Mechanisms Underlying the Costs of Egg Production

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Female birds incur costs associated with increased egg production, including reductions in chick provisioning ability, in future fecundity, in survival, and in egg and chick viability. It should be possible to identify the components of the physiological system underlying reproduction, or the specific reproductive traits themselves, that explain these costs, but this has proved to be difficult, in part because of marked, but unexplained, individual variation in these traits. Resolving the physiological and evolutionary consequences of this individual variation represents an exciting challenge for the future. Several mechanisms have been proposed for the cost of egg production (e.g., protein depletion and impaired flight muscle function; immunosuppression), which assume relatively simple resource-allocation trade-offs. I argue that such mechanisms provide an unsatisfactory explanation for costs that can occur over months or even years. A more productive approach for future research will be to focus on hormonally mediated, non-resource-based costs of egg production caused by pleiotropic effects of reproductive hormones that can operate over the longer time scales at which costs of reproduction are expressed.

Keywords: egg production, costs of reproduction, hormones, individual variation, pleiotropy

There has long been a dichotomy of opinion as to whether egg production is biologically costly or not. On the one hand, many research articles have included statements such as "Egg production is energetically or nutritionally demanding," based mainly on early theoretical estimates of energy and protein requirements (Carey 1996). In contrast, Lack (1947) set a different path for avian reproductive biology by suggesting that clutch size—the number of eggs a bird lays—is "far below the potential limit of egg production." Lack thought that clutch size was not determined by a physiological inability of females to produce more eggs, but rather that it evolved in relation to the number of young that the parents could successfully rear. A legacy of Lack's ideas has been that most avian studies have continued to focus on reproductive limitations operating during chick rearing, particularly in altricial species whose parents feed their young in the nest (but see Reid and colleagues [2002] for a summary of costs of incubation). Lack (1968) recognized that in precocial species, where young are self feeding, clutch size might be explained by factors different from those in altricial species (e.g., the availability of food for laying females), but few studies have addressed physiological, or life-history, costs of egg production in either altricial or precocial species to date. Consequently, the idea that costs of egg production per se are relatively minor, and unimportant for understanding the evolution of clutch size and the fitness costs of reproduction in birds, has continued to dominate the literature until very recently (Monaghan and Nager 1997).

In this article, I will first review the recent evidence that females can and do incur costs (*sensu* Williams 1966) associated specifically with reproductive effort during egg production. I will then consider what biologists currently know about the physiological mechanisms underlying these costs of egg production. In particular, I will focus on two types of costs: (1) costs associated with resource-allocation decisions and (2) non-resource-based costs. Resource-allocation costs are significant only if egg production is energetically or nutritionally demanding, if resources are limited, and if some individuals pay costs in meeting these requirements (e.g., energy required for egg production might be taken away from other physiological functions, resulting in negative effects on an individual's overall fitness). Of the non-resource-based costs, I will focus in particular on those associated with multiple physiological effects of reproductive hormones (i.e., pleiotropy; Ketterson and Nolan 1999). Throughout the overview, I will stress the importance of understanding individual or intraspecific variability in physiological and reproductive traits. Individuals that produce more or larger eggs, or that can produce eggs earlier in the season, should have higher fitness. Thus, in theory, one can distinguish "high-quality" birds (large eggs, many eggs) from "low-quality" birds (small eggs, few eggs), and it should be possible to identify the physiological traits that vary between them, that is, specific traits that underlie physiological costs associated with individual variation in reproductive output.

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Life-history costs of egg production

Many birds (called "indeterminate layers") will lay additional eggs if eggs are removed from the nest during laying, and this has been one of the main arguments against there being physiological limitations on egg production. However, it is now clear that females do incur costs associated specifically with reproductive effort during egg production, though studies are limited to seabirds and one or two passerine species to date. Reproduction can be manipulated by giving parents an extra chick (increasing only rearing costs) or by using egg removal to make females lay an additional egg (increasing both egg production costs and rearing costs). Birds with increased egg production costs have chicks with lower hatching mass (independent of egg size), reduced chick growth, increased early mortality, and decreased chick survival relative to those with only additional rearing costs (Monaghan et al. 1995). These effects are mediated through the parents' reduced brood-rearing or provisioning capacity (Monaghan et al. 1998). Furthermore, in several species, the females that are made to lay additional eggs (through egg removal) also have lower local return rates the following year (Nager et al. 2001, Visser and Lessells 2001). Although birds can lay additional eggs in response to egg removal, egg quality decreases rapidly with increasing egg number above the normal clutch size. For example, lesser black-backed gulls (*Larus fuscus*) will lay almost three times the normal clutch size of three eggs in response to continuous egg removal, but eggs laid late in the experimental laying sequence have 50% lower fledging success (Nager et al. 2000). This decrease in egg quality is due to changes in egg composition that are independent of changes in egg size (see also Williams and Miller 2003). Increased reproductive effort during egg production therefore clearly induces a cost of reproduction in terms of a female's capacity for chick rearing, her body condition, and her future fecundity and survival; and females have a limited ability to maintain egg quality when laying more than their normal clutch size. Given this mounting evidence for substantial life-history costs of egg production, what is the mechanistic basis of these costs? I will start by considering the idea that egg production is energetically or nutritionally demanding (or both) and that it is simply resource limitation that underlies the costs of reproduction.

