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REVIEW

Testing the immunocompetence handicap hypothesis: a review of the evidence

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The immunocompetence handicap hypothesis was formulated 12 years ago in an attempt to offer a proximate mechanism by which female choice of males could be explained by endocrine control of honest signalling. The hypothesis suggested that testosterone has a dual effect in males of controlling the development of sexual signals while causing immunosuppression. Our purpose in this review is to examine the empirical evidence to date that has attempted to test the hypothesis, and to conduct a meta-analysis on two of the assumptions of the hypothesis, that testosterone reduces immunocompetence and increases parasitism, to ascertain any statistical trend in the data. There is some evidence to suggest that testosterone is responsible for the magnitude of trait expression or development of sexual traits, but this is by no means conclusive. The results of many studies attempting to find evidence for the supposed immunosuppressive qualities of testosterone are difficult to interpret since they are observational rather than experimental. Of the experimental studies, the data obtained are ambiguous, and this is reflected in the result of the meta-analysis. Overall, the meta-analysis found a significant suppressive effect of testosterone on immunity, in support of the hypothesis, but this effect disappeared when we controlled for multiple studies on the same species. There was no effect of testosterone on direct measures of immunity, but it did increase ectoparasite abundance in several studies, in particular in reptiles. A funnel analysis indicated that the results were robust to a publication bias. Alternative substances that interact with testosterone, such as glucocorticoids, may be important. Ultimately, a greater understanding is required of the complex relationships that exist both within and between the endocrine and immune systems and their consequences for mate choice decision making.

The immunocompetence handicap hypothesis (ICHH; Folstad & Karter 1992) was suggested some 12 years ago as a model explaining the expression of male sexual signals involving the interaction of the endocrine system and immune function. It states that as testosterone is responsible for the production of male secondary sexual traits and is simultaneously immunosuppressive, the cost of being able to express sexual traits is decreased immune function. Immunosuppression should result in greater vulnerability to pathogen or parasite attack; therefore, only high-quality males could afford to display sexual characteristics fully without suffering large parasite loads (Folstad & Karter 1992). The model incorporates both Zahavi's handicap hypothesis (Zahavi 1975), in that individuals that express male epigamic traits are handicapped by a reduced immune response, and Hamilton & Zuk's parasite model (Hamilton & Zuk 1982), since only males possessing 'good genes' can fully express such displays without suffering reduced fitness from pathogen or parasite attack. Therefore, a male's display is an honest signal of genetic quality to the female. According to the ICHH, cheaters could not invade, since their immunocompetence would be compromised to such an extent by the effects of testosterone that their lifetime reproductive success would be reduced (Folstad & Karter 1992).

In the intervening years the ICHH has attracted much attention (Folstad & Karter 1992 is among the top 10 of cited papers in ecology in the past 10 years), although the results of many of the studies of the ICHH have been

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ambiguous if not contradictory. Our aim in this review is to consider whether, 12 years on from the publication of the ICHH (Folstad & Karter 1992), the research has progressed sufficiently for us to support or discount it as a possible mechanism for the developmental control of exaggerated sexual traits. The ICHH predicts that each male should have his own optimum level of testosterone allowing maximum trait expression, while minimizing immunosuppression. As such, the strongest evidence in support of the ICHH should be found from studies that have manipulated individual levels of testosterone. We examine qualitatively the evidence in support of the ICHH (specifically the predicted immunosuppressive effect of testosterone) and provide a quantitative summary of experimental studies.

Testosterone and Sexual Signal Development

Several studies have documented the role of testosterone in controlling sexual behaviour (Wingfield et al. 1990; Owens & Short 1995; Salvador et al. 1996; Enstrom et al. 1997; Hunt et al. 1997; Hagelin & Ligon 2001; Schlinger et al. 2001). Male dark-eyed juncos, *Junco hyemalis*, implanted with testosterone outperform controls in courtship displays and are more attractive to females (Enstrom et al. 1997), whereas testosterone-implanted male Lapland longspurs, *Calcarius lapponicus*, sing more but are not more aggressive than controls (Hunt et al. 1997). In the lizard *Sceloporus jarrovi*, testosterone implantation increases territorial aggression (Marler et al. 1995).

In addition to evidence for testosterone-mediated sexual behaviour, there is also evidence that testosterone affects the expression of sexual ornaments in birds. For example, the frontal shield of moorhens, *Gallinula chloropus*, increases in thickness, size and colour when testosterone is elevated (Eens et al. 2000). Testosterone is positively related to badge size in house sparrows, *Passer domesticus* (Evans et al. 2000; Buchanan et al. 2001; Gonzalez et al. 2001), and experimental results suggest that differences in feather abrasion, related to testosterone levels, also affect seasonal exposure of the badge (Gonzalez et al. 2001). Testosterone is involved in the induction of prenuptial moults in superb fairy-wrens, *Malurus cyaneus* (Peters et al. 2000). Experimentally elevated testosterone increases wattle size (a sexually selected trait) and male aggressiveness in pheasants, *Phasianus colchicus* (Briganti et al. 1999). In addition, comb size in the closely related junglefowl, *Gallus gallus*, is related to levels of testosterone (Zuk et al. 1995). In lizards, testosterone enhancement increases the intensity of nuptial skin coloration (Salvador et al. 1996); and in white-tailed deer, *Odocoileus virginianus*, testosterone is positively correlated with antler size (Ditchkoff et al. 2001). Other studies, however, have not shown similar effects. Elevated levels of testosterone cause plumage to become dull in male house finches, *Carpodacus mexicanus* (Stoehr & Hill 2001), and little correlation has been found between testosterone and secondary sexual traits in male red-winged blackbirds, *Agelaius phoeniceus* (Weatherhead et al. 1993).

