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REVIEW

Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis

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The challenge hypothesis (Wingfield et al. 1990, *American Naturalist*, **136**, 829–846) predicts varying androgen responses to mating, breeding or territorial behaviour in avian males. At the interspecific level, the highest androgen responsiveness has been observed in males from monogamous species with paternal incubation, and the lowest in males from promiscuous, nonpaternal species. Studies of a number of vertebrate species have discussed the extension of the challenge hypothesis predictions to nonavian vertebrates, but a general 'vertebrate consensus' has not been achieved. For this quantitative review we included data from all vertebrate species available in the literature into several meta-analyses. We distinguished between the effects of androgens on sexual, aggressive and paternal behaviour and the effects of behaviour on androgen levels and compared these effects between taxa, mating systems and types of parental care. We observed large variations between taxa in all data sets. Nevertheless, at the vertebrate level the challenge hypothesis predictions originating from the avian literature were confirmed for the modulation of androgen responsiveness (1) to sexual behaviour by paternal care but not by mating system, and (2) to paternal behaviour by mating system but not the degree of paternal care. In contrast, our results provide (3) no support for the predicted modulation of androgen levels in response to agonistic interactions by mating and parental care system at the vertebrate level. Furthermore, our meta-analyses suggest that the effects of exogenous testosterone on sexual and agonistic behaviour, as a rule of thumb, may be expected to be larger in nonpaternal than in paternal systems.

Androgens prominently regulate the conserved vertebrate reproductive axis (i.e. hypothalamic-pituitary-gonadal axis). Conversely, androgen levels may also be modulated by an individual's social environment and behaviour. Based on results from avian studies a conceptual framework for the social modulation of androgens known as the 'challenge hypothesis' has been proposed (Wingfield 1984; Wingfield et al. 1990, 2000). As a measure of an individual's androgen responsiveness, Wingfield et al. (1990) introduced the concepts of the constitutive nonbreeding androgen level (a), the breeding baseline (b; i.e. the average androgen level throughout all breeding phases) and the physiological maximum response to a territorial challenge (c). From these measures the androgen responsiveness, expressed as the ratio (c – a)/(b – a), allows the comparison of the androgen responsiveness to territorial challenges of individual males independent of nonbreeding baseline androgen levels. The challenge hypothesis combines the modulatory roles of male territorial aggression, mating and paternal behaviour on androgen responsiveness. It predicts variation of a male's androgen responsiveness to social challenges depending on the mating system and the degree of paternal investment. Thus, a monogamous and biparental male bird is predicted to respond at higher rates than a male from a polygynous and nonpaternal species. In addition to mating and parental care systems, other parameters of the social environment may also be involved in the variability of androgen responsiveness. Consequently, several questions have derived from the challenge hypothesis, such as how breeding dispersal, group density, social status (Beletsky et al. 1992; Hirschenhauser et al. 2003), alternative

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life histories (Moore 1991; Ketterson & Nolan 1992), and the predictability of the physical environment (Wingfield et al. 1993, 2000) modulate males' androgen levels, in particular breeding baseline levels (Hirschenhauser et al. 2003) and, therefore, androgen responsiveness.

The challenge hypothesis paved the way for studies on the social modulation of androgens also in nonavian vertebrate species. However, across the taxa the results of these studies were equivocal and there is no general consensus. In various teleost species, for example, high androgen levels were observed in response to sexual interactions, territorial challenges and at high population densities (Kindler et al. 1989; Cardwell & Liley 1991; Pankhurst & Barnett 1993; Oliveira et al. 1996, 2001b, 2002, 2003; Hirschenhauser et al. 2004), and decreased androgen levels were reported in paternal males (Kindler et al. 1989; Pankhurst & Barnett 1993; Oliveira et al. 2001a). However, paternal care, which is the most common mode of parental care among teleosts, was not always associated with the predicted low androgen levels. Male plainfin midshipman fish, *Porichthys notatus*, caring for eggs had similar androgen levels to courting males, but males caring for embryos in later stages of development had undetectable androgen levels (Knaap et al. 1999). In contrast, male black-chinned tilapia, *Sarotherodon melanotheron*, had low androgen levels during egg incubation, but not during later brooding phases, when the eggs had hatched (Kishida & Specker 2000). In the closely related St Peter's fish, *Sarotherodon galilaeus* (Ros et al. 2003) as well as in the rock-pool blenny, *Parablennius parvicornis* (Ros et al. 2004) male androgen patterns were not related to paternal care at any breeding stage. In amphibian species, behaviourally evoked androgen responsiveness was typically absent, as the challenge hypothesis predicts for promiscuous, nonpaternal species. However, in some toad species of the genus *Bufo* sexual and aggressive interactions resulted in profound elevations of male testosterone levels (Orchinik et al. 1988; Houck & Woodley 1995; Houck et al. 1996). Testosterone-implanted male green treefrogs, *Hyla cinerea*, showed an increased motivation for spontaneous mating calls; however, socially stimulated (in response to a chorus) implanted males called even less than intact males (Burmeister & Wilczynski 2001). Also in paternal Puerto Rican coqui, *Eleutherodactylus coqui*, males, testosterone implantation did not alter brooding behaviour (Townsend et al. 1991). Among reptiles the predicted small androgen responsiveness to (staged) male–male agonistic encounters for promiscuous species with no paternal care has been observed in most cases (Thompson & Moore 1992; Schuett et al. 1996; Klukowski & Nelson 1998), but in green anole lizards, *Anolis carolinensis*, winners responded with dramatically higher androgen levels than did losers (Greenberg & Crews 1990). In mammalian studies, results supporting the predictions of the challenge hypothesis have focused on male androgen responsiveness to sexual and aggressive interactions (Cavigelli & Pereira 2000; Buck & Barnes 2003; Goymann et al. 2003; Muller & Wrangham 2004). However, the complex social organization of some mammalian species seemed to result in contradictory testosterone patterns (Creel et al. 1993; Lynch et al. 2002). Studies of mammalian paternal care and testosterone also produced heterogeneous results (Ziegler et al. 1996; Creel et al. 1997; Reburn & Wynne-Edwards 1999; Nunes et al. 2000; Trainor & Marler 2001). The equivocal conclusions of the above-cited studies may be explained by methodological differences and the lack of a uniform test protocol or, alternatively, may reflect particular differences between taxa or social systems.

