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Orchestration of avian reproductive effort: an integration of the ultimate and proximate bases for flexibility in clutch size, incubation behaviour, and yolk androgen deposition

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ABSTRACT

How much effort to expend in any one bout of reproduction is among the most important decisions made by an individual that breeds more than once. According to life-history theory, reproduction is costly, and individuals that invest too much in a given reproductive bout pay with reduced reproductive output in the future. Likewise, investing too little does not maximize reproductive potential. Because reproductive effort relative to output can vary with predictable and unpredictable challenges and opportunities, no single level of reproductive effort maximizes fitness. This leads to the prediction that individuals possessing behavioural mechanisms to buffer challenges and take advantage of opportunities would incur fitness benefits. Here, we review evidence in birds, primarily of altricial species, for the presence of at least two such mechanisms and evidence for and against the seasonal coordination of these mechanisms through seasonal changes in plasma concentrations of the pituitary hormone prolactin. First, the seasonal decline in clutch size of most bird species may partially offset a predictable seasonal decline in the reproductive value of offspring. Second, establishing a developmental sibling-hierarchy among offspring may hedge against unpredictable changes in resource availability and offspring viability or quality, and minimize energy expenditure in raising a brood. The hierarchy may be a product, in part, of the timing of incubation onset relative to clutch completion and the rate of yolk androgen deposition during the laying cycle. Because clutch size should influence the effects of both these traits on the developmental hierarchy, we predicted and describe evidence in some species that females adjust the timing of incubation onset and rate of yolk androgen deposition to match clutch size. Studies on domesticated precocial species reveal an inhibitory effect of the pituitary hormone prolactin on egg laying, suggesting a possible hormonal basis for the regulation of clutch size. Studies on the American kestrel (*Falco sparverius*) and other species suggest that the seasonal increase in plasma concentrations of prolactin may regulate both a seasonal advance in the timing of incubation onset and a seasonal increase in the rate of yolk androgen deposition. These observations, together with strong conceptual arguments published previously, raise the possibility that a single hormone, prolactin, functions as the basis of a common mechanism for the seasonal adjustment of reproductive effort. However, a role for prolactin in regulating clutch size in any species is not firmly established, and evidence from some species indicates that clutch size may not be coupled to the timing of incubation onset and rate of yolk androgen deposition. A dissociation between the regulation of clutch size and the regulation of incubation onset and yolk androgen deposition may enable an independent response to the predictable and unpredictable challenges and opportunities faced during reproduction.

Key words: clutch size, egg laying, hatching asynchrony, life-history traits, maternal androgens, maternal effects, parental care, prolactin, seasonal breeding, yolk androgens.

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I. INTRODUCTION

For an individual that breeds more than once, few of its decisions are as important from a fitness perspective as those related to reproductive effort. Reproductive effort is proportional to the parental energy invested in reproduction, and it is determined for a single reproductive bout by the quantity of offspring raised to independence. Over half a century ago, David Lack (1947) argued that, in a single bout of reproduction, parents should produce the maximum number of offspring for which they can provide sufficient resources. Nearly 20 years later, George Williams (1966*a*, *b*) explained how variation in reproductive effort might influence fitness (see also Stearns, 1992) by combining Lack's ideas with one of the central tenets of life-history theory, that current reproduction comes at a cost of future reproduction (Fisher, 1930). The more an individual invests in any single bout of reproduction – that is, the greater its reproductive effort – the less it can invest in future bouts (Ricklefs, 1977; Drent & Daan, 1980; Reznick, 1985; Deerenberg *et al.*, 1995; Daan, Deerenberg & Dijkstra, 1996*a*; Monaghan & Nager, 1997; Stevenson & Wilson, 2001; Visser & Lessells, 2001; Ardia, Schat & Winkler, 2003; Barnes & Partridge, 2003; Hõrak, 2003). Therefore, an individual should not necessarily produce the maximum quantity of offspring it can raise in a single bout of reproduction (Lack, 1947). Rather, it should pace itself relative to its probable reproductive lifespan in order to maximize its future fecundity, longevity, and lifetime reproductive success (Williams, 1966*b*).

The physiological mechanisms for the cost of reproduction and how variation in reproductive effort leads to variation in its cost are not well understood (Williams, 2005). However, recent studies have advanced the idea that accumulation of free-radicals from oxidative metabolism may, in part, mediate the cost of reproduction in some species. Reproduction elevates both basal and field metabolic rates, and these increases may give rise to an elevation in oxidative stress through increased accumulation of DNA-, lipid-, and protein-damaging free-radicals that can cause senescence and impair reproductive competence (Alonso-Alvarez *et al.*, 2004; Wiersma *et al.*, 2004). As reproductive effort increases, one would expect increases in oxidative stress and the damaging effects of free-radicals on reproductive competence.

Of the myriad variations in behavioural and physiological phenomena that occur as a result of reproduction, which among them affects energy expenditure enough to affect fitness costs and therefore influences reproductive effort? What does our current understanding of these phenomena tell us about their physiological regulation, and what, in turn, does our understanding of the mechanisms inform us about the evolution of this putative suite of traits that, when regulated together, influence reproductive effort and therefore the life-history trade-off between current and future reproduction?

In this review we integrate the ultimate, adaptive bases with the proximate, mechanistic bases for variation in reproductive effort in birds, particularly altricial species. In doing so, we focus on the multiple strategies available to female birds which may bestow upon them the reproductive flexibility necessary to cope with both the predictable and the unpredictable challenges and opportunities they may face in any given reproductive bout so that their lifetime reproductive success may be maximized. We initially describe major determinants of variation in reproductive effort, that is, the multiple physiological and behavioural traits in female birds that affect the quantity of offspring raised to independence and hence the trade-off between current and future reproductive success. These determinants include clutch size (Williams, 1966*b*) and the establishment of a sibling developmental hierarchy through the early onset of incubation (Lack, 1954; Magrath, 1990; Stoleson & Beissinger, 1995; Stenning, 1996) and potentially the differential deposition of maternally derived yolk androgens (Schwabl, 1993, 1996*b*; Sockman & Schwabl, 2000). Probably contributing to variation in reproductive effort, as well, are variation in egg size (Williams, 1994; Wiebe & Bortolotti, 1995*a*, 1996; Williams, Reed & Walzem, 2001) and brood sex ratio (Dijkstra, Daan & Buker, 1990; Drummond *et al.*, 1991; Wiebe & Bortolotti, 1992; Daan, Dijkstra & Weissing, 1996*b*; Nishiumi *et al.*, 1996; Greenwood, 1997; Nager *et al.*, 1999; Korpimäki *et al.*, 2000; Aparicio & Cordero, 2001), in species in which the cost of producing one sex differs from that of producing the other (Trivers & Willard, 1973). Moreover, the interaction of some of these variables and their relative differences across eggs within a clutch are likely to influence reproductive effort, as well; a male egg may exact different effects on reproductive effort than a female egg in the first but not the second, third, and fourth eggs of a clutch (Badyaev, Hill & Beck, 2003*b*; Blanco *et al.*, 2003; Young & Badyaev, 2004). Importantly, the variation in energy expended as a result of variation in these behavioural and physiological traits may not manifest when the behavioural and physiological phenomena occur but rather several days or weeks later when their effects take hold. For example, as we discuss in Section II.4.b, the acts of making and depositing yolk androgens may not appreciably alter energy expenditure at the time they occur (Eising *et al.*, 2003) but instead may have profound effects on the energetics of post-hatching parental care due to the potential effects of these yolk androgens on growth and survival rates of nestlings (Schwabl, 1996*b*; Sockman & Schwabl, 2000; Eising *et al.*, 2001). Although variation in clutch size probably does result in variation in the energetic costs of egg production and laying (Williams, 2005), the more significant energetic variation resulting from clutch-size variation, in most species, may be a product of feeding nestlings. With all other factors equal, five nestlings hatching from five eggs will require more energy to feed than four nestlings hatching from four eggs.

Some of these components (e.g. yolk androgen deposition, brood sex ratio) have spawned entire fields that, to varying extents, focus on the one component in relative isolation. However, a goal of this review is to articulate the interconnectedness of these components (clutch size, timing of incubation onset, yolk androgen deposition, *etc.*), their dependence on one another, and the need to investigate them not as isolated phenomena but rather as an integrated suite of behavioural and physiological traits that may serve some common endpoint, namely the optimization of reproductive effort in a given bout of reproduction.

In making this case for an integrative approach, we then discuss how variation in these traits may be orchestrated in the individual. To that end, we describe the evidence for and against a possible physiological mechanism that regulates these traits as a suite, a mechanism that some evidence suggests may be based, at least in part, on the functionality of a single pituitary hormone, prolactin. The evidence related to prolactin's potential role is based on work we have conducted on free-living and laboratory populations of the American kestrel (*Falco sparverius*) and on work conducted by others on American and European kestrels (*Falco tinnunculus*) and on a diversity of other bird species, from Galliformes to gulls and songbirds. We illustrate how an understanding of the ultimate bases for variation in the components of reproductive effort can shed light on their proximate mechanistic bases and how an understanding of the proximate mechanisms can contribute to our knowledge of the evolution of these life-history traits and potential for trade-offs.

The effects of sibling developmental hierarchies on mediating both sibling competition and the energetics of brood rearing is one of our foci and is primarily relevant to the altricial developmental mode, which characterises the vast majority of bird species. Nonetheless, many of our considerations apply to varying extents across a diversity of taxa, including precocial bird species and non-avian taxa. For example, another focus of this review, brood size, is probably the most important determinant of reproductive effort in most animal species in which brood size can vary, regardless of developmental mode. Many organisms are likely to adjust brood size according to environmental conditions and the changing reproductive value of the offspring (Bernardo, 1996; McInroy, Brousmiche & Wynne-Edwards, 2000). A question central to life-history theory is how individuals do this, be it through control of the number of ovulated follicles (clutch size in birds) or through secondary adjustment (brood reduction in altricial birds, selective implantation failure or abortion in mammals, *etc.*). As we will demonstrate, brood-size adjustment may be facilitated in female altricial birds by at least two mechanisms in addition to the adjustment of clutch size. These mechanisms are based on the timing of incubation onset and the

deposition rate of yolk androgens. Both mechanisms represent forms of differential offspring investment that result in mothers favouring or handicapping some offspring, concepts which readily apply to many taxa. For example, exposure to maternal androgens varies among sibling rodents (Ryan & Vandenbergh, 2002) and reptiles (Bowden *et al.*, 2001; Lovern & Wade, 2001, 2003*a*, *b*; Bowden *et al.*, 2002), and these androgens may selectively confer advantages or handicaps to some offspring relative to others. In addition, the notion that multiple determinants of reproductive effort may be under the regulatory control of prolactin secretion is not limited to birds. The role prolactin plays in various aspects of reproduction that contribute to reproductive effort, such as milk production, parental care, brooding, and anti-gonadal effects, are well known in a diversity of vertebrates, from mammals to fishes (Clarke & Bern, 1980).

Many unknowns and inconsistencies remain regarding the ultimate and proximate bases for adaptive flexibility in reproductive effort, some of which we will also discuss. In addition, we limit our review to reproductive effort in females, because they determine much of the effort put into a single bout of reproduction, though we recognise a need to understand better the ultimate and proximate control of the various components of male reproductive effort, such as mate-attraction (Sockman *et al.*, 2005*a*) and paternal behaviour (Sockman, 1998; Wynne-Edwards & Reburn, 2000; Sockman, Schwabl & Sharp, 2004*a*).

II. DETERMINANTS OF REPRODUCTIVE EFFORT

(1) Number of offspring *versus* quantity of offspring

Reproductive effort is often estimated as the number of propagules initially produced in a single bout of reproduction, as in the *number* of eggs in an individual bird's clutch (i.e. the clutch size) (Williams, 1966*b*). For some organisms this is probably an accurate estimation. But for most, this proxy of reproductive effort is oversimplified and imprecise. If female control over reproductive effort were limited to the number of propagules she initially produced, she would have little ability to make fine adjustments in effort. For example, in a single-brooded passerine bird that lays a modal clutch size of four eggs, initial adjustments in reproductive effort, if solely determined by the number of eggs laid, would be available in increments of only 25 % (one egg of a four-egg clutch). But in terms of factors that influence reproductive effort, there is substantial evidence now that females may modulate much more than simply the number of propagules, and these factors likely influence female energy expenditure either directly or through indirect effects on such variables as offspring feeding effort (see Section II.4). The continuously variable size of an egg is an example of one such factor; a four-egg clutch of larger eggs could require greater reproductive effort than a five-egg clutch of smaller eggs not only because large eggs constitute larger investments in yolk and other egg constituents but also because large eggs may give rise to large young that require more food (Williams, 1994). As such, restricting analyses of reproductive effort to the *number* of offspring fails to capture significant sources of variation in reproductive effort, in that parentally dependent offspring differ from one another, either innately or as a result of differences in parentally provided resources. These differences among dependent offspring may be associated with differences in parental reproductive effort. Hence, in keeping with the concepts that reproductive effort reflects the proportion of available resources directed towards reproduction (Clutton-Brock, 1984; Reznick, 1985) or the level of commitment to reproduction (Williams, 1966*b*; Ricklefs, 1977), we define the determinant of reproductive effort as the *quantity* of offspring raised to independence in a single bout of reproduction. Below we distinguish this definition from one characterised by the *number* of offspring either initially produced (e.g. a clutch of eggs) or raised to independence.

The term "quantity" reflects not only the number of propagules, but also variation among individuals in their size or other traits that may affect production expenses. When all other factors are equal, a larger offspring is quantitatively "more offspring" and therefore more expensive to raise to independence than a smaller offspring. This is not to say that larger offspring are necessarily more valuable but simply that they generally require more reproductive effort. *Quantity* reflects the continuum of variation among individual offspring and the continuum of within- and between-parent variation in reproductive effort; it is the quantity, not number, of offspring raised to independence that most accurately reflects resource use and applies to the life-history trade-off between current and future reproductive output. For example, parents may adjust their investment in individual offspring as a function of the energetic requirements of the offspring (Whittingham & Robertson, 1993; Wiebe & Bortolotti, 1994*a*; Anderson, Reeve & Bird, 1997; Smiseth, Amundsen & Hansen, 1998). Indeed, under a given set of environmental conditions, raising three large offspring may require greater parental effort and therefore have a larger impact on future reproductive potential than raising four small offspring. But to conclude that reproductive effort measured solely as the number of offspring would be lower for the latter than for the former brood would be incorrect. In species exhibiting sexual size dimorphism, the energetic requirements of offspring may be greater for males than for females, and, therefore, the cost or effort to produce the larger male would be more than that to produce the smaller female.

Thus, the quantification of reproductive effort should subsume some measure more precise and informative than the number of propagules initially produced. The more quantitative measure of reproductive effort, offspring *quantity*, takes into account not just the number of offspring but also factors that account for their size and other attributes up to the point of independence that more accurately reflect the fitness cost of producing and raising them.

(2) Indirect and direct costs of reproduction

Although much of the discussion above centres on the costs of rearing offspring in the sense of feeding them, depending

on the species other costs likely factor in as well. For example, a female bird can incur a cost of reproduction not only from feeding young but also from forming (Williams, 2005) and incubating eggs, and these costs have been shown in some cases to affect future reproductive output (Moreno & Sanz, 1994; Gloutney, West & Clark, 1996; Monaghan & Nager, 1997; Monaghan, Nager & Houston, 1998; Thomson, Monaghan & Furness, 1998; Stevenson & Wilson, 2001; Reid, Monaghan & Nager, 2002). Indeed, costs of reproduction can be direct and indirect and are often products of complex interactions between number, type, and size of offspring and the relative distribution of offspring type or size within a brood.

(3) Clutch size

Notwithstanding the presence of a continuum of variation in reproductive effort, probably the most important determinant of reproductive effort in birds is clutch size (Williams, 1966*b*). Clutch size establishes the maximum number of offspring produced and therefore sets the upper limit for parental effort in terms of the number of eggs laid and incubated and the number of young brooded and fed in a single bout of reproduction (Thomson *et al.*, 1998). Clutch size is an important determinant of reproductive effort in all bird species, precocial or altricial, that lay eggs in bouts, including obligate brood parasites (Hauber & Dearborn, 2003) and some communal nesters which may not lay all their eggs in one nest (Vehrencamp, 2000). However, other components of reproductive effort, for example those related to parental care, may be less applicable to some of these latter, less typical species.

