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Mitochondrial function, ornamentation, and immunocompetence

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ABSTRACT

Understanding the mechanisms that link ornamental displays and individual condition is key to understanding the evolution and function of ornaments. Immune function is an aspect of individual quality that is often associated with the expression of ornamentation, but a general explanation for why the expression of some ornaments seems to be consistently linked to immunocompetence remains elusive. We propose that condition-dependent ornaments may be linked to key aspects of immunocompetence through co-dependence on mitochondrial function. Mitochondrial involvement in immune function is rarely considered outside of the biomedical literature, but the role of mitochondria as the primary energy producers of the cell and the centres of biosynthesis, the oxidative stress response, and cellular signalling place them at the hub of a variety of immune pathways. A promising new mechanistic explanation for correlations between a wide range of ornamental traits and the properties of individual quality is that mitochondrial function may be the 'shared pathway' responsible for links between ornament production and individual condition. Herein, we first review the role of mitochondria as both signal transducers and metabolic regulators of immune function. We then describe connections between hormonal pathways and mitochondria, with implications for both immune function and the expression of ornamentation. Finally, we explore the possibility that ornament expression may link directly to mitochondrial function. Considering condition-dependent traits within the framework of mitochondrial function has the potential to unify central tenets within the study of sexual selection, eco-immunology, oxidative stress ecology, stress and reproductive hormone biology, and animal physiology.

Key words: mitochondria, display trait, hormones, oxidative phosphorylation, OXPHOS, innate immune function, adaptive immune function, ROS.

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I. INTRODUCTION

Hamilton & Zuk (1982) proposed the novel hypothesis that the key feature of an individual that is signalled by ornamentation is genetically inherited capacity to resist parasites. Since the publication of that seminal paper, biologists interested in sexual selection and mate choice have explored the idea that some ornamental traits may be signals of immune system function and the degree to which individuals are infected by pathogens (Westneat & Birkhead, 1998; Baeta *et al.*, 2008). Thirty years of empirical work in a variety of animals have shown that infecting an individual with a pathogen can reduce ornament expression (reviewed in Møller, Christe & Lux, 1999; Hill, 2006) and that individuals with well-executed ornaments can have better immune system function (Kelly *et al.*, 2012; Whittingham *et al.*, 2015) and better resistance to pathogens (Lindström & Lundström, 2000; Hill & Farmer, 2005) compared to individuals with poorly executed ornaments. Correlative as well as experimental evidence for these relationships has been found for a wide range of ornaments in studies spanning disparate taxa, such as insects (Ryder, 2000; Rantala *et al.*, 2003), lizards (Martín *et al.*, 2007; López, Gabirot & Martín, 2009), arachnids (Ahtiainen *et al.*, 2004), and birds (Saks, Ots & Hõrak, 2003). Despite widespread indications of connections between immune function and ornamental traits, the physiological and biochemical mechanisms that can make the expression of these ornaments honest signals of immune system function remain contentious (Sild *et al.*, 2011; Hill, 2014).

One of the first detailed explanations for how immune system function might link to the expression of ornamentation was the immunocompetence handicap hypothesis (Folstad & Karter, 1992), which proposed that hormonal mediation was the key to honest signalling. The immunocompetence handicap hypothesis proposed specifically that ornaments depend on high levels of circulating testosterone for production, but that elevated testosterone suppresses immunocompetence. Hence, only individuals that can withstand down-regulation of the immune system – individuals in good condition – can produce full ornamentation. This hypothesis has since been refined (Westneat & Birkhead, 1998; Buchanan, 2000), but the immunocompetence handicap hypothesis has not succeeded as a general explanation for how ornaments may serve as honest indicators of health; in particular, it cannot account for the many ornaments that are not regulated by testosterone or other steroid hormones (Kimball & Ligon, 1999) and the fact that testosterone is not universally immunosuppressive (Olsen & Kovacs, 1996; Ros, Groothuis & Apanius, 1997; Hasselquist *et al.*, 1999; Peters, 2000; Bilbo & Nelson, 2001; Lindström *et al.*, 2001; Roberts, Buchanan & Evans, 2004). Nevertheless, it has become clear that hormones do play key roles in regulating the immune system and that they also frequently affect ornament production, so the immunocompetence handicap hypothesis has remained important to the discussion for how ornaments may serve as honest signals of health (Rantala *et al.*, 2012; Desprat *et al.*, 2015).

In recent years, explanations for how ornaments may be reliable indicators of immune system function have shifted to the idea of resource allocation (Morehouse, 2014). Under the resource trade-off hypothesis, only individuals with large pools of resources can afford to allocate resources toward ornamentation as opposed to self-maintenance processes like immune function (Lozano, 1994; von Schantz *et al.*, 1999; Møller *et al.*, 2000; Alonso-Alvarez *et al.*, 2008; McGraw *et al.*, 2010). However, it is difficult to make predictions based on this hypothesis because the resources in question – and, consequently, their availability and pathways of allocation – are often challenging to define and to measure experimentally. An alternative to resource trade-offs is the hypothesis that individual condition arises from core system functionality, which is a product of genetic, somatic, and epigenetic state (Hill, 2011). Under this shared pathway hypothesis, interdependencies on the same core pathways, rather than resource trade-offs, can explain correlations between the quality of ornamental traits and core system functions, including immunocompetence (Hill, 2011, 2014). However, the specific shared pathways and biochemical connections between condition-dependent ornaments and immune system function remain to be explored.

Here, we propose that mitochondrial function may be essential for both production of ornamentation and immunocompetence (Hill, 2011, 2014; Johnson & Hill, 2013; Fig. 1). We put forth this mitochondrial function hypothesis because a large literature indicates that the physiological pathways that dictate immune function converge at the mitochondrion, with close integration with hormonal pathways and potential connections to ornament production. Herein, we synthesize the extensive biomedical literature describing the interconnectedness of mitochondrial pathways and mechanisms of immune function, including innate mechanisms of antiviral and antibacterial response and adaptive processes of memory and effector lymphocyte differentiation and activity. We then describe the bidirectional interactions between mitochondria and steroid and thyroid hormones, and we outline hypotheses for how condition-dependent ornamental traits may also be linked to mitochondrial function. Our goals are to highlight how mitochondria mediate a wide range of aspects of immune function that are of interest to behavioural ecologists and eco-immunologists and how shared mechanisms centred in mitochondria may explain associations between immunocompetence, hormones, and condition-dependent ornamentation.

II. MITOCHONDRIAL MEDIATION OF THE IMMUNE SYSTEM

Mitochondria are best known as the primary sites of ATP production, primarily *via* oxidative phosphorylation

