading, Fig. 3) within all functional groups. These sex-specific biases occur in mean trait sizes and also in our measures of trait variability. There were strong positive relationships between mean and variance across traits ($r > 0.94$ on the log scale; SI

Appendix Fig. S2.1), and therefore, we report the results of lnCVR, which controls for differences in means, in the main text. Results on lnVR are presented in the electronic supplementary material (SI Appendix Fig. S5.1 and S5.2).

There was no consistent pattern in which sex has more variability (lnCVR) in the here examined traits (left panel in Fig. 3A). Our meta-analytic results also did not support a consistent pattern of either higher male variability or higher female variability (see Fig. 3B, left panel: "All" indicates that across all traits and functional groups, there was no significant sex bias in variances; lnCVR = 0.005, 95% confidence interval, 95% CI = [-0.009 to 0.018]). However, there was high heterogeneity among traits (I^2 = 76.5 %, SI Appendix Table S6.4; see also SI Appendix Table S6.5), indicating sex differences in variability are trait-dependent, corroborating our general observation that variability in some traits was male-based but others female-biased (Fig. 3A).

As expected, specific functional trait groups showed significant sex-specific bias in variability (Fig. 3B). The variability among-traits within a functional group was lower than that of all the traits combined (SI Appendix Table S6.4). For example, males exhibited an 8.05% increase in CV relative to females for morphological traits (lnCVR = 0.077; CI = [0.041 to 0.113], I^2= 67.3%), but CV was female-biased for immunological traits (6.59% higher in females, lnCVR = -0.068, CI =[-0.098 to 0.038], I^2 = 40.8%) and eye morphology (7.85% higher in females, lnCVR = -0.081, CI =[-0.147 to (- 0.016)], I^2 = 49.8%).

The pattern was similar for overall sexual dimorphism in mean trait values (here, a slight male bias is indicated by larger "turquoise" than "orange" areas; Fig. 3A, right and Fig. 3B, lnRR: "All", lnRR = 0.012, CI = [-0.006 to 0.31]). Trait means (lnRR) were 7% larger for males (lnRR = 0.067; CI = [0.007 to 0.128]) in morphological traits and 15.3 % larger in males for metabolic traits (lnRR = 0.142; CI = [0.036 to 0.248]). In contrast, females had 5.59 % [lnRR = 0.057, CI = [-0.107 to (-0.007)] larger means than those of males for immunological traits. We note that these meta-analytic estimates were accompanied by very large between-trait heterogeneity values (morphology I^2 = 99.7%, metabolism I^2 = 99.4%, immunology I^2 = 96.2; see SI Appendix Table S6.4), indicating that even within the same functional groups, the degree and direction of sex-bias in the mean was not consistent among traits.

[Figure 4 here]

DISCUSSION

We tested competing predictions from the two hypotheses for why sex-biases in trait variability exist. Neither the 'greater male variability' hypothesis nor the 'estrus-mediated variability' hypothesis explain the observed patterns in sex-biased trait variation on their own. Therefore, our results add further empirical weight to calls that question the basis for the routine exclusion of one sex in biomedical research based on the estrus-mediated variability hypothesis (3, 5–7).

Greater male variability vs. estrus-mediated variability?

Evolutionary biologists commonly expect greater variability in the heterogametic sex than the homogametic sex. In mammals, males are heterogametic, and hence are expected to exhibit higher trait variability compared to females, which is also consistent with an expectation from the theory of sexual selection (24). Our results provide only partial support for the greater male variability hypothesis because the expected pattern only manifested for morphological traits (see Fig. 3 & 4). This result corroborates a previous analysis across animals, which found that the heterogametic sex was more variable in body size (24). However, our data do not support the conclusion that higher variability in males occurs across all traits including within the class of morphological traits).

