



Both sexes suffer increased parasitism and reduced energy storage as costs of reproduction in the brown anole, *Anolis sagrei*

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Sexual selection theory proposes that males suffer reduced immune function and increased parasitism as costs of expressing sexual signals. Life-history theory proposes that females suffer the same costs because of inherent trade-offs between reproduction and self-maintenance. Mechanistically, each theory invokes an energetic trade-off, although few experiments have directly compared these costs of reproduction between the sexes as a result of fundamental sex differences in the nature of reproductive investment and a tendency for each theory to focus on a single sex. To test whether males and females experience comparable costs of reproduction in terms of energetics, immune function, and parasitism, we used gonadectomy to eliminate most aspects of reproductive investment in wild brown anole lizards (*Anolis sagrei*) of both sexes. We compared these nonreproductive males and females with intact, reproductive controls with respect to stored energy (fat bodies), immune function (swelling response to phytohemagglutinin), and the prevalence and intensity of infection by four types of parasite (gastric nematodes, intestinal nematodes, faecal coccidia, and ectoparasitic mites). Gonadectomized anoles experienced dramatic increases in fat storage that were accompanied by decreases in the prevalence of intestinal nematodes and in the intensity of coccidia infection. These costs of reproduction were comparable between males and females, although neither sex exhibited the predicted increase in immune function after gonadectomy. Our results suggest that, despite fundamental sex differences in the nature of reproductive investment, both male and female anoles experience similar costs of reproduction with respect to energy storage and some aspects of parasitism. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **00**, 000–000.

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INTRODUCTION

As a result of fundamental differences in their reproductive biology, males and females invest resources in different aspects of reproduction (Trivers, 1972; Clutton-Brock & Parker, 1992; Rolff, 2002; Maklakov & Lummaa, 2013). Males of many species maximize their reproductive success by increasing their mating opportunities, and so they often invest heavily in ornaments, weaponry, mate searching, courtship, and territory defence (Bateman, 1948; Jones,

Arguello & Arnold, 2002; Bonduriansky *et al.*, 2008). Females are limited by the time and energy required to provision an embryo, and so they are inherently predisposed to allocate resources to vitellogenesis, egg or embryo production, and parental care (Queller, 1997; Kokko & Jennions, 2008). Despite these fundamental sex differences, total reproductive investment is predicted to be equal for males and females when the adult sex ratio is equal. This is because, at the population level, each sex is engaged in intrasexual competition for the same overall fitness benefit: half of the genes that will be passed on to the next generation (Fisher, 1930; Queller, 1997).

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However, this prediction is often difficult to assess in wild populations because of inherent sex differences in the nature and seasonal timing of reproductive investment (Cox, 2014). Consequently, the costs associated with reproduction are rarely considered simultaneously in both sexes (Queller, 1997; Bon-duriansky *et al.*, 2008; Chu & Lee, 2012).

One reason for this lack of integration between sexes stems from the historical separation of sexual selection theory, which has focused on costs of reproduction that enforce honest signalling in males (Zahavi, 1975; Folstad & Karter, 1992), and life-history theory, which grew from an interest in costs that structure the evolution of demography and population growth via female reproduction (Cole, 1954; Gadgil & Bossert, 1970). This distinction can be illustrated by considering immunosuppression and parasitism as proximate costs of reproduction (Cox, 2014). As an extension of sexual selection theory, the immunocompetence handicap hypothesis predicts that male vertebrates should suffer increased parasitism resulting from the immunosuppressive effects of androgens that coordinate the expression of secondary sex traits, which serve as honest indicators of health and quality (Folstad & Karter, 1992). Although males exhibit lower immune function and higher parasite loads than females in some taxa (Moore & Wilson, 2002; Nunn *et al.*, 2009), the reverse is true in others (McCurdy *et al.*, 1998), and general tests for male-biased parasitism and immunosuppression yield equivocal results (Roberts, Buchanan & Evans, 2004). One reason may be that reproductive females, similar to males, also experience costs of reduced immune function and increased parasitism (Nordling *et al.*, 1998; Ardia, Schat & Winkler, 2003; French, DeNardo & Moore, 2007; Cox *et al.*, 2010; McKean & Lazzaro, 2011). Life-history theory assumes that such physiological costs structure the evolutionary trade-off between reproduction and survival (Rose & Charlesworth, 1981), and reduced immunocompetence in females is considered to result from energetic trade-offs between reproduction and self-maintenance (Sheldon & Verlhust, 1996; Martin, Scheuerlein & Wikelski, 2003; Martin, Hawley & Ardia, 2011). These energetic trade-offs provide a mechanism that is common to both sexes because the immunosuppressive effects of androgens invoked by the immunocompetence handicap hypothesis are considered to derive from their role in coordinating energy allocation between reproduction and immune defence (Wedekind & Folstad, 1994; Cox, 2014). Therefore, both sexes are predicted to face pronounced energy allocation trade-offs during reproduction, which could result in similar costs with respect to reduced immune function and increased parasitism. However, the extent to which these costs

are comparable between the sexes is generally unknown.

Experimental manipulations of reproductive investment are often necessary to provide conclusive evidence for proximate costs of reproduction (Reznick, 1992; Landwer, 1994; Cox, 2006). However, experiments can be complicated by the fact that reproductive investment occurs at different times, involves different aspects of reproduction, and incorporates different regulatory pathways in each sex (Cox, 2014). As a simplified example, male vertebrates tend to incur large respiratory (i.e. metabolic) costs related to androgen-mediated courtship, mate-searching, and territory defence at the outset of the breeding season, whereas female vertebrates tend to incur large production (i.e. biosynthetic) costs related to oestrogen- and progesterone-mediated provisioning of eggs and embryos later in the season (Michener & Locklear, 1990; Hoffman *et al.*, 2008; Cox, 2014). Experimental comparisons of reproductive costs are therefore most straightforward in situations where the timing and nature of reproductive investment are closely aligned for males and females. For example, in birds with biparental care, brood manipulations (egg removals and additions) have demonstrated that a loss of body mass and increased parasitism are costs incurred by one or both sexes during offspring provisioning (Allander, 1997; Velando & Alonso-Alvarez, 2003; Christe *et al.*, 2012). However, brood manipulations only address a single aspect of reproductive investment, and their generality is unclear beyond the relatively small proportion of species that provide biparental care (Cox, 2014). Other studies have directly compared the costs of reproduction between the sexes by manipulating mating frequency or the duration of exposure to mates (Kotiaho & Simmons, 2003; Fedorka, Zuk & Mousseau, 2004; Dugas, Wamelink & Richards-Zawacki, 2015), although the costs associated solely with mating likely represent different fractions of total reproductive investment for each sex. An ideal approach would manipulate total reproductive investment and directly compare the magnitude of its associated costs between the sexes.