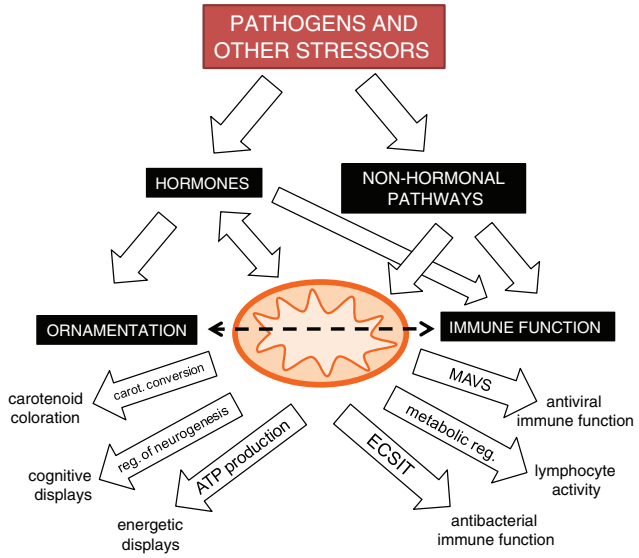

Fig. 1. Diagram outlining proposed hypotheses for connections among hormones, ornamentation, immunocompetence, and mitochondrial function. Exposure to pathogens or other stressors stimulates hormonal and non-hormonal (e.g. immune receptor) signalling pathways that rely directly on mitochondrial proteins and processes for effective response. For example, several mitochondrial proteins [mitochondrial antiviral signalling proteins (MAVS), evolutionarily conserved signalling intermediate in Toll pathways (ECSIT)] and mitochondrial mediation of oxidative phosphorylation and biosynthesis are critical to innate and adaptive immune function (other pathways not shown). Mitochondria are also centres for the synthesis of steroid hormones, and the activities of both steroid and thyroid hormones rely on mitochondrial regulation of energy production, which in turn influences both immune system function and the expression of ornamentation. Moreover, recent hypotheses propose that the production of ornamental display traits also requires proper mitochondrial function; for example, enzymes necessary for the conversion of ornamental carotenoid (carot.) pigments may reside in the inner mitochondrial membrane in close association with oxidative phosphorylation complexes, and carefully regulated ATP production as well as other mitochondrial signals may be essential to the regulation (reg.) of neurogenesis and expression of cognitively complex or athletic displays. Mitochondria therefore represent the 'shared pathway' through which correlations emerge among these processes – particularly immunocompetence and ornamentation (dashed arrow).

(OXPHOS) carried out by the electron transport chain. The energy produced from mitochondria is essential to the production of proteins and fuelling of processes required for mounting an immune response (Demas, 2004; Lane & Martin, 2010; Demas *et al.*, 2012), but energy and protein production are not the only connections between mitochondria and immune function. A growing body of empirical research has implicated mitochondria as key mediators of both innate and adaptive immune processes. Because mitochondria are both the source for the energy required to mount an immune response as well as the targets of many pathogens (West, Shadel & Ghosh, 2011*b*; Weinberg, Sena & Chandel, 2015), it is essential for immune systems to be responsive to the state of mitochondria and hence to have mitochondria intimately involved in immune signalling (Lartigue & Faustin, 2013). Indeed, a variety of immune signal cascades have been found to involve mitochondrial proteins, placing mitochondria at the centre of the regulation of immune responsiveness. In this section, we describe these mechanisms through which mitochondria are involved in mounting an effective immune response. We briefly describe each aspect of immune system function and provide empirical evidence for mitochondrial involvement in that function. By outlining the specific biochemical interactions between mitochondria and different branches of immune function, we aim both to provide a detailed source of information for eco-immunologists investigating particular aspects of immunocompetence and to substantiate the claim that strong mitochondrial function is essential for highly functional immune system activity.

(1) Mitochondrial regulation of innate immune function

The innate immune response comprises both constitutive and rapidly induced components that enable immediate response to pathogen exposure and tissue damage (Janeway & Medzhitov, 2002; Beutler, 2004; Akira, Uematsu & Takeuchi, 2006; Kawai & Akira, 2006; Takeuchi & Akira, 2010). Innate defences against pathogens are triggered when pathogen-associated molecular patterns (PAMPs) are detected by the pattern recognition receptors (PRRs) of dendritic cells, macrophages, and a variety of nonprofessional immune cells (Akira *et al.*, 2006; Takeuchi & Akira, 2010). Recognition of a PAMP by a PRR initiates complex signalling cascades that promote the rapid elimination of pathogens through inflammation and other innate immune processes. Signalling molecules from the innate response can have both localized and systemic effects, and cytokines produced downstream of PAMP recognition stimulate the activation of more specific and longer-term adaptive immune responses to pathogen infection (Fearon & Locksley, 1996; Medzhitov & Janeway, 1997; Akira, Takeda & Kaisho, 2001; Le Bon & Tough, 2002). Stimulating and/or measuring aspects of the innate immune response, such as by measuring inflammatory swelling after injection of a foreign substance (e.g. Blount *et al.*, 2003; Alonso-Alvarez *et al.*, 2009), injecting the isolated PAMP bacterial lipopolysaccharide (LPS), or quantifying acute innate immune protein production (Millet *et al.*, 2007), is an important and widespread component of eco-immunology.

The nature of the innate immune response depends on the properties of the activated PRR and its corresponding PAMP, so the signals that are induced will differ depending on the type of pathogen indicated by the molecular patterns detected. For example, the presence of viral or bacterial PAMPs triggers specific (although sometimes overlapping) signalling cascades to induce an appropriate response, and both pathways have been traced through mitochondrial

proteins at one or more steps. Recent research has found that proper mitochondrial activity is necessary for the transduction of these signalling pathways and for mounting an effective innate immune response.

(*a*) *Mitochondria in innate immune responses to viral infection*

The antiviral immune response is triggered by the recognition of viral PAMPs, such as virus-specific nucleic acids, by one of two classes of PRRs: toll-like receptors (TLRs) or retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). TLRs, found primarily within specialized immune cells, are transmembrane proteins that can recognize several types of viral PAMPs on the cell's surface or in cytoplasmic vacuoles formed after phago- or endocytosis; RLRs are present in a wide variety of cell types and detect cytoplasmic viral RNA (Takeuchi & Akira, 2009; Dixit & Kagan, 2013). Both initiate signalling cascades that converge to stimulate the production of type I interferons and other proinflammatory cytokines that facilitate both immediate innate and long-term adaptive antiviral defences (Kawai & Akira, 2006; Takeuchi & Akira, 2009).

RLRs, particularly RIG-I and melanoma differentiated-associated gene 5 (MDA5), are specific to the antiviral immune response and are critical for detecting replicating viral double-stranded RNA (dsRNA) within infected cells (Dixit & Kagan, 2013). Both RIG-I and MDA5 are produced constitutively at low levels within most cell types, but are inactive until ligand binding causes a conformational change that exposes the caspase-activated recruitment domains (CARDs) needed for downstream signalling (Dixit & Kagan, 2013). Though RIG-I and MDA5 differ in ligand specificity (short or long dsRNA, respectively; Takeuchi & Akira, 2010), the CARDs of both interact with the CARD of the mitochondrial antiviral signalling protein (MAVS), a nuclear-encoded protein that is produced in most animal cells and that localizes to the outer mitochondrial membrane (Seth *et al.*, 2005; Horner *et al.*, 2011; Koshiba, 2013; Weinberg *et al.*, 2015). Once stimulated by RLR binding, MAVS recruits a variety of other signalling molecules, including tumor necrosis factor receptor-associated factor 6 (TRAF6), which together induce the production of nuclear factor-kB (NF-kB) and interferon regulatory factors (IRF3 and IRF7; West *et al.*, 2011*b*). NF-kB stimulates the transcription of genes for a variety of proinflammatory cytokines, and IRF3 and IRF7 enhance transcription of type I interferon (IFN-I) genes, both of which boost antiviral defences (Seth *et al.*, 2005; Takeuchi & Akira, 2009; Koshiba, Bashiruddin & Kawabata, 2011*a*; Dixit & Kagan, 2013; Koshiba, 2013; Fig. 2).

