Relating endocrinology, physiology and behaviour using species with alternative mating strategies

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Summary

1. Recent reviews demonstrate that genetically determined alternative mating strategies (AMS) are widespread and typically consist of morphs that are recognized by morphological or colour traits. Despite well-established behavioural differences associated with each morph, and evidence that androgens are involved in the induction of morphs, few studies have examined whether morphs also vary in whole-organismal performance traits, which may affect dominance status, resource holding potential (RHP) or mate attraction.

2. Our survey revealed a link between androgens and physiological performance traits that are associated with territorial or courtship displays across vertebrate taxa, although the number of species in the sample is limited. Experimental elevation of testosterone alters muscular contractile properties, swimming performance, sprint speed and endurance in males. Whether morphs differ in physiological capacities is relatively unexplored, although recent studies have found that males with high dominance status also exhibit greater physiological capacities (locomotor performance, call duration).

3. Multiple studies support the hypothesis that elevated testosterone results in fitness trade-offs. Potential costs of testosterone include impaired immune function, higher parasite loads, greater energetic requirements and ultimately reduced survival. Long term studies of *Uta stansburiana* highlight the trade-offs among life-history traits induced by variation in testosterone. Circumstantial evidence suggests a role of testosterone in depressing immune function in species with AMS.

4. We argue that hypotheses regarding the role of trade-offs in shaping selection on functional modules, which are involved in sexual selection, are best developed by manipulative studies on discrete morphs. Our review highlights the need to measure multiple traits to provide additional insights into the roles of sexual selection and physiological epistasis in maintaining intraspecific variation in reproductive phenotypes. The integration of endocrine control of behaviour, physiology and performance is rarely attempted in most studies and may be facilitated by analyses that focus on estimating correlational selection.

Key-words: Alternative mating strategies, correlational selection, immunocompetence, performance, sexual selection, testosterone

Integration of androgens, physiology and behaviour

Circumstances where mating opportunities are skewed such that a small proportion of males in a population acquire mates, and there is substantial intrasexual variation in sexually selected traits, subordinate males may evolve alternative morphologies or behaviours to enhance their fitness (Brockmann 2001; Shuster & Wade 2003; Oliveira 2004). In some cases, subordinate alternative types may specialize in exploiting weaknesses of the most successful dominant males, thereby gaining some or even high fitness. Subordinate vs dominant alternative phenotypes may be continuous or discrete, and/or labile or fixed. Continuous variation

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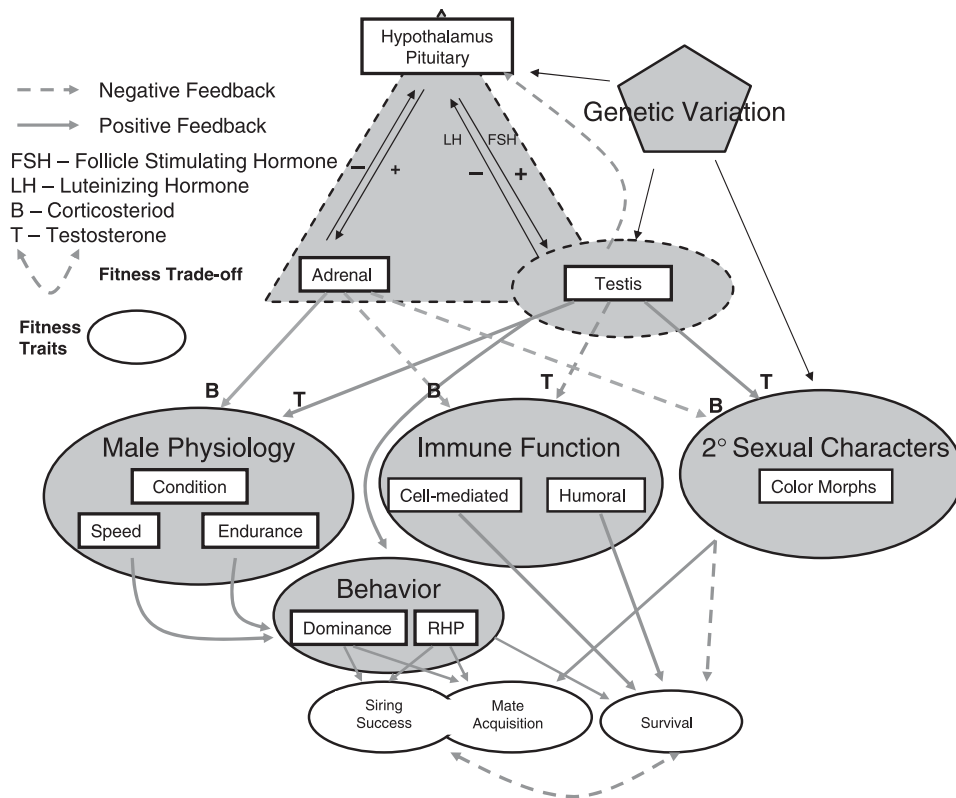
Fig. 1. The integration of sexual selection, hormones, performance and behaviour. The diagram highlights the synergism between the endocrine system and functional modules for morphology, physiology, immune function, behaviour and fitness. The triangle represents the hypothalamic-pituitary-gonadal (HPG) axis and hypothalamic-pituitary-adrenal (HPA) axis. An endocrine cascade begins with the secretion of gonadotropins by the pituitary gland, and is regulated by gonadotropin releasing hormone (GnRH) by the hypothalamus. The gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), critically affect reproduction by regulating gonadal function. The secretion of testosterone (T) by the testes is stimulated by LH. The arrows illustrate the pervasive effects across multiple functional modules that determine male reproductive behaviour and fitness, with solid arrows designating positive influences and dashed arrows signifying negative effects. Genetic variation perturbs the response of each module to the endocrine cascade, which in turn generates trade-offs among traits. Genotypic differences among morphs (e.g. throat colour, plumage pattern) governs the endocrine cascade as well as the coupled behavioural (Resource Holding Potential *vs* Resource Defence Potential) and physiological complexes (endurance, sprint speed, song displays) characterizing each male reproductive strategy. Androgens may enhance physiological performance, but do so at a cost in terms of suppressed immunocompetence, or higher daily energy expenditures and ultimately reduced survivorship. Figure modified from Sinervo & Calsbeek (2003).