[Figure 4]

The estrus-mediated variability hypothesis was, at least until recently (6, 12), regularly used as a rationale for including only male subjects in many biomedical studies. So far, we know very little about the relationship between hormonal fluctuations and general trait variability within and among female subjects. Our results are consistent with the estrus-mediated variability hypothesis for immunological traits only. Immune responses can strongly depend on sex hormones (32, 33), which may explain higher female variability in these traits. However, if estrus status affects traits through variation in hormone levels, we would expect to also find higher female variability in physiological and hematological traits. This was not the case in our dataset. Interestingly, however, eye morphology (structural traits, which should fluctuate little across the estrus cycle) also appeared to be more variable in females than males, but little is known about sex differences in ocular traits in general (34, 35). Overall, we find no consistent support for the female estrus-mediated variability hypothesis.

In line with our findings, recent studies have refuted the prediction of higher female variability (6, 12, 18, 28, 29). For example, several rodent studies have found that males are more variable than females (6, 12, 28, 29, 36, 37). Further studies should investigate whether higher female variability in immunological traits is indeed due to the estrus cycle, or generally because of greater between-individual variation (cf. Fig. 2).

In general, we found many traits to be sexually dimorphic (Fig. 4), in accordance with previous studies (4). More specifically, males are larger than females, while females have higher

192 immunological parameters (see Fig. 4). Notably, most sexually dimorphic trait means also
193 show the greatest differences in trait variance (Fig. 3 & Fig. 4). Indeed, theory predicts that
194 sexually selected traits (e.g., larger body size for males due to male-male competition) are
195 likely more variable, as these traits are often condition dependent (38). This relationship may
196 explain why male-biased morphological traits are larger and more variable.

197 **Eco-evolutionary implications**

198 We have used lnCVR values to compare phenotypic variability (CV) between the sexes. When
199 lnCVR is used for fitness-related traits, it can signify sex differences in the 'opportunity for
200 selection' between females and males (38). If we assume that phenotypic variation (i.e.
201 variability in traits) has a heritable basis, then large ratios of lnCVR may indicate differences
202 in the evolutionary potential of each sex to respond to selection, at least in the short term
203 (41). We note, however, that in our study, lnCVR reflects sex difference in trait variability
204 within strains, so that the observed variability differences are mainly due to phenotypic
205 plasticity.

206 Sex-specific differences could lead to sex-skewed populations if fitness-related traits exhibit
207 strong sex-bias in variability. For example, disease outbreaks or the ability to deal with
208 changing temperatures could affect one sex more severely than the other. Changes in sex-
209 ratios, in turn, can the influence mating systems (42, 43) with potential downstream effects
210 on population dynamics. In addition, sex-specific variation and differences in evolutionary
211 potential may also have important implications for modelling population dynamics, where
212 such sex-specific differences are not normally taken into account. Explicitly modelling sex
213 difference in trait variability could lead to different conclusions compared to existing models
214 (cf. 44) .

215 **Statistical and practical implications**

216 It is now mandatory to include both sexes in biomedical experiments and clinical trials funded
217 by the NIH, unless there exists strong justification against the inclusion of both sexes (45). In
218 order to conduct meaningful research and make sound clinical recommendations for both
219 male and female patients, it is necessary to understand not only how trait means, but also
220 how trait variances differ between the sexes. If one sex is systematically more variable in a
221 trait of interest than the other, then experiments should be designed to accommodate
222 relative differences in statistical power between the sexes (which has not been considered
223 before, see 3, 5–7). For example, given a limited number of animal subjects in an experiment
224 measuring immunological traits, a balanced sex ratio may not be optimal. Female
225 immunological traits are generally more variable (i.e. higher CV and SD). If we assume that
226 responses to an experimental treatment will be similar between the sexes for this functional
227 trait group, we will require more females to achieve the same statistical power as for the
228 males.