In conclusion, whereas there is a substantial amount of evidence that testosterone alters behaviour, the evidence that it controls the degree of sexual ornamentation is far from conclusive (Owens & Short 1995; Kimball & Ligon 1999). However, this may be in part because the wrong emphasis has been placed on measuring trait quality. Testosterone may control variation in the degree of plumage trait quality (Evans et al. 2000), rather than the overall presence or absence of the trait (Owens & Short 1995). Furthermore, because of the large seasonal differences in testosterone levels, it is essential that the timing of testosterone control of ornament production is understood and that variation in individual hormone levels is tested at the correct time. It is worth considering that the involvement of testosterone control might vary between intra- and intersexually selected traits; the ICHH predictions were made specifically for traits used in mate choice. In contrast, as indicated in the examples above, the evidence for testosterone control of the development of traits used in male–male interactions seems stronger, although it is also true that many sexual traits are probably used in both contexts.

Testosterone and Immunity

Testosterone is a gonadal steroid hormone produced principally by the testes, but also in small amounts by the ovaries and adrenal glands, allowing females to have low circulating levels of testosterone (Nelson 2000). Testosterone influences sexual development in males, as evidenced by the effects of castration (e.g. Nelson 2000). The evidence that testosterone is immunosuppressive is based on several observations. For example, after castration male rats exhibit increased thymus and spleen mass, organs that are known to be involved in immune defence (Grossman 1985). In addition, the sexes differ in their immune response: immunoglobulin production is greater in females than in males for example (Grossman 1985); and the organs responsible for immune response often have specific receptors for gonadal steroids (Grossman 1990). Observations that females are often more susceptible to autoimmune disorders have, for example, been taken as support for the immunosuppressive nature of testosterone (Grossman 1990). Certainly testosterone has been shown in vitro to affect the proliferation of thymic T cells, and to suppress antibody release by B cells at the cellular level, while castration in mice, *Mus musculus*, causes gross changes to both thymic tissue mass and structure (Grossman 1990). Androgen metabolites, dehydroepiandrosterone and dihydrotestosterone, also appear to play a role in immunomodulation and the senescence of the immune system, although they seem capable of stimulating immune activity in certain circumstances (Marsh 1996). In addition, it is also clear that oestrogens can modulate immune activity and, as castration affects levels of both testosterone and oestrogens, their effects are difficult to separate. To complicate matters, it is also true that the immune system can modulate hormone levels through the production of cytokines which interact with the neuroendocrine system, as well as through the

production of lymphoid organ-specific hormones (Marsh 1996); and certain immunological parameters can change seasonally or at different stages of an animal's annual cycle (Paz Nava et al. 2001). In conclusion, it not possible to say that increased androgen levels would always lead to immunosuppression, as this appears to depend on complex interactions between the neuroendocrine and immune systems (Marsh 1996).

Testosterone and Signalling

Observational work

Correlations between individual measures of immunocompetence and signal quality have been interpreted as support for the ICHH (e.g. Kortet et al. 2003). However, the ICHH can act to reinforce signalling honesty only if increases in testosterone cause a decrease in individual immunocompetence. Without manipulations, both positive and negative correlations between signal quality, testosterone and immunocompetence could be regarded as support for the ICHH. In particular, seasonal changes in testosterone have been used to examine how changes in testosterone could affect immune function. Although testosterone was not measured directly, some studies have found seasonal differences in immunocompetence correlated with testis size (Merila & Sheldon 1999) and sexually selected areas of plumage (Gonzalez et al. 1999). Direct measurements of testosterone that correlate negatively with immunity have been found in European starlings, *Sturnus vulgaris* (Duffy et al. 2000; Duffy & Ball 2002). Weatherhead et al. (1993) found a positive correlation between testosterone titre and mite infestation in red-winged blackbirds. They also reported a lack of covariation between testosterone, other parasites and sexually selected traits such as song and plumage colour, except epaulette size (Weatherhead et al. 1993). Antler size in white-tailed deer is correlated with testosterone levels and antler size and nematode infection are negatively related; however, there is no direct correlation between testosterone and nematode load (Ditchkoff et al. 2001).

Another method of inferring a role for testosterone-mediated immunosuppression from observational work has been to compare the immune function or parasite load of males and females. Sex differences in hormone profiles may well explain sex differences in immunocompetence (Grossman 1990) and identify why many more women than men are prone to certain autoimmune disorders, whereas males show greater susceptibility to parasitic infection (Klein 2000). In a meta-analysis of 33 studies, McCurdy et al. (1998) found the opposite in birds: infection by *Haemoproteus* (a blood parasite) is more prevalent in females, both overall and within only polygynous species (McCurdy et al. 1998). However, across taxa, males tend to have reduced immune function and higher parasite loads (Poulin 1996; Schalk & Forbes 1997; Klein 2000), although there is considerable variation between species (Moore & Wilson 2002). These results are difficult to interpret because behavioural and genotypic factors might also differentially affect sex differences in immunocompetence and parasite exposure (Klein 2000). If male behaviour is more 'risky', increasing the chance of infection to gain fitness benefits, the 'cost' of mounting an immune response may differ for males and females (Zuk & Stoehr 2002). In addition, oestrogens, which occur in higher levels in females, enhance antibody production (Grossman 1990), suggesting that sex differences in immunocompetence may not be solely caused by the actions of testosterone. In a house finch population, male-biased mortality occurred after an outbreak of mycoplasmal conjunctivitis between 1994 and 1996 (Nolan et al. 1998). Male survivors had significantly redder plumage than males that died, suggesting that only fit males could resist the epidemic (Nolan et al. 1998). However, the expression of red plumage in this species may not be dependent upon testosterone (Stoehr & Hill 2001).

