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Distinguishing seasonal androgen responses from male-male androgen responsiveness – revisiting the Challenge Hypothesis

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Abstract

Androgen levels show strong patterns throughout the year in male vertebrates and play an important role in the seasonal modulation of the frequency, intensity, and persistence of aggression. The Challenge Hypothesis (Wingfield et al. 1990) predicts that seasonal patterns in androgen levels vary as a function of mating system, male-male aggression and paternal care. Although many studies have addressed these predictions, investigators have often assumed that the ratio of the breeding season maximum and breeding baseline concentrations (termed “androgen responsiveness”) reflects hormonal responses due to social stimulation. However, increasing evidence suggests that seasonal androgen elevations are not necessarily caused by social interactions between males. Here, we separate the seasonal androgen response ($R_{seasonal}$) and the androgen responsiveness to male-male competition ($R_{male-male}$) to begin to distinguish between different kinds of hormonal responses. We demonstrate that $R_{seasonal}$ and $R_{male-male}$ are fundamentally different and should be treated as separate variables. Differences are particularly evident in single-brooded male birds that show no increase in plasma androgen levels during simulated territorial intrusions (STIs), even though $R_{seasonal}$ is elevated. In multiple-brooded species, STIs typically elicit a rise in androgens. We relate these findings to the natural history of single - and multiple-brooded species and suggest a research approach that could be utilized to increase our understanding of the factors that determine different types of androgen responses. This approach does not only include $R_{seasonal}$ and $R_{male-male}$, but also the androgen responsiveness to receptive females ($R_{male-female}$) and to non-social environmental cues ($R_{environmental}$), as well as the physiological capacity to produce and secrete androgens ($R_{potential}$). Through such studies, we can begin to better understand how social and environmental factors may lead to differences in androgen responses.

Key words: aggression, androgen responsiveness, birds, Challenge Hypothesis, corticosterone, estradiol, resident-intruder paradigm, simulated territorial intrusion, testosterone

Introduction

Androgens such as testosterone are intimately associated with reproduction and affect a variety of morphological, physiological and behavioral traits. Therefore, they have been the focus of many integrative studies on vertebrate physiology, life-history, ecology and evolution (e.g. Marler et al., 1988; Ketterson and Nolan, 1992; Ketterson et al., 1999; Sinervo et al., 2000; Ricklefs and Wikelski, 2002). For example, androgens induce muscle hypertrophy and are responsible for the development of accessory reproductive organs and some secondary sex characters. They regulate spermatogenesis and provide a negative feedback signal for gonadotropin and GnRH secretion. Furthermore, androgens affect sexual and aggressive behaviors, at least in reproductive contexts (reviewed in Wingfield et al., 2001).

Wingfield (1984b) proposed that androgen levels above breeding baseline function to increase the frequency and intensity of aggression in males, especially when hierarchies are being established or when dominance relationships are challenged. Although it is now clear that androgens can strongly influence the behavior of males, behavioral and social cues can also feed back to the brain to trigger the release of androgens and other hormones. Studies have demonstrated rapid effects of social interactions on plasma concentrations of androgens in a wide array of vertebrate taxa, such as fish, amphibians, reptiles, birds, and mammals including humans (for recent reviews see Oliveira, 2004; Archer, 2006; Hirschenhauser and Oliveira, 2006). For example, in association with experimentally-induced social challenge, plasma androgen levels of males have been found to increase to adjust the readiness and intensity of agonistic and sexual display behavior (e.g. Wingfield and Wada, 1989; Gwinner et al., 2002; Hirschenhauser et al., 2004; for a review on all vertebrate taxa see Hirschenhauser and Oliveira, 2006). In line with these data, seasonally breeding birds with a high degree of male-male competition show a correlation between high plasma androgens and periods of social instability and/or when females are receptive (Fig. 1). In contrast, high concentrations of circulating androgens are virtually absent in species that do not compete for territories or mates, such as in Western gulls (*Larus occidentalis;* Fig. 1) or in seabirds with long-term pairbonds (e.g. Lormée et al. 2000; Goymann et al. 2004). Due to such observations it was presumed that elevations in circulating androgens above the breeding baseline (Level B, for a definition see below) were for the most part associated with temporal variations in aggressive and sexual behavior, rather than with basal reproductive physiology. This prediction led to the formulation of the Challenge Hypothesis (Wingfield et al., 1990), which states that circulating levels of androgens correlate with aggression only during periods of social instability, and that male-male interactions over social status and access to females, as well as the presence of fertile females, tend to increase plasma androgens.

Wingfield et al. (1990) postulated three levels at which testosterone or other androgens may be present in the circulation (see Fig. 2): (1) A constitutive homoeostatic 'Level A' which represents the basal secretory activity of Leydig cells during the non-breeding season. This level is presumed to maintain feedback regulation of both GnRH and gonadotropin release. (2) A regulated (periodic) breeding season baseline 'Level B', which represents constitutive secretory activity stimulated by environmental cues, e.g. day length. Level B is sufficient for spermatogenesis, the development of some secondary sexual characters and accessory organs, and the expression of reproductive behaviors. And, finally, (3) a maximum 'Level C' that is achieved through social stimulation from competing males or via interactions with receptive females. Theoretically, Level C represents the physiological maximum, but because it is obtained from seasonal profiles it may not represent the absolute maximum to which plasma androgens may increase. The increase in androgens to Level C can be short or long in duration, and small or great in magnitude. In contrast to the increase from Level A to Level B, which periodically occurs at the onset of the breeding season, the increase from Level B to Level C is considered facultative, i.e. is triggered mostly by social stimulation (Wingfield et al., 1990, 2000).

Another important cornerstone for the Challenge Hypothesis is based on the observation that high levels of testosterone (at Level C) often interfere with male parental care in birds (e.g. Silverin, 1980; Hegner and Wingfield, 1987; Ketterson et al., 1992; Beletsky et al., 1995; Saino and Moeller, 1995; Schoech et al., 1998; Moreno et al., 1999; De Ridder et al., 2000; Stoehr and Hill, 2000). Therefore, Wingfield et al. (1990) hypothesized that temporal patterns of plasma androgens are the result of a trade-off between the degree to which male parental care is necessary for reproductive success as compared to the necessity to express aggressive behavior (Fig. 1). In sum, socially monogamous species with a high degree of male parental care are predicted to show an increase in androgens to Level C only during periods of territory establishment, during male-male challenges, or when females are fertile, so that paternal care is not compromised. In contrast, androgen levels in polygynous species with little or no paternal care should be close to the breeding season maximum Level C throughout breeding due to intense and continued interactions between males and the regular availability of receptive females. However, polygynous males that do contribute to duties at the nest should show an androgen pattern between those two extremes. To test these predictions Wingfield et al. (1990, 2000) conducted an interspecific comparison of seasonal androgen patterns in birds. Wingfield et al. (1990) also introduced the term "androgen responsiveness", or "R", which they defined as "a hormonal responsiveness to social interactions". Further, they established a measure for R taking into consideration the interspecific variation in baseline and maximum testosterone levels, i.e. R = (Level C – Level A) / (Level B – Level A). Because Level A is usually (but not always) non-detectable, this equation can typically be simplified to R = Level C / Level B. These interspecific comparisons confirmed the existence of a relationship between mating system, importance of male parental care, and degree to which androgen levels respond to competing males and fertile females. A more recent analysis by Hirschenhauser et al. (2003) controlled for phylogenetic relationships and obtained similar results, with the exception that the effect of paternal care was not observed. Indeed, recent studies have shown that testosterone does not always suppress male parental care (Van Duyse et al., 2000; Lynn et al., 2002; Lynn et al., 2005), leading to the formulation of the essential male parental care hypothesis. According to this hypothesis, males should become insensitive to testosterone when male parental care is absolutely essential for the success of a nest (Lynn et al., 2002, 2005).

To date, most studies have calculated androgen responsiveness using seasonal androgen profiles, although Hirschenhauser et al. (2003) have additionally incorporated androgen levels following experimentally-induced male-male interactions. As already mentioned, Wingfield et al. (1990) defined androgen responsiveness as "a hormonal responsiveness to social interactions". Hirschenhauser et al. (2003) called it "an androgen responsiveness to social challenges". However, because androgen responsiveness in these publications is largely based on seasonal androgen profiles a more accurate term would be "seasonal androgen response" ($R_{seasonal}$, a term we use for the remainder of this paper, see Table 1). We propose this term not only because it is formally more accurate, but also because we suggest that the seasonal androgen response ($R_{seasonal}$) should be differentiated from immediate androgen responsiveness to social instability, e.g. the short-term change in circulating androgens experienced by territorial males that are challenged with a conspecific intruder, such as during a simulated territorial intrusion (STI). Immediate androgen responsiveness can be measured by taking the ratio between androgen levels in challenged males and unchallenged controls. We refer to this type as male-male androgen responsiveness ($R_{male-male}$; Table 1).

The necessity for a clear distinction between $R_{seasonal}$ and $R_{male-male}$ is exemplified by recent findings in blue tits. Male blue tits (*Cyanistes caeruleus*) show a pronounced seasonal increase in circulating testosterone during the breeding season: from an undetectable Level A during non-breeding to a detectable Level B of 0.6 ng/ml during the parental phase and, finally, to a maximum Level C of 5.4 ng/ml during egg-laying. This change in androgen concentrations results in a high calculated value for $R_{seasonal}$ (Level C/Level B = 5.4/0.6 = 9). However, when males of this species are challenged with direct male-male competition trials (STIs), testosterone concentrations decline. Thus, the seasonal androgen response ($R_{seasonal}$) and responsiveness to male-male interactions ($R_{male-male}$) do not necessarily correspond in the blue tit (Landys et al., in press) and may also show no correlation in other species, such as the great tit (*Parus major*; Van Duyse et al., 2004).