One way of experimentally assessing the total cost of reproduction is to eliminate most aspects of reproductive investment via gonadectomy. In female brown anole lizards (*Anolis sagrei*), this method has been used to demonstrate pronounced costs of reproduction with respect to growth, body condition, energy storage, hematocrit, immune function, and survival (Cox & Calsbeek, 2010a; Cox *et al.*, 2010; Cox, Lovern & Calsbeek, 2014b). In principle, castration can also be used to eliminate direct costs of gamete production in males and to reduce or elimi-

nate other indirect costs as a result of androgen-mediated behaviour and physiology. Castration of males abolishes some reproductive and territorial behaviours completely, and reduces the frequency or elevates the stimulus threshold necessary for others (Barfield, Busch & Wallen, 1972; Arnold, 1975; Adkins, 1977; Harding, Sheridan & Walters, 1983; Tokarz, 1986; Tokarz *et al.*, 2002). In the present study, we used gonadectomy to reduce or eliminate most aspects of reproductive investment in a wild population of brown anoles. We then directly tested whether males and females experience similar costs of reproduction with respect to energy storage (wet mass of fat bodies), immune function (swelling response to a novel antigen, phytohemagglutinin), and the prevalence (percentage of infected individuals) and intensity (number of parasites per infected individual) of infection by four different parasites: gastric nematodes (Physalopteridae), intestinal nematodes (Atractidae), faecal coccidia (Eimeriidae), and ectoparasitic mites (Trombiculidae). We predicted that reduction of reproductive investment via gonadectomy would increase energy storage and immune response at the same time as decreasing the prevalence and/or intensity of parasites in both sexes. In accordance with the general prediction that overall reproductive investment of males and females should be equivalent, we predicted that treatment effects would be similar in both sexes (Rolff, 2002; Bonduriansky *et al.*, 2008).

MATERIAL AND METHODS

STUDY SPECIES

The brown anole (*A. sagrei*) is a sexually dimorphic lizard that is native to Cuba and The Bahamas. Adult males from the closed island population that we studied at Regatta Point on Great Exuma in The Bahamas (23°30'N, 75°45'W) are 32% larger than females, on average, in terms of snout–vent length and 150% larger in terms of mass (Cox & Calsbeek, 2010b). Males fight and display to establish territories encompassing multiple females, and their seasonal reproductive investment extends approximately from the onset of testicular recrudescence and elevated testosterone levels in February until the cessation of mating around September (Tokarz, 1985, 1998; Tokarz *et al.*, 1998). Females repeatedly produce single-egg clutches approximately every 10 days from April to as late as October, with follicular maturation and ovulation continuously alternating between right and left ovaries (Lee *et al.*, 1989; Cox & Calsbeek, 2010a). Genetic analyses reveal a high incidence of multiple paternity across successive eggs produced by

individual females (Calsbeek *et al.*, 2007), such that both sexes are polygamous. Because opportunities for fertilization are continuously available and females are constantly gravid, the overall timing of reproductive investment in this species is broadly similar between sexes across most of the lengthy breeding season.

EXPERIMENTAL DESIGN AND SURGICAL PROCEDURES

We used a hand-held noose to capture 220 female and 120 male *A. sagrei* adults early in the reproductive season (23 May to 4 June), well after the onset of mating activity (February/March) and shortly after the onset of regular oviposition (April/May) but 4–5 months prior to the cessation of mating activity and oviposition (September/October). We measured snout–vent length (nearest 1 mm) and body mass (nearest 0.01 g) for each lizard. We then marked each animal with a unique toe clip and randomly assigned it to one of two treatment groups: (1) bilateral gonadectomy (GDX: removal of both ovaries or testes, $N = 110$ females, 60 males) or (2) control surgery (gonads manipulated but left intact, $N = 110$ females, 60 males). During surgery, we confirmed that all experimental animals were in reproductive condition, as determined by enlarged testes with visible seminiferous tubules or enlarged ovaries with vitellogenic follicles. We followed previously reported surgical protocols (Cox *et al.*, 2009, 2010, 2014b; Cox & Calsbeek, 2010a) that began with the administration of local anaesthesia and analgesia (2–4 μ L injection of 0.25% bupivacaine HCl; Hospira Inc.). We then immobilized animals with a 5–8 min exposure to 4 °C and conducted surgeries on top of a chemical ice pack with a slightly thawed boundary layer. For all surgeries, we made a single 5–8-mm ventral incision into the coelomic cavity, and then ligated, ablated, and cauterized each gonad for the gonadectomy treatment. For control surgeries, we briefly exteriorized the gonads and returned them to the body cavity intact. We closed incisions with Vet-Close cyanoacrylate surgical glue (Butler Schein Animal Health). We allowed animals to recover overnight in individual containers, and then released each animal at its exact site of capture the next day.

We returned to Regatta Point 10 weeks later (6–15 August) to recapture 50 of 120 males (17 GDX; 33 control) and 51 of 220 females (23 GDX; 28 control), which were used to assess sex and treatment effects on energy storage, immune function, and parasite loads (see below). Although we have previously documented an increase in survival of GDX females, relative to controls, in a nearby population (Cox & Calsbeek, 2010a; Cox *et al.*, 2010), we did not detect any treatment effect on female survival in the

present study (logistic regression: $\chi^2 = 0.41$, $P = 0.52$), and survival rates of both GDX and control females (21% and 25%, respectively) were at the low end of natural variation across six previous years at Regatta Point (mean 33%, range 17–46%). By contrast, survival of control males (55%) was significantly higher than that of GDX males (28%) in the present study (logistic regression: $\chi^2 = 9.31$, $P < 0.01$), and higher than in any of nine previous years of study on unmanipulated males at Regatta Point (mean 38%, range 24–46%), whereas survival of GDX males was within the lower range of natural variation across previous years. Consequently, we found a significant sex difference in survival in the present study (logistic regression: $\chi^2 = 11.36$, $P < 0.001$), driven primarily by the atypically high survival of control males ($\chi^2 = 3.56$, $P = 0.059$), as well as an overall treatment effect on survival ($\chi^2 = 7.46$, $P < 0.01$). Because this treatment effect was in the opposite direction predicted from previous studies of GDX and control females (Cox & Calsbeek, 2010a; Cox *et al.*, 2010), we do not interpret these results in the context of survival costs of reproduction. It is unclear whether this indicates that our gonadectomy procedure itself detrimentally affected the survival of males, or that survival of control males was atypically high by chance, or both. Consequently, we focus on inferring costs of reproduction from measures of energy storage, immune function, and parasite levels of recaptured animals.

ENERGY STORAGE

Anoles and other lizards store energy in paired, abdominal fat bodies (Derickson, 1976). This energy is used for gonadal recrudescence, egg provisioning, and nutrition during periods of low food availability (Chapman & Chapman, 1964; Sexton *et al.*, 1971; Lin, 1979). The size of these fat bodies cycles seasonally in anoles, increasing as reproductive activity decreases toward the end of the breeding season (Licht & Gorman, 1970). Analysis of stomach contents at multiple time points throughout the year suggests that, rather than being driven by patterns of food intake, changes in the mass of fat bodies are instead driven by reproductive activity as stored energy is mobilized to fuel reproduction (Lee *et al.*, 1989). Although the fat bodies are not the sole sites of fat storage, they are a useful index of energetic savings associated with the cessation or elimination of reproduction (Cox *et al.*, 2010, 2014b). To assess the energetic cost of reproduction, we compared the wet mass of abdominal fat bodies dissected from a subset of recaptured males and females in each treatment ($N = 23$ total: 6 GDX males, 5 control males; 7 GDX females, 5 control females).