Using an elegant comparative analysis approach to examine the role of parasites in mediating sexual selection pressures in mammals, Moore & Wilson (2002) have shown an association between sexual size dimorphism and male-biased parasitism across taxa. This association was also true for male-biased parasitism and degree of polygyny, when mating system was used as an index of sexual selection pressure, implying a role for parasites in evolution under sexual selection. In addition, Moore & Wilson showed that male-biased mortality is higher in taxa with higher degrees of sex-biased parasitism, suggesting mortality associated with parasitic infection is a real fitness cost and that parasites have played an important role in the evolution of species under strong sexual selection pressure. Their study could not positively identify the proximate mechanism for these effects. These trends could be caused by a link between immunosuppression and enhanced androgen levels in males, particularly in highly sexually selected, sexually dimorphic species. However, data on body size and sex dimorphism suggest an alternative scenario: that the effect of parasitic infection on somatic investment may be more likely than androgen immunosuppression to mediate the cost of parasitism (Moore & Wilson 2002).

In support of the ICHH, but not testing the assumptions of the hypothesis directly, have been a number of comparative studies that have reported higher immunocompetence in sexually dichromatic bird species than in monochromatic species (using spleen size as a surrogate of immunocompetence); males have smaller spleens than females, and male plumage brightness is negatively related to the size of the spleen in dichromatic but not in monochromatic species (Møller 1997; Møller et al. 1998a, b). In addition, Hosken & O'Shea (2001) found a negative correlation between spleen size and testis mass in two Australian bats (*Chalinolobus morio* and *Nyctophilus geoffroyi*). These results all provide some support for the ICHH, although it is questionable whether spleen size is a positive indicator of immunocompetence, or whether testis size is a reliable surrogate for testosterone levels.

Experimental Work

Because of the complexity of the ICHH, experimental manipulations are necessary to test differentially the factors influencing immune function. A number of

approaches have been tried including manipulating testosterone levels through castration and implantation, and using testosterone antagonists which suppress testosterone production. The effects on both immune function and parasite load have been tested. One of the best experimental approaches to test the ICHH has been to produce selected lines of animals varying in immunocompetence. Verhulst et al. (1999) found that male chickens, *Gallus gallus domesticus*, selected for low levels of antibody response had relatively larger combs (a sexually selected trait) and higher testosterone levels than males selected for high immune response. This is perhaps the most convincing evidence to date of a genetic link between testosterone and immune function which could mediate an immunosuppressive cost of developing a sexual trait (Verhulst et al. 1999).

META-ANALYSIS

To quantify the results of the studies testing the ICHH in a statistically meaningful way, it is necessary to carry out a meta-analysis. Meta-analyses can account for sample size and error differences between studies and quantify the effects of treatment, and have recently become widely used in ecology (for example Hamilton & Poulin 1997; Osenberg et al. 1997; Gontard-Danek & Møller 1999; Palmer 1999; Arnqvist & Nilsson 2000; Gates 2002). The role of testosterone in signal development and female choice was not addressed in all of the studies that we included in the meta-analysis; however, the analysis was explicitly intended to ascertain only whether testosterone increased parasite intensity and decreased various measures of direct immunity, not to test other assumptions of the ICHH.

Selection Criteria

Studies included in the meta-analysis had to fulfil certain criteria.

(1) They must be experimental in nature. Correlational, observational studies were omitted, because the results of such studies are difficult to interpret in terms of the ICHH. Studies were chosen in which males were subject to manipulation of their circulating testosterone levels by the use of silastic implants. Unfortunately, few studies were on males castrated before implantation, so studies in which implantation but not castration was carried out are included. Castration removes any confounding effect of endogenous testosterone production which may result in differing plasma levels of testosterone between individuals. The immune parameters and parasite loads of unimplanted control males were compared with the same variables in testosterone-implanted males. Most studies included in the meta-analysis measured testosterone levels after implantation to ensure that the hormone was circulating at the desired level.

(2) Males tested must be adult. This removes any effect of age in confounding the effect size.

(3) Since the meta-analysis was comparing two groups (control and treated) in different studies, we used Hedges' d as the effect size measure (Rosenberg et al. 2000). To calculate this statistic, we needed means and standard errors or standard deviations and sample sizes from the raw data. Studies that did not quote these were therefore omitted.

Data Collection

We collected the data by searching the biological databases Web of Science, BIDS and the Science Citation Index. Searches were also carried out on the World Wide Web using several search engines, and in some cases data were obtained directly from authors. A total of 36 separate data sets were found and treated as independent, although a number of them came from single studies. Twenty-two studies fulfilled the selection criteria, which is comparable to other meta-analyses that have compared effect sizes (e.g. Hamilton & Poulin 1997). These studies are listed in Table 1.

Statistical Methodology

As stated, Hedges' d was used as a measure of effect size; this statistic is appropriate for the nature of the data used, since it summarizes the difference between control and treatment groups (Rosenberg et al. 2000). This model estimates effect sizes that range from $-\infty$ to $+\infty$, where 0 corresponds to no difference in effects between the control and treatment groups. Negative values signify control groups attaining a higher value for the trait in question than the experimental groups, and positive values signify experimental groups attaining higher values than control groups (Rosenberg et al. 2000). Effect sizes of zero therefore provide support for the null hypothesis, while positive values provide support for the ICHH. Negative values would suggest that testosterone has an immuno-enhancing effect. For the parasite load and immune trait analyses, positive values would indicate larger parasite loads and decreased immunocompetence, respectively. Significance was determined by measuring the 95% confidence intervals (CI) around d: if the 95% CI straddled 0, then the effect was not significant.

