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## Plasma corticosterone in American kestrel siblings: effects of age, hatching order, and hatching asynchrony

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### Abstract

Although it is well documented that hatching asynchrony in birds can lead to competitive and developmental hierarchies, potentially greatly affecting growth and survival of nestlings, hatching asynchrony may also precipitate modulations in neuroendocrine development or function. Here we examine sibling variation in adrenocortical function in postnatally developing, asynchronously hatching American kestrels (*Falco sparverius*) by measurements of baseline and stress-induced levels of corticosterone at ages 10, 16, 22, and 28 days posthatching. There was a significant effect of hatching order on both baseline and stress-induced corticosterone levels during development and these effects grew stronger through development. First-hatched chicks exhibited higher baseline levels than later-hatched chicks throughout development and higher stress-induced levels during the latter half of development. Furthermore, there was significant hatching span (difference in days between first- and last-hatched chicks) × hatching order interaction on both baseline and stress-induced corticosterone levels during development. Hatching span was also positively correlated with both measures of corticosterone and body mass in first-hatched chicks, but was negatively correlated with these factors through most of the development in last-hatched chicks. It is known that hatching asynchrony creates mass and size hierarchies within kestrel broods and we suggest that hierarchies in adrenocortical function among siblings may be one physiological mechanism by which these competitive hierarchies are maintained.

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**Keywords:** Corticosterone; Stress response; Hypothalamic–pituitary–adrenal (HPA) axis; Sibling variation; Development; Hatching asynchrony; American kestrel

Hatching asynchrony is common in altricial birds (Clark and Wilson, 1981; Magrath, 1990) and has been suggested to have evolved to adjust brood size to the prevailing conditions during rearing of nestlings (Lack, 1954, 1968; Magrath, 1989), resulting in a brood with chicks of different ages and sizes (Clark and Wilson, 1981; Mock, 1984). These age differences among siblings can lead to competitive and developmental hierarchies (Mead and Morton, 1985; Evans, 1996; Nilsson and Svenson, 1996; Stoleson and Beissinger, 1997). Older, and characteristically larger, siblings can dominate in competition for parentally provided resources and through a positive feedback loop fur-

ther enhance their competitive ability and, hence, resource gain. Hatching asynchrony can therefore greatly affect growth and survival of nestlings (Howe, 1976; Zach, 1982; Bortolotti, 1986; Sockman and Schwabl, 2000). Schwabl (1999) proposed that hatching asynchrony may also precipitate modulations in neuroendocrine development or function, potentially giving rise to differences in adrenocortical development among siblings.

Adrenocortical function is characterized by the secretion of corticosterone, the primary glucocorticosteroid released by the adrenal gland in birds (Kitaysky, Piatt, Wingfield, and Romano, 1999). Variations in baseline and acute stress-induced levels of corticosterone are known to correlate with unpredictable nonpathogenic events such as harsh weather (Wingfield, 1994), in addition to predictable events such as seasonal cycles, reproductive season, and migration (Romero, Ramenofsky, and Wingfield, 1997). In adult birds, the

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induction of the stress response increases foraging behavior, mobilizes stored energy reserves, and can trigger irruptive migration and abandonment of reproduction (Astheimer, Buttemer, and Wingfield, 1992; Bray, 1993; Wingfield, Breuner, and Jacobs, 1997). These behavioral responses can improve adult survival during food shortages (Astheimer et al., 1992). Our present understanding of the function, adaptive significance, and proximate sources of adrenocortical variation in developing birds is very limited. Studies in developing birds to date have examined associations with dietary restriction (Le Ninan, Cherel, Sardet, and Le Maho, 1988; Kitaysky et al., 1999), body condition (Heath and Dufty, 1998), timing of nest departure (Heath, 1997; Kern, Bacon, Long, and Cowie, 2001), sibling dominance–subordination relationships (Núñez-de la Mora, Drummond, and Wingfield, 1996; Ramos-Fernández, Núñez-de la Mora, Wingfield, and Drummond, 2000), and ontological changes in relation to varying reproductive modes (Holmes and Kelley, 1976; Wentworth and Hussein, 1985; Carsia, Morin, Rosen, and Weber, 1987; Holmes, Cronshaw, Collie, and Rohde, 1992; Schwabl, 1999; Love, Bird, and Shutt, 2003). A study of wild American kestrel (*Falco sparverius*) chicks where the authors removed the entire brood and sampled each individual once, in sequential order, found no difference among siblings (Sockman and Schwabl, 2001). However, since corticosterone increases over time after capture, comparison among siblings in regard to corticosterone levels was not possible in this study since chicks were sampled at different times after removal from their nest.