Is egg production energetically costly?

What evidence is there that egg production is energetically demanding and that birds might have trouble meeting these energy costs while also maintaining the function of other physiological systems? Do egg-producing birds have higher energy requirements than nonlaying birds, and is reproductive energy expenditure positively correlated with egg or clutch size? Researchers have made surprisingly little progress in directly addressing these questions, in part because of the focus on chick rearing rather than on egg production. Early estimates of basal metabolic rate (BMR) or resting metabolic rate (RMR) during egg production were calculated from theoretical models using information on the chemical energy content of egg components and reproductive organs, and on the pattern and duration of yolk and albumen formation. These models predicted that the energy costs of egg production would represent approximately 45%–60% of BMR for passerines, 80%–130% for shorebirds and galliforms, and more than 200% for waterfowl (Carey 1996). However, recent empirical measurements of RMR in egg-laying birds actually suggest lower energy costs of egg production, at least for passerines. Nilsson and Raberg (2001) measured the RMR of great tits (*Parus major*) at different breeding stages and observed a 27% increase over wintering RMR in egg-laying females. Vézina and Williams (2002), using an alternate approach of measuring changes in RMR through the complete cycle of ovarian follicle development and ovulation, reported a 22% increase in RMR from the beginning of the prelaying period to the point where birds had developed a complete follicle hierarchy. Clearly, more data are required for nonpasserines, but the conflicting results of theoretical and empirical studies suggest that costs are met through reallocation of resources among different physiological systems, rather than in an additive manner, and therefore that integrated measurements such as BMR will not reflect true costs.

A similar problem arises with measurements of daily energy expenditure (DEE) during egg production. Since DEE integrates all activities, females might reduce energy expenditure in one activity (e.g., locomotion), reallocate this energy to reproduction, and show no net increase in measured energy expenditure. The DEE during egg laying can be comparable to that during incubation and chick rearing (Ward 1996), and in birds laying large eggs the DEE can be close to four times the BMR (Stevenson and Bryant 2000). Yet in swallows (*Hirundo rustica*), DEE was not correlated with clutch size or with the energy content of yolk or whole eggs (Ward 1996). In great tits, there was a positive relationship between DEE and egg mass in one year (consistent with the idea that larger eggs are more costly; Stevenson and Bryant 2000), but in a second year this relationship was negative. This highlights the complexity of interpreting integrated measures of energy expenditure in relation to egg production. Perhaps one overall conclusion from these data is that absolute energy expenditure during egg production is at least as high as that during other stages of breeding. This is potentially very important if resources are less available earlier in the breeding season to meet these demands, such that the *relative* energy demands of egg production might be much higher than those of chick rearing (see below).

What is the basis of increased energy costs during egg production?

Despite some interpretational problems, the studies above clearly show that egg production is associated with non-negligible increases in energetic expenditure. What is the physiological basis of this increased energy demand? One possibility is that egg formation requires substantial increases in the mass, or in the metabolic activity, of organs such as the liver (which produces yolk precursors) or the reproductive

organs (the ovary and oviduct). Hypertrophy of these organs might, in turn, increase RMR by increasing maintenance energy costs. In a comprehensive study, Vézina and Williams (2003) reported marked variation in body composition in European starlings (*Sturnus vulgaris*), but for most organs there was no consistent pattern of change in organ size, or in organ metabolic capacity (measured as citrate synthase activity; Vézina and Williams 2005), in relation to specific stages of reproduction. Furthermore, changes in the size of nonreproductive organs did not parallel the cycle of growth and regression of the reproductive organs, as would be predicted if these were adjusted to temporal variation in demands for egg production. In egg-laying birds, the only organ whose mass was related to RMR was the oviduct, which explained 18% of total variation in RMR, suggesting that the cost of oviduct function contributes to the cost of egg production (see also Williams and Ames 2004). The data indicating that birds do not up-regulate their "metabolic machinery," such as the heart, kidneys, and liver, during egg production contrast with the data for pregnant and lactating small mammals, but would be consistent with a relatively low energetic cost of egg production. The cause of the increase of approximately 20% in RMR, which has been consistently reported for egg-producing birds in three studies (see above), therefore remains unknown. However, a more important point is that no studies so far have demonstrated that such short-term, transient increases in energy expenditure can have long-term effects, over weeks or even months, that might underpin the costs of reproduction.

Is egg production nutritionally demanding?