These findings prompted us to re-examine some of the interspecific predictions of the Challenge Hypothesis. Our aim is to show (1) that $R_{seasonal}$ and $R_{male-male}$ represent different measures of androgen responses, (2) that these measures may not necessarily be additive, and (3) that differences between $R_{seasonal}$ and $R_{male-male}$ may be related to differences in the flexibility in the timing of life-cycle stages. To this end, we compared the seasonal androgen response ($R_{seasonal}$) with androgen responsiveness to male-male interactions ($R_{male-male}$) in birds for which data are currently available and related these measures to the natural history. Although androgens also play a role in female behavior (e.g. Staub and deBeer, 1997; Ketterson et al. 2005; Wingfield et al., 2006) our analyses were restricted to males. The results lead us to propose that at least three additional measures of androgen responsiveness should be considered in future investigations, i.e. the androgen responsiveness of males to receptive females $R_{male-female}$, the androgen responsiveness to non-social environmental cues $R_{environmental}$, and the physiological potential to release androgens into the circulation $R_{potential}$, which can be measured via injections of GnRH (Table 1).

Methods

We compiled data from all breeding birds that have been examined to our knowledge for changes in plasma testosterone – the principal androgen in birds – during male-male territorial interactions. From these data, we determined the androgen responsiveness to male-male interactions ($R_{male-male}$) for each species by calculating the ratio between plasma testosterone of birds caught after an STI trial and testosterone levels of unstimulated control birds. For great tits, blue tits, Gambel's white-crowned sparrows, rufous-collared sparrows, rufous whistlers and pied flycatchers, data for $R_{male-male}$ were available for more than one sub-stage of breeding (territory establishment and parental phases). Sample size was too limited to conduct separate analyses for all sub-stages. However, with the exception of the pied flycatcher, the directionality of responses were similar for different sub-stages in all species. Thus, we calculated the mean $R_{male-male}$ across sub-stages. For each species included in our comparison of $R_{male-male}$ we also collected information on the seasonal androgen response ($R_{seasonal}$, defined as the ratio between the highest mean testosterone level observed in the breeding season profile Level C, and the breeding baseline Level B). It is important to note that a Level C estimated from breeding season profiles does not necessarily represent the maximum level to which testosterone may increase, as levels generated during male-male interactions may reside above the respective breeding season profile. Nevertheless, we found that $R_{seasonal}$ was generally larger than $R_{male-male}$, as the latter is a ratio usually based on androgen levels located between Levels B and C. For this reason and because the comparison of ratios in statistical tests is problematic (Atchley et al. 1976; Packard and Boardman, 1988), we decided to employ a meta-analytical approach with standardized effect sizes (or standardized effect differences) instead of comparing the absolute magnitude between the two androgen responsiveness measures. Standardized effect sizes (or Cohen's d, defined as the difference between two means divided by the pooled standard deviation for those means; Cohen, 1988) represent a measure of the magnitude of the difference between Level B and C, or between control and STI-challenged birds, respectively, on a standardized scale. Thus, they allow a direct comparison of the magnitude of a response independent of absolute differences in the respective measurements. Standardized effect sizes for $R_{seasonal}$ and $R_{male-male}$ and their 95% confidence intervals were calculated using the program ESCIdelta (Cumming and Finch, 2001; for recent reviews on the usefulness of effect sizes see (Cohen, 1990; Cumming and Finch, 2001; Thompson, 2002; Nakagawa and Foster, 2004).

As per the original Challenge Hypothesis (Wingfield et al., 1990), we ranked males on a scale from 1 to 3 according to their degree of male-male aggression (1 = low aggression: males may show some aggression at the beginning of the breeding season, but only for a brief period; 2 = moderate aggression: males interact aggressively for a large part of the breeding season, but the frequency of aggression abates once the parental phase starts; 3 = high aggression: males interact aggressively throughout the entire breeding season). Males were also ranked for their contribution to parental duties (1 = low parental care: males function as an escort or in predator-defense, or may provide no care at all; 2 = high parental care: males provide direct parental care by actively incubating and/or feeding young at rates equal to or even greater than those of their mates). Ranks for both traits were utilized to generate an index of male-male aggressiveness over male parental care according to the ratio between those two traits, as in the original paper by Wingfield et al. (1990). We are aware that there are statistically more sophisticated methods to look at effects of male-male aggression and male investment in parental care (for example see Hirschenhauser et al., 2003) and that the use of ratios is problematic (e.g. Atchley et al. 1976; Packard and Boardman, 1988). However, we used the aggressiveness/parental care ratio as originally presented in Wingfield et al. (1990), so that we could compare androgen responsiveness patterns in our data (derived from $R_{male-male}$) to those of the original data set (based on $R_{seasonal}$). However, we should point out that we use this ratio mainly for illustrative purposes, as most of the analyses presented do not include and are independent of this ratio.

All statistical comparisons were conducted with Systat 11 (Systat Software, Erkrath, Germany). Tests were two-tailed and the significance level was set to $\alpha=0.05$. Due to limited sample size, phylogenetic corrections were not possible. However, the main purpose of this contribution is conceptual rather than analytical, which in our view justifies such an approach.

Results

Males with a low aggression/parental care ratio (i.e. males that show little aggression but provide parental care) also expressed a large $R_{seasonal}$, whereas males with a high aggression/parental care ratio showed a low $R_{seasonal}$. Although we made sure to include data on $R_{seasonal}$ (such as Wingfield et al., 1990; Wingfield et al., 2000), our results are in accordance with a larger interspecific comparisons of androgen responsiveness that bases androgen responsiveness not only on $R_{seasonal}$ but also to some extent on $R_{male-male}$ (Hirschenhauser et al., 2003; Fig. 3a).

When we replaced $R_{seasonal}$ with $R_{male-male}$ the relationship between androgen responsiveness and the aggression/parental care ratio disappeared (Fig. 3b), suggesting that $R_{seasonal}$ and $R_{male-male}$ relate to aggressiveness and male parental care in different ways. Interestingly, single-brooded species (i.e. species that raise only one brood per breeding season) showed the highest $R_{seasonal}$ but the lowest $R_{male-male}$, whereas multiple-brooded species showed the reverse. In a first analytical step, we directly tested for a relationship between $R_{seasonal}$ and $R_{male-male}$. Overall, the two variables showed no significant correlation (Spearman's rho=0.159, p<0.3). However, when we considered single- and multiple-brooded species separately, there was a significant positive correlation between $R_{seasonal}$ and $R_{male\text{-}male}$ in single-brooded birds (Pearson's r=0.836, p=0.02), but not in multiple-brooded birds (Pearson's r=0.512, p=0.2; Fig. 4).

To further evaluate and compare differences between $R_{seasonal}$ and $R_{male\text{-}male}$, we calculated the standardized effect sizes for each of these measures ($dR_{seasonal}$ and $dR_{male\text{-}male}$, respectively). Standardized effect sizes allowed us to directly compare $R_{seasonal}$ and $R_{male\text{-}male}$ in standardized units independent of differences in the absolute magnitude of the measures. We compared standardized effect sizes using a mixed model with $dR_{seasonal}$ and $dR_{male\text{-}male}$ as the wrapped dependent variable. To identify the two different kinds of androgen response measures, the new wrapped dependent variable was labelled $R_{seasonal}$ or $R_{male\text{-}male}$ and the label was included as a fixed factor. "Number of broods" (single- or multiple-brooded) represented a second fixed factor, and "species" was included as a random factor (because most species contributed two measurements, one for $dR_{seasonal}$ and one for $dR_{male\text{-}male}$). The random factor "species" did not explain a significant proportion of the variance (Z = 0.703, p=0.24). However, $dR_{seasonal}$ was significantly larger than $dR_{male\text{-}male}$ (Z = 8.455, p < 0.0001; Fig. 5). The number of broods raised per breeding season did not have an overall effect (Z=0.234, p = 0.82), but the interaction between brood number and type of androgen response ($R_{seasonal}$ or $R_{male\text{-}male}$) was significant (Z = 3.017, p = 0.003): the difference between $dR_{seasonal}$ and $dR_{male\text{-}male}$ was larger in single-brooded than in multiple-brooded species (Fig. 5), indicating that $R_{male\text{-}male}$ explains a relatively larger proportion of $R_{seasonal}$ in multiple-brooded birds. The fact that the $dR_{male\text{-}male}$ of multiple brooded species was positive and the 95% confidence interval did not cross zero suggests that multiple-brooded species respond to male-male interactions with a significant increase in circulating androgens (Fig. 5). In contrast, the $dR_{male\text{-}male}$ of single-brooded species was close to zero, suggesting that male-male interactions do not lead to significant changes in androgen concentrations in single-brooded males.

The effect sizes $dR_{seasonal}$ and $dR_{male\text{-}male}$ were not correlated (Pearson's r^2 = -0.02, p = 0.93, Fig. 6), a result that did not change when analyzing single- and multiple-brooded species separately (single-brooded: r^2 = 0.03, p = 0.95; multiple-brooded: r^2 = 0.37, p = 0.37; Fig. 6). This suggests that the magnitude of the seasonal change in androgen concentrations does not reflect the magnitude of the change in androgen concentrations during male-male encounters.

Discussion

Results from the original Challenge Hypothesis and subsequent comparative studies suggested that patterns of androgen secretion during the breeding season are related to mating system, degree of male-male aggression, and – with limitations – to patterns of male parental care (Wingfield et al., 1990, 2000; Hirschenhauser et al., 2003; Garamszegi et al., 2005). Although most of the androgen data used in these studies represent what we now call the seasonal androgen response ($R_{seasonal}$), some of these studies also included data based on immediate androgen responsiveness to staged male-male interactions ($R_{male\text{-}male}$), with the assumption that $R_{seasonal}$ is basically a reflection of $R_{male\text{-}male}$ or vice versa (see also Fig. 1). Our distinction between these two measures has shown that the relationship between $R_{seasonal}$ and $R_{male\text{-}male}$ is more complex than previously assumed. $R_{seasonal}$ may even be unrelated to $R_{male\text{-}male}$. For example, unlike $R_{seasonal}$, $R_{male\text{-}male}$ can display a value of less than 1, i.e. androgen levels may actually decline during male-male interactions (e.g. in blue tits and great tits). Only when single- and multiple-brooded species were considered separately did we find a positive correlation between these two androgen response measures – albeit with a shallow slope and only in single-brooded species (Fig. 4). A larger sample may reveal that the relationship between $R_{seasonal}$ and $R_{male\text{-}male}$ may be significant also in multiple-brooded species. If so, Fig. 4 predicts that the slopes of the relationships will be vastly different in single- and multiple-brooded bird species. In any case, our results suggest that the relationship between $R_{seasonal}$ and $R_{male\text{-}male}$ in single- and multiple-brooded species may be based on completely different factors (see discussion below).