In the present study, we wished to investigate corticosterone levels in American kestrel siblings during postnatal development as done by Sockman and Schwabl (2001), however, using a repeated sampling procedure which controls for variation in corticosterone secretion over time (Wingfield, 1994), thereby allowing sibling comparisons. The American kestrel is a cavity-nesting raptor which exhibits hatching asynchrony leading to pronounced developmental hierarchies (Bortolotti and Wiebe, 1993; Wiebe and Bortolotti, 1994, 1995; Sockman and Schwabl, 2000). Since variation in corticosterone levels has been related to begging in one species (Kitaysky, Wingfield, and Piatt, 2001), correlated with hierarchies in both birds (Rohwer and Wingfield, 1981; Schwabl, Ramenofsky, Schwabl-Benzinger, Farnier, and Wingfield, 1988) and mammals (see Creel, 2001, for review), and given the existence of developmental hierarchies in kestrel broods, it is reasonable to hypothesize that corticosterone may be correlated with hatching and or laying order in this species. In the present study, we wished to examine chicks whose parents had access to unrestricted food during chick rearing in order to examine possible correlations of corticosterone levels with hatching order and hatching span while removing the known effects of food restriction on corticosterone levels. The objectives were (1) to investigate the dynamics of sibling adrenocortical function through measures of corticosterone during postnatal development and (2) to determine whether natural variation

in hatching asynchrony was related to variation in adrenocortical function among chicks in a nest. Baseline and acute stress-induced levels of corticosterone were measured in captive sibling kestrels from nests of four siblings only, during four successive postnatal developmental stages.

## Materials and methods

### *Species and reproductive parameters*

The study was conducted at the Avian Science and Conservation Centre of McGill University from April to July 1999 using captive birds reared from captive-raised adults. We adhered to the standards of the McGill University Institutional Animal Care and Use Committee for the humane treatment of our subjects. Twenty-five pairs of banded adult kestrels of known pedigree were removed from same-sex communal flight pens and placed in outdoor breeding pens measuring  $3.5 \times 5.5 \times 3.0$  m (l  $\times$  w  $\times$  h). Pairs were genetically unrelated within the last seven generations and, depending on pairs, egg laying began within 10–13 days following pairing. Eggs were individually marked to keep track of laying order and hatching order was determined by checking nest boxes for hatching three times daily. Once hatched, chicks were marked with nontoxic colored felt markers daily for individual identification until 16 days of age, at which time they were marked with a colored plastic leg band. Parents were fed a diet of three day-old cockerels until hatching, at which time they were fed this in addition to 1.5 day-old cockerels per nestling, per day. At no time during the experiment was the food supply exhausted by parents raising chicks. Birds were supplied with water for drinking and bathing daily.

Nest boxes were checked each morning between 08:00 and 09:00 to assign lay dates with the prior knowledge that kestrels lay one egg every 2 days (Bortolotti and Wiebe, 1993). Egg length and width were measured only upon clutch completion using digital calipers so as not to disturb the laying female unnecessarily. Egg volume was calculated based on Hoyt (1979) using the equation  $0.51 lw^2$ . Since Wiebe and Bortolotti (1994) found a strong relationship between hatching span and relative difference in nestling mass in American kestrels (RDNM, the difference between the smallest and largest nestling divided by the mean nestling mass), we chose to use hatching span as a measure of hatching asynchrony (difference in days between first- and last-hatched chicks within a nest). In this study, only 3 of 64 chicks showed a hatching order discrepancy from laying order, and therefore in this study these terms were considered interchangeable. We chose to use the term “hatching order” in order to examine sibling differences.

Captive chicks hatch after 28–30 days of incubation and depend on adults for the delivery of prey and feeding. Eyes are partially open by the first or second day (Roest, 1957; Bird and Palmer, 1988) and thermoregulation is accom-

plished by age 8 to 10 days (Bird and Palmer, 1988). By 7 days they begin to show stereotypical defense behavior of rolling on their backs and presenting talons and are fully able to defend against nest box intruders by 16 to 18 days of age. They reach adult body mass by 22 days, fledge between 26 and 28 days in captivity, and in the wild are dependent on parents for food for about 12–14 days (Bird and Palmer, 1988; Varland, Klass, and Loughin, 1991).

