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REVIEW AND SYNTHESIS

Sexual dimorphism in immunity across animals: a meta-analysis

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Abstract

In animals, sex differences in immunity are proposed to shape variation in infection prevalence and intensity among individuals in a population, with females typically expected to exhibit superior immunity due to life-history trade-offs. We performed a systematic meta-analysis to investigate the magnitude and direction of sex differences in immunity and to identify factors that shape sex-biased immunocompetence. In addition to considering taxonomic and methodological effects as moderators, we assessed age-related effects, which are predicted to occur if sex differences in immunity are due to sex-specific resource allocation trade-offs with reproduction. In a meta-analysis of 584 effects from 124 studies, we found that females exhibit a significantly stronger immune response than do males, but the effect size is relatively small, and became non-significant after controlling for phylogeny. Female-biased immunity was more pronounced in adult than immature animals. More recently published studies did not report significantly smaller effect sizes. Among taxonomic and methodological subsets of the data, some of the largest effect sizes were in insects, further supporting previous suggestions that testosterone is not the only potential driver of sex differences in immunity. Our findings challenge the notion of pervasive biases towards female-biased immunity and the role of testosterone in driving these differences.

Keywords

Immune response, immunity, life-history, sexual dimorphism.

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INTRODUCTION

Infectious diseases are widespread across the animal kingdom, but patterns of infection vary among populations and among individuals in a population. Heterogeneity in infection patterns is driven by many factors that are broadly classified into exposure or susceptibility, where the former represents differential contact with parasites and pathogens through behavioural or ecological differences, and the latter typically represents post-exposure susceptibility differences driven by factors such as age, hormones or stress (Nunn & Altizer 2006). Often, it is difficult to know if exposure or susceptibility are driving a correlation with some phenotypic characteristic of the host. For example older individuals may be more likely to be infected because of declining immune function (e.g. Adamo *et al*. 2001; Zerofsky *et al*. 2005), or because they have been exposed to more infectious agents (Nunn *et al*. 2014).

Sex differences in life histories are also thought to shape sex-differences in infection prevalence and intensity (Klein & Flanagan 2016), with males typically considered to be 'the sicker sex' (Zuk 2009), at least in mammals and birds (Schalk & Forbes 1997; Moore & Wilson 2002; Robinson & Klein 2012). Although sex differences in exposure may occur, more often male-biased parasitism is attributed to superior female immunity (Zuk 1990, 2009; Zuk & McKean 1996; Rolff 2002). At the proximate level, sex differences in immunocompetence – defined here in very general terms as overall resistance to infection based on immune defenses – could arise from the negative effects of male sex hormones on the immune system, positive effects of female sex hormones, or both (Folstad & Karter 1992; but see: Roberts *et al*. 2004; Nunn *et al*. 2009; Foo *et al*. 2017). Ultimately, sex differences in immunocompetence could reflect variation in resource-based trade-offs between immunity and other fitness-related traits (Zuk 1990, 2009; Sheldon 1996; Zuk & Stoehr 2002; Nunn *et al*. 2009; Jacobs & Zuk 2011). Specifically, some authors have argued that males should invest fewer resources into immunity than females: the benefits of increased mating success (via increased allocation to sexually selected traits) in males are expected to outweigh the costs of reduced lifespan due to disease, whereas the cost of reduced lifespan is argued to be greater for females (Zuk 1990; Rolff 2002; Zuk & Stoehr 2002; but see Stoehr & Kokko 2006). Superior female immunocompetence could also arise from ecological feedback dynamics that are in addition to, or independent of, sexual

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selection (Restif & Amos 2010; Bacelar *et al*. 2011). Measured immune levels may also be higher in females of internal fertilisers following a direct immunological response to male ejaculates (Morrow & Innocenti 2012).

Despite widespread acceptance of these arguments, the empirical evidence for female-biased immunocompetence is mixed. Although several studies across a wide array of animal taxa report superior female immunocompetence (Nunn *et al*. 2009; Schmid-Hempel 2011; Robinson & Klein 2012), examples of male-biased immunocompetence, or no difference between the sexes, continue to accumulate (e.g. Zuk *et al*. 2004; Stoehr 2007; Kelly & Jennions 2009; Kelly 2016). Even when sex differences in immunocompetence do arise via different resource allocation strategies, such strategies themselves may be plastic in their magnitude and direction, depending on, for example the availability of fitness-limiting resources (e.g. Zuk *et al*. 2004; McKean & Nunney 2005).

Theoretical studies have also provided some arguments against the generality of these earlier expectations for ubiquitous female-biased immunity. For example, if infection substantially reduces male mating success and, at the same time resources invested into immunocompetence become unavailable for offspring production in females, no sex differences or even a male-biased investment into immunity in the face of strong sexual selection can be expected (Stoehr & Kokko 2006). The evolution of parasite adaptations that are specific to males or females could also shape differences in infection between the sexes, regardless of whether or not they inherently differ in immunocompetence (Duneau & Ebert 2012; Duneau *et al*. 2012; Ubeda & Jansen 2016).