IMMUNE FUNCTION

We assayed immune function by measuring the localized swelling response to challenge with a novel antigen, phytohemagglutinin (PHA), for a subset of recaptured individuals ($N = 65$ total: 10 GDX males, 22 control males; 12 GDX females, 21 control females). Injection of PHA induces both innate and acquired immune defences (Kennedy & Nager, 2006), including the influx of lymphocytes, heterophils, thrombocytes, basophils, and macrophages, manifesting as localized swelling at the site of injection (Martin *et al.* 2006). The extent of localized swelling in response to PHA is typically interpreted as a measure of immunocompetence, with greater swelling indicative of a more robust immune response (Goto *et al.*, 1978; Smits, Bortolotti & Tella, 1999; Calsbeek, Bonneaud & Smith, 2008; but see Kennedy & Nager, 2006). We used a dial caliper to measure the thickness of each animal's right hind foot to the nearest 0.1 mm between the first and fifth digits, and then subcutaneously injected 0.1 mg of PHA (Sigma-Aldrich Inc., St Louis, MO, USA), dissolved in 0.01 mL of phosphate-buffered saline, at this same location. We measured the thickness of the foot again 24 h after injection of PHA and calculated the proportional increase in the thickness of the foot as the difference between initial and final thickness divided by initial thickness. We used the mean of three consecutive measurements per individual at each time point in our analysis.

PARASITE INFECTION

We used a hand lens to count external mite parasites (Trombiculidae) on a subset of recaptured individuals ($N = 80$ total: 15 GDX males, 24 control males; 19 GDX females, 22 control females). We then held all animals for 24 h in sanitized plastic containers to collect a faecal sample for later quantification of coccidian oocysts ($N = 55$ individuals provided fecal samples: 8 GDX males, 19 control males; 11 GDX females, 17 control females). We measured the wet mass of each faecal sample and stored it in 1 mL of 10% formalin. We subsequently placed each fecal sample into a Fecalyzer (EVSCO Pharmaceuticals) with 8 mL of Fecasol (Vétoquinol), ground each faecal sample into solution, and added additional Fecasol (approximately 7 mL) until a meniscus formed on the top of the tube. We then placed a coverslip on the meniscus and let each sample stand for 17 min to allow oocysts to float to the surface before transferring the coverslip to a clean microscope slide for examination at $100\times$ magnification. We recorded the number of coccidian oocysts on the entire coverslip for each individual and expressed this number

per mg of feces. We identified all oocysts as belonging to Eimeriidae, a family of intracellular protozoan parasites within the Coccidia subclass of the phylum Apicomplexa. Coccidians induce cell damage in their hosts, although the degree to which they can be considered pathogenic in wild reptiles is largely unknown (Greiner, 2003).

To quantify both intestinal and gastric nematodes, we dissected the gastrointestinal tracts from the same subset of individuals that we killed to assess fat storage ($N = 23$ total: 6 GDX males, 5 control males; 7 GDX females, 5 control females). We stored the entire gastrointestinal tract in 10% formalin, then sectioned lower gastrointestinal tracts into 1-cm pieces and counted the total number of rectal nematodes (Atractidae) in all sections under a dissection scope. Atractid nematodes are viviparous, undergo direct development in the lower intestine of their host, and have a venereal mode of transmission between individual hosts (Norval *et al.*, 2011; Langford, Willobee & Isidoro, 2013). For the same subset of animals, we dissected the stomach and counted all visible nematodes (Physalopteridae). Physalopterids parasitize all vertebrate classes and their attachment to gastric mucosa can result in inflammation and excessive mucus production (Levine, 1968; Goldberg & Bursey, 1989).

STATISTICAL ANALYSIS

To test for effects of sex and reproductive investment on energy storage, we used analysis of variance (ANOVA) with mass-specific fat mass [fat mass/(body mass – fat mass)] as the response variable and main effects of sex and treatment with a sex-by-treatment interaction. To test for effects of reproductive investment on immune function, we used ANOVA with proportional swelling response to PHA [(final thickness – initial thickness)/initial thickness] as the response variable and main effects of sex and treatment with a sex-by-treatment interaction. To account for scaling of fat mass or swelling response with body mass, we also performed analysis of covariance with either absolute fat mass or swelling as the response variable, treatment as the main effect, and body mass as a covariate. We conducted these analyses separately within each sex because males and females do not overlap in the covariate (body mass). To compare the prevalence of infection by each parasite (i.e. the proportion of lizards infected) between sexes and treatment groups, we used nominal logistic models with the presence of each parasite type (0 or 1) as the response variable, sex and treatment as main effects, and a sex-by-treatment interaction. Because parasite count data were non-normally distributed, we used generalized linear models to

compare the intensity of infection by each parasite (i.e. number of parasites per infected individual) between sexes and treatment groups (Alexander, 2012) with untransformed parasite counts (O'Hara & Kotze 2010) as the response variable, fitted with a Poisson distribution and an over-dispersion parameter estimated as the Pearson chi-squared value divided by degrees of freedom. For each of these models, we tested for effects of sex, treatment, and their interaction.

RESULTS

ENERGY STORAGE AND IMMUNE FUNCTION

Experimental reduction of reproductive investment via gonadectomy dramatically increased the wet mass of fat bodies (treatment: $F_{1,19} = 21.99$, $P < 0.001$) (Fig. 1A). The treatment effect did not differ between sexes (sex \times treatment: $F_{1,19} = 0.08$,

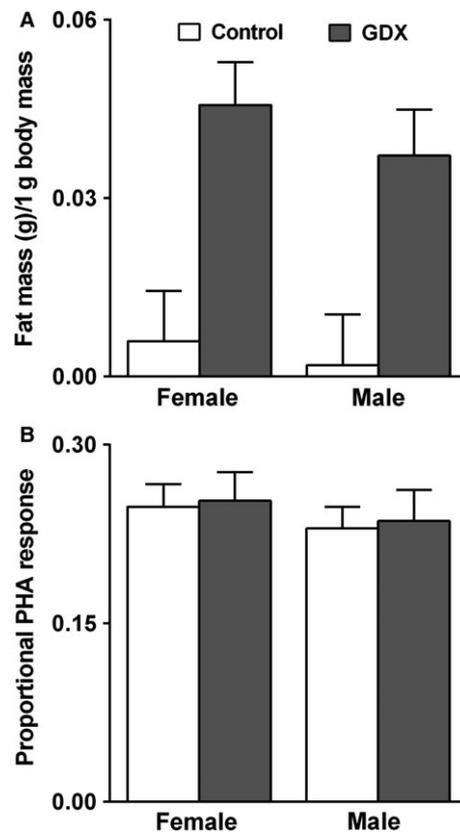


Figure 1. Gonadectomy (GDX) dramatically increased energy storage (wet mass of fat bodies) for both sexes (A). Gonadectomy did not affect the immune response to phytohemagglutinin (PHA), which was measured as the proportional increase in the thickness of the foot from pre- to post-treatment with PHA (B). All data are the mean \pm SE.