#### *Blood sampling and general procedure*

Blood samples were taken from chicks at the ages of 10, 16, 22, and 28 days posthatching between 10:00 and 15:00 to minimize time of day effects on circulating plasma corticosterone levels (Wingfield, Vleck, and Moore, 1992). These ages were chosen to cover development sufficiently as well as focus on major stages of muscular and behavioral development. Sixteen broods of chicks were used in this experiment involving a total of 64 chicks, with each chick being blood sampled during each of the four age classes. To control for differences between clutches, only broods of four eggs were used. Birds underwent a standardized capture, handling, and restraint protocol known to elicit an increase in the circulating hormone corticosterone (Wingfield et al., 1992). Birds were removed from their nest boxes and a stopwatch was used to record the time of first contact when the nest box door was opened. Birds were transported in  $45 \times 35 \times 40$  cm (l  $\times$  d  $\times$  h) insulated plastic coolers with 10-mm holes drilled for ventilation. Ten-day-old chicks were kept warm using a hot-water bottle covered with a towel placed at the bottom of the cooler. Individual birds were first weighed to determine maximal amounts of blood to be sampled based on total body mass. Between 25 and 75  $\mu$ l (depending on the age of the chick) of whole blood was collected from the brachial vein as soon as possible after removal of the bird from its pen ( $\sim$ 1–2 min) using a heparinized 27-gauge needle and 1-ml syringe. Additional samples were collected at 5, 10, 30, and 45 min after removal from the nest. All initial samples were taken in under 2 min following capture and linear regression analyses showed no effect of time after capture (within 0- to 2-min interval) on plasma levels of corticosterone in initial blood samples ( $r^2 = 0.026$ ,  $F_{1,62} = 0.641$ ,  $P = 0.5762$ ). Thus, initial blood samples were considered to reflect baseline levels of corticosterone.

Staggering in same-brood sampling was possible due to asynchrony in the age of chicks within a nest. If two chicks were of the same age in one nest, baseline samples were still obtained within the above-mentioned 2-min limit. Between blood collections, birds were placed back in their individual coolers which were located in a quiet and dark location. Following the sampling procedure, beak and tarsus length were measured. Whole blood samples were centrifuged at 10,000 rpm for 10 min and plasma was removed and stored frozen at  $-30^\circ\text{C}$  for radioimmunoassay analysis. The sex of each bird was identified based upon dimorphic plumage

which is clearly visible by the age of 12 days (Bird and Palmer, 1988).

#### *Corticosterone radioimmunoassay analysis*

Plasma levels of corticosterone were measured using a specific radioimmunoassay (RIA) at the National Wildlife Research Centre, Hull, Quebec. Plasma samples were thawed, vortexed, and diluted at a 1:10 ratio of plasma to steroid diluent using RIA kits (ICN Biomedicals, Inc., Costa Mesa, CA, Cat. No. 07-120103) designed for mouse and rat corticosterone analysis. Kits were validated in-house for avian plasma using standard RIA analysis techniques described by Wingfield, Smith, and Farner, (1982). Briefly, we used dextran-coated charcoal to strip the plasma and then determined plasma corticosterone interferences by measuring a range of plasma: (1) keeping the plasma volume constant and (2) with the plasma volume varied. We also ran range-finding experiments to determine the dilutions of stress-induced and baseline plasma which generated corticosterone concentrations in the linear portion of the standard curve. Finally, we checked the inter- and intraassay variation using a Herring gull (*Larus argentatus*) plasma pool.

During the assay of kestrel samples, 100  $\mu$ l of each diluted plasma sample was added in duplicate to labeled assay tubes. A total of 200  $\mu$ l each of  $^{125}\text{I}$ -labeled corticosterone and then corticosterone anti-serum were added to each tube, vortexed, and allowed to incubate for 2 h. Then 500  $\mu$ l of steroid precipitant was added, vortexed, and centrifuged for 15 min at 2300 rpm. The supernatant was removed and analyzed with a Packard Cobra II Auto Gamma-counter (Model No. E5002). Standard concentrations of corticosterone were used to develop a standard binding curve before each day's analysis. Recovery values ranged from 84 to 96% and were used to adjust assayed concentrations of corticosterone. Quality control samples were run with every set and intra- and interassay coefficients of variation were 9.4 and 11.6%, respectively.

#### *Data analysis*

Baseline and stress-induced corticosterone data were analyzed using a repeated measures analysis of variance (ANOVA) with age, hatching order, and hatching span in the model. In addition, to examine possible interactions between hatching order and hatching span on baseline and stress-induced corticosterone levels, separate ANOVAs were performed for each age category. Sibling comparisons of stress-induced corticosterone levels were calculated as a maximal increase (value at 10 min after capture-baseline value) to account for significant differences between siblings in baseline corticosterone levels. These values are referred to as stress-induced corticosterone levels from this point onward. Since altricial nestlings are in a very rapid state of growth and development, the use of a body condi-

tion measure based on a relationship between body mass and size may be inappropriate. Potentially then, the use of body mass alone is a better measure of nestling condition for a rapidly growing altricial nestling. However, because of the strong covariation between body mass and age, it is inappropriate to include both as independent variables in a single model (Sockman and Schwabl, 2001). Therefore, regression analysis was used to examine the relationship between baseline and stress-induced corticosterone levels and mass during development. During statistical analyses initial data were tested for homoscedastic assumptions required by a parametric statistical test according to Sokal and Rohlf (1995). At no time were any of these assumptions violated, and therefore data transformation was not necessary. Preliminary analysis indicated that baseline and stress-induced corticosterone did not vary with time of day, season, nestling condition, or sex. We therefore excluded these variables from subsequent models.