in male morphs may be a consequence of condition-dependent traits (Knapp *et al.* 2003). Examples of continuous variation include body size or shape (Leary *et al.* 2005; Cook & Bean 2006) or colour differences (McGraw & Hill 2000) among males. In some species, condition-dependent traits are age related (Oliveira 2004, but see Leary *et al.* 2005). An increasing number of studies have identified behavioural differences associated with each morph, and that testosterone is involved in the induction of morphs. However, few studies have examined how variation in testosterone levels is associated with whole-organismal performance traits among morphs, which may affect dominance status, resource holding potential (RHP) or mate attraction. Furthermore, despite evidence that elevated levels of testosterone may have a negative influence on survival, the physiological mechanisms underlying this trade-off are obscure.

In this paper, our goal is to highlight the synergistic effects of androgens on traits that are favoured by sexual selection (Fig. 1). Our focus is on vertebrate species with genetically determined morphs, because more studies are available on interactions among the components of the endocrine system and traits under sexual selection. Variation among morphs arises through feedback loops within the hypothalamic-pituitary-gonadal axis (HPG) that initiate a hormonal cascade involving luteinizing hormone (LH) and follicle stimulating hormone (FSH), which ultimately affects level of plasma testosterone secreted by the testes (Fig. 1, Rhen & Crews 2002; Nelson 2005). Differences in morphological (secondary sexual signals) and behavioural traits linked with resource holding potential (RHP) and dominance status, for example, displays, territorial contests, singing, push-ups, among morphs should be modulated by inter-individual variation in performance capacities, for example, endurance, speed or bite force. One goal of this review is to determine whether varying levels of testosterone are associated with physiological traits that are tied to RHP. Fitness differences from mate acquisition

and siring success should be enhanced by allocation of resources to traits that enhance dominance and RHP. However, males that enhance their reproductive success via RHP are expected to experience a survival trade-off due to increased allocation of energy and time to costly behaviours. A second goal is to review the potential physiological pathways associated with fitness trade-offs. We also discuss how sexual selection acting on suites of traits generates trade-offs among functional modules, for example, morphological traits, physiological, behavioural and life-history traits (Sinervo & Svensson 2002).

Studies based on discrete morphs provide unique opportunities to investigate the functional bases of sexual selection that integrate physiology, behaviour and fitness trade-offs while controlling for extraneous variation, such as condition and age (Sinervo & Calsbeek 2003). Examining morphotypic variation therefore allows the characterization of the effects of androgens on sexual ornaments and behaviour; cascading effects on integrated functional trait modules like locomotor performance, and their role in generating life-history trade-offs. Because morphs are often the outcome of sexual selection (Andersson 1994; Brockmann 2001; Taborsky 2001; Rhen & Crews 2002), experiments using species with alternative mating strategies (AMS) provide the elucidation of how sexual selection can be constrained by trade-offs and yet generate distinct adaptive solutions in traits that segregate within a single population. Focusing on variation among morphs presents an opportunity to evaluate steroid-mediated effects on Darwinian fitness and the interplay between natural selection and sexual selection.

The natural history of alternative mating strategies (AMS)

PATTERNS AND PREVALENCE

AMS typically are manifested as intrasexual variation in either morphology, behaviour or both (Taborsky 2001). Recent surveys highlight how common AMS are among invertebrate (Insecta, Crustacea and Araneae) and vertebrate taxa (Osteichthyes, Amphibia, Reptilia and Aves) (Moore, Hews & Knapp 1998; Brockmann 2001; Shuster & Wade 2003; Zamudio & Sinervo 2003; Jukema & Piersma 2004; Oliveira 2004). Most AMS systems entail two morphs. One morph is a dominant male that defends a territory or nest, and displays to females to acquire copulations. The alternative morph may be a 'satellite' male or a 'sneaker' male. The former morph abandons territorial defence or lekking behaviour and instead may remain adjacent to the territory of a dominant male, whereas the latter morph evolves female mimicry behaviour in order to surreptitiously copulate with already mated females.

Conspicuous morphs are uncommon in mammals; however this may be because most have limited colour vision, which makes colour morphs unlikely. Nevertheless, Sinervo & Zamudio (2002) points out that morphs may be common in olfactory sensory modalities, particularly in rodents. A profitable avenue would be to analyze levels of various steroids secreted in urine or faeces, which serves as a key signal in mammals. Examples of morphs are found in higher primates. For example, the orangutan (*Pongo pygmaeus*) exhibits an aggressive and dominant male morph with enlarged facial fat pads compared to a subordinate morph with reduced facial fat pads (Maggioncalda, Sapolsky & Czekala 1999). Analysis of steroid levels in the urine revealed that dominant males had elevated testosterone relative to the subordinate males (Maggioncalda *et al.* 1999). Levels of testosterone in urine samples in primates would provide inference on the role of steroids in modulating this kind of dramatic AMS.