To help researchers adjust their sex-specific sample size to achieve optimal statistical power, we provide a tool (ShinyApp; https://bit.ly/sex-difference/). This tool may serve as a starting point for checking baseline variability for each sex in mice. The sex bias (indicated by the % difference between the sexes) is provided for separate traits, procedures, and functional groups. These meta-analytic results are based on our analyses of more than 2 million rodent data points, from 27,147 individual mice. We note that, however, variability in a trait measured in untreated individuals maintained under carefully standardized environmental conditions, as reported here, may not directly translate into the same variability when measured in experimentally treated individuals, or individuals exposed to a range of environments (i.e. natural populations or human cohorts).

Relevantly, when two groups (e.g., males and females) show difference in variability, we violate an important statistical assumption, the homogeneity of variance or homoscedasticity. Such violation is detrimental (i.e., leading to a higher Type I error rate), especially when the two groups have different sample sizes, for which we advocate above. Therefore, we should consider incorporating heteroscedasticity (different variances) explicitly or using robust estimators of variance (also known as 'the sandwich variance estimator') to prevent a higher Type I error rate (46).

Conclusion

We have shown that sex biases in variability occur in many mouse traits but that the direction of those biases differs between traits. Neither the 'greater male variability' nor the 'estrus-mediated variability' hypothesis provides a general explanation for sex-differences in trait variability. Instead, we have found that the direction of the sex bias varies across traits and among trait types (Fig. 3 & 4). Our findings have important ecological and evolutionary ramifications. If the differences in variability correspond to the potential of each sex to respond to changes in specific environments, this sex difference needs to be incorporated into demographic and population-genetic modelling. Moreover, in the (bio-)medical field, our results should inform decisions during study design by providing more rigorous power analyses that allow researchers to incorporate sex-specific differences for sample size. We believe that taking sex-differences in trait variability into account will help avoid misleading conclusions and provide new insights into sex differences across many areas of biological and bio-medical research. Ultimately, such considerations will not only better our knowledge, but also close the current gaps in our biased knowledge (47).

METHODS

Data selection and process

The IMPC (International Mouse Phenotyping Consortium) provides a comprehensive catalogue of mammalian gene function for investigating the genetics of health and disease, by systematically collecting phenotypes of knock-out and wild type mice. To investigate

differences in trait variability between the sexes, we only considered the data for wild-type control mice. We retrieved the dataset from the IMPC server in June 2018 and filtered it to contain non-categorical traits for wildtype mice. The initial dataset comprised over 2,500,000 data points for 340 traits. In cases where multiple measurements were taken over time, data cleaning started with selecting single measurements for each individual and trait. In these cases, we selected the measurement closest to "100 days of age". We excluded data for juvenile and unsexed mice (SI Appendix Fig. S1.1 A; this data set and scripts can be found on https://bit.ly/code-mice-sex-diff; raw data: https://doi.org/10.5281/zenodo.3759701).

Grouping and effect size calculation

We created a grouping variable called "population" (SI Appendix Fig. S1.1 B). A population comprised a group of individuals belonging to a distinct wild-type strain maintained at one particular location (institution); populations were identified for every trait of interest. Our data were derived from 11 different locations/institutions, and a given location/institution could provide data on multiple populations (see SI Appendix Table S6.1 for details on numbers of strains and Institutions). We included only populations that contained data points for at least 6 individuals, and which had information for members of both sexes; further, these populations for a particular trait had to come from at least two institutions to be eligible for inclusion. After this selection process, the dataset contained 2,300,000 data points across 232 traits.

We used the function *escalc* in the R package, *metafor* (48) to obtain lnCVR, lnVR and lnRR and their corresponding sampling variance for each trait for each population; we worked in the R environment for data cleaning, processing and analyses (R Core Team 2017 (49); version 3.6.0; for the versions of all the software packages used for this article and all the details and code for the statistical analyses, see the electronic supplements).