## Results

### Baseline levels of corticosterone

Baseline levels of corticosterone increased significantly during postnatal development (age effect:  $F_{3,144} = 11.5$ ,  $P < 0.001$ ; Fig. 1) were significantly related with hatching order (hatching order effect:  $F_{3,144} = 6.7$ ,  $P < 0.05$ ; Fig. 2) and were significantly related to hatching asynchrony (hatching span effect:  $F_{6,144} = 7.8$ ,  $P < 0.01$ ; Fig. 1). There also existed significant interactions between all factors (age  $\times$  hatching order:  $F_{9,144} = 20.2$ ,  $P < 0.0001$ ; age  $\times$  hatching span:  $F_{18,144} = 6.35$ ,  $P < 0.05$ ; hatching order  $\times$  hatching span:  $F_{18,144} = 10.1$ ,  $P < 0.05$ ; and age  $\times$  hatching order  $\times$  hatching span:  $F_{54,144} = 21.5$ ,  $P < 0.01$ ). When examining within each age group, hatching order was significant at all ages (all  $P < 0.05$ ), and hatching span was significant at all ages except 10 days (all  $P < 0.05$ ). Interaction between hatching order and span was significant at 16, 22, and 28 days (all  $P < 0.05$ ) (Fig. 1).

### Acute stress-induced levels of corticosterone

Chicks of all four age stages responded to the standardized handling protocol with a rapid increase in the secretion of corticosterone (Fig. 2). Stress-induced levels of corticosterone increased significantly during postnatal development (age effect:  $F_{3,144} = 12.3$ ,  $P < 0.01$ ; Figs. 2 and 3) were significantly related with hatching order (hatching order effect:  $F_{3,144} = 13.5$ ,  $P < 0.01$ ; Figs. 2 and 3) and were significantly related to hatching asynchrony (hatching span effect:  $F_{6,144} = 7.4$ ,  $P < 0.05$ ; Fig. 3). There also existed significant interactions between all factors (age  $\times$  hatching order:  $F_{9,144} = 23.6$ ,  $P < 0.01$ ; age  $\times$  hatching span:  $F_{18,144} = 11.6$ ,  $P < 0.001$ ; hatching order  $\times$  hatching

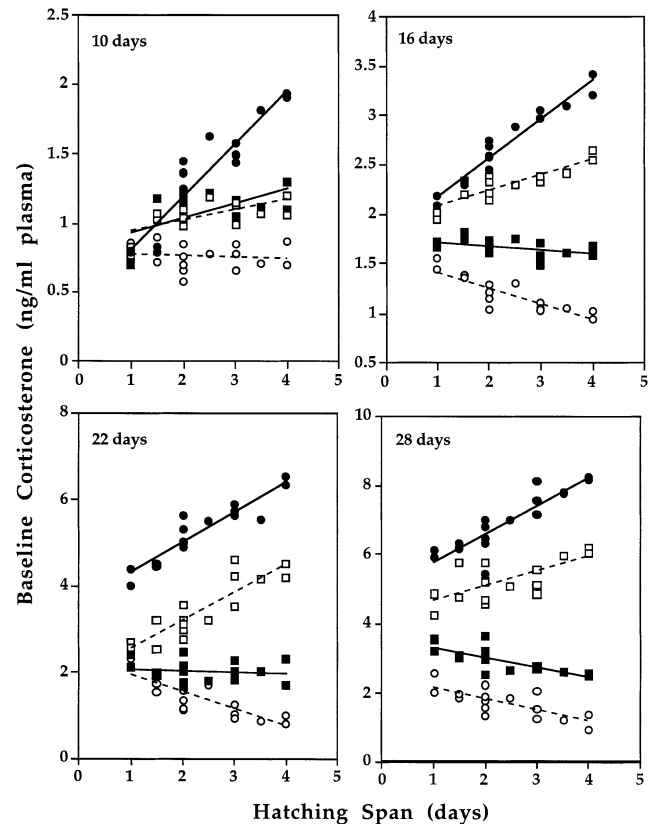


Fig. 1. Baseline corticosterone levels in sibling captive American kestrel chicks during postnatal development in relation to hatching span (difference between first- and last-hatched chick in days).  $N = 16$  broods for each age class. First-hatched chicks (—●—), second-hatched chicks (—□—), third-hatched chicks (—■—), and fourth-hatched chicks (—○—).