Discrete morphs are often genetic and fixed throughout life (Rhen & Crews 2002). The genetic basis of discrete morphs in some systems has been assessed by elaborate breeding studies (Shuster 1989; Lank *et al.* 1995; Shuster & Sassaman 1997; Sinervo, Bleay & Adamopoulou 2001; Lindholm, Brooks & Breden 2004). The maintenance of discrete genetic morphs has been attributed to several mechanisms including niche specialization, neutral polymorphism, frequency-dependent selection and overdominant selection (Roulin 2004). Recent studies support hypotheses that morphs are maintained by negative frequency-dependent selection in which male types gain an advantage when rare (Zamudio & Sinervo 2003; Sinervo & Calsbeek 2006), or by high fitness of heterozygotes compared to homozygotes, that is, overdominant selection (Turelli 1981; Sinervo & Zamudio 2002). These explanations are not mutually exclusive; overdominant heterozygous types may experience lower fitness if common, or homozygous genotypes may experience elevated fitness when rare.

Manifestations of phenotypic differences among AMS include body size or shape (Wikelski, Carbone & Trillmich 1996; Clark 1997) or colour (Rand 1992; Lank *et al.* 1995; Pryke & Griffith 2006; Spinney, Bentley & Hau 2006; Whiting *et al.* 2006) or both (Sinervo & Lively 1996, Clark & Biesiadecki 2002; Franck, Müller & Rogmann 2003). Larger males tend to be aggressive and territorial, whereas smaller males adopt either a sneaker strategy or a satellite strategy. For example, males of the marine amphipod *Jassa marmorata* exhibit a polymorphism involving size of the body and the propodus ('thumb'). Large males or 'majors' have larger thumbs than the small males or 'minors'. Clark (1997) showed that the male morphs employ different mating strategies: majors aggressively exclude other males to obtain exclusive access to receptive females compared to minors. In other species such as the Lusitanian toadfish (*Halobatrachus didactylus*), morphs are dichotomized by territorial behaviour as well as sound production capacity (Modesto & Canário 2003). Type I, nest defender, males have hypertrophied sonic muscles attached to the swim bladder, which acts as a resonator. Type II cuckolder males lack the increase in the mass of the sonic muscle.

Trimorphic male mating systems also appear to be widespread and occur in invertebrates, fish, reptiles and birds. For example, Galápagos marine iguanas (*Amblyrhynchus cristatus*) have three phenotypes, territorial males, satellite males and sneaker males, which correspond with variation in age and size (Wikelski *et al.* 2004). The mating strategy adopted by a given male is conditional on the number of available territories and frequency of large males (Wikelski *et al.* 2004). Male side-blotched lizards (*Uta stansburiana*) can be categorized into one of three morphs based on throat colour (orange, blue or yellow). Laboratory crosses and field release of offspring (Sinervo *et al.* 2001), field pedigree data (Zamudio & Sinervo 2000), gene mapping studies in the field pedigree with transmission linkage decay (Sinervo *et al.* 2006) and theoretical models (Sinervo *et al.* 2001) support the observation that inheritance of colour segregates as a single Mendelian gene. The three colour morphs correspond to AMS: males expressing the orange throat colour phenotype (O = *oo*, *bo*, *yo* genotypes) are large, aggressive (territory usurpers, Calsbeek & Sinervo 2002), and have large territories that overlap the home ranges of many females (Zamudio & Sinervo 2000). Blue males (B = *bb*) cooperatively defend territories (Sinervo & Clobert 2003; Sinervo *et al.* 2006), which is effective in limiting advances of O males (however, one B male altruistically defends his partner and achieves zero fitness) as well as completely excluding sneakers (both B males obtain mutualistic benefits when sneakers are common). Yellow males (Y = *by*, *yy*) abandon territoriality and adopt a strategy of crypsis, characteristic of a sneaker. In addition, Y males sire many progeny on later clutches, obtaining posthumous fertilizations late in the season (Zamudio & Sinervo 2000). Morph competition is intense and drives multi-year cycles in morph frequency and a rock-paper-scissors (RPS) dynamic: sneakers beat usurpers, cooperators beat sneakers, and usurpers beat cooperators. The same locus in females results in two phenotypes (orange and yellow), which vary in how they allocate resources to offspring (egg size-clutch size trade-off) (Sinervo, Svensson & Comendant 2000b). Polymorphisms may be still more extreme, for example, one live bearing fish, *Poecilia parae* has five genetically determined male colour morphs (Lindholm *et al.* 2004).

Hormones, morphs, performance and behaviour – consequences of variation in androgens

Linking sexual selection and physiological performance requires information on two aspects of phenotype. First, evidence must be available demonstrating that testosterone is critical in the organization of different phenotypes (badges) and behaviours which covary with morph status of males. Behavioural traits associated with elevated testosterone include increased aggressiveness (Marler & Moore 1988), higher dominance status and expanded territories (Wingfield *et al.* 1990; Sinervo *et al.* 2000a). Testosterone should enhance performance traits that are tied to RHP, such as push-up displays (Brandt 2003), head butting, singing or calling. Second, androgens must contribute to differences in physiological performance. Focusing on species with AMS facilitates the opportunity to build links between hormones, badges and performance. For example, the ability to acquire and defend territories or engage in prolonged courtship displays should correspond with high physiological capacity of an individual. Aggressive interactions are energetically expensive (Castro *et al.* 2006) and require modifications of muscle tissue and metabolic capacity for extended displays.