Adding a control for phylogeny in a recent re-examination of the challenge hypothesis in birds confirmed the robust effect of mating system on androgen responsiveness in an enlarged avian sample. The effect of paternal care, however, remained significant only in species with paternal investment during incubation, but the effects of paternal care on androgen responsiveness during later breeding phases, that is, feeding offspring, had disappeared (Hirschenhauser et al. 2003). In addition, the effect of mating system on androgen responsiveness has been confirmed in teleosts, although the effect of paternal investment turned out to be more complex (Oliveira et al. 2001c, 2002; Hirschenhauser et al. 2004). However, at the interspecific level, tests of the modulatory roles of mating system and the degree of paternal care on androgen responsiveness are restricted to taxa with sufficient variation of social systems. In contrast to birds and fish, the prevalence of promiscuity and nonpaternal care in amphibians, reptiles and mammals is reflected in the available literature on testosterone and behaviour, which has not allowed quantitative tests of the challenge hypothesis among these groups so far.

Therefore, we attempted to search for a vertebrate-wide concept for the social modulation of male androgens, by using meta-analysis. Meta-analysis is a quantitative approach to summarize a body of research by examining the magnitude and the generality of a predicted pattern, while taking sample sizes into account. It also provides a way to investigate the heterogeneity among studies, and to identify modulatory variables that may account for such heterogeneity (Hedges & Olkin 1985; Rosenthal 1991; Møller & Ninni 1998; Roberts et al. 2004). We analysed the existing literature on male androgen patterns in response to sexual and paternal behaviour across all vertebrates. Studies that tested the effects of territorial/agonistic behaviour on androgens in the various vertebrate taxa were more heterogeneous. We succeeded in collating sufficient numbers of studies examining the effects of group density and of dominance status on male androgen levels. Table 1 lists the particular predictions that were tested with separate meta-analyses. We also tested for variation between studies sampled during specific breeding phases and predicted that the effect of paternal effort on testosterone would be larger during incubation than during later breeding phases, that is, feeding or brooding (Hirschenhauser et al. 2003). All observed effect size patterns were subsequently tested for differences between the modulatory variables, that is, between taxa, mating systems and parental care systems.

METHODS

We carried out a literature survey collecting male androgen and behavioural data from various vertebrate species

Table 1. One-tailed predictions to be tested with meta-analyses of the existing specific literature in vertebrates

	Behavioural effects on T	Effects of exogenous T on behaviour
Sexual context	Did a male respond with increased T levels to the presence of or the interaction with a receptive female(s)?	Did T+ males show higher frequencies of sexual behaviour than C_0? Did T− decrease frequencies of male sexual behaviour compared with C_0?
Paternal context	Did paternal investment decrease male T levels compared with nonpaternal phases/males?	Did T+ males invest less in paternal care than C_0? Did T− increase paternal investment compared with C_0?
Agonistic context	Did dominant males have higher T levels than subordinate males?	Did T+ increase frequencies of agonistic behaviour?
	Did males living at higher group densities have higher T levels than conspecific males living in smaller groups or alone?	Did T− decrease frequencies of agonistic behaviour?

T: Testosterone. T+: males with experimentally elevated T levels; T−: experimental suppression of T levels; C_0: control males.

on specific questions deriving from the predictions of the challenge hypothesis. We distinguished between studies measuring the behavioural effects of androgen manipulations focusing on aggressive, sexual and paternal behaviour, from the reciprocal approach of studies measuring the effects of group density, dominance/subordinance, presence of or interaction with females and paternal behaviour on androgen levels. The specific questions were organized in separate meta-analysis data sets (Table 1). From each study sample sizes, arithmetic means and standard errors were required for a control and an experimental group. When the data needed were not given in the text or in tables, the published figures were scanned and the data extrapolated using the UnGraph software package (Biosoft 1998). A total of 196 articles on 168 vertebrate species provided sufficient information to be included in this study; the combined sample size for these was 7317 (studies and effect sizes are listed in the Supplementary material).

Meta-analyses

Meta-analysis quantitatively combines and evaluates the heterogeneity of a body of research data on a specific subject. It also offers the opportunity to explore the variability of effect sizes, both within and between categories of study or subject characteristics. Initially, a measure of the effect size (d) and the 95% confidence interval around this effect size are needed for each study. The effect size is calculated as the difference between the means of two studies (typically, an experimental and a control group), divided by their pooled standard deviation. Thus, the effect size is the difference in standard deviation units between the experimental and the control groups and, therefore, is independent of sample sizes and of the scales of measurement used in the experiments (Rosenberg et al. 1997). The 'overall effect size' ($d+$) is the cumulative weighted average of the effect size estimates for all studies included (Rosenberg et al. 1997). Conventionally the magnitudes of effect sizes are interpreted as small ($0.2 < d \leq 0.5$), moderate ($0.5 < d \leq 0.8$), large ($0.8 < d \leq 1$) and very large ($d > 1$; Cohen 1969). One of the assumptions of meta-analysis is the normal distribution of effect sizes. In cases where we observed a skewed effect size distribution (as determined by one-sample Kolmogorov–Smirnov tests: $P < 0.05$) we continued the analyses with arcsine-transformed data.