We used a fixed-effects categorical model, because the studies selected had to fulfil the stringent criteria listed above. In particular, the fact that all the studies were manipulative and were carried out in a similar manner reduces the possibility of any random effects confounding the result. The studies were categorized into Class (birds, reptiles and mammals); immune trait measured (white blood cells, antibodies, phytohaemagglutinin (PHA) response); type of parasite (ecto- or endo-); species; and whether the experimental males had been castrated or not. Heterogeneity (Q) of effect sizes was compared between and within categories; the larger the value of Q, the greater the heterogeneity. This was used to determine the consistency of results across studies. The value of Q was then tested against a χ^2 distribution to obtain a P value to identify significant differences between studies

Table 1. Studies included in the meta-analysis

Species	Immune trait	Parasite type	Castrated?	Effect size	Source
Reptilia					
Psammodromus algirus		Endo	No	0.0286	Veiga et al. 1998
Lacerta agilis		Ecto	No	0.6890	Olsson et al. 2000
Psammodromus algirus	White blood cells		No	0.8805	Salvador et al. 1996
Psammodromus algirus		Ecto	No	0.8731	Salvador et al. 1996
Psammodromus algirus		Ecto	No	0.0893	Salvador et al. 1997
Sceloporus undulatus hyacinthus		Ecto	No	0.7722	Klukowski & Nelson 2001
Aves					
Passer domesticus	White blood cells		No	−0.9087	Puerta et al. 1995
Carpodacus mexicanus		Endo	Yes	0.3605	Duckworth et al. 2001
Licherostomus penicillatus		Endo	No	0.3267	Buttemer & Astheimer 2000
Carduelis chloris		Endo	No	0.1614	Lindstrom et al. 2001
Lanis ridibundus	Antibodies		No	0.4473	Ros et al. 1997
Carduelis chloris	Antibodies		No	0.1426	Lindstrom et al. 2001
Passer domesticus		Ecto	Yes	0.2256	Poiani et al. 2000
Sturnus vulgaris	Antibodies		No	0.7660	Duffy et al. 2000
Sturnus vulgaris	PHA		No	1.9101	Duffy et al. 2000
Junco hyemalis	Antibodies		No	1.7775	Casto et al. 2001
Junco hyemalis	PHA		No	1.0350	Casto et al. 2001
Junco hyemalis	PHA		No	1.2408	Casto et al. 2001
Passer domesticus	Antibodies		Yes	−1.0926	Evans et al. 2000
Agelaius phoeniceus	Antibodies		No	−1.0305	Hasselquist et al. 1999
Hirundo rustica	White blood cells		No	−0.0928	Saino et al. 1995
Hirundo rustica	White blood cells		No	−0.0939	Saino et al. 1995
Hirundo rustica		Ecto	No	0.7666	Saino et al. 1995
Hirundo rustica		Ecto	No	0.1851	Saino et al. 1995
Hirundo rustica		Ecto	No	0.1561	Saino et al. 1995
Hirundo rustica		Ecto	No	−0.2946	Saino et al. 1995
Passer domesticus	Antibodies		Yes	0.3535	Buchanan et al. 2003
Passer domesticus	PHA		Yes	0.1826	Buchanan et al. 2003
Malurus cyaneus	Antibodies		No	0.6421	Peters 2000
Gallinula chloropus	White blood cells		No	−1.639	Eens et al. 2000
Gallinula chloropus		Ecto	No	0.5710	Eens et al. 2000
Mammalia					
Clethrionomys glareolus		Endo	Yes	1.4033	Hughes & Randolph 2001a
Phodopus sungorus	Antibodies		Yes	−0.1105	Bilbo & Nelson 2001
Clethrionomys glareolus		Ecto	Yes	0.1987	Hughes & Randolph 2001b
Apodamus sylvaticus		Ecto	Yes	0.5297	Hughes & Randolph 2001b
Phodopus sungorus	PHA		Yes	−2.1310	Bilbo & Nelson 2001

PHA: phytohaemagglutinin.

in effect sizes. For the analysis we used the Metawin 2.0 statistical package (Rosenberg et al. 2000).

Results

When we combined all categories (taxa, immune tests, parasite types and whether castration was used) of the model, there was a significantly positive mean effect size ($d = 0.239$, 95% CI: 0.104, 0.373). Therefore, overall, experimental males with testosterone implants suffered higher parasite loads and reduced immune function compared to controls, in support of the ICHH. However, there was a large amount of heterogeneity in effect size between studies ($Q_{35} = 92$, $P < 0.0001$). It is possible that some species were overrepresented in the analysis, since several studies were conducted on the same species (Table 1), which also results in a nonindependence of studies conducted by the same researchers. To counter this, we combined the effect sizes of individual species to give a mean value for each species. The confidence intervals of the means were then used to ascertain whether any of the mean effect sizes were significant. Importantly, combining studies to give mean values for each species resulted in an overall effect size that was still positive, but nonsignificant at the 5% level ($d = 0.318$, 95% CI: −0.038, 0.675).

To attempt to ascertain whether differences existed in immune parameters measured between studies, we ran a categorical model. Control males tended to have lower numbers of white blood cells than testosterone-implanted males ($d = -0.141$, 95% CI: −0.592, 0.310), but higher PHA responses ($d = 0.406$, 95% CI: −0.224, 1.036) and higher numbers of antibodies ($d = 0.157$, 95% CI: −0.165, 0.480). None of these results was significant; indeed the overall effect size of all the immune parameters combined was not significant ($d = 0.098$, 95% CI: −0.104, 0.299; Fig. 1). We found higher heterogeneity within categories of studies (for example only studies measuring white blood cells) than between categories (between studies measuring white blood cell counts, PHA response and antibodies). Overall, the heterogeneity was high between