HORMONES AND MALE MORPHS

In most vertebrates, gonadal steroids like testosterone have been implicated in the expression of secondary sexual traits and specifically behavioural traits used in male–male competition and female attraction (Brantley, Marchaterre & Bass 1993; Salvador *et al.* 1996; Oliveira *et al.* 2001a; Oliveira, Canario & Grober 2001b; Cox *et al.* 2005; Lee & Bass 2005). Testosterone is implicated in the development of wattles, pigmentation on the skin, aggressive behaviours and courtship displays (Owens & Short 1995; Blas *et al.* 2006). An interaction between testosterone and autosomal genes is presumed to generate sexually dichromatic plumage in birds, for example (Lank, Coupe & Wynne-Edwards 1999, see also Ros 1999; Peters *et al.* 2000). Manipulation of androgens, either through castration or exogenous testosterone, has demonstrated the role of testosterone in the development of male morphs, though other components of the endocrine system are likely involved (Bass 1993; Moore *et al.* 1998; Knapp *et al.* 2003; Knapp 2004).

ANDROGENS AND PHYSIOLOGY

Given the suite of traits that vary among morphs, what is the evidence that testosterone coordinates the expression of badges, aggressive behaviours, and enhanced physiological capacity necessary for displays, agonistic interactions and courtship behaviours (Lailvaux & Irschick 2006)? The inclusion of endocrine effects expands the discussion of how morphology and performance affect social behaviour (Garland & Losos 1994), or how behaviour drives evolution of physiology and morphology (Huey, Hertz & Sinervo 2003). Higher plasma testosterone benefits males via exaggerated ornaments (Berglund, Bisazza & Pilastro 1996) that are reinforced with increased aggressiveness, higher display rates, dominance status, larger territory or home range size and greater reproductive success. Recent work has shown that sprint speed and endurance may be important components of dominance behaviours in males (Garland, Hankins & Huey 1990; Kodric-Brown & Nicoletto 1993; Robson & Miles 2000; Perry *et al.* 2004). However, the association has not been found in other species (*Lacerta monticola*, Lopez & Martin 2002; *Gallotia galloti*,

Huyghe *et al.* 2005). In addition, comparisons between the sexes have revealed significant sexual dimorphism in ecologically relevant whole organismal performance traits, for example, bite force, sprint speed and endurance (Snell *et al.* 1988; Cullum 1998; Miles, Snell & Snell 2001; Lappin, Hamilton & Sullivan 2006; Royle, Metcalfe & Lindstrom 2006). Finally, locomotor performance has been shown to correlate with survivorship (Miles 2004) and number of offspring, as in the lizard *Crotaphytus collaris* (Husak *et al.* 2006). Thus, the covariation between sexually selected signals, for example, exaggerated colouration and dominance behaviour, with fitness is likely to be mediated by physiological performance.

MUSCULAR PHYSIOLOGY AND PERFORMANCE

Studies on vertebrate taxa have demonstrated the role of androgens in mediating a diverse array of muscular and physiological traits (Supplementary Table S1). What is the evidence that testosterone induces changes in traits likely to affect intrasexual competition and male mating success? A recent review concluded that elevated levels of testosterone are associated with skeletal muscle hypertrophy and an increase in the size of motor neurons, which may be critical in performing complex displays for an extended duration (Herbst & Bhasin 2004). In addition, experimentally elevating androgens induced changes in muscles involved in swimming in fish. Implantation of 11-ketotestosterone in immature rainbow trout (*Oncorhynchus mykiss*) resulted in greater ventricular mass relative to body mass and an increase in the cross-sectional area of lateral red muscle (Thorarensen, Graham & Davie 1996) and the size of the same traits in mature males. Thorarensen *et al.* (1996) hypothesized that one role for androgens in rainbow trout was to enhance endurance in reproductive males which would enhance display behaviours.

Acoustical signals are used to advertize male quality in multiple taxa. For example, in several species of fish specialized muscles attached to the swim-bladder wall (sonic muscles) generate calls used to attract females. Implanting testosterone into non-breeding males increased the mass and cross-sectional area of sonic muscles (Brantley *et al.* 1993; Connaughton & Taylor 1995; Connaughton, Fine & Taylor 2002; Modesto & Canário 2003). Calling in frogs, the main signal used by males to attract females, is energetically costly (Taigen & Wells 1985). High contractile speed of oblique muscles, which is induced by testosterone, is critical for the production of rapid pulsed calls that attract females. Girgenrath & Marsh (2003) demonstrated that the contractile properties of the external oblique muscles are modified by testosterone. The oblique muscles of post-breeding males have a lower proportion of fast- *vs* slow-twitch fibres and mass. Treatment of post-breeding males and females with testosterone results in muscles having similar mass and contractile characteristics

observed in breeding males (Girgenrath & Marsh 2003). Non-breeding males and females do not vocalize, and their muscles have a higher proportion of slow-twitch fibres. Testosterone also alters characteristics of another sexually dimorphic muscle, the flexor carpi radialis, which is a forelimb muscle used during amplexus (Regnier & Herrera 1993a). Testosterone implants resulted in larger average fibre size, greater cross-sectional area and changes in contractile properties (Regnier & Herrera 1993a, 1993b; Dorlöchter, Astrow & Herrera 2004). The proportion of such muscles in satellite males, a common AMS in anuran amphibians, has not been characterized.