Additivity of androgen responsivenesses

Ideally, $R_{seasonal}$ should represent the maximum to which androgen levels of a species increase during the breeding season. However, $R_{seasonal}$ does not always equal the physiological maximum of testosterone secretion, as can be elicited using a GnRH challenge (for a detailed discussion of this topic see the section ‘Future directions’ below). According to the 3-level (A, B, C) model of androgen secretion (Wingfield et al., 1990; see introduction), the seasonal androgen response consists of androgen responsiveness to male-male interactions, as well as androgen responsiveness to other environmental or social cues. Thus, the effect size of the seasonal androgen response $dR_{seasonal}$ should be the sum of the effect sizes of male-male androgen responsiveness $dR_{male\text{-}male}$ and possible androgen responsiveness to other cues, such as to the presence of receptive females, which we refer to as $dR_{male-female}$. However, to date, most tests of the Challenge Hypothesis have focussed only on male interactions, sometimes without distinguishing between $R_{seasonal}$ and $R_{male-male}$. Male-female interactions have received much less attention in studies of free-ranging bird species, even though receptive females or additional reproductive cues have been demonstrated to strongly impact on androgen secretion patterns in males. For example, Feder et al. (1977) and O'Connell et al. (1981) found that when male ring doves (*Streptopelia risoria*) were exposed to females, plasma levels of testosterone increased. Moore (1983) showed that male Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelli*) paired with estradiol-implanted females expressed androgen concentrations about two times higher than those of males paired with untreated females (see Table 3), and Johnsen (1998) found that during the late breeding season, free-ranging male red-winged blackbirds maintained higher levels of testosterone when receptive females were present. In accordance with these data, males of many bird species show a seasonal peak in plasma androgens during the period of nest-building and egg-laying, when females are most fertile (for a recent example see Goymann et al., 2006). This effect of females is not specific to birds. There is ample evidence that females have an impact on the secretion of gonadotrophins and androgens also in fish, amphibians, reptiles, and mammals, including humans (e.g. Bronson and Desjardins 1982, James and Nyby, 2002; Goymann et al. 2003; Stacey, 2003; Archer, 2006; Dloniak et al. 2006; reviews in Wingfield et al., 1994). Further, Gwinner et al. (2002) demonstrated that non-social reproductive cues may also trigger a full androgen response: male European starlings show similar androgen elevations when presented with a combination of a male competitor and a nestbox, a female and a nestbox, or a nestbox alone. Androgen responsiveness to such non-social environmental cues could be termed $R_{environmental}$. Furthermore, male starlings housed in groups without nextboxes had lower levels of luteinizing hormones and testosterone during the breeding season than males of groups housed with nestboxes (Gwinner et al. 1987).

Presently, we do not know whether $R_{seasonal}$ represents a composite of androgen responses to various reproductive cues. If so, the effect sizes of the different types of androgen responsiveness measures should be additive, i.e. $dR_{seasonal} = dR_{male-male} + dR_{male-female} + dR_{environmental}$. To our knowledge, the Gambel's white-crowned sparrow and the red-winged blackbird are the only species for which such information is available at present. In Gambel's white-crowned sparrows the effect sizes $dR_{male-male}$ and $dR_{male-female}$ almost add up to $dR_{seasonal}$ (Table 3). This is not the case for red-winged blackbirds:

$dR_{\text{male-male}}$ is slightly negative and $dR_{\text{male-female}}$ does not compensate for this negative effect (Table 3). However, because $dR_{\text{male-female}}$ is similar in magnitude as dR_{seasonal}, $R_{\text{male-female}}$ alone may be responsible for the expression of a full R_{seasonal} in male red-winged blackbirds, but only at times when they are not challenged by other males. Thus, at least in some species, the different kinds of androgen responsiveness may be non-additive, i.e. their effect sizes may not sum up ($dR_{\text{seasonal}} \neq dR_{\text{male-male}} + dR_{\text{male-female}} + dR_{\text{environmental}}$). If the latter is true, this leaves us with two possibilities: Either is R_{seasonal} not a direct function of the hormonal responsiveness to social interactions ($R_{\text{male-male}}$ and $R_{\text{male-female}}$) and non-social environmental cues ($R_{\text{environmental}}$). More likely, the expression of a full R_{seasonal} in some species may be a function of mainly one androgen responsiveness measure ($R_{\text{male-male}}$, $R_{\text{male-female}}$, $R_{\text{environmental}}$), and its full expression may require the absence of the other factors. This latter idea is supported by evidence from red-winged blackbirds (described above). The androgen decrease in blue tits and great tits subjected to male-mael interactions (Van Duyse et al., 2004; Landys et al., in press) also hints at the existence of such non-additive effects. In fact, Fig. 5 suggests that $R_{\text{male-male}}$ contributes little, if anything to R_{seasonal} in single-brooded species. Thus, it is likely that $R_{\text{male-female}}$ or $R_{\text{environmental}}$ are responsible for most of R_{seasonal} in these species (as already proposed for parids; Landys et al. in press). The potential consequences of this for seasonal androgen concentrations in cavity nesting species, such as blue tits and great tits, are especially interesting. If androgen patterns are driven mainly by $R_{\text{male-male}}$, high density populations that breed in nest-boxes (in which males frequently encounter each other) should have a lower R_{seasonal} than natural low-density populations. However, if male tits strongly respond to females ($R_{\text{male-female}}$) or to the nest-boxes themselves ($R_{\text{environmental}}$), R_{seasonal} should be higher in the artificially-created nest-box populations.

Typically, androgen concentrations of free-living male birds are higher than those of captive conspecifics. It has been suggested that low androgen levels of captive males are the result of restricted access to the full repertoire of environmental stimuli usually experienced during breeding, e.g. the ability to interact with other males or with receptive females (Wingfield et al., 1990). However, at the Max Planck Institute in Andechs, an interesting exception to this rule was recently found: although androgen concentrations are indeed higher in free-living as compared to captive multiple-brooded European stonechats (*Saxicola torquata*), the opposite is observed in single-brooded stonechats from Siberia (*Saxicola maura*): captive male Siberian stonechats held singly in cages without visual access to other males or females have significantly higher levels of testosterone than free-living males. In free-living Siberian stonechats, testosterone levels are often undetectable, even during the territory establishment phase or when females are most fertile (M. Raess and E. Gwinner, unpublished data). In combination with the documented $R_{male-male}$ of blue tits and great tits, the Siberian stonechat data suggest that the release of androgens may sometimes be suppressed rather than increased by social interactions – a pattern that may be more common in single-brooded than in multiple-brooded birds. Thus, in such species, the expression of a full seasonal androgen response may require the absence, rather than the presence, of at least some social stimuli.

Number of broods and $R_{male-male}$

Our analysis suggests that there is a relationship between $R_{male-male}$ and the number of broods raised per breeding season (or another variable closely related to brood number, see also Landys et al., in press): regardless of reproductive stage, the effect size of $R_{male-male}$ ($dR_{male-male}$) was smaller for single-brooded species than for multiple-brooded birds. Our meta-analysis showed that males of multiple-brooded species generally respond to male-male interactions with a significant increase in circulating androgens. In contrast, male-male interactions in single-brooded species do not lead to significant changes in androgen concentrations (see effect size measures for $R_{male-male}$ in Fig. 5). Differences between multiple- and single-brooded species may arise as a consequence of differences in the flexibility of reproductive timing programs. The finite state machine theory describes an organism’s life cycle as a series of life-history stages (e.g. non-breeding, spring migration, breeding, moult, fall migration), and, further predicts that flexibility in timing and the hormonal control of the transition between life-cycle stages may differ depending on their number and duration (Jacobs and Wingfield, 2000; Wingfield, 2005). Reproduction in single-brooded species is often characterized by a short breeding season (due to climatic conditions and/or dependence on specific food resources available only during limited periods) and a high breeding synchrony. Thus, the timing of breeding may be comparatively more constrained in single-brooded as compared to multiple-brooded birds and, hence, may be regulated by different mechanisms. In single-brooded species, androgens may only prime male-male aggression at the start of breeding, rather than maintain it throughout the season. For example, in blue tits, testosterone treatment does not increase male-male aggression or song, but may increase interactions with females (Foerster and Kempenaers, 2005; Kunc et al., 2006). The only two other single-brooded species for which comparable data are available – the Gambel's white-crowned sparrow and the great tit – also show a lack of behavioral sensitivity to external testosterone, with the exception that testosterone stimulates singing in great tits (Van Duyse et al., 2000; Meddle et al., 2002; Van Duyse et al., 2002). From a more ultimate perspective, a low $R_{male\text{-}male}$ in single-brooded species may represent a strategy to minimize any negative behavioral and/or physiological consequences of high androgen levels (Hillgarth and Wingfield, 1997; Wingfield et al., 2001).

Landys et al. (in press) found that in contrast to multiple-brooded birds, males of single-brooded species show an increase in circulating corticosterone when they are challenged with a territorial intruder. Hence, differences in hormonal responses during male-male interactions between single- and multiple-brooded species are not restricted to androgens. Changes in plasma corticosterone during male-male interactions may play a critical and perhaps even more important role than acute changes in androgens, e.g., through energy provisioning, stimulation of activity, or promotion of aggression (Landys et al., in press).