$P = 0.78$) and there was no overall sex difference in fat storage per unit body mass (sex: $F_{1,19} = 0.61$, $P = 0.44$). Separate models that included body mass as a covariate also showed that treatment reduced fat body mass for both sexes (females: $F_{1,11} = 14.77$, $P < 0.005$; males: $F_{1,10} = 24.98$, $P < 0.005$). Swelling response to PHA did not differ as a function of sex ($F_{1,65} = 0.67$, $P = 0.42$), treatment ($F_{1,65} = 0.06$, $P = 0.81$) or their interaction ($F_{1,65} < 0.01$, $P = 0.98$) (Fig. 1B). Separate analyses with body mass included as a covariate also indicated no treatment effect on swelling response to PHA for either sex (females: $F_{1,32} = 2.58$, $P = 0.12$; males: $F_{1,31} = 0.39$, $P = 0.54$).

PREVALENCE AND INTENSITY OF PARASITE INFECTION

Experimental reduction of reproductive investment via gonadectomy resulted in a significant decrease in the prevalence (presence or absence) of atractid nematodes in the intestine but did not affect the prevalence of physalopterid nematodes in the stomach, eimeriid coccidia in the faeces or trombiculid mites on the ectoderm (Fig. 2, Table 1). Males and females did not differ in the prevalence of infection by atractid nematodes, physalopterid nematodes,

eimeriid oocysts or trombiculid mites (Fig. 2, Table 1). Treatment effects on the prevalence of infection did not differ by sex for any class of parasite (Fig. 2, Table 1).

Experimental reduction of reproductive investment via gonadectomy resulted in a significant decrease in the intensity of infection (parasites per infected host) by eimeriid coccidia but did not affect the intensity of infection by physalopterid nematodes, atractid nematodes or trombiculid mites (Fig. 3, Table 1). Infected males had a greater number of mites than infected females (Fig. 3, Table 1), although we did not detect any other sex differences in the intensity of infection, nor did treatment effects on the intensity of infection differ by sex for any class of parasite (Fig. 3, Table 1).

DISCUSSION

Reproductive investment is predicted to be equivalent in males and females because, at the population level, mean reproductive success should be equal for each sex when the adult sex ratio is balanced (Fisher, 1930; Queller, 1997). Hence, each sex stands to gain comparable genetic benefits from

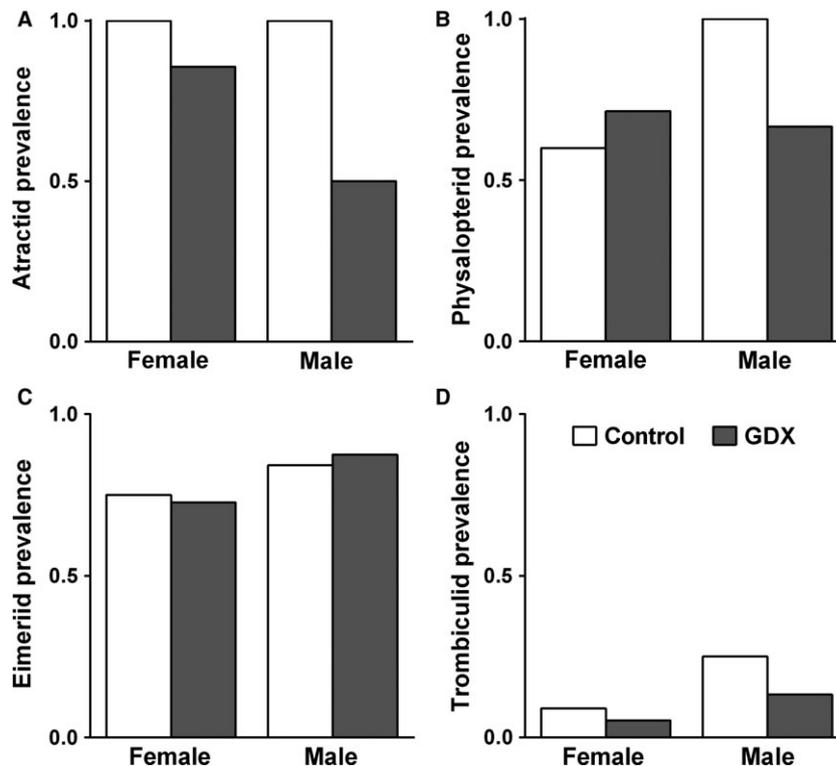


Figure 2. Gonadectomy (GDX) reduced the prevalence of infection by atractid nematodes for individuals of both sexes (A). Treatment did not affect the prevalence of physalopterid nematodes (B), eimeriid coccidia (C) or trombiculid mites (D).

Table 1. Summary of statistical tests for effects of host sex, reproductive treatment, and their interaction on the prevalence (presence or absence) and intensity (number) of four parasites

Parasite family	Model effect	Prevalence (\pm)			Intensity (number)		
		d.f.	χ^2	<i>P</i>	d.f.	χ^2	<i>P</i>
Atractidae	Sex	19	0.01	0.99	15	2.83	0.09
	Treatment		4.51	0.038		0.45	0.50
	Sex \times Treatment		0.01	0.99		1.22	0.27
Physalopteridae	Sex	19	2.07	0.15	15	1.35	0.24
	Treatment		1.47	0.23		0.56	0.46
	Sex \times Treatment		2.46	0.10		0.01	0.99
Eimeriidae	Sex	51	0.98	0.32	40	2.04	0.15
	Treatment		0.01	0.96		4.26	0.039
	Sex \times Treatment		0.10	0.76		0.38	0.54
Trombiculidae	Sex	80	2.21	0.14	7	4.50	0.034
	Treatment		0.82	0.36		0.02	0.87
	Sex \times Treatment		0.01	0.09		0.02	0.87

Significant ($P < 0.05$) effects are shown in bold.