Several studies have examined the effects of testosterone on locomotor performance and locomotor activity. Klukowski, Jenkinson & Nelson (1998) implanted testosterone in *Sceloporus undulatus* males and induced a 17% increase in endurance and a 24% increase in sprint speed relative to controls, but at a cost of continued growth in body size. In *Uta* males, Sinervo *et al.* (2000a) found that testosterone enhanced endurance by 49% relative to controls. In addition, O male *Uta* with naturally high testosterone also exhibited 50% higher endurance relative to B or Y males. In contrast, in a correlational study, Husak *et al.* (2006) reported no association between testosterone levels and sprint speed in the lizard *C. collaris*. In birds, castration and implants revealed the role of testosterone in increasing locomotor activity (Wada 1982, 1986). In mammals, testosterone has been linked with indices of locomotor activity in male meadow voles (*Microtus pennsylvanicus*), principally distance travelled and percent time moving (e.g. Perrot-Sinal *et al.* 1998). Anabolic steroids also enhanced running performance in male rats. Experimental male rates given anabolic steroids were shown to run 41% longer compared to rats given saline implants (Van Zyl, Noakes & Lambert 1995). Paradoxically, exposure to predator odours (red fox, *Vulpes vulpes*, urine) inhibited locomotor activity in males with high levels of testosterone.

Deriving links between testosterone and locomotor performance in birds is challenging, given the specialized equipment necessary for measuring flight capacities (but see Møller 1991). Yet, birds also use elaborate displays and vocal communication to resolve inter-male contests and signal male quality to females. Therefore, one approach to examining physiological consequences of testosterone in birds is to quantify changes in display rates and estimate metabolic costs of signalling (Ketterson & Nolan 1999). Those studies that manipulated testosterone levels in birds, either through implantation or castration have noted an increase in male displays (Beani *et al.* 2000; Reed *et al.* 2006). In addition, testosterone alters the acoustic characteristics of calls, yielding longer and lower pitched calls (Wada 1982, 1986; Beani *et al.* 2000). Testosterone also increased aggressive behaviours, raising the RHP of males, which in turn enhanced their success in the acquisition of territories (Veiga *et al.* 2001).

TESTOSTERONE, PHYSIOLOGICAL VARIATION
AMONG MORPHS AND MATE CHOICE

Variable levels of plasma testosterone among morphs should result in predictable rank order differences in physiological performance. With respect to condition-dependent ornaments, signal intensity and performance should be positively correlated. Bright blue coloured, territorial Pecos pupfish males (*Cyprinodon pecosensis*) have higher swimming capacities than pale, non-territorial conspecifics (Kodric-Brown & Nicoletto 1993). In *P. reticulata* swimming performance is assumed to have a positive effect on survivorship as well as display behaviour. Male guppies have three ornaments, tail shape, dorsal fin length and carotenoid pigment density, which are involved in mate choice (Nicoletto 1991). Females may select males based on one of these ornaments, which would be an indicator of a male's ability to avoid predators. Carotenoid density is likely to be influenced by testosterone as well as being a condition-dependent trait. Only one ornament, carotenoid pigment density, was positively and significantly correlated with swimming performance (Nicoletto 1991; Nicoletto & Kodric-Brown 1999). It is also possible that swimming performance may be tied to display behaviour; however this was not tested in the study. Nevertheless, females use intensity of carotenoid pigmentation on males in mate choice (Kodric-Brown 1993; Nicoletto 1993), providing indirect evidence that performance may be under intersexual selection. In a separate study, Blows, Brooks & Kraft (2003) analyzed female preference for male guppy colouration and found that females exhibit a striking pattern of three distinct colour preferences that were under strong correlational selection. This suggests that colour preferences of females favour three quite divergent colour syndromes. Similar methods could be used to estimate multivariate female preference functions for colour, morphological traits listed above and performance.

The behavioural differences observed in male morphs of *Uta* also suggest an underlying association with performance. O males have the highest endurance, Y-males the lowest and B-males intermediate endurance (Sinervo *et al.* 2000a). The observed difference in performance was not a consequence of variation in body size among the morphs (Sinervo *et al.* 2000a). Implanting exogenous testosterone into males with B or Y genotypes resulted in an increase in endurance, which approximates the high values recorded in O males.

It is possible that differences in performance among morphs may be confounded by a trait that is genetically correlated with an ornament. Such is the case in the swordtail *Xiphophorus nigrensis*. Three size morphs occur in nature, large, intermediate and small males, which are determined by the P locus on the Y chromosome. Ryan (1988) demonstrated significant differences in swimming capacity among morphs; larger males had greater endurance. A nested analysis of variance revealed a significant effect for genotype, but size

(standard length), attributed to the P locus, was a better predictor of endurance. A potentially confounding factor involves species that have multiple badges (size or signals) or multiple cues used for announcing dominance status. There may be circumstances where multiple badges covary with performance; conversely, performance may be related to only one signal. In the lizard *Urosaurus ornatus,* Meyers *et al*. (2006) found no difference in sprint performance among throat colour morphs. However, bite force was strongly related to the area of the ventral blue belly patches (Meyers *et al.* 2006). This suggests that care must be taken to ensure that the signal and performance measurement are suitably matched.