Meta-analyses: overview

We conducted meta-analyses at two different levels (SI Appendix Fig. S1.1 C-J). First, we conducted a meta-analysis for each trait for all three effect size types (lnRR, lnVR and lnCVR), calculated at the 'population' level (i.e. using population as a unit of analysis). Second, we statistically amalgamated overall effect sizes estimated at each trait (i.e. overall trait means as a unit of analysis) after accounting for dependence among traits. In other words, we conducted second-order meta-analyses (50). We used the second-order meta-analyses for three different purposes: A) estimating overall sex biases in variance (lnCVR and lnVR) and mean (lnRR) in the nine functional groups (for details, see below) and in all these groups combined (the overall estimates); B) visualizing heterogeneities across populations for the three types of effect size in the nine functional trait groups, which complemented the first set of analyses (SI Appendix Fig. S1.1 I, Table S6.6); and C) when traits were found to be significantly sex-biased, grouping such traits into either male-biased and female-biased traits, and then, estimating overall magnitudes of sex bias for both sexes again for the nine

functional trait groups. Only the first second-order meta-analysis (A) directly related to the testing of our hypotheses, we report the method detail and the results of B and C in SI Appendix.

Meta-analyses: population as an analysis unit

To obtain degree of sex bias for each trait mean and variance (SI Appendix Fig. S1.1 C), we used the function *rma.mv* in the R package *metafor* (48) by fitting the following multilevel meta-analytic model (sensu 51):

$$ES_i \sim 1 + (1 \mid Strain_j) + (1 \mid Location_k) + (1 \mid Unit_i) + Error_i,$$

where 'ES_i' is the *i*th effect size (i.e. lnCVR, lnVR and lnRR) for each of 232 traits, the '1' is the overall intercept (other '1's are random intercepts for the following random effects), '$Strain_j$' is a random effect for the *j*th strain of mice (among 9 strains), '$Location_k$' is a random effect for the *k*th location (among 11 institutions), '$Unit_i$' is a residual (or effect-size level or 'population-level' random effect) for the *i*th effect size, '$Error_i$' is a random effect of the known sampling error for the *i*th effect size. Given the model above, meta-analytic results had two components: 1) overall means with standard errors (95% confidence intervals), and 2) total heterogeneity (the sum of the three variance components, which is estimated for the random effects).

We excluded traits which did not carry useful information for this study (i.e. fixed traits, such as number of vertebrae, digits, ribs and other traits that were not variable across wildtype mice; note that this may be different for knock-down mutant strains) or where the meta-analytic model for the trait of interest did not converge, most likely due to small sample size from the dataset (14 traits, see SI Appendix, for details: Meta-analyses; 1. Population as analysis unit). We therefore obtained a dataset containing meta-analytic results for 218 traits at this stage, to use for our second-order meta-analyses (SI Appendix Fig. S1.1 D).

Meta-analyses: accounting for correlated traits

Our dataset of meta-analytic results included a large number of non-independent traits. To account for dependence, we identified 90 out of 218 traits, and organized them into 19 trait sub-groups (containing 2-10 correlated traits, see SI Appendix Fig. S1.1 E). For example, many measurements (i.e. traits) from hematological and immunological assays were hierarchically clustered or overlapped with each other (e.g., cell type A, B and A+B). We combined the meta-analytic results from 90 traits into 19 meta-analytic results (Fig. 3F) using the function *robu* in the R package, *robumeta* with the assumption of sampling errors being correlated with the default value of r = 0.8 (52). Consequently, our final dataset for secondary meta-analyses contained 147 traits (i.e. the newly condensed 19 plus the remaining 128 independent traits, see SI Appendix Fig 1.1 , Table S6.2), which we assume to be independent of each other.

Second-order meta-analyses: trait as an analysis unit

We created our nine overarching *functional groups* (SI Appendix Fig. S1.1 G) by condensing the IMPC's 26 procedural categories into related clusters (see SI Appendix Table S6.3 for details on clustering of traits, procedures and grouping terms).