Nutrition might limit reproduction generally, through food availability, but also because there are specific macro- or micronutrient requirements for egg production (e.g., specific amino acids, calcium). Nutrient (or energy) limitation might be compounded by any temporal variation in food availability. Most temperate and higher-latitude birds time their breeding season such that chicks are growing in the nest during the seasonal peak in food availability. However, egg production is often initiated well in advance of any seasonal increase in food availability (see figure 1 in Williams 1999). Before young birds hatch, their parents must undergo extensive physiological and behavioral preparation for breeding (e.g., maturation of the reproductive system, finding a nest and mate, and laying and incubating eggs), and these events can take 6 to 8 weeks to complete. One way to address the role of nutrition in limiting reproduction is to provide birds with more energy (i.e., food) and see if this advances or increases reproductive effort. In most cases, food supplementation does significantly advance the laying date (though some groups, such as gulls, do not appear to respond to food supplementation), suggesting that food availability directly influences the timing component of egg production, but egg size and clutch size are more or less independent of food availability. In studies of 24 different species, almost no food-supplemented females laid larger eggs or more eggs than unsupplemented ones, independent of the effect of earlier laying (Meijer and Drent 1999).

Fewer studies have considered nutritional quality in supplementary feeding experiments, but these suggest that different macronutrients might have specific effects on egg and clutch size. In a range of species, providing females with extra protein, but not with extra lipid, causes an increase in egg size, clutch size, or both, supporting the idea that egg production can be protein limited (Bolton et al. 1993, Reynolds et al. 2003). Houston and colleagues (1995) speculated that egg production could be limited not by overall protein availability, but by the availability of specific sulfur-rich or essential amino acids, such as lysine, tyrosine, or methionine. In blue tits (*Parus caeruleus*), birds that received a supplementary diet containing an amino acid balance close to that of egg protein laid larger clutches (by 18%), whereas those that were given a similar diet but without an optimal amino acid balance did not increase egg production (Ramsey and Houston 1998). Houston and colleagues (1995) suggested that small passerines might be able to meet the requirements for egg production by accumulating specific protein stores containing elevated levels of these limiting amino acids, but Cottam and colleagues (2002) found no evidence to support this. Rather, any removal of protein from pectoral muscle during egg production is of a general nature, not restricted to any specific proteins.

There is still relatively little known about more specific nutritional requirements that might limit egg production in birds (Carey 1996). Calcium availability has been shown to limit egg production in some habitats, such as those with acidified or base-poor soils. Calcium intake increases markedly in laying females, specifically coupled to the timing of shell formation; and females with an inadequate calcium supply often produce smaller eggs and clutches, and have a higher incidence of eggshell defects and irregular laying (Graveland et al. 1994), although these effects can be species or population specific (Mand and Tilger 2003). More recently, attention has focused on the importance of carotenoids and other antioxidants, such as vitamin E, as components of the diet for breeding females (Bortolotti et al. 2003). Carotenoids are lipid-soluble pigments that cannot be synthesised *de novo* by animals, and that are deposited in eggs (which is why yolks are yellow; Blount et al. 2000), where they function as antioxidants to reduce tissue damage caused by free radicals and to enhance immune function in developing embryos and hatchlings (Surai et al. 2001). This is a rapidly developing area of study, but there is little evidence so far to suggest that laying females are carotenoid limited, or that supplementing females with additional carotenoids increases egg mass, yolk mass, or clutch size (Bortolotti et al. 2003), though Blount and colleagues (2004) did find a higher level of renesting in carotenoid-supplemented females. Carotenoid-supplemented females lay eggs with higher yolk carotenoid concentrations (Bortolotti et al. 2003), and Saino and colleagues (2003) have shown that elevated yolk carotenoids enhance T cell–mediated immune function in barn swallow nestlings. Thus,

again, although numerous studies suggest that certain specific nutrients might limit current reproduction, there is little or no evidence that nutrient availability explains long-term (future) costs of egg production. Nevertheless, given the intuitive appeal of resource-allocation trade-offs, it is worth asking what mechanisms might link current resource availability to long-term costs.

Physiological mechanisms for resource-based costs of egg production

It is certainly plausible that high resource demands for egg laying mean that resources must be taken away from other critical physiological functions. But what functions? And exactly how are these functions impaired or compromised? While studies of energy and nutrient demands tell us something about the currency of egg production (i.e., energy and nutrients), they do not really inform us about the mechanism of costs (i.e., how energy debts might be incurred and paid). As an example, although calcium availability clearly limits egg production in some situations (see above), there is little evidence that this involves a physiological mechanism that might generate a cost of egg production. Some species store calcium in bone before laying (Reynolds 1997), but calcium withdrawal from bone appears to contribute relatively little to total calcium requirements during laying, especially in small birds. Moreover, calcium is stored as medullary bone (i.e., in the lumen of bones), and there is no evidence that reallocation of calcium from bone to shell formation has any negative consequences (e.g., osteoporosis-like effects, such as bone fragility, or effects on general calcium homeostasis), in contrast to bone loss in poultry or mammals. However, two specific mechanisms for resource-based costs of reproduction have been proposed, and I will discuss these in turn (a third mechanism, a trade-off involving molt, feather quality, and thermoregulatory ability, has not been investigated in relation to egg production; Dawson et al. 2000).

