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Battle of the sexes: analysis of sex bias in host use and reporting practices in parasitological experiments

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ABSTRACT

Experimental approaches are among the most powerful tools available to biologists, yet in many disciplines their results have been questioned due to an underrepresentation of female animal subjects. In parasitology, experiments are crucial to understand host-parasite interactions, parasite development, host immune responses, as well as the efficacy of different control methods. However, distinguishing between species-wide and sex-specific effects requires the balanced inclusion of both male and female hosts in experiments and the reporting of results for each sex separately. Here, using data from over 3600 parasitological experiments on helminth-mammal interactions published in the past four decades, we investigate patterns of male versus female subject use and result reporting practices in experimental parasitology. We uncover multiple effects of the parasite taxon used, the type of host used (rats and mice for which subject selection is fully under researcher control versus farm animals), the research subject area and the year of publication, on whether host sex is even specified, whether one or both host sexes have been used (and if only one then which one), and whether the results are presented separately for each host sex. We discuss possible reasons for biases and unjustifiable selection of host subjects, and for poor experimental design and reporting of results. Finally, we make some simple recommendations for increased rigour in experimental design and to reset experimental approaches as a cornerstone of parasitological research.

1. Introduction

One of the basic principles of experimental biology is that, whatever the study species, the subjects used are representative of the population at large (Selwyn, 1996). If not, i.e., if the subjects capture only a particular section of the population, the experimental results and their implications may not apply more generally to the entire population or species. Yet this simple principle has been frequently violated in biomedical research, where there has been consistent underrepresentation of female animal subjects in experimental studies across all subdisciplines (Beery and Zucker, 2011; Yoon et al., 2014). Incredibly, for many human diseases that are more prevalent among women than men worldwide, a majority of studies using animal models include only male subjects (Zucker and Beery, 2010). This has no doubt resulted in poorer treatment outcomes for women, leading the National Institutes of Health (USA) to develop a policy to encourage the equal use of male and female animal subjects in experimental studies and consider sex as a key biological variable in the research they fund (Clayton and Collins, 2014).

For sex-specific research questions (e.g., the influence of diet on pregnancy, or the efficacy of various treatments against testicular cancer), the use of subjects of one sex only is obviously justified. In studies addressing issues that are not sex-specific, however, the ideal design should include equal representation of both sexes. The underrepresentation of female subjects in many such studies may simply reflect a subconscious bias; it does not have to be deliberate, as most scientists may not even be aware of the consequences of subject sex for their conclusions. However, in many cases researchers have been deliberately avoiding female subjects, due to the perceived complicating issue of hormonal changes during the menstrual cycle (Beery, 2018). The underlying assumption is that if female subjects are in different stages of their oestrous cycle, data obtained for females will show greater variability and therefore confound the results. This assumption has been debunked, with a large empirical dataset compiled across several

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biomedical disciplines showing that traits measured in males and females are similarly variable (Beery, 2018).

The importance of avoiding sex bias in subject selection extends to other disciplines such as parasitology. A huge body of research in medical and veterinary parasitology has been conducted on experimental mammalian model species such as rats, mice and sheep. For mammals, it is particularly inappropriate to use male-only subjects in experiments as the 'default' representatives for a species, and then extrapolate the findings to the species as a whole. It would be equally inappropriate to use only female subjects. The reason is that there are profound differences between conspecific male and female mammals in key physiological and life history traits such as growth rates and lifespan, as well as many other phenotypic traits (Neigh and Mitzelfelt, 2016; Karp et al., 2017; Lemaître et al., 2020). More importantly, sex differences in immune responses have been well documented across all mammalian species studied to date (Fischer et al., 2015; Klein and Flanagan, 2016; Roved et al., 2017). These differences have long been known to make male hosts generally more susceptible to infection than female hosts (Poulin, 1996a; Zuk and McKean, 1996; Schalk and Forbes, 1997), even in some cases making males better hosts for parasite growth and development (Poulin, 1996b). The outcome of infection, whether for the host or the parasite, depends on the sex of the host. It should therefore be clear that whatever the area of research, studies in experimental parasitology aiming to uncover general effects applicable to the entire species must account for sex differences and include both male and female hosts in their design.

Here we conduct an analysis of male versus female subject use in parasitological studies from the past four decades, to answer questions such as: Are both male and female hosts equally represented in parasitological research? If there is a sex bias, is it worse within some research areas than others? Has this sex bias changed over time? We address these and other questions using a large dataset compiled from published studies involving experiments on mammalian model species infected by helminth parasites. We consider experimental studies only, i.e., studies in which researchers have some control over what animals they use. These include studies where animals are experimentally exposed to or infected with parasites, or where previously infected animals are assigned to various experimental treatments such as the administration of different drugs. Our results reveal prevalent biases and unjustifiable selection of host subjects in experimental parasitology, as well as lapses in experimental design and reporting of results. We end with some recommendations for future experimental research in parasitology, aimed at redressing any bias and achieving not only a more complete understanding of host-parasite interactions, but one that is nuanced by the sometimes subtle influence of host sex.

2. Materials and methods

2.1. Data compilation

We compiled a dataset by searching every issue of the following eight journals published between 1980 and 2021 for data on experiments involving helminth parasites (nematodes, trematodes, cestodes) and mammalian hosts: *International Journal for Parasitology*, *International Journal for Parasitology: Drugs and Drug Resistance*, *Journal of Helminthology*, *Journal of Parasitology*, *Parasitology*, *Parasitology International* (available from 1997–2021 only), *Parasitology Research*, and *Veterinary Parasitology*. Experiments using acanthocephalans in mammalian hosts were too few for these parasites to be included in our study. We considered only studies on the following six host species, which are among the most frequently studied by parasitologists: sheep, cattle, goats, horses, laboratory rats, and mice. Some of these actually comprise different breeds or subspecies; however, since our focus is on experimental design rather than on result interpretations, we do not separate them further. The studies retrieved during our search fall into five broad research areas: (i) immunology, which includes any study measuring some aspect of host immune responses; (ii) anthelmintic efficacy, including studies testing the efficacy of any compound against infection or the resistance of parasites against such compounds; (iii) factors affecting infection or transmission, including studies of environmental factors such as temperature, pasture type, host density, etc.; (iv) pathology, health impact and loss of productivity, comprising studies on the impact of parasites on any component of host fitness, health or productivity such as milk yield or wool growth; (v) parasite biology, including studies focusing on the parasite rather than the host, such as studies of parasite growth, reproductive output, etc.