(*a*) *The role of offspring reproductive value and the cost of reproduction in the seasonal decline in clutch size*

In their seminal work in the 1980s and 1990s on the European kestrel, Serge Daan and co-workers (Beukeboom *et al.*, 1988; Daan *et al.*, 1988, 1996*a*; Daan, Dijkstra & Tinbergen, 1990) described and found strong empirical support for a theoretical framework for one of the most ubiquitous phenomena among birds, the seasonal decline in clutch size. In the vast majority of single-brooded species, females lay more eggs when they initiate clutches early in the season than when they initiate clutches late in the season (Klomp, 1970). The American kestrel is no exception (Bird & Laguë, 1982; Sockman & Schwabl, 2001*a*). Data collected on wild, free-living American kestrels over three breeding seasons revealed that females initiating clutches early in the season tend to lay five- or six-egg clutches, whereas those initiating clutches late in the season tend to lay four-eggs or fewer (Sockman & Schwabl, 2001*a*). According to the work of Daan and colleagues, the ultimate basis for this phenomenon is related to seasonal changes in forage yield (and therefore the number of young which can be raised), seasonal changes in the reproductive value (i.e. the potential for future reproduction) of the eggs, and the relationship between clutch size and the reproductive value of the parents and clutch (Fig. 1). Regardless of season, the reproductive value of the clutch increases with its size;

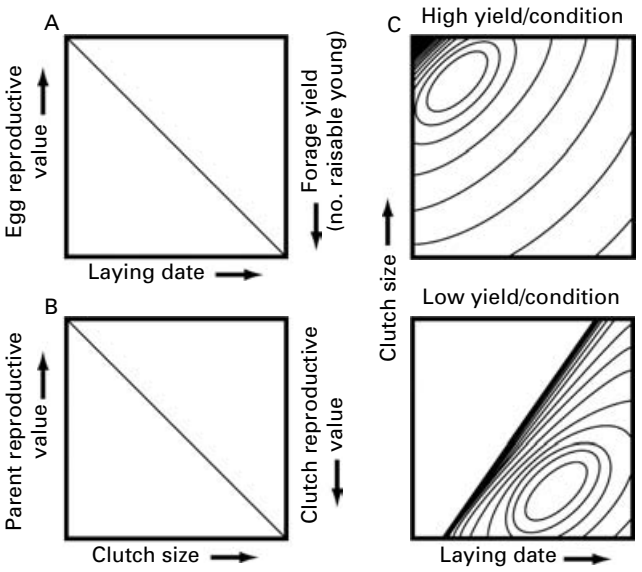

Fig. 1. Hypothesis for the seasonal decline in clutch size ultimately based on the effects of clutch size on the cost of reproduction and on the seasonal decline in offspring reproductive value. Arrows indicate direction of increasing axis values. (A) The relationship between laying date and reproductive value of an individual egg or offspring and between laying date and forage yields, which influences the number of young which can be raised. (B) Clutch size *versus* reproductive value of the parent and clutch. (C) Fitness contours, reflecting the sum of the clutch and parent reproductive values, empirically determined from data collected on wild European kestrels (Daan *et al.*, 1990). Fitness varies depending on the combination of clutch size and laying date. When forage yields and parental condition are relatively high, fitness is maximized (inner contour) by laying relatively early and large clutches. When forage yields and parental condition are relatively low, fitness is maximized by laying relatively late and small clutches. Modified from Daan *et al.* (1990) with permission.

the more eggs laid the more potential for future reproduction by the individuals hatching from those eggs and eventually reproducing themselves. However, the reproductive value of the parent producing that clutch of eggs declines as the clutch size increases. The basis for this ties to the central component of life-history theory described above. The more an individual invests in any given bout of reproduction, the less that individual will be able to invest in future bouts (Fig. 1B).

As the breeding season progresses, forage yields increase due to a general increase in the food supply (Fig. 1A). Therefore, raising a given sized clutch early in the season requires more energy and therefore a better-condition female than raising the same-sized clutch later in the season. Also as the season progresses, the reproductive value of an individual egg declines, because individuals hatching from eggs laid late in the breeding season are less likely to survive and reproduce themselves in subsequent seasons (Daan *et al.*, 1988). Thus, although a late-season clutch of a given size is easier to rear than an early-season one, each of its eggs is worth less and therefore exerts a different contribution to

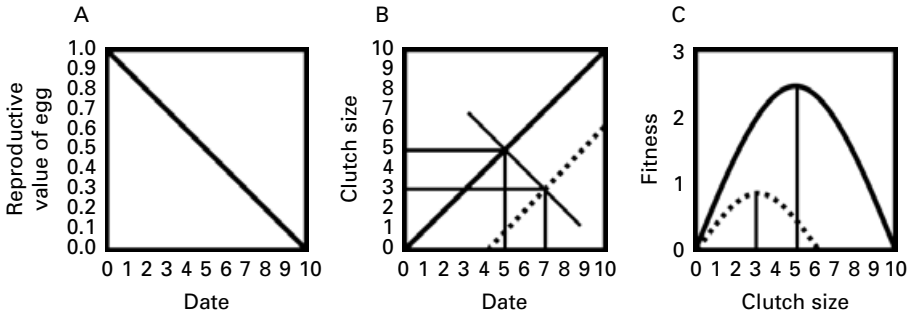

Fig. 2. Hypothesis for the seasonal decline in clutch size ultimately based on the seasonal decline in the reproductive value of the eggs and on variation in female quality or condition. (A) The reproductive value of an egg declines seasonally because the probability of offspring recruitment declines with hatch date. (B) To amass condition necessary to lay a given clutch size requires time, the more of which is available, the greater the clutch size possible. At a given date, females of higher quality or in better condition (thick solid line) lay more eggs than those of lower quality or in worse condition (broken line). (C) The product of the reproductive value of a single egg and the number of eggs yields a fitness curve maximized by some intermediate clutch size. The clutch size which maximizes fitness is smaller for a late-breeding (broken line) than for an early breeding (solid line) female, giving rise to a seasonal decline in the optimal clutch size (thin, black, diagonal line in B). Modified from Rowe *et al.* (1994) with permission.

the trade-off between reproductive effort and value of the parent than does an early-season clutch. Daan *et al.* (1990) empirically determined the fitness (combined reproductive value of a clutch and parent) of a continuum of clutch-size/laying date combinations using data from the European kestrel and found that the worse the energy or condition circumstance, the more the optimal solution shifts towards later laying and fewer eggs, culminating in a total lack of breeding under very poor energetic circumstances (Fig. 1C). Therefore, in a population of individuals which vary in quality, one will expect to find early breeders laying more eggs and late breeders laying fewer eggs (Klomp, 1970).

In multi-brooded species such as the mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*), sizes of clutches initiated mid-season are often larger than both late-season and the very earliest clutches (Morton, 2002). Ultimately, this may be a consequence of the fact that the earliest nesters are the most likely to produce multiple broods within a season and would therefore need to temper their early-season effort by laying fewer eggs than a single-brooded female might somewhat later in the season. For example, an early-laying, multi-brooded female may lay a four-egg clutch on 1 April and then a two-egg clutch on 1 June. A relatively early-laying, single-brooded female may lay a five-egg clutch on 1 May, and a late-laying, single-brooded female may lay a three-egg clutch on 1 June. Although the earliest layer does not lay as many eggs in her first nest as one somewhat later, her total egg-output for the year is greater than the other females, owing to additional reproductive effort in her second brood. In this case, perhaps it is not clutch size that necessarily declines with season but rather the yearly total of eggs that declines with the date at which reproductive competence for the season first emerges.

Daan and colleagues found general agreement between their model for the ultimate control of the seasonal decline in clutch size in birds and data collected over several years on their free-living study population of European kestrels (Daan *et al.*, 1990). Still, the applicability of this model to other species likely varies and some have found only limited support for some aspects of the model, even in the European kestrel (Aparicio, 1994; Korpimäki & Wiehn, 1998). However, unlike the studies of Daan and co-workers, these latter studies lack data on some of the model's essential elements and therefore cannot be considered complete tests of the model.

(*b*) *The role of offspring reproductive value and female quality in the seasonal decline in clutch size*

Although clutch size is a major determinant of a female bird's reproductive effort and probably affects the life-history trade-off between current and future reproduction, costs in terms of future reproduction may not be necessary to explain the seasonal decline in clutch size. Rowe, Ludwig & Schluter (1994) have provided a theoretical framework for the seasonal decline in clutch size that does not rely on the cost of reproduction as an explanatory factor. Their model suggests that a seasonal decline in the reproductive value of the offspring combined with individual variation in female condition is sufficient to drive the seasonal decline in clutch size (Fig. 2). This model assumes first a positive relationship between clutch size and the body or energetic condition necessary to produce that clutch. Second, it assumes that individuals vary in the time of the season they reach the level of body or energetic condition necessary to produce a clutch of eggs. Individuals that gain condition relatively rapidly, perhaps because they are high-quality individuals that possess high-quality territories with plentiful food resources or because they breed in a bountiful year, more rapidly reach the condition threshold for producing a clutch. Similarly, individuals that begin the breeding season in relatively good condition because, for example, they are high-quality individuals or because they experienced plentiful food conditions on wintering grounds and faced favourable migration conditions, would reach the condition threshold first and therefore are those which initiate laying relatively early in the breeding season. Those that reach the threshold

later lay later in the season, and these are presumably individuals of lower quality, on lower quality territories, or experiencing a lean year in terms of food availability. Their model also assumes that the probability of offspring survival and recruitment as breeders in subsequent years (i.e. offspring reproductive value) is negatively correlated with hatch date, an assumption with considerable support from studies on multiple species (Perrins, 1965; Daan *et al.*, 1990; Morton, 1992; Dzus & Clark, 1998; Visser & Verboven, 1999; Morton *et al.*, 2004; Smith, 2004). Finally, the model assumes that production of large clutches requires greater condition than production of small ones and that females that build better condition by delaying laying can lay more eggs.

The combination of how these components of the model fit together – within- and between-individual variation in time to reach threshold condition, additional time amassing condition enabling larger clutch sizes, and the seasonal decline in the reproductive value of the eggs – determines the optimal clutch size-laying date combination. Early in the season, the reproductive value of each egg in a clutch is high and therefore the probability of an individual offspring surviving is high. Every additional day a female uses to build condition and lay more eggs is a day unavailable for offspring to build their own condition prior to the end of the breeding season (e.g. Morton, 1992). Thus, a delay in laying enables the female to lay more eggs but simultaneously reduces the survival probability of the offspring. At a particular time point, the rate of seasonal decline in offspring value exceeds the rate of seasonal increase in clutch size due to amassing condition. At that point, it is no longer worth the cost of lost offspring value to gain another egg.

As an example, consider a high-quality female that may amass enough condition early in the season to lay six eggs, each with a future reproductive probability of 0.50. This female could wait, however, and amass more reserves over additional days that enable her to produce a clutch of eight eggs. However, due to the seasonal decline in the reproductive value of the offspring, each of these eggs might therefore have a reproductive probability of 0.25. With the early six-egg clutch, on average she would have produced three reproducing young. But with the late eight-egg clutch, on average she produces two reproducing young. Hence, by waiting so that she could lay more eggs, this female reduced her probable fitness.

Similarly, a female on a poor territory may not be able to reach threshold until mid-season, at which point her condition enables her to produce four eggs, each with a reproductive value of 0.25. If she delayed laying to amass more condition and therefore produce six eggs, the delay would lead to reduced reproductive value of those eggs, each with a reproductive probability of 0.10, for example. Hence, the mid-season four-egg clutch will likely yield 1 reproducing offspring, whereas the late-season six-egg clutch will likely yield none.

When you apply these phenomena predictions to a population of females that vary in territory quality and hence condition, the result should be a population in which high-condition females lay early, large clutches and low-condition females lay late, small clutches. Were any of the females to have waited longer to amass the condition necessary for a larger clutch, they would have reduced their fitness due to the rapid seasonal decline in the reproductive value of the egg (e.g. Smith, 2004).

(*c*) *Concluding remarks on clutch size, its seasonal variation and the reproductive value of eggs*

The model of Rowe *et al.* (1994) was tested experimentally in snow geese (*Chen caerulescens atlantica*) and the validity of the model largely confirmed (Bêty, Gauthier & Giroux, 2003). However, amassing body condition may not be a prerequisite for laying in some species such as small songbirds (Williams, 1996, 1999*b*). Therefore, the generality of Rowe's model to all species needs further evaluation, and a number of investigators have proposed other ultimate explanations for the seasonal decline in clutch size.

One of these additional, ultimate explanations for the seasonal decline in clutch size relates to thermal factors during egg laying and incubation that influence egg viability and the energetics of incubation (Cooper *et al.*, 2005). According to the "clutch cooling hypothesis" of Reid and co-workers (Reid, Monaghan & Ruxton, 2000; Reid *et al.*, 2002), large clutches retain heat longer than small ones and therefore reduce the energetic costs of incubation more in cool (i.e. early season) than in warm conditions. Although large clutches require more incubation effort to warm and to maintain their temperature during on-bouts of incubation than do small clutches, large clutches cool much more slowly during off-bouts than do small clutches (Vleck & Hoyt, 1980; Reid *et al.*, 2002; reviewed in Cooper *et al.*, 2005) and therefore depart the optimal temperature range for embryonic development much more slowly. Small clutches in cool conditions would therefore require greater incubation attentiveness for a given rate of embryonic development and could cut into foraging time of females. The greater thermal inertia of large clutches would therefore favour them in the cool ambient temperature conditions found early in the season.

With their "egg viability hypothesis," Stoleson & Beissinger (1999) proposed a complementary explanation for the seasonal decline in clutch size. Under warm conditions, eggs laid early in the sequence of a large clutch before the onset of incubation may be exposed to temperatures between 26 and 36 °C, which can desynchronize tissue growth (Webb, 1987; Deeming & Ferguson, 1991) or subject the embryo to microbial infections (Cook *et al.*, 2003, 2005), both of which can induce developmental abnormalities or impair egg viability. Eggs remain viable for long periods at temperatures below 26 °C, which is why large clutches in cooler conditions are less vulnerable. In warm conditions, females might protect eggs laid early in the sequence of a large clutch by initiating incubation early, but early onset of incubation relative to clutch completion likely incurs its own costs by limiting the time she would otherwise have to forage during the potentially energetically demanding egg-production phase and by inducing suboptimally asynchronous hatching patterns (See Section II.4). Thus, the warmer conditions of the late season would favour smaller clutches, because eggs laid early in the sequence of a

small clutch would incur less exposure to suboptimal temperatures than would those of a large clutch (Cooper *et al.*, 2005).

The possibility that thermal constraints on egg viability might select for a seasonal decline in clutch size has some empirical support (Cooper *et al.*, 2005). It is also appealing because it may explain an additional phenomenon among birds, the latitudinal increase in clutch size (Lack, 1968; Klomp, 1970; Ricklefs, 2000; Cooper *et al.*, 2005). Indeed, seasonal changes in ambient temperatures tend to mirror latitudinal changes, and thus the notion of a thermal constraint on clutch size may be applicable to both the seasonal and latitudinal axes of clutch size variation. However, one of the limitations of thermal constraints as a universal predictor of clutch size variation is its apparent inability to explain the situation in double-brooded species, in which often the very earliest clutches of the season are smaller than those laid somewhat later (e.g. Morton, 2002).

Nonetheless, despite questions concerning the accuracy of the models of Daan *et al.* (1990), Rowe *et al.* (1994), or Cooper *et al.* (2005), two well-supported phenomena emerge from this discussion. First, clutch size declines seasonally in many bird species (Klomp, 1970). Second, although a cost of reproduction paid in future reproductive success may not be a component of the seasonal decline in clutch size, this cost likely does influence clutch size irrespective of its seasonal decline (Deerenberg *et al.*, 1995; Daan *et al.*, 1996*a*). In other words, good-condition females may lay, for example, six-egg clutches early in the season and poor-condition females may lay three-egg clutches late in the season. But if these reproductive bouts were not particularly costly in terms of future reproduction, the clutch sizes for each of these females may be, respectively, seven and four eggs, still showing a seasonal decline. Moreover, numerous factors in addition to season account for variation in clutch size, including genotype and environmental factors related to resource availability (Gwinner, König & Haley, 1995). Still, for a large proportion of species, season appears to account for much of the variation in clutch size. Therefore, one of the primary axes of variation in reproductive effort is season, which gives rise to the decline in the reproductive value of offspring. By reducing clutch size seasonally, females of at least some species cope, in part, with this predictable reproductive challenge.

(4) Sibling developmental hierarchy

Like many organisms, female birds face a variety of unpredictable reproductive situations, some of which are challenges and others opportunities. Any one of these unpredictable situations could lead a female to under- or overestimate optimal reproductive effort. Either too many offspring in an unexpectedly lean year or too few offspring in an unexpectedly propitious year may exact costs on female fitness.

Once the clutch is complete, the machinery involved in egg production, including the oviduct and ovaries, regresses (Etches, 1996; Christians & Williams, 1999*b*; Williams & Ames, 2004), perhaps at a time when little information is available to that female regarding resource availability or offspring viability days later during incubation or weeks later during nestling or fledgling development. Therefore, should a female unexpectedly experience a surge in resource availability or the partial loss of her clutch (due to egg infertility or a congenital defect, for example), she cannot compensate by supplementing her initial investment in the clutch with another egg, at least not until the first brood is partially reared (for some species). Even if a female tracking improving ecological conditions were physiologically capable of adding eggs to her clutch after an initial bout of laying, it would make little sense from an ultimate perspective for her to do so (Forbes & Mock, 1996), because the young from those additional eggs would be at a very different stage of development and would not likely survive in the presence of siblings that may be as much as 10 times their mass. Or, worse, she may be faced with simultaneously having to incubate and care for fledglings. This uncertainty, both in offspring viability and resource availability, has led some to suggest that a female should produce an oversized, optimistic clutch to enable her to take advantage of an unexpected windfall in resources (Lack, 1947, 1948, 1954; Pijanowski, 1992) or as insurance against the partial loss of her clutch (Forbes & Lamey, 1996; Forbes *et al.*, 1997; Forbes & Mock, 1998; Forbes, Grosshans & Glassey, 2002). With an optimistically sized clutch, she would maximize her fitness in those cases in which the resources required for those extra young are present or when one young is of poor viability or quality.

However, overproduction can have negative consequences just as underproduction can. In altricial species, a female enjoying a bounty of food during egg production may face limited food in the coming weeks when energy is most needed to raise nestlings and fledglings (Walsberg, 1983). Limited food during offspring development, due to overproduction of offspring or due to an optimistic strategy in reproductive effort, can lead to competition among offspring which, from the parent's perspective, may not only waste the energy that is in short supply but also lead to offspring of poor condition (Ricklefs, 1983; O'Conner, 1984). Because, in most species, condition affects male more than female fitness (Trivers & Willard, 1973), overproduction of male offspring in poor condition is particularly undesirable from the perspective of optimizing reproductive effort. In situations of offspring overproduction, the parents or their offspring would likely expend considerably more effort than they would otherwise with a smaller brood (Emms & Verbeek, 1991; Conrad & Robertson, 1993; Deerenberg *et al.*, 1995; Wiehn & Korpimäki, 1997; Dawson & Bortolotti, 2003).