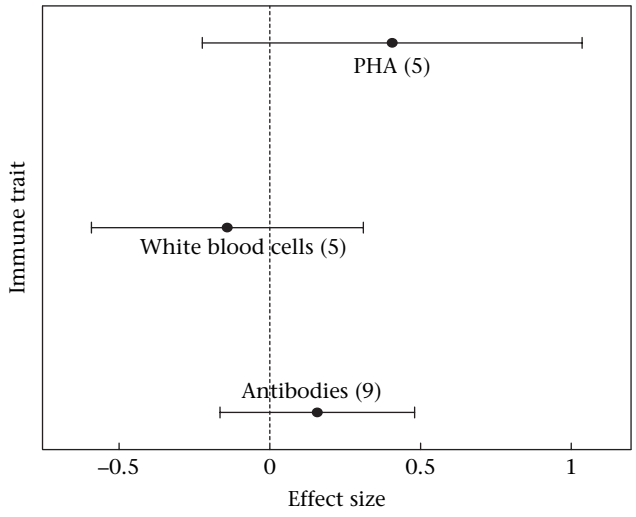

Figure 1. Effect sizes and 95% confidence interval plots of effect of testosterone implantation on immune traits. Number of studies is given in parentheses. PHA: phytohaemagglutinin.

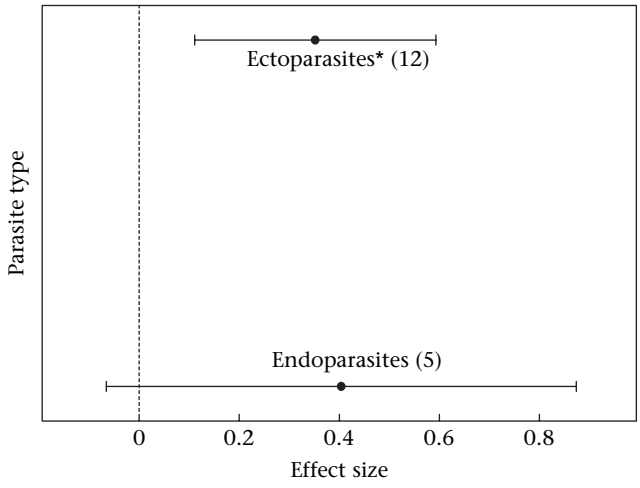

Figure 3. Effect sizes and 95% confidence interval plots of effect of testosterone implantation on parasite loads. *$P<0.05$. Number of studies is given in parentheses.

studies measuring immune parameters ($Q_{18}=69$, $P<0.0001$). When we combined studies at the species level and compared mean effect sizes for the different immune parameters, there was no difference in either the significance or the direction of trends from the above analyses.

When Classes of taxa were compared, the effect sizes were all positive (birds: $d=0.178$, 95% CI: 0.020, 0.337; mammals: $d=0.269$, 95% CI: −0.406, 0.945; reptiles: $d=0.481$, 95% CI: 0.075, 0.886; Fig. 2). In reptiles and birds the effect sizes were significantly different from zero, indicating that in these groups experimental manipulation of testosterone had a significantly immunosuppressive effect. This was not true for studies on mammals. The effect of the tests on reptiles and birds caused the overall effect size to be significantly positive ($d=0.285$, 95% CI: 0.104, 0.373), but the heterogeneity of effect sizes was again higher within than between taxa (Fig. 2). When we combined studies at the species level and compared mean effect sizes, the only significant effect of testosterone treatment in support of the ICHH was found in reptiles.

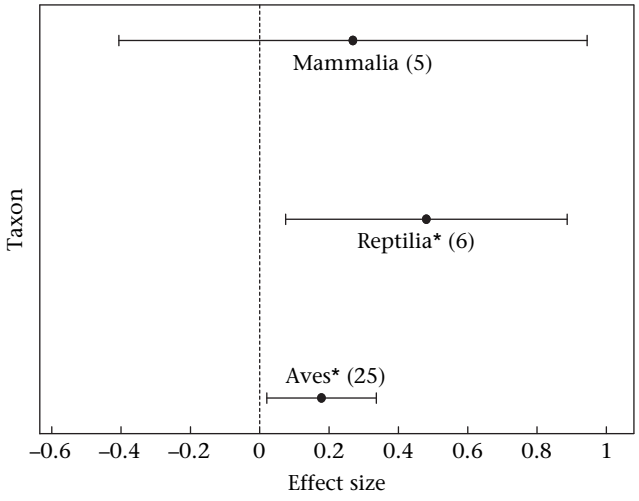

Figure 2. Effect sizes and 95% confidence interval plots of effect of testosterone implantation in three classes of vertebrate. *$P<0.05$. Number of studies is given in parentheses.

Experimental manipulation had a nonsignificant positive effect on the number of endoparasites ($d=0.404$, 95% CI: −0.066, 0.874). The number of ectoparasites was significantly higher on experimental males than on control males ($d=0.352$, 95% CI: 0.111, 0.593). Overall (studies that had looked at the effect of testosterone manipulation on parasite load and combining endo- and ectoparasites) the effect size was significant ($d=0.367$, 95% CI: 0.173, 0.562; Fig. 3). There was much less heterogeneity between and within studies, and the overall heterogeneity between studies was low ($Q_{16}=20$, $P=0.238$). When we combined studies at the species level and compared mean effect sizes, there was no difference in either the significance or the direction of trends from the original analysis.

We also compared studies in which males were castrated and other studies in which no castrations were performed. There was a nonsignificant positive effect size in those studies that castrated males ($d=0.108$, 95% CI: −0.224, 0.440), but in studies in which castration was not carried out the effect was significantly positive ($d=0.272$, 95% CI: 0.119, 0.425; Fig. 4). This indicates that studies that did not use castration were more likely to demonstrate an immunosuppressive effect of testosterone. When we combined studies at the species level and compared mean effect sizes, there was a nonsignificant positive effect of testosterone within studies that did not castrate males, in contrast to the above analysis.