$R_{male\text{-}male}$ and the essential paternal care hypothesis

Although our dataset is currently too sparse for a meaningful statistical evaluation, our results regarding $R_{male\text{-}male}$ in combination with the essential paternal care hypothesis (Lynn et al., 2005) prompts us to propose a modification of one of the predictions of the Challenge Hypothesis. The essential paternal care hypothesis predicts that insensitivity to androgens should "arise in any environment in which reduced assistance by males in response to high levels of androgens may lead to a significant reduction in reproductive success" (Lynn et al., 2005, p. 171). We predict that organisms that have evolved behavioral insensitivity to androgens during the parental phase should by default also avoid an increase in androgens during male-male challenge. Initial results indicate that this really may be the case (Lynn and Wingfield, 2005; see also Table 1 in Landys et al., in press). If so, then species with essential male parental care should keep androgen levels low during challenge (close to Level B), resulting in a low $R_{male\text{-}male}$. In species without any male parental care androgens should circulate at high concentrations (close to level C) throughout the breeding season, and, as a consequence also produce a low $R_{male\text{-}male}$. In fact, only species in which males contribute to parental care but are not essential for the success of a clutch should express a large $R_{male\text{-}male}$. This scenario predicts a bell-shaped curve for $R_{male\text{-}male}$ during the parental phase depending on the presence and importance of male parental care (Fig. 7). Additional data from more species are required to determine whether the differences of $R_{male-male}$ of single- and multiple-brooded species are related to differences in the importance of male parental care. It should be recognized, however, that blue tits and great tits, and possibly other single-brooded species show a low $R_{male-male}$ already before the initiation of the parental phase, suggesting that male parental care patterns cannot account for all differences in $R_{male-male}$ between single- and multiple-brooded species.

Future directions

To better understand and address how brood-number contributes to observed differences in the various androgen responsiveness measures ($R_{seasonal}$, $R_{male-male}$, $R_{male-female}$ etc.), they should be evaluated in species comprised of both single and multiple-brooded populations. This would allow for testing of the ‘broodedness’ effect irrespective of phylogenetic constraints. Further, a larger data set would allow a refined evaluation of the potential impact of different mating systems and especially the importance of male parental care on $R_{male-male}$.

While little is known about $R_{male-male}$, even less is known about $R_{male-female}$ or androgen responsiveness to non-social environmental cues ($R_{environmental}$), such as ownership of a nestbox (as seen in starlings; Gwinner et al., 1987, 2002). To investigate to what degree $R_{seasonal}$ is a combination of other androgen responsiveness measures, experiments that measure androgen responsivenesses to different kinds of stimuli – both environmental and social – are required. To this end, future investigations should always include (1) the determination of a seasonal androgen profile which allows an estimation of $R_{seasonal}$, (2) a measure of $R_{male-male}$ via use of STIs, and (3) an estimation of $R_{male-female}$ via presentation of estradiol-primed females (Moore, 1983) or through use of more sophisticated methods. For example, Patricelli et al. (2002, 2006) have used robotic females to measure behavioral responses of males to copulation solicitations. Such robotic females may have the potential to become a standardized method to investigate $R_{male-female}$.

A complete picture of androgen responsiveness in a species should also include a GnRH-challenge during breeding to establish the physiological capacity of an organism to produce androgens (see e.g. Wingfield et al., 1991; Moore et al., 2002; Goymann and Wingfield, 2004). Androgen levels obtained with GnRH-challenges may represent a more consistent estimate of Level C androgen concentrations than androgen concentrations obtained from seasonal hormone profiles, especially when the seasonal profile is based on a small number of samples. As mentioned in the introduction, Wingfield et al. (1990) defined Level C as the physiological maximum that is achieved during social stimulation from competing males or from interactions with receptive females. In the current paper, we propose that a full androgen response in some species may require the absence of such social cues. Hence, in contrast to the original definition, Level C in such species could only be achieved through *absence* of social stimulation. Assuming that males of all bird species respond with a maximum release of androgens when injected with a sufficient dose of GnRH, one could use this technique to estimate the maximum potential of an organism to mount an androgen response ($R_{potential}$, i.e. the ratio between circulating androgens after stimulation with GnRH as compared to circulating androgens before such stimulation, ideally at Level B). Thus, $R_{potential}$ would represent a standardized measure for the physiological capacity of an organism to produce a maximum androgen response. We have listed data for $R_{potential}$ in Table 3. In the white-browed sparrow weaver and the rufous-collared sparrow $R_{potential}$ and $R_{seasonal}$ are rather similar. In others, such as the African black coucal, the Gambel's white-crowned sparrow and the dark-eyed junco, $R_{seasonal}$ is higher than $R_{potential}$, whereas in Pudget sound white-crowned sparrows and red-winged blackbirds $R_{potential}$ is higher than $R_{seasonal}$. Part of the variance probably stems from the fact the androgen response to GnRH may vary between breeding sub-stages, as has been demonstrated for the dark-eyed junco (Jawor et al., 2006). In the black coucal, for example, the androgen response to GnRH was measured during the parental phase, but possibly GnRH injections may have elicited a stronger response during the mating phase (Goymann and Wingfield, 2004).

Throughout this paper we have referred to changes in circulating androgen concentrations as a means whereby behavioural or physiological effects may be produced. It should be pointed out, however, that there are many other levels at which hormone action can be regulated. Ultimately, it is the number and distribution of androgen and estrogen receptors that transduce plasma levels of androgens into behavioral or physiological effects. Even before a steroid binds to its receptor, other factors, such as binding globulins, receptor co-factors, and conversion enzymes may modulate the action of the hormone (for a recent review on the complex interaction of steroid hormones with such factors see Hau, 2007). Thus, there are many mechanistic layers at which the signalling value of a particular concentration of plasma hormones may be altered. These different layers are the basis for a biodiversity of mechanisms that organisms have evolved to modify the actions of androgens or other hormones. This biodiversity of mechanisms will always limit our possibilities to generalize patterns in levels of circulating hormones.

In addition, we suggest that a larger incorporation of the ethological approach would greatly benefit the field of behavioral endocrinology. Throughout this paper we have referred to the androgen response of males toward STIs as male-male androgen responsiveness, or $R_{\text{male-male}}$. However, although STIs may be useful in establishing $R_{\text{male-male}}$, at least three issues may potentially complicate interspecific comparisons of this measure. First, different methodologies employed by different studies may to some extent confound interpretations (addressed in more detail in Landys et al., in press). For example, Wikelski et al. (1999) stimulated spotted antbirds with playback for two hours and found changes in plasma levels of testosterone only after this time. In contrast, other species (e.g. Astheimer et al., 2000; McDonald et al., 2001; Meddle et al., 2002) were exposed to simulated territorial intrusions lasting only 10 minutes and significant changes in circulating testosterone were not observed.

Second, Wingfield and Wada (1989) found a testosterone response in song sparrows only when both a live decoy and playback of conspecific song was presented. In contrast, free-living blue tits showed a strong albeit negative endocrine response to the presentation of a caged decoy alone (Landys et al., in press). Hence, not only may hormonal responses to STIs in and of themselves differ among species, but also the kind and duration of stimuli that are required to elicit hormonal responses may show interspecific variation.

Third, we assume that STIs effectively mimic intrusions from competing conspecific males. However, the different ways in which such social situations are perceived may modulate androgen responsiveness (reviewed in Oliveira, 2004). For example, the territorial cichlid fish *Oreochromis mossambicus* responds to a live intruder with an increase in circulating androgens (Hirschenhauser et al., 2004), but shows no change in androgen levels when presented with its mirror image, even though vigorous aggressive displays are expressed (Oliveira et al., 2005). Thus, at least in the case of this cichlid, androgen responsiveness depends on appropriate behavioral feedback from the opponent.

Our own experience with STIs suggests that also the behavior of caged intruder birds may influence the behavioral response of territory owners. For example, female

European robins typically respond less strongly to a caged female intruder that crouches in a corner as compared to a caged intruder sitting on a perch (W. Goymann, pers. obs.). Furthermore, in male European robins we found that the corticosterone levels of territoriy owners differed when exposed to STIs with a stuffed dummy as compared to STIs with a live decoy (M. Scriba and W. Goymann, unpublished data), although androgen concentrations did not differ. Hence, subtle differences in experimental setups may produce large consequences for hormonal responses.

Future investigations of $R_{male-male}$ and $R_{male-female}$ may benefit not only from the standardization of experimental protocols, but also from the incorporation of more realistic situations. For one, the behavior of live decoys is hard to control. Even though stuffed dummies may offer a methodological alternative to live decoys, as they can be made to pose in a standardized threatening manner, such dummies do not exhibit other behaviors. In an effort to better reproduce the suite of behaviors a live animal might express, Balsby and Dabelsteen (2002), Narins et al. (2003) and Patricelli et al. (2002, 2006) pioneered the use of robotic models for the behavioral research of wild vertebrates. Robotic male intruders could be programmed to “behave” either more submissively or more offensively. The hormonal response of the territory owner may change depending on such subtle cues. Furthermore, as mentioned above, a robotic female that mimics the copulation solicitaton display of a species may provide an elegant way to measure $R_{male-female}$.

In summary, our study has shown that it is important to separate the seasonal androgen response ($R_{seasonal}$) from the androgen responsiveness to male-male interactions ($R_{male-male}$). $R_{male-male}$ cannot be predicted from $R_{seasonal}$, and it is currently not possible to evaluate how other kinds of androgen responsiveness measures, such as $R_{male-female}$ or $R_{potential}$, relate to $R_{seasonal}$. To our knowledge, a distinction among different kinds of androgen responsiveness measures has not previously been made, but may be essential for a better understanding of hormone-behavior interactions. Furthermore, we have demonstrated that $R_{seasonal}$ and $R_{male-male}$ differ between single-brooded and multiple-brooded species, suggesting that the number of broods a pair can raise per breeding season (or a correlated variable) represents an important ecological factor that may shape differences in the evolution of hormone-behavior relationships.

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Figure legends

Fig. 1. Schematic representation of interspecific variation in seasonal profiles of plasma testosterone in birds. Black bars refer to periods of frequent male-male interactions or mate guarding. With respect to Western gulls territories and mates are available in excess, hence there is little or no competition (see Wingfield et al. 1982). Redrawn from Wingfield et al. (1987).