span:  $F_{18,144} = 29.3$ ,  $P < 0.0001$ ; and age  $\times$  hatching order  $\times$  hatching span:  $F_{54,144} = 14.2$ ,  $P < 0.001$ ). When examining within each age group, hatching order, hatching span, and the interaction between hatching order and span were significant at 16, 22, and 28 days (all  $P < 0.05$ ) (Fig. 3).

### Mass, corticosterone, and hatching order/span

When controlling for age, ANOVA indicated that mass was significantly related to hatching order, hatching span, and the interaction between hatching order and span at 16, 22, and 28 days (all  $P < 0.05$ , Fig. 4). In addition, linear regression analysis indicated that age-corrected body mass was significantly positively correlated with baseline corticosterone among siblings (chick 1:  $r^2 = 0.23$ ,  $P < 0.05$ ; chick 2:  $r^2 = 0.25$ ,  $P < 0.05$ ; chick 3:  $r^2 = 0.32$ ,  $P < 0.01$ ; chick 4:  $r^2 = 0.36$ ,  $P < 0.001$ ). Finally, linear regression analysis indicated that age-corrected body mass was also significantly positively correlated with stress-induced corticosterone levels among siblings (chick 1:  $r^2 = 0.12$ ,  $P < 0.05$ ; chick 2:  $r^2 = 0.18$ ,  $P < 0.05$ ; chick 3:  $r^2 = 0.29$ ,  $P < 0.01$ ; chick 4:  $r^2 = 0.31$ ,  $P < 0.01$ ).

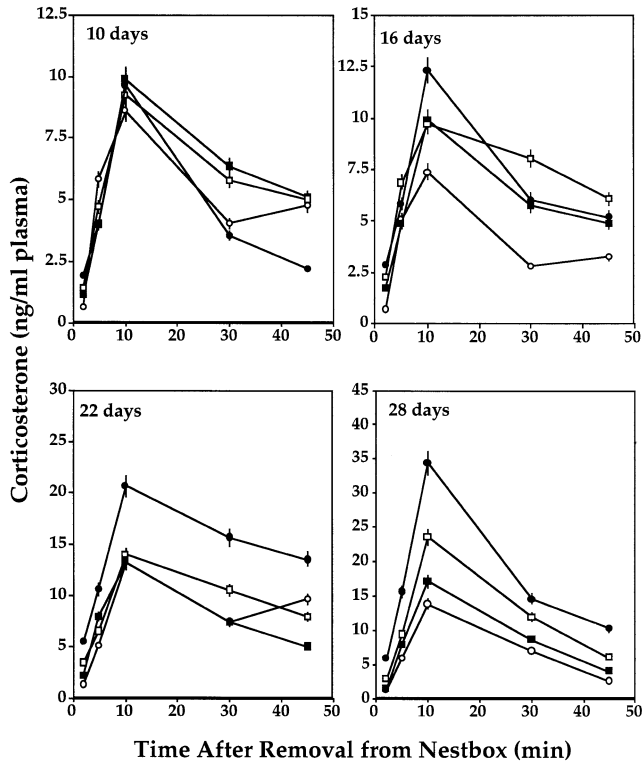


Fig. 2. Plasma profiles of corticosterone secretion (mean  $\pm$  SE) in response to capture and handling in sibling captive American kestrel chicks during postnatal development in relation to hatching span (difference between first- and last-hatched chick in days).  $N = 16$  broods for each age class. First-hatched chicks (—●—), second-hatched chicks (—□—), third-hatched chicks (—■—), and fourth-hatched chicks (—○—).

**Discussion**

Our results indicate that both hatching order and hatching span can significantly affect baseline and stress-induced corticosterone levels in captive sibling American kestrels, with earlier-hatched chicks exhibiting higher levels than later-hatched chicks throughout postnatal development. In addition, whereas first-hatched chicks exhibit increasing corticosterone levels in relation to increasing hatching span through development, fourth-hatched chicks exhibit decreasing levels with increasing hatching span. To our knowledge, this is the first known study to show that hatching asynchrony has an effect on sibling variation in adrenocortical function.