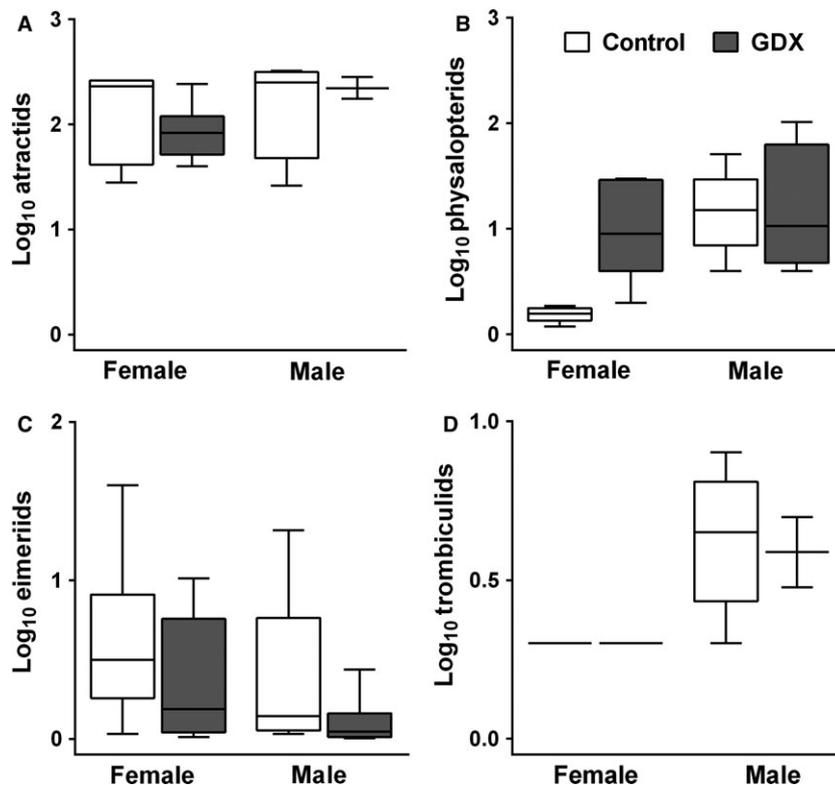


Figure 3. Treatment had no effect on the intensity of infection by attractid nematodes (A) or physalopterid nematodes (B). Gonadectomy (GDX) reduced the number of eimeriid oocysts for infected individuals of both sexes (C). Treatment had no effect on the number of trombiculid mite parasites. However, infected males had more mites than did infected females (D). Because the distributions of parasite counts are non-normal, all data are expressed as medians (bar) with 25–75% interquartiles (box) and the total range (whiskers).

reproduction. Whether this means that the myriad costs of reproduction should also tend to be similar in each sex is less clear, particularly when these

costs are measured in units (e.g. stored energy, intensity of parasitism) that do not translate directly into fitness (Cox, 2014). In the present study, we

found comparable costs of reproduction in each sex with respect to energy storage and several measures of parasite infection, which we view as broadly consistent with the prediction that both sexes should invest similarly in reproduction. In female brown anoles, gonadectomy has previously been used to demonstrate physiological costs of reproduction including reduced energy storage, immune function, and parasite tolerance (Cox *et al.*, 2010; Cox & Calsbeek, 2011). Our experiment extends those results by demonstrating that males also experience similar costs of reproduction. Despite dramatic differences in the behavioural and physiological basis of reproductive investment in each sex, males and females experienced costs of reproduction that were comparable with respect to energy storage and parasite load, although we note that small sample sizes reduced the power of our statistical tests for sex-by-treatment interactions for several parasites (Table 1). An additional caveat to the present study is that our experimental design limited our assessment of costs of reproduction to animals that survived to be recaptured, which could complicate our inferences about treatment effects if, for example, individuals with particularly high or low levels of energy storage or parasitism were more likely to survive in one treatment group than another.

The similar increase in the mass of fat bodies for both sexes following gonadectomy suggests that, for both male and female brown anoles, the energy invested in reproduction is diverted from storage over the course of the breeding season. However, given the divergent reproductive strategies of each sex, different mechanisms probably underlie these otherwise comparable energetic responses. For females, the biosynthetic demands of provisioning eggs are likely to drive the total cost of reproduction. The mass of a single egg is typically close to 10% of the body mass of an adult female brown anole, and individual females repeatedly lay single eggs approximately once every 10 days. Across a 6-month breeding season, a female anole may therefore have a total reproductive output far exceeding its own body mass (Andrews & Rand, 1974). Removal of the ovaries eliminates this large biosynthetic cost and likely results in a substantial energetic savings. However, we cannot rule out additional, non-exclusive explanations, such as an increase in the abdominal space available for food and fat storage, direct effects of ovarian hormones on fat storage and metabolism or indirect effects of ovarian hormones on behaviours that influence energy acquisition and/or expenditure (Cox *et al.*, 2010). Although we cannot rigorously address most of these possibilities, GDX and control females do not differ in their aggressive responses to staged territorial intrusions by females, nor does exogenous oestradiol

(which may be reduced by gonadectomy) have any discernable effect on display behaviour in captive females (Cox *et al.*, 2014a, b; E. Parker, N. Brown, R. Cox & R. Calsbeek, unpubl. data).

For male anoles, competitive interactions with other males and the associated metabolic costs of activity and territory defence are likely to contribute heavily to the overall cost of reproduction. Male anoles are highly aggressive towards each other and engage in frequent displays and occasional combat throughout their lengthy breeding season (Evans, 1938; Stamps, 1977; Jenssen, Greenberg & Hovde, 1995). Surgical and chemical castration of male anoles abolishes some aggressive behaviours at the same time as reducing the frequency others (Tokarz, 1986, 1995; Tokarz *et al.*, 2002; Cox *et al.*, 2009), and these behavioural aspects of male reproductive investment have demonstrable metabolic costs in other lizards (Marler *et al.*, 1995; Cox, Skelly & John-Alder, 2005). Mechanistically, many of these energetic effects are likely mediated by a reduction in circulating levels of androgens such as testosterone, which we have previously confirmed as a consequence of our gonadectomy procedure (Cox & John-Alder, 2005; Cox *et al.*, 2005, 2009). These effects could occur directly, given that testosterone reduces body fat in many vertebrate species (Ketterson *et al.*, 1991; Cox *et al.*, 2014a) or indirectly because of the elimination of androgen-mediated behaviours with energetic costs (Marler *et al.*, 1995; Cox *et al.*, 2005). As in the case of females, we cannot rule out additional, non-exclusive explanations for increased energy storage in GDX males, such as an increase in the abdominal space available for food and fat storage (the testes comprise a substantial portion of the coelomic cavity during the breeding season) or the energetic costs associated with maintaining enlarged testes and supporting spermatogenesis. Indeed, one advantage of gonadectomy is that it provides a holistic assessment of the summed contributions of all of these aspects of reproductive investment in each sex.