To analyse the effects of the modulating variables, we assigned all d per species/study to categories (i.e. taxa: amphibians, fishes, birds and mammals; social variables such as mating and parental care systems: monogamous, polygynous, promiscuous; and maternal, biparental, paternal or nonpaternal versus paternal). To compare these categories we used the between-class homogeneity statistic Q to test whether at least one of the true effect sizes differed from the rest. Q has approximately a χ^2 distribution ($k - 1$ degrees of freedom, k being the number of categories) and an associated P value (Hedges & Olkin 1985; Rosenthal 1991; Rosenberg et al. 1997; Archer et al. 1998; Roberts et al. 2004). The greater the observed value of Q, the greater was the heterogeneity of d among comparisons. When heterogeneity among modulator variables was evident, d differences between categories were evaluated based on confidence intervals: we interpreted a difference as significantly nonrandom ($P < 0.05$) if the 95% confidence intervals (CI) were not overlapping (chi-square test; Cohen 1969; Rosenberg et al. 1997; Book et al. 2001). Where there were only a few studies in a category ($N < 3$), these were either combined with other categories or dropped from the analysis. Numbers of studies in comparisons among modulatory variables may vary, because meta-analyses cannot be conducted if a category contains only one study (for example, exogenous testosterone effects on paternal behaviour contained only one polyandrous species and one mammalian study, which had to be excluded for comparisons between mating systems and between taxa, respectively; Table 2). All analyses were conducted following the methods outlined in Rosenthal (1991) and using the MetaWin 1.0 software (Rosenberg et al. 1997).

To control for the interaction between taxa and mating system or parental care we used two-way ANOVA of the effect sizes weighted by the reciprocal of the effect sizes' sampling variance (Cooper & Hedges 1994; Jennions et al. 2001).

Table 2. Effects of modulatory variables (taxum, mating system and parental care system) on the observed effects of behaviour on testosterone (T), as well as of the effects of exogenous T on behaviour with regard to the sexual, paternal and agonistic contexts

Modulator variables	Categories	Heterogeneities (*df*)	No. of species	Effect sizes per category *d* (95% CI)	
Sexual context					
Sexual behaviour effect on T					
Between taxa		75.48*** (4)	56		
	Amphibians		4	−0.023 (−0.579; 0.533)	a
	Reptiles		3	1.605 (1.241; 1.969)***	bd
	Fish		13	1.213 (0.929; 1.497)***	bc
	Birds		12	0.527 (0.146; 0.909)***	c
	Mammals		24	2.210 (1.909; 2.510)***	d
Between mating system		4.52 (2)	56		
	Monogamous		12	1.027 (0.683; 1.371)***	a
	Polygynous		22	1.491 (1.230; 1.751)***	a
	Promiscuous		22	1.368 (1.131; 1.604)***	a
Between parental care systems		17.46*** (1)	56		
	Nonpaternal		40	1.551 (1.366; 1.735)***	a
	Paternal		16	0.813 (0.520; 1.106)***	b
Overall mean effect size (*d*+)			56	1.342 (1.186; 1.498)	
Exogenous T on sexual behaviour					
Between taxa		171.12*** (3)	25		
	(Amphibia)		(1)	[−0.596 (−1.677; 0.585)]	
	Reptiles		4	7.586 (6.736; 8.436)	a
	Fish		6	1.040 (0.461; 1.619)***	b
	Birds		7	1.856 (1.370; 2.341)***	b
	Mammals		8	3.049 (2.424; 3.675)***	c
Between mating system		2.84 (1)	21		
	Polygynous		12	3.001 (2.552; 3.450)***	a
	Promiscuous		9	3.614 (2.552; 3.450)***	a
Between parental care systems		231.37*** (3)	25		
	No parental care		4	7.586 (6.736; 8.436)	a
	Maternal		10	4.480 (3.828; 5.133)***	b
	Biparental		5	1.422 (0.943; 1.902)***	c
	Paternal		6	0.673 (0.104; 1.241)***	c
Overall mean effect sizes (*d*+)					
Taxa; Parental care system			25	2.621 (2.322; 2.920)	
Mating system			21	3.243 (2.894; 3.592)	
Paternal care context					
Paternal behaviour effect on T					
Between taxa		107.13*** (2)	68		
	Fish		10	0.659 (0.284; 1.035)***	a
	Birds		49	2.469 (2.301; 2.637)***	b
	Mammals		9	0.942 (0.553; 1.332)***	a
Between mating system		55.53*** (2)	65		
	Monogamous		48	2.214 (2.055; 2.372)***	a
	Polygynous		11	0.872 (0.425; 1.319)***	b
	Promiscuous		6	0.635 (0.092; 1.177)***	b
Between parental care systems		0.0009 (1)	64		
	Biparental		51	1.978 (1.821; 2.135)***	a
	Paternal		13	1.984 (1.611; 2.357)***	a
Overall mean effect sizes (*d*+)					
Taxa			68	2.002 (1.859; 2.145)	
Mating system			65	1.963 (1.819; 2.107)	
Parental care system			64	1.979 (1.834; 2.124)	
Exogenous T on paternal behaviour					
Between taxa		6.470 (1)	14		
	Fish		4	1.427 (0.682; 2.171)***	a
	Birds		10	2.512 (2.131; 2.893)***	a
Between mating system		0.306 (1)	14		
	Monogamous		7	2.079 (1.588; 2.571)***	a
	Polygynous		7	2.285 (1.745; 2.825)***	a
Between parental care systems		47.299*** (1)	15		
	Biparental		9	3.246 (2.786; 3.706)***	a
	Paternal		6	0.877 (0.383; 1.372)***	b
Overall mean effect sizes (*d*+)					
Taxa			14	2.287 (1.948; 2.626)	
Mating system			14	2.173 (1.809; 2.536)	
Parental care system			15	2.148 (1.811; 2.484)	

Table 2 (*continued*).