Protein depletion, muscle function, and flight ability

One potential mechanism for costs associated with the high nutrient demands of egg production is the impact of depletion of protein or lipid from organs or tissues during laying. In some precocial species, such as geese and ducks, a substantial proportion of the nutrients invested in eggs can come from body reserves, and it seems clear that endogenous reserves at the time of laying do limit egg production (e.g., birds will delay onset of laying to accumulate more nutrients at the expense of survival rate of juveniles; Gauthier et al. 2003). However, few studies have investigated the functional consequences of depletion of body reserves as a potential basis for long-term costs. One exception is the depletion of protein reserves from the pectoral (flight) muscle, which results in decreased pectoral muscle mass during egg production (Houston et al. 1995). It has been suggested that this might result in decreased chick-rearing ability through negative effects on flight performance (Monaghan et al. 1998, Veasey et al. 2001). Compromised flight performance of fecund females might also lead to higher predation risk, which could constitute a behavioral cost of egg production (Lee et al. 1996, Kullberg et al. 2002). However, while it is clear that in many species pectoral muscle mass does decrease during egg production, it is less clear that this is directly related to the protein demands of egg formation and to the direct transfer of proteins from muscle to eggs (Williams 1996, Cottam et al. 2002). Although both muscle mass loss and reproductive output can show correlated responses to diet manipulations (Selman and Houston 1996, Blount et al. 2004), this does not confirm a causal, mechanistic link between these traits. It is possible that changes in muscle mass are a response to some other trait, which is itself independently linked to either dietary intake or egg production. Some birds are capable of producing normal clutches without any decreases in body mass or pectoral muscle mass (Williams 1996, Woodburn and Perrins 1997), and mass loss during laying can be independent of variation in fecundity (Williams 1996). Furthermore, in some birds, such as the European starling, increased egg production in response to egg removal does not increase depletion of pectoral muscle mass (Christians 2000); and even though muscle mass in European starlings does decrease through laying, this decrease continues during the later stages of breeding. Therefore, changes in pectoral muscle mass are not restricted to the phase of egg production (Vézina and Williams 2003). Finally, even in altricial species, nutrients can be depleted from various tissues during egg production, not just from pectoral muscle (Williams and Martyniuk 2000), suggesting that the utilization of endogenous nutrient sources by laying birds is more complex than currently thought (Cottam et al. 2002). Alternative hypotheses for changes in pectoral muscle mass during breeding should be investigated, such as atrophy due to disuse through reduced locomotion, or an adaptive change to maintain wing loading in relation to varying body mass. Similarly, changes in flight performance might be independent of changes in flight muscle mass; for example, they might reflect a change in the center of gravity in females with eggs or with enlarged gonads (Lee et al. 1996).

Resource availability and immune function

A second commonly cited mechanism for a resource-allocation trade-off that might underlie a cost of egg production is the trade-off between reproduction and immune function or resistance to parasite infection (Gustafsson et al. 1994). Egg-laying females appear to have a higher susceptibility to parasite infection. In collared flycatchers (*Ficedula albicollis*), late-laying birds and those laying smaller clutches had higher levels of various serological parameters indicative of infection (e.g., white blood cell counts, immunoglobulins, and the proportion of heterophiles; Gustafsson et al. 1994). In great tits, females laying larger clutches had a higher prevalence of *Plasmodium* infection, and females made to lay one additional egg through egg removal also had a higher prevalence of infection when measured during chick rearing (Oppliger et al. 1997). This highlights a problem of discerning cause and effect (e.g., does increased reproductive effort

itself cause higher parasite load, or are infected individuals in poorer condition and as a result likely to lay smaller eggs?). A resource-based trade-off between reproduction and immune function (immunocompetence) could operate through general energy or nutrient reallocation, or through reallocation of a specific limiting factor that plays a key role in regulating immune function in embryos or chicks as well as in the adult female (e.g., carotenoids or immunoglobulins), but few studies have addressed this issue in egg-producing birds. Martin and colleagues (2003) estimated the cost of an elevated cell-mediated immune response as 29% of RMR in captive, nonbreeding house sparrows (*Passer domesticus*), which they equated to a theoretically determined cost of production of half an egg. However, experimentally elevated primary antibody production during egg formation had no effect on egg or clutch size in free-living European starlings (Williams et al. 1999).

Eggs contain substantial amounts of various immune factors (carotenoids, immunoglobulins, lysozymes, etc.), which might be important in determining offspring fitness through effects on immune function in developing embryos or chicks. If laying females deplete their own stores of these factors, they might compromise their own ability to mount an immune response. Few studies have considered the effects of such factors on laying females, but in female barn swallows, plasma lysozyme activity does decline during the prelaying and laying periods (Saino et al. 2002). However, although plasma immunoglobulin concentrations increased during egg laying, postlaying plasma concentrations of immunoglobulins were similar to concentrations before laying, suggesting that females did not compromise their own immune system in order to allow passive transfer of immunity to offspring for this immune factor (Saino et al. 2001). In summary, although reproductive effort can lead to a decrease in immune function, only one study to date has linked long-term changes in immune function to future survival (i.e., to a measurable cost of reproduction), and this was in chick-rearing, not egg-producing, birds (Ardia et al. 2003). As with studies showing increases in energy demand during egg production, there is no evidence to show that transient, short-term decreases in immune function can have long-term consequences for future fecundity or survival.