Furthermore, when the sexes differ in how they utilise resources important for immune defence, any sex differences in immunocompetence that are found may depend on the immune system component that is investigated (Lee 2006). In arthropods, for example, sexual dichromatism in melanin-based coloration or in other uses for melanin (e.g. egg tanning; Li *et al*. 1993; Fuchs *et al*. 2014) could result in sex differences in the melanin-based components of immunity (Stoehr 2010). Lee (2006) argued that in vertebrates, females should have stronger adaptive and non-inflammatory immune responses, whereas males should have stronger innate and inflammatory immune responses. This effect is proposed to arise because adaptive and low-inflammatory responses are thought to be less costly, and therefore will have less impact on the high costs of reproduction in females (Lee 2006). Likewise, females are expected to invest more in inducible and less in constitutive defences than males (Lee 2006).

Sex differences in immunity may also depend upon the taxon being considered, in part because of the very different immunological mechanisms that different species use. Such differences can therefore occur at the deepest taxonomic divisions due to variation in fundamental immunological mechanisms (e.g. vertebrates vs. arthropods), yet differences can also occur in more closely related species or among populations due to life-history differences (e.g. in relation to patterns of parental care). If sex differences in immunocompetence are largely driven by trade-offs with reproduction, life-stage should also affect patterns of immunity, with larger sex differences in adults than in immature animals (e.g. Adamo *et al*. 2001). Finally, methodological differences among studies may affect sex differences in immunity or the ability to detect these differences, particularly if the differences affect resource abundance. For example, laboratory-based experimental studies may manipulate or control resources in ways that field-based correlational studies cannot or do not.

Given the conflicting empirical evidence – together with the identification of theoretical cases in which males are expected to exhibit stronger immune defences – a great need exists to assess the evidence for sex differences in immunity, and to identify factors that explain variation among studies. This is particularly true as we continue to expand immunology beyond the more well-studied laboratory models, such as the mouse; i.e. into the growing field of ecological immunology (Pedersen & Babayan 2011; Brock *et al*. 2014). Here, we perform a systematic meta-analysis to determine the magnitude and direction of sex differences in immunity across many animal species, including vertebrates and invertebrates. We also investigate the role of moderating factors in explaining the patterns of dimorphism reported in the literature. We achieve this by examining whether sex differences in immunity are related to (1) the type of assay used to assess immune response, closely corresponding to the component of immunity being considered (e.g. innate or adaptive; constitutive or inducible); (2) the animal taxon studied; (3) whether the study is experimental or correlational; and (4) whether the research was conducted in the laboratory or field. We also consider (5) life stage (adults vs. juveniles), predicting that effects will be stronger in adults than immature animals if sex differences are driven by sexual selection and associated sex-specific investment in mating effort or reproduction. Thus, in addition to expanding the taxonomic breadth (and sample size) relative to a previous meta-analysis of sex differences in immunity (Nunn *et al*. 2009), we also expand the treatment of potentially important factors expected to influence variation in effect sizes.

METHODS

Search protocol

The main goal of our search protocol was to locate as many studies as possible on sex differences in immunity, while minimising sampling biases. We searched SCOPUS to obtain an initial pool of articles (see Figure S1). SCOPUS indexes 1.4 billion research articles from 22 000 journals dating back to 1970, and includes major journals in fields such as evolution, ecology, behaviour, pest management and medicine. We searched SCOPUS up to May 2016 for articles [i.e. DOCTYPE(ar)] within the 'agriculture' subject area [i.e. SUBJAREA(AGRI)] using the following search string entered into the 'topic' window (i.e. in title, abstract or keywords): ((immunity OR immunocompetence OR immune) AND (sex* OR gender) AND (dimorph* OR differ*)) AND DOCTYPE(ar) AND SUBJAREA(AGRI) AND (LIMIT-TO(SUBJAREA, 'AGRI')). We then inspected the abstracts to exclude papers that were irrelevant or highly unlikely to contain suitable studies for the meta-analysis. Papers that potentially contained relevant data, or which had to be read more closely to

assess their suitability, were then examined to see if they met our inclusion criteria (see below).

Criteria for study inclusion or exclusion of studies

To be included, we required that the study investigate a difference between the sexes in at least one standard phenotypic measure of immunity (see Table S1), and that data on the sex difference were extractable as an effect size. In addition, our goal was to include studies in systems that would most likely uncover effects (only) due to evolved life-history differences; those sex differences of most interest to the field of 'ecological immunology' (Schmid-Hempel 2011). Therefore, we excluded studies performed on knockout/transgenic or domesticated animals (including highly inbred models such as 'the laboratory mouse'), studies on humans and studies conducted on populations exposed to nuclear contamination (e.g. Chernobyl). For humans, it would be hard to find studies in which one could confidently argue that various medical (or social) interventions were not at play. For laboratory animals, particularly rodents, genetic lines are bred in the laboratory for many generations for the express purpose of reducing genetic variation, such as the 'laboratory mouse' (Viney *et al*. 2015). We therefore acknowledge that many studies in the biomedical literature were excluded. While these laboratory-based studies using 'non-wild type' organisms are critical for elucidating mechanisms responsible for sex differences in immunity, the reasons described above also limit their relevance for understanding broad taxonomic and evolutionary trends, particularly if they are combined with more 'natural' studies and study organisms. Furthermore, we know that laboratory and wild mice may differ immunologically in important ways (Abolins *et al*. 2017).