Modulator variables	Categories	Heterogeneities (*df*)	No. of species	Effect sizes per category *d* (95% CI)	
Agonistic context					
Dominance effect on T					
Between taxa		27.63*** (3)	42		
	Reptiles		3	0.498 (−0.118; 1.115)	ab*
	Fish		12	1.082 (0.749; 1.416)***	ac
	Birds		10	0.249 (−0.054; 0.553)	b*
	Mammals		17	1.296 (1.012; 1.581)***	c
Between mating system		18.04*** (2)	41		
	Monogamous		10	0.307 (−0.005; 0.620)	ab
	Polygynous		13	1.175 (0.923; 1.428)***	a
	Promiscuous		18	0.768 (0.421; 1.115)***	b*
Between parental care systems		31.67*** (3)	42		
	No parental care		5	1.010 (0.501; 1.591***	ab
	Maternal		21	1.117 (0.845; 1.389)***	a
	Biparental		11	0.323 (0.057; 0.590)***	b
	Paternal		5	1.832 (1.290; 2.374)***	a
Overall mean effect sizes (*d*+)					
Taxa; Parental care system			42	0.854 (0.685; 1.024)	
Mating system			41	0.817 (0.646; 0.988)	
Group density effect on T					
Between taxa		182.60*** (4)			
	Amphibia		5	0.885 (0.331; 1.438)***	a
	Reptiles		2	−1.322 (−2.026; −0.618)***	b
	Fish		6	1.713 (1.283; 2.143)***	a
	Birds		8	1.271 (0.965; 1.578)***	a
	Mammals		5	4.485 (3.935; 5.035)***	c
Between mating system		29.95*** (2)			
	Monogamous		11	1.806 (1.332; 2.280)***	ab
	Polygynous		8	1.135 (0.880; 1.391)***	a
	Promiscuous		7	2.553 (2.099; 3.008)***	b
Between parental care systems		7.41 (1)			
	Nonpaternal		15	1.746 (1.494; 1.999)***	a
	Paternal		11	1.164 (0.829; 1.499)***	a
Overall mean effect size (*d*+)			26	1.536 (1.334; 1.740)	
Exogenous T on aggressive behaviour					
Between taxa		94.042*** (3)			
	Reptiles		3	3.277 (2.768; 3.787)***	a
	Fish		12	1.037 (0.629; 1.444)***	b
	Birds		10	0.385 (0.098; 0.672)***	b
	Mammals		9	1.013 (0.642; 1.384)***	b
Between mating system		125.519*** (2)			
	Monogamous		11	0.492 (0.145; 0.839)***	a
	Polygynous		13	0.397 (0.124; 0.670)***	b
	Promiscuous		10	2.847 (2.483; 3.211)***	b
Between parental care systems		112.975*** (3)			
	No parental care		4	3.379 (2.871; 3.886)***	a
	Maternal		10	1.175 (0.886; 1.463)***	b
	Biparental		12	0.340 (0.016; 0.664)***	c
	Paternal		8	0.115 (−0.394; 0.623)	c*
Overall mean effect size (*d*+)			34	1.055 (0.870; 1.240)	

The overall mean effect sizes *d*+ (95% confidence intervals, CI), and the mean effect sizes *d* (95% confidence intervals) for each category of the modulatory variables are summarized. With regard to the paternal context, note that positive effect sizes indicate the confirmation of the predicted decreasing effect of paternal behaviour on T. Asterisks indicate the significance of the heterogeneity and the effect within categories (chi-square test: $P < 0.001$). Different letters indicate significantly different effect sizes between the categories, asterisks next to letters signify ranges of confidence intervals overlapping zero. The studies used for each variable are listed in the Supplementary material.

RESULTS

All observed overall mean effect sizes were classified as very large ($d+ > 1$; Table 2). On average the predicted behavioural effects on testosterone (T) were observed in $76 \pm 7\%$ ($\overline{X} \pm \mathrm{SEM}$) of all studies on behavioural effects on T included in the meta-analyses ($N = 194$; Supplementary material, Table S1), and the expected effects of exogenous T on behaviour were reported in $79 \pm 8\%$ of the 74 studies included (Supplementary material, Table S2). In five of the seven meta-analysis data sets, d values did not differ significantly from a normal distribution (Kolmogorov–Smirnov tests: NS), except for the data in 'exogenous T effect on sexual behaviour' and in 'paternal effort effect on T' ($Z = 1.6$, $N = 27$, $P < 0.01$ and $Z = 1.9$, $N = 70$, $P < 0.001$, respectively), which were normally distributed after arcsine transformation ($Z = 1.9$, $N = 27$, NS and $Z = 1.2$, $N = 70$, NS, respectively).

Sexual Context

The set of effect sizes of sexual behaviour on T was heterogeneous between taxa and was due to paternal care, but not mating systems (Table 2). The observed behavioural effects on T were larger in mammals (dominated by promiscuous nonpaternal systems) than in all other taxa; the mean effect size among amphibians, however, was close to zero. No differences resulting from mating strategies were observed (Table 2). As would be predicted by the challenge hypothesis, the effects of sexual behaviour on T were smaller in paternal species (including $N = 14$ biparental and $N = 2$ paternal systems) than in nonpaternal species (including $N = 9$ species with no care and $N = 31$ maternal species; Table 2). This indicates that independent of mating system, high T levels were strongly associated with high frequencies of sexual behaviour, with the largest effects observed in nonpaternal vertebrate species, particularly in mammals.

The effects of exogenous T on sexual behaviour also varied to a high degree both among taxa and resulting from paternal care (Table 2). The effect size magnitudes were largest in reptiles (all of which lack parental care), and were larger in mammals than in fish and birds. Across parental care systems this pattern was also present: the effects of exogenous T on sexual behaviour were larger in nonpaternal species (no parental care or maternal brooders) than in biparental or paternal species. Effects of T on sexual behaviour were homogeneous in the two mating systems included in this sample (Table 2). However, no differences between the sexual responsiveness to exogenous T of polygynous and promiscuous species would have been predicted by the challenge hypothesis.

We controlled for the interaction of the independent variables taxa and parental care by using a weighted two-way ANOVA on effect size. The heterogeneity between categories was significant for the exogenous T effects on sexual behaviour but not for the sexual behaviour effects on T (Table 3). This indicates that the pattern of T effects on sexual behaviour between different taxa was not independent of the parental care pattern, whereas the effect size patterns of sexual behaviour on T resulting from parental care were probably robust to the variation between taxa.