Individual variation and the cost–benefit paradigm

A common theme in this overview so far has been that egg production, or some of the physiological processes associated with it, is in some sense costly. In general, if this is true, it would be expected that the capacity of these physiological systems should be tightly coupled to demand (*sensu* Diamond and Hammond 1992). For example, if yolk production is costly, then "high-quality" individuals that can maintain high plasma levels of yolk precursors should produce larger yolks or eggs, with consequent benefits for offspring fitness. Conversely, unless there is some associated benefit, individuals should not produce excessive amounts of yolk precursors beyond the amount needed to maximize rates of egg production. One might expect, therefore, to find positive correlations between physiological components of the reproductive system and traits such as egg or clutch size. At one level, there is some evidence to support these ideas. Plasma levels of 17β-estradiol, which regulates egg production (Williams et al. 2004a), and of yolk precursors (Challenger et al. 2001) increase very rapidly at the onset of follicular development and decrease rapidly as soon as the last yolky follicle is ovulated. Similarly, growth and regression of the oviduct occur very rapidly (within 24 hours) at the start and end of egg development, respectively, such that full oviduct size is only maintained during the period of albumen secretion (Williams and Ames 2004). All this is consistent with the hypothesis that the costs of egg production are sufficiently high to select against early onset of reproductive development in advance of egg formation, or against maintenance of a fully developed reproductive system after cessation of egg formation (even though there might clearly be benefits to a more prolonged period of reproductive maturity in terms of flexibility of onset of laying, renesting ability, or both).

Nevertheless, at the individual (intraspecific) level, there is much less support for the idea that costly components of the reproductive system are correlated with measures of reproductive output. All components of the reproductive system underlying egg production show marked variation among individual birds (figure 1), which is often unrelated to individual variation in reproductive output, even though there should be clear functional relationships between these traits according to our knowledge of the mechanisms underlying egg production. For example, estrogens play a fundamental role in regulating female reproduction (stimulating yolk-precursor production and oviduct development), yet in European starlings, individual variation in plasma 17β-estradiol is unrelated to the plasma levels of the main yolk precursor vitellogenin (figure 1a) or to the total mass of yolky follicles developing at the time of blood sampling (Williams et al. 2004a). Similarly, lipoprotein yolk precursors (vitellogenin and very low-density lipoprotein [VLDL]), which are synthesized and released by the liver in response to estrogens, circulate in the plasma and are taken up by developing follicles to form yolks. Yet in zebra finches, variation in the size of developing yolky follicles, or in the total mass of developing follicles, is independent of individual variation in yolk precursor levels (figure 1b). Furthermore, in European starlings, these traits are actually negatively correlated (Challenger et al. 2001). These results are particularly striking given, for example, that yolk precursor levels vary eightfold and plasma E2 levels vary tenfold among different females. Thus, for any given level of plasma estradiol or plasma vitellogenin there can be three- to fourfold variation in the putative response trait (i.e., vitellogenin or total follicle mass, respectively; figure 1a, 1b). Variability in physiological components of egg production does, however, appear to have an impact on some aspects of reproduction. Christians and Williams (2001) reported a positive correlation between yolk mass and uptake rate of vitellogenin into the ovary (figure 1c), and there is also

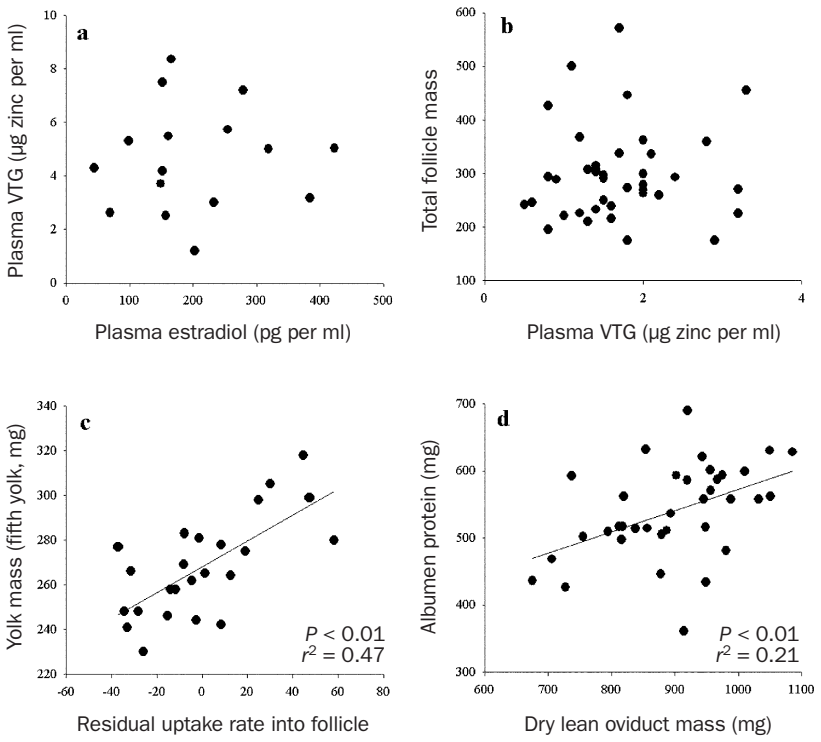

Figure 1. Variation in physiological and reproductive traits among individual birds, showing relationships between (a) plasma estradiol and plasma vitellogenin (VTG), (b) plasma VTG and total follicle mass, (c) residual VTG uptake rate into follicle and yolk mass, and (d) dry lean oviduct mass and albumen protein. The traits in each of these relationships would be predicted to be functionally linked.

a positive relationship between albumen content of eggs and oviduct mass (figure 1d). Furthermore, *experimental* manipulation of yolk precursor levels, using the antiestrogen tamoxifen, does affect egg size, though even here a 50% decrease in yolk precursors levels reduces egg size by only 10% (Williams 2001), consistent with a buffered capacity for precursor production.