Fig. 2. Graphical representation of the 3-Level model of androgens in male birds. Level A represents the non-breeding androgen baseline required for feedback regulation of GnRH and gonadotropin release. Level B represents the androgen baseline during breeding induced by environmental cues such as an increase in day length. Level B is sufficient for spermatogenesis to proceed, as well as for the expression of secondary sexual characters and reproductive behaviours. Level C represents the physiological testosterone maximum that can be achieved during interactions with other males or receptive females. The increase from level A to level B occurs seasonally at the onset of the breeding season, while the increase from level B to level C is facultative, i.e. only triggered by social stimulation or challenge during the breeding season. Redrawn from Wingfield et al. (1990).

Fig. 3. (a) Relationship between the seasonal androgen response ($R_{seasonal}$) and the index of male-male aggression and male parental care (from low aggression and high male parental care to high aggression and low male parental care; see (Wingfield et al., 1990) and main text for further explanation) in bird species for which data on male-male androgen responsiveness ($R_{male-male}$) are available. The stipled regression line in (a) represents the original regression line by Wingfield et al. (1990) and is included for comparative illustrative purposes only. (b) Relationship between the male-male androgen response ($R_{male-male}$) and the ratio of male-male aggression and paternal care of all bird species for which data on male-male androgen responsiveness are available. Species that raise only one clutch per breeding season are represented by solid circles; species that raise more than one clutch per breeding season are represented by open circles. The line in (b) represents the regression line for $R_{male-male}$.

Fig. 4. Correlation between the seasonal androgen response ($R_{seasonal}$) and male-male androgen responsiveness ($R_{male-male}$) in single-brooded (solid circles; Pearson's r = 0.836, p = 0.02; regression line y = 0.13 x - 0.083) and multiple-brooded birds (open circles; Pearson's r = 0.512, p = 0.2) listed in Table 2. Single-brooded species show a relatively higher $R_{seasonal}$ and a lower $R_{male-male}$ than multiple-brooded birds.

Fig. 5. Mean (± 95% confidence intervals) standardized effect sizes of the seasonal androgen response ($dR_{seasonal}$, white bars) and male-male androgen responsiveness ($dR_{male-male}$, grey bars) in single- and multiple-brooded species from Table 2. $dR_{seasonal}$ is significantly larger than $dR_{male-male}$ in both single- and multiple-brooded species. Further, $dR_{male-male}$ is significantly larger in multiple- than in single-brooded species. Numbers above bars refer to sample sizes.

Fig. 6. Correlation between effect sizes (± 95% confidence intervals for both variables) of the seasonal androgen response ($dR_{seasonal}$) and male-male androgen responsiveness ($dR_{male-male}$) in single- and multiple-brooded species from Table 2. The area shaded in grey represents negative values for $dR_{male-male}$, i.e. circles in this area indicate species that decrease testosterone levels in response to male-male interactions. Species with confidence intervals crossing the dotted lines do not show significant changes in the respective androgen response. All species show a significantly positive seasonal androgen response. Most single-brooded species show either a negative or a lacking male-male androgen responsiveness, see also Fig. 5. The effect sizes $dR_{seasonal}$ and $dR_{male-male}$ are not correlated.

Fig. 7. Predicted relationship between the importance of male parental care and the androgen responsiveness to male-male interactions $R_{male-male}$ during the parental phase. When males do not provide parental care, $R_{male-male}$ should be low because androgen levels remain high (at Level C) throughout the breeding season. When male parental care is essential, $R_{male-male}$ should be low because males should not mount a testosterone response above Level B when they are challenged by an intruder.

Table 1. Definition of the different kind of androgen responses (R). Please mind that statistical comparisons are more appropriately done with the effect sizes (dR) rather than with the actual R-ratios.

Type of androgen response	Definition
$R_{seasonal}$ and its effect size $dR_{seasonal}$	Seasonal androgen response, which is obtained by comparing Level C (seasonal maximum) with Level B androgen levels (breeding baseline)
$R_{male\text{-}male}$ and its effect size $dR_{male\text{-}male}$	Androgen responsiveness to male-male interactions during the breeding season, which can be established by comparing the androgen concentrations of males measured after a simulated territorial intrusion with those of control males
$R_{male\text{-}female}$ and its effect size $dR_{male\text{-}female}$	Androgen responsiveness of males to the presence of receptive females during the breeding season. This can be tested, for example, by pairing males to females that are treated with estradiol (and therefore engage in frequent copulation solicitation display) and compare the androgen levels of these males to those of males paired with control females (that do not solicit copulations).
$R_{environmental}$ and its effect size $dR_{environmental}$	Androgen responsiveness to non-social environmental cues, such as the presence of nest-boxes, suitable nesting sites, or food and water. This response to environmental cues is tested by comparing androgen levels of males after the introduction of the environmental cue to those of control males.
$R_{potential}$ and its effect size $dR_{potential}$	Physiological capacity of an organism to mount an androgen response. This capacity is tested by injecting a sufficient dose of GnRH. The androgen levels after such a GnRH-challenge are then compared with control values obtained before the injection or with those of individuals injected with saline.

Table 2. List of male birds for which data on the seasonal androgen response ($R_{seasonal}$) and male-male androgen responsiveness ($R_{male\text{-}male}$) from plasma testosterone data are available

Species	mating system	number of broods	seasonal T level B mean ± se (ng/ml)	seasonal T level C mean ± se (ng/ml)	$R_{seasonal}$ (C/B)	$dR_{seasonal}$ mean [95%CI]	male-male T control (CO) mean ± se (ng/ml)	male-male T experim. (E) mean ± se (ng/ml)	$R_{male\text{-}male}$ (E/CO)	$dR_{male\text{-}male}$ mean [95%CI]	references
Red jungle fowl Gallus gallus	Polyg.	2+					0.8 ± 0.2	1.1 ± 0.2	1.4	0.54 [-0.41;1.48]	Johnsen and Zuk 1995
Spotted antbird Hylophylax naevioides	monog.	2+	0.2 ± 0.1	1.6 ± 0.6	8.0	1.59 [0.17;2.95]	0.2 ± 0.1	0.9 ± 0.2	4.5	2.09 [0.86;3.29]	Wikelski et al. 1999; 2003; Hau et al. 2000
White-bellied antbird Myrmeciza longipes	monog.	1					0.3 ± 0.1	0.3 ± 0.1	1.0	0.00 [-1.24;1.24]	Fedy and Stutchbury 2006
Rufous whistler Pachycephala rufiventris	monog.	1	0.15 ± 0.00	1.7 ± 0.5	11.3	0.85 [-0.70;2.37]	0.9 ± 0.3	1.1 ± 0.3	1.4	0.22 [-1.01;1.44]	McDonald et al. 2001
Pied flycatcher Ficedula hypoleuca	Polyg.	1	0.3 ± 0.1	3.4 ± 0.4	11.3	3.80 [2.32;5.29]	1.1 ± 0.3	2.6 ± 0.6	1.8	0.52 [-0.33;1.37]	Silverin 1980, 1993, 1998
Great tit Parus major	monog.	1	0.3 ± 0.05	1.1 ± 0.1	3.7	2.97 [1.47;4.41]	0.5 ± 0.2	0.2 ± 0.0	0.5	-0.58 [-1.44;0.30]	Röhss and Silverin 1983; Van Duyse et al. 2004
Blue tit Cyanistes caeruleus	monog.	1	0.6 ± 0.5	5.4 ± 0.6	9.0	2.80 [0.76;4.75]	3.4 ± 0.7	2.1 ± 0.1	0.6	-1.09 [-2.11;-0.04]	Landys et al. in press; Landys et al., unpublished data
European nuthatch Sitta europaea	monog.	1	0.4 ± 0.2	3.8 ± 1.7	9.5	2.07 [0.70;3.38]	3.0 ± 1.0	2.7 ± 1.0	0.9	-0.16 [-1.54;1.24]	Landys et al., unpublished data
White-browed sparrow weaver Plocepasser mahali	Coop.	2+	0.1 ± 0.02	0.3 ± 0.1	3	1.39 [-0.24;2.92]	0.2 ± 0.7	0.2 ± 0.5	1.0	-0.01 [-1.10;1.09]	Wingfield et al. 1991, 1992, Wingfield and Lewis 1993
Eastern song sparrow Melospiza melodia melodia	monog.	2+	1.4 ± 0.2	5.8 ± 2.0	4.1	0.71 [-0.24;1.63]	1.3 ± 0.3	2.8 ± 0.6	2.3	0.74 [-0.04;1.51]	Wingfield 1984a, 1984b, 1985, Wingfield and Wada 1989
Western song sparrow Melospiza melodia morphana	monog.	2+	1.7 ± 0.3	9.5 ± 2.0	5.6	2.80 [1.16;4.37]	0.4 ± 0.1	2.1 ± 1.0	5.3	1.00 [-0.23;2.18]	Wingfield and Hahn 1994
Gambel's white-crowned sparrow Zonotrichia leucophrys gambelii	monog.	1	0.3 ± 0.08	4.5 ± 1.1	13.6	1.76 [0.47;3.00]	1.7 ± 0.4	2.1 ± 0.6	1.5	0.60 [-0.45;1.63]	Wingfield and Farner 1978a; Meddle et al. 2002
Puget Sound white-cr. Sparrow Zonotrichia leucophrys pugetensis	monog.	2+	1.1 ± 0.1	5.5 ± 0.5	5.0	3.04 [2.08;3.97]	0.4 ± 0.2	0.8 ± 0.2	2.0	0.86 [-0.35;2.03]	Wingfield and Farner 1978b, Wingfield and Hahn 1994
Rufous-collared sparrow Zonotrichia capensis	monog.	2+	1.3 ± 0.5	9.7 ± 1.8	7.8	1.63 [0.48;2.74]	2.8 ± 0.4	3.2 ± 1.1	1.2	0.12 [-0.52;0.77]	Moore et al. 2004a, b
American tree sparrow Spizella arborea	monog.	1	0.2 ± 0.05	2.5 ± 0.5	12.5	3.94 [1.74;6.08]	2.5 ± 0.1	4.7 ± 1.7	1.9	0.55 [-0.69;1.76]	Astheimer et al. 2000
European starling Sturnus vulgaris	Polyg.	2+	0.3 ± 0.1	2.4 ± 0.4	8	2.08 [1.11;3.01]	0.9 ± 0.2	2.6 ± 0.2	2.9	1.42 [0.89;1.93]	Ball and Wingfield 1987, Gwinner et al. 2002
Red-winged blackbird Agelaius phoeniceus	Polyg.	2+	1.8 ± 0.2	3.2 ± 0.7	1.8	0.93 [-0.16;1.99]	4.1 ± 0.5	2.9 ± 0.6	0.7	-0.47 [-1.11;0.19]	Beletsky et al. 1989, 1992

Birds are listed according to phylogeny. We indicate number of broods raised per season and mating system (socially monogamous – if less than 15% of individuals are polygynous, polygynous, and cooperative). The seasonal androgen response ($R_{seasonal}$) was calculated by dividing level C by level B;. Male-male androgen responsiveness ($R_{male-male}$) was calculated by dividing experimental (E) testosterone levels by control (CO) levels using data from simulated territorial intrusion experiments. We did not include studies that examined effects of breeding density on plasma testosterone. Density may suggest how testosterone levels change with intensity of male-male competition, but density studies do not investigate effects of male-male competition directly and thus should be interpreted with caution.