The unpredictability of viable offspring and of resource shortages might select for a mechanism that would enable the secondary reduction of brood size, in particular by the culling of poor-condition offspring, once resource shortages and offspring viability become realized or once they exert their effects (Forbes *et al.*, 2001). This leads to the question of how a female should cull the brood most efficiently and which offspring should be removed. The female could consistently feed some offspring but not others until one dies. However, because food restriction is unlikely to take effect instantly, the efficiency of this strategy would depend on the

female's ability to discriminate between offspring or on the presence of a sibling competitive hierarchy in which some nestlings but not others consistently acquire food so that her feeding efforts are consistently focused on a subset of offspring. For example, a female facing limited food availability may benefit by reducing a brood from five to four nestlings. In the absence of an initial ability to discriminate between offspring and recognize one from the other or in the absence of a sibling competitive hierarchy, the ability to reduce a brood by selectively feeding some but not others would depend on drift-like mechanisms. This is because the female's only option would be randomly to feed some offspring but not others until, by chance, she had avoided feeding one individual enough times in a row that it became recognizable by its smaller size. Only then would she consistently be able to neglect it or eliminate it by some other fashion. The presence of a pre-existing hierarchy, however, would greatly increase the efficiency of brood reduction when it is needed, because either competitive asymmetries among young would dictate who got fed or because the female, from the outset, could recognize and therefore avoid feeding one nestling (the runt) (Lack, 1947, 1954; Drummond, González & Osorno, 1986; Forbes, 1993). Alternatively, when the brood is too large for available resources, the female could remove one nestling chosen at random, but this carries the disadvantage of potentially removing a nestling that may be of relatively higher value than another. The hierarchy provides the additional advantage of enabling the female to focus efforts on those nestlings in which she has already invested the most and which may be worth the most.

David Lack (1954, 1968) and numerous subsequent investigators (Magrath, 1990; Ricklefs, 1997; Rabouam, Thibault & Bretagnolle, 1998) have argued that, in birds, the sibling developmental hierarchy, produced in the vast majority of bird species by the asynchronous development and hatching of the eggs within a clutch, enables females to lay an oversized clutch, the size of which could be efficiently adjusted downward when resources are scarce or when all offspring are viable (Forbes *et al.*, 2002). Under limited resources, the developmental hierarchy becomes a competitive hierarchy in which the oldest, most developed offspring are the most competitive and likely to survive (Mock & Forbes, 1995; Mock & Parker, 1997, 1998). The youngest become the least likely to survive, and the brood is efficiently culled to match parental effort more closely with resource availability.

In cases of resource abundance, satiated older siblings may yield to younger, smaller siblings, enabling them to catch up in development and the female to match reproductive effort more closely to these elevated resources. Alternatively, a developmental hierarchy among siblings during periods of resource abundance may still result in brood reduction, with the youngest and least developed nestling failing to survive (Pijanowski, 1992). Indeed, in good resource years, success of all offspring can be greater under synchronous than under asynchronous hatching (Forbes *et al.*, 2002). However, good years may be unpredictable, and this disadvantage to hatching asynchrony in good years can be offset by the advantages during unexpectedly bad years (Pijanowski, 1992; Forbes *et al.*, 2002), when the benefits of the developmental hierarchy take effect.

This explanation for hatching asynchrony and the presence of a sibling developmental hierarchy is an intuitive modification of Lack's brood reduction hypothesis (Lack, 1947; Pijanowski, 1992), but it is insufficient to account for the presence of sibling developmental hierarchies generally in birds. For instance, in species with obligate brood reduction, loss of the developmentally disadvantaged nestling is a virtual certainty, with or without variation in resource availability (Mock, Drummond & Stinson, 1990). Moreover, several field studies have yielded equivocal support for Lack's brood reduction hypothesis and have prompted a call for alternative explanations for sibling developmental hierarchies (Mead & Morton, 1985; Magrath, 1990; Stoleson & Beissinger, 1995; Stenning, 1996). Forbes *et al.* (2002) have addressed these concerns, with a long-term examination of this complex phenomenon, and have proposed a multifaceted basis for the functional significance of hatching asynchrony. In short, they suggest that the advantages of hatching asynchrony may arise under both resource and developmental uncertainty, the latter of which is based on the notion that some offspring may be unviable, suffer from congenital defects, or be of unexpectedly low quality for other reasons. In the situation of their loss, competition in the nest is reduced and resources can be directed to the remaining young, which may include the so-called insurance nestlings hatching asynchronously. Should no such defects occur, the brood is efficiently culled to a quantity that presumably benefits the parents from a cost-of-reproduction perspective.

Still other explanations for hatching asynchrony abound (Stenning, 1996). In addition to corollaries of Lack's brood reduction hypothesis (Pijanowski, 1992) and the concept of surplus offspring as insurance against developmental mishaps or the low quality of some offspring (Kozlowski & Stearns, 1989; Forbes & Lamey, 1996; Forbes & Mock, 1998), investigators have proposed several other advantages of hatching asynchrony. Among them include hypotheses related to the energetic efficiency of raising a brood (Hussell, 1972; Hahn, 1981) and the "ice box" hypothesis (Alexander, 1974), in which the developmentally disadvantaged offspring serve as a living larder for their older siblings in the event resources become limited.

In American kestrels, developmental hierarchies among nest mates are ubiquitous and frequently lead to brood reduction (Balgooyen, 1976; Wiebe & Bortolotti, 1995*b*; Sockman & Schwabl, 2000). As in the case for Forbes's work, it appears that the benefits of these hierarchies in American kestrels may arise through different mechanisms, depending on the situation (Wiebe & Bortolotti, 1994*a*, *b*, 1995*b*; Wiebe, 1995, 1996). For example, parent kestrels appear to benefit from sibling developmental hierarchies because these hierarchies lower sibling rivalry and energy waste and because offspring or even parents might survive brief, unpredictable reductions in food availability by cannibalising the developmentally disadvantaged young (Bortolotti, Wiebe & Iko, 1991; Wiebe, 1996). Despite the possibility of multiple advantages of sibling developmental hierarchies, these advantages are all related to one another

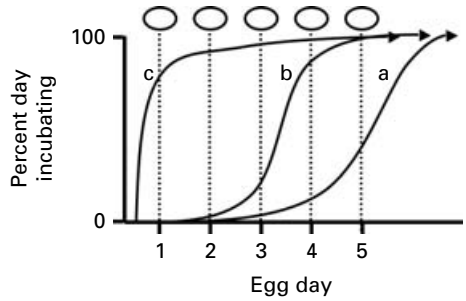

Fig. 3. Hatching asynchrony induced by timing of incubation onset. Array of ovals represents the temporal pattern in which females successively lay each egg of a single clutch. Incubation levels (percent day incubating) are shown by curves a (rise after clutch completion), b (rise during laying) and c (rise at clutch initiation). Egg day is the day on which each successive egg of a clutch is laid.

in that they each may benefit the female by maximizing the reproductive value of her brood relative to her reproductive effort.

(*a*) *Timing of incubation onset and the establishment of a sibling developmental hierarchy*

Developmental and hatching asynchrony is primarily controlled by the timing of incubation onset relative to the completion of the clutch (Clark & Wilson, 1981; Mead & Morton, 1985; Wiebe, Wiehn & Korpimäki, 1998). Therefore, the mechanisms for adaptive modification of reproductive effort through the establishment of a sibling developmental hierarchy would primarily concern the control of incubation onset.

To illustrate how timing of incubation onset controls the degree of hatching asynchrony and hence the sibling developmental hierarchy, consider a hypothetical situation in which eggs are laid on subsequent days until the clutch is complete at five eggs, at which point incubation behaviour increases rapidly to reach full expression (Fig. 3, curve a). Because incubation behaviour is minimal or non-existent over the five days of egg laying, the embryos within those eggs are not developing or at most developing only slightly (Deeming, 2002). Once incubation behaviour increases at the time of clutch completion, embryonic development begins simultaneously for all five embryos. Therefore, all five will hatch roughly synchronously, giving rise to little or no developmental hierarchy and therefore little or no mechanism for efficient brood reduction should resources become limited or should all young prove viable and of high quality. There would be less relief from the energetic expense of raising young with similar competitive abilities.

Alternatively, incubation could begin with clutch initiation. In this hypothetical scenario, the embryo within each egg would begin developing at approximately the time its egg is laid (Fig. 3, curve c), resulting in complete hatching asynchrony. Complete hatching asynchrony is not common in birds, possibly because too much of a hierarchical relationship among siblings would handicap some nestlings so much that it might all but ensure their demise even under conditions when it would not optimize the female's reproductive effort (Cooper *et al.*, 2005).

Typically, incubation begins during egg laying, often around the time the penultimate egg of a clutch is laid (Fig. 3, curve b). In the hypothetical five-egg clutch, this would result in the first four eggs hatching simultaneously and the fifth hatching asynchronously, producing a "runt" offspring that could be efficiently culled in the unexpected event resources became limited or all young proved viable.

Thus, the timing of incubation onset relative to clutch completion is the principle regulator of hatching asynchrony. The earlier the onset of incubation occurs relative to clutch completion, the greater the degree of hatching asynchrony and the greater the developmental hierarchy among the young. This is not to say that the advantages of establishing a sibling developmental hierarchy are necessarily the principal selective forces driving early onset of incubation. Depending on species, there is evidence for other benefits of early incubation onset. One is the notion that early incubation onset shortens the total nest cycle by reducing the time elapsed between laying and fledging, which may reduce the exposure of the nest to predators (Clotfelter & Yasukawa, 1999; Conway & Martin, 2000). In addition, incubation delayed too long after laying may subject eggs to suboptimal temperatures that compromise egg viability by desynchronizing embryonic tissue growth (Webb, 1987; Deeming & Ferguson, 1991) and elevating the risk of infection by microorganisms (Cook *et al.*, 2003, 2005). Whatever forces might select for incubation onset before clutch completion (and there are likely to be many), a preponderance of evidence, detailed above, indicates that the result, hatching asynchrony, can benefit the female by minimizing reproductive effort in unpredictable environments. It is possible, however, that the *degree* of hatching asynchrony, determined by incubation behaviour, may still be suboptimal from the perspective of optimizing the sibling developmental hierarchy. Thus, additional mechanisms for fine-tuning that hierarchy may exist, as explained below (Section II.4.b).

(*b*) *Yolk androgen deposition and its effects on the sibling developmental hierarchy*

(*i*) *Introduction to yolk androgen deposition.* Although timing of incubation onset probably plays a major role, it may not be the only factor influencing the nature of the sibling developmental hierarchy in some species. Several recent studies now suggest an additional means by which a mother bird might modulate the sibling developmental hierarchy and thus her own reproductive effort, and this may augment or mitigate against incubation behaviour in its influence on the hierarchy. Specifically, females deposit androgen hormones into the yolks of their eggs as the yolks are forming on the ovarian follicles (Hackl *et al.*, 2003). Maternal deposition of androgen hormones into egg yolks, first described by Schwabl (1993) for the canary (*Serinus canaria*), is now known to occur in a large diversity of bird species (Gil, 2003). In most of these, androgen concentrations vary reliably and systematically between sibling yolks, typically increasing (e.g. Schwabl, 1993; Sockman & Schwabl, 2000; Eising *et al.*, 2001) but sometimes decreasing (Schwabl, Mock & Gieg,

1997; Gil *et al.*, 1999; Reed & Vleck, 2001) in concentration with respect to the egg's laying order within a clutch (hereafter laying order). These yolk androgens can affect nestling growth and survival (Schwabl, 1993; Lipar & Ketterson, 2000; Sockman & Schwabl, 2000; Eising *et al.*, 2001), and therefore, when they are elevated in concentration, they presumably influence the female's energy investment in the offspring and thus her reproductive effort.

The study of yolk hormone deposition in birds is quite new, and, as a consequence, we know little about the physiological mechanisms regulating this phenomenon. For example, it is not known whether androgens pass into the yolk from surrounding thecal and granulosa cells of the adjacent ovarian tissue by way of passive diffusion into the yolk's lipophilic matrix or by active or receptor-mediated uptake. Further, we know little about what happens to these androgens once they are in the yolk, how they are metabolised by the developing embryo, and what their activational and organisational effects on the offspring are.

Because the concentrations of androgens can vary among sibling yolks, it might seem that some insight into the mechanisms of their deposition and function would come from the classic studies by vom Saal and co-workers, who investigated the effects of intrauterine position on androgen exposure in foetal mammals (vom Saal, 1981*a*, *b*, *c*, 1984; vom Saal *et al.*, 1990, 1999; Clark, vom Saal & Galef, 1992; Nonneman *et al.*, 1992; Zielinski, vom Saal & Vandenbergh, 1992). This series of studies, spanning more than a decade, demonstrated that a male or female foetus flanked by two male foetuses in the uterine horn is exposed to blood concentrations of testosterone approximately 1/3 greater than the same-sex foetus flanked by two female foetuses (vom Saal, 1981*a*). A male or female foetus flanked by two female foetuses in the uterine horn is exposed to blood concentrations of estradiol approximately 1/3 greater than the same-sex foetus flanked by two male foetuses. These differences in steroid exposure during foetal development affect a range of adult traits, such as sensitivity to steroids, aggression, home range size, and brood sex ratio (vom Saal, 1981*c*, 1984; Zielinski & Vandenbergh, 1991; Clark *et al.*, 1992; Nonneman *et al.*, 1992; Zielinski *et al.*, 1992). Moreover, the differences in steroid exposure caused by the sex of the adjacent foetuses can be eliminated by maternal stress, which elevates plasma concentrations of maternal testosterone and therefore foetal testosterone levels in all foetuses, not just those surrounded by two male foetuses (vom Saal *et al.*, 1990; Zielinski, Vandenbergh & Montano, 1991).

Variation between sibling mammalian foetuses in their exposure to androgen hormones would seem highly relevant to the study of yolk androgen deposition in birds and may inform avian biologists of possible effects of this variable exposure. However, there are some major differences between the mammalian and avian systems. For instance, the primary source of this variation in mammals is a product of foetal sex and the fact that sibling foetuses partially share their surrounding steroid milieu. In birds, simultaneously growing sibling follicles adjacent to one another on the ovary also share their surrounding steroid environment. But this sharing occurs well before the formation of a zygote and therefore well before sex determination, which, in domestic chickens (*Gallus gallus domesticus*), occurs at about the time the follicle leaves the shared steroid milieu of the ovary when it is ovulated (Etches, 1996). In birds, the source of variation in sibling steroid exposure is the mother; embryonic steroidogenic tissue has not yet developed at this point. Therefore, the mechanisms that give rise to variation in embryonic androgen exposure are probably quite different between mammals and birds. In addition, because vom Saal *et al.* (1999) found considerable differences between mammalian species in the effects of variation in foetal androgen exposure, it would not be surprising to find significant differences between mammals and birds in these effects.

Despite the gaps in basic knowledge on mechanisms of deposition, mechanisms of embryonic metabolism, and the function of yolk androgens in birds, the very rapid growth in this field has yielded a number of insights. Lipar *et al.* (1999) determined that, immediately after laying, egg yolk in red-winged blackbirds (*Turdus iliacus*) and dark-eyed juncos (*Junco hyemalis*) is not a homogenous blend of maternal steroids. Rather, individual steroids change in concentration with distance from the yolk centre, as predicted by known temporal changes in steroidogenesis in maturing follicles of the domestic chicken. These findings are consistent with a mechanism for yolk androgen deposition based, at least in part, on simple diffusion, and they also raise the possibility that the developing embryo is exposed to multiple concentration gradients that, depending on the hormone, may increase or decrease over the time course of yolk uptake or metabolism.

It is possible, however, that these gradients do not persist long into embryonic development. Two days after injecting peanut oil (a vehicle commonly used for yolk steroid injections) coloured with dye, Navara, Hill & Mendonça (2005) found that the yolks of eastern bluebird (*Sialia sialis*) eggs were homogenous in their incorporation of the dye, suggesting that steroids sharing lipophilic properties similar to the oil would diffuse throughout the yolk in a similar manner. Within individual yolks of American coots (*Fulica americana*) sampled within 24 h of laying, Reed & Vleck (2001) found no concentration gradient in testosterone.

In addition, when and at what rate yolk androgens might be metabolized or sequestrated by the developing embryo is not clear for the majority of species. In Japanese quail (*Coturnix coturnix*), however, 10 days of incubation results in a decrease in yolk androgen concentrations to between 1/5 and 1/10 values at laying (Pilz, Adkins-Regan & Schwabl, 2005). Embryonic metabolism or sequestration of yolk androgens appears to occur in a similar fashion in the American kestrel, for which yolks sampled approximately half-way into the incubation period (14 out of 28 days) did not contain detectable levels of androgens (K. W. Sockman and H. Schwabl, personal observations).

In the American kestrel, androstenedione and testosterone concentrations in recently laid, unincubated eggs are significantly lower in the yolks of first-laid eggs than in the yolks of later-laid eggs within the clutch; that is, concentrations of yolk androgens increase with laying order (Sockman & Schwabl, 2000; Sockman, Schwabl & Sharp, 2001). This pattern of increasing concentrations with laying order is similar to that originally reported in the canary

(Schwabl, 1993) and since reported in a number of species, and it raised the possibility that yolk androgen deposition might augment or ameliorate the ubiquitous sibling developmental hierarchy by exerting differential effects on the growth and survival of sibling embryos and nestlings. To test this idea in American kestrels, Sockman & Schwabl (2000) injected into the first-laid egg of a clutch either a cocktail of androstenedione and testosterone dissolved in the vehicle sesame oil or sesame oil alone as a control. They left all other eggs in the clutch unmanipulated. The investigators took care to ensure that the final concentration of androgens (endogenous plus injected levels) in the yolks of these first-laid eggs with naturally low endogenous concentrations was within the range that occurs naturally in later-laid eggs. This enabled them to reduce greatly the possibility of effects due to embryonic exposure to unnaturally high androgen concentrations. Regardless of treatment level (androgen or vehicle), the injected egg hatched in all nests in which at least one egg (injected or not) hatched (Sockman & Schwabl, 2000), reducing the possibility that manipulations biased the sample of nestlings subsequently analysed for growth and survival. Prior to the study on kestrels, the only study showing effects of experimentally elevated androgen concentrations in the yolks of bird eggs revealed an enhancement of the begging behaviour and growth of laboratory-housed nestling canaries whose parents have *ad libitum* access to food (Schwabl, 1996*b*). So it was surprising to find for wild, free-living kestrels that, in fact, young from eggs with experimentally elevated yolk androgen concentrations grow more slowly, have elevated concentrations of the stress hormone corticosterone, and exhibit higher mortality rates than young from control-injected eggs (Sockman & Schwabl, 2000, 2001*b*).