In general, these observations seem to question the idea of an "efficient" egg production system that has evolved through natural selection to match the capacity of physiological traits to variation in reproductive output. Moreover, no studies have found any relationship between variation in these physiological traits and clutch size (fecundity). If receptor-mediated uptake of yolk into follicles does represent a "costly" component of egg production (figure 1c), why would individuals vary in their ability to synthesize receptors? Are there developmental pathways that link lipoprotein receptor expression, such that upregulation of vitellogenin receptors causes upregulation of receptors in other tissues with potentially negative consequences? We know virtually nothing about the costs of the individual components of the reproductive system. However, two recent studies suggest that elevated yolk precursor and hormone synthesis are not associated with elevated energy expenditure in laying birds (Eising et al. 2003, Vézina et al. 2003).

This lack of a systematic relationship between many physiological and reproductive traits, which should be functionally linked, is not simply due to the fact that variation in the physiological trait is "neutral" or random. Plasma vitellogenin concentration in laying birds is a highly repeatable trait (Challenger et al. 2001), even though it has a low degree of genetic determination (Nestor et al. 1996). In other words, there appear to be "high-vitellogenin" and "low-vitellogenin" phenotypes (albeit at the ends of a continuum), but this phenotypic variation in lipoprotein physiology is unrelated to variation in egg size or clutch size. So why do some birds secrete tenfold higher estradiol or vitellogenin levels compared with other birds, when this has no apparent benefit in terms of increased reproductive output? Resolving the functional consequences of this large-scale, interindividual physiological variation in the reproductive system represents an exciting challenge for the future in terms of our understanding of costs of egg production, both from a physiological and an evolutionary perspective. In this context, it is also important to note that at the intraspecific level, interrelationships between "core" life-history traits (egg size, female size, incubation period, etc.) are often weak, or even nonexistent, whereas these relationships can be strong at the interspecific level (see figure 4.2 in Bennett and Owens 2002). This highlights a problem in applying results from traditional interspecific or comparative analyses to an understanding of intraspecific variation (another example is the very different mass exponents obtained for allometry of body composition from between- versus within-species studies; Guglielmo and Williams 2003). It is clear that we have to better understand the variability in physiological systems involved in egg production if we are to link these systems, and other physiological processes, to life-history costs of egg production.

Physiological mechanisms for non-resource-based costs of egg production

Most studies, and therefore much of this review, have focused on resource-based trade-offs as the basis of physiological costs of egg production. In general, much less attention has been paid to non-resource-based costs, even though these can often lead to very different interpretations. For example, it has been suggested that immunosuppresion during laying might be adaptive if it prevents inappropriate autoimmune responses analogous to protection of the fetoplacental unit in mammals (Raberg et al. 1998). Potential mechanisms for non-resource-based costs of egg production come from the broad pleiotropic effect of reproductive hormones (Ketterson and Nolan 1999). Egg formation involves large cyclical changes

in many hormones, associated with reproductive behaviors, ovarian and oviductal growth and regression, and onset of incubation. Steroid hormones and gonadotropins increase rapidly to a peak at the time of courtship, territoriality, and egg laying, and then rapidly decrease, whereas prolactin increases at onset of incubation and is high through incubation (see figure 7 in Williams 1999). Many, if not all, hormones involved in reproduction have widespread (pleiotropic) effects on a wide range of physiological systems, both positive and negative, which could provide a basis for costs of egg production. For example, estrogens (and progestins) have many effects on nonreproductive physiological systems in females, including vaso- and neuroprotection, angiogenesis, and immune function. These nonreproductive effects have been relatively well documented in mammals, but far less so in birds. Elevated androgen levels might also incur costs, such as decreased body condition, immunosuppression, and oncogenic effects that reduce lifetime fitness (Wingfield et al. 2001). However, most of this work has focused on males or nonbreeding birds, and we know very little about patterns of androgen secretion, or functions of androgens, during egg production in females. Pleiotropic effects of reproductive hormones need not be restricted to the egg-producing female herself, since there is abundant evidence for transfer of maternal hormones (e.g., steroids, glucocorticoids, thyroid hormones) to yolks, which can then have wide-ranging effects on offspring development. Many studies have interpreted this as an "adaptive" phenomenon (i.e., females utilize or control transfer of maternal steroids to yolks to optimize offspring phenotype). However, if yolk steroid levels are influenced by variation in maternal hormone levels, it is possible that there are trade-offs between the female's own requirements for hormonal regulation of reproductive function (vitellogenesis, etc.) and negative effects on offspring phenotype. As one example, estradiol treatment of laying females or chicks can be associated with male-biased offspring mortality, and with impairment of gonadal development and reproductive performance of offspring at maturity (Williams 1999, Millam et al. 2001). Thus, despite the clear requirement for gonadal steroids for regulation of reproductive function, there are myriad potential physiological mechanisms—most of them unexplored—that could underlie costs of egg production via negative nonreproductive effects of gonadal steroids. I will focus in more detail on two potential mechanisms underlying such nonresource-based costs of egg production.