Four studies to date have examined potential sibling variation in adrenocortical activity. Schwabl (1999) reported variation in baseline levels of corticosterone in the altricial canary (*Serinus canaria*): first-hatched, fledged chicks aged 23 days had higher baseline corticosterone levels than third- and fourth-hatched chicks. In fact, a trend for higher levels in first-hatched chicks was already established at 15 days posthatching, just before young usually fledge. Sockman and Schwabl (2001) found no effect of hatching order on corticosterone levels in wild American kestrels; however, the authors were unable to collect blood

samples in the standardized manner necessary to control for the effects of time after capture on corticosterone secretion, making comparisons among siblings difficult. Ramos-Fernández et al. (2000) used manipulation experiments to examine endocrine correlates in sibling dominance relationships in the blue-footed booby (*Sula nebouxi*) and found that dominants had significantly higher corticosterone compared with subordinates. Finally, Núñez-de la Mora et al. (1996) found that under a food-restricted manipulation, subordinate blue-footed booby chicks had significantly higher baseline corticosterone levels than dominants.

*Potential sources for sibling variation in corticosterone levels*

Sibling variation in adrenocortical function may be attributed to several factors. Differences in egg size within a clutch may contribute to variation in size among siblings, which compete for food (Howe, 1976; O'Connor, 1978; Ojanen, Orell, and Vaisanen, 1981; but see Stokland and Amundsen, 1988; Williams, 1994), potentially resulting in differential development of adrenocortical function. However, egg volume did not vary significantly either within or

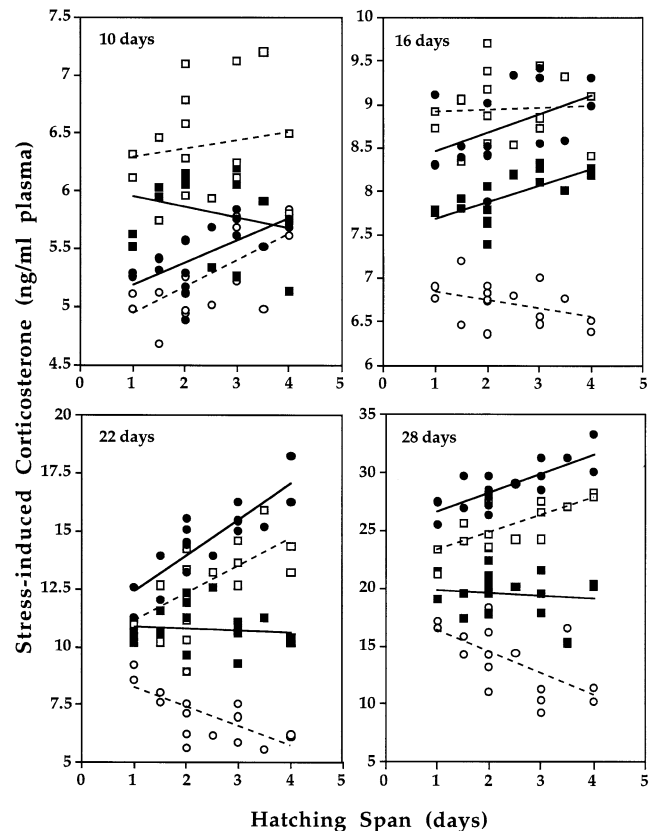


Fig. 3. Stress-induced corticosterone levels in sibling captive American kestrel chicks during postnatal development in relation to hatching span (difference between first- and last-hatched chick in days).  $N = 16$  broods for each age class. First-hatched chicks (—●—), second-hatched chicks (—□—), third-hatched chicks (—■—), and fourth-hatched chicks (—○—).