Table 3: List of male birds with data on $R_{seasonal}$, or $R_{male\text{-}male}$ in combination with $R_{male\text{-}female}$, or $R_{potential}$. $R_{male\text{-}female}$ was calculated by dividing plasma testosterone levels of experimental males (E) exposed to receptive females by testosterone levels of control (CO) males. $R_{potential}$ was calculated by dividing testosterone levels of GnRH challenged males (E) by testosterone levels of saline injected control males or control levels before injection of GnRH (CO). For $R_{male\text{-}male}$ see Table 2.

Species	$R_{seasonal}$ (C/B)	$dR_{seasonal}$ mean [95%CI]	$R_{male\text{-}male}$ (E/CO)	$dR_{male\text{-}male}$ mean [95%CI]	$R_{male\text{-}female}$ (E/CO)	$dR_{male\text{-}female}$ mean [95% CI]	$R_{potential}$ (E/CO)	$dR_{potential}$ mean [95% CI]	references
African black coucal Centropus grillii	3.7	1.14 [0.42;1.85]					2.5	1.98 [0.53;3.36]	Goymann and Wingfield 2004
White-browed sparrow weaver Plocepasser mahali	3.0	1.39 [-0.24;2.92]	1.0	-0.01 [-1.10;1.09]			3.0	0.77 [-0.54;2.05]	Wingfield et al. 1991, 1992, Wingfield and Lewis 1993
Gambel's white-crowned sparrow Zonotrichia leucophrys gambelii	13.6	1.76 [0.47;3.00]	1.5	0.60 [-0.45;1.63]	2.47	0.97 [0.09; 1.84]	2.84	3.60 [1.93;5.22]	Wingfield and Farner 1978a; Meddle et al. 2002; Moore 1983; Moore et al. 2002
Puget Sound white-cr. Sparrow Zonotrichia leucophrys pugetensis	5.0	3.04 [2.08;3.97]	2.0	0.86 [-0.35;2.03]			7.4	1.99 [0.78;3.15]	Wingfield and Farner 1978b, Wingfield and Hahn 1994; Moore et al. 2002
Rufous-collared sparrow Zonotrichia capensis	7.8	1.63 [0.48;2.74]	1.2	0.12 [-0.52;0.77]			7.2	1.52 [0.48;2.53]	Moore et al. 2002, 2004a, b
Dark-eyed junco Junco hyemalis	3.8	1.94 [0.85;3.01]					2.3	1.79 [1.46;2.12]	Chandler et al. 1997; Jawor et al. 2006
Red-winged blackbird Agelaius phoeniceus	1.8	0.93 [-0.16;1.99]	0.7	-0.47 [-1.11;0.19]	2.45	0.84 [-0.05;1.70]	3.1	5.54 [4.18;6.88]	Beletsky et al. 1989, 1992 ; Lacombe et al. 1991 ; Johnson 1998

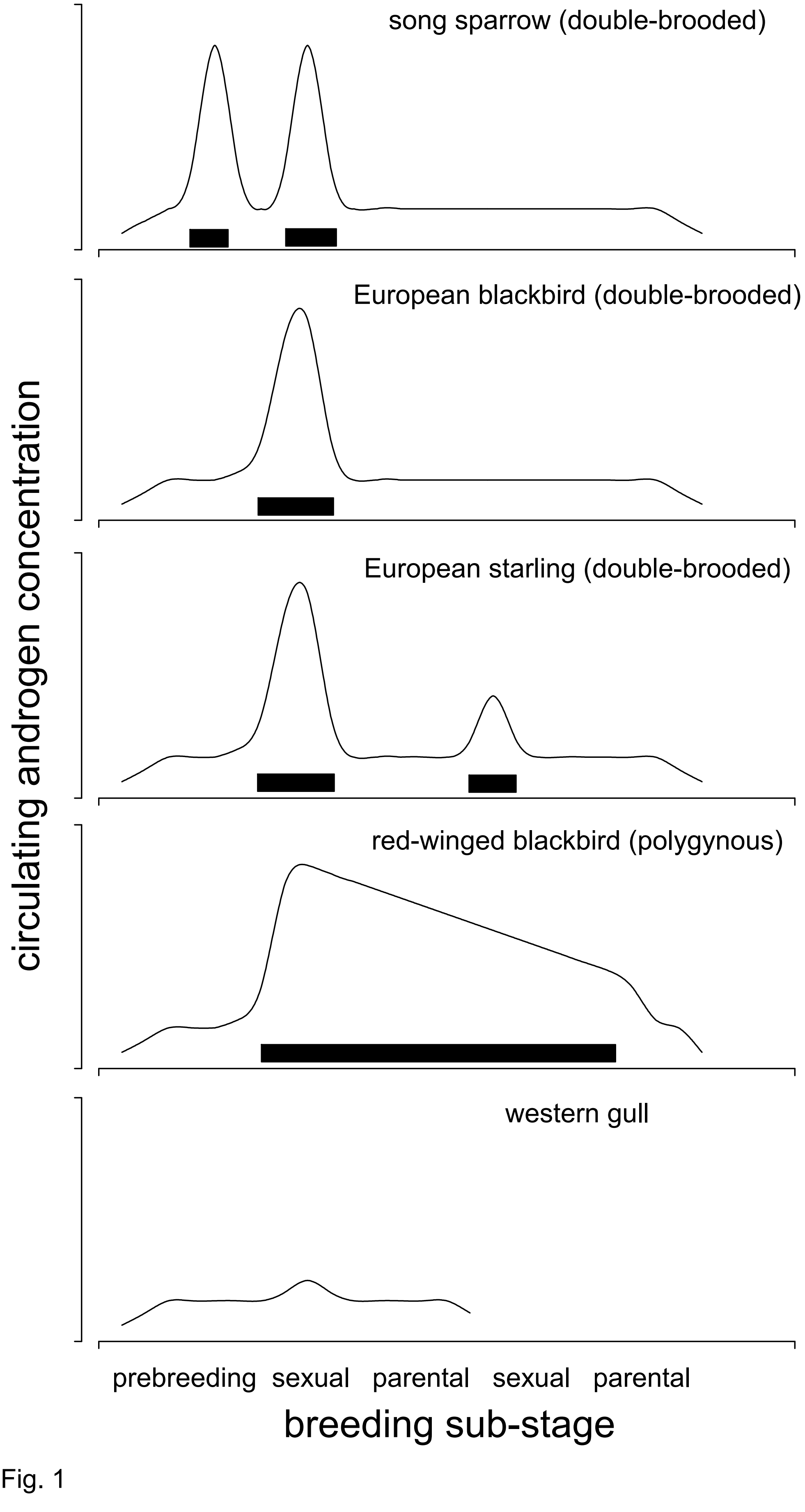
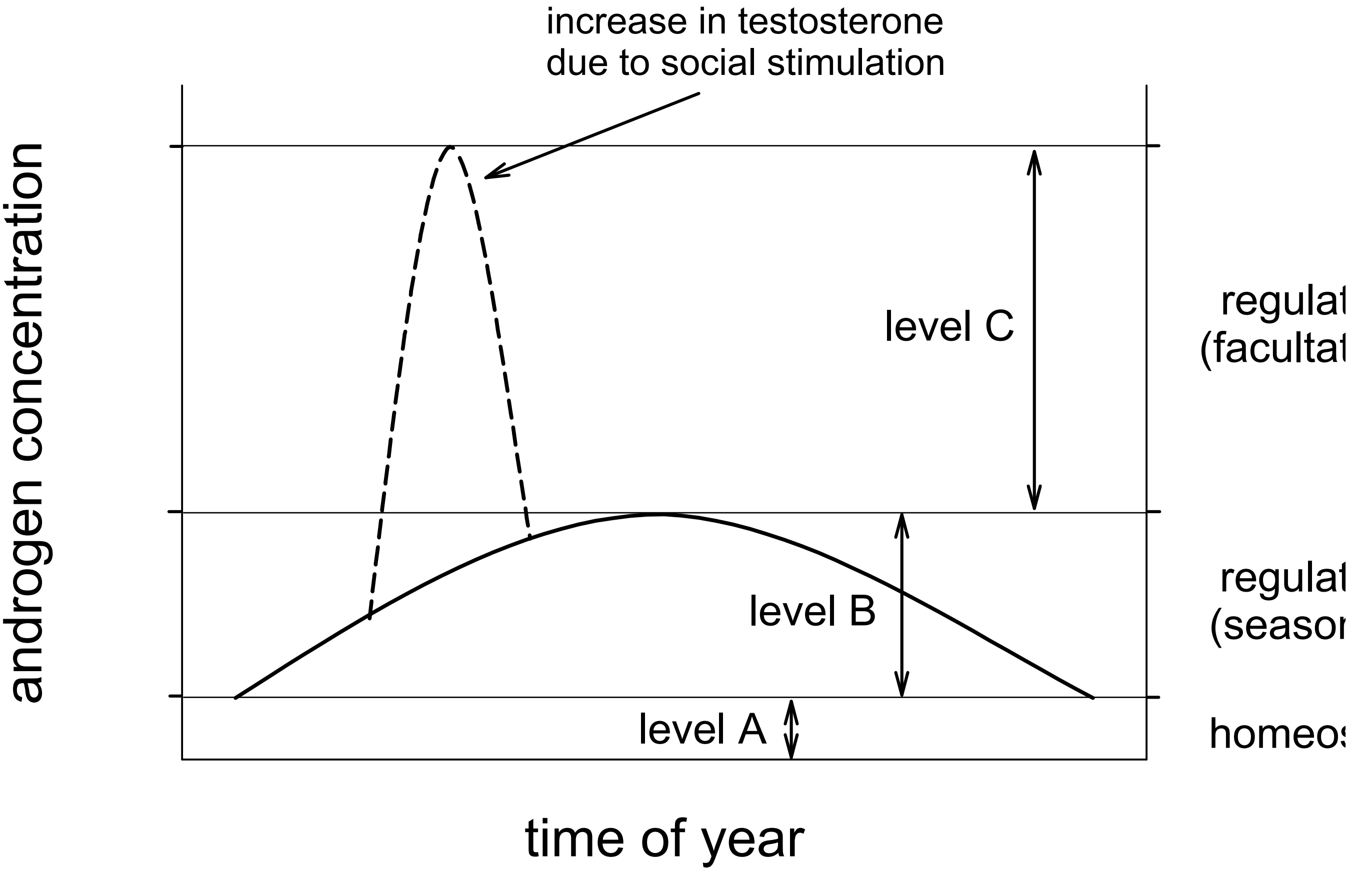