Despite being popular proxies for immune function, we also excluded studies scoring naturally occurring levels of infection with parasites and pathogens because infection level is confounded by variation in exposure to, resistance of and tolerance to parasites and pathogens. However, we did include studies that assayed immunity using metrics such as survival following controlled inoculation (e.g. Adamo *et al*. 2001; Gershman 2008), as these were assumed to control for exposure and to reliably assess the effectiveness of the immune response. Studies measuring the size of organs associated with immune response, such as the spleen, were excluded because they are indirect measures of immune investment.

We accepted a large number of standard measures of immunity (Table S1). In particular, many studies examined white blood cells (WBC) or their subtypes (e.g. basophils, lymphocytes or heterophils). If the study reported counts of subtypes of WBCs as well as total WBC, we used WBC counts only. However, if total WBC was not given then we accepted, in decreasing priority, heterophil:lymphocyte ratio, proportion of lymphocytes, heterophils and basophils.

Even when a study satisfied the 'design' criteria, in several cases we were still unable to include it because the results were presented ambiguously and prevented us from confidently extracting an effect size. This is a common problem in most meta-analyses and we erred on the side of caution (Curtis *et al*. 2013), which is why some studies that might seem to be appropriate were excluded. For example we excluded studies (or sets of data within studies) in which we could not be absolutely certain about the direction of the effect size or the sample size, or when only interaction effects were provided. We were unable to extract reliable effect sizes from models containing covariates because this required knowing the mean for each factor-level and the slope between each factor-level and the covariate (Lajeunesse 2013); these data were not reported in the population of papers that we extracted during our literature search.

Data extraction

We retrieved data directly from the text or tables or indirectly by extracting data from figures using GraphClick (Arizona Software). We used the R package *compute.es* (Del Re 2015) to convert the collected study statistics into the standard effect size measure, Hedges' g [often referred to as Hedges' d in the literature (Nakagawa & Cuthill 2007; Borenstein *et al*. 2009; Rosenberg *et al*. 2013)]. In addition to estimating the standardised difference between means of two groups, Hedge's g also controls for an upward bias caused by small sample size, unlike other metrics such as Cohen's d (Nakagawa & Cuthill 2007; Del Re 2015). Throughout, differences refer to the female value relative to the male value, with positive g indicating a higher immune response in females. Variance for Hedge's g was computed following Del Re (2015).

Because different immune components can be more effective for different types of infectious organisms – and because this may vary across host species, individual ages and setting – we also recorded a number of additional variables. Specifically, for each extracted effect size, we also recorded: (1) the type of assay used to measure the immune response (Table S1), (2) the higher-level taxonomic group (mammal, fish, lizard, bird, spider, insect, decapods and molluscs), (3) method of inference (experimental or correlational), (4) source of the individuals tested (laboratory, field or semi-natural populations, with the last category including subjects reared in conditions such as aviaries, fenced-off areas outdoors or mesocosms, where they generally experienced natural conditions with some human intervention) and (5) age category (adult, juvenile or both if adults and juveniles were pooled). These variables were used as moderators in our meta-regression analyses (see below).

Categories of immune defence

The vertebrate immune system is comprised of two systems of immunity – innate and adaptive – and different components of these systems are required for controlling different types of parasites and pathogens. The immune system has also been categorised by Schmid-Hempel & Ebert (2003) as non-specific or specific, which corresponds to innate and adaptive respectively (Habig & Archie 2015). The innate response is the body's first line of defence and is broadly effective against many pathogens. In contrast to the innate immune system, the adaptive immune response is more specific to particular infectious agents. It is this specificity that makes the adaptive immune system capable of immunological memory (Coico & Sunshine 2015): upon subsequent exposure to the same or a

similar pathogen, the adaptive immune system mounts a rapid and heightened response.

In addition to being classified as innate or adaptive, immune defences can be categorised as constitutive or inducible. Constitutive immune defences comprise cellular (e.g. heterophils) and humoral (e.g. natural antibodies) components that are present in the absence of an immune challenge. In contrast, inducible defences (e.g. the antibody response to an antigen challenge) are called to action upon exposure to a pathogen (Tollrian & Harvell 1999). We first used two separate models to assess whether constitutive and induced responses differed within innate and adaptive categories and then we assessed whether innate and adaptive categories exhibited a global sex difference in immune response. We classified immune components (Table S1) following Habig & Archie (2015), Lee (2006) and Schmid-Hempel (2005), but acknowledge that categorisation of constitutive and induced may vary with the biology of the organism. For example some immune responses switch from inducible to constitutive under conditions such as nutritional stress (Becker *et al*. 2010; Price *et al*. 2015; Adamo *et al*. 2016).

Statistical analyses

All statistical analyses were performed in the *R* environment (R Development Core Team 2017). All meta-analyses were run on effect sizes weighted by their variance using linear mixed models, fitted using restricted maximum likelihood (REML) in the *R* package *metafor* (Viechtbauer 2010). Although we modelled multiple effect sizes from the same study and species by including 'study' and 'species' as random factors using the function 'rma.mv' in *metafor*, there remains the possibility of non-independence among effect sizes since different immune measures are likely to be correlated when measured from the same group of individuals (Noble *et al*. 2017). Because correlations among related effect sizes are nearly never reported, the multilevel models that we report in the main text assume correlations of zero. We also report as supplementary material on multilevel models that conservatively assume all correlations to be 0.5 (Tables S2 and S3, Figure S2). Both sets of analyses give qualitatively identical results. We imputed the covariance matrices for our meta-analyses using the *R* package *clubSandwich* (Pustejovsky 2018). All of our models included a residual error term.