While MAVS is not found exclusively on mitochondrial membranes, the signals it produces differ depending on subcellular location. Peroxisomal MAVS induces the expression of interferon-stimulated genes but not IFN-1 itself, causing a more transient antiviral response; a maximal antiviral response requires both mitochondrial and peroxisomal MAVS activation (Dixit & Kagan, 2013; Odendall *et al.*, 2014; Weinberg *et al.*, 2015). MAVS-enriched

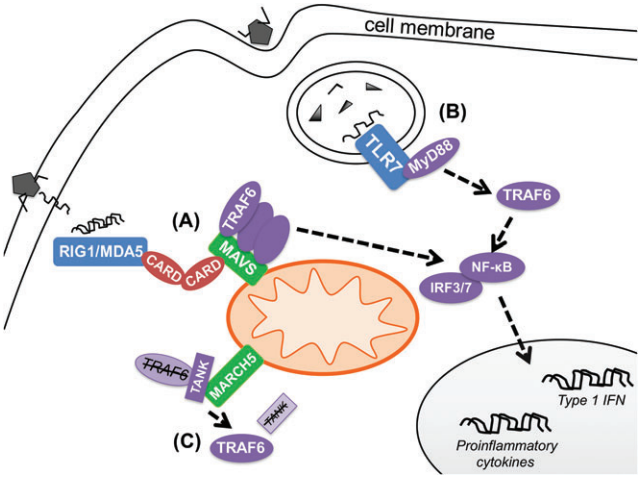

Fig. 2. Main pathways of antiviral innate immune response that pass through the mitochondria. (A) Retinoic acid-inducible gene 1/melanoma differentiated-associated gene 5 (RIG1/MDA5)-like receptors (RLRs) recognize viral double-stranded RNA in the cytosol, and their caspase-activated recruitment domains (CARDs) interact with the CARDs of mitochondrial antiviral signalling proteins (MAVS). MAVS recruits signalling molecules, including tumor necrosis factor receptor-associated factor 6 (TRAF6), which initiate a signalling cascade culminating in transcription factors that stimulate the production of type 1 interferon (IFN) and proinflammatory cytokines. (B) Toll-like receptor 7 (TLR7), present in endosomal membranes, detects viral single-stranded RNA contained in vesicles and recruits the myeloid differentiation primary response gene 88 (MyD88) protein as the first step in a signalling cascade that ultimately overlaps with the RLR pathway. (C) Membrane-associated RING-CH 5 (MARCH5) releases the signalling intermediate TRAF6 from the inhibitor TRAF-associated NF-kB activator (TANK), potentiating the antiviral response; MARCH5 is particularly important for TLR7 activity (Shi *et al.*, 2011). Other antiviral TLR pathways not shown. Purple boxes represent signalling intermediates; blue boxes represent pattern recognition receptors (PRRs); red boxes represent CARD domains; and green boxes represent mitochondrial proteins. IRF3/7, interferon regulatory factors 3/7; NF-kB, nuclear factor kB.

mitochondria further enhance defences against viruses by surrounding intracellular sites of viral replication, and failure to do so handicaps the antiviral response (Onoguchi *et al.*, 2010; West *et al.*, 2011*b*; Dixit & Kagan, 2013). These findings emphasize the importance of MAVS to the antiviral innate response, and the importance of mitochondrial MAVS specifically – indeed, mitochondria with MAVS inhibited by RNA interference (RNAi) do not induce proper IRF3 activity, and MAVS-deficient mice do not mount a normal IFN-1 response (Sun *et al.*, 2006; Arnoult *et al.*, 2011; West *et al.*, 2011*b*; Dixit & Kagan, 2013). Moreover, functional mitochondria are required for a strong antiviral response in general: damaged mitochondrial networks or mitochondria lacking membrane potential (a critical component of mitochondrial activity) decrease function of antiviral signalling pathways

(Castanier *et al.*, 2010; Koshiba *et al.*, 2011*b*; Weinberg *et al.*, 2015).

Independently of the RLR pathways, TLRs can also induce antiviral innate immune responses (West, Koblansky & Ghosh, 2006; Takeuchi & Akira, 2009; Koshiba, 2013). Herein, we reference the TLRs of mammals (TLR1, TLR2, etc.), which are best known and exemplify the diversity of TLR function, although homologous pathways are present in other taxa (Hoffmann *et al.*, 1999; Beutler, 2004; Roach *et al.*, 2005; Werling *et al.*, 2009). Among the TLRs involved in the antiviral response, TLR2 and TLR4 are present on the plasma membrane of the cell and recognize viral envelope proteins (along with other, non-viral PRRs), while TLR3, TLR7, TLR8, and TLR9 are present on endoplasmic reticulum (ER) and other endosomal membranes and detect viral nucleic acids (Akira *et al.*, 2006; Kawai & Akira, 2011). Like the RLR pathways, the TLR response induces increased production of IFN-I and other proinflammatory cytokines to combat viral infection (Kawai & Akira, 2006; Takeuchi & Akira, 2009); the pathways initiated by TLR3 recognition of viral dsRNA in fact converge with RLR signalling pathways at the point of NF-kB and IRF production (Takeuchi & Akira, 2009; Koshiba, 2013; Fig. 2).

Mitochondrial involvement in the antiviral TLR pathways is less clear than in the RLR pathways, and several responses may overlap with the signalling cascades of antibacterial TLRs (see Section II.1*b*). However, a uniquely antiviral response mediated by mitochondrial involvement has recently been elucidated for the TLR7 pathway, which is stimulated exclusively in dendritic cells upon recognition of viral single-stranded RNA (Kawai & Akira, 2006). A protein expressed constitutively in the mitochondrial membrane, membrane-associated RING-CH 5 (MARCH5), boosts the antiviral effects of TLR7 by deactivating an inhibitor called TRAF-associated NF-kB activator (TANK), which normally keeps the signalling intermediate TRAF6 in an inactive state (Shi *et al.*, 2011; Fig. 2). Importantly, MARCH5 is nonfunctional when mislocalized to the cytoplasm or the nucleus, emphasizing the necessity of mitochondrial involvement in the TLR7 antiviral pathway (Shi *et al.*, 2011).

(*b*) *Mitochondria in innate immune responses to bacterial infection*

Mitochondria are involved in the innate immune response to bacterial infection at several levels, including the regulation of antibacterial reactive oxygen species (ROS) production and the mediation of metabolic shifts within phagocytes to address their changing energetic needs (West *et al.*, 2011*b*; Cloonan & Choi, 2013; Weinberg *et al.*, 2015). Interestingly, TLRs are associated with an increase in mitochondrial ROS (mROS) production in response to bacterial but not viral recognition (West *et al.*, 2011*a*), indicating the potential role of mROS in the antibacterial (but not antiviral) response. ROS are known to be important to breaking down bacteria that have been engulfed by phagocytic cells into endosomes, where a process called 'respiratory burst' kills internalized bacteria with a flood of highly reactive superoxide anions produced predominately by NADPH oxidases in the endosomal membrane (Underhill & Ozinsky, 2002; Lambeth, 2004; West *et al.*, 2011*b*). mROS stimulated by TLR signals have also been implicated as an important component of the respiratory burst response (West *et al.*, 2011*b*). Similar TLR activity is also important to eliminating fungal pathogens, although the specific role of mitochondria in these responses is not well understood (West *et al.*, 2006; Netea & Maródi, 2010; Kawai & Akira, 2011; Romani, 2011).

In one of the most definitive experimental evaluations of the role of mitochondria and mROS in bacterial defence, West *et al.* (2011*a*) examined the response of isolated mouse macrophages to TLR stimulation by bacterial PAMPs, as well as the significance of specific mitochondrial components in ultimately killing live bacterial pathogens. In an initial set of experiments, West *et al.* (2011*a*) found increased mitochondrial recruitment to phagosomes as well as increased mROS generation in macrophages with experimentally stimulated bacteria-sensitive TLRs (TLR1, TLR2, and TLR4). To understand better the molecular pathways responsible for these responses as well as their significance to successful antibacterial defence, West *et al.* (2011*a*) then examined the activity of two critical mediators in the antibacterial TLR pathways: the signalling intermediate TRAF6 and the evolutionarily conserved signalling intermediate in Toll pathways (ECSIT). ECSIT was originally described as a cytosolic intermediate in TLR pathways (Kopp *et al.*, 1999), although it has also been found to also be important in the bone morphogenic protein (BMP) pathway in mouse embryogenesis (Xiao *et al.*, 2003); importantly, ECSIT has recently been found to play a role in Complex I assembly in the electron transport chain (Vogel *et al.*, 2007; West *et al.*, 2011*a*,*b*), implicating the significance of ECSIT activity to mitochondria.