Paternal Care Context

The magnitudes of paternal care effects on T were heterogeneous across taxa and among mating systems, but not with regard to the degree of paternal care (Table 2). Large effects of paternal behaviour on T were observed in birds (dominated by monogamous and biparental systems), but were less pronounced in fish and mammals. As would be predicted by the challenge hypothesis, the effects of paternal care on T were larger in monogamous than in polygynous or promiscuous systems (Table 2). On the other hand, an effect of the degree of paternal investment, that is between biparental and paternal species, on T was absent in our sample (Table 2). However, a weighted two-way ANOVA of effect sizes by taxa and mating system indicated that the observed patterns of these two variables were not independent of each other (Table 3).

To underline recent results on the specific contribution of paternal incubation to the interspecific variation of androgen responsiveness (Hirschenhauser et al. 2003), we also compared the variation of effect sizes resulting from the specific breeding phase sampled (Supplementary material, Table S1). Throughout all vertebrates, the reported effect of paternal effort on T was larger if sampled during incubation ($N = 14$; $d = 3.65$; CI = 3.25, 4.05) than during feeding phases ($N = 16$; $d = 3.17$; CI = 2.78, 3.56). In addition, within the avian subsample the reported effect of paternal effort on T was larger if sampled during incubation

Table 3. Summary of the results of a weighted two-way ANOVA to control for the interaction between observed modulatory variables, that is taxa and mating system or parental care

Context	Data set (Modulator variables)	Q_b	df	F	P
Sexual context	Sexual behaviour effect on T (Taxa+parental care)	30.35	7	2.06	(0.067)
	Exogenous T on sexual behaviour (Taxa+parental care)	24.02	8	3.12	0.025
Paternal care context	Paternal behaviour effect on T (Taxa+mating system)	38.96	10	3.0	0.004
	Exogenous T on paternal behaviour (Taxa+parental care)	10.84	2	3.15	(0.083)
Agonistic context	Dominance effect on T (Taxa+parental care)	16.77	9	0.67	NS
	Group density effect on T (Taxa+mating system)	9.80	11	0.81	NS
	Exogenous T on aggressive behaviour (Taxa+mating system)	18.15	7	0.93	NS

Q_b are heterogeneities between categories as indicated by the sums of squares from the ANOVA.

($N = 8$; $d = 4.32$; CI = 3.66, 4.99) than during the feeding offspring phase ($N = 16$; $d = 3.17$; CI = 2.78, 3.56).

Only one of the tested modulatory variables, the degree of paternal care, significantly explained the observed variation of the effects of exogenous T on paternal behaviour: exogenous T decreased paternal behaviour more in biparental than in paternal species (Table 2). Heterogeneity was not significant across taxa and among mating systems (Table 2), although the pattern tended to reflect the larger behavioural responsiveness in birds than in teleost species, which was previously observed for the effects of paternal behaviour on T. However, 95% confidence intervals were overlapping, as variation was large and sample sizes rather small in this sample ($N = 14$ with only 4 fish species; Table 2). Yet, a weighted two-way ANOVA indicated no significant interaction between T effects on paternal behaviour resulting from taxa and parental care system (Table 3).

Agonistic Context

For the agonistic context, three data sets were analysed in separate meta-analyses, that is, the effects of dominance and of group density on T, and the effects of exogenous T on frequencies of agonistic interactions. In all three analyses the effect sizes were heterogeneous across taxa, between mating systems, and resulting from paternal care, except for the density effect on T by parental care (Table 2). In all of the agonistic context data sets the observed effect size patterns resulting from mating system or parental care were sufficiently robust to explain the variation between taxa (Table 3).

Higher T in dominant than subordinate males was observed in fish and mammals. Among birds the mean effect size was close to zero and, as in reptiles, its range overlapped zero (Table 2). In monogamous and biparental systems the observed effects of dominance status on T were also only moderate and close to zero (Table 2). These categories were dominated by avian species; however, they also included some teleost and mammalian species (see Supplementary material, Table S1: *Neolamprologus pulcher*, *Tilapia zillii*, *Homo sapiens* and *Meriones unguiculatus*). The largest effects of dominance status on T were found in systems with paternal care (Table 2).

The predicted increasing effects of high group density on T were dramatically larger in mammals than in any of the other taxa (Table 2). In reptiles (although containing only two species) the direction of the observed effects was negative, which represents the converse to the test prediction, that is, T levels at high group densities were even lower than in low-density situations. Altogether this resulted in one of the largest values of Q_b observed in this study (Table 2). The assumption of higher T at higher group densities was only moderately confirmed in studies with polygynous species; however, there was no difference between monogamous and promiscuous systems (Table 2). No d differences were observed between nonpaternal (including no care and maternal) and paternal (including biparental) vertebrate species (Table 2).

Reinforcing effects of exogenous T on male aggressiveness were relatively homogeneous among nonreptilian taxa (Table 2). The smallest effect sizes came from avian studies. The effects observed in the three reptile species, however, were dramatically larger than in all nonreptilian taxa (Table 2). Even though the reptile category has a low sample size ($N = 3$ species), it may be relevant to report this curious pattern. Exogenous T effects on agonistic behaviour were larger in promiscuous mating systems than in polygynous and monogamous species. Corresponding to the mating system pattern, larger effects were observed in nonpaternal systems than in biparental and paternal systems, both of which had even effect sizes close to zero (Table 2).

DISCUSSION

Publication Bias

Publication bias is the result of missing studies that might exist in a meta-analysis and the effect that these studies might have had on its outcome. This bias occurs whenever the strength or direction of the results of published and unpublished studies differs (Møller & Jennions 2001). To identify publication bias we used funnel plots (Fig. 1; Gates 2002) and the associated rank correlation test of Begg & Mazumdar (1994).