Each entry in the dataset included, when available, the sample sizes for each host sex and the total host sample size used in an experiment, obtained by adding up the number of host individuals used across all control and treatment groups. We defined an experiment as a manipulative study (controlled infection, exposure to drugs or various environmental conditions, etc.) on a group of host animals pre-selected by the researchers. If a published study used more than one host species, or reported the outcomes of distinct experiments, we considered each separately, i.e. some studies comprised more than one experiment.

For each experiment, in addition to the total host sample size, we also recorded: (i) the host species used, considering only the six species identified above; (ii) the parasite higher taxon (nematodes, trematodes, or cestodes); (iii) which of the five broad research areas listed above the experiment fell into; (iv) whether the host individuals used were of one sex only or both, in which case we recorded sample sizes for each sex, or whether host sex was not specified; (v) whether at least some findings were reported separately for each host sex, in the case of experiments that considered both sexes; (vi) the year of publication; and (vii) the journal in which the study was published.

For analysis (see below), the six host species were split into two categories: those for which the researchers generally have total control over which animals are included in an experiment because the animals are obtained from commercial breeding facilities (rats and mice), and those for which the researchers usually have only partial control as they are constrained by what animals are available on the farms included in the study (cattle, sheep, goats and horses).

2.2. Data analysis

Our analyses addressed several questions, all answered using generalized linear mixed models (GLMMs), using the *lme4* package (Bates et al., 2015) in the R computing environment (R Core Team, 2022). For binary response variables, data were modelled with a binomial distribution, and for continuous response variables (i.e. relative sample size differences, see below) with a Gaussian distribution. We used the same four predictors (fixed factors) in all GLMMs: parasite taxon (three levels: nematodes, trematodes, or cestodes), host taxon (two levels: taxa for which researchers have total control and those for which they have partial control over subjects included), research subject area (five levels: see section 2.1), and year of publication. All GLMMs included the journal as a random factor, to account for any variation among journals in the type of studies they published.

The questions addressed were the following, each answered with a separate GLMM:

(i) Many studies did not specify the sex of the animals used. Was host sex more likely to be specified in certain host or parasite taxa, in certain research areas, or over time?
(ii) In experiments where sex of subjects used was indicated, many used only one host sex. Was this more likely in certain host or parasite taxa, in certain research areas, or over time?
(iii) In experiments where only one host sex was used, was it more or less likely to be males in certain host or parasite taxa, in certain research areas, or over time?
(iv) In experiments where both male and female animals were used (and their respective sample sizes were given), were their sample sizes equal, or more or less likely to be male-biased in certain host or parasite taxa, in certain research areas, or over time? Here, the response variable was the number of males used minus the number of females used, divided by total sample size. Positive values indicate a male bias, and negative ones indicate a female bias; the greater the absolute value, the greater the bias.
(v) In experiments where both male and female animals were used, results were sometimes presented separately for each sex. Was this more likely to be done in certain host or parasite taxa, in certain research areas, or over time?

3. Results

In total, our dataset included 3612 entries, each representing a separate experiment (see Supplementary Table S1). The most frequently used parasites were nematodes (62.4% of experiments), followed by trematodes (25.4%) and cestodes (12.2%) (Table 1). By far the most widely used host taxa were mice (40.1% of exper-

Table 1
Number of separate experiments and (in parentheses) mean total number of individual hosts used per experiment, for all combinations of host and parasite taxa covered by our dataset.

Host taxon	Nematodes	Trematodes	Cestodes	TOTAL
Rats	225 (78.9)	143 (57.9)	90 (77.4)	458 (72.2)
Mice	613 (96.0)	547 (79.3)	289 (90.3)	1449 (88.6)
Cattle	411 (99.9)	77 (53.4 [a])	18 (120.5)	506 (93.8 [a])
Sheep	732 (102.0)	124 (43.4)	29 (158.5)	885 (96.5)
Goats	160 (106.9)	26 (23.4)	7 (19.4)	193 (93.2)
Horses	113 (78.2)	2 (7.0)	6 (114.5)	121 (78.8)
TOTAL	2254 (97.5)	919 (66.4 [a])	439 (93.1)	3612 (89.4 [a])

[a] Excluding one extreme outlier.