West *et al.* (2011*a*) found that cytosolic ECSIT localizes specifically to the outer mitochondrial membrane upon interaction with TRAF6 during antibacterial TLR stimulation, where it causes an increase in mROS generation (Fig. 3). Hypothesizing that ECSIT might play a role in mROS generation because of its interactions with Complex I, West *et al.* (2011*a*) used genetic techniques to reduce ECSIT expression in either isolated macrophages or live mice and found significantly reduced mROS production in both. Importantly, macrophages with knocked-out ECSIT function were found to contain significantly more bacteria (*Salmonella typhimurium*) after experimental exposure than control cells at all time points examined (West *et al.*, 2011*a*), indicating the ultimate importance of mitochondrial ECSIT and mROS in defence against bacterial infection. These results are critical to establishing not only the importance of mROS to killing bacteria, but also the pivotal role of mitochondrial ECSIT to mounting an effective antibacterial response. Further, they demonstrate an important aspect of mitochondrial dynamics in mounting antibacterial defence by finding increased mitochondrial localization to macrophage phagosomes after TLR stimulation, indicating that mitochondria are specifically recruited to generate

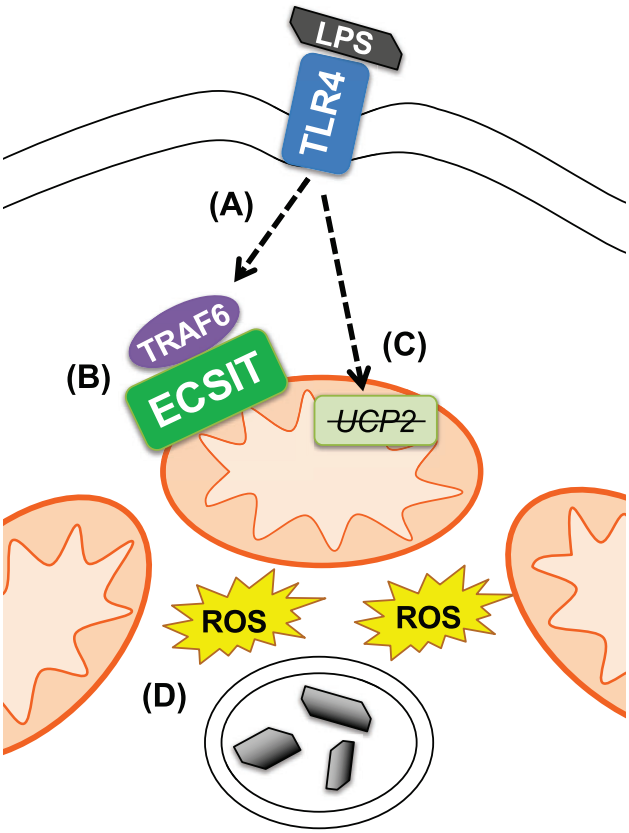

Fig. 3. Mitochondrial activity in the antibacterial innate response. (A) Surface Toll-like receptors (TLRs) TLR1, TLR2 and TLR4 detect bacterial pathogen-associated molecular patterns (PAMPs); for example, TLR4 recognizes bacterial lipopolysaccharide (LPS) and stimulates tumor necrosis factor receptor-associated factor 6 (TRAF6). (B) TRAF6 interacts with evolutionarily conserved signalling intermediate in Toll pathways (ECSIT), which recruits mitochondria around sites of intracellular bacteria (such as phagosomes) and increases reactive oxygen species (ROS) production from the mitochondrial electron transport chain. (C) TLR4 stimulation also down-regulates the expression of a mitochondrial uncoupling protein (UCP2), which causes an additional increase in mitochondrial ROS (mROS) production. (D) mROS aids in defence against the intracellular bacteria.

mROS in proximity to ingested pathogenic bacteria (West *et al.*, 2011*a*; Fig. 3).

Other studies have isolated additional aspects of mitochondrial dynamics that are important both to mROS generation and the antibacterial response. For example, bacterial pathogens that specifically target mitochondrial function have been found to induce a mitochondrial unfolded protein response, which both stabilizes mitochondria during stress and promotes a defensive innate immune response (Pellegrino *et al.*, 2014; Pellegrino & Haynes, 2015). In addition, a mitochondrial uncoupling protein located on the inner mitochondrial membrane, UCP2, has been found to mediate the antibacterial response by adjusting mROS production from OXPHOS such that decreasing expression of mitochondrial UCP2 during bacterial challenge increases production of mROS to be used in antibacterial defence (West *et al.*, 2011*b*; Fig. 3). Experimental evidence supports this relationship: mice with fully knocked-out UCP2 production are more resistant to challenge from an intracellular bacterium, and wild-type macrophages decrease UCP2 expression and consequently increase mROS production during LPS exposure (Kizaki *et al.*, 2002; Emre *et al.*, 2007; West *et al.*, 2011*b*). These studies are testament to the importance of carefully mediating mROS production to defending against bacterial pathogens. Inefficient or otherwise inhibited mitochondria producing too much or too little mROS during bacterial challenge run the risks of either self-inflicting oxidative damage or failing to eliminate a bacterial challenge, respectively. Fine control of mitochondrial ROS production through UCP2 and innate immune signalling cascades is therefore an important component of an organism's ability to defend against infection.

The central role of mitochondria in metabolism also places them at the crux of several processes important to proper innate immune cell effector functions across pathogen types. For example, mitochondria mediate the metabolic changes that differentiate two main classes of macrophages: classically activated (M1), which primarily utilize aerobic glycolysis, and alternatively activated (M2), which utilize mitochondrial OXPHOS (Huang *et al.*, 2014; Weinberg *et al.*, 2015). The different polarizations of macrophages specialize for different roles within the immune system: M1 macrophages promote inflammation and are associated with response to microbial pathogens and tumors, while M2 macrophages are involved with tissue repair, metabolic homeostasis, and response to parasitic helminths (Huang *et al.*, 2014). Shifts in metabolism also characterize the maturation of dendritic cells, which rapidly upregulate glycolysis and suppress mitochondrial respiration in order to meet the anabolic demands of the differentiating cell (Everts *et al.*, 2014; Weinberg *et al.*, 2015). Metabolic intermediates themselves can also serve functions that extend beyond their roles in energy production. For example, succinate, an intermediate in the tricarboxylic acid (TCA) cycle occurring in the mitochondrial lumen, increases in concentration in macrophages stimulated by TLR4 recognition of bacterial LPS and enhances the production of proinflammatory cytokines (Wang, Malo & Hekimi, 2010; Tannahill *et al.*, 2013; Chouchani *et al.*, 2014; Mills & O'Neill, 2014; Weinberg *et al.*, 2015).