Because the effect of yolk androgens on offspring growth and survival was inhibitory in this study on the American kestrel, the pattern of yolk androgen deposition increasing with laying order seems, in this case, to augment the developmental hierarchy already established by asynchronous onset of incubation and provide even more capacity by the female to cull the brood in the unexpected event that food resources become limited or that all offspring prove viable (Forbes & Lamey, 1996; Sockman & Schwabl, 2000; Forbes *et al.*, 2002). It would seem to further enhance developmental differences among young, reduce sibling rivalry, and therefore reduce energy demands of the brood (Wiebe & Bortolotti, 1994*a*). We would predict that, under less-than-optimal resource conditions or when all siblings prove viable (that is, when the surplus offspring are a burden), the late-laid offspring would be removed from the brood, either actively through neglect, infanticide, or siblicide or passively through starvation induced by competition with siblings. Reproductive effort would presumably be tuned more toward the optimum than would otherwise occur in the absence of an androgen-facilitated sibling hierarchy. Thus, the effects of the androgens and therefore the hierarchy are potentially beneficial to the mother, even when they are not to the offspring.

These results from studies in which yolk androgens are experimentally manipulated must be interpreted cautiously, however. Natural variations in yolk androgen concentrations may co-vary with natural variations in other aspects of the egg, such as its concentrations of endogenous antioxidants (Royle, Surai & Hartley, 2001) or other steroids (Reed & Vleck, 2001), that themselves may interact with the androgens to influence offspring phenotype. When investigators experimentally elevate levels of one androgen in the absence of manipulating other yolk components that naturally co-vary with the androgen, they may draw erroneous conclusions regarding the effects of the manipulated androgen, because, after the manipulation, its concentration relative to the concentration of other yolk components may be unnaturally skewed. To some extent, injections of cocktails of known, co-varying yolk constituents (Sockman & Schwabl, 2000; Eising *et al.*, 2001) might allay this concern. Still, it will always be difficult to know if there are important yolk constituents that are not in the cocktail but which might interact with the androgens being injected.

In the lesser black-backed gull (*Larus fuscus*), concentrations of yolk androgens increase, whereas concentrations of yolk antioxidants decrease with laying order (Royle *et al.*, 2001), as yolk antioxidants also do in the barn swallow (*Hirundo rustica*) (Saino *et al.*, 2002). Yolk antioxidants could buffer against the damaging effects of oxidative stress produced by a yolk-androgen-induced increase in metabolic rate (Royle *et al.*, 2001). Consequently, variation in the deposition of yolk antioxidants might constitute another determinant of avian reproductive effort. In late-laid eggs, high yolk androgen concentrations together with low antioxidant levels would seem to conspire against the offspring hatching from those eggs. Perhaps, as the authors suggest, it is for this reason that late-laid eggs in the lesser black-backed gull tend to produce the least viable offspring. Presumably young hatching from them cannot sustain the metabolic costs of high androgen exposure, in part, because they lack the protection of high antioxidant levels.

In the canary (Schwabl, 1993), black-headed gull (*Larus ridibundus*) (Eising *et al.*, 2001), European starling (*Sturnus vulgaris*) (Pilz *et al.*, 2003), and great tit (*Parus major*) (Tschirren, Richner & Schwabl, 2004), yolk androgens increase in concentration with laying order. For each of these species, investigators experimentally elevated concentrations of yolk androgens in early-laid eggs of the clutch to bring levels to or above those found naturally in late-laid eggs of a clutch. In certain situations that vary depending on the study, these investigators found that nestlings hatching from these eggs tended to grow faster than control nestlings, whose yolks were injected with vehicle (Schwabl, 1996*b*; Eising *et al.*, 2001; Pilz *et al.*, 2004; Tschirren *et al.*, 2005). Within-clutch patterns of antioxidant deposition were not measured in these species. However, if patterns resemble those described above for the lesser black-backed gull, as is the case for yolk androgen deposition, and if antioxidants protect embryos from oxidative damage made worse by high yolk androgen exposure, then these growth-enhancing effects of elevated yolk androgens may have been permitted by high antioxidant levels that would be found in early-laid eggs but not in the late-laid eggs the investigators were trying to mimic. Moreover, in the starling and great tit studies, as well as a similar study on eastern bluebirds that also found growth-enhancing effects of yolk androgen

elevation (Navara *et al.*, 2005), enough androgens were injected probably to raise levels above those that naturally occur in later-laid eggs, exposing embryos to unnaturally high levels of androgens. Therefore, these results should be interpreted with caution. In the American kestrel studies cited above, however, final yolk androgen levels were probably within the range that naturally occurs, but, again, the pattern of yolk antioxidant deposition was not measured. Nonetheless, if the pattern is similar to that in the lesser black-backed gull, as is the pattern of yolk androgen deposition, then the detrimental effects of yolk androgens found in the kestrel would probably not have occurred *because* of the unnatural ratio of androgens to antioxidants but, rather, *in spite* of it.

This is not to say that high levels of yolk androgens in late-laid eggs with low antioxidant concentrations should necessarily be detrimental to offspring growth. First, we do not know how ubiquitous the within-clutch pattern of antioxidant deposition is and in what other species it decreases with laying order. Second, we do not know if low levels found in late-laid eggs are low enough to compromise nestling growth or even if they have any effect at all on nestling growth.

If we ignore the possibility that growth-enhancing effects of yolk androgens could be an artefact of an unnatural androgen-antioxidant ratio, it might be argued for these species that deposition patterns that increase with laying order would mitigate against the pre-existing developmental hierarchy (e.g. Schwabl, 1996*b*; Eising *et al.*, 2001). But decades of research (see above) suggests that females often derive adaptive benefits from this hierarchy, raising questions as to what the female is mitigating against. The possibility in the American kestrel that elevated yolk androgens augment the hierarchy by inhibiting growth and survival of late-laid offspring would be consistent with the adaptive benefits of this hierarchy. However, a consistent handicapping effect of yolk androgens on offspring growth would be difficult to reconcile with theoretical predictions related to parent-offspring conflict (Hamilton, 1964; Trivers, 1974). That is, if yolk androgens necessarily handicap offspring, one would predict the evolution of offspring strategies that reduce these negative effects, such as the downregulation of androgen receptors at critical moments during development. This would lead to the evolution of higher deposition concentrations by females to offset offspring loss in androgen sensitivity, which itself would lead to the evolution of still lower offspring sensitivity. Ultimately, the offspring might win the game by reducing sensitivity to zero. However, empirical observations detailed above reveal that nestling birds are sensitive to these yolk androgens, suggesting that this evolutionary race is not occurring. In short, considering what we currently know about yolk androgen deposition in several bird species, it is difficult to reconcile both a consistent effect of yolk androgens favouring late-laid offspring with adaptive benefits of sibling hierarchies and a consistent effect of yolk androgens handicapping late-laid offspring with inherent conflict between the mother and her offspring. However, yolk androgen deposition as an evolutionary strategy might stabilize if the effects of elevated yolk androgens vary from favouring to handicapping offspring growth as a function of conditions that are unpredictable. Below we describe a hypothesis for condition-dependent yolk androgen effects that we call the Offspring Value Hypothesis for yolk androgen deposition.

(*ii*) *The offspring value hypothesis for yolk androgen deposition.* Depending on the bird species, the reproductive value of an individual offspring may vary with a number of factors, such as its position in the sibling hierarchy, environmental or parental condition, sex, and interactions between these factors (Mock & Parker, 1997). In facultative brood-reducing species, for which conditions would usually range from somewhat favourable to somewhat poor, the value of early-laid offspring is likely to be relatively high. The value of late-laid offspring is likely to depend on the range of conditions frequently experienced, being relatively high and similar to that of early-laid offspring when conditions are favourable, to relatively low when conditions are moderate, to very low when conditions are poor. In very poor conditions, even the early-laid offspring may have low reproductive value, a situation in which nest abandonment might be favoured. In light of this range of possible, unpredictable conditions, a female would seemingly benefit from a mechanism that would enable her to capitalize on good conditions, during which all offspring are of relatively high value, but hedge against bad conditions, when the value of some offspring is low. To some extent, the presence of the sibling hierarchy set up by early incubation onset probably provides such a mechanism, as we have argued above. At the very least, the hierarchy, by definition, identifies a marginal offspring, based on size differences, that may later be culled. But, as mentioned above, the nature of this hierarchy may be constrained by egg viability and other factors that influence the timing of incubation onset, and therefore, the hierarchy may not be optimally shaped for the most efficient reduction of the brood, were it necessary. Also as we mentioned above, the presence of a hierarchy can be detrimental in good conditions, by promoting brood reduction in situations when the female might not benefit from it. Additional mechanisms for adjusting the sibling developmental hierarchy might enable the female to augment it under poor conditions and mitigate against it when conditions are favourable. Here, we suggest that yolk androgen deposition might facilitate such processes. With the Offspring Value Hypothesis, we propose that the benefits of elevated yolk androgens to offspring growth and survival are proportional to the reproductive value of the offspring (Fig. 4).

Androgens are widely thought to be anabolic in their effects on growth (Herbst & Bhasin, 2004). Yolk testosterone concentrations positively correlate with size of the hatching muscle of red-winged blackbird hatchlings, and controlled injections of testosterone into the yolks of red-winged blackbird eggs increase hatching muscle mass (Lipar & Ketterson, 2000). Of course, testosterone is not an energy source, so it alone cannot induce elevated growth rates. Sustained anabolism is only possible when the energetic building blocks are available. This leads to the prediction that under good conditions, androgens direct energy resources towards muscle and body growth and perhaps away from storage. However, promoting growth over storage would seemingly have a negative

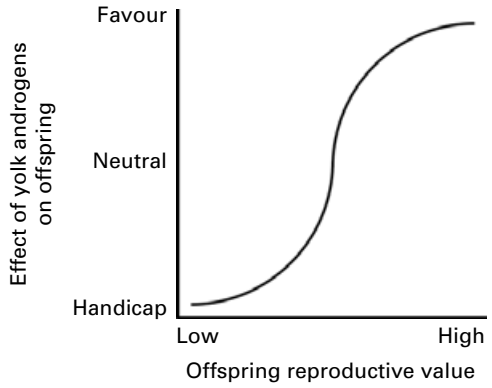

Fig. 4. Offspring Value Hypothesis for yolk androgen deposition. As the reproductive value of the offspring increases, the effects of yolk androgens change from handicapping offspring growth to favouring offspring growth.

impact under poor conditions because resources would be rapidly consumed and provide little buffer against acute food shortages. Royle *et al.* (2001) argued that food availability specifically should play a major role in whether androgens in the yolks of later-laid eggs should favour or handicap the growth of nestlings hatching from those eggs. They predict that under limited food conditions, high yolk androgen concentrations in later-laid eggs will give rise to high metabolic costs and oxidative stress and thus reduced growth and survival rates of late-laid nestlings. Under favourable conditions, the metabolic costs and oxidative stress would be reduced in these nestlings from late-laid, high-androgen eggs, and the beneficial effects of androgens, such as enhanced growth and survival, would partially offset or even overcome the detrimental effects of hatching late as a result of hatching asynchrony (Royle *et al.*, 2001).

Few investigators have studied the interactive effects of condition and yolk androgens on offspring growth. Nonetheless, food availability is known to limit reproductive success in American kestrels (Dawson & Bortolotti, 2002), and the growth-inhibiting effects of elevated yolk androgens observed in American kestrels (Sockman & Schwabl, 2000) occurred in a year in which food conditions were much worse than in previous years (K. W. Sockman, M. S. Webster, V. Talbot, J. Weiss & H. Schwabl, unpublished data). The poor food conditions may explain why growth inhibition by yolk androgen injection occurred in nestlings of first-laid eggs, nestlings that, under moderate conditions, would be predicted to fair well from elevated androgen levels. The growth-enhancing effects observed in the canary occurred under conditions in which parents had *ad libitum* access to food (Schwabl, 1996*b*). Neither of these studies were tests of the present hypothesis, because neither simultaneously examined multiple food regimens. Still, the Offspring Value Hypothesis may reconcile these otherwise conflicting findings.

Importantly, the reproductive value of any individual offspring may be relatively unpredictable at laying, because it is, in part, a product of unpredictable conditions and laying order. This unpredictability of offspring value would seem to stabilize the opposing selective forces on the parent's pattern of yolk androgen deposition and on offspring sensitivity to these androgens. An increase in offspring sensitivity would benefit it in good conditions but handicap it in bad conditions. Similarly, a reduction in sensitivity would benefit it in bad conditions but handicap it in good conditions. In either case, because conditions are unpredictable, evolution of offspring sensitivity away from the parental optimum would not, on average, improve the offspring's own prospects and may even reduce its inclusive fitness (Hamilton, 1964) because the whole family unit would be expected to suffer when sensitivity deviates from the optimum.

The possibility that, in some species, the effects of yolk androgens might be sex-specific would be an interesting corollary to the Offspring Value Hypothesis for yolk androgen deposition. Building on the ideas of Fisher (1930), Trivers & Willard (1973) argued that because male fitness is typically more sensitive than female fitness to variation in condition, parents that produce relatively more males under good conditions and relatively more females under bad conditions would tend to invest more in the sex that would have the larger pay-off relative to production cost (Clutton-Brock, 1986). There is now evidence in a variety of bird species that manipulation of the offspring sex ratio does occur (Clutton-Brock, 1986; Drummond *et al.*, 1991; Krackow, 1995; Daan *et al.*, 1996*b*; Godfray & Werren, 1996; Greenwood, 1997; Hardy, 1997; Komdeur, 2002; Pike & Petrie, 2003), and, in many cases, this manipulation is consistent with these adaptive predictions. Some of the clearest examples of brood sex-ratio manipulation occur in raptors, such as European, lesser (*Falco naumanni*), and American kestrels (Dijkstra *et al.*, 1990; Wiebe & Bortolotti, 1992; Daan *et al.*, 1996*b*; Smallwood & Smallwood, 1998; Korpimäki *et al.*, 2000; Aparicio & Cordero, 2001; Blanco *et al.*, 2003; Laaksonen, Lyytinen & Korpimäki, 2004). In some bird species (Kilner, 1998; Komdeur, Magrath & Krackow, 2002; von Engelhardt *et al.*, 2004; Correa, Adkins-Regan & Johnson, 2005), brood sex-ratio is biased by manipulation of egg sex (i.e. manipulation of the primary sex ratio), which carries the advantage of tipping initial production towards the more profitable sex, compared to adjustments to the secondary sex-ratio which occur after oviposition through, for example, sex-biased nestling mortality. However, manipulation of the secondary sex ratio might be a better strategy for tracking unpredictable resources such as food, simply because females may not have sufficient information about resources around the time of ovulation, when the sex of an egg is determined (Etches, 1996).

Could differential patterns of yolk androgen deposition provide a mechanism for the adaptive adjustment of secondary sex ratios in some species and thus a mechanism for the optimization of reproductive effort according to unpredictable conditions? The Offspring Value Hypothesis predicts that yolk androgens will favour the growth and survival (to independence) of offspring proportionally to their reproductive value. On a population level, the mean reproductive value of females must equal that of males. However, if *variation* in reproductive value is less in females than in males, as Trivers & Willard (1973) predict, then we would predict yolk androgens to have relatively less effect on

female than on male offspring, in either good or bad conditions, simply because variation in conditions would have less effect on female value than on male value. In moderate conditions, we would predict little effect in either sex.

Few investigators have examined sex-specific effects of yolk androgens on nestling growth and survival. However, in the American kestrel, evidence suggests that male fitness is more variable than female fitness (Smallwood & Smallwood, 1998), and, consistent with our prediction, the growth-inhibiting effects of yolk androgens during poor conditions are specific to male but not female nestling kestrels (Sockman *et al.*, 2005*b*).

The mechanisms by which yolk androgens might affect male but not female offspring are theoretically straightforward, though they have not yet been tested empirically. For yolk androgens to have effects in one sex but not the other, one might predict different expression levels of androgen receptor during critical stages of offspring development. Because males would usually be the sex expected to be most sensitive, it is not unreasonable to predict that androgen receptors in important growth-regulating regions might be more prevalent in male than in female offspring during the critical periods. A careful examination of embryonic zebra finches (*Taeniopygia guttata*) revealed no evidence for sex differences in androgen expression in some hindbrain and syringeal regions (Godsave *et al.*, 2002), however whether or not sex differences might be found elsewhere in regions more closely associated with body growth is not known.

In species with a sex difference in sensitivity to conditions and yolk androgens, we predict male eggs should not differ from female eggs in their yolk androgen concentrations. Our rationale is that, first, there is probably little or no direct energetic cost to the mother in making or depositing yolk androgens (Eising *et al.*, 2003). Second, although androgen deposition should benefit laying females by exerting condition-dependent effects on males, there should be little fitness cost to deposition in female eggs due to the insensitivity of female offspring that we predict. Therefore, there would not be selection for a mechanism that would confer different concentrations to female than to male eggs in species in which males are more sensitive than females to condition.

Some investigators have claimed to find sex differences in yolk androgen concentrations (Petrie *et al.*, 2001; Müller *et al.*, 2002; Rutstein *et al.*, 2004; Gilbert *et al.*, 2005). However, in each of these cases, the investigators sampled yolk for androgen measurement at least three days into the incubation period. But three days of embryonic development may be sufficient to cause a noticeable change in yolk androgen concentrations due to embryonic metabolism or sequestration (Pilz *et al.*, 2005). If the sexes differ in their rate of androgen metabolism or sequestration, this difference could be reflected in the concentration of androgens remaining in the yolk after three days. On those occasions in which androgens were measured in freshly laid yolk when only the most minimal embryonic development would have occurred, no sex differences in concentrations were detected (Schwabl, 1993; Lipar & Ketterson, 2000; Pilz *et al.*, 2005).