If the effect sizes derive from a random sample of studies using similar research methods, small studies should generate a greater range of variance than larger studies, resulting in a funnel-shaped relation between effect size and sample size (Light & Pillemer 1984; Møller & Jennions 2001). In four of the seven data sets the funnel-shaped relation between effect size and sample size was observed (sexual behaviour and dominance effect on T, and exogenous T effects on paternal and aggressive behaviours; Fig. 1a, d, e, g), which indicates a robust sample for these analyses. The plots of exogenous T effects on sexual behaviour and of group density effects on T, however, showed an outlier each (Fig. 1b, f). In both outlier studies, the effect size (d) differed from the overall mean effect size ($d+$) even though the study was based on a large sample (Table 2). The funnel plot of effects of paternal effort on T resulted in the most asymmetric pattern (Fig. 1c). This indicates a possible reporting bias, or the presence of bias from low methodological quality of smaller studies on the effects of paternal behaviour on T (Begg & Mazumdar 1994). However, Tang & Liu (2000) criticized the simplistic conclusion that a meta-analysis is biased based on the informal observation of an asymmetric funnel plot and suggested that one should interpret it as a ‘precision-related’ heterogeneity.

Therefore, we also applied the associated formal rank correlation test of Begg & Mazumdar (1994) to test statistically for asymmetry of the data. This method predicts that if there is publication bias, effect size is expected to weaken as sample size increases. Despite the patterns observed by graphical funnel shape inspection, in none of the data sets were the sample sizes significantly correlated with the standardized effect sizes (Begg & Mazumdar correlations: $r_s < 0.2$, NS in all contexts). This suggests that small trials had similar relative effects to those of large trials. Thus, the effects of paternal effort on T, for example, may

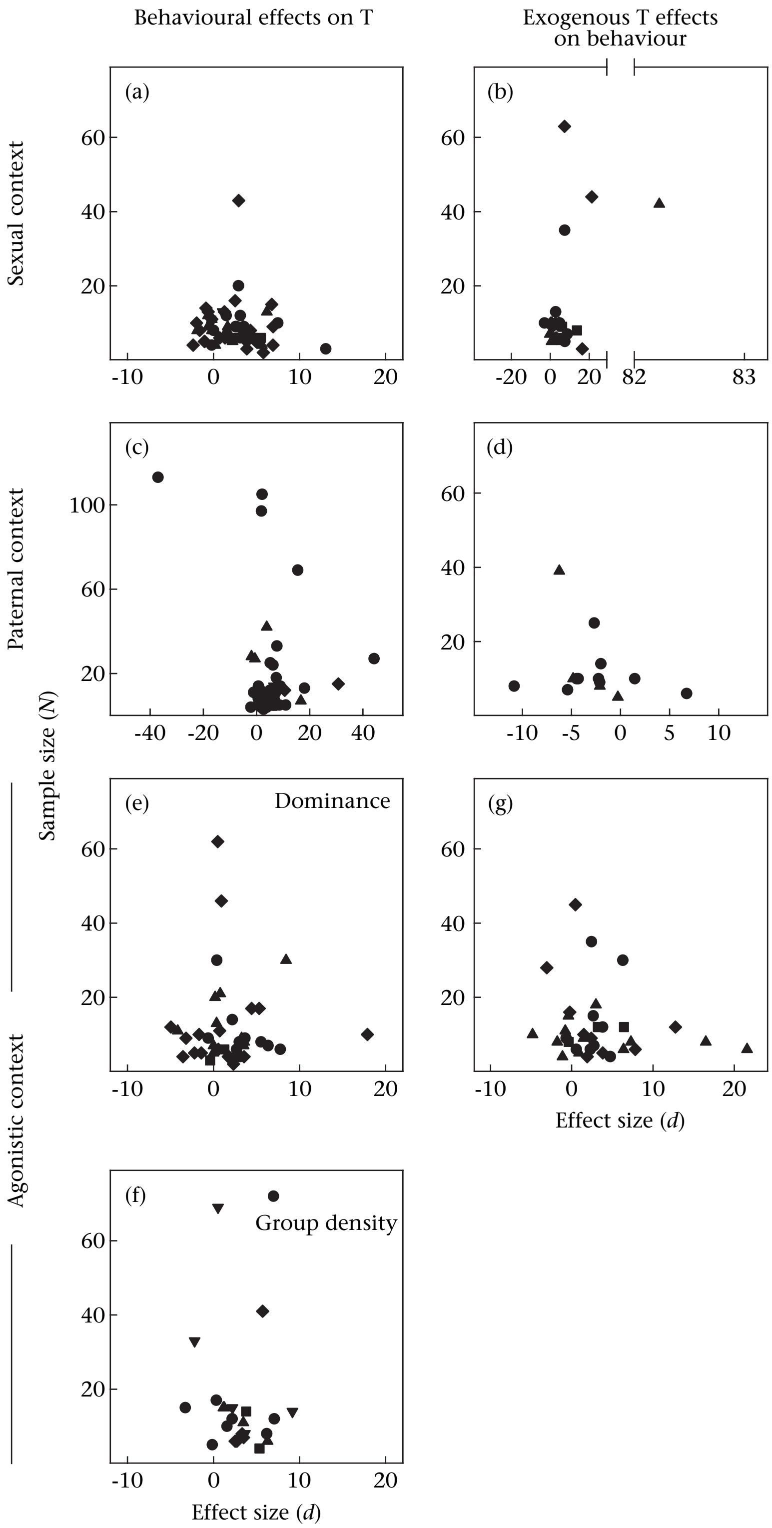

Figure 1. Funnel plots of effect size according to sample size of individual studies for detection of publication bias in the meta-analyses (Møller & Jennions 2001; Gates 2002). Behavioural effects on T and exogenous T effects on behaviour are shown. (a, b) Sexual context, (c, d) paternal context, (e, f, g) agonistic context. ▼: Amphibians; ■: reptiles; ▲: fish; ●: birds; ◆: mammals.

still be considered as a representative data set, despite the observed asymmetric funnel shape.