In summary, mitochondria are involved in several key processes related to innate immune function, including: MAVS-mediated induction of inflammatory cytokines in response to antiviral stimulation of RLRs; MARCH5 on the mitochondrial membrane promoting the antiviral signalling of TLR7; mitochondrial ECSIT mediating increased mROS production surrounding sites of intracellular bacterial infection; and metabolic shifts and biosynthesis within mitochondria influencing immune cell differentiation and aiding in effector functions. These connections between mitochondria and the innate immune response are also

important to ensuring proper activation of adaptive immunity, providing a more long-term defence against persistent or repeated infections. Many innate immune responses are critical for the proper induction of an adaptive immune response. For example, IFN-I produced by RLR and TLR pathways – both of which have direct connections to mitochondrial function (Cloonan & Choi, 2013) – can trigger the maturation of and stimulate the activity of dendritic cells, which in turn promote adaptive T cell activation through antigen presentation (Le Bon & Tough, 2002). However, the primary role of mitochondria in mounting an adaptive immune response lies in mediating the metabolic shifts necessary for the differentiation of adaptive immune cells.

(2) Mitochondrial involvement with adaptive immune function

Adaptive immune function is largely mediated by the actions of B and T lymphocytes, each of which comprises several specialized groups of cell types that initiate recognition and removal of specific extracellular pathogens (e.g. through antibodies produced from humoral B cells) or host cells infected with intracellular pathogens (e.g. through cytotoxic T cells), or that remain dormant in preparation for future defence against a previously encountered pathogen (memory cells; Ahmed & Gray, 1996; Barry & Bleackley, 2002; McHeyzer-Williams & McHeyzer-Williams, 2005). While a detailed discussion of the various components that function within the adaptive immune system is beyond the scope of this review, it is important to note that adaptive immunity largely involves a vast pool of inactive (naïve) T and B lymphocytes that, upon recognition of a non-host antigen, must rapidly differentiate into one of several mature forms, each requiring a metabolism fine-tuned to carrying out a particular effector function (Fox, Hammerman & Thompson, 2005; Jones & Thompson, 2007; Windt & Pearce, 2012; Walker *et al.*, 2014; Weinberg *et al.*, 2015). Given how essential adaptive immunity is to preventing long-term infection and to resisting infection of a pathogen upon repeated exposures, adaptive immune function is also an important component of eco-immunology; for example, studies may assess antibody production after vaccinating animals with a novel antigen (e.g. Svensson *et al.*, 1998; Hasselquist *et al.*, 1999). Interestingly, a study of house sparrows (*Passer domesticus*) used a morphological measurement of adaptive immune system function – the size of the bursa of Fabricius, the site of B cell development in young birds – and found a relationship with the size of a sexually selected plumage ornament, indicating a potential link between adaptive immunity and the expression of display traits (Møller, Kimball & Erritzøe, 1996).

As with the metabolic changes noted in maturing macrophages and dendritic cells in innate immunity, mitochondria are intimately involved in how naïve T and B cells alter energetic and biochemical needs upon activation and maturation. Here, we primarily discuss the specific metabolic transitions of T cells, although comparable metabolic transitions occur in B cells (Garcia-Manteiga *et al.*, 2011; Wheeler & DeFranco, 2012; Caro-Maldonado *et al.*, 2014; Walker *et al.*, 2014; Weinberg *et al.*, 2015). Resting, naïve T cells require energy and biosynthesis only for basal cell maintenance and circulation through lymphoid tissue; as such, inactive T cells rely primarily on oxidative phosphorylation for energy production (Fox *et al.*, 2005; Jones & Thompson, 2007; Pearce, 2010; MacIver, Michalek & Rathmell, 2013). Upon antigen recognition and T cell activation, T cells rapidly up-regulate glycolysis and biosynthetic pathways and down-regulate oxidative phosphorylation to meet increased energetic needs while shifting from a primarily catabolic to anabolic state. To support the structural changes associated with T cell differentiation into effector forms, mitochondria become primary energy producers as well as centres of biosynthesis through products of glycolysis and the TCA cycle (Fox *et al.*, 2005; Jones & Thompson, 2007; Pearce, 2010; MacIver *et al.*, 2013; Fig. 4).

Beyond participating in metabolic shifts, mitochondria are also directly involved in the signals initiating and sustaining the transition of a naïve T cell into a mature effector T cell upon recognition of an antigen. Mitochondria localize to the immune synapse between a T cell receptor and an antigen on an antigen-presenting cell and appear critical to signal transduction *via* calcium ion flux and mROS generation (Jones & Thompson, 2007; Quintana *et al.*, 2007; Kamiński *et al.*, 2010; Gill & Levine, 2013; Sena *et al.*, 2013; Martín-Cófreces, Baixauli & Sánchez-Madrid, 2014; Weinberg *et al.*, 2015). mROS production in particular has emerged as an important cellular signal during T cell differentiation; for example, mice with knock-out mutations that reduce T cell mROS generation cannot initiate rapid T cell differentiation and proliferation upon antigen exposure (Sena *et al.*, 2013).

Even after initial activation, mitochondrial dynamics are important to meeting the different metabolic needs of varying T cell types. Regulatory T cells, for example, rely on increased oxidative phosphorylation compared to helper T17 cells, which instead exhibit increased glycolytic activity to meet biosynthetic needs (Michalek *et al.*, 2011; Walker *et al.*, 2014). In addition, memory T cells have increased mitochondrial density compared to naïve T cells despite similar energetic needs between the two resting cell types (Fig. 4); the large number of mitochondria in memory T cells gives them a 'spare respiratory capacity', which allows them rapidly and drastically to upregulate ATP production upon future antigen stimulation in order to mount an immediate response against a repeated pathogen exposure (Pearce, 2010; van der Windt *et al.*, 2012; Walker *et al.*, 2014). Moreover, mitochondria play a direct role in providing energy to activated lymphocytes migrating towards sites of pathogen infection: mitochondrial fission and localization to the uropod region of activated lymphocytes is required for proper chemotaxis (Campello *et al.*, 2006).

Overall, mitochondria are most important to adaptive immunity through their central functions in ATP production