Irrespective of the exact causative factors underlying the energetic costs of reproduction, our results are consistent with the idea that reproductive anoles have less energy available for functions such as immune defence, which may leave reproductive anoles of either sex vulnerable to parasites if there is an energetic cost associated with parasite defence. Reduced immune defence against parasites and pathogens is frequently implicated as a cost of reproductive investment mediating the ubiquitous trade-off between reproduction and survival (Gustafsson *et al.*, 1997; Lochmiller & Deerenberg, 2000; Norris & Evans, 2000; Harshman & Zera, 2007). By contrast to previous work in this species, we did not

detect compromised immune function (reduced swelling response to PHA) as a cost of reproduction (Cox *et al.*, 2010; Cox & Calsbeek, 2011), potentially because our sample sizes were relatively small and the PHA assay is subject to considerable measurement error (Smits *et al.*, 1999). Despite this, we observed reductions in the prevalence or intensity of parasite infection for two of the four parasite types that we quantified (attractid nematodes, eimeriid oocysts), which builds on previous studies of lizards demonstrating that gonadectomy decreases ectoparasite loads in male striped plateau lizards (Cox & John-Alder, 2007) and female brown anoles (Cox *et al.*, 2010). The fact that, in the present study, this treatment effect was seen for some endoparasites but not for ectoparasites is consistent with the ideas that immune responses to parasites can be highly specific and achieved by a variety of underlying mechanisms (Vass, Nappi & Carton, 1993; Schmid-Hempel & Ebert, 2003), and that different modes of defence can vary in the costs that they impose on the host (Couston & Chevillon, 2000; Rigby, Hechinger & Stevens, 2002). The decrease in the prevalence of attractid nematodes that we observed in nonreproductive males and females also highlights the importance of sexually transmitted parasites as a frequently overlooked cost of reproduction (Hurst *et al.*, 1995; Lockhart, Thrall & Antonovics, 1996). To the extent that castration may have eliminated or reduced the frequency of copulation, castrated animals may have been sheltered from continued reinfection by attractid nematodes (Langford *et al.*, 2013). Although few sexually transmitted parasites are studied in wild populations, animals with high degrees of promiscuity and overlapping generations are particularly likely to harbour such parasites (Webberley *et al.*, 2004).

Although we found that gonadectomy increased energy storage and reduced infection by some parasites, we cannot conclusively determine the causality in this relationship. The reduced energy stores of reproductive individuals could leave them more vulnerable to parasitism or, alternatively, reproduction could directly lead to higher levels of parasitism (e.g. exposure to sexually transmitted nematodes), thereby placing greater demands on the immune system and, in turn, reducing energy stores. Although gonadectomy had no effect on immune function as measured in the present study with the PHA assay, the relationship between immunocompetence and host-parasite interactions is highly complex, and we caution against a simplistic interpretation of our data as evidence against a relationship between immune function and parasite loads (Owen & Clayton, 2007). Regardless of any causal links that may exist between reduced energy storage and increased

parasite loads, our results show that both occur as costs of reproduction for male and female brown anoles. This is broadly consistent with the theoretical prediction that total reproductive investment should be comparable for males and females, and it supports the emerging view that males and females share many of the same costs of reproduction (Fedorka *et al.*, 2004; Paukku & Kotiaho, 2005; Penn & Smith, 2007; Hoffman *et al.*, 2008; Cox, 2014; Dugas *et al.*, 2015). The present study also presents a new experimental framework for directly comparing costs of reproduction between sexes by using the same measures of cost and manipulations of total reproductive investment over the same period of time.

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REFERENCES

- Adkins EK. 1977.** Effects of diverse androgens on the sexual behavior and morphology of castrated male quail. *Hormones and Behavior* **8**: 201–207.
- Alexander N. 2012.** Review: analysis of parasite and other skewed counts. *Tropical Medicine & International Health* **17**: 684–693.
- Allander K. 1997.** Reproductive investment and parasite susceptibility in the great tit. *Functional Ecology* **11**: 358–364.
- Andrews R, Rand AS. 1974.** Reproductive effort in anoline lizards. *Ecology* **55**: 1317–1327.
- Ardia DR, Schat KA, Winkler DW. 2003.** Reproductive effort reduces long-term immune function in breeding tree-swallows (*Tachycineta bicolor*). *Proceedings of the Royal Society of London Series B, Biological Sciences* **270**: 1679–1683.
- Arnold AP. 1975.** The effects of castration and androgen replacement on song, courtship, and aggression in zebra