Sexual Context

The effects of sexual behaviour on T, as well as the reverse effects of exogenous T on sexual behaviour, were largest in nonpaternal species, which was particularly well reflected among mammals and reptiles (Table 2). In both directions, we observed a clear modulatory role for parental care system, in which effect sizes decreased the more paternal care was involved (Table 2). Although the asymmetric funnel plot indicated some bias in this sample (Fig. 1b), these results complement a recent experimental study using five closely related cichlid species with divergent mating systems. In cichlids, androgen responses to an interaction with ovulating females were observed in males from maternal but not biparental species (Hirschenhauser et al. 2004). Furthermore, in a literature survey on 59 teleost species the interspecific patterns of androgen responsiveness related systematically with variation among breeding baseline androgen levels (i.e. the average androgen level throughout all breeding phases as defined by Wingfield et al. 1990) rather than among the observed physiological maximum levels in response to territorial challenges (Oliveira et al. 2001c). In line with this, the results of the present meta-analyses add evidence for a systematic variation in the magnitude of the androgen responsiveness to females with a parental care pattern.

Mating system was not related to the interaction between mating behaviour and T in either direction (Table 2). However, in the case of exogenous T effects on sexual behaviour the variation in mating strategies (which included promiscuous and polygynous systems) was not optimally covered in the current sample. Originally, we had also scanned the data from three monogamous species (Supplementary material, Table S2: *Colinus virgianus texanus*, *Zonotrichia leucophrys gambelii* and *Homo sapiens*). However, because the sample was small and heterogeneous, and the range of effect sizes overlapped zero (indicating a nonsignificant effect; Rosenthal 1991; Rosenberg et al. 1997), we excluded these from the mating system comparisons post hoc (Table 2). A larger sample of data on the effects of exogenous T on sexual behaviour in monogamous species would be desirable to clarify this pattern. For the remaining comparison between polygynous and promiscuous species, behavioural responsiveness to exogenous T was homogenous (Table 2), as the challenge hypothesis predicts. In sum, our results indicate that, independent of the mating system, high T levels corresponded robustly to high frequencies of sexual behaviour, with the largest effects being observed in nonpaternal species, particularly in mammals. In the case of sexual behaviour effects on T, the patterns caused by taxa and parental care were independent of each other (Table 3). However, the large effects of exogenous T on sexual behaviour in nonpaternal systems reflected the large effects in reptiles and mammals (Table 3).

Paternal Care Context

The effect size patterns observed for the paternal behaviour effects on T did not clearly correspond with the inverse effects of exogenous T effects on paternal behaviour (Table 2). Tests for publication bias in the 'paternal effort effects on T' were inconclusive: the informal funnel plot indicated a lack of studies with nonsignificant or contradictory results (Fig. 1c), whereas the formal Begg & Mazumdar correlation for asymmetry was far from significant ($r_s = 0.01$, $N = 70$, $P = 0.916$). In the absence of a consensus on the existence of publication bias, the biological relevance of the results, that is, the matching of the meta-analysis patterns with the predictions of the challenge hypothesis based on a large number of studies ($N = 70$) included in this data set, is self-explanatory.

The decreasing effect of paternal investment on T varied with mating system, but not with parental care system. However, this result was probably biased by the dominating proportion of bird species in the category 'monogamous' as indicated by the significant interaction of the modulatory variables taxa and mating system (Table 3). In addition, in a literature survey of 83 bird species the relation between paternal investment and androgen responsiveness (Wingfield et al. 1990) was not present when data were controlled for phylogenetic bias, whereas the effect of mating strategy remained significant (Hirschenhauser et al. 2003). Hirschenhauser et al. (2003) suggested that, if any, paternal investment during incubation, rather than during later breeding phases, contributed to the evolution of different androgen responsiveness patterns. The present meta-analyses confirmed the specifically large effect of paternal behaviour during incubation throughout all vertebrates, as well as within the avian subsample.

In contrast, the exogenous effects of T on paternal behaviour varied not with mating system, but with the degree of paternal care, with effect sizes three-fold larger in biparental than in paternal species (Table 2). This pattern seemed to reflect the abundance of specific parental care systems in the two taxa included in this file: the large effects in species with biparental care (i.e. the prevalent system among birds) compared with only moderate effects among species with exclusive paternal care (i.e. the most abundant parenting system among teleosts). However, for the exogenous effects of T on paternal behaviour there was no significant interaction between the modulatory variables taxa and degree of parental care (Table 3). In the biparental California mouse, *Peromyscus californicus*, T maintained paternal behaviour with a very large effect size and aggressive behaviour was not reduced by castration (Trainor & Marler 2001; Supplementary material, Table S2). Unfortunately, this was the only available experimental study of a biparental mammal to be included in the analysis. Thus, the current meta-analysis cannot directly elucidate the role of T in mammalian paternal behaviour. However, the observed effects of mammalian paternal behaviour on T were less pronounced than in birds and the predicted modulatory roles of paternal care and mating system were lacking (Table 2). In addition, in amphibians the effect of T on paternal behaviour remains to be resolved (Townsend & Moger 1987; Townsend et al. 1991). However, the sparse sample of studies included in our meta-analyses generally suggests only moderate T responsiveness to behaviour in amphibians (Table 2). To sum up, more data are needed for the exogenous effects of T on paternal behaviour from monogamous species, particularly from nonavian taxa.

Agonistic Context

The patterns of exogenous effects of T on male aggression seemed to be dominated by the curiously large effect sizes in reptiles (Table 2), of which all were promiscuous with no parental care. To control for the influence of these outlier data points, we repeated the analysis without them and found that the larger effects in promiscuous and nonpaternal systems remained robust even when the reptiles were excluded. Therefore, we suggest that T implantation studies may be expected to result in larger effects on male aggression in promiscuous and nonpaternal species than in monogamous and paternal species.

In two meta-analyses of the agonistic context (dominance effects on T and exogenous T effects on male aggression), only moderate effect sizes of the interactions between agonistic behaviour and T were observed in monogamous and biparental systems and consequently also in birds. The largest effects of dominance status on T, however, were observed in paternal species (Table 2). The effects of group density on T in mammals were the largest observed in all of the presented meta-analyses ($d = 4.485$), which also contributed to the large group density effects on T in promiscuous mating systems. However, in both data sets no significant interaction between the modulatory variables was observed (Table 3). Altogether, the present sample failed to confirm a consistent pattern of agonistic behaviour effects on T, even though the funnel plots (Fig. 1e, g, f) showed that the literature samples used for the meta-analyses of the agonistic context were largely symmetrical and, thus, robust.