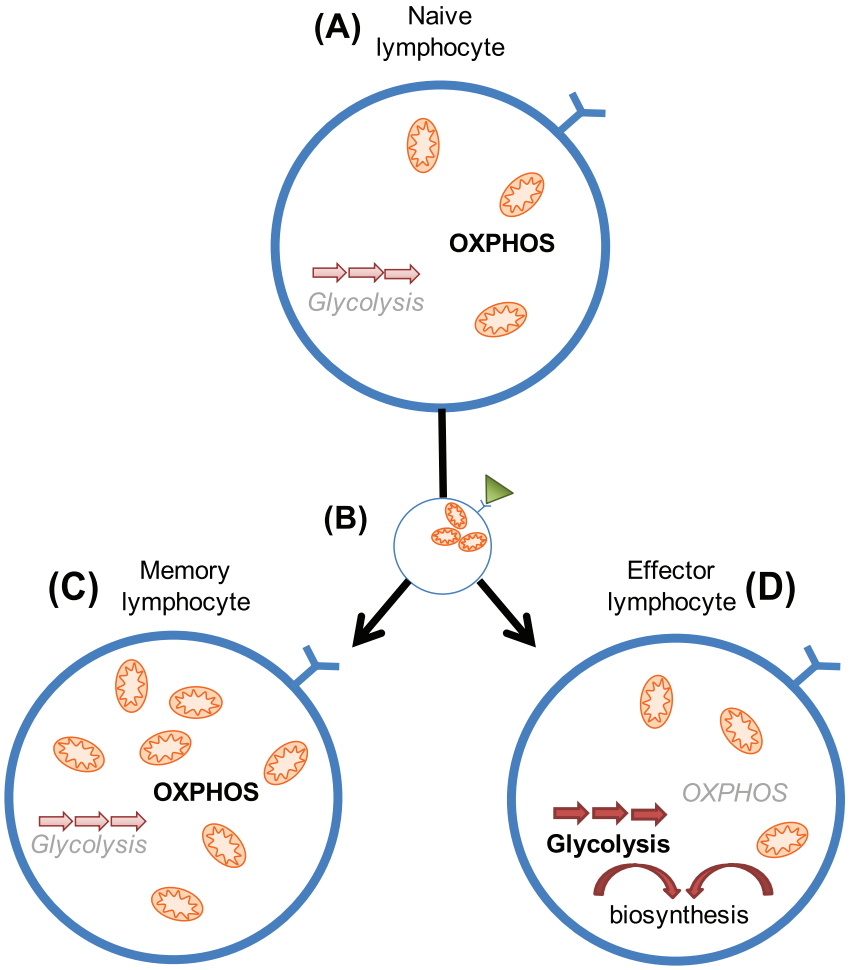

Fig. 4. Changes in mitochondrial activity related to the maturation and activation of lymphocytes. (A) Naïve lymphocytes use primarily oxidative phosphorylation (OXPHOS) to produce ATP for general maintenance. (B) During activation, mitochondria localized around the immune synapse help stimulate differentiation. (C) Memory lymphocytes prioritize OXPHOS for general maintenance, similarly to naïve lymphocytes; however, memory lymphocytes also possess an excess of mitochondria, which enables rapid up-regulation of energy production and biosynthesis upon activation. (D) Effector lymphocytes prioritize biosynthesis through glycolysis as well as the tricarboxylic acid cycle within mitochondria.

and biosynthesis, critical for the effector function of differentiated cells as well as the successful maturation of naïve cells. When considered in tandem with the innate immune signalling pathways passing directly through mitochondrial intermediates and the other roles of effective mitochondrial activity in innate defences, it is clear that mitochondria play an integral role in a wide variety of immune system pathways. An understanding of how mitochondria are central to immune activity offers interesting future directions for study into how variation in the function of this organelle may have cascading effects on multiple aspects of immune defences – and on an animal's overall immunocompetence, which is important to both eco-immunology and to the study of the condition dependence of traits. The biomedical literature is rapidly advancing our understanding of the subcellular biochemical interactions that define mitochondrial involvement in immune function; however, extending these findings to explore their potential downstream effects on organism immunocompetence, condition-dependent display production, and individual fitness is essential to providing ultimate significance to these mitochondrial functions.

Key to understanding the importance of mitochondria to these aspects of whole-organism biology is that the synthesis and effector functions of hormones also have critical connections to mitochondrial function. Given the involvement of sex steroids and other hormones in aspects of both ornament production and immunocompetence, the role of mitochondria in hormone activity provides a critical mechanistic link between the mitochondrial–immune connections described in this section, and the potential immune–ornament connections that are important to behavioural ecology. In Section III, we briefly review these connections among hormones, ornamentation, and immune function, and describe how the role of mitochondria in hormonal pathways may be important to maintaining these connections.

III. HORMONES, ORNAMENTATION, AND THE IMMUNE SYSTEM

Physiological ecologists have long recognized that hormones can affect both ornament production (Møller, 1995; Owens & Short, 1995) and immune system function (Braude, Tang-Martinez & Taylor, 1999; Verhulst, Dieleman & Parmentier, 1999). Early work on the interactions among hormones, ornaments, and immune function was largely based within the framework of the immunocompetence handicap hypothesis (see Section I), which posits that ornamentation serves as a signal of immunocompetence because of the dual effects of testosterone in stimulating ornament production while depressing the immune system (Folstad & Karter, 1992). Critics of the immunocompetence handicap hypothesis point out that many secondary sexual traits and behaviours are not controlled by androgens (Wingfield & Farner, 1993; Kimball & Ligon, 1999), and so this hypothesis is therefore limited only to testosterone-dependent signals. In addition, the fundamental assumption of this hypothesis is that testosterone suppresses immune function, but many studies report either no relationship or immune-enhancing effects of testosterone (Olsen & Kovacs, 1996; Ros *et al.,* 1997; Hasselquist *et al.,* 1999; Peters, 2000; Bilbo & Nelson, 2001). Further confusing the relationship between testosterone and immunity is the finding that the stage of development may play a large role in the degree of the interactions (Schuurs *et al.,* 1992).

We propose that the links among hormones, immunocompetence, and ornamentation may be better explained by hormonal mediation of key mitochondrial pathways and mitochondrial roles in hormone synthesis, which in turn affect aspects of both ornamentation and immune function (Hill, 2014). Mitochondria are a primary site of action for steroid and thyroid hormones, which have been shown to have intimate relationships with immunocompetence and ornamentation in a variety of vertebrate taxa (Silva, 1999; Wingfield, Lynn & Soma, 2001; Klein, 2004; Hau, 2007; Husak & Moore, 2008; Dhabhar, 2009). Briefly, steroid hormones include the sex hormones that are important to the physiological and behavioural changes associated with reproduction, and also stress-response hormones, like glucocorticoids, that mediate redistributing energy towards processes supporting immediate survival and away from non-essential processes. These hormones have varied effects on immune function and/or ornament production. For example, the sex hormone testosterone is often linked to increased intrasexual aggression (i.e. territory defence or competition over mates; Hau, 2007) and production of ornaments as varied as antlers in deer (Bartos, Bubenik & Kuzmova, 2012) and bill redness in zebra finches (Ardia, Broughton & Gleicher, 2010), although this relationship is not without complications (see Section I). In addition, glucocorticoids are generally immunosuppressive in nature, interacting with the nuclear genome to inhibit the synthesis, release, or efficacy of immune mediators like cytokines and immunoglobulins (Sapolsky, Romero & Munck, 2000). Thyroid hormones are also closely linked with immune activity: low levels of thyroid hormones have been associated with decreased thymic activity, splenic and lymph node changes, and depressed humoral and cell-mediated immune responses (reviewed in Klecha *et al.,* 2006). At the centre of these complex interactions among hormones, immune function, and ornamentation are mitochondria (Fig. 1).

The interactions between mitochondria and hormones are bidirectional: mitochondria play a role in the biosynthesis and control of steroid hormones (Bose, Lingappa & Miller, 2002), and thyroid and steroid hormones regulate the expression of DNA involved in OXPHOS (Psarra, Solakidi & Sekeris, 2006). It is important for organisms to have this direct connection between hormonal activity and mitochondrial function because steroid and thyroid hormones are generally involved in energy-demanding metabolic, growth, and developmental processes; hormones can modify mitochondrial activity to fuel physiological processes, while the role of mitochondria in hormone production ensures that energetically expensive processes can only be triggered if there is adequate mitochondrial function to support such processes (Demonacos *et al.,* 1996; Gavrilova–Jordan & Price, 2007; Manoli *et al.,* 2007; Du *et al.,* 2009).