- finches (*Poephila guttata*). *Journal of Experimental Zoology* **191**: 309–325.
- Barfield R, Busch D, Wallen K. 1972.** Gonadal influence on agonistic behavior in the male domestic rat. *Hormones and Behavior* **3**: 247–259.
- Bateman AJ. 1948.** Intra-sexual selection in *Drosophila*. *Heredity* **2**: 349–368.
- Bonduriansky R, Maklakov A, Zajitschek F, Brooks R. 2008.** Sexual selection, sexual conflict and the evolution of ageing and life span. *Functional Ecology* **22**: 443–453.
- Calsbeek R, Bonneaud C, Prabhu S, Manoukis N, Smith TB. 2007.** Multiple paternity and sperm storage lead to increased genetic diversity in *Anolis* lizards. *Evolutionary Ecology Research* **9**: 495.
- Calsbeek R, Bonneaud C, Smith TB. 2008.** Differential fitness effects of immunocompetence and neighbourhood density in alternative female lizard morphs. *Journal of Animal Ecology* **77**: 103–109.
- Chapman B, Chapman R. 1964.** Observations on the biology of the lizard *Agama agama* in Ghana. *Proceedings of the Zoological Society of London* **143**: 121–132.
- Christe P, Glaizot O, Strepparava N, Devevey G, Fumagalli L. 2012.** Twofold cost of reproduction: an increase in parental effort leads to higher malarial parasitaemia and to a decrease in resistance to oxidative stress. *Proceedings of the Royal Society of London Series B, Biological Sciences* **279**: 1142–1149.
- Chu CYC, Lee RD. 2012.** Sexual dimorphism and sexual selection: a unified economic analysis. *Theoretical Population Biology* **82**: 335–363.
- Clutton-Brock TH, Parker GA. 1992.** Potential reproductive rates and the operation of sexual selection. *Quarterly Review of Biology* **67**: 437–456.
- Cole LC. 1954.** The population consequences of life history phenomena. *Quarterly Review of Biology* **29**: 103–137.
- Coustau C, Chevillon C. 2000.** Resistance to xenobiotics and parasites: can we count the cost? *Trends in Ecology & Evolution* **15**: 378–383.
- Cox RM. 2006.** A test of the reproductive cost hypothesis for sexual size dimorphism in Yarrow's Spiny Lizard, *Sceloporus jarrovi*. *Journal of Animal Ecology* **75**: 1361–1369.
- Cox RM. 2014.** Integrating costs of reproduction between the sexes. In: Martin LB, Woods LB, Ghalambor C, eds. *Integrative organismal biology*. Chichester: Wiley Scientific.
- Cox RM, Calsbeek R. 2010a.** Severe costs of reproduction persist in *Anolis* lizards despite the evolution of a single-egg clutch. *Evolution* **64**: 1321–1330.
- Cox RM, Calsbeek R. 2010b.** Sex-specific selection and intraspecific variation in sexual size dimorphism. *Evolution* **64**: 798–809.
- Cox R, Calsbeek R. 2011.** An experimental test for alternative reproductive strategies underlying a female-limited polymorphism. *Journal of Evolutionary Biology* **24**: 343–353.
- Cox RM, John-Alder HB. 2005.** Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.) with opposite patterns of sexual size dimorphism. *Journal of Experimental Biology* **208**: 4679–4687.
- Cox RM, John-Alder HB. 2007.** Increased mite parasitism as a cost of testosterone in male striped plateau lizards *Sceloporus virgatus*. *Functional Ecology* **21**: 327–334.
- Cox RM, Skelly SL, John-Alder HB. 2005.** Testosterone inhibits growth in juvenile male eastern fence lizards (*Sceloporus undulatus*): implications for energy allocation and sexual size dimorphism. *Physiological and Biochemical Zoology* **78**: 531–545.
- Cox RM, Stenquist DS, Henningsen JP, Calsbeek R. 2009.** Manipulating testosterone to assess links between behavior, morphology, and performance in the brown anole *Anolis sagrei*. *Physiological and Biochemical Zoology* **82**: 686–698.
- Cox RM, Parker EU, Cheney DM, Liebl AL, Martin LB, Calsbeek R. 2010.** Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. *Functional Ecology* **24**: 1262–1269.
- Cox CL, Hanninen AF, Reedy AM, Cox RM. 2014a.** Female anoles retain responsiveness to testosterone despite the evolution of androgen-mediated sexual dimorphism. *Functional Ecology* **29**: 758–769.
- Cox RM, Lovern MB, Calsbeek R. 2014b.** Experimentally decoupling reproductive investment from energy storage to test the functional basis of a life-history trade-off. *Journal of Animal Ecology* **83**: 888–898.
- Derickson WK. 1976.** Lipid storage and utilization in reptiles. *American Zoologist* **16**: 711–723.
- Dugas MB, Wamelink CN, Richards-Zawacki CL. 2015.** Both sexes pay a cost of reproduction in a frog with biparental care. *Biological Journal of the Linnean Society* **115**: 211–218.
- Evans LT. 1938.** Cuban field studies on territoriality of the lizard *Anolis sagrei*. *Journal of Comparative Psychology* **25**: 97.
- Fedorka KM, Zuk M, Mousseau TA. 2004.** Immune suppression and the cost of reproduction in the ground cricket, *Allonemobius socius*. *Evolution* **58**: 2478–2485.
- Fisher R. 1930.** *The genetical theory of natural selection*. Clarendon Press, Oxford.
- Folstad I, Karter AJ. 1992.** Parasites, bright males, and the immunocompetence handicap. *American Naturalist* **139**: 603–622.
- French SS, DeNardo DF, Moore MC. 2007.** Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *American Naturalist* **170**: 79–89.
- Gadgil M, Bossert WH. 1970.** Life historical consequences of natural selection. *American Naturalist* **104**: 1–24.
- Goldberg SR, Bursey CR. 1989.** *Physaloptera retusa* (Nematoda, Physalopteridae) in naturally infected sagebrush lizards, *Sceloporus graciosus* (Iguanidae). *Journal of Wildlife Diseases* **25**: 425–429.
- Goto N, Kodama H, Okada K, Fujimoto Y. 1978.** Suppression of phytohemagglutinin skin response in thymectomized chickens. *Poultry science* **57**: 246–250.
- Greiner EC. 2003.** Coccidiosis in reptiles. *Seminars in Avian and Exotic Pet Medicine* **12**: 49–56.
- Gustafsson L, Nordling D, Andersson M, Sheldon B, Qvarnström A. 1997.** Infectious diseases, reproductive

- effort and the cost of reproduction in birds. In: Hamilton WD, Howard JC, eds. *Infection, polymorphism and evolution*. New York, NY: Springer, 53–61.
- Harding CF, Sheridan K, Walters MJ. 1983.** Hormonal specificity and activation of sexual behavior in male zebra finches. *Hormones and Behavior* **17**: 111–133.
- Harshman LG, Zera AJ. 2007.** The cost of reproduction: the devil in the details. *Trends in Ecology & Evolution* **22**: 80–86.
- Hoffman CL, Ruiz-Lambides AV, Davila E, Maldonado E, Gerald MS, Maestripieri D. 2008.** Sex differences in survival costs of reproduction in a promiscuous primate. *Behavioral Ecology and Sociobiology* **62**: 1711–1718.
- Hurst GD, Sharpe RG, Broomfield AH, Walker LE, Majerus TM, Zakharov IA, Majerus ME. 1995.** Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*. *Ecological Entomology* **20**: 230–236.
- Jenssen TA, Greenberg N, Hovde KA. 1995.** Behavioral profile of free-ranging male lizards, *Anolis carolinensis*, across breeding and post-breeding seasons. *Herpetological Monographs* **9**: 41–62.
- Jones AG, Arguello JR, Arnold SJ. 2002.** Validation of Bateman's principles: a genetic study of sexual selection and mating patterns in the rough-skinned newt. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 2533–2539.
- Kennedy MW, Nager RG. 2006.** The perils and prospects of using phytohaemagglutinin in evolutionary ecology. *Trends in Ecology & Evolution* **21**: 653–655.
- Ketterson ED, Nolan V Jr, Wolf L, Ziegenfus C, Dufty AM Jr, Ball GF, Johnsen TS. 1991.** Testosterone and avian life histories: the effect of experimentally elevated testosterone on corticosterone and body mass in dark-eyed juncos. *Hormones and Behavior* **25**: 489–503.
- Kokko H, Jennions MD. 2008.** Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology* **21**: 919–948.
- Kotiaho JS, Simmons LW. 2003.** Longevity cost of reproduction for males but no longevity cost of mating or courtship for females in the male-dimorphic dung beetle, *Onthophagus binodis*. *Journal of Insect Physiology* **49**: 817–822.
- Landwer A. 1994.** Manipulation of egg production reveals costs of reproduction in the tree lizard (*Urosaurus ornatus*). *Oecologia* **100**: 243–249.
- Langford GJ, Willobee BA, Isidoro LF. 2013.** Transmission, Host Specificity, and Seasonal Occurrence of *Cyrtosomum penneri* (Nematoda: Atractidae) in Lizards from Florida. *Journal of Parasitology* **99**: 241–246.
- Lee JC, Clayton D, Eisenstein S, Perez I. 1989.** The reproductive cycle of *Anolis sagrei* in southern Florida. *Copeia* **4**: 930–937.
- Levine ND. 1968.** *Nematode parasites of domestic animals and of man*. Minneapolis, MN: Burgess Pub. Co.
- Licht P, Gorman GC. 1970.** *Reproductive and fat cycles in Caribbean Anolis lizards*. Oakland, CA: University of California Press.
- Lin EJ. 1979.** Fatbody and liver cycles in two tropical lizards *Chamaeleo hohneli* and *Chamaeleo jacksoni* (Reptilia, Lacertilia, Chamaeleonidae). *Journal of Herpetology* **13**: 113–117.
- Lochmiller RL, Deerenberg C. 2000.** Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**: 87–98.
- Lockhart AB, Thrall PH, Antonovics J. 1996.** Sexually transmitted diseases in animals: ecological and evolutionary implications. *Biological Reviews* **71**: 415–471.
- Maklakov AA, Lummaa V. 2013.** Evolution of sex differences in lifespan and aging: causes and constraints. *BioEssays* **35**: 717–724.
- Marler CA, Walsberg G, White ML, Moore M, Marler C. 1995.** Increased energy expenditure due to increased territorial defense in male lizards after phenotypic manipulation. *Behavioral Ecology and Sociobiology* **37**: 225–231.
- Martin LB, Scheuerlein A, Wikelski M. 2003.** Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society of London Series B, Biological Sciences* **270**: 153–158.
- Martin LB, Han P, Lewittes J, Kuhlman JR, Klasing KC, Wikelski M. 2006.** Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immunological technique. *Functional Ecology* **20**: 290–299.
- Martin LB, Hawley DM, Ardia DR. 2011.** An introduction to ecological immunology. *Functional Ecology* **25**: 1–4.
- McCurdy DG, Shutler D, Mullie A, Forbes MR. 1998.** Sex-biased parasitism of avian hosts: relations to blood parasite taxon and mating system. *Oikos* **82**: 303–312.
- McKean KA, Lazzaro BP. 2011.** The costs of immunity and the evolution of immunological defense mechanisms. In: Flatt T, Heyland A, eds. *Mechanisms of life history evolution*. Oxford: Oxford University Press, 299–310.
- Michener GR, Locklear L. 1990.** Differential costs of reproductive effort for male and female Richardson's ground squirrels. *Ecology* **71**: 855–868.
- Moore SL, Wilson K. 2002.** Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* **297**: 2015–2018.
- Nordling D, Andersson M, Zohari S, Gustafsson L. 1998.** Reproductive effort reduces specific immune response and parasite resistance. *Proceedings of the Royal Society of London Series B, Biological Sciences* **265**: 1291–1298.
- Norris K, Evans MR. 2000.** Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology* **11**: 19–26.
- Norval G, Bursey CR, Goldberg SR, Mao J-J, Slater K. 2011.** Origin of the helminth community of an exotic invasive lizard, the brown anole, *Anolis sagrei* (Squamata: Polychrotidae), in southwestern Taiwan. *Pacific Science* **65**: 383–390.
- Nunn CL, Lindenfors P, Pursall ER, Rolff J. 2009.** On sexual dimorphism in immune function. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **364**: 61–69.
- O'Hara RB, Kotze DJ. 2010.** Do not log-transform count data. *Methods in Ecology and Evolution* **1**: 118–112.
- Owen JP, Clayton DH. 2007.** Where are the parasites in the PHA response? *Trends in Ecology & Evolution* **22**: 228–229.