To test our two hypotheses about how trait variability changes in relation to sex, we estimated overall effect sizes for nine functional groups by aggregating meta-analytic results via a 'classical' random-effect models using the function *rma.uni* in the R package *metafor* (48). In other words, we conducted three sets of 10 second-order meta-analyses (i.e. meta-analyzing 3 types of effect size: lnRR, lnVR and lnCVR for 9 functional groups and one for all the groups combined, SI Appendix Fig. S1.1 H).

Author contributions

SN conceived the initial idea, and all contributed to furthering the idea and the design of the study. SRKZ, along with FZ, led the analyses and writing with inputs from all authors. DWAN created the Shiny application. Apart from SRKZ, FZ, DWAN and SN, all authors have contributed equally, yet uniquely, and are listed in alphabetical order.

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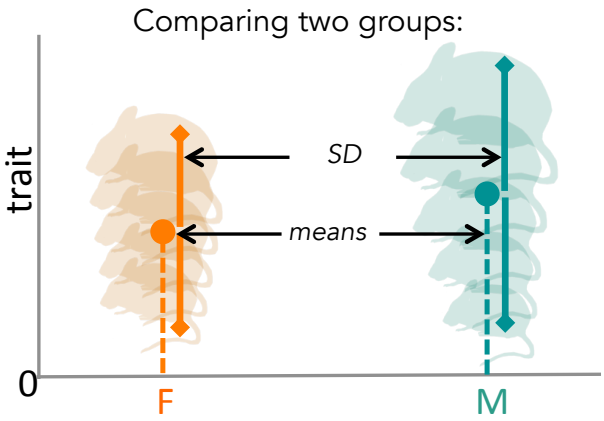
Fig. 1.

Overview of meta-analytic methods used to detect differences in means and variances in any given trait (e.g., body size in mice). The orange shaded mice represent females (F), turquoise shaded mice stand for males (M). The solid "dot" represents a mean trait value within the respective group. Solid lines represent standard deviation, with upper and lower bounds indicated by diamond shapes. Below, we present three types of effect sizes that can be used for comparing two groups, along with the respective formulas and interpretations. Compared to lnVR, lnCVR provides a more general measure of the difference in variability between two groups (mean-adjusted variability ratio).



Which group has larger mean value?

Response Ratio:

$lnRR = \ln\left(\frac{\text{M}}{\text{F}}\right)$

lnRR > 0 ➔ *male-biased mean trait values*

Which group is more variable?

Variability Ratio:

$lnVR = \ln\left(\frac{\text{M}}{\text{F}}\right)$

lnVR > 0 ➔ *traits more variable in males*

Which group is more variable when controlling for the means?

Coefficient of Variation Ratio:

$lnCVR = \ln\left(\frac{\text{M}}{\text{F}}\right)$

lnCVR > 0 ➔ *male-biased mean trait values*

Fig. 2.

The two hypotheses ("Greater Male Variability" vs "Estrus Mediated variability") have different underlying predictions on how variabilities influence total observed phenotypic variance (V_{total} in the figure). For Greater Male Variability, the within-subject [or within-trait] variation V_{within} could be potentially negligible or is equal in males and females. This is illustrated as the shaded distributions around each individual mean (dashed vertical lines), which are of equal area for the males (turquoise) and females (orange). The greater value of V_{total} is driven by wider distribution of mean trait values in males compared to females (i.e. $V_{between}$, represented by a thick horizontal bar). The estrus-mediated variability hypothesis, in contrast, assumes that within-subject [or within trait] variability is much higher in females than in males (broader orange-shaded trait distributions than blue-green distributions), while the variability of the means between individuals stays the same (thick horizontal bars).