Up to this point in our case for the Offspring Value Hypothesis for yolk androgen deposition, we have not addressed variation in the pattern of androgen deposition but instead focused on what seems to be the usual pattern, an increase in concentrations with laying order (e.g. Schwabl, 1993; Sockman & Schwabl, 2000; Eising *et al.*, 2001). In some species, however, yolk androgen concentrations decrease with laying order (e.g. Schwabl *et al.*, 1997; Gil *et al.*, 1999; Reed & Vleck, 2001). In these species, it seems conceivable that laying females may take an alternative strategy that may still make use of the effects of yolk androgens based on offspring value, as predicted by the Offspring Value Hypothesis. Specifically, in these species, we suggest that elevated yolk androgen concentrations in early-laid, high-value eggs augment the sibling hierarchy beyond that which would occur as a result of incubation behaviour alone. If incubation behaviour alone does not establish the degree of sibling hierarchy which would benefit females the most under the widest array of possible conditions, females may augment that hierarchy with elevated androgens in early-laid eggs, which would confer even greater nestling growth and survival benefits owing to their high reproductive value. Currently, however, the effects of yolk androgens on nestling growth have not been tested in species in which yolk androgen concentrations decline with laying order.

It is also not clear to what extent the Offspring Value Hypothesis would apply across the altricial-precocial continuum. With increasing precociality, there is decreasing parental care and therefore a decline in the need for brood reduction to trim uneconomical costs of reproductive effort. However, in most precocial species, some post-incubation parental care does occur, for example, in the form of protection of young chicks. If effort expended in or risk incurred from this form of care is proportional to the number of chicks present, then females might gain from the efficient elimination of chicks of relatively low value through deposition of yolk androgens.

As discussed above, the Offspring Value Hypothesis for yolk androgen deposition would seem to provide a possible basis for the efficient adjustment of brood size according to the reproductive value of individual offspring. If accurate, it would seem to resolve at least some reports in the literature that were heretofore irreconcilable. However, there are many components of the hypothesis to be empirically tested. In particular, testing the interaction between food conditions and yolk androgen exposure on nestling growth and survival would be of primary concern. Additionally, how yolk androgens differentially affect male *versus* female offspring and whether concentrations differ with egg sex should be tested in more species. Finally, the hypothesis does not account for potential interactive effects of other yolk constituents, such as antioxidants and other steroid hormones, although the presence of such interactive effects does not necessarily discount the plausibility of the hypothesis. Still, interactive effects of other yolk constituents might make assigning an effect of yolk androgens more challenging.

Regardless of the accuracy of the Offspring Value Hypothesis and what the primary function of yolk androgen

deposition might be, it is apparent that yolk androgens can differentially affect sibling growth and potentially influence the sibling hierarchy and the probability of brood reduction (Sockman & Schwabl, 2000). In the American kestrel, the within-clutch pattern of deposition (increasing with laying order) combined with the strong growth-inhibiting effects on male nestlings during poor conditions suggests that yolk androgen deposition can augment the developmental hierarchy during poor conditions (Sockman & Schwabl, 2000). Under situations of limited food availability or when all siblings are viable, this hierarchy will likely result in the elimination of the smallest, least competitive male nestling and hence a beneficial reduction in reproductive effort to match the particular conditions and ultimately to enhance lifetime reproductive success of the female. Under highly favourable conditions or in the event an egg is lost, such competition will be less likely to ensue, and we predict the detrimental effects of yolk androgens may turn beneficial (Schwabl, 1996*b*; Royle *et al.*, 2001) (Fig. 4) and that the youngest male nestling will more likely survive, enabling the parent to take full advantage of the conditions and maximize her reproductive success.

(5) Concluding remarks on the determinants of reproductive effort

We argue that the three phenomena described above – variation in clutch size, timing of incubation onset, and rate of yolk androgen deposition – contribute to female flexibility in reproductive effort. Variation in clutch size, primarily through seasonal variation, enables females to cope with predictable reproductive challenges, namely, the predictable decline in the reproductive value of the offspring. Timing of incubation onset and rate of yolk androgen deposition, we argue, ultimately influence the developmental hierarchy among sibling young. In non-precocial species, establishing a developmental hierarchy among sibling young through asynchronous incubation onset and through yolk androgen deposition might enable mothers to cope, at least in part, with unpredictable reproductive challenges, such as limited resource availability, and unpredictable reproductive opportunities, such as a windfall in resources or the ability to use an egg or nestling as insurance against the loss of another in the clutch (Evans, 1996, 1997; Forbes & Lamey, 1996; Wiebe, 1996; Aparicio, 1997; Forbes *et al.*, 1997; Clifford & Anderson, 2001).

Additional factors that probably contribute to reproductive effort as well include variation in egg size, not only within clutches but also between clutches (Howe, 1976; Williams, 1994; Wiebe & Bortolotti, 1995*a*; Bernardo, 1996), and variation in the deposition of other yolk components, such as corticosterone (Eriksen *et al.*, 2003; Hayward & Wingfield, 2004; Hayward, Satterlee & Wingfield, 2005) and antioxidants (Royle *et al.*, 2001; Saino *et al.*, 2002). As mentioned previously, in sexually size-dimorphic species, modification of the brood primary sex ratio is likely to influence reproductive effort insofar as broods biased towards the more expensive sex would require greater reproductive effort than those without such a bias. In birds, females are the heterogametic sex, and modification of the brood primary sex ratio would require some control in individual ova over sex determination. The possible mechanistic bases for this were recently reviewed (Pike & Petrie, 2003). Additionally, the length of the interval between successively laid eggs in a single clutch will influence the duration of the laying cycle, which would influence the effects of timing of incubation onset and rate of yolk androgen deposition. Variability in laying interval is likely to have considerable influence not just on individual offspring and their developmental relationships with siblings but also on the total hatching spread and therefore the reproductive effort of the parents (Astheimer, 1985; Watson, Robertson & Cooke, 1993; Wiebe & Martin, 1995; Oppenheimer, Pereyra & Morton, 1996). Further, the potential for complex interactive effects between a variety of factors, including the position in the laying sequence, offspring sex, yolk androgen deposition, incubation timing, laying interval, and egg size, may affect reproductive effort as well (Blanco *et al.*, 2003; Young & Badyaev, 2004). As for variation in egg size and other yolk constituents, little is known about the mechanistic bases behind female flexibility in the expression of these traits (Williams *et al.*, 2001; Williams, 2005). In fact, in some species, females may not have much flexibility in egg-size determination (Christians, 2002). Most of this review will focus on the roles of clutch size, timing of incubation onset, and the rate of yolk androgen deposition. We briefly describe some of these additional components in our discussion on the hormonal orchestration of the various components of reproductive effort.

III. SEASONAL COORDINATION OF THE SIBLING HIERARCHY WITH CLUTCH SIZE

It is convenient to discuss yolk androgens in terms of their rate of deposition and define this by how rapidly yolk androgen concentrations rise when the time scale is measured as consecutive eggs laid in a clutch. By this definition, a slow rate of deposition would result in relatively low concentrations in all eggs, whereas a rapid rate would result in high concentrations in all eggs. In either case, differences among sibling yolks would be low, but it is precisely these differences which may contribute to the developmental hierarchy. Thus the rate of yolk androgen deposition, where time is measured as consecutively laid eggs in a clutch, should be critical for establishing the differences among sibling yolks that contribute to the developmental hierarchy.

The production of a sibling developmental hierarchy is itself dependent on clutch size because of the relationship between the duration of the egg-laying period, which is positively correlated with clutch size, and the timing of incubation onset and rate of yolk androgen deposition. In the American kestrel, which typically lays one egg every two days, the duration of the laying period for a six-egg clutch is roughly 10 days, whereas that for a four-egg clutch is six days (Balgooyen, 1976; Sockman & Schwabl, 1998). To illustrate this interdependence between clutch size and the sibling hierarchy, consider an early-season clutch of six eggs and compare that to a late-season clutch of four (Fig. 5).

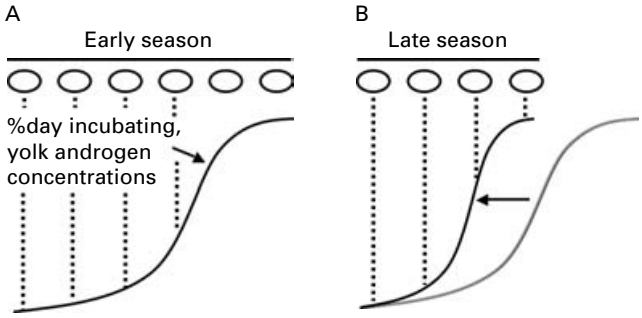

Fig. 5. Seasonal advance in the timing of incubation onset and increase in the rate of yolk androgen deposition ultimately based on the seasonal decline in the reproductive value of the eggs (seasonal decline in clutch size) and the benefits of establishing a developmental hierarchy among sibling nest mates. Array of ovals represents the temporal pattern in which females successively lay each egg of a single clutch. In large, early-season clutches (A), incubation (curved solid line) may increase to reach maximal levels of expression around the time the penultimate egg of a clutch is laid. Yolk androgen concentrations (also represented by the curved solid line) are relatively low in the first four eggs, before reaching relatively high levels in eggs 5 and 6, reflecting a slow "rate" of deposition (where egg-days, defined as the days on which each successive egg of a clutch is laid, are the unit of time measurement) for a large, early-season clutch. Because there is less laying time in a small, late-season clutch (B), the rate at which both incubation behaviour increases and yolk androgens are deposited must increase so that incubation begins before clutch completion and so that yolk androgen concentrations rise from the low levels of early eggs to high levels by eggs 3 and 4.

Although the function describing change in incubation behaviour over the course of the laying cycle can assume a variety of shapes (Wiebe *et al.*, 1998), typically it might look sigmoidal, with very low levels through much of laying but then a rapid increase to full expression around the time the penultimate egg of a clutch is laid (Zerba & Morton, 1983*a*, *b*; Mead & Morton, 1985; Morton, 2002). In a six-egg clutch, this will result in the more-or-less synchronous hatching of eggs 1–5 and the asynchronous hatching of the sixth egg, giving rise to a sibling developmental hierarchy in which the youngest nestling is somewhat of a runt and most likely not to survive but the first five nestlings are all roughly similar in size and thus represent the core offspring.

This function illustrating change in incubation behaviour across the laying period of a six-egg clutch (Fig. 5A) might simultaneously serve to illustrate the rate of yolk androgen deposition. With a relatively slow rate in a six-egg clutch, high levels of yolk androgens are reached late relative to the onset of laying such that eggs 5 and 6 would have high levels but eggs 1–4 relatively low levels. If a late-laying female producing a four-egg clutch (Fig. 5B) applies the behavioural incubation strategy (i.e. the sigmoidal function describing change in incubation relative to progress in the laying cycle) and the physiological yolk-deposition strategy shown for a typical six-egg clutch (Fig. 5A), she would not produce the developmental hierarchy among her young that may enable her to cull the brood under limited food conditions or under situations when all the eggs are viable. Consequently, with the seasonal decline in clutch size, there should be a concomitant seasonal advance in the timing of incubation onset and also a seasonal elevation in the rate of yolk androgen deposition. This seasonal change in these behavioural and physiological traits would result in an earlier onset of full incubation behaviour (e.g. on the day egg 3 is laid) and more rapid yolk androgen deposition (such that eggs 3 and 4 have high concentrations) for late-season four-egg nests compared to early-season six-egg nests. Across the entire range of seasonally declining clutch sizes, females would establish developmental hierarchies among their young. Such behavioural and physiological plasticity would enable the female adaptively to tune clutch size to the predictable seasonal decline in the reproductive value of the young but simultaneously cope (at least in part) with unpredictable challenges or opportunities (limited food, full viability of clutch) by culling the brood. The presumed benefits of maintaining a sibling developmental hierarchy in the presence of a seasonally dynamic clutch size leads to the hypothesis that as clutch size declines seasonally, timing of incubation onset (relative to the onset of egg laying) should advance and rate of yolk androgen deposition should increase (Fig. 5).

In order to explore the merits of this hypothesis for the American kestrel, we can formulate predictions regarding seasonal change in incubation behaviour on the days specific eggs of a clutch are laid and seasonal change in yolk androgen concentrations for those same eggs. In taking this approach, we do not address yolk androgen concentrations in eggs 5 and 6 or incubation behaviour on the days egg 5 and 6 are laid, because clutches with 5 or 6 eggs are generally restricted to the early breeding season. For eggs 1 and 2, we predict according to our model (Fig. 5) that yolk androgen concentrations are relatively low and that incubation behaviour on the days those eggs are laid is likewise low, regardless of when in the season those clutches are initiated. However, for eggs 3 and 4, we predict that yolk androgen concentrations and incubation behaviour are relatively low in early-season nests but relatively high in late-season nests. Thus, we predict the most noticeable seasonal increase in both incubation behaviour and yolk androgen concentrations to occur on the days eggs 3 and 4 are laid.

For both incubation and yolk androgen deposition in the American kestrel, this is exactly what was found. There is no evidence of seasonal change in incubation behaviour quantified on the days eggs 1 and 2 are laid, but there are seasonal increases in incubation behaviour if quantified on the days eggs 3 and 4 are laid (Sockman & Schwabl, 2001*a*). Indeed, seasonal patterns in timing of incubation onset similar to these in the American kestrel have been documented in other species as well (Beukeboom *et al.*, 1988; Meijer, Daan & Hall, 1990; Müller *et al.*, 2004). Additionally, there is no evidence for seasonal change in androgen concentrations if quantified in the yolks of eggs 1 and 2, but there are reliable seasonal increases if quantified in the yolks of eggs 3 and 4 (Sockman *et al.*, 2001). In the canary, concentrations of yolk androgens are lower in late-season clutches than in early-season clutches (Schwabl, 1996*a*), but in the black-headed gull, seasonal increases in

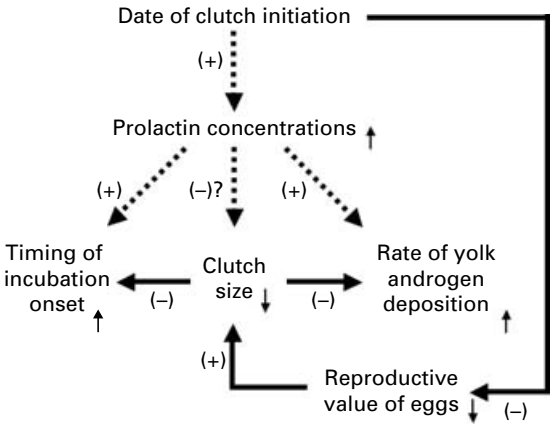

Fig. 6. Hypothetical integration of the ultimate (solid lines) and proximate (broken lines) bases for the modulation of reproductive effort in birds according to the seasonal decline in the reproductive value of the eggs and the need to maintain the sibling developmental hierarchy to cope with unpredictable reproductive challenges and opportunities. Positive (+) and negative (−) signs reflect the relationship between the two flanking factors, and small arrows indicate the direction (up or down) of change in a factor with increasing date of clutch initiation.

yolk androgen concentrations similar to those in the American kestrel occur (Müller *et al.*, 2004). So, the pattern of seasonal increases clearly varies among species. Moreover, seasonal patterns in timing of incubation onset and rate of yolk androgen deposition relative to laying onset have been examined in only a few species. Examination of these phenomena in additional species would be valuable. Nonetheless, it appears that there is at least initial support for the hypothesis that in order to maintain a sibling developmental hierarchy in the presence of a seasonal decline in clutch size, females can adjust their behavioural and physiological strategies by advancing the timing of incubation onset and by increasing the rate of yolk androgen deposition relative to clutch initiation.

Based on the American kestrel studies, we can begin to assemble a model for the seasonal modulation of reproductive effort (Fig. 6). Increasing date ultimately drives the seasonal decline in the reproductive value of the eggs, which drives the seasonal decline in clutch size. Because clutch size declines seasonally but preparedness for unpredictable reproductive challenges and opportunities should be present throughout the season, females should advance the timing of incubation onset relative to clutch initiation and accelerate the rate of yolk androgen deposition to maintain the presence of a sibling developmental hierarchy under a seasonally declining clutch size. Thus, a suite of behavioural and physiological traits – clutch size, timing of incubation onset, and rate of yolk androgen deposition – should be modulated in concert on a seasonal basis, and this would presumably enable females to contend more effectively with the predictable challenge of the seasonal decline in an egg's reproductive value and with unpredictable challenges and opportunities such as variation in food resources or offspring viability. Moreover, the presumed adaptive functionality of this suite of traits, the likelihood that they all function together toward meeting some common end (optimization of reproductive effort), and the fact that seasonal changes in these three traits are coordinated provides a framework for postulating a mechanistic basis for their coordinated regulation. That is, the ultimate bases for changes in these traits suggest something about their proximate regulation. Because these traits seem to vary in a fashion that is coordinated with season or increasing date and because they seem to serve a common function, a reasonable hypothesis would be that modulation of these traits is controlled by a similar or single mechanism.

IV. ORCHESTRATION OF REPRODUCTIVE EFFORT BY PLASMA PROLACTIN

Clutch-size determination, timing of incubation onset, and rate of yolk androgen deposition have several properties suggestive of a shared regulatory control mechanism. The fact that they are all major determinants of reproductive effort, occur simultaneously (during egg laying), and vary in a coordinated fashion over the breeding season might select for a single, common, hormonal mechanism for their regulation (Mead & Morton, 1985). Their sharing a single regulatory hormonal mechanism would not be surprising considering that individual hormones often perform a suite of functions that serve a common functional endpoint. For example, a seasonal rise in plasma concentrations of testosterone in breeding male birds may not only support spermatogenesis but also the territorial and courtship behaviours that must accompany it for successful reproduction (Wingfield, 1984, 1994; Wingfield *et al.*, 1990; Wingfield & Hahn, 1994; Ball & Bentley, 2000; Dawson *et al.*, 2001; Ball & Balthazart, 2002). Similarly, an elevation in plasma concentrations of the steroid corticosterone in response to environmental stimuli perceived as stressors elevates heart rate, mobilizes glucose stores, and elevates locomotor activity all to serve the common end of preparing the individual to cope with the stressor (Astheimer, Buttemer & Wingfield, 1992; Wingfield, 2003). Some evidence suggests that the pituitary hormone prolactin might be central to the regulation of the suite of traits that determine reproductive effort in birds (Fig. 6), and below we review this evidence. First, however, we provide some background on the synthesis, structure, secretion, and function of this hormone.