To assess whether particular species differed in their immunocompetence in response to testosterone, we compared effect sizes between species. There were no significant effect sizes (either positive or negative) in any of the species studied (Fig. 5). Owing to the nature of the meta-analysis, species for which only one study was available were excluded from this analysis.

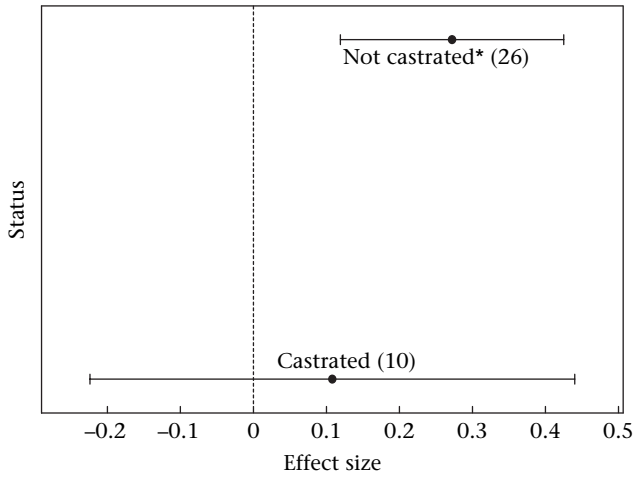

Figure 4. Effect sizes and 95% confidence interval plots of effect of testosterone in studies in which experimental males were castrated before testosterone implantation and those in which no castrations were performed. *$P<0.05$. Number of studies is given in parentheses.

Interpretation of Results

When we used individual studies as the unit for the meta-analysis, the experimental studies carried out since 1992 appear to have succeeded in finding testosterone immunosuppressive. However, when we controlled for the nonindependence of studies and conducted the analysis at the species level, this effect disappeared. Overall therefore there is at best weak evidence in support of the ICHH to date. However, combining studies for species-level analysis may result in a Type II error, owing to a decrease in sample size. No significant effect of testosterone was found on several immune parameters; indeed there was a trend for testosterone-treated males to have more white blood cells than controls. Considering that these studies are all manipulative, this is contrary to the assumptions of the ICHH. At the species level of analysis, the only taxonomic group to show a significant effect of testosterone was reptiles. Testosterone had a significant effect on ectoparasite loads but not on endoparasite loads. It should be borne in mind when interpreting these results that the immune parameters and parasite types measured were not evenly distributed between taxa. For example, numbers of ectoparasites were measured in most of the reptile species in the analysis, whereas in only one species were endoparasites measured. The difference between the significant (ectoparasites) and nonsignificant (endoparasites) results may be caused in part by the taxa included rather than the parasites themselves. The studies in which males were castrated (and so more experimentally rigorous) failed to find a significant effect of testosterone, whereas in studies that did not castrate their males, there was a significant effect indicating an immunosuppressive effect of testosterone. This may be caused in part by the maintenance of the natural endogenous production of testosterone and consequent feedback within the endocrine system, or by the stress of the castration procedure masking differences in immunocompetence between experimental groups.

To test for publication bias, we used a funnel graph to evaluate the data (Fig. 6). Funnel graphs are useful tools for finding publication bias, because if the effect sizes derive from randomly sampled studies using similar research methods, a funnel shape should emerge when effect size is plotted against sample size (Rosenberg et al. 2000; Møller & Jennions 2001). Indeed, Gates (2002) recommended that funnel plots be used as the main diagnostic tool for publication bias. The funnel graph indicated a funnel-shaped relation between effect size and sample size (Fig. 6). In addition, there was no significant relation between effect size and sample size ($R^2 = 0.003$, $F_{1,35} = 0.097$, $P = 0.758$). Although the shape of the funnel graph indicates that the overall result of the meta-analysis is robust (Fig. 6), caution should be exercised in interpretation of the findings. The large contribution that the significant effects of testosterone on ectoparasite loads and in reptiles in particular make on the

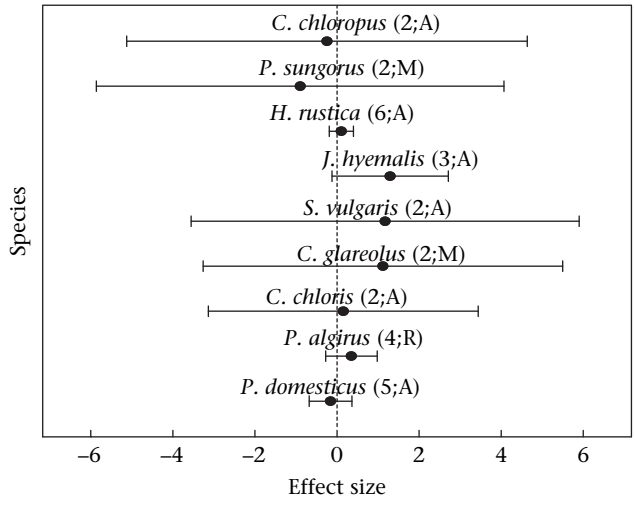

Figure 5. Effect sizes and 95% confidence interval plots of effects of testosterone implantation in different species. Number of studies and taxon are given in parentheses (A: Aves; M: Mammalia; R: Reptilia).