- Paukku S, Kotiaho JS. 2005.** Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity. *Journal of Insect Physiology* **51**: 1220–1226.
- Penn DJ, Smith KR. 2007.** Differential fitness costs of reproduction between the sexes. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 553–558.
- Queller DC. 1997.** Why do females care more than males?. *Proceedings of the Royal Society of London Series B, Biological Sciences* **264**: 1555–1557.
- Reznick D. 1992.** Measuring the costs of reproduction. *Trends in Ecology and Evolution* **7**: 42–45.
- Rigby MC, Hechinger RF, Stevens L. 2002.** Why should parasite resistance be costly? *Trends in Parasitology* **18**: 116–120.
- Roberts ML, Buchanan KL, Evans MR. 2004.** Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour* **68**: 227–239.
- Rolff J. 2002.** Bateman's principle and immunity. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 867–872.
- Rose MR, Charlesworth B. 1981.** Genetics of life history in *Drosophila melanogaster*. I. Sib analysis of adult females. *Genetics* **97**: 173–186.
- Schmid-Hempel P, Ebert D. 2003.** On the evolutionary ecology of specific immune defence. *Trends in Ecology & Evolution* **18**: 27–32.
- Sexton OJ, Ortleb EP, Hathaway LM, Ballinger RE, Licht P. 1971.** Reproductive cycles of three species of anoline lizards from the Isthmus of Panama. *Ecology* **52**: 202–215.
- Sheldon BC, Verlhust S. 1996.** Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11**: 317–321.
- Smits J, Bortolotti GR, Tella JL. 1999.** Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology* **13**: 567–572.
- Stamps J. 1977.** The relationship between resource competition, risk, and aggression in a tropical territorial lizard. *Ecology* **58**: 349–358.
- Tokarz RR. 1985.** Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*). *Animal Behaviour* **33**: 746–753.
- Tokarz RR. 1986.** Hormonal regulation of male reproductive behavior in the lizard *Anolis sagrei*: a test of the aromatization hypothesis. *Hormones and Behavior* **20**: 364–377.
- Tokarz RR. 1995.** Importance of androgens in male territorial acquisition in the lizard *Anolis sagrei*: an experimental test. *Animal Behaviour* **49**: 661–669.
- Tokarz RR. 1998.** Mating pattern in the lizard *Anolis sagrei*: implications for mate choice and sperm competition. *Herpetologica* **54**: 388–394.
- Tokarz RR, McMann S, Seitz L, John-Alder HB. 1998.** Plasma corticosterone and testosterone levels during the annual reproductive cycle of male brown anoles (*Anolis sagrei*). *Physiological Zoology* **71**: 139–146.
- Tokarz RR, McMann S, Smith LC, John-Alder H. 2002.** Effects of testosterone treatment and season on the frequency of dewlap extensions during male-male interactions in the lizard *Anolis sagrei*. *Hormones and Behavior* **41**: 70–79.
- Trivers R. 1972.** Parental investment and sexual selection. In: Campbell B, ed. *Sexual selection and the descent of man, 1871-1971*. Chicago: Aldine-Atherton, 136–179.
- Vass E, Nappi AJ, Carton Y. 1993.** Comparative study of immune competence and host susceptibility in *Drosophila melanogaster* parasitized by *Leptopilina bouvardi* and *Aso-bara tabida*. *Journal of Parasitology* **79**: 106–112.
- Velando A, Alonso-Alvarez C. 2003.** Differential body condition regulation by males and females in response to experimental manipulations of brood size and parental effort in the blue-footed booby. *Journal of Animal Ecology* **72**: 846–856.
- Webberley KM, Hurst GD, Husband RW, Schulenburg J, Sloggett JJ, Isham V, Buszko J, Majerus ME. 2004.** Host reproduction and a sexually transmitted disease: causes and consequences of *Coccipolipus hippodamiae* distribution on coccinellid beetles. *Journal of Animal Ecology* **73**: 1–10.
- Wedekind C, Folstad I. 1994.** Adaptive or nonadaptive immunosuppression by sex hormones? *American Naturalist* **143**: 936–938.
- Zahavi A. 1975.** Mate selection – a selection for a handicap. *Journal of Theoretical Biology* **53**: 205–214.