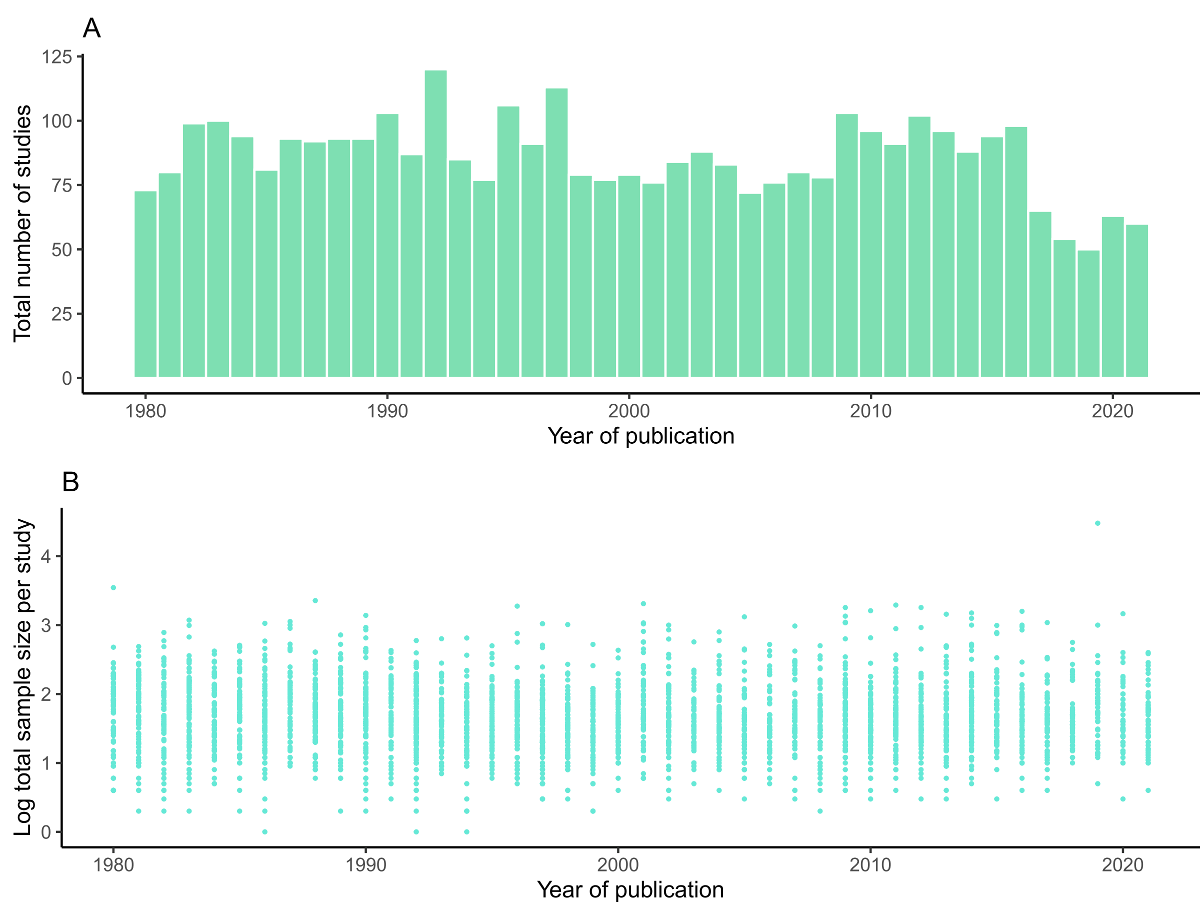

Fig. 1. Overview of the dataset on experimental studies in parasitology used here. (A) Numbers of experimental studies published each year between 1980 and 2021 in the journals covered. (B) Log_{10}-transformed total sample sizes, i.e. total number of host individuals used, per study as a function of year of publication, for the 3267 studies where sample size was stated.

iments) and sheep (24.5%) (Table 1). Based on our categorisation, the most common research area was immunology (32% of experiments), followed by anthelmintic efficacy (29.7%) and pathology (15.1%).

At a broad level, no strong temporal trend was apparent in the dataset. The annual number of experimental studies published remained approximately consistent over time, across all journals (Fig. 1). Total sample size, i.e., the total number of individual hosts used per experiment, was stated in 3267 (90.4%) out of all studies in our dataset, with older studies more likely not to report the sample size used. Among studies reporting it, the total host sample size per experiment showed no tendency to increase or decline across the four decades covered in our study (Fig. 1). Based only on those 3267 experiments reporting total sample size, an overall total of 322,147 animals were used in parasitological experiments published between 1980 and 2021 in the eight target journals, consisting of 28,377 rats, 107,769 mice, 76,132 cattle, 82,880 sheep, 17,609 goats and 9380 horses.

The results of GLMMs addressing our main questions are summarised in Table 2 and the main patterns are illustrated in Figs. 2–5. In our first analysis, we found that across all 3612 experiments, 1010 (28%) did not even specify the sex of the host animals used.

Table 2
Results of generalized linear mixed models testing the effects of four predictors, i.e., parasite taxon, host taxon (rats and mice for which researchers control what subjects are included versus taxa for which only partial control is possible), research area, and year of publication, on the five questions addressed. For categorical predictors, the reference level chosen is the one most distinct from the others in pairwise comparisons (parasite taxon = trematodes, research area = anthelmintic efficacy). The journal in which an experiment was reported (random factor) accounted for 15% of unexplained variance in the first analysis, but no more than 2% in the other analyses. Significant effects (NB: based on uncorrected *P*-values) are shown in bold.