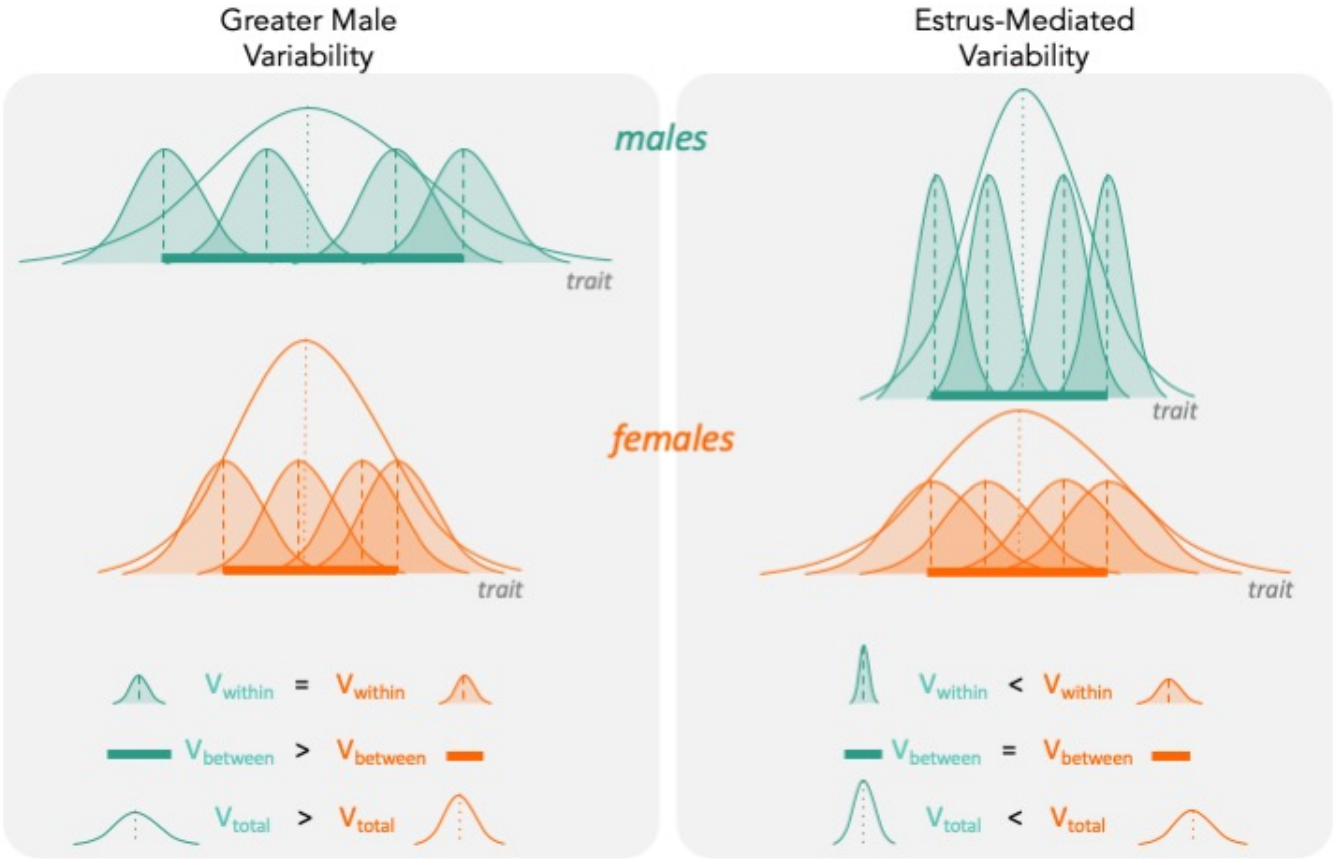


Fig. 3:

Panel A shows the numbers of traits across functional groups that are either male-biased (turquoise) or female-biased (orange; as in SI Appendix Fig. S1.1 D). The x-axes in Panel A show the overall percentages of traits, coloured shading is indicative of direction of sex-bias sex (if meta-analytic means < 0, then they are female-based whereas if they are > 0, male-based). White numbers in the turquoise bars represent numbers of traits that show male-bias within a given group of traits, number in the orange area the number of female-biased traits. Panel B shows effect sizes and 95% CI from separate meta-analysis for each functional group (SI Appendix Fig. S1.1 H). Traits that are male biased in Panel B are shifted towards the righthand side of the zero-midline (near the turquoise male symbol), whereas female bias is shifted towards the left (near orange symbol).