We estimated the between-studies variance in our population of studies by calculating tau (the estimated standard deviation of underlying true effects across studies) and used Cochran's Q statistic to test whether the heterogeneity among effect sizes was greater than that expected by sampling error alone (Borenstein *et al*. 2009). We also calculated the ratio of true heterogeneity to the total variation observed across effect estimates (I^2), which serves as an index of signal-to-noise (Borenstein *et al*. 2009). Following Nakagawa & Santos (2012), we partitioned the proportion of unknown variation that is not attributable to sampling variance (i.e. I^2; sampling variance is equal to 100% minus I^2) into the contribution from each random factor. We therefore calculated the variance in effect sizes due to phylogenetic relatedness ($I^2_{\text{phylogeny}}$), differences among studies (I^2_{study}), differences among species (I^2_{species}) and differences in within-study variation (i.e. residual variation in traditional mixed models; $I^2_{\text{effect size}}$). The four percent-values of variation sum to give I^2_{total}. Higgin *et al*. (2003) proposed that I^2 values of 25, 50 and 75% indicate low, moderate and high levels, respectively, of inconsistency among studies.

We conducted phylogenetically controlled meta-analyses by first constructing a phylogeny of our 105 species using the Interactive Tree of Life online tree generator (iTOL) (http://itol.embl.de/). The iTOL uses data from the National Center for Biotechnology Information taxonomy database to create phylogenies. We relied on published phylogenies (Jansa & Weksler 2004; Pons *et al*. 2005; Wang *et al*. 2012; Guo *et al*. 2013; Regier *et al*. 2013; Misof *et al*. 2014; Torres-Pachón *et al*. 2017) to resolve polytomies in our tree. We then entered the phylogeny as an unstructured variance-covariance matrix in our models, assuming that phylogenetic correlations were the product of a Brownian motion model of evolution (Lajeunesse 2009; Lajeunesse *et al*. 2013). We calculated phylogenetic heritability H^2 as an index of phylogenetic signal (Nakagawa & Santos 2012). Nakagawa & Santos (2012) define H^2 as the proportion of phylogenetic variance relative to the sum of all other variance components not including sample error variance. Together with $I^2_{\text{phylogeny}}$, H^2 indicates the magnitude of phylogenetic signal in the data (Nakagawa & Santos 2012; : see also Booksmythe *et al*. 2017).

Meta-regression models were used to test whether variation among effect sizes was associated with any of the five moderator variables listed above (i.e. moderator variables are equivalent to covariates in traditional statistical analyses). We ran separate single-factor meta-regressions for each moderator to calculate whether each moderator level differed from zero, thus enabling us to discern how effects differed by immunoassays, taxonomic group and types of immune response. We also used log likelihood ratio tests to determine (and retain) the best meta-regression model by assessing the fit and plausibility of a full model to one with non-significant factors deleted. Following Foo *et al*. (2017), we tested the significance of the moderators that were retained in the final model using the Q test, which is an omnibus test for each moderator and more conservative than z-values. We interpreted the levels of the moderators retained in the final model using the parameter estimates obtained from the single-factor models. Means for each moderator level are presented with ± 95% confidence intervals (unless stated otherwise) and p-values that are based on z-values in *rma.mv* model output. The null hypothesis for each analysis was that the mean effect size equalled zero.

In addition to including the immunoassay type as one of the moderators, we also used separate meta-regression models to address whether sex bias in immunity may be related to two types of general response (i.e. constitutive vs. induced) within the two major categories of defence (i.e. innate vs. adaptive) that the assay measured (Table S1). Most immune parameters were included in multiple meta-analyses of the major immune categories. For instance, baseline immunoglobulin levels measure adaptive and constitutive immunity and so were included in tests of these immune categories. Heterophil:lymphocyte (n = 21), neutrophil:lymphocyte (n = 1) and monocyte:lymphocyte (n = 1) ratios reflect differences in

innate relative to adaptive cells and were thus excluded from these analyses. Studies that assessed survival after controlled injection of live bacteria ($n = 8$) were also excluded from this set of analyses because it was not clear which component was being stimulated.

We did not conduct a formal analysis of publication bias for two reasons. First, analyses based on funnel plot symmetry (Duval & Tweedie 2000) assume no relationship between the effect size under study and the sample size used to study it, which is violated when, for example researchers collect larger sample sizes to investigate effect sizes that are more difficult to detect (Simonsohn 2017). Second, given the large number of effects that we had access to, imputation of any missing effects would have little effect on our analyses. We do, however, investigate how effect sizes vary with year to test whether studies with weaker effects are increasingly more likely to be published over time (Simmons *et al*. 1999; Jennions & Møller 2002). This was accomplished by entering year of publication together with our other five moderators into the full model to determine whether year was retained in the final model (see above).

RESULTS

Our literature search yielded 1610 studies, of which 124 met the criteria for our meta-analysis and provided usable data (Figure S1). From these 124 studies on 105 species we extracted 597 effects, which was reduced to 584 effects from 104 species (Table S4) after one study (Martin *et al*. 2008) with very large and positive (i.e. greater female immunity) effects was removed from our analysis (a model run with this study was a substantially poorer fit than one without it $\Delta AIC = 1976.98$).