song sparrow (double-brooded)
European blackbird (double-brooded)
European starling (double-brooded)
red-winged blackbird (polygynous)
western gull
circulating androgen concentration
prebreeding
sexual
parental
sexual
parental
breeding sub-stage

Fig. 1


increase in testosterone due to social stimulation
androgen concentration
level C
level B
level A
regulat
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(seasor
homeos
time of year

Fig. 2

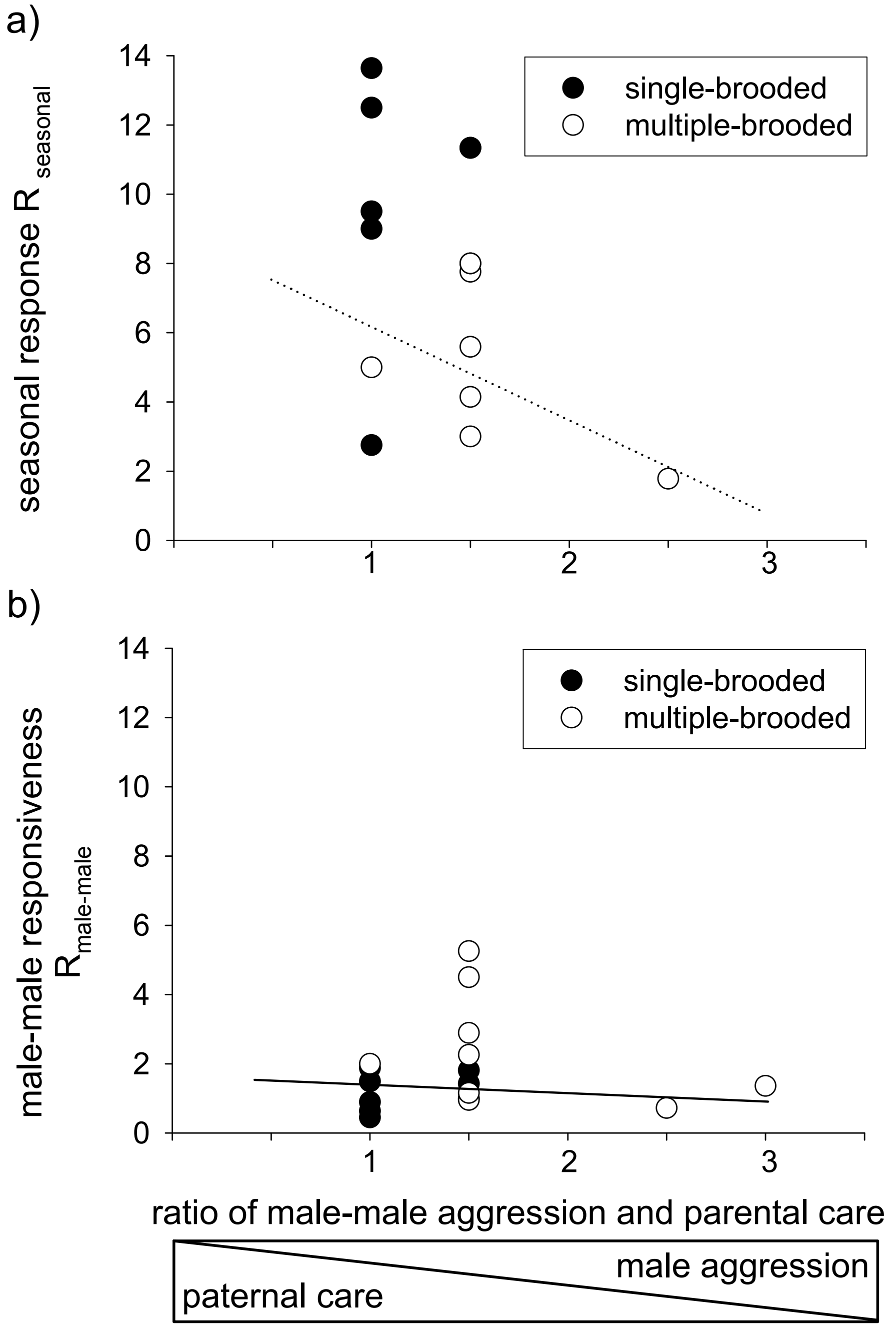

a)
seasonal response $R_{seasonal}$
14
12
10
8
6
4
2
0
1
2
3
● single-brooded
○ multiple-brooded
b)
male-male responsiveness $R_{male-male}$
14
12
10
8
6
4
2
0
1
2
3
● single-brooded
○ multiple-brooded
ratio of male-male aggression and parental care
male aggression
paternal care