Predictor	Estimate	Standard error	z-value	*P*
1- When was the sex of hosts used more likely to be specified? (n = 3612)				
Intercept	**0.823**	**0.200**	**4.108**	**<0.0001**
Parasite taxon: Nematode	**0.278**	**0.096**	**2.907**	**0.0037**
Parasite taxon: Cestode	**0.294**	**0.138**	**2.132**	**0.0330**
Host taxon: taxa for which only partial control of selected animals is possible	**−1.054**	**0.107**	**9.781**	**<0.0001**
Research area: Factors affecting tramsmission or infection	**0.517**	**0.135**	**3.832**	**0.0001**
Research area: Immunology	0.062	0.102	0.609	0.5425
Research area: Parasite biology	**−0.628**	**0.138**	**4.564**	**<0.0001**
Research area: Pathology and impacts on health or productivity	**0.432**	**0.129**	**3.357**	**0.0008**
Year of publication	**0.016**	**0.003**	**4.600**	**<0.0001**
2- When were both host sexes more likely to be used? (n = 2602)				
Intercept	**−1.424**	**0.187**	**7.593**	**<0.0001**
Parasite taxon: Nematode	**0.328**	**0.139**	**2.354**	**0.0186**
Parasite taxon: Cestode	**0.521**	**0.187**	**2.791**	**0.0053**
Host taxon: taxa for which only partial control of selected animals is possible	**1.043**	**0.139**	**7.504**	**<0.0001**
Research area: Factors affecting tramsmission or infection	−0.225	0.150	1.502	0.1332
Research area: Immunology	**−0.262**	**0.131**	**2.003**	**0.0452**
Research area: Parasite biology	−0.310	0.205	−1.510	0.1309
Research area: Pathology and impacts on health or productivity	**−0.534**	**0.158**	**3.374**	**0.0007**
Year of publication	**−0.022**	**0.004**	**4.828**	**<0.0001**
3- When was the only host sex used more likely to be male? (n = 2002)				
Intercept	−0.134	0.166	0.804	0.4214
Parasite taxon: Nematode	**0.532**	**0.114**	**4.660**	**<0.0001**
Parasite taxon: Cestode	0.169	0.153	1.103	0.2701
Host taxon: taxa for which only partial control of selected animals is possible	0.264	0.140	1.887	0.0592
Research area: Factors affecting tramsmission or infection	**−0.381**	**0.156**	**2.438**	**0.0148**
Research area: Immunology	−0.052	0.123	0.428	0.6688
Research area: Parasite biology	0.130	0.180	0.726	0.4680
Research area: Pathology and impacts on health or productivity	0.086	0.140	0.612	0.5404
Year of publication	**−0.011**	**0.004**	**−2.586**	**0.0097**
4- When were the host sample sizes used more strongly male-biased? (n = 301)				
Intercept	0.147	0.082	1.781	0.0759
Parasite taxon: Nematode	**−0.148**	**0.062**	**2.385**	**0.0177**
Parasite taxon: Cestode	−0.115	0.084	1.369	0.1722
Host taxon: taxa for which only partial control of selected animals is possible	−0.048	0.051	0.928	0.3544
Research area: Factors affecting tramsmission or infection	0.078	0.063	1.246	0.2139
Research area: Immunology	0.021	0.055	0.381	0.7034
Research area: Parasite biology	−0.007	0.082	0.086	0.9315
Research area: Pathology and impacts on health or productivity	−0.032	0.064	0.502	0.6163
Year of publication	0.001	0.002	0.131	0.8958
5- When were the results obtained more likely to be presented separately for each host sex? (n = 600)				
Intercept	**−1.734**	**0.369**	**4.693**	**<0.0001**
Parasite taxon: Nematode	**0.918**	**0.287**	**3.204**	**0.0014**
Parasite taxon: Cestode	0.402	0.364	1.105	0.2692
Host taxon: taxa for which only partial control of selected animals is possible	**−0.718**	**0.225**	**3.195**	**0.0014**
Research area: Factors affecting tramsmission or infection	**1.303**	**0.279**	**4.666**	**<0.0001**
Research area: Immunology	**1.157**	**0.259**	**4.460**	**<0.0001**
Research area: Parasite biology	**0.889**	**0.392**	**2.265**	**0.0235**
Research area: Pathology and impacts on health or productivity	**1.617**	**0.299**	**5.391**	**<0.0001**
Year of publication	−0.003	0.008	0.303	0.7620

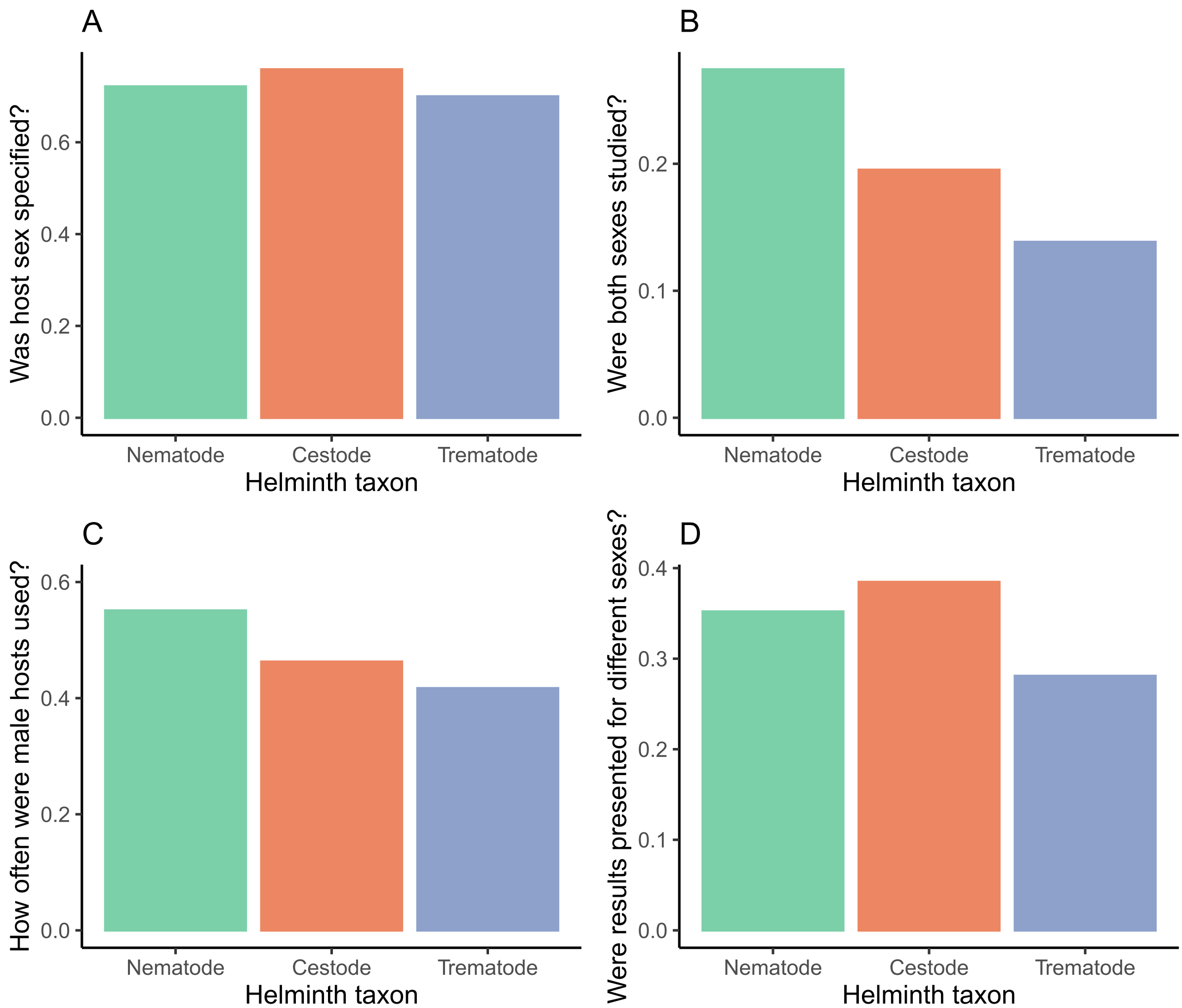

Fig. 2. Proportion of experimental studies in our dataset in which (A) the sex of host animals used was specified, (B) host sex was specified and both male and female hosts were used, (C) only one host sex was used and they were males, and (D) both host sexes were used and the results were presented separately for each sex. The proportions are shown separately for each of the three parasite taxa considered. See Table 2 for sample sizes and significant effects.