In order to coordinate an increase in energy production within the cell, steroid and thyroid hormones stimulate the up-regulation of nuclear and mitochondrial OXPHOS genes. Specifically, steroid and thyroid hormones belong to the nuclear receptor superfamily characterized by a central DNA-binding domain, which targets the receptor to specific DNA sequences in the genome (hormone response elements; HREs; Beato, Herrlich & Schütz, 1995; Mangelsdorf *et al.,* 1995; Chrousos & Kino, 2005). HREs for steroid and thyroid hormones have been found on OXPHOS genes both within the nucleus (Pillar & Seitz, 1997; Scarpulla, 1997; Weber *et al.,* 2002; Scheller & Sekeris, 2003) and the mitochondrion (Ioannou, Tsawdaroglou & Sekeris, 1987; Demonacos *et al.,* 1995; Tsiriyotis, Spandidos & Sekeris, 1997; Psarra *et al.,* 2006). Moreover, receptors for glucocorticoids (Psarra *et al.,* 2003; Scheller, Seibel & Sekerist, 2003; Sionov *et al.,* 2006), sex steroid hormones (Chen *et al.,* 2004; Solakidi *et al.,* 2005; Pedram *et al.,* 2006; Jönsson *et al.,* 2007), and thyroid hormones (Sterling, Campbell & Brenner, 1984; Ardail *et al.,* 1993; Scheller *et al.,* 2003; Morrish *et al.,* 2006) have been found on the mitochondrial membrane. The mitochondrial transcription factors regulated by these hormones have downstream effects on the biogenesis of mitochondria (Tome *et al.,* 2012) as well as on mitochondrial fission (Lee *et al.,* 2013; Picard, Juster & McEwen, 2014), which are important to mediating mitochondrial energy and protein production. Consequently, steroid and thyroid hormones provide transcriptional control over mitochondrial mechanisms to enable a rapid response to changing energetic needs.

Even as hormones regulate mitochondrial activity, mitochondria also play a role in regulating the production of hormones, particularly steroids. Because steroidogenic cells have little capacity to store hormones, steroid hormone

levels are controlled primarily at the stage of synthesis, much of which takes place in mitochondria (Bose *et al.*, 2002). Generally, steroid hormone synthesis initiates in the mitochondria, moves to the endoplasmic reticulum, and then is finalized within the mitochondria of the cell (Rosol *et al.*, 2001). Biosynthesis of steroids therefore requires the coordination of uptake, transport, and utilization of cholesterol, the molecular basis of steroid hormones, in the mitochondria, as most cells do not synthesize cholesterol for steroids *de novo* (Papadopoulos & Miller, 2012). Once cholesterol has reached the inner mitochondrial membrane, a set of enzymes within the mitochondria is responsible for further steroid hormone biosynthesis, which is tissue-specific (reviewed in Stocco, 2001; Miller, 2013). These hormones can then be used to transduce signals throughout the organism.

Understanding the importance of mitochondria in hormone synthesis and response adds new perspective to the discussion of relationships between endocrine systems, other aspects of physiology, and behaviour. For example, the sex steroid testosterone, which has been central to the immunocompetence handicap hypothesis (Folstad & Karter, 1992), has been proposed not only as a potential immunodepressant and stimulant of male ornament production, but also as a cause of increased ROS production (Alonso-Alvarez *et al.*, 2007; Mougeot *et al.*, 2009). Indeed, high testosterone levels may be linked to increased metabolism (e.g. Buchanan *et al.*, 2001) and locomotion (e.g. Wikelski *et al.*, 1999). If mitochondria are functioning properly, then a higher metabolic rate and increased muscular activity may impose no change – or even a decrease – in ROS production from OXPHOS (Murphy, 2009; Jastroch *et al.*, 2010). However, inefficient or otherwise dysfunctional mitochondria produce ROS at higher rates than healthy mitochondria (Lane, 2011), so a testosterone-mediated boost in activity levels and ATP demand may exacerbate potential oxidative damage caused by poor mitochondrial function. With mitochondrial control of both synthesis of and response to sex steroids like testosterone, an organism could potentially avoid such costs because dysfunctional mitochondria would likely not properly facilitate sex steroid production and activity in the first place, adding new mechanistic insight into why different individuals may vary in hormone response and the costs of that response. However, these connections remain to be tested directly; robust, but separate, literatures describe the interactions between mitochondria and hormones *or* hormones and immune function as well as ornament production. Future empirical study into the specific mechanistic effects of mitochondrial mediation of hormone signalling on particular aspects of immune function and/or sexual display production will be important to further substantiating these important relationships.

In summary, properly functioning mitochondria are essential in both the response to hormone signals and the production of steroid hormones themselves, with consequential effects on the hormonal mediation of immune activity and ornament expression. If mitochondrial activity is disrupted through inefficient OXPHOS or other dysfunction, then the hormone-mediated up-regulation of energy production as well as the biosynthesis of steroids will be interrupted, resulting in improper function of the myriad processes that respond to hormonal signals. Interactions between mitochondria and hormones therefore add indirect connections between mitochondrial function, immunocompetence, and expression of ornamentation, in addition to the direct mechanistic pathways described in Section II and proposed in Section IV.

IV. ORNAMENTATION AS A SIGNAL OF CELLULAR RESPIRATION

Hormone signalling provides an indirect but powerful link between mitochondrial function and ornament production, given how sex hormones are often involved in the expression of sexual displays. However, we propose that ornamentation itself may also be directly linked to mitochondrial function, independently of hormonal control (Johnson & Hill, 2013). Mechanisms relating to the expression of a host of condition-dependent ornaments, including carotenoid metabolic conversion (Johnson & Hill, 2013; Lopes *et al.*, 2016), neural development and cognitive function (Cheng, Hou & Mattson, 2010; Wallace & Fan, 2010; Massaad & Klann, 2011; Morava & Kozicz, 2013), motor function (Podos, 1997), chemiluminescent light displays (Buck & Case, 2002; Carlson, 2004), and large protein structures (Ohlsson *et al.*, 2002; Rands, Evans & Johnstone, 2011), can all be plausibly linked to the efficiency of cellular respiration and hence to mitochondrial function (Hill, 2014). Critically, mitochondrial function may be reflected in ornamentation both because OXPHOS produces the ATP needed to fuel energetically expensive processes and to synthesize and fold proteins (Lane & Martin, 2010) and because ornament expression can be sensitive to the redox homeostatic state of the organism, which is largely mediated by mitochondria (Ježek & Hlavatá, 2005; Cecarini *et al.*, 2007; Hill & Johnson, 2012).

These latter relationships between oxidative stress and ornamentation are a growing component in the field of oxidative stress ecology (Monaghan, Metcalfe & Torres, 2009; McGraw *et al.*, 2010; Costantini, 2014); here, we focus on recent hypotheses that have proposed how the production of certain displays may be sensitive directly to mitochondrial function. In particular, the specific mechanisms that may connect mitochondrial activity to the expression of an ornamental trait are perhaps most directly described in carotenoid-based red colouration. Because animals cannot synthesize carotenoid pigments *de novo*, carotenoids used for coloration are acquired from the diet. The diets of most animals contain predominantly yellow rather than red carotenoids (McGraw, 2006), so red-colour displays require the metabolic conversion of yellow dietary carotenoids into red carotenoids *via* ketolation reactions (Brush, 1990). Recent evidence suggests that carotenoid ketolation occurs in mitochondria in association with complexes of the electron transport chain (Johnson & Hill, 2013; Ge *et al.*, 2015;

Lopes *et al.*, 2016). The ability of animals to generate red *versus* yellow colouration may therefore be directly dependent on the oxidative state and membrane potential of mitochondria, such that mitochondrial dysfunction disrupts ketolation and causes a loss of red ornamentation. In addition, energetic constraints during neural development have been proposed to be a key determinant in the quality of birdsong, a sexually selected trait whose expression can be linked directly to neurological quality (Peters, Searcy & Nowicki, 2014); given that the production of ATP needed to support the energetically expensive process of neurogenesis depends almost exclusively on mitochondrial OXPHOS, mitochondrial function may also be key to condition dependence in displays like birdsong. Indeed, mitochondrial quality has been shown within biomedical literature to be a key determinant of cognitive ability (Cheng *et al.*, 2010; Wallace & Fan, 2010; Massaad & Klann, 2011; Morava & Kozicz, 2013), and birdsong quality has been proposed to be directly dependent on cognitive function (Peters *et al.*, 2014). However, explicit empirical links between mitochondria, neural development, and sexually selected displays remain to be tested.