Trade-offs and the cost of testosterone

Testosterone promotes the expression of secondary sexual traits and behaviours, but at the cost of elevated mortality. Two pathways may generate the observed trade-offs (Partridge 1992). Testosterone may directly or indirectly suppress immune function, which increases mortality (Folstad & Karter 1992). Alternatively, testosterone may induce an escalation in aggressive behaviour and activity, which raises the risk of mortality through exposure to predators, parasitic infestations and elevated metabolic costs (Marler & Moore 1988; Dufty 1989; Salvador *et al.* 1996; Buchanan, Evans & Goldsmith 2003; Reed *et al.* 2006). Energy allocated into morphological, physiological or behavioural traits which enhance reproductive success because of higher secretion of testosterone, necessitates lower energy to support maintenance activities that favour growth, adult survival or incur costs in terms of directed aggression from territorial neighbours (Buchanan *et al.* 2001). Furthermore, greater activity levels are likely to necessitate higher metabolic rates and concomitantly higher energetic requirements, which generates life-history trade-offs (Zera & Harshman 2001).

The immunocompetence handicap hypothesis (ICHH)

In their discussion of the costs of honest signals, Folstad & Karter (1992) suggested that testosterone directly acted to suppress immune function. A trade-off between elevated testosterone and components of immune function (e.g. cell-mediated, humoral or leukocyte counts) has mixed empirical support (Casto, Nolan & Ketterson 2001; Roberts *et al.* 2004; Buchanan *et al.* 2003; Owen-Ashley, Hasselquist & Wingfield 2004; Deviche & Cortez 2005; Greives *et al.* 2006). Most evidence comes from studies that measure immune function after manipulating plasma testosterone. Many studies have produced evidence supporting the ICHH, yet a cautionary interpretation of the role of testosterone in suppressing the immune system was suggested by Roberts *et al.* (2002). Considered in isolation, most studies cited by Roberts *et al.* (2002) concluded elevated testosterone

levels were immunosuppressive. However, a meta-analysis only weakly supported the ICHH (Roberts *et al.* 2002). In contrast, there are systems in which mating tactics are unrelated to immunocompetence as in the comparison among female morphs in the coenagrionid damselfly species *Coenagrion puella* (e.g. Joop *et al.* 2006). Nevertheless results from recent publications tend to support the immunosuppressive effects of elevated testosterone (Lozano & Lank 2003; Belliure, Smith & Sorci 2004; Greives *et al.* 2006, Reed *et al.* 2006).

Testosterone may entail costs through pathways other than by directly suppressing the immune response (Wedekind & Folstad 1994; Belliure *et al.* 2004; Owen-Ashley *et al.* 2004; Ahtiainen *et al.* 2005). An unresolved question is the mechanistic basis of alternative trade-offs. Do elevated testosterone levels impair the immune response by re-allocating energy or nutrients from the immune system towards self-maintenance (Wedekind & Folstad 1994) or through other mechanisms, unrelated to energy- or nutrient limitation? Owen-Ashley *et al.* (2004) reviewed the immunosuppressive effects of testosterone and highlighted stress-induced immunosuppression and aromatization as two additional indirect pathways. Stress-induced immunosuppression would occur as a consequence of the correlated elevation of corticosterone levels after manipulation of testosterone. The second hypothesized indirect pathway involves estradiol-induced immunosuppression as a result of the conversion of testosterone into estradiol by the enzyme aromatase (Owen-Ashley *et al.* 2004).

In fact, there is little evidence that immune responses are energetically costly (Råberg *et al.* 1998; Svensson *et al.* 1998). Although limitation of certain nutrients, such as carotenoids (see further below), may have a role, the energetic costs of mounting immune responses are very low, and at most a few percent of the basal metabolic rates in birds (Svensson *et al.* 1998). This observation has led some authors to argue instead that the major costs of mounting immune responses are not constrained by energy- or nutrient limitation, but rather attributable to the immunopathological consequences related to the difficulty from distinguish 'self' from 'non-self' tissue, that is, the risks of autoimmune damage (Råberg *et al.* 1998). Alternatively, fever and inflammation accompanying injury may raise the risk of mortality through impaired performance (Millet *et al.* 2007).

One prediction of ICHH is the expected trade-off between plasma levels of testosterone, available carotenoids and immune response. Carotenoids have a dual role in being a key component of secondary sexual signals and immune function, both of which would be affected by diet quality. Specific theories of sexual selection posit that carotenoids may be important cofactors in immune function and/or free radical elimination (e.g. superoxide dismutase), and thus bright carotenoid colours could be used as a signal of male quality (Blount *et al.* 2003; Grether *et al.* 2004; McGraw 2005). Blas *et al.* (2006) manipulated testosterone levels and availability of dietary carotenoids to examine direct and indirect contributions of testosterone to immunocompetence. Their experiment demonstrated that in red-legged partridges (*Alectoris rufa*) carotenoids affect secondary sexual traits. Exogenous testosterone increased carotenoid concentrations in the plasma and liver, and higher residual concentrations of carotenoids were significantly and positively correlated with cell-mediated immune response.