Estrogens, lipoprotein metabolism, and oxidative stress

Estrogens play a fundamental role in regulating many aspects of female reproduction. In egg-producing females, there are marked estrogen-dependent changes in lipid metabolism, including variation in the types and absolute concentrations of circulating lipoproteins, with especially high levels of VLDL and vitellogenin, and in the macromolecular structure of the predominant lipoproteins (Walzem 1996). As we have seen (figure 1), lipoprotein concentrations can be highly variable among individuals (though in a systematic, repeatable manner), but they do not correlate with simple measures of reproductive effort, such as egg size. Recent studies in humans have suggested a link between lipoprotein phenotype and longevity (Barzilai et al. 2003), and in bees (*Apis mellifera*), the high-density lipoprotein vitellogenin may play a role in regulatory control of aging (Amdam et al. 2004). Thus, the marked estrogen-dependent changes in lipoprotein metabolism in laying birds might indeed be linked to key components of the cost of reproduction, such as survival, through as yet unknown pathways. Other functional linkages between estrogens and immune function also seem likely; in mammals, females generally have higher serum immunoglobulin levels, higher antibody titers in response to antigens, and higher incidence of autoimmune diseases, which have been related to gender differences in gonadal steroids (Olsen and Kovacs 1996). Both positive and negative effects of estrogens on immune function have been reported in poultry and mammals, and given the marked interindividual variation in plasma 17β-estradiol levels (figure 1a), these effects should again be individually variable. It seems possible, for example, that the estrogen-dependent increased production of lipid-rich yolk precursors in egg-producing birds might be associated with increased production of reactive oxygen species via lipid peroxidation, and with accumulation of oxidative stress, generating nonspecific negative effects. Given the role of oxidative stress in aging in many taxa, and the fact that the reproductive system can influence this process (Hsin and Kenyon 1999) via insulin or insulin-like growth factor signaling pathways, it would be surprising if this were not ultimately revealed as a component of mechanisms underlying costs of egg production in oviparous vertebrates such as birds.

Anemia and aerobic performance as a cost of egg production

Egg production in birds is routinely associated with a reduction in hematocrit—that is, a decrease of 9% to 20% in the number of circulating red blood cells (figure 2a; Williams et al. 2004b). The initial decrease in hematocrit is caused by an increase in blood volume associated with osmoregulatory adjustments to elevated levels of lipoprotein yolk precursors (Kern et al. 1972). However, hemodilution can not be the only factor involved, because hematocrit does not return to prebreeding levels at clutch completion (figure 2a), even though yolk precursor levels have decreased to zero by this stage (Challenger et al. 2001). Reduced hematocrit can persist in some years through incubation, chick rearing, or both; in European starlings, in one year 20% of chick-rearing birds were clinically anemic (based on hematocrit values more than two standard deviations lower than prebreeding values; Williams et al. 2004b). In quail, 17β-estradiol suppresses bone hematopoiesis (Clermont and Schrar 1979); in mice, it causes atrophy of the thymus (Olsen and Kovacs 1996); and both bone and thymus are important sites for erythropoiesis in adult birds. In quelea (*Quelea quelea*), Kendall and Ward

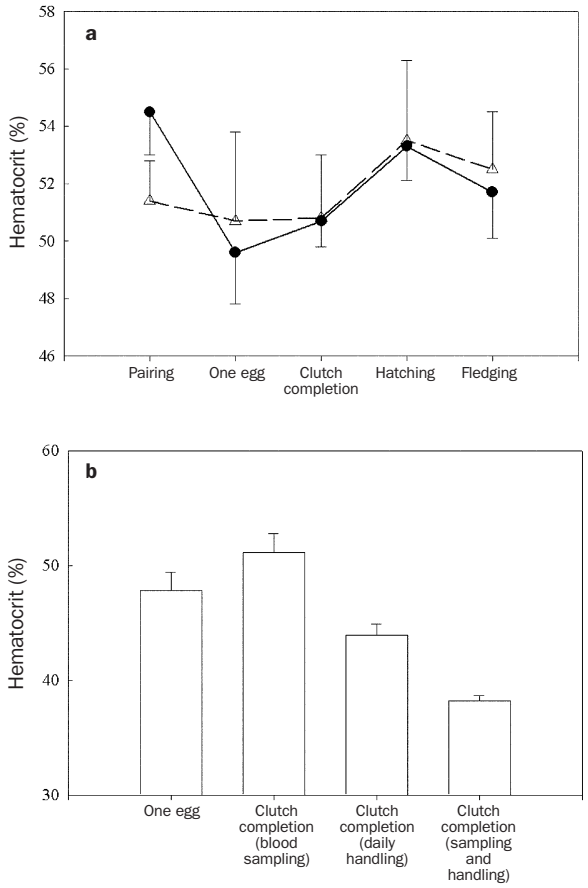

Figure 2. Reduction in hematocrit (red blood cell volume as a percentage of whole blood volume) during egg production. (a) Changes in hematocrit throughout the breeding cycle in female (filled circles) and male (open triangles) zebra finches; (b) hematocrit in laying birds after producing one egg compared with the hematocrit at clutch completion after exposure to additional stressors (blood sampling and daily handling).