Host sex was slightly but significantly more likely to be specified when the parasites studied were nematodes and cestodes than when they were trematodes (Fig. 2A), and much more likely to be specified when researchers had total control over the animals selected, i.e. when the experiments used rats or mice (Fig. 3A). There were also differences among research areas, in particular with studies focused on parasite biology being less likely to indicate the sex of hosts used (Fig. 4A). Finally, the likelihood of host sex being specified increased significantly over time, i.e. recent studies were more likely to indicate host sex than older studies (Fig. 5A).

Second, in the 2602 experiments specifying the sex of the animals used, 2002 (77%) used only one host sex. Inclusion of both host sexes was more likely when the experiment involved nematodes or cestodes than trematodes (Fig. 2B), and much more likely when researchers had little control over animal selection, i.e. when subjects were farm animals such as cattle, sheep, goats or horses (Fig. 3B). Again, there were differences among research areas, with studies investigating anthelmintic efficacy being more likely to include hosts of both sexes than studies on other topics (Fig. 4B). We also observed a temporal decline in the probability that a study included hosts of both sexes, with studies from the past few years being more likely to focus on a single host sex (Fig. 5B).

Third, in the 2002 experiments using only one host sex, only males were used in 1005 (50.2%) cases and only females in 997 (49.8%) cases. Despite these almost perfectly even frequencies of single-sex studies between male and female hosts, there were dissimilarities among the different study categories. The use of only male hosts was more likely in studies of nematode parasites (Fig. 2C), it was not influenced by the host taxon used (Fig. 3C), and it was less likely in studies investigating factors affecting transmission and infection processes (Fig. 4C). More than half of the experiments published in the 1980s where only one host sex was used included male hosts only; however that proportion has declined significantly since then (Fig. 5C).

Fourth, in the 301 experiments where both male and female animals were used and where sample sizes were given for both sexes separately, we found equal sample sizes in 75 (24.9%) cases, male-biased sample sizes in 121 (40.2%) cases, and female-biased sample sizes in 105 (34.9%) cases. There was very little evidence of a bias in sample sizes based on host sex, and the magnitude of any bias in sample sizes was almost the same across all types of experiments (see Table 2). Indeed, apart from male-biased sample sizes being a little less pronounced in studies of nematode parasites, the extent of male-biased sample sizes was not affected by the host taxa used, the research area, or the year of publication.