Inclusion of study identity (likelihood ratio test:$X^2_1 = 4547.58$, $P < 0.0001$) and species identity (likelihood ratio test: $X^2_1 = 4542.62$, $P < 0.0001$) significantly improved the model when entered individually. Simultaneous inclusion of both factors (i.e. study and species) improved the model over including just species identity (likelihood ratio test: $X^2_1 = 4.96$, $P = 0.026$) but not just study identity (likelihood ratio test: $X^2_1 = 0.00$, $P = 1.0$). On the basis of these results, we included both random factors in our model.

An intercept-only random-effects model estimates the overall mean effect size while accounting for study identity and species identity, but without considering any moderating variables. Our intercept-only model – based on $n = 584$ effect sizes – showed significant female bias in immunity when phylogeny was not incorporated (mean ± SE, $g = 0.1626 \pm 0.063$, $z = 2.58$, $P = 0.01$, $n = 584$ effects; Fig. 1). Controlling for phylogenetic descent improved the fit of our model (likelihood ratio test: $X^2_1 = 5.15$, $P = 0.023$); however, its inclusion also increased the standard error of the meta-analytic mean, and thus eliminated the significant female bias in immune response ($g = 0.2104 \pm 0.138$, $z = 1.52$, $P = 0.13$, $n = 584$ effects; phylogenetic meta-analytic mean, Fig. 1).

Our data set exhibited a high degree of heterogeneity (non-phylogenetically controlled: $I^2 = 90.67$, $\tau^2 = 0.18$; phylogenetically controlled: $I^2 = 90.74$, $\tau^2 = 0.14$; Table 1) based on the cutoffs of Higgin *et al*. (2003). The different constituents of variation not due to sampling error were very similar between the simple and phylogenetic meta-analyses (Table 1). In both cases, the proportion of variance at the effect-size level ($I^2_{\text{effect size}}$) was approximately twice that of the study level (I^2_{study}). Species made a very small contribution (< 1.0%), perhaps due to the low repeatability within species (only 19 of 123 species were represented in more than one study) (cf. Booksmythe *et al*. 2017). Phylogeny accounted for a relatively small proportion of variation in the phylogenetic meta-analysis ($I^2_{\text{phylogeny}} = 4.26\%$; $H^2 = 4.69\%$) (Table 1).

The large heterogeneity in our data warranted the inclusion of moderator variables in our models. A reduced meta-regression model with the non-significant factors 'taxonomic group', 'life stage', 'inference' and 'study type' removed from the full model was significantly worse fit than a full model with all moderators included (likelihood ratio test: $X^2_1 = 36.39$, $P < 0.001$) (Tables S5 and S6), so the full model was retained. Because phylogeny significantly improved the fit of our model, we controlled for phylogeny in all subsequent analyses.

Separate phylogenetically controlled meta-regressions for each moderator revealed a significant effect of assay type/immune measure only ($Q = 164.90.13$, d.f. = 20, $P < 0.0001$), with both female and male biases. Significant female biases (positive effect sizes) were associated with assays involving bactericidal capacity and melanisation. Significantly male-biased immune measures (negative effect sizes) involved interleukin-1β response to immune stimulant, interleukin-6 response to immune stimulant and TNF-α response to immune stimulants (Table 2; Fig. 1). Meta-regression (i.e. intercept included) showed no significant heterogeneity among life stages ($Q = 4.08$, d.f. = 2, $P = 0.13$); however, adults had relatively greater female-biased immunity than non-adults ($z = 2.02$, $P = 0.044$), whereas situations in which adults and non-adults were pooled (i.e. both) did not differ from either adults ($z = 0.20$, $P = 0.84$) or non-adults ($z = 0.24$, $P = 0.81$) (Table 2; Fig. 1). In other words, although the three life stage categories differed significantly from each other, they did not differ from zero. Meta-regression suggested little heterogeneity among study locations (i.e. laboratory, field or semi-natural; $Q = 0.63$, d.f. = 2, $P = 0.73$), inference types (i.e. experimental or correlational; $Q = 1.68$, d.f. = 1, $P = 0.19$), or the eight taxonomic groups ($Q = 3.19$, d.f. = 7, $P = 0.87$).

We found that among innate immune responses, induced responses ($g = 0.395 \pm 0.18$, 95% CI 0.042 to 0.748, $P = 0.03$), but not constitutive responses ($g = 0.169 \pm 0.15$, 95% CI −0.122 to 0.460, $P = 0.26$), were significantly female-biased; but they did not differ significantly from each other ($Q = 2.01$, d.f. = 1, $P = 0.16$; Fig. 2). Among adaptive responses, constitutive and induced responses did not differ significantly ($Q = 1.31$, d.f. = 1, $P = 0.25$) and neither effect size differed from zero (constitutive: $g = 0.309 \pm 0.24$, 95% CI −0.156 to 0.775, $P = 0.19$; induced: $g = 0.052 \pm 0.15$, 95% CI −0.249 to 0.353, $P = 0.66$; Fig. 2). In analyses that pooled constitutive and induced responses, adaptive and innate components did not differ significantly from each other ($Q = 0.88$, d.f. = 1, $P = 0.35$) and neither are significantly female-biased (innate: $g = 0.171 \pm 0.18$, 95% CI −0.182 to 0.525, $P = 0.34$; adaptive: $g = 0.308 \pm 0.19$, 95% CI −0.069 to 0.686, $P = 0.11$). Finally, pooled adaptive and innate