The critical role of mitochondria in many of the processes related to the production and maintenance of sexually selected displays, including ATP production, hormonal signalling, and potentially processes like carotenoid conversion, suggests that highly functional mitochondria may be necessary for the full expression of ornamental traits such that the quality of ornaments reliably signals mitochondrial function (Hill, 2014). These connections form the final link between ornamentation and immunocompetence: if efficient OXPHOS and effective mitochondrial signalling are important both to mounting a strong immune response and to expressing a high-quality ornament, then mitochondrial function can be considered the core condition that mediates the honesty of sexual display traits. The bidirectional links between hormone signalling and mitochondrial function serve to substantiate this relationship further by providing additional connections between mitochondria and hormonally controlled aspects of immunocompetence and ornamentation.

Importantly, however, many of the specific mechanisms linking sexually selected traits to mitochondrial function remain to be tested experimentally. Future empirical study into the role of mitochondria in the expression of ornamentation is imperative to assessing whether these hypothesized direct connections – in addition to the indirect connections mediated *via* hormones – substantiate the correlations between immune performance and ornament quality.

V. MITOCHONDRIA AS THE SHARED PATHWAY

A trade-off in the allocation of resources is the current, widely embraced explanation for the often-observed links between ornamentation and immune function (López-Rull *et al.*, 2015). At its most basic conception, this resource trade-off hypothesis proposes that animals maximize the accumulation of energy in the body and then distribute this pool of resources between body maintenance and ornamentation (Rowe & Houle, 1996). We propose that while this hypothesis recognizes the basic elements that dictate both ornament production and immune function, a simple trade-off of resources does not capture the mechanisms that link stress, condition, immune function and ornamentation. Biochemists, immunologists, and biomedical researchers have identified the mitochondrion as the central regulator of both innate and adaptive immunity, but this literature has thus far remained largely unknown to ecologists. By hypothesizing that the production of many condition-dependent ornaments is critically dependent on the same aspects of mitochondrial function that directly affect immune system function, we offer a new mechanistic explanation to close the circle between core system function, immunocompetence, and display traits.

Having mitochondria at the centre of pathways involved in immune function is advantageous because mitochondria create the energy and proteins needed for an immune response (Lane & Martin, 2010). It is critical that the state of mitochondria and hence the availability of proteins and energy be taken into account when an individual initiates an immune response so that the response is within the scope of the body's capabilities (Cloonan & Choi, 2012; Lartigue & Faustin, 2013). Mitochondrial dysfunction may cause unpredictable mROS production and otherwise handicap the metabolic and signalling capabilities of the mitochondria (Lane, 2011), interrupting critical immune signal cascades or disrupting lymphocyte function. If production of ornamentation is also dependent on mitochondrial function – through mitochondrial control of hormone signalling or through direct mechanistic pathways – then any impairment in mitochondrial function necessarily impacts ornamentation while it also necessarily impacts immune function.

This new framework for understanding the connections between immune function and ornamentation offers a logical explanation for how females may benefit from assessing male ornamentation. If females were only assessing a male's resource pool through his display traits, then a male could – at least in the short term – manipulate his internal allocation of resources to produce a quality ornament that was a dishonest indicator of his true condition (Getty, 2006; Morehouse, 2014). On the other hand, if ornament production depended on the functionality of a fundamental cellular process like OXPHOS, then females that assess ornamentation would be assessing the core functionality of a prospective mate; there would no potential for dishonest signalling because a male's ability to produce an ornament would be inescapably constrained by the limits of his cellular physiology (Hill, 2014). This mitochondrial function hypothesis also provides an explanation for the often-observed associations between hormone levels, immune function, and ornamentation, as steroid and thyroid hormones are central regulators of mitochondrial function,

linking perception of stressors like pathogens or extreme temperatures to the up- or down-regulation of mitochondrial protein components (Psarra *et al.,* 2006; Psarra & Sekeris, 2009). Thus, Folstad & Karter (1992) were correct in pointing out key connections between hormones, ornaments, and immune function, but they focused on the signals rather than on the biochemical pathways that are the targets of the signals. By recognizing the central place for mitochondria in this interconnectivity, the benefits for females assessing honest signals of mitochondrial function become more clear.

Altering the framework by which we examine the condition dependence of ornamental traits has important ramifications for how we interpret signal form, function, and evolution. The relationships among mitochondrial pathways, hormones, the immune system, and ornamentation provide new, testable predictions for how environmental stressors or genetic incompatibilities may impact an animal's performance – in terms of fitness, health, and ability to produce attractive displays. Not all display traits are necessarily condition dependent or objects of female mate choice (Prum, 2010); even within ornamental traits considered to be honest signals of male quality, variation often exists in what physiological parameters that trait appears to signal, and the potential relevance of those parameters to choosing females (e.g. Dunn *et al.,* 2010; Freeman-Gallant *et al.,* 2010). Whether or not females are assessing males according to mitochondrial quality, the consistent and widespread links between mitochondrial function and various other aspects of physiological quality are intriguing. As biomedical techniques continue to advance understanding of core processes like OXPHOS efficiency (Weissig & Edeas, 2015), empirical study into the relationships between these core underlying processes and the outcome parameters important to studies of eco-immunology, oxidative stress ecology, and sexual selection may offer new insight into currently observed patterns – and may present new patterns altogether. Isolating the key mechanisms driving variability in ornamentation and physiological condition is an important step toward uniting the wide range of correlational studies examining condition-dependent traits.

VI. CONCLUSIONS

(1) Many empirical studies have shown associations between ornamentation and immune system function, but the mechanisms that give rise to such relationships remain uncertain. While resource trade-offs are commonly invoked to explain condition-dependent ornaments, the size of pools of resources and differential resource allocation strategies are difficult to measure and do not consistently predict relationships between the expression of display traits and measurements of individual condition. A shared dependence on mitochondrial pathways between physiological systems offers a new mechanistic explanation for correlations between sexually selected traits and immunocompetence.

(2) The foundation of this mitochondrial function hypothesis is the rich biomedical literature linking immune system signalling pathways and effector functions to mitochondrial activity. We review the evidence that the function of mitochondrial biosynthesis, energy production, and signalling is essential to innate immune defences, specifically against viral and bacterial pathogens, and adaptive immune cell differentiation and activity.

(3) Steroid and thyroid hormones can further influence both immune system activity and the expression of ornamental traits, and regulate and/or are regulated by mitochondrial pathways. Specifically, the synthesis of steroid hormones takes place largely within the mitochondria, and the downstream physiological changes induced by hormone signalling rely on functional changes in mitochondrial activity. The bidirectional relationship between mitochondrial function and hormone signalling provides a secondary link between mitochondria and immunocompetence, as well as influencing ornamentation.

(4) We propose that the mechanisms responsible for the production of many display traits may also depend on proper mitochondrial function, through the activity of redox-sensitive pathways and the need for efficient energy production and biosynthesis. Notably, the enzymatic conversion of dietary yellow to ornamental red carotenoid pigments may occur in close association with complexes of the electron transport chain, and the energy requirements as well as many cellular changes associated with neurogenesis and neural activity – needed for complex cognitive or athletic displays – require proper mitochondrial function.

(5) A greater understanding of mitochondrial involvement provides new, testable predictions for the interrelationships between immune, hormone, and ornamentation pathways. Because proper mitochondrial function is essential for many immune and hormone activities and may also be directly involved in the production of display traits, an organism's mitochondrial genotype and phenotype can potentially link internal physiological condition to external sexual signals. We encourage future investigation into this promising new avenue of research to pursue clarification to longstanding questions within behavioural ecology.

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