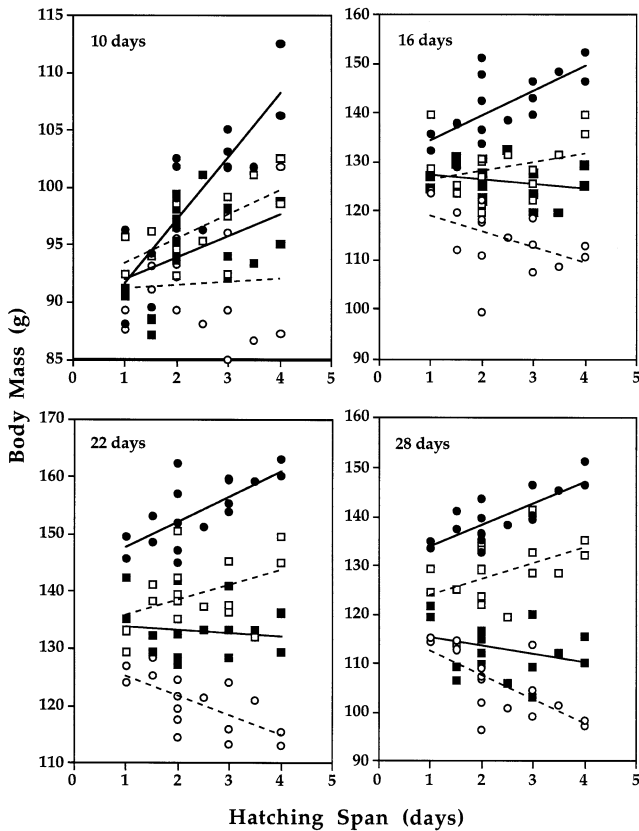


Fig. 4. Mass changes in sibling captive American kestrel chicks during postnatal development in relation to hatching span (difference between first- and last-hatched chick in days).  $N = 16$  broods for each age class. First-hatched chicks (—●—), second-hatched chicks (-□-), third-hatched chicks (—■—), and fourth-hatched chicks (-○-).

between clutches in our study. Body condition is also known to have a significant effect on both baseline and stress-induced levels of corticosterone (Smith, Wingfield, and Veit, 1994; Heath and Dufty, 1998; Kitaysky et al., 1999; Sockman and Schwabl, 2001). A study in wild blue-footed boobies found that later-hatched chicks exhibited higher corticosterone levels which were associated with measures of poor body condition (Nunez-de la Mora et al., 1996). In the present study, captive chicks came from relatively small clutches and parents were fed enough food to successfully raise their chicks, resulting in no overall relationship between nestling condition and corticosterone levels. Although later-hatched chicks in this study showed lower body masses than their earlier-hatched siblings, these were still well above those considered normal during development for the species (I. Ritchie, personal communication). Past studies have also found that chicks fed energy-restricted diets have elevated baseline corticosterone levels due to poor body condition (Freeman, Manning, and Flack, 1981; Holmes, Cronshaw, and Redondo, 1990; Kitaysky et al., 1999). Since all birds were not food restricted and were considered well within the developmental mass range for the species, we believe the elevated levels in this study are

indicative of a more competent HPA axis, rather than a secondary response due to food restriction.

Schwabl (1999) proposed that maternal androgenic hormones, which are differentially deposited in early and late eggs (Schwabl, 1993; Schwabl, Mock, and Gieg, 1997; Lipar, Ketterson, and Nolan, 1999; Sockman and Schwabl, 2000), may influence the neuroendocrine development and function of siblings, since neonatal androgens can have organizational effects on the HPA axis (McCormick, Furey, Child, Sawyer, and Donohue, 1998). The neuroendocrine effects of maternal yolk-based androgens remain largely untested; however, Sockman and Schwabl (2000) reported that the yolks of late-laid American kestrel eggs had higher levels of testosterone (T) and androstenedione ( $A_4$ ) than those of first-laid eggs. Furthermore, Sockman and Schwabl (2001) found that treatment of American kestrel eggs with maternal androgens produced somewhat elevated posthatching corticosterone levels in chicks. Through this androgenic effect then, elevated corticosterone levels should be observed in later-hatched kestrel chicks; however, in the present study the reverse pattern of sibling corticosterone secretion occurred. Although it is known that yolk androgen levels in captive birds are comparable with those of wild birds (Sockman, Schwabl, and Sharp, 2001), it is possible that differences in clutch size between the two studies may be responsible for differences in adrenocortical patterns. One of the most intriguing possible remaining sources for the sibling corticosterone patterns observed in the present study is the effects of (1) sibling social hierarchies and (2) the direct developmental effects of hatching asynchrony itself.

#### *Competition and costs of dominance*

Recently it has been shown that mammals and birds living in social hierarchies show distinct patterns of stress-hormone levels in relation to their social rank (Creel, 2001). Although early captive work showed that subordinates often showed increased glucocorticoid (GC) secretion compared with dominant individuals in mammals (Bronson and Eleftheriou, 1964; Louch and Higginbotham, 1967; Manogue, 1975), recent studies of cooperative breeders in the wild have shown that dominant individuals exhibit elevated GCs more often than do subordinates (Creel, Creel, and Monfort, 1996; see Creel, 2001, for multitaxa review). Elevated levels in dominants may represent a cost to being at the head of a cooperative breeding (Creel, 2001) or nest hierarchy (Kern et al., 2001), and it is possible that the age hierarchy in kestrel nests in the present study produced by hatching asynchrony is costing dominant chicks in terms of elevated corticosterone levels. However, there are potential problems with this hypothesis in relation to American kestrels in the present study. First, although a number of raptorial species such as eagles (*Aquila*) do exhibit extreme sibling competition and even siblicide (Newton, 1979), there is no evidence for overt aggression occurring in nest-