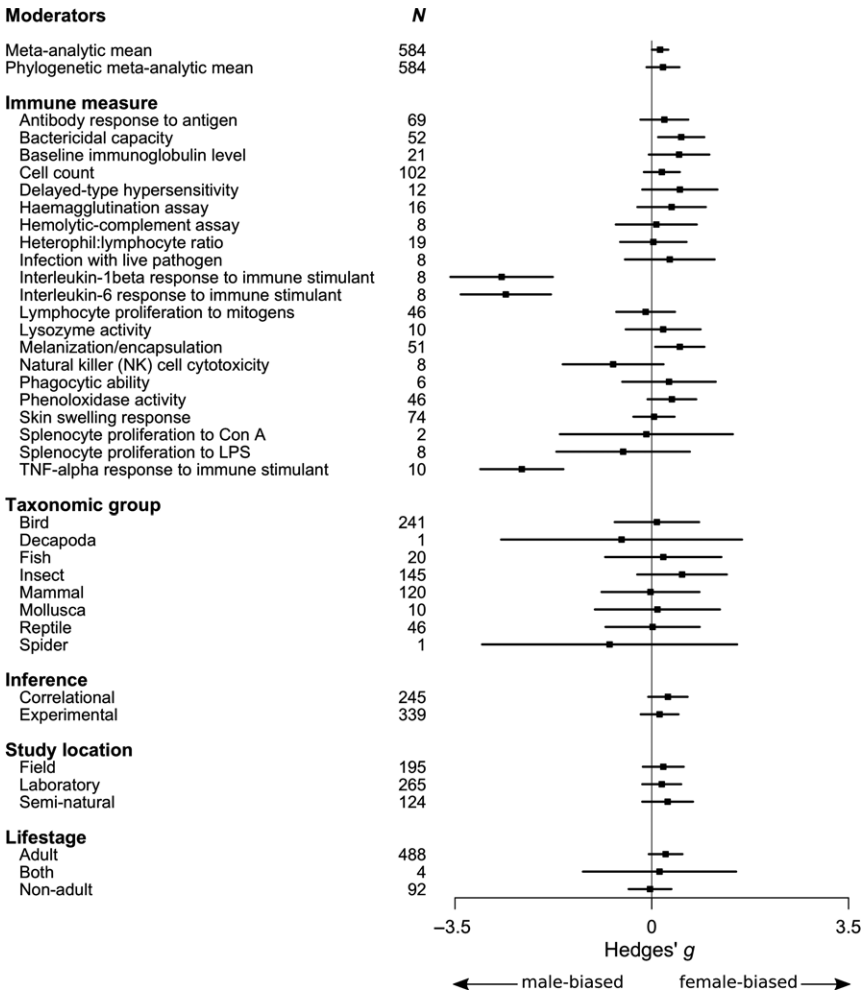

Fig. 1 Mean (± 95% CI) effect size (Hedges' *g*) for each level of five moderating factors. Each moderating factor was analysed using a separate meta-regression assuming correlations among related effect sizes to be zero. A meta-analytic mean (all $n = 583$ effect sizes pooled) was calculated without including any moderating factors.

Table 1 Results from multilevel and phylogenetic (random effects) meta-analyses (no moderator variables included in model)

Type	*k*	*m*	*n*	Mean (*Z*)	Lower CI (2.5%)	Upper CI (97.5%)	I^2_{study} (%)	$I^2_{species}$ (%)	$I^2_{effect\ size}$ (%)	$I^2_{phylogeny}$ (%)	I^2_{total} (%)	H^2(%)
Multilevel (non-phylogenetic)	584	104	123	0.1626	0.039	0.286	16.51	< 1.0	74.16	–	90.68	–
Phylogenetic	584	104	123	0.2104	−0.068	0.482	12.95	< 1.0	73.53	6.43	90.74	4.69

constitutive responses were not significantly different from pooled adaptive and innate induced responses ($Q = 0.04$, d.f. = 1, $P = 0.85$): neither constitutive responses (0.237 ± 0.16, 95% CI −0.067 to 0.541, $P = 0.13$) nor induced responses (0.213 ± 0.16, 95% CI −0.092 to 0.517, $P = 0.17$) were significantly female-biased.

A model having only standardised publication year as a moderator suggests that there is no significant relationship between effect size and date of publication (estimate ± SE: −0.097 ± 0.064, $z = 1.52$, $P = 0.13$; Figure S3). Similarly, including standardised publication year in our final model showed no effect of time (estimate ± SE: −0.111 ± 0.076, $z = 1.46$, $P = 0.15$) on immunological sexual dimorphism after controlling for fixed (i.e. moderators) and random (study, species and phylogenetic descent) effects (Table S6).