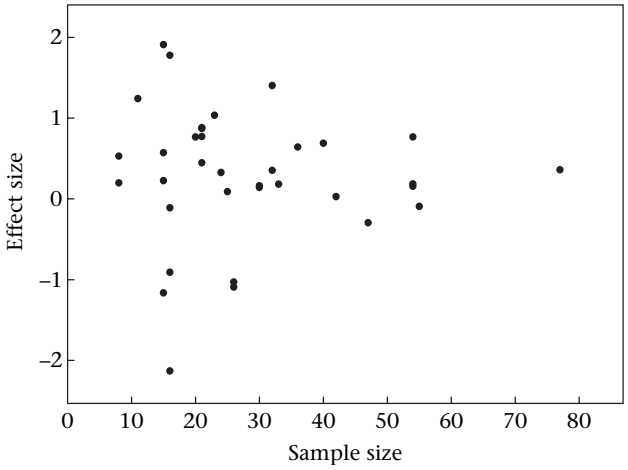

Figure 6. Funnel graph of all studies included in the meta-analysis. See text for details.

overall result should not be underestimated; and the fact that most studies finding a significant effect were not properly addressing the problem of endogenous testosterone production by castrating the experimental males before implantation should not be overlooked. Another caveat is the possibility that pseudoreplication may have confounded the result. In several studies, different measurements of immune parameters and parasite loads were carried out on the same individual birds, leading to an overestimate of sample size. In summary, the result of the meta-analysis gives only weak support to the ICHH, and only for certain taxa and certain indirect measures of immunocompetence, such as ectoparasite burdens.

THEORETICAL ADVANCES

A considerable number of published articles have explored the theoretical implications of the ICHH, and indeed the handicap paradigm generally. Immunosuppression may be expensive, but there is currently limited empirical evidence to suggest that it reduces survival or reproductive success. In the light of this, Kotiaho (2001) argued that from an evolutionary perspective immunosuppression is not costly per se, so may not be a true handicap (but see Saino et al. 1997). He also emphasized the point that differential costs incurred by high- and low-quality males should be taken into account in any test of the ICHH (Kotiaho 2001). Getty (2002) stated that high-quality signallers are more efficient than lower-quality individuals. He suggested that since they obtain only a marginal fitness gain from an incremental increase in signalling, the assumption that fit males will have fewer parasites than inferior males is flawed. Theoretically, fit males can have greater health and more parasites than poorer males, so measuring parasite loads as a way of testing the ICHH is unproductive (Getty 2002).

An important paper by Braude et al. (1999) postulated that rather than being immunosuppressive, testosterone affects the deployment of different arms of the immune system. This would explain the inconclusive results of many studies testing the immunosuppressive ability of testosterone. Testosterone may appear to decrease the number of circulating leukocytes, but in fact the cells have merely been redeployed to target tissues. They also suggest that testosterone may interact with corticosteroids that in turn affect the distribution of immune cells; as opposed to immunosuppression, Braude et al. coined this process as immunoredistribution. If testosterone is not immunosuppressive, then no cost is incurred by the signaller. This hypothesis is not therefore compatible with the ICHH. Kurtz et al. (2000) suggested a way in which immunoredistribution could be fitted into a handicap model. Under stressful conditions, tissue damage (caused by extreme physical exercise and increased metabolic rate resulting in increases in toxic by-products) may draw resources away from parasite defence. Immune cells would be redirected to eliminate damaged tissue, and so a trade-off occurs between different components of the immune system (Kurtz et al. 2000). This revised immunoredistribution model is compatible with the ICHH since honest signalling is maintained by the cost of parasite resistance in stressful conditions (i.e. during the breeding season).

Another model along the lines of the ICHH was proposed by Poiani et al. (2000). They suggested that in expressing both handicapping traits and 'badges of status' (intrasexual traits of dominance), hormones can be both immunosuppressive and immuno-enhancing either simultaneously or sequentially, depending on the hormone titre. The integrated immunocompetence model involves several hormones ensuring the honesty of a particular ornament (Poiani et al. 2000).

On the whole, most theoretical work carried out since 1992 has attempted to find alternative models to the ICHH because of the lack of conclusive empirical evidence to support the supposedly immunosuppressive nature of testosterone. Cost should be quantified from an evolutionary perspective, and the differential costs for high-quality and low-quality males examined. The direct measurement of parasite loads may be considerably less useful than direct measures of immunity (Getty 2002). Theoretical advances have focused on the possibility that testosterone may not be immunosuppressive directly, which might in part explain why so many studies have failed to find effects. If ultimately testosterone is found not to affect immunity in any way, then a resource allocation/body condition model (Wedekind & Folstad 1994) may be the best explanation for the maintenance of honest signals.

CONCLUSIONS

The results from the meta-analysis suggest a causal link between elevated testosterone levels and immunosuppression, but only within certain taxa and for certain measures of immunocompetence. Furthermore, this overall effect disappeared when we controlled for the nonindependence of studies and for the fact that some species are overrepresented. We would suggest, therefore, that while studies to date have provided support for the ICHH, this result should be treated with caution. There is at best weak evidence to suggest that testosterone directly influences immune function in males according to the ICHH. The high heterogeneity between studies suggests that the most reliable results come from the analysis conducted at the species level, which do not show overall support for the ICHH. Whether testosterone is even responsible for the production of many male secondary sexual characteristics is also controversial, although there is little doubt that testosterone is often involved in the stimulation of male sexual displays.

The results from the meta-analysis (conducted at the individual study level) clearly demonstrate support for the ICHH within reptiles and birds. The effect was not found in mammals, but this may be caused by the small number of studies included in the analysis. When we conducted the analysis at the species level, only the reptile studies provided evidence supporting the ICHH, and the reasons for this are not clear. Hillgarth & Wingfield (1997) suggested that, as testosterone levels are generally lower in birds than in mammals and show only short-term seasonal elevations, birds may not be the best group in

which to test the ICHH. Relevant to this is the observation that reptiles appear to possess higher seasonal concentrations of plasma testosterone than birds (e.g. in reptiles: Marler & Moore 1988; Thompson & Moore 1992; Diaz et al. 1994; Dunlap & Schall 1995; Knapp & Moore 1997; Tokarz et al. 1998; in birds: Charles et al. 1992; Cockrem & Seddon 1994; Saino & Møller 1995; Malecki et al. 1998; Peters 2000; Peters et al. 2001; Moore et al. 2002). We would suggest, therefore, that the stronger support for the ICHH in reptiles may be a result of higher levels of testosterone in this taxon, which might provide the scope for more efficient experimental manipulations. Our meta-analysis also provided support for studies investigating the effect of testosterone on ectoparasites, but not on endoparasites. This may be because levels of parasitism are easier to quantify for ectoparasites than endoparasites or because the aspects of the immune system that fight infection at the skin are more susceptible to suppression by testosterone. Certainly there is some evidence that endocrine levels can cause a redistribution of immune resources which might be particularly relevant to repelling ectoparasites (Braude et al 1999). Finally, testosterone may have an effect only on behaviour, and not directly on immunological variables. This would explain why increased testosterone had an effect on parasite loads (increased exploratory behaviour resulting in greater exposure to parasites), but not on any direct measure of immunity.