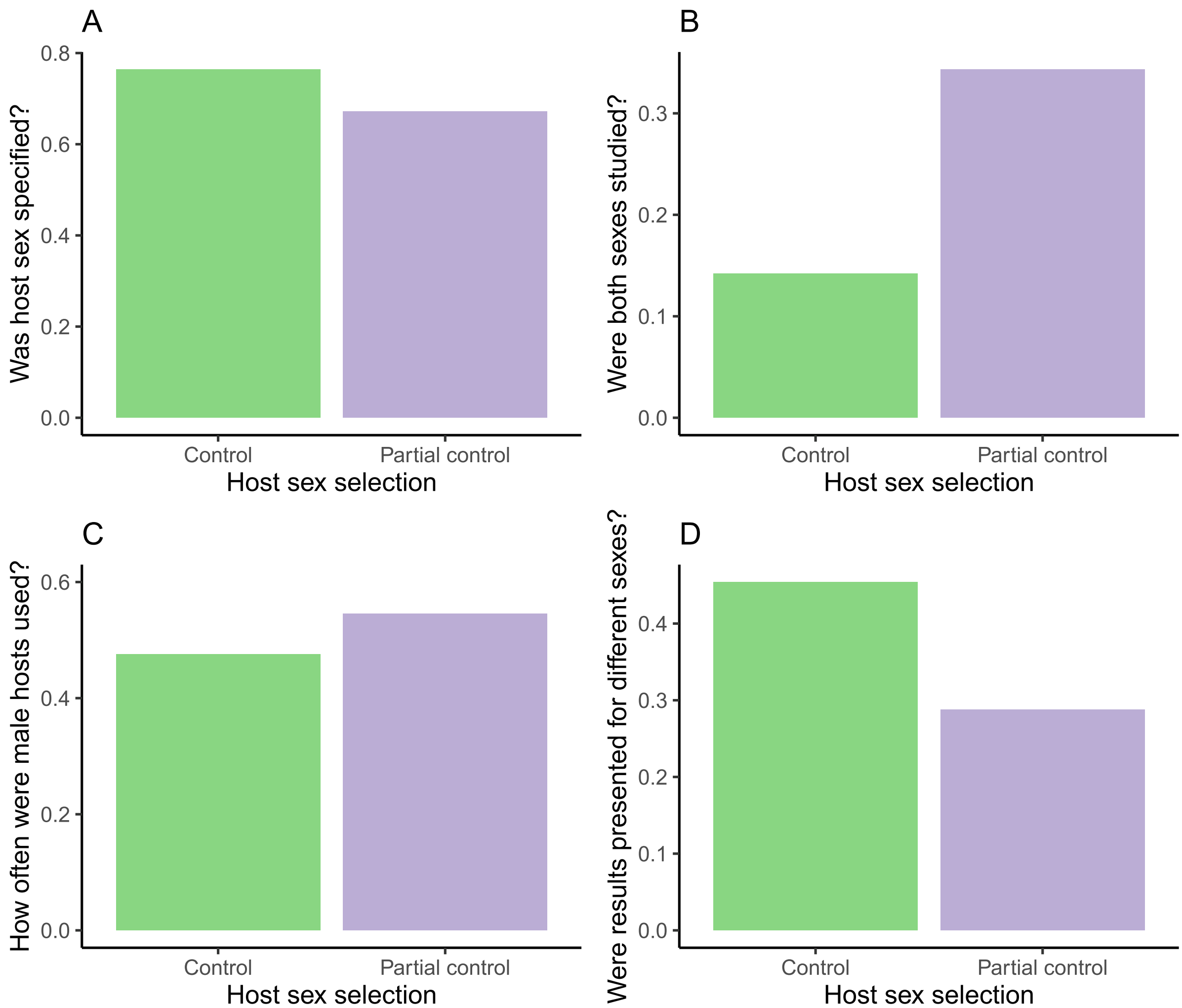

Fig. 3. Proportion of experimental studies in our dataset in which (A) the sex of host animals used was specified, (B) host sex was specified and both male and female hosts were used, (C) only one host sex was used and they were males, and (D) both host sexes were used and the results were presented separately for each sex. The proportions are shown separately for host taxa where researchers have total control over which animals are included in an experiment (rats and mice), and those for which researchers usually have only partial control (cattle, sheep, goats and horses). See Table 2 for sample sizes and significant effects.

Finally, in the 600 experiments where both male and female animals were used (whether or not sample sizes were given for each sex separately), results were presented separately for each sex in 207 (34.5%) cases. Results were less likely to be given separately for each sex when the experiment involved trematodes (Fig. 2D), and when researchers had little control over animal selection, i.e. when subjects were farm animals (Fig. 3D). Notably, results were much less likely to be separated by host sex in experiments testing for anthelmintic efficacy (Fig. 4D). Finally, although the likelihood of results being presented separately for each host sex declined slightly over time, this trend was not significant (Fig. 5D and Table 2).

4. Discussion

The use of experimental mammalian model species in parasitology has been, and continues to be, an extremely powerful approach to elucidate various aspects of host-parasite interactions as well as the efficacy of different control methods (see Holland, 2021; Simwela and Waters, 2022; Sitali et al., 2022). The value of animal models and the experiments using them rests entirely on how representative these models are and whether the findings they yield can be generalised. This in turns requires an unbiased selection of subjects that captures the full variability of the model population or species. Here, we identify previously unrecognised links between the host and parasite taxa used in experiments, the research subject area, and the year of publication, and whether the sex of hosts used is specified, whether both sexes are used, and if not which one is used, and whether findings are reported separately for each sex. Our analysis of over 3600 parasitological experiments reveals the existence of biases in the make-up of host cohorts and result reporting in experimental parasitology, and hints at some of the likely root causes.

The experimental approach has remained popular across the four decades covered by our analysis. Indeed, the number of experimental studies published per year has remained approximately consistent over time. This may be due to the larger number of articles published annually in most journals; in relative terms, the experimental approach may have lost some of its appeal in the face of competing approaches, such as the rise in the use of molecular and genomic tools in the last 25 years (Selbach et al., 2019). Surprisingly, despite the tightening of ethical constraints regarding the use of live animals for experimentation, the total host sample size per experiment also showed no tendency to decrease across