DISCUSSION

Our meta-analysis of sex differences in immunity across animal species was unable to reject the null hypothesis of no difference between the sexes once we accounted for phylogenetic non-independence. While our non-phylogenetic meta-analysis revealed a statistically significant female-biased (i.e. superior) effect size, this effect was small and became non-significant

Table 2 Mean Hedges' g (95% C.I.) for the levels of each of five moderating factors

	Mean	95% Confidence interval Lower	Upper	P
Immune measure				
Antibody response to antigen	0.182	−0.229	0.593	0.386
Bactericidal capacity	**0.479**	**0.090**	**0.868**	**0.016**
Baseline immunoglobulin level	0.466	−0.059	0.990	0.082
Cell count	0.180	−0.127	0.487	0.250
Delayed-type hypersensitivity	0.545	−0.109	1.200	0.103
Haemagglutination assay[a]	0.413	−0.171	0.996	0.166
Haemolytic-complement assay[a]	0.003	−0.681	0.687	0.993
Heterophil:lymphocyte ratio[a]	0.039	−0.544	0.622	0.896
Infection with live pathogen	0.390	−0.400	1.179	0.333
Interleukin-1β response to immune stimulant[b]	**−2.751**	**−3.550**	**−1.951**	**< 0.0001**
Interleukin-6 response to immune stimulant[b]	**−2.705**	**−3.459**	**−1.952**	**< 0.0001**
Lymphocyte proliferation to mitogens	0.010	−0.502	0.522	0.969
Lysozyme activity	0.169	−0.502	0.841	0.621
Melanization/encapsulation	**0.501**	**0.072**	**0.929**	**0.022**
Natural killer (NK) cell cytotoxicity[c]	−0.512	−1.359	0.334	0.236
Phagocytic ability	0.299	−0.523	1.121	0.476
Phenoloxidase activity[d]	0.393	−0.033	0.820	0.070
Skin swelling response to injection with mitogens/antigens	0.062	−0.293	0.416	0.733
Splenocyte proliferation to Con A[b]	−0.069	−1.580	1.442	0.929
Splenocyte proliferation to LPS[b]	−0.641	−1.792	0.511	0.276
TNF-α response to immune stimulant[b]	**−2.461**	**−3.177**	**−1.745**	**< 0.0001**
Taxonomic group				
Bird	0.129	−0.706	0.963	0.763
Decapoda	−0.492	−2.702	1.718	0.662
Fish	0.181	−0.917	1.280	0.746
Insect	0.546	−0.336	1.428	0.225
Mammal	0.049	−0.903	1.002	0.919
Mollusca	0.110	−1.069	1.289	0.855
Reptile	−0.020	−0.931	0.890	0.965
Spider	−0.781	−3.211	1.649	0.529
Method of inference				
Correlational	0.298	−0.027	0.623	0.072
Experimental	0.141	−0.177	0.458	0.385
Study type				
Field	0.218	−0.130	0.567	0.219
Laboratory	0.176	−0.161	0.514	0.306
Semi-natural	0.328	−0.095	0.751	0.128
Lifestage				
Adult	0.256	−0.026	0.538	0.075
Both	0.123	−1.171	1.418	0.852
Non-adult	−0.036	−0.405	0.332	0.847

A separate random effects meta-regression (without intercept) model was fitted for each moderator assuming a within-study correlation of $r = 0$ among effects. Statistically significant ($P < 0.05$) moderator levels are in bold type.

Some immune measures were tested in one taxon only: a=birds, b=mammals, c=reptiles, d=insects

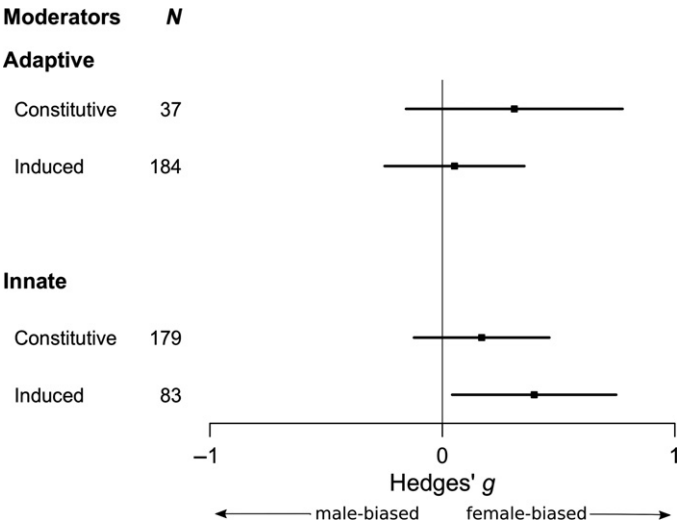

Fig. 2 Mean (± 95% CI) effect size (Hedges' g) for constitutive and induced immune components within adaptive (specific) and innate (non-adaptive) immune responses assuming correlations among related effect sizes to be zero.

when we controlled for phylogeny, in a model that was strongly supported over the non-phylogenetic model. Previous studies have revealed that non-phylogenetic methods can produce elevated Type I error rates (Lajeunesse *et al.* 2013), which may explain the difference in our results.

The lack of a strong overall (i.e. meta-analytic mean) sex difference in immunocompetence is an important finding because it is in contrast with much of the literature suggesting that greater female immunocompetence is, or should be, widespread (e.g. Zuk 1990; Rolff 2002). While the magnitude and direction of sex biases in immunocompetence are predicted to depend upon the strength of sexual selection on the sexes (Zuk 1990), a factor we did not include, the expectation of superior female immunity arises based on the reasonable assumption that, overall (i.e. across all of our sampled taxa), sexual selection is going to be stronger on males. Thus, our large and taxonomically broad meta-analysis suggests that sex differences in immunity are either not widespread and strong, or perhaps more likely, not consistent among animals. A small and non-significant mean effect size could result from effect sizes being generally close to zero. However, the large degree of heterogeneity (i.e. I^2) in our data set, combined with our small, non-significant mean effect size, suggests that more variable effect sizes that include similar numbers of positive (i.e. female-biased immunocompetence) and negative (i.e. male-biased immunocompetence) effect sizes better explain our primary finding.