(1) Prolactin synthesis, structure, secretion and function

Several reviews provide details on prolactin synthesis, structure, function, and regulation of secretion (Clarke & Bern, 1980; Hall, Harvey & Chadwick, 1986; Dutt *et al.*, 1994; Freeman *et al.*, 2000; Sharp & Blache, 2003). Prolactin belongs to the prolactin-growth hormone-placental lactogen family, the genes for which evolved by duplication of a single ancestral gene approximately 400 million years ago (Freeman *et al.*, 2000). Prolactin is

composed of a single polypeptide chain 199 amino-acid residues in length, with three intramolecular disulfide bonds between six cysteine residues (Freeman *et al.*, 2000). Within mammals alone, there is substantial variation in sequence homology, from 97% among primates to 56% between primates and rodents. In birds, there is high identity between the predicted prolactin amino-acid sequences from different species; for example, compared with chicken prolactin, domestic duck (*Anas platyrhynchos*), ring dove (*Streptopelia risoria*) and European starling prolactins are 90, 91, and 89% homologous, respectively. The greatest difference occurs between residues 140 and 150, which are not directly involved in the binding of prolactin to its receptor (Haworth, Goldsmith & Sharp, 1994), and, consequently, mutational changes in the region of the prolactin gene encoding these residues are least likely to have functional significance. Functional changes in the primary structure of avian prolactin, driven by evolutionary pressure on avian life histories, thus appear to be unlikely. However there may be species differences in prolactin isoforms generated by post-translational modification, including truncated or variably glycosylated and phosphorylated isoforms (Corcoran & Proudman, 1991; Bédécarrats *et al.*, 1999*b*), and these could be subjected to functionally significant evolutionary change.

The structure of the avian prolactin receptor is similar to that of mammals, except for an extracellular domain with a repeated WSXWS motif that is homologous with a portion of the ligand-binding domain in the mammalian prolactin receptor (Kelly *et al.*, 1991). At least two major forms of prolactin receptor occur in mammals (Kelly *et al.*, 1991), and multiple forms occur in the chicken and turkey (*Meleagris gallopavo*) (Ohkubo *et al.*, 1998; Pitts *et al.*, 2000). The presence of multiple forms of prolactin receptor is likely to be widespread in birds and may form a part of the basis for the evolution of differences in the actions of prolactin at the tissue, individual, or species level and thus a basis for flexibility in how or whether prolactin orchestrates some components of avian reproductive effort.

Most prolactin is synthesised in specialized cells of the adenohypophesis (anterior pituitary) called lactotrophs, although there is evidence in mammals (Freeman *et al.*, 2000) and birds (Berghman *et al.*, 1992; Ramesh, Keunzel & Buntin, 2000) that prolactin may also be synthesised at extra-pituitary sites in the brain. In mammals, lactotrophs have spontaneously high secretion rates of prolactin and are primarily under the inhibitory control of dopamine secreted into the portal vasculature by neurons originating in the paraventricular and arcuate nuclei of the hypothalamus. The situation in birds is different because avian lactotrophs do not secrete prolactin spontaneously at a high rate. In birds, prolactin secretion is predominantly under the stimulatory control of vasoactive intestinal polypeptide (VIP), the avian prolactin-releasing hormone, which is secreted into the portal vasculature from hypothalamic VIP neurons (Macnamee *et al.*, 1986; El Halawani, Silsby & Mauro, 1990; El Halawani, Youngren & Pitts, 1997; Sharp, Dawson & Lea, 1998; Maney *et al.*, 1999*b*; Vleck & Patrick, 1999). It should be noted however that an element of prolactin secretion in birds may be under the inhibitory control of dopamine (El Halawani *et al.*, 1991*b*), but the physiological circumstances in which this control mechanism is significant have not been established.

The functions of prolactin in vertebrates are diverse (Clarke & Bern, 1980; Freeman *et al.*, 2000), and in birds this is suggested by the occurrence of prolactin receptor binding sites and mRNA in many central nervous and peripheral tissues (Buntin & Ruzycki, 1987; Fechner & Buntin, 1989; Lea, Georgiou & Sharp, 1994; Buntin, 1996; Ohkubo *et al.*, 1998). The functions of prolactin in birds have been most extensively investigated in the Columbiformes (pigeons and doves), in which the hormone facilitates parental behaviours, particularly those associated with feeding young with crop milk. In this group of birds, plasma prolactin concentrations increase after the onset of incubation, and is thought to play a role in maintaining the behaviour (Lehrman & Brody, 1961; Buntin & Tesch, 1985; Buntin, Becker & Ruzycki, 1991).

Some evidence supports a role for prolactin in the seasonal modulation of reproductive effort in non-columbiform birds, specifically in the regulation of two of the three primary components of reproductive effort outlined above – clutch size and timing of incubation onset (Eisner, 1960; Mead & Morton, 1985; Meijer *et al.*, 1990). Following an examination of this evidence, we will present relatively new but still limited evidence that prolactin also plays a role in regulation of the third primary component of reproductive effort outlined above – rate of yolk androgen deposition.

(2) Prolactin and the regulation of incubation onset and clutch size

A role for prolactin in the expression of parental behaviours in birds is well established (Riddle, Bates & Lahr, 1935; Nalbandov, 1945; Buntin, 1996; Vleck, 2002). In many species, plasma prolactin concentrations in females rise during egg laying (Etches, Garbutt & Middleton, 1979; Burke & Dennison, 1980; Lea *et al.*, 1981; Bluhm, Phillips & Burke, 1983; Hall & Goldsmith, 1983; Silverin & Goldsmith, 1983; Sharp *et al.*, 1988; Sockman, Schwabl & Sharp, 2000; Young & Badyaev, 2004; Badyaev *et al.*, 2005), a period coinciding with the development of incubation behaviour during an individual bout of reproduction. Typically, prolactin concentrations are higher after than immediately before the onset of incubation (El Halawani, Burke & Dennison, 1980; Dawson & Goldsmith, 1982; Goldsmith, 1982; Silverin & Goldsmith, 1983; Dawson & Goldsmith, 1985; Hall, 1986; Meijer, 1988; Sharp *et al.*, 1988; Gratto-Trevor *et al.*, 1990; Seiler *et al.*, 1992; Williams & Sharp, 1993; Cherel *et al.*, 1994; Buntin, 1996; Brown & Vleck, 1998; Bédécarrats *et al.*, 1999*a*). In the domestic chicken and turkey, administration of exogenous prolactin promotes incubation (Riddle *et al.*, 1935; El Halawani *et al.*, 1986; Sharp *et al.*, 1988; Youngren *et al.*, 1991), whereas immunization against prolactin or the avian prolactin-releasing-factor, VIP, inhibits incubation (Sharp *et al.*, 1989; March *et al.*, 1994; El Halawani *et al.*, 1995; Crisóstomo *et al.*, 1997; Crisóstomo *et al.*, 1998). When prolactin is administered to ovariectomised turkeys, it does not induce incubation behaviour, unless it is

administered following estradiol and progesterone treatment (El Halawani *et al.*, 1986). Similarly, prolactin facilitates incubation behaviour in the ovariectomised budgerigar (*Melopsittacus undulatus*) provided it is given in combination with estrogen (Hutchison, 1975). These observations demonstrate that the effectiveness of prolactin in facilitating incubation behaviour in these species depends on ovarian steroid "priming." In this context, it should be noted that incubation behaviour is considered to be persistent nesting behaviour. Nesting behaviour motivates birds to lay at a nest site and is induced by an interaction between estrogen and progesterone originating from preovulatory, yellow-yolky follicles (Gilbert & Wood-Gush, 1976). The function of increased plasma prolactin concentrations, it would seem, is to reinforce this behaviour (El Halawani *et al.*, 1986).

Studies on many bird species, with the notable exception of the Columbiformes, have revealed temporal correlations between circulating prolactin levels and the timing of incubation onset (see citations above). However, until recently, the only experimental evidence suggesting that concentrations of circulating prolactin are somehow causally or permissively related to this propensity to initiate incubation came from three species, the domestic chicken, turkey, and budgerigar (see citations above). Our research on the American kestrel added a fourth species to this list (Sockman *et al.*, 2000). Consistent with findings from other species, we found in American kestrels that plasma concentrations of prolactin rise in conjunction with the development of incubation behaviour, suggesting a possible role for the hormone in stimulating or permitting onset of incubation. Additionally, when we experimentally elevated prolactin levels prematurely in the laying cycle, we found that, when compared to control females receiving vehicle only, females with experimentally elevated prolactin levels develop higher expression of incubation behaviour early during the laying cycle (Sockman *et al.*, 2000).

Studies on other species suggest that elevated prolactin concentrations depress secretion of luteinizing hormone (LH) (Buntin, Lea & Figge, 1988; Sharp *et al.*, 1988; El Halawani *et al.*, 1991*a*; Buntin *et al.*, 1999), at least in part by a direct inhibitory effect at the level of the anterior pituitary gland (You *et al.*, 1995). Conversely, treatment of incubating domestic hens with prolactin antiserum increases plasma concentrations of LH (Lea *et al.*, 1981). Because LH is thought to play a role in ovarian development and steroid secretion, elevated plasma concentrations of prolactin may therefore regulate clutch size by depressing secretion of LH. The observation that passive immunization with LH antibody in laying chickens leads to rapid ovarian regression and cessation of laying lends further support to this view (Sharp, Scanes & Gilbert, 1978). Further, the administration of prolactin to laying quail (Alexander & Wolfson, 1970; Camper & Burke, 1977) and turkeys (Youngren *et al.*, 1991) depresses or inhibits egg production and, in the Puget Sound white-crowned sparrow (*Zonotrichia leucophrys pugetensis*), inhibits photoinduced ovarian growth (Bailey, 1950). This effect of prolactin could be mediated at the level of the ovary, because, in the white-throated sparrow (*Zonotrichia albicollis*), prolactin administration blocks the stimulatory effect of exogenously administered gonadotropins (Meier, 1969). However, in several other species, this anti-gonadal effect of prolactin has not been confirmed (Meier & Dusseau, 1968). Nonetheless, plasma prolactin concentrations in females of many bird species rise over the course of laying a clutch (see above citations) and, consequently, may be a causal factor in terminating egg laying by depressing plasma LH levels. However, it has not been established that the experimental elevation of plasma prolactin concentrations inhibits LH secretion sufficiently to inhibit egg laying, or conversely, that the experimental reduction of plasma concentrations of prolactin increases clutch size.

Lea *et al.* (1981) suggested a mechanism by which prolactin promotes incubation and inhibits laying. They suggested that tactile information from an accumulating clutch of eggs stimulates neural pathways emanating from the brood patch and which terminate on hypothalamic VIP neurons, thereby stimulating VIP release into the hypophyseal portal vasculature and consequently elevating prolactin secretion. The resulting increase in plasma concentrations of prolactin is thought to promote further nest attendance through prolactin receptors in the hypothalamus (Buntin, Ruzycki & Witebsky, 1993) controlling the expression of incubation behaviour (see below). Consistent with this view, plasma prolactin levels drop when investigators remove incubating birds from their nests (El Halawani *et al.*, 1980; Goldsmith, Burke & Prosser, 1984; Ramsey, Goldsmith & Silver, 1985; Sharp *et al.*, 1988), and denervating (Book *et al.*, 1991) or anaesthetizing (Hall & Goldsmith, 1983) the brood-patch reduces nest attendance and prolactin levels.

Lea *et al.* (1981) further propose that the increase in plasma prolactin levels induced by nesting associated with a growing clutch eventually reaches a saturation threshold for the full expression of incubation behaviour. However, prolactin levels may continue to rise with prolonged incubation to reach a higher and second threshold which, if exceeded, results in inhibition of gonadotropin secretion. This idea is supported by a study on the turkey (Lea & Sharp, 1983), where it was observed that the expression of full incubation behaviour and egg laying can occur simultaneously. However, the concentration of circulating prolactin associated with full incubation behaviour was lower than that associated with the termination of egg laying. In the domestic chicken (Lea *et al.*, 1981), as in the American kestrel (Sockman & Schwabl, 2001*a*), the onset of incubation behaviour is gradual and is associated with a progressive increase in plasma prolactin levels presumably triggered by each bout of nesting behaviour.

In support of the mechanism proposed by Lea *et al.* (1981), experimental addition or removal of eggs before or during laying suggests for some species, including the American kestrel, that eggs in the nest may provide the tactile stimulus that terminates their production (Porter, 1975; Beukeboom *et al.*, 1988; Haywood, 1993*a*, *b*). In the absence of a threshold number of eggs in the nest, due to experimental egg-removal, laying continues far beyond the number normally laid. Likewise, eggs experimentally added to a nest early in the laying cycle can prematurely terminate laying. However, even when very little tactile stimulation from eggs is available, females at some point cease laying

eggs in egg-removal experiments, and therefore tactile stimuli alone are insufficient to terminate laying (Williams & Miller, 2003). Rather, variation in clutch size is probably a product of variation in the extent to which tactile stimulation from eggs disrupts follicular growth. Females laying large clutches would be less sensitive to stimulation and, in egg-removal experiments, may even lay almost indefinitely if tactile stimulation is minimal. Females laying small clutches would be more sensitive and, when tactile stimulation is minimal due to egg removal, they would still cease laying at some point (Williams & Miller, 2003).

One means by which prolactin may then play a role in clutch-size variation is by elevating sensitivity to tactile stimulation. However, this probably does not characterize the mechanistic basis for clutch-size control by prolactin in species such as the domesticated turkey or duck, in which prolactin levels change as a function of tactile input from the brood patch (see citations above). Thus, it seems that for these species, if prolactin is causally or permissively tied to clutch-size regulation, then it is not so much regulating sensitivity to tactile stimulation from the eggs as it is mediating the effects of tactile stimulation on clutch size. Whether turkeys, chickens, or ducks demonstrate significant variation in sensitivity to tactile stimulation from eggs is not known.

If prolactin does regulate clutch size, it probably does so in the days preceding termination of egg laying, either by inhibiting recruitment of primordial follicles into the hierarchy of follicles which will eventually undergo rapid yolk deposition, by inhibiting development and growth of follicles already recruited, or by inhibiting ovulation of large yolky follicles (Porter, 1975; Mead & Morton, 1985). Although the mechanism by which prolactin would inhibit egg production would differ for each of these three possible modes and although the precise timing of prolactin's functionality would likewise differ by a few days for each, relatively high prolactin levels would nonetheless need to occur between recruitment and ovulation of the last follicle one to several days before the last egg is laid. Therefore, the timing of this event relative to clutch *initiation* would itself depend on (i.e. determine) the eventual clutch size.

In our studies on the American kestrel, we found a negative correlation between the concentration of prolactin circulating in the female's blood plasma early during laying (when late-laid ovarian follicles are selectively recruited for rapid yolk deposition followed by ovulation and laying) (Porter, 1975) and her clutch size (Sockman *et al.*, 2000). Females with high prolactin concentrations on the day the first egg of a clutch was laid had smaller clutches than those with low concentrations on this day, consistent with the idea that the timing of the relative rise in prolactin levels during laying itself may determine how many additional follicles are recruited, yolked, ovulated, and eventually laid. However, when we experimentally elevated prolactin concentrations using osmotic minipumps on the day egg 2 of a clutch was laid, we did not observe any effect on clutch size (Sockman *et al.*, 2000).

This negative result is difficult to interpret for the following reasons. First, due to its availability, we used ovine prolactin instead of kestrel or even avian prolactin. Ovine prolactin administered to birds stimulates parental behaviours (Riddle *et al.*, 1935; El Halawani *et al.*, 1986; Sharp *et al.*, 1988; Pedersen, 1989; Youngren *et al.*, 1991). In the female American kestrel, it induces incubation behaviour (Sockman *et al.*, 2000) and can affect concentrations of yolk testosterone (Sockman *et al.*, 2001) (see below). But its effectiveness in clutch size regulation may be small, perhaps due to tissue-specific sensitivity. Second, the timing of this experimental elevation in prolactin concentrations may have been inappropriate, particularly in light of the possibility that the implanted osmotic minipumps may require two or three days after implantation to become fully effective. This raises the possibility that the time point at which females experienced a large surge in exogenous prolactin concentrations was well after each had already experienced an endogenous surge. For example, females which were predisposed to lay four or five eggs may be unresponsive to further rises in prolactin levels at the two- or three-egg stage because the mechanisms for restricting the clutch to four or five eggs may have already been initiated (Porter, 1975; Beukeboom *et al.*, 1988). It is possible that the only females that would have been affected by this treatment were the few predisposed to lay six eggs, probably too few for us to notice an effect. In other words, it is possible that clutch size was already physiologically determined in most of our females at the time our prolactin manipulation began to take effect.

In a second attempt to manipulate clutch size, we conducted an experiment in which we initiated exogenous prolactin administration on the day the first egg of a clutch was laid (Sockman *et al.*, 2001). Again, we observed no effect on clutch size. If clutch size is determined by prolactin-induced inhibition of follicular recruitment, this may still have been too late in the follicular cycle. Unfortunately, it was not feasible for us to anticipate onset of laying and implant pumps before clutch initiation. Until we are able to do so or can experimentally reduce prolactin levels early in the follicular cycle, we do not have strong evidence for a causal role of prolactin in the regulatory control of clutch size in the American kestrel.

It is also quite possible that prolactin plays little if any regulatory role in the control of clutch size in American kestrels, despite a putative role in stimulating or permitting onset of incubation. In fact, we found considerable variation within individual females in timing of incubation onset (relative to clutch completion) in the absence of variation in clutch size (Sockman & Schwabl, 2001*a*), suggesting that the mechanisms regulating these traits differ. When females that were allowed to lay a complete clutch early during a photoinduced breeding period were forced to renest later in this subjective breeding season, the time spent incubating early during the laying cycle for renests was more than twice that for first nests. However, clutch size did not differ between first and renests. Thus, we observed considerable variation between first and renests in the rate at which incubation developed relative to the termination of laying. A temporal dissociation such as this suggests the presence of separate mechanisms for the regulation of these two traits. Seasonal shifts in the sensitivity or threshold levels of prolactin necessary to exert an effect on clutch size may give rise to the temporal dissociation. Or, prolactin may

not be the primary factor involved in the regulation of clutch size. Other species, from gulls to songbirds, also show a dissociation between the timing of incubation onset and the cessation of laying (Sockman & Schwabl, 1999; Badyaev, Hill & Whittingham, 2002; Badyaev *et al.*, 2003*b*, 2005; Müller *et al.*, 2004), suggesting the possible presence of different mechanisms for clutch size determination and for regulation of incubation onset in these species. In fact, despite some plasticity in timing of incubation onset relative to clutch completion, individual females may display relatively little plasticity in clutch size (Christians, Evanson & Aiken, 2001), possibly due to the presence of a strong genetic or otherwise innate component to clutch size in some species (Gwinner *et al.*, 1995). In short, there is some support for prolactin playing a causal or permissive role in regulating egg laying in the turkey, quail, and white-crowned sparrow, as detailed above, but support for this role of prolactin in the American kestrel and other species is, at present, relatively weak.