Testosterone and parasites

Parasite loads have been shown in field and experimental studies to covary with testosterone in males of many species (Saino, Møller & Bolzern 1995; Salvador *et al.* 1996; Olsson *et al.* 2000; Klukowski & Nelson 2001; Mougeot *et al.* 2004; Mougeot *et al.* 2005; Perez-Orella & Schulte-Hostedde 2005), but causal relationships are uncertain. Disentangling whether an observed increase in parasite loads due to testosterone has a physiological origin (immunocompetence hypothesis; resources are diverted from the immune system to secondary sexual traits) or a behavioural origin (encounter hypothesis; exposure mediated parasite loads) is difficult. Studies with exogenous testosterone provide indirect support that males may encounter more parasites, through enlarged territories, longer periods of activity and greater risk of interaction with infected individuals. A limitation of using exogenous implants to manipulate testosterone levels is the difficulty of separating the relative contribution of each factor given that testosterone alters both physiology and behaviour. One of the few studies that separated behaviour from physiological mechanisms involved the use of flutamide and aromatase inhibitor (FA) treatments (Mougeot *et al.* 2005). Male red grouse (*Lagopus lagopus scoticus*) were implanted with either FA implants or sham controls. FA implants allow circulating testosterone levels to rise, but prevents the associated behavioural changes. Experimental males did not exhibit a difference in home range size, pairing success or breeding success despite having higher testosterone concentrations relative to controls. Higher parasite infestations in FA treatment males supported the hypothesis of infection being a consequence of increased physiological stress and higher susceptibility. To date we know of no studies demonstrating differences in parasite load in male morphs, though linkages between immune function and morphs are being measured, as recently demonstrated for colour morphs of the wall lizard (*Podarcis muralis*) (Sacchi *et al.* 2007).

DO HIGHER PARASITES LOADS COMPROMISE PERFORMANCE CAPACITIES? SUPPORT FOR ICHH

One potential consequence of the immunosuppressive effects of testosterone is an increase in parasite loads that alters the energy balance of a male and has a concomitant decrease in both male condition and the ability

to sustain costly locomotor activities. A few notable examples have been recently published that demonstrate that parasites decrease endurance and sprint speed. Parasites were implicated in impaired stamina in juvenile toads, *Bufo bufo* (Goater, Semlitsch & Bernasconi 1993). However, reduced performance was inferred to be a secondary effect of the lung nematode, *Rhabdias bufonis*. Body size and stamina were found to be positively correlated (Goater *et al.* 1993). Infection by the parasite reduced growth rates of metamorphs by 26% and resulted in smaller adult toads with lower stamina. In another experiment, male sand lizards (*L. agilis*) implanted with testosterone had higher parasite loads and lost more weight than controls (Olsson *et al.* 2000). Parasite loads were unrelated to condition, which favours the hypothesis of a direct immunosuppressive effect of testosterone. Because male sand lizards with exogenous testosterone also moved further, parasite infestations could be a consequence of higher encounter rates. No association between parasite load and activity was detected (Olsson *et al.* 2000). Clobert *et al.* (2000) also found a negative association between endurance and parasite load in the lizard, *L. vivipara*. They suggested that lizards with low endurance increased their risk of parasitic infection, rather than the parasite causing decreased performance. Experimental manipulations of tick infestations in Australian sleepy lizards (*Tiliqua rugosa*) resulted in an alteration of both behavioural and locomotor performance. Adults with enhanced tick infestations had reduced home ranges and shorter movement distances than lizards whose ticks were removed (Main & Bull 2000). Furthermore, adults with high tick loads had lower endurance than uninfested individuals. Also, juvenile lizards with ticks were slower than uninfested siblings. Marden & Cobb (2004) investigated how gregarine gut parasites might diminish flight performance, RHP and mating success in the male dragonfly, *Libellula pulchella*. In ponds where parasites were rare, Marden & Cobb (2004) demonstrated a positive association between muscle power output, territory acquisition and mating success. In ponds where parasites were common, males segregated into two groups. One group consisted of territorial males lacking parasites and showed a positive relationship between muscle performance and territorial ownership. These males also had higher fat content that affected muscle power output. The second group of males consisted of submissive satellites, with lower fat content and high parasite infestation. The relationship between muscle output and mating success was not significant in the parasitized males. Marden & Cobb (2004) inferred that the decrease in muscle power output was a consequence of disruption of energy homeostasis and alteration of signalling pathways by gregarine gut parasites.

Testosterone and metabolism

Enhanced activity capacities of high quality males, induced by testosterone are presumed to entail a metabolic cost, either through resting or activity metabolic rate such as energy expended in prolonged behavioural interactions (Marler & Moore 1989). Therefore, one prediction is that a positive correlation should exist between elevated testosterone and metabolic rate. The limited number of manipulative studies has generated equivocal results. In one group of studies, experimentally increasing testosterone did not produce the predicted increase in resting or basal metabolic rate (Wikelski *et al.* 1999; Buttemer & Astheimer 2000). Paradoxically, white-crowned sparrows, *Zonotrichia leucophrys*, exhibited a lower metabolic rate after administration of testosterone (Wikelski *et al.* 1999). Other studies have shown a positive correlation between metabolic rate and elevated testosterone (Marler *et al.* 1995; Buchanan *et al.* 2001; Ros *et al.* 2004), which supports the conclusion that elevated androgens result in an energetic cost. The metabolic effects of testosterone may occur through an increase in active metabolic rate. Marler *et al.* (1995) found that increased activity in *S. jarrovi*, induced by testosterone, was accompanied by a 31% rise in daily energy expenditures. Similarly, common wall lizards (*P. muralis*) exhibited an increase in average metabolic rate after receiving testosterone implants (Oppliger *et al.* 2004). It is unknown whether daily energy expenditures differ among male morphs. Given their high activity patterns and use of energetically expensive display behaviours, dominant males should have higher active metabolic rates or higher daily energy expenditures (as measured by doubly labelled water) than either satellite males or sneaker males.