The lack of a significant difference among eight broad taxonomic groups is also notable because much of the earliest literature on sex differences in immunity focused on proximate physiological mechanisms and, in particular, on the potentially immunosuppressive effects of testosterone in male vertebrates (Roberts *et al.* 2004; Foo *et al.* 2017). We found, however, that sex differences in immunity in vertebrates were not different from zero (i.e. no bias) and also not different from effects in invertebrates. Indeed, although also not statistically different from zero, we found the largest female bias in immunity in the insects (which lack testosterone). It is possible

that other hormones with sex-specific expression patterns in insects (De Loof 1998, 2006; Bear & Monteiro 2013) may at times result in a female-bias in immune response (e.g. Villanueva *et al*. 2013).

Although we emphasise here the lack of a 'universal' or taxonomic sex difference in immunocompetence, this is not to suggest that sex differences never occur or are not important. In fact, our meta-regressions revealed interesting effects of certain moderators that may help guide future research. In particular, we found that when parsed by assay type or immune measure, a few components of immune defence are female-biased, whereas others are male-biased, and several show no bias. Our results for 21 different immune measures highlight the need to develop mechanism-specific hypotheses of immune-response sexual dimorphism. For example, we found significant female bias in studies of melanisation/encapsulation, which is only one component of arthropod immune defence. Phenoloxidase (PO) activity, a related component of arthropod immune defence, was not quite significantly ($P = 0.07$) female-biased (see also Nunn *et al*. 2009). In female insects, egg-chorion hardening (Christensen *et al*. 2005) also involves the use of phenoloxidase and melanin. Immune challenges have been shown to decrease egg laying in Wellington tree weta (Kelly 2011) and in *Aedes aegypti* mosquitoes (Li *et al*. 1993), suggesting that PO might function in both immunity and reproduction. In female *Anopheles gambiae* mosquitoes, silencing a gene (phenylalanine hydroxylase) involved in the conversion of the amino acid phenylalanine into tyrosine (a precursor of melanin; Christensen *et al*. 2005), effectively shutting down melanin production, impaired both melanotic encapsulation and egg production (Fuchs *et al*. 2014). Perhaps female insects have higher standing concentrations of PO because they are investing in egg production. Female-biased PO-based immunity might, therefore, arise not as a direct effect of selection for resistance but rather as a consequence of females investing in fecundity.

Lee (2006) argued that we should generally expect more female-biased immunity among adaptive and inducible components of immunity and less female-biased (or even male-biased) immunity among innate and constitutive components. Our findings are generally not consistent with Lee's (2006) predictions. While we did find that among those components classified as innate, induced responses were significantly female biased and constitutive responses were not, these categories did not differ from each other. Furthermore, across other categorical comparisons (e.g. adaptive vs. innate), we found little evidence for consistent sex biases in either direction. There is some arbitrariness to some of these categories and some components of immunity span multiple categories (e.g. Adamo *et al*. 2016), and thus we are cautious in our conclusions. While our findings are in contrast with several of Lee's (2006) predictions, we agree with the spirit of Lee's (2006) argument, which is that an integration of the theory of life-history trade-offs with a greater emphasis upon the specific physiological details of the varied components of immune defence is a fruitful path forward. Our findings also strengthen the arguments of Adamo (2004) and others (Brock *et al*. 2014) that multiple assays testing different arms of the immune system are needed in any given study if one hopes to make specific empirical claims about sex differences in overall 'immunocompetence'; this term should be reserved for only the most general of uses, for example as we use here or in the context of theoretical models.

We also considered the possibility that aspects of study design, such as mode of inference or study location, might explain some of the variation among effect sizes. These moderators were retained in the final meta-regression model but were not statistically significant. We expected stronger effects from experimental studies than correlational ones because controlled experiments generally have lower variance and thus should reveal a biological effect if one exists. However, we found no effect of study design on sexual dimorphism in immune response. In addition, study location had little effect on the direction of sex bias in immune response. We also did not find a significant relationship between effect size and year of publication, a finding in contrast with Jennions & Møller's (2002) conclusion that more recently published studies in ecology and evolution report weaker findings (Møller 1998; Møller & Alatalo 1999; Simmons *et al*. 1999).

In conclusion, our meta-analysis failed to find general support for the widely assumed female-biased sex difference in immunity (Zuk 1990; Zuk & McKean 1996; Rolff 2002; Zuk & Stoehr 2002), although we did find sex differences in some subsets of the data. These differences, when they occurred, were both male- and female-biased and less likely to depend on study methodology or taxon, but effects were sensitive to the particular immune measure being assayed. As a result, we suggest that nuanced, mechanism-specific trade off hypotheses may prove more fruitful in understanding variation in immunity than more general hypotheses relying on widespread and consistent life-history differences between the sexes.

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AUTHOR STATEMENT

CDK collected the data, performed the meta-analysis and co-wrote the manuscript. AMS collected the data and co-wrote the manuscript. CN co-wrote the manuscript. KNS and ZMP collected the data and contributed to revisions.

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