(3) Prolactin and the regulation of yolk androgen deposition

Currently, the only evidence for a role of plasma prolactin in the regulation of the third component of reproductive effort described above – yolk androgen deposition – exists for the American kestrel. To our knowledge, however, the American kestrel is the only species in which evidence for this role has been sought, although circumstantial evidence exists for the black-headed gull, as well (see below).

As indicated above, prolactin concentrations in female American kestrels rise abruptly over the course of egg laying, with relatively low concentrations early in the laying cycle giving way to highly elevated concentrations by midway on through the latter portion of the laying cycle when incubation behaviour is developing (Sockman *et al.*, 2000). Also, as indicated above, concentrations of yolk androgens increase over the course of laying each successive egg in a clutch, with low concentrations for the first-laid egg leading to high concentrations for later eggs. Thus, there exists a temporal association between the rise in prolactin levels and the rise in yolk androgen concentrations over the course of egg laying. But do these rising prolactin levels *cause* the rise in yolk androgens over the course of laying a clutch? Experimental evidence from American kestrels suggests the answer to this question is yes, at least with respect to yolk testosterone. In the experiment in which we administered exogenous prolactin by subcutaneously implanting osmotic minipumps on the day the first egg of a clutch was laid (see above), the yolks of subsequently laid eggs of females with experimentally elevated prolactin levels contained two to three times the concentration of testosterone that the yolks of eggs from control females contained (Sockman *et al.*, 2001). We did not see an effect of exogenous prolactin administered to laying females on yolk androstenedione concentrations, raising the possibility for a dissociation between regulation of yolk testosterone and yolk androstenedione concentrations and for different functionality of the two androgen hormones (H. Schwabl and K. W. Sockman, unpublished data). In black-headed gulls, as the timing of incubation onset relative to clutch initiation advances, concentrations of yolk testosterone but not androstenedione increase, consistent with a possible dual role of prolactin in the stimulation of incubation behaviour and elevation of yolk testosterone concentrations (Müller *et al.*, 2004). In sum, the evidence supports the hypothesis that the rapid rise in prolactin concentrations over the course of egg laying promotes an increase in yolk testosterone levels with each successive egg in a clutch. It further suggests that females initiating clutches with low levels of prolactin (i.e. those most likely to lay large clutches and initiate incubation late relative to clutch initiation) are those which will deposit relatively low concentrations of androgen into the yolks of the eggs laid early in the laying sequence of a clutch. Of course, these are the females that are typically laying earliest in the season, suggesting, perhaps, that these females therefore have the lowest basal levels of plasma prolactin. Below we detail the evidence that the seasonal change in reproductive effort, which is determined by the seasonal decline in clutch size, the seasonal advance in incubation onset, and the seasonal increase in rate of yolk androgen deposition, is orchestrated by seasonal changes in prolactin concentrations.

(4) Seasonal regulation of reproductive effort by prolactin

As detailed above, clutch size declines seasonally in numerous bird species (Klomp, 1970). Females which initiate clutches early in the season lay more eggs than those which initiate clutches late in the season. In the American kestrel and other species, there is also a seasonal advance in the timing of incubation onset relative to clutch initiation. That is, maximal expression of incubation behaviour occurs sooner after onset of laying in late-season clutches than in early-season clutches (Sockman & Schwabl, 2001*a*). Also in American kestrels, the rate of yolk androgen deposition increases seasonally (Sockman *et al.*, 2001). High concentrations of prolactin circulating in the female's plasma apparently drive or at least permit onset of incubation, high concentrations of yolk androgens, and, in the domestic turkey and quail but probably not the American kestrel, the termination of egg laying. This suggests then that prolactin concentrations should increase seasonally, and, in fact, the evidence for an increase in basal concentrations of prolactin over the course of a breeding season is substantial and occurs in a diversity of bird species (Burke & Dennison, 1980; Dawson & Goldsmith, 1982; Wingfield & Goldsmith, 1990; Goldsmith, 1991; Chakraborty, 1995; Malecki *et al.*, 1997; Silverin & Goldsmith, 1997; Dawson & Sharp, 1998; Sreekumar & Sharp, 1998; Maney *et al.*, 1999*a*), including both the European (Meijer *et al.*, 1990) and the American kestrel (Sockman *et al.*, 2001). That is, females initiating clutches early in the breeding season are doing so when basal concentrations of plasma prolactin are relatively low, and those initiating clutches late in the breeding season are doing so when basal concentrations of plasma prolactin are relatively high (Sockman *et al.*, 2001). This suggests that some components of the seasonal modulation of reproductive effort, ultimately induced by the seasonal decline in

the reproductive value of the eggs, is proximally orchestrated, at least in part, by a seasonal increase in the female's plasma prolactin concentrations and the effects of these elevated concentrations on each of the determinants of reproductive effort detailed above – timing of incubation onset, rate of yolk androgen deposition, and, in the turkey and quail, cessation of laying (Fig. 6).

The above analyses of clutch size, timing of incubation onset, and rate of yolk androgen deposition reveal that the respective, seasonal changes in these three traits are temporally associated and that they vary in a coordinated fashion as a suite of traits. This, combined with our current, although still limited, understanding of the function of variation in these traits suggests that each is a component of reproductive output. Their temporal integration and common functionality led us to the hypothesis that a mechanism based on the functionality of a single hormone orchestrates their variation, and, indeed, some evidence suggests that changes in plasma prolactin concentrations form at least part of the basis of that mechanism. The model depicted in Fig. 6 illustrates how the ultimate, adaptive bases of variation in reproductive effort may be integrated with their possible regulatory mechanisms.

(5) Mechanism for effects of prolactin on reproductive effort

(*a*) *How prolactin may stimulate incubation onset*

A hypothetical mechanism by which prolactin may exert its effects on reproductive effort is depicted in Fig. 7. Because the timing of incubation onset is a behavioural phenomenon, at some level, it is necessarily controlled by the brain. Therefore, it would be reasonable to expect prolactin, presumably crossing the blood-brain barrier at the choroid plexus (Buntin, 1993), to bind to receptors located in regions of the avian brain that control incubation behaviour.

Several studies on domesticated species support the hypothesis that prolactin binds to receptors and stimulates brain regions important in regulating incubation and parental behaviour in general. Metabolic activity in many brain regions is greater in incubating than in non-incubating ring doves, and this increase in metabolic activity can be induced by exogenous treatment with prolactin (Lea *et al.*, 1994). In the turkey, lesions to the preoptic area and to the lateral hypothalamus and ventromedial nucleus of the hypothalamus prevent the onset of incubation but do not block increasing prolactin secretion associated with increasing day lengths (Youngren *et al.*, 1989). These lesions do block the rapid increase in prolactin concentrations that accompanies incubation, which would be expected if the behaviour of sitting on eggs is part of a positive feedback system which elevates further prolactin secretion (Lea *et al.*, 1981). Youngren *et al.* (1989) also found evidence for a neural pathway from the preoptic area through the lateral hypothalamus and ventromedial nucleus of the hypothalamus to the median eminence, where factors regulating pituitary prolactin release would be secreted. This would suggest a role in one or all three of these preoptic or hypothalamic regions for the action of prolactin on initiating

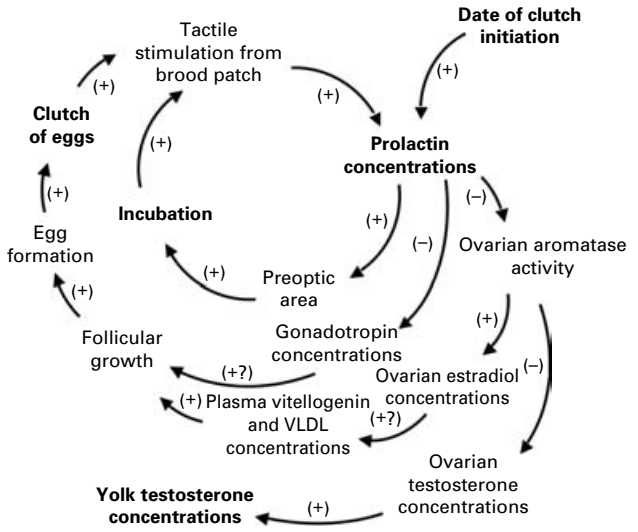

Fig. 7. Hypothetical mechanism by which seasonal changes in prolactin levels drive seasonal change in clutch size, timing of incubation onset, and rate of yolk-testosterone deposition (bold font). Positive (+) and negative (−) signs indicate the relationship between the two flanking factors. Photostimulation drives the seasonal increase in prolactin, which, in laying females, binds to the preoptic area of the diencephalon to induce incubation, which increases tactile input from eggs in the nest, which drives further increases in prolactin levels in a positive feedback loop that eventually results in full expression of incubation behaviour. Prolactin inhibits gonadotropin secretion from the anterior pituitary, which may inhibit follicular growth and therefore egg formation. Prolactin inhibits ovarian aromatase, the enzyme which irreversibly converts testosterone to estradiol. Inhibition of ovarian aromatase may have two principal effects. One is a reduction in concentrations of ovarian estradiol, which is thought to elevate circulating vitellogenin and very low density lipoprotein (VLDL) concentrations necessary for follicular growth. The other is an increase in the substrate for aromatase, testosterone. The lipophilic yolk then sequesters this accumulating testosterone. Despite the traditional notions that variation in gonadotropin concentrations give rise to variation in follicular growth and that variation in estradiol concentrations give rise to variation in plasma vitellogenin and VLDL concentrations, there is little evidence, at least in some groups of birds, for either of these putative phenomena, raising doubts about the role of prolactin in the regulation of clutch size.

incubation behaviour, although more conclusive evidence might come from experiments demonstrating that lesioned birds provided with exogenous prolactin do not initiate incubation. As expected, prolactin receptors are found in abundance in these areas in the brain of the domestic chicken (Ohkubo *et al.*, 1998), as well as the brains of ring doves and several songbird species (Buntin, 1993). In fact, the preoptic area specifically binds prolactin in all bird species so far examined in this way (Buntin, 1996), lending support to the notion that the preoptic area may be crucial for mediating the effects of prolactin on incubation behaviour in general and on onset of incubation in non-columbiform birds in particular (Fig. 7).

(b) How prolactin may elevate yolk androgen deposition and simultaneously inhibit egg production

Notwithstanding the limited evidence, if we tentatively assume that prolactin does regulate clutch size, what might be the mechanism and what might be the mechanistic basis behind the effects of prolactin on yolk androgen deposition? That the effects of prolactin on these two traits are mediated at the level of the ovary would be a reasonable hypothesis, because the ovary is proximally in control of follicular development, ovulation, and presumably steroid deposition into the yolk (Hackl *et al.*, 2003).

Prolactin has long been known to inhibit the ovary and oviduct itself (Clarke & Bern, 1980), but to understand how it might do this, it is important to first understand how the ovary synthesises some steroids. Along the pathways of steroid biosynthesis, the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) irreversibly converts dehydroepiandrosterone to androstenedione. 17β-hydroxysteroid dehydrogenase (17β-HSD) reversibly converts testosterone and androstenedione, and aromatase irreversibly converts testosterone to estradiol and androstenedione to estrone. Prolactin inhibits the activity of aromatase in rats (Dorrington & Gore-Langton, 1981; Tsai-Morris *et al.*, 1983; Papadopoulos, Drosdowsky & Carreau, 1986) and enhances the activity of both 3β-HSD and 17β-HSD in the bonnet monkey *Macaca radiata* (Gunasekar, Kumaran & Govindarajulu, 1988). If the same were true for birds, if the conversion of ovarian androstenedione to testosterone is limited by 17β-HSD activity, and if androstenedione is abundant, as suggested by the much higher androstenedione than testosterone levels in yolks (Sockman & Schwabl, 2000), then a prolactin-induced elevation of 17β-HSD and inhibition of aromatase might lead to a net accumulation of testosterone in the ovary and its developing yolky follicles (Zelinski-Wooten *et al.*, 1993). Yolk androstenedione levels would seemingly change little under the ovarian effects of prolactin, because they may be replenished by prolactin-induced inhibition of aromatase and prolactin-enhanced activity of 3β-HSD (Sockman *et al.*, 2001). In short, one means by which prolactin might elevate yolk testosterone deposition is by inhibition of ovarian aromatase activity (Fig. 7). To our knowledge, however, there is no direct evidence of this in birds, and, therefore, the regulation of yolk androgen concentrations by prolactin-induced inhibition of ovarian aromatase is only a hypothesis at the moment.

In species such as the quail, for which there is evidence that prolactin regulates follicular recruitment, development, and ovulation (Alexander & Wolfson, 1970; Camper & Burke, 1977) and hence egg production, what might be the mechanism? Follicular development is, in part, a product of follicular sequestration of yolk precursor proteins such as vitellogenin and very low density lipoprotein (VLDL) (Etches, 1996). Vitellogenin and VLDL are produced in the liver and secreted into the plasma under hepatic stimulation by ovarian estradiol (Christians & Williams, 1999*a*; Williams, 1999*a*, *c*). The plasma then caries vitellogenin and VLDL to ovarian follicles, which consequently and necessarily enlarge prior to ovulation (Challenger *et al.*, 2001). Vitellogenin and VLDL then serve as two of the major yolk resources for the developing embryo (Burley & Vadehra, 1989). Because the conversion of testosterone to estradiol by aromatase is irreversible, the inhibition of ovarian aromatase that may lead to an increase in yolk testosterone levels would simultaneously reduce ovarian estradiol synthesis. Under prolactin-induced reductions in estradiol, one might therefore expect a reduction of vitellogenin and VLDL levels, a reduction of follicular development that leads to ovulation, and hence a mechanism for the cessation of laying (Fig. 7).

In addition to its possible effects on ovarian aromatase, prolactin also inhibits secretion of LH in birds (Buntin *et al.*, 1988, 1999; Sharp *et al.*, 1988; You *et al.*, 1995; Dawson & Sharp, 1998). Thus, this may be an additional means by which the domestic turkey reduces estradiol concentrations and hence vitellogenin- and VLDL-induced follicular development. Still, as indicated above, whether or not this prolactin-induced inhibition of LH is sufficient to alter egg production is not known. Moreover, experimental evidence that prolactin can regulate egg laying (Alexander & Wolfson, 1970; Camper & Burke, 1977; Youngren *et al.*, 1991) and possibly clutch size is limited to the domestic turkey and quail.

V. EVOLUTION, CONSTRAINT AND DISSOCIATION OF MECHANISMS

The idea that changing concentrations of a single hormone might regulate multiple components of reproductive effort (at least in some species) may shed light on the evolution of phenotypic or inter-individual plasticity in these components. In fact, the existence of a regulatory mechanism based on a single chemical signal (prolactin) would raise the intriguing hypothesis that the evolution of any one of these traits may influence the evolution of the others. That is, seasonal change in timing of incubation onset and yolk androgen deposition may evolve as correlated traits, and thus, a change in one may drive change in the other. For example, if seasonal changes in both components are regulated by seasonal change in the concentrations of plasma prolactin, the evolution of the seasonal change in timing of incubation onset would require the evolution of the mechanism that gives rise to that trait (the seasonal change in prolactin levels and the effects of prolactin on incubation onset) and the simultaneous evolution of seasonal change in rate of yolk androgen deposition because it is also influenced by seasonal change in prolactin concentrations. Comparative studies on species that differ in the rate of seasonal change in either of these components of reproductive effort might be useful in this regard, for example, to determine if the other component shares comparable differences in its rate of seasonal change.

It is possible that a shared mechanism for variation in these traits impedes the independent evolution of one trait due to the consequences of changing another. A shared mechanism would prevent adaptive change in one trait without potentially maladaptive change in another. Thus, natural selection might favour individuals with seasonal

plasticity in timing of incubation onset (relative to clutch completion) in the absence of seasonal plasticity in clutch size. That is, natural selection might favour the independent physiological regulation of some of these traits.

As mentioned above, seasonal change in timing of incubation onset (relative to clutch completion) without change in clutch size has been observed in a diversity of bird species, from falcons (Sockman & Schwabl, 2001*a*), to gulls (Müller *et al.*, 2004) and songbirds (Sockman & Schwabl, 1999; Badyaev *et al.*, 2002, 2003*b*, 2005). In canaries, incubation onset is tightly associated with a profound reduction in estradiol concentrations, as one would expect if prolactin induces or permits onset of incubation and simultaneously inhibits ovarian aromatase activity (Sockman & Schwabl, 1999) (Fig. 7). Similar findings have been reported for European starlings (Williams, Kitaysky & Vézina, 2004). However, neither timing of incubation onset nor estradiol levels at the onset of incubation are closely associated with clutch size in canaries (Sockman & Schwabl, 1999). In other words, estradiol levels drop precipitously at the moment incubation begins, regardless of clutch size. In short, there is an apparent dissociation between clutch size and prolactin-induced timing of incubation onset (relative to clutch completion) in some species, and, in the canary, prolactin seems to also shut down estradiol secretion. These studies suggest that, in some species, prolactin may not directly regulate clutch size specifically because prolactin-induced variation in estradiol levels may not be involved in the regulation of clutch size.