Fig. 3

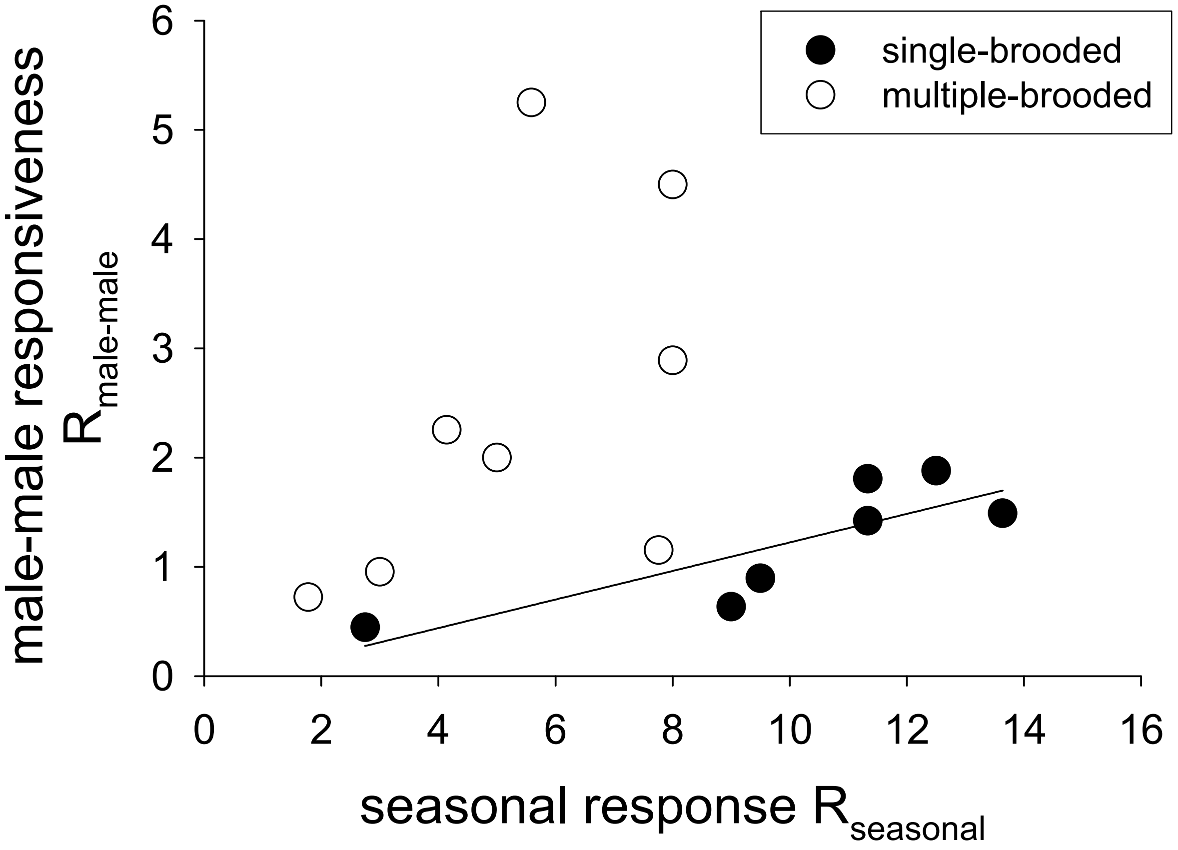
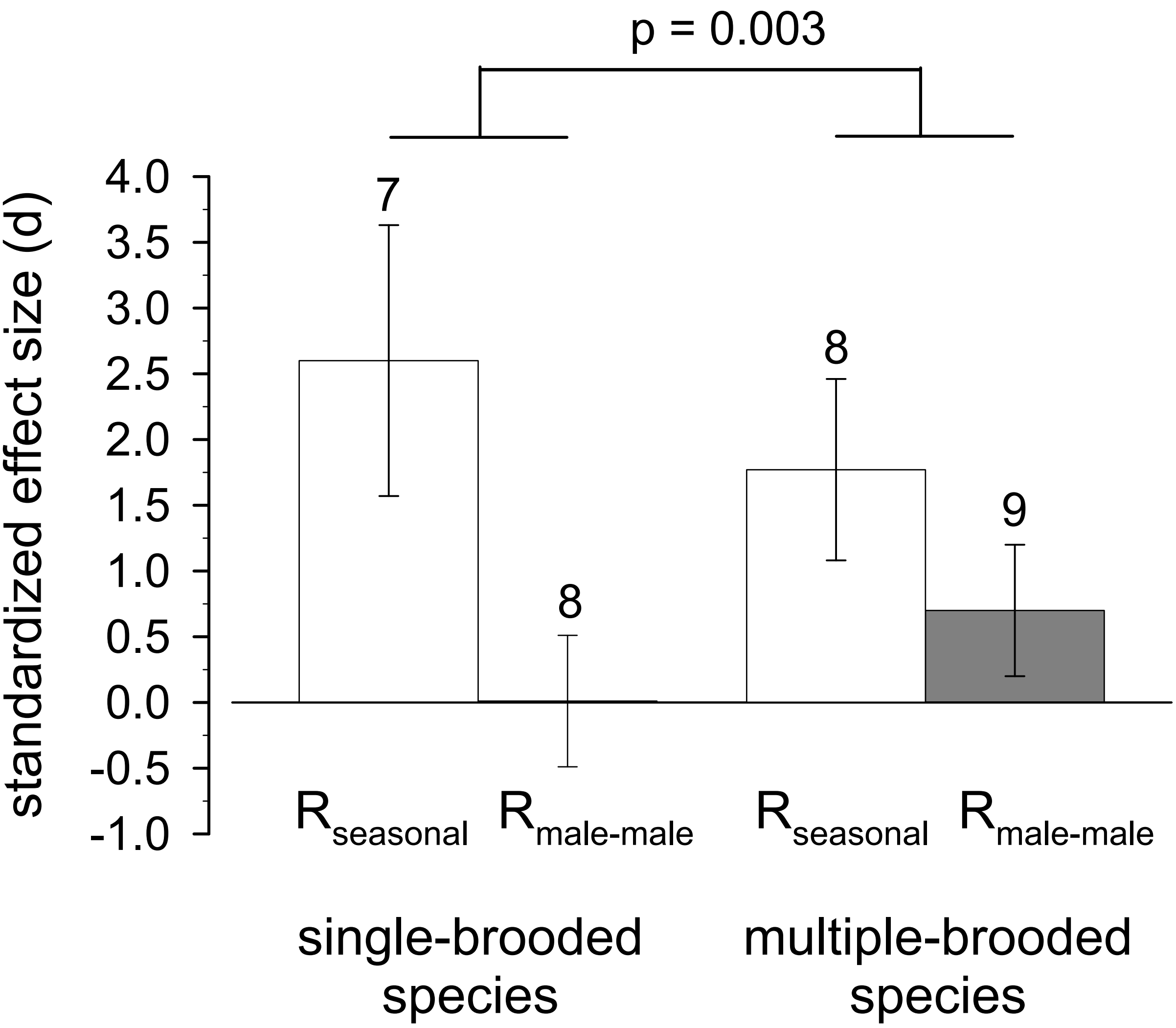
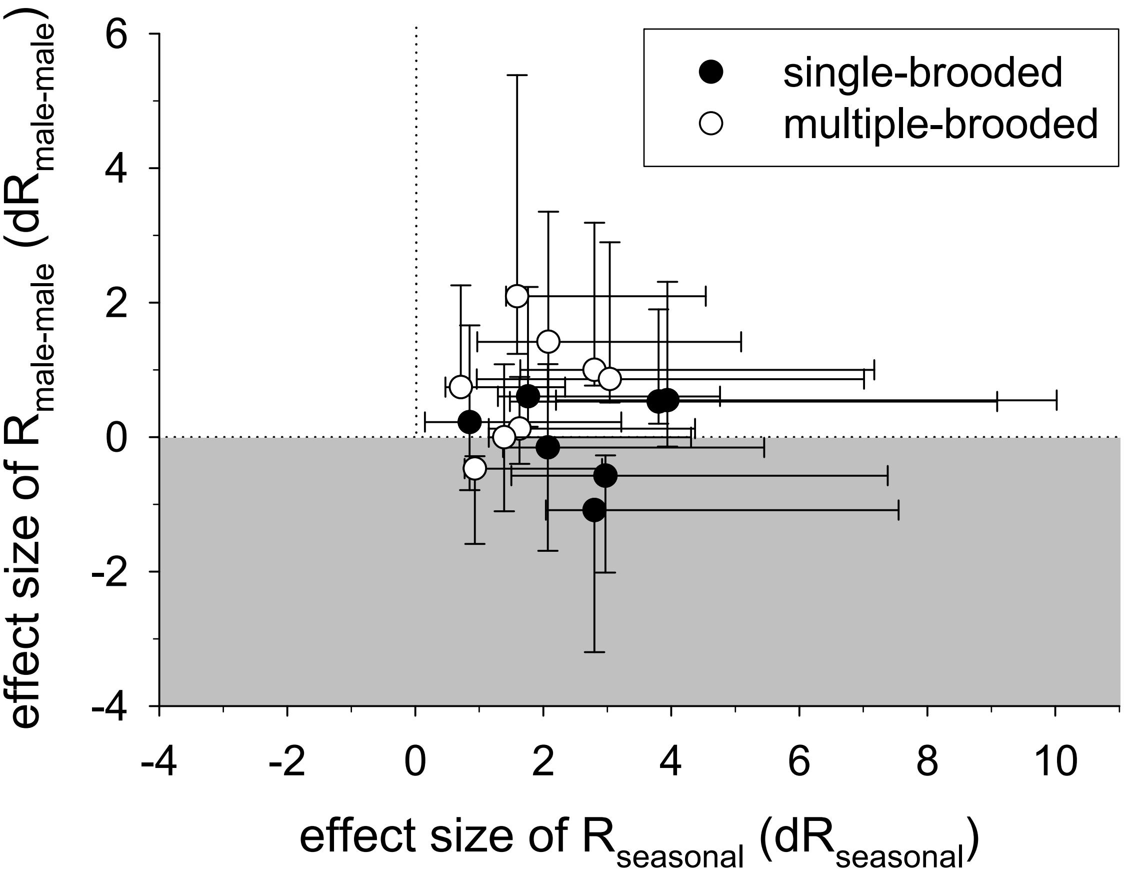

6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
male-male responsiveness $R_{male-male}$
seasonal response $R_{seasonal}$
● single-brooded
○ multiple-brooded

Fig. 4



p = 0.003
standardized effect size (d)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
7
8
8
9
$R_{seasonal}$
$R_{male-male}$
$R_{seasonal}$
$R_{male-male}$
single-brooded species
multiple-brooded species

Fig. 5


6
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-4
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10
● single-brooded
○ multiple-brooded
effect size of $R_{male\text{-}male}$ ($dR_{male\text{-}male}$)
effect size of $R_{seasonal}$ ($dR_{seasonal}$)

Fig. 6

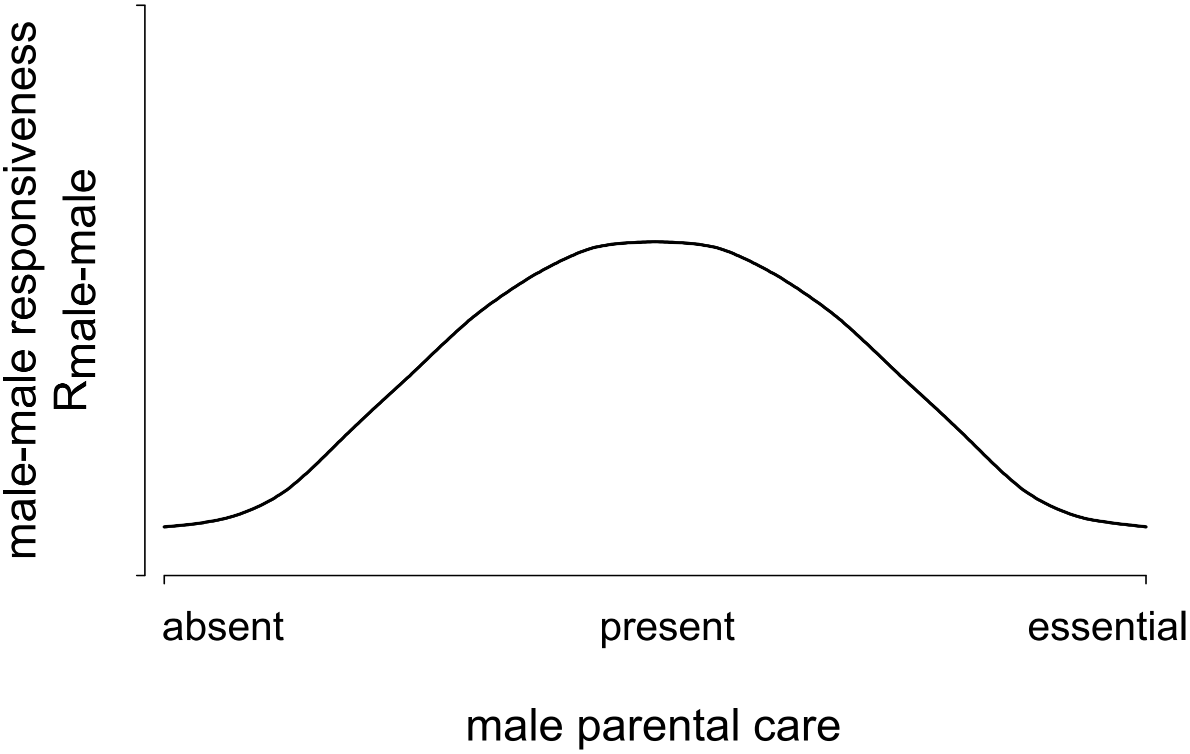
male-male responsiveness
$R_{\text{male-male}}$
absent
present
essential
male parental care

Fig. 7