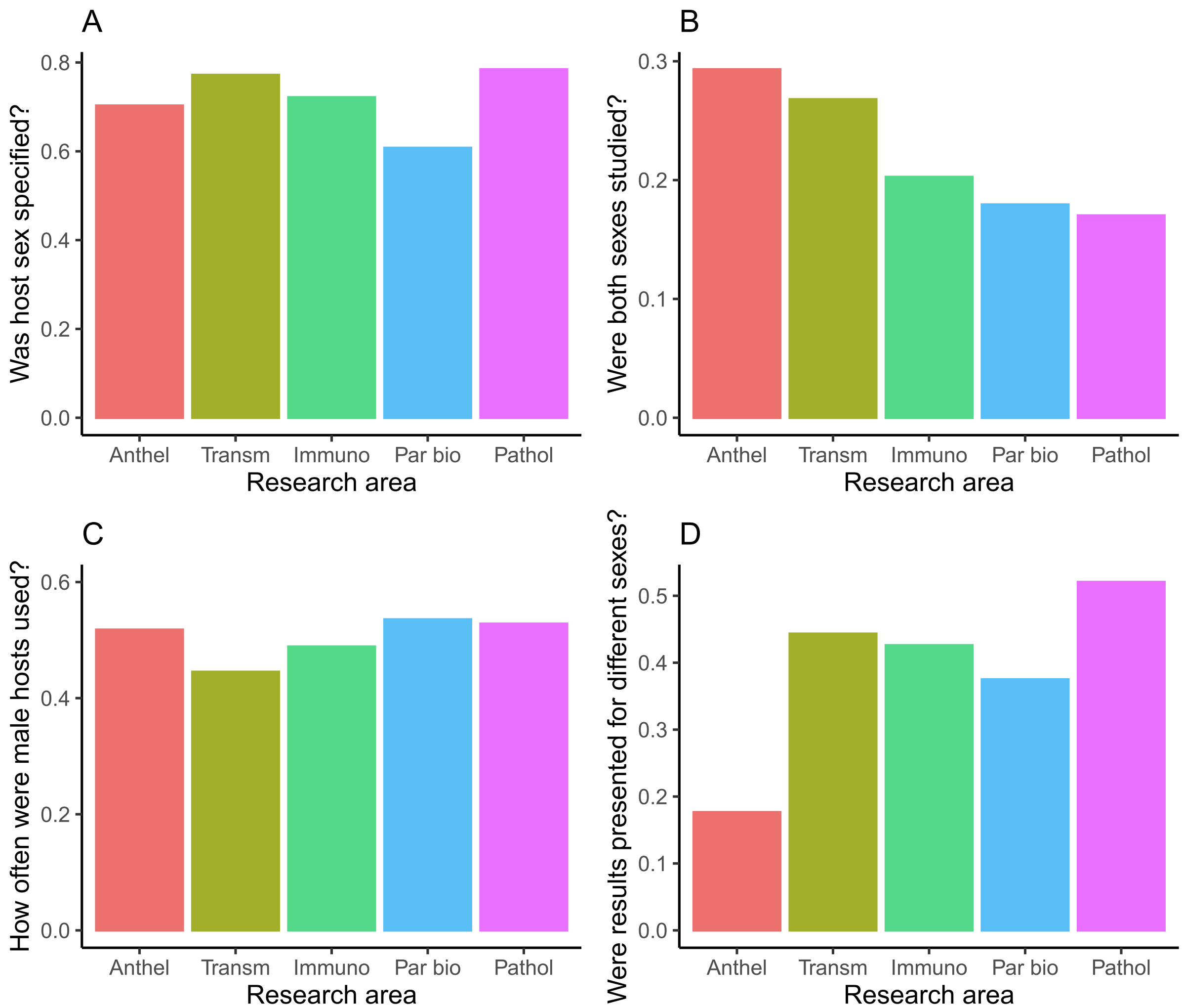

Fig. 4. Proportion of experimental studies in our dataset in which (A) the sex of host animals used was specified, (B) host sex was specified and both male and female hosts were used, (C) only one host sex was used and they were males, and (D) both host sexes were used and the results were presented separately for each sex. The proportions are shown separately for each of the five research areas considered: anthelmintic efficacy (Anthel), factors affecting infection or transmission (Transm), immunology (Immuno), parasite biology, i.e. studies focusing on the parasite rather than the host (Par bio), and pathology studies on the impact of parasites on host health or productivity (Pathol). See Table 2 for sample sizes and significant effects.

the four decades, indicating that the statistical power of recent experimental studies has not been compromised.

The research question addressed by an experiment appears to have influenced whether individual hosts of one or both sexes were included and how the results were reported. Notably, studies focused on parasite biology were less likely to indicate the sex of host animals used. This may be because the focus of such studies is on the parasite and not the host; however, the latter represents the immediate environment of the parasite, and host sex is known to affect parasite infectivity, growth and survival (Poulin, 1996b; Zuk and McKean, 1996). Studies on anthelmintic efficacy were more likely to include hosts of both sexes than studies on other topics, perhaps because researchers in this area are often forced to use any available animals on the farm. However, results of experiments investigating anthelmintic efficacy were much less likely to be presented separately for each host sex than those of experiments in other areas. Lumping together results for males and females can mask important interactions with host sex, and limit the usefulness of research findings for the development of parasite control strategies.

Similarly, there were differences among experiments regarding the inclusion of one or both sexes and how the results were reported based on the host and parasite taxa used in experiments. Among the main patterns observed, inclusion of both host sexes was more likely when the experiment involved nematodes than other parasites. This may be a consequence of the fact that nematodes are the most frequently studied parasites in experiments on anthelmintic efficacy, which are more likely to include hosts of both sexes than studies on other topics (see above). However, when only one host sex was included in an experiment, use of males only was more likely in studies on nematode parasites. The reasons for this are unclear. With respect to host taxa, not surprisingly host sex was much more likely to be specified in studies where researchers had total control over the animals selected (i.e. for rats or mice), since the sex of experimental subjects was deliberately selected prior to the experiment. Furthermore, on the one hand, when both host sexes were used, results were also more likely to be presented separately for each sex in experiments using rats or mice. On the other hand, experiments using rats or mice were much more likely to use only one host sex. Presumably, this