bound kestrels (Newton, 1979). Second, Creel (2001) noted that such patterns are most likely produced if either a hierarchy is unstable or dominants fight more often than subordinates. Since parent kestrels in the present study were fed adequate amounts of food for raising relatively small broods of chicks which hatched without extreme amounts of hatching asynchrony, it is unlikely that hierarchies were unstable. Third, although baseline levels of corticosterone were consistently higher in first-hatched chicks compared with later-hatched siblings, these levels are not unrealistically high and are well within ranges reported during postnatal development for the species (Sockman and Schwabl, 2001). In fact, levels for later-hatched chicks are quite low. Furthermore, it is not currently understood whether elevated corticosterone causes aggression or subordination in siblings since it has been shown to be correlated with both within the same species (Ramos-Fernández et al., 2000, and Núñez-de la Mora et al., 1996, respectively).

#### *Direct effects of hatching asynchrony on development*

Schwabl (1999) proposed that hatching asynchrony can precipitate modulations in neuroendocrine development or function, potentially giving first-hatched chicks adrenocortical, along with implicit growth and survival, advantages over their siblings. Hatching asynchrony causes pronounced developmental hierarchies in sibling wild American kestrels (Bortolotti and Wiebe, 1993; Wiebe and Bortolotti, 1995) and is largely under facultative female control (Wiebe and Bortolotti, 1994). We propose that most of the sibling variation in corticosterone levels seen in the present study can be attributable to the differential developmental effects originating from hatching asynchrony, since its effects on growth and survival are so pronounced (Magrath, 1992). Increasing hatching span in the present study resulted in pronounced developmental delays in later-hatched siblings as seen by slower mass gain and lower corticosterone secretion. Hatching span is known to be positively correlated to the relative difference in nestling mass between sibling American kestrel (Wiebe and Bortolotti, 1994; Fig. 4.). However, in the present study, early-hatched chicks were not in better condition compared with younger siblings, but rather were just developing at a faster rate. We believe that the effect of hatching order on corticosterone levels is largely due to the fact that all broods hatched asynchronously to some degree, probably resulting in competitive hierarchies at even low degrees of hatching span. This is supported by the significant hatch order  $\times$  hatch span interactions seen in the present study, with the hatching order effect being most obvious in broods with a large hatching span.

#### *Conclusion*

Experimentally elevated levels of corticosterone within baseline ranges increase begging behavior in free-living

Black-legged kittiwake chicks (*Rissa tridactyla*) and parents respond to the change in begging by providing more food (Kitaysky et al., 2001). These authors suggest that parent kittiwakes assess the physiological condition of their chicks by monitoring begging. Variation in begging rates among siblings would allow efficient distribution of limited food resources, helping parents maximize the number of offspring that have a reasonable chance of surviving to adulthood (Alcock, 1998). It is possible that first-hatched kestrel chicks in this study may therefore be able to obtain increased amounts of food from parents compared with their siblings, with this effect increasing with increasing hatching span. We suggest that hierarchies in adrenocortical function among siblings may be one physiological mechanism by which these developmental competitive hierarchies are maintained, without the costs associated with chronic, acute-stress-induced levels of corticosterone (Sapolsky, 1992; Sapolsky, Krey, and McEwen, 1986). Potentially then, begging rates in captive kestrels under regular daily conditions could be mediated instead by differences in baseline corticosterone levels, a point which has recently been raised (Wingfield and Kitaysky, 2002).

Finally, in addition to elevated baseline levels of corticosterone demonstrated in this study, first-hatched chicks in all age groups exhibited higher stress-induced levels of corticosterone than successive chicks. These higher levels may provide first-hatched American kestrel higher survival chances during food shortages associated with the often unpredictable food resources wild kestrels face (Wiebe and Bortolotti, 1994). In fact, first-hatched chicks showed greater change from 10 to 28 days of stress-induced levels of corticosterone than in successive chicks. Temporary increases in corticosterone levels are known to facilitate locomotory (Astheimer et al., 1992; Smith et al., 1994) and foraging behavior (Bray, 1993) and an enhanced adrenocortical response system at fledging may stimulate foraging behavior once they are exposed to an environment where they are more likely to be preyed upon (Varland, Klass, and Loughin, 1993). An enhanced adrenocortical response system in earlier-hatched chicks at this stage may even increase their chances of survival over their siblings. Potentially, female American kestrels may be trading off short-term survival of the clutch against long-term postfledging survival, which they have less control over. These potential effects of among-sibling variation in adrenocortical function on long-term survival and reproductive fitness remain to be studied, especially in wild birds.

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