Correlational selection

In males with AMS, fitness trade-offs may arise as a consequence of the conflict between allocating energy into mating success at the expense of future survival. Experimental manipulations of testosterone demonstrate an increase in aggressive behaviours (Marler & Moore 1988) and enlarged territories (Sinervo *et al.* 2000a), which increases the number of potential mating opportunities. However, the increase in the proportion of energy and time devoted reproduction generates trade-offs with survival (Salvador *et al.* 1996; Reed *et al.* 2006). For example, our studies on *U. stansburiana* show that O-males increase the number of mating opportunities by expanding the size of their territory and reproductive opportunities (Zamudio & Sinervo 2000), however at the cost of lower survival (Sinervo & Lively 1996; Sinervo *et al.* 2000a). In birds, males implanted with testosterone spend more time in territorial defence at the expense of care for the young in the nest, which results in a reduction in fledging success (e.g. Vleck & Brown 1999).

When the covariation of a trait and fitness is contingent on multiple traits, selection operating on a trait is correlational, that is, the selection coefficients depend on the product of two traits (Sinervo & Svensson 2002; McGlothlin *et al.* 2005). One consequence of

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correlational selection, either natural or sexual, is the functional integration of traits (Sinervo & Svensson 2002; Sinervo & Calsbeek 2003). Frequency-dependent selection is also correlational because it involves a sender (signal) and receiver; genes governing the sender trait(s) are invariably different from genes governing signal reception (Sinervo & Calsbeek 2006). Morph-specific secondary sexual traits may reliably signal high RHP, because of the correlation among morphological and physiological traits, and aggressive behaviours. As a result, correlational selection results in the generation of genetic correlations among traits (Whitlock *et al.* 1995; Lynch & Walsh 1998; Sinervo & Calsbeek 2003) that convey quality of a male, which in turn facilitate the evolution of honest signals. Given the expectation of correlational selection, we should see physiological epistasis in which multiple interacting physiological pathways affect expression of hormonal cascades and the traits governed by hormonal cascades. Despite its central role in structuring trait integration, the role of correlational selection in AMS is currently poorly understood (Sinervo & Svensson 2002; Sinervo & Calsbeek 2006).

Unresolved issues and future research directions

In this review we evaluated the pathways by which testosterone may affect multiple traits both in terms of benefits and costs (Fig. 1). Species with AMS are model systems for understanding the link between physiological traits and the components of sexual selection. Each strategy requires decisions regarding the allocation of time or energy or both to competing activities to maximize fitness (Brockmann 2001).

Our survey revealed only a few examples of whole-organismal physiological performance differences induced by testosterone. Most testosterone manipulations resulted in enhanced performance in several traits, including muscle performance, locomotor activity and calling performance. It is unresolved whether enhancement of physiological performance results in larger territories or greater access to potential mates. In addition, far fewer studies performed manipulations of steroids with the express purpose of ascertaining the hormonal dependence of physiological performance, especially of traits that are tied to dominance behaviours and RHP. Such studies are critical, given the discussion made by past analyses regarding the role of testosterone in modulating display behaviours and performance (e.g. Garland *et al.* 1990; Perry *et al.* 2004). Even more surprising is the limited data available on variation in performance capacities among male morphs. Whether behavioural differences among morphs are a consequence of individual variation in physiological capacities is an unresolved question. One promising avenue of research would be the manipulation of androgens among morphs with the express purpose of ascertaining the hormonal dependence of physiological performance in traits that are tied to dominance

behaviours and RHP. More studies are needed to determine the connection between morph-specific variation in androgens and the physiological performance traits that may influence a males' RHP. Elevated levels of testosterone incurs a cost, which may be realized as reduced survival through various avenues, including direct immunosuppression or indirectly through exposure-mediated parasite loads, increased energetic costs and decreased body condition. Yet, we lack evidence linking a trade-off between endocrine enhanced physiological performances, for example, endurance, with a cost in terms of lowered immunocompetence. Because testosterone affects physiological and behavioural traits, causal pathways generating observed trade-offs are difficult to resolve. Recent advances in manipulating testosterone levels without inducing changes in behaviour are one way to disentangle causal factors.

Furthermore, sexual selection involves multiple suites of traits interacting to affect fitness; hence studies should encompass functionally integrated trait modules as well as analyses that take into correlational selection into account (Fig. 1). It is well known that testosterone may induce changes in phenotypic traits spanning multiple modules. Thus, it is imperative that future studies include more than one response variable. Testosterone may result in enhanced sexual ornaments or physiological performance, yet such change is likely to require re-allocation of energy or time. Redistribution of resources is known to result in trade-offs among target traits. However, it is possible for testosterone to result in complex interactions among traits that may mask the expected enhancements to performance or behaviour, which will generate unexpected results. For example, males given a testosterone implant may initially realize a performance advantage; a concomitant induced behavioural shift to greater territoriality and aggression can lower energy acquired and hence yield a seasonal decline in body condition. The timing of measures of physiological performance by an investigator may lead to contradictory insights into effects of androgens on physiological performance. Parasites may also induce metabolic pathway changes that constrain performance. While many studies have elucidated the increase in parasites as a consequence of elevated testosterone, the joint impact of testosterone and parasites on physiological performance is largely unexplored.

Finally, while this review has focused on testosterone other components of the endocrine system may also play roles in induction and mediation of AMS. The type or quantity of hormone receptors, binding enzymes, or steroidogenic enzymes may vary among morphs, possibly leading to differing effects of testosterone and other hormones even if circulating hormone levels are similar. Additionally, effects of peptide hormones such as arginine vasotocin (AVT) on male reproductive behaviour may differ among morphs. These effects would likely interact with those of testosterone, and should be integrated into studies on the endocrine control of AMS.

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Supplementary material

The following supplementary material is available for this article.

Table S1. Examples of the effects of testosterone (T) on physiological traits

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2435.2007.01304.x

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