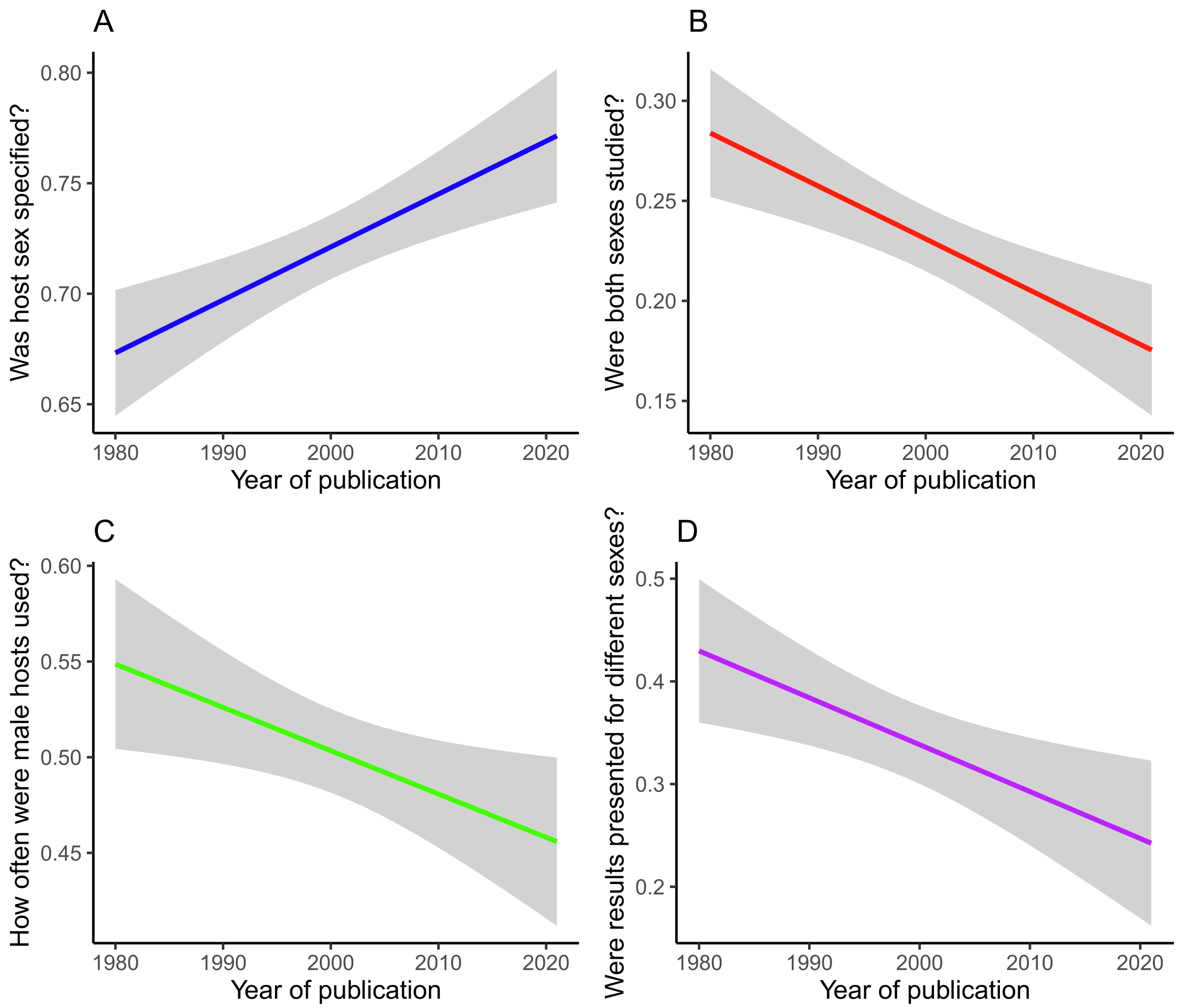

Fig. 5. Model-predicted relationships (and 95% confidence intervals) between the proportion of experimental studies in which (A) the sex of host animals used was specified, (B) host sex was specified and both male and female hosts were used, (C) only one host sex was used and they were males, and (D) both host sexes were used and the results were presented separately for each sex, as a function of the year in which they were published. See Table 2 for sample sizes and significant effects.

reflects attempts by researchers to limit inter-individual variability among subjects when they can precisely choose those subjects prior to an experiment, something only possible when they can be ordered from commercial suppliers.

Finally, our analysis revealed some temporal trends regarding whether individual hosts of one or both sexes were included and how the results were reported. Notably, recent studies were more likely to specify the sex of hosts used than older studies. At the same time, the proportion of studies including both males and females has decreased slightly over time in favour of single-sex studies. This goes against a trend observed in a large survey of experiments published in biological journals, where the proportion of experiments including both male and female animals has increased in the past decade (Willingham, 2022). In our dataset, however, the weak sex bias in those single-sex studies has reverted over time: whereas more than half of single-sex studies involved male hosts 40 years ago, this has shifted to a slight bias in favour of female hosts in recent years, for reasons that are unclear.

Factors other than those considered here may also account for biases in the selection of hosts for experimental studies. For instance, the identity or gender of the researcher may also affect the choice of host subjects. However, it is impossible to test this with our data. Nearly all studies have multiple authors; not only is it not always possible to assign a gender to particular authors, but also it is impossible to determine which author played the key role in choosing the subjects. Despite a possible role for other influential factors, our analysis has clearly demonstrated that simple ones such as the subject area or the host species used can affect whether both host sexes are used and whether results are reported for each sex separately.

Overall, much is left to be desired in the design and reporting of experimental studies in parasitology. Based on over 3600 experiments published in the past four decades, 28% did not report the sex of the host animals they used, and 10% did not report sample sizes. Among those that specify the sex of hosts used, three-quarters used a single host sex, although only a tiny fraction of those investigated sex-specific factors such as pregnancy. Therefore, host sex-biased results are the norm in experimental parasitology and they lack justification. In experiments where both males and females are used, only one-third report the results separately for each sex. Although the slight bias has reverted from male-only studies predominating 40 years ago to female-only studies more recently, on the positive side overall there have been approximately equal numbers of single-sex studies using males and females. And when both sexes were used, the sample sizes (number of individuals used) were about the same for both sexes.

Our results lead to a few simple recommendations for future studies. First, unless a study focuses on sex-specific processes (e.g., the influence of pregnancy), it should include both male and female host subjects if researchers have control over host sex. Otherwise, extrapolating from male-only or female-only data to obtain species-level mean values or trends is almost certain to lead to erroneous conclusions. Essentially, using single-sex experimental samples represents a failure to properly account for half of a species. We acknowledge the logistical difficulties involved in using both sexes (e.g., female and male mice may need to be housed separately), however this should remain the preferred experimental design. Second, equal numbers (or nearly equal numbers) of males and females should be used, again to avoid the overall findings being disproportionately influenced by one particular host sex. Third, all experimental studies should report sex-disaggregated descriptive data and results of statistical analyses, even if only in supplementary material, to enable both species-wide and sex-specific conclusions to be drawn, and to allow use in a future meta-analysis. Much can be learned from sex-disaggregated data (i.e., data analysed and reported separately for males and females), because sex-based differences in hormone profiles or gene expression can influence host responses to infection, parasite development, or the efficacy of anthelmintic treatment (Willingham, 2022). The above recommendations agree with the broader Sex and Gender Equity in Research (SAGER) guidelines (Heidari et al., 2016) endorsed by the *International Journal for Parasitology* in its instructions to authors.

Experimental approaches have a long history in parasitology (Holland, 2021; Simwela and Waters, 2022; Sitali et al., 2022), but they are not necessarily free of limitations. For example, the often necessary use of immunosuppressed or immunodeficient individuals in many experimental studies (McKerrow and Ritter, 1993) already raises questions about the applicability of their findings to natural host populations. Nevertheless, live animal experimentation cannot easily be replaced by more ethical alternatives for a full understanding of host-parasite interactions (Eckert, 1997). Therefore, it is imperative to maximise the usefulness and reliability of findings obtained from the sacrifice of experimental animals. We hope that the empirical assessment of past practices presented here and the recommendations we make will help ensure that experimental studies deliver the most rigorous results possible and remain a cornerstone of parasitological research.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2023.03.002.

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