(1974) reported an increase in thymus gland size during incubation and chick rearing—that is, after plasma estradiol would have returned to basal levels, which they argued facilitated a recovery of hematocrit through increased erythropoiesis. A prolonged decrease in hematocrit might therefore be due to a direct estradiol-dependent inhibition of red blood cell production (Kern et al. 1972, Williams et al. 2004b). The relatively long life span of avian red blood cells (30–42 days) supports the idea that a transient inhibition of erythropoiesis could induce a prolonged reduction in hematocrit. Females are particularly vulnerable to further decreases in hematocrit when exposed to additional stressors during egg production (figure 2b), and this is associated with a reduction in breeding success. Since hematocrit can affect flight ability and aerobic performance in general (Carpenter 1975, Hammond et al. 2000), decreased hematocrit might provide an explanation for long-term costs of egg production, such as decreased chick-provisioning ability, renesting probability, or survival.

Conclusions

It is clear that birds can and do incur costs of reproduction specifically associated with egg production, although comprehensive studies have so far been limited to a few taxa (seabirds and some passerines). If egg production is indeed costly, it should be possible to identify the components of the physiological system underlying reproduction, or the specific reproductive traits themselves, that explain these costs. This has proved to be surprisingly difficult, primarily because of the marked individual variation that occurs in reproductive and physiological traits (perhaps suggesting that these traits are not costly and that they are not under strong selection). Many studies continue to focus on factors, such as food availability, social environment, and temperature, that actually contribute relatively little to variability in reproductive traits (e.g., less than 10% of variability in egg size) compared with the much greater true variability among individuals (e.g., variability of 60%–100% in egg size; Christians 2002). Although egg and clutch size are, to varying degrees, genetically determined, the mechanism or mechanisms through which genotype determines intraspecific, phenotypic variation are unknown. We need a much better characterization of this intraspecific variability, and descriptive and correlational studies will be informative, although experimental manipulations of physiological mechanisms are really needed (Williams 2000, 2001). The many recent studies on other aspects of egg quality, such as egg composition, egg carotenoid content, and egg hormone content, have simply added more evidence of unexplained phenotypic variation rather than advancing our knowledge of the mechanisms underlying this variation or their associated costs.

Several mechanisms have been proposed for the cost of egg production (e.g., protein depletion and impaired flight muscle function; immunosuppression), but the focus to date has been on resource-based costs. These proposed mechanisms assume that animals have finite resources and that the increased resource demand for egg production reduces the resources available for other functions. It is clear that the energy and nutrient demands of egg production are nonnegligible, but recent empirical data suggest that these demands might actually be lower than earlier theoretical estimates. However, it is important to dissociate the many studies that have investigated energy or nutrient limitations of egg production from the far fewer studies of the actual mechanisms of physiological costs. Few, if any, studies have demonstrated that transient shortfalls in energy or nutrients can have long-term consequences with negative effects on future fecundity and survival (for a rare example in chick-rearing birds, see Daan and colleagues [1996]). Even if females invest more in additional or larger eggs, why can they not simply recover this investment very rapidly during the postlaying period, or at least within the same breeding attempt, by increasing food intake? Similarly, even if laying females are immunosuppressed, they should not incur long-term costs if immune function is restored rapidly at clutch completion. The relatively long time frames over which the costs of reproduction are medi-

ated (weeks to months or years) surely argue against a simple resource-allocation basis for trade-offs. Instead, I suggest that a more productive approach for future research will be to focus on non-resource-based costs of egg production, such as those mediated by the pleiotropic effects of reproductive hormones. These generate fundamental questions with regard to mechanisms, such as why individuals cannot dissociate positive and negative effects of hormones through tissue-specific receptor expression. But I believe that non-resource-based mechanisms (e.g., lipoprotein phenotype, accumulation of oxidative stress, and estrogen-induced anemia) are more consistent with the longer time scales over which biological costs operate. They might also explain why costs can be related to reproduction in an all-or-nothing way—that is, they are related to egg production per se but are independent of the level of variation in fecundity (Ladyman et al. 2003). Finally, there has been something of a disconnect between the studies of costs of reproduction in birds and those in other taxa. This is, in part, because much of this work has been carried out in an ecological or evolutionary context (e.g., by field behavioral ecologists) rather than a genetic, physiological, or molecular biology context. Work on reproduction and life span in model species (e.g., *Caenorhabditis elegans, Drosophila*) is far more mechanistically sophisticated and should inform future studies in nonmodel species, such as free-living birds.

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