Our model for the mechanisms by which prolactin might regulate reproductive output (Fig. 7) assumes for clutch-size regulation that variation in estradiol concentrations leads to variation in vitellogenin or VLDL secretion and subsequent accumulation of vitellogenin and VLDL in follicles. But this close link between circulating estradiol concentrations and vitellogenin and VLDL may not exist in some species. Although experimental elevation of estradiol levels in non-breeding zebra finches elevates vitellogenin and VLDL levels to concentrations observed in breeders, elevation of estradiol concentrations in breeders has no affect on vitellogenin, VLDL, clutch size, or egg size (Williams, 1999*c*). In female European starlings all at a similar stage of follicular development, tenfold variation in circulating estradiol concentrations does not correlate with variation in plasma vitellogenin levels or with the total mass of yolky follicles developing at the time of blood sampling (Williams *et al.*, 2004). Further, experimental elevation of estradiol concentrations elevates circulating vitellogenin and VLDL concentrations in starlings, but it does not increase egg or clutch size (Christians & Williams, 1999*a*). This suggests that although estradiol is important in multiple aspects of female gonadal function, ovarian activity and yolk deposition apparently proceed normally under highly variable levels in some species. In addition, prolactin can inhibit secretion of gonadotropins, such as luteinizing hormone, in European starlings, but large differences in concentrations of luteinizing hormone produce, at most, very small differences in follicular growth (Sockman *et al.*, 2004*b*).

Experimental evidence suggests that prolactin has an inhibitory role in the control of ovarian function in some species and hence control of clutch size, but in many other species there is no direct evidence that prolactin plays a role in the regulation of clutch size. The consensus view would be that prolactin stimulates or permits onset of incubation or maintains the behaviour once it is established, but that it does not invariably play a role in the regulation of clutch size. One important caveat here is that the dissociation between termination of egg laying and onset of incubation described above assumes incubation behaviour is accurately quantified. Problems identifying variation in incubation intensity could lead to the conclusion that incubation onset and cessation of laying are temporally dissociated. Nonetheless, we are still without strong evidence for a clutch-size regulation factor for birds in general, and, in fact there may not be one. That is, clutch size may be regulated differently, depending on the species.

What then explains the close temporal association between prolactin concentrations and the cessation of laying if prolactin is not causally involved? Of course, any answer to this would be speculative at this point. But based on the evidence provided in this review, it would seem that prolactin functionality during laying may be tied to the regulation of incubation onset and rate of yolk androgen deposition. We suggest that evolution of independent regulation of the determinants of reproductive effort gives females a relatively high level of control over reproductive effort and therefore flexibility to contend with predictable and unpredictable reproductive challenges and opportunities.

VI. GAPS AND FUTURE DIRECTIONS

This review only begins to address the ultimate and proximate bases for variation in reproductive effort in birds. Indeed, there exist many gaps in our knowledge. For example, we have concentrated on clutch size, timing of incubation onset, and rate of yolk androgen deposition as determinants of reproductive effort, but there are certainly many more determinants, such as timing of breeding onset, egg size, egg-laying interval, brood sex ratio, and yolk corticosterone (Eriksen *et al.*, 2003; Hayward & Wingfield, 2004; Hayward *et al.*, 2005) and antioxidant (Royle *et al.*, 2001; Saino *et al.*, 2002) deposition. Little is known regarding the mechanistic control of these other factors, although a recent review on the control of offspring sex-determination in birds suggests a role for prolactin, as well (Pike & Petrie, 2003). Still, this role included variation in yolk androgen deposition as a mediating factor (Petrie *et al.*, 2001), but the role of yolk androgens in sex determination has been questioned (Müller *et al.*, 2002; Pilz *et al.*, 2005), and it appears that, if yolk androgens do influence offspring sex, other factors must modulate their influence.

Prolactin concentrations may regulate offspring sex in a manner that is independent of yolk androgen deposition, as suggested by recent findings in the house finch (*Carpodacus mexicanus*) (Badyaev *et al.*, 2003*b*, 2005). In this sexually size-dimorphic (Badyaev *et al.*, 2002, 2003*a*) songbird species, females which initiate incubation with the laying of the first

egg produce clutches in which offspring sex depends, in part, on laying order within the clutch. Females initiating incubation sometime later produced no such sex-biased laying order in their clutches. If prolactin regulates timing of incubation onset in house finches, as it apparently does in other bird species, then prolactin may regulate the additional component of reproductive effort, offspring sex (Badyaev *et al.*, 2003*b*).

A major gap in our knowledge concerns the proximate basis for variation in how the multiple components of reproductive effort are orchestrated. The presence of prolactin isoforms and multiple forms of prolactin receptor suggests a potential for the functions of prolactin to change rapidly in response to selective forces. Comparative examination of species with different life histories and of individuals with different reproductive strategies in their circulating prolactin isoforms and in their tissue-specific expression of different forms of prolactin receptor may enhance our understanding of the proximate basis for the orchestration of reproductive effort.

Of particular interest would be first to determine the mechanistic basis for clutch-size regulation in birds. In fact, given its central importance as a determinant of reproductive effort, it is surprising more headway has not been made towards this end. Understanding how clutch size is regulated might elucidate the adaptive significance for a putative dissociation between its regulation and the regulation of other components of reproductive effort, such as egg size (Williams, 2001; Williams *et al.*, 2001), timing of incubation onset, and rate of yolk androgen deposition. Initial studies involving correlational evidence between prolactin levels and clutch size were promising, but experimental support is lacking. Consequently, it might prove fruitful to examine physiological mechanisms not directly linked to incubation or prolactin levels, such as mechanisms related to the newly characterized gonadotropin inhibitory hormone (Bentley *et al.*, 2003; Osugi *et al.*, 2004; Ubuka *et al.*, 2005) or to seasonal changes in photoperiodically dependent ''hypothalamic drive'' on neurons secreting gonadotropin-releasing hormone (GnRH), which travels *via* the portal vasculature to stimulate the pituitary secretion of the gonadotropins LH and follicle-stimulating hormone (FSH) (Sharp & Blache, 2003). The term ''hypothalamic drive'' refers to the aggregate of stimulatory and inhibitory inputs to GnRH neurones, and has been shown to decrease as a function of time exposed to the long-day photoperiods of spring. Evidence in support of this view comes from studies in birds that terminate breeding through the development of photorefractoriness (insensitivity to the stimulatory effects of long-day photoperiods) and on the LH response to castration (Stokkan & Sharp, 1984; Follett, 1988) or to testosterone treatment (Wilson, 1986) after exposure to a photostimulatory day length for various periods.

Although it seems LH is unlikely as a mediation factor between GnRH and clutch size (see above), an examination of FSH, its role in clutch size regulation, and how its secretion might be regulated independently of LH would be worthwhile. A. L. Johnson and co-workers have proposed that, for the domestic chicken, FSH is necessary for the maintenance of pre-hierarchical follicle viability and for the initiation of granulosa cell differentiation following selection of follicles into the hierarchy (Johnson *et al.*, 1996; Johnson, Bridgham & Jensen, 1999; Johnson & Bridgham, 2001; Woods & Johnson, 2005). Therefore, it is plausible that a decline in FSH levels might initiate pre-hierarchical follicular atresia and limit the number of follicles available for ovulation. Plasma FSH concentrations measured in incubating pied flycatchers (*Ficedula hypoleuca*) using a heterologous assay do not correlate with clutch size (Silverin & Goldsmith, 1983), but whether this is also true for laying individuals is not known. And, although porcine FSH administered to laying zebra finches does not affect clutch size (Christians & Williams, 2002), ovine FSH administered to tree pies (*Dendrocitta vagabunda*) elevates ovarian mass and follicle size (Chaudhuri & Maiti, 1998). Equivocal findings involving the use of heterologous assays and protein hormones from other species are difficult to interpret. Indeed, the role of the species-specific form of FSH on clutch size merits testing in any bird species.

In addition, the experimental evidence that prolactin can induce or permit onset of incubation behaviour is relatively scant and exists for only four groups of birds: kestrels and the domestic chicken, turkey, and budgerigar. Although correlational evidence for a role of prolactin in incubation onset exists for numerous other species, including a number of songbirds, the lack of experimental evidence weakens the case for prolactin as a general incubation factor in birds. In addition, prolactin probably does not induce incubation onset in Columbiformes. Instead, it seems that progesterone serves the purpose of triggering incubation onset and prolactin serves to maintain incubation (once it has already begun) and stimulate production of crop milk and parental feeding (Lehrman, 1958; Lehrman & Brody, 1961; Buntin & Tesch, 1985; Janik & Buntin, 1985; Buntin *et al.*, 1991; Buntin, 1996; Wang & Buntin, 1999).

Evidence that prolactin is involved in the mediation of yolk testosterone deposition exists for only the American kestrel, although circumstantial evidence exists for black-headed gulls as well. Of course, to our knowledge, the effects of prolactin on yolk testosterone deposition have been tested only in kestrels, so of immediate interest would be for more studies on other species for this putative role, as well as the role of prolactin on the deposition of other yolk steroids, such as androstenedione. Because deposition of androstenedione and testosterone into yolks are apparently under different regulatory control mechanisms (Sockman *et al.*, 2001; Müller *et al.*, 2004), it is important to determine whether and what the separate functions of these two yolk androgens might be. To date, nobody has examined the role of yolk androstenedione in the absence of yolk testosterone. Although androstenedione is thought to bind only to a single androgen receptor to which testosterone also binds and may serve simply as a reservoir for the synthesis of testosterone, it is also possible that it serves functions independent of those served by testosterone, particularly if its affinity for the androgen receptor differs from that of testosterone.

For some species, any potential role for prolactin in the regulation of yolk-androgen deposition may not be as straightforward as it appears to be in the kestrel. For example, in canaries, yolk androgen concentrations show a

seasonal decline (Schwabl, 1996*a*), despite the species' seasonal increase in prolactin levels (Goldsmith, 1982). It is possible that prolactin does not regulate yolk androgen concentrations in this species. Alternatively, prolactin may serve the same role as in the kestrel but be supplemented by other factors. For example, in the hypothetical absence of a seasonal increase in prolactin levels, yolk testosterone concentrations in the canary might show an even steeper seasonal decline than they do in the presence of a seasonal increase in prolactin. In the cattle egret (*Bubulcus ibis*) (Schwabl *et al.*, 1997), the zebra finch (Gil *et al.*, 1999), and the American coot (Reed & Vleck, 2001), levels of yolk androgens are higher in first- than in later-laid eggs of the clutch. Whether prolactin concentrations during the follicular cycles of these species track these changes in yolk androgen concentrations would be of interest.

The mechanistic basis for timing of the onset of egg laying is a result of an interaction between the birds' photoperiodic response and non-photoperiodic environmental factors (Wingfield, Hahn & Doak, 1993; Hahn, 1995; Hahn *et al.*, 1997; Ball & Bentley, 2000; Ball & Balthazart, 2002). But what is not clear is how this mechanism is related to clutch size, incubation timing, rate of yolk androgen deposition, and other factors that might affect reproductive effort, such as brood sex ratio. It is of particular interest because early breeders tend to have higher reproductive success than late breeders. Unfortunately, virtually nothing is known about the mechanisms giving rise to the fact that some individuals breed early in the season and others late, though recent evidence suggests a role for priming of the hypothalamo-pituitary-gonadal axis by experience with photostimulation (Sockman *et al.*, 2004*b*). Additionally, little is known about the physiological mechanisms that link body condition with seasonal timing of breeding or about the basis that might give rise to the vast between-individual (but within-population) variation in reproductive parameters such as clutch size, timing of incubation onset, and rate of yolk androgen deposition. Towards that end, one might begin looking for such a basis by examining tissue-specific variation in prolactin sensitivity with a thorough examination of prolactin receptor expression.

As mentioned above, in many species there is large within-population variation in the timing of breeding onset and parameters of reproductive output such as clutch size. Much of this variation is associated with female age; in most iteroparous species, female reproductive output increases with age, particularly between the first and second year of breeding (Clutton-Brock, 1988; Sæther, 1990; Rowe *et al.*, 1994; Forslund & Part, 1995). According to life-history theory, an age-related increase in reproductive effort is due to the fact that the probability of future reproduction declines with age (Williams, 1966*a*). Therefore, in older individuals, a given level of effort has less impact on future reproductive success than it does in younger individuals. On a proximate level, some investigators attribute the age-related increase in reproductive output to variation in reproductive experience (Williams, 1966*a*; Pugesek, 1981; Sæther, 1990; Forslund & Part, 1995; Sockman *et al.*, 2004*b*), but what experiences are relevant and how are they physiologically transduced to effect an increase in reproductive effort? A recent study on European starlings suggests that one important experience that potentially gives rise to age-related increases in reproductive effort is experience with photostimulation (Sockman *et al.*, 2004*b*). A temperate-zone female breeding for the first time in her life is experiencing photostimulation for the first time, whereas an older female has had prior experience with photostimulation. In starlings, photo-experience primes the hypothalamo-pituitary-gonadal axis such that it responds more rapidly and robustly upon second exposure to photostimulation (Sockman *et al.*, 2004*b*). Could such a physiological priming effect be a basis for the age-related increase in reproductive effort? An increase in clutch size is one way to increase reproductive effort, but are more subtle changes also apparent? For example, females could increase effort by investing more in late-laid offspring, and one way in which they might do this would be to hatch them more synchronously with siblings from other eggs and consequently feed them more. This leads to the prediction that older females should delay the onset of incubation, which itself might be reflected in a delayed rise in prolactin concentrations.

Many unanswered questions related to the ultimate and proximate bases for flexibility in avian reproductive effort remain. Still, we feel we have provided compelling evidence for the presence of a coordinated suite of traits that, when regulated together, can serve the common function of optimizing reproductive effort by preparing females for the predictable seasonal decline in the reproductive value of their eggs and, simultaneously, an aseasonal unpredictability of resources and offspring viability. The traits on which we focused, clutch size, timing of incubation onset, and deposition of yolk androgens, have usually been examined in isolation without consideration of their interrelatedness. Because it is possible that the effects of yolk androgens on nestling development depend heavily on a pre-existing nestling hierarchy induced principally by timing of incubation onset and because the effects of timing of incubation onset depend heavily on the duration of the laying cycle and hence clutch size, examining these traits as components of a larger trait, reproductive effort, is likely to yield greater understanding of each individual component.

VII. CONCLUSIONS

(1) How much effort to expend during a given bout of reproduction is one of the most important decisions made by an individual which breeds more than once in its lifetime. This is because reproduction comes at a cost in terms of future reproduction. The more effort expended in any given reproductive bout, the less the individual will be able to spend in future bouts. Thus, an individual should pace its reproductive effort throughout its probable reproductive lifespan in order to maximize its lifetime reproductive success.

(2) In birds, reproductive effort is largely determined by clutch size. Clutch size sets the maximum number of offspring produced in any given bout of reproduction. A major source of variation in clutch size is based on time in the

breeding season. In the vast majority of single-brooded species, clutch size declines seasonally, and this is ultimately due to the predictable seasonal decline in the reproductive value of the offspring.

(3) Establishment of a developmental hierarchy among sibling nest mates may also be an important determinant of reproductive effort. The presence of a sibling developmental hierarchy can increase the efficiency of culling a brood too large for an unexpectedly low supply of resources, can increase the efficiency of culling a brood in which more young develop successfully than can easily be reared, and can reduce energy costs of raising a brood. The establishment of the sibling developmental hierarchy therefore may help female birds cope, in part, with unpredictable reproductive challenges or opportunities.

(4) The developmental hierarchy is a product of the timing of incubation onset relative to clutch completion and possibly differences in yolk androgen concentrations between sibling eggs. The first-laid eggs of a clutch are the first exposed to development-inducing incubation temperatures if incubation begins before clutch completion. Consequently, they will be the first to hatch, giving rise to offspring that are developmentally advanced relative to their later-hatching siblings. Yolk androgens can affect the rate of nestling growth, and, therefore, different concentrations among sibling yolks can affect how quickly nestlings grow relative to their siblings. Yolk androgens may therefore affect the sibling developmental hierarchy together with timing of incubation onset.

(5) The mechanisms by which a female bird establishes a developmental hierarchy, through the timing of incubation onset and possibly the rate of yolk androgen deposition, depend on the duration of the laying cycle and hence the clutch size. Thus, the seasonal decline in clutch size may ultimately give rise to a seasonal advance in the timing of incubation onset and a seasonal increase in the rate of yolk androgen deposition. This presumably enables the female to contend with unpredictable reproductive challenges and opportunities while simultaneously adjusting reproductive output according to the predictable seasonal decline in the reproductive value of the offspring.

(6) Evidence that prolactin inhibits ovarian function and hence egg laying is inconsistent across species, but there is consistent, although limited, evidence that prolactin plays a role in either the timing of incubation onset or in the maintenance of incubation, and in the rate of yolk androgen deposition over the course of the laying cycle. Further, prolactin concentrations in numerous bird species increase seasonally, potentially providing a regulatory signal for the changes in timing of incubation onset and rate of yolk androgen deposition described above. Therefore, there is some evidence for the presence of a mechanism, based on the functionality of a single hormone, that orchestrates some of these behavioural and physiological traits and which enables female birds to tune reproductive effort according to predictable and unpredictable reproductive challenges and opportunities.

(7) A proposed mechanism by which a rising concentration of plasma prolactin might simultaneously inhibit egg production (in a few species), stimulate incubation onset, and elevate yolk androgen deposition is as follows. Prolactin levels increase due to tactile stimulation from eggs in the nest, and this prolactin binds to receptors in the preoptic area of the diencephalon which itself stimulates incubation behaviour, further tactile stimulation from the eggs, and more prolactin release. We hypothesize that prolactin also inhibits ovarian aromatase activity and therefore elevates testosterone levels and simultaneously reduces estradiol production. The yolky follicle surrounding the steroidogenic thecal and granulosa cells of the ovary sequester this accumulating testosterone. Lack of estradiol may reduce secretion of the primary yolk precursors vitellogenin and very low density lipoprotein (VLDL), which may lead to the cessation of follicular development and hence egg production.

(8) Evidence from some species suggests that prolactin does not invariably trigger cessation of laying, possibly due to a lack of relationship between concentrations of plasma estradiol and vitellogenin and VLDL, the primary yolk precursors. This raises the question as to why, from an adaptive perspective, these behavioural and physiological traits should be regulated independently. We suggest the independent regulation of clutch size and other determinants of reproductive effort gives females greater control of reproductive effort and therefore greater flexibility to contend with predictable and unpredictable reproductive challenges and opportunities.

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