The data on agonistic behaviour in the various vertebrate species were the most heterogeneous sample, which made generalizations and simplifications especially difficult in this context. The studies included in our meta-analyses were all on male–male aggression; however, aggressive behaviour might still have been the result of conflicts over territories, females, food or nest defence behaviour. Therefore, we conducted additional comparisons to identify the confounding effect of the context of aggression in our sample, that is, we classified the agonistic context of the studies included and compared the mean effect sizes. With regard to dominance effects on T, higher T in territory owners than in nonterritorial males (for example floaters) was observed with larger effect sizes ($d = 2.20$, $N = 7$, CI = 1.77, 2.64) than the observed differences in studies comparing winners with losers ($d = 0.61$, $N = 35$, CI = 0.43, 0.80). Increasing effects of high group density on T were larger in studies focusing on aggressive behaviours ($d = 3.13$, $N = 7$, CI = 2.73, 3.53) than in studies with focus on courtship behaviour ($d = 0.88$, $N = 10$, CI = 0.60, 1.15). Exogenous T affected territorial male–male aggression ($d = 1.19$, $N = 28$, CI = 0.99, 1.39) with significantly larger effect sizes than nest defence studies ($d = 0.24$, $N = 6$, CI = −0.25, 0.73). Thus, the different contexts and measures of aggression have confounded the results of all three meta-analyses on the interaction between agonistic behaviour and T. In addition, the stimulus intensity of a territorial challenge (i.e. duration or number of actors) may affect the resulting androgen responses (Hau et al. 2004), which may be another confounding variable in the present literature sample.

How do the meta-analyses patterns across all vertebrates fit into the challenge hypothesis prediction, that is, androgen responsiveness to territorial challenges should be larger in monogamous and paternal species than in polygynous and nonpaternal species (Wingfield et al. 1990)? The predicted larger androgen responsiveness in monogamous and biparental species is based on lower breeding baseline T levels in monogamous than in polygynous species (birds: Wingfield et al. 1990; teleost fish: Oliveira et al. 2001c). Starting from lower baseline levels, the (situational) short-term T increase in response to a territorial challenge should result in a larger androgen responsiveness in monogamous than in polygynous species. The data collected for the group density effects were indirect measures of 'agonistic behaviour', which we assume to be, in fact, measures of the social environment rather than direct effects of situational agonistic conflicts. These allow the assumption of secondarily involved higher levels of male aggressiveness throughout the breeding season. The meta-analysis of group density effects was, therefore, investigating the effects of high levels of agonistic interactions on baseline androgen levels. In contrast, the dominance–subordinance effects on T may have recorded effects of situational aggression as short-term T responses. However, the agonistic behaviour effects on T (baseline or situational) failed to confirm the challenge hypothesis predictions (Table 2). In most vertebrate species, elevated T concentrations do not activate aggression per se, but rather increase the frequency of aggressive behaviour and promote more intensive fighting among males (Wingfield et al. 1999). The potential measures of the intensity of an aggressive behaviour may be a highly species-specific issue and it is therefore likely to be impossible to compare such measures, for example, between mice and geese. Furthermore, in tropical bird species with year-round territorial aggression the association between T and aggression is not always consistent, which suggests the involvement of possible 'nontraditional' hormonal mechanisms, such as aromatization to 17β-oestradiol or dehydroepiandrosterone as an androgen precursor of adrenal origin, in the regulation of territorial aggression (Wingfield 1994; Hau et al. 2000, 2004; Wingfield et al. 2001; Soma et al. 2002).

CONCLUSION

Taken together, in all contexts there were large differences in the effect size magnitudes of androgen responsiveness between taxa. Androgen responsiveness to sexual behaviour and to high group densities was observed, with the largest effect sizes in reptiles and mammals, whereas androgen responsiveness to paternal involvement had the largest effect sizes in birds. Overall, at the vertebrate level the challenge hypothesis predictions originating from the avian literature were confirmed for the previously suggested modulation of androgen responsiveness to sexual behaviour by paternal care but not by mating system (as observed in cichlids, Hirschenhauser et al. 2004), and to paternal behaviour by mating system but not the degree of paternal care (as observed in birds, Hirschenhauser et al. 2003). However, the current results provide no support for the predicted modulation of either baseline or situational androgen levels in response to high levels of agonistic interactions by mating and parental care system (Wingfield et al. 1990, 2000) at the vertebrate level. Furthermore, our meta-analyses suggest that studies focusing on the effects of exogenous T on sexual and agonistic behaviour may, as a rule of thumb, expect larger effects in nonpaternal than in paternal systems (Table 2).

The agreement in patterns of behavioural effects on T with T effects on behaviour was reasonable in the sexual context and to some degree in the agonistic context (with regard to group density effects, but not with the patterns from dominance effects). Therefore, the observed interactions between T and sexual or agonistic behaviours may be considered as mutual. Nevertheless, the paternal context results differed because of the studied direction of the interaction: decreasing effects of paternal behaviour on T were related to mating system, but not parental care, whereas the decreasing effects of exogenous T on paternal behaviour were related to the degree of paternal care, but not to mating system (Table 2).

The proposed conclusions based on meta-analyses across all vertebrates should be taken with caution. In the future these conclusions may be confirmed, criticized and enriched by new empirical studies, particularly on species in those categories that suffer from small sample sizes and possible publication bias in this study. Generalizations such as these are predestined to overlook the specific natural history details that make the research on a particular species so rewarding. However, 'mixing "apples and oranges" may be appropriate if we wish to generalize to the level of the "fruit"' (Rosenthal 1991, page 129).

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version at doi:10.1016/j.anbehav.2005.04.014.

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