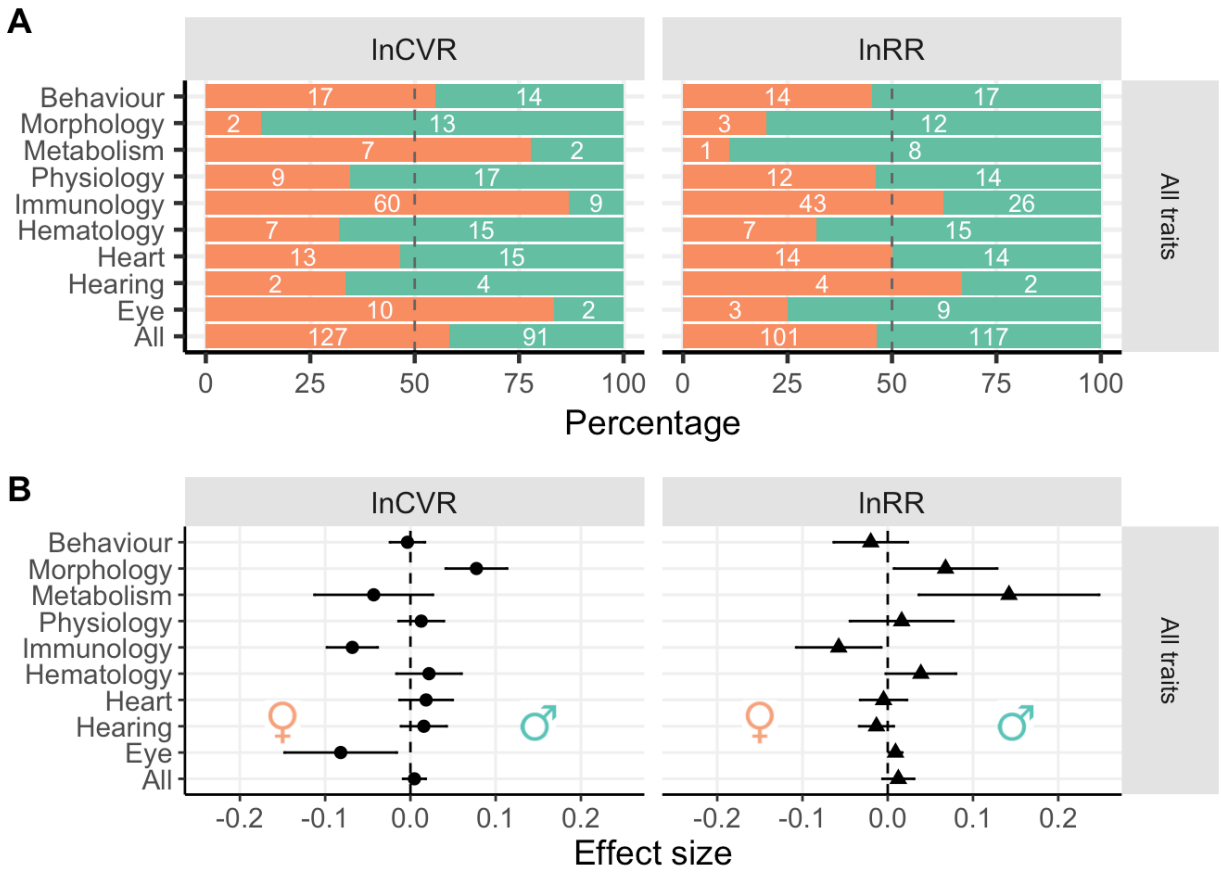


Fig. 4.

Summary of sex-differences in the mean trait values (lnRR) and variances (lnCVR) across ten functional trait groups.