One issue that may be of concern here is whether all indexes of immune function are of equal value. As different immune tests test different aspects of the immune system, using a combination of tests is highly desirable in terms of estimating overall immunocompetence (Norris & Evans 2000). White blood cell counts are useful when measured in response to an experimental challenge, but initial counts alone could not differentiate between an immunocompetent individual and an immunochallenged individual (Sheldon & Verhulst 1996). All of the studies we considered in the meta-analysis that measured white blood cell counts attempted to do so after testosterone manipulation, but none of these studies measured an increase in white blood cell count in relation to an immune challenge. If increasing testosterone levels leads to an increase in access to dietary resources and condition, it is entirely plausible that testosterone might appear to be immunostimulating in these circumstances, rather than suppressive, as suggested by the results from the meta-analysis. We would suggest, therefore, that surveys of white blood cell counts used in this way are not a robust test of the ICHH. There is considerable difficulty in evaluating particular immune tests and whether they produce biologically meaningful results. Perhaps the best tests are those that produce a response to a biologically relevant antigen (Westneat et al. 2003). The use of multiple immune tests seems desirable (Norris & Evans 2000) but raises the potential problem of an increase in the likelihood of Type I errors.

While Folstad & Karter (1992) suggested that testosterone could mediate immunosuppression, they also concluded that other biologically active substances could mediate the ICHH indirectly. This idea has been followed up by a range of researchers (Møller 1995; Hillgarth & Wingfield 1997; Buchanan 2000; Evans et al. 2000). There is considerable potential for corticosterone to mediate the ICHH. If this route were relevant it might explain why so many studies have failed to find evidence to support the ICHH, because variation in the relation between testosterone and corticosterone introduces error into the analysis. Testosterone has been found to correlate with circulating corticosterone in several species (Schoech et al. 1999; Duffy et al. 2000; Evans et al. 2000; Poiani et al. 2000; Sockman & Schwabl 2001). However, although there is ample evidence that chronically elevated levels of glucocorticoids are immunosuppressive (Wingfield et al. 1997; Råberg et al. 1998; Buchanan 2000; but see Rodriguez et al. 2001), a number of studies have failed to show any link between testosterone and corticosterone titres (Wikelski et al. 1999; Astheimer et al. 2000; Buttemer & Astheimer 2000). This may be in part because levels of pulsatile hormones such as corticosterone are difficult to quantify, or because absolute circulating levels of these hormones are less important than the percentage of the hormone that is bound and inactive. In the dark-eyed junco, exogenous testosterone treatment increased the levels of plasma corticosteroid-binding globulins (CBG), which not only bind corticosterone but also testosterone, albeit with lower affinity (Klukowski et al. 1997; Deviche et al. 2001). This is important, since it suggests that testosterone and corticosterone may 'compete' for binding sites; an increase in exogenous testosterone elevates corticosterone but it also increases CBG, thereby slowing the corticosterone clearance rate (Klukowski et al. 1997). Conversely, an increase in plasma corticosterone could free up testosterone by binding to CBG, leading to increased free plasma testosterone (Deviche et al. 2001).

Robust tests of the hypothesis require well-designed manipulations of testosterone within natural variation in physiological levels, combined with biologically relevant tests of immunity. As this requires extremely careful experimental planning, it may be part of the reason for the lack of strong support for the ICHH to date. It is entirely plausible that the supposed immunosuppressive qualities of testosterone occur indirectly, making the effect difficult to demonstrate. In the future, new tests may involve the use of other measures of individual immune potential including the major histocompatibility complex (Mhc), a highly polymorphic set of genes involved in antigen presentation which has been linked to mate choice (for a review in birds see Zelano & Edwards 2002). There seems to be a potential association between Mhc type and testosterone production suggesting that certain Mhc types may allow individual males to bear the cost of elevated testosterone levels (Ditchkoff et al. 2001). In addition, the importance of dietary quality in influencing the response to immune function appears to be considerable (Klasing 1996) and in particular the restriction of the immune system by levels of essential amino acids (Klasing & Calvert 1999). This has been somewhat neglected in field studies to date and may be the source of substantial error in the assessment of immune function. Confirming this conclusion, carotenoids have recently been advocated in mediating both immunity and trait expression (Blount

et al. 2003; Faivre et al. 2003). Behaviour promoted by high testosterone levels, such as increased dominance over subordinates leading to better access to food, may explain why several studies have detected an immunoenhancing effect of testosterone (Evans et al. 2000). Greater access to food may result in better body condition and consequently increased immunocompetence (Evans et al. 2000). The results from the meta-analysis suggest that the argument in support of the ICHH will progress best by research branching into species that are as yet unstudied. In conclusion, the current weight of evidence provides weak support for the ICHH in its simplest form. However, we would suggest this may be in part because of experimental problems and the small number of studies (particularly in some taxa) that have addressed the hypothesis in a robust manner. Despite 12 years of active investigation, the ICHH seems far from an established solution to the evolution of complex sexual signals. Indeed, studies to date seem to have raised more questions than they have answered.

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