Life-history Evolution, Abiotic Constraints, and Climate Adaptability of Burrowing Owls (*Athene cunicularia*) Breeding Along a Latitudinal Gradient

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by
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Authorization to Submit Dissertation

This dissertation of Carl G. Lundblad, submitted for the degree of Doctor of Philosophy with a Major in Natural Resources and titled "Life-history Evolution, Abiotic Constraints, and Climate Adaptability of Burrowing Owls (*Athene cunicularia*) Breeding Along a Latitudinal Gradient," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Two of the most prominent contemporary goals of ecology are to: 1) understand how populations are regulated through variation in the rates of births and deaths and, 2) to elucidate, anticipate, and mitigate the impacts of climate change and other environmental changes on wildlife populations. These two objectives are inherently linked because to fully understand how populations will respond to climate change, we first need an improved understanding of how they are currently adapted to their thermal environment. However, the extent to which fitness is directly linked to the abiotic environment and the specific mechanisms that mediate those relationships are often poorly understood. Therefore, we need a more detailed understanding of the patterns and drivers of life-history variation and the ways in which such variation is driven by thermal effects.

I examined intraspecific patterns and causes of life-history variation within a single widespread species, the Burrowing Owl (*Athene cunicularia*), breeding along a 1400-km latitudinal gradient in western North America from 2015 – 2018. My overall objectives were to: 1) document latitudinal and other patterns of variation in a large suite of life-history traits and breeding behaviors, 2) test mechanistic hypotheses to explain that variation with an emphasis on examining abiotic and thermal mechanisms, and 3) understand the complex trade-offs and interactions among traits, activities, and allocation decisions occurring at different stages of the avian reproductive cycle. We used motion-activated video recording inside of occupied Burrowing Owl nests to gain detailed data on the reproductive behaviors of Burrowing Owls across all stages of the reproductive cycle.

Chapter Two documents latitudinal patterns in a suite of traits related to hatching patterns and hatching asynchrony, tests a thermally-based hypothesis to explain the cause of asynchronous hatching, and considers whether the same mechanism responsible for asynchronous hatching might also explain the latitudinal variation in clutch size. We found that most traits varied clinally with latitude, but all the traits were more strongly associated with individual nest microclimates than with latitude, and all in the directions predicted by the *egg viability hypothesis*. These results suggest that abiotic conditions in the nest drive an important life-history tradeoff and that thermal gradients are sufficient to account for observed biogeographic and seasonal patterns in life-history strategies.

Chapter Three documents latitudinal variation in egg-laying behaviors and tests hypotheses to explain variation in laying interval. Burrowing Owls laid eggs at all times of day but laid more eggs in the morning hours, and that tendency was strongest among first eggs in a clutch (i.e., nest initiation times). Laying interval duration varied dramatically, was negatively correlated with latitude, and was associated with burrow temperature and with numerous behavioral and life-history traits. Our results indicate that laying interval is not a fixed constraint on avian reproduction but rather should be considered an important life-history trait in future studies.

Chapter Four documents latitudinal variation in incubation behavior, examines patterns and sources of variation in nest attentiveness, and uses a hypothetico-deductive approach to test a pair of alternative hypotheses to explain variation in incubation period length. Daily nest attentiveness, cumulative nest attendance, and incubation period duration all increased with increasing latitude, but that variation could be accounted for by predicted life-history tradeoffs. Burrowing Owls reduced their daily attentiveness on days that the average burrow temperature was within the range that is optimal for embryo development. Finally, variation in incubation periods was best explained by cumulative nest attendance instead of daily nest attentiveness suggesting that variation in incubation periods is driven by differential selection on neonate quality, as suggested by the *neonate quality hypothesis*.

Chapter Five reviews the historical development of “Ashmole’s hypothesis” of clutch size evolution, which is based in a simple abiotic mechanism driven by seasonality of productivity. I summarize the empirical evidence in support and against Ashmole’s hypothesis, and then discuss and clarify numerous sources of confusion in the existing literature surrounding the hypothesis. As a whole, this dissertation documents numerous biogeographic patterns in avian life-histories that have heretofore been unknown or poorly documented, it provides new insights into the drivers of that variation in into the drivers of life-history variation generally, and it identifies mechanisms by which avian reproduction is adapted to the abiotic environment and may therefore respond to climate change.

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Dedication

This work is dedicated to my maternal grandmother, Maryetta Sutton who passed away on 3 May 2020, and to the rest of my supportive family for whom I have boundless love.

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Chapter 1: Introduction

Two of the most prominent contemporary goals of ecology are to: 1) understand how populations are regulated through variation in the rates of births and deaths and, 2) to elucidate, anticipate, and mitigate the impacts of climate change and other environmental changes on wildlife populations. These two objectives are inherently linked because to fully understand how populations will respond to climate change, we first need an improved understanding of how they are currently adapted to their thermal environment. However, the extent to which fitness is directly linked to the abiotic environment and the specific mechanisms that mediate those relationships are often poorly understood. Previous attempts to model the impacts of climate change on wildlife populations have relied heavily on extrapolations from climate envelope models (Peterson et al. 2002, Hijmans and Graham 2006, Duncan et al. 2009) that typically do not capture all of the indirect mechanisms by which animals may be impacted by changes to their thermal environment. Therefore, we need a more detailed understanding of the patterns and drivers of life-history variation and the ways in which such variation is driven by thermal effects.

The concept of life-history refers to the evolved strategies through which animals allocate their limited resources between reproduction and self-maintenance in a way that maximizes their lifetime inclusive fitness (Roff 2002). Life-history strategies interact with environmental variation to generate the demographic rates found in life tables (fecundity and survival), and hence, uncovering the factors responsible for life-history variation is key to understanding the mechanisms that regulate animal populations (Ricklefs 2000a). Similar to the dramatic and awe-inspiring variation that we observe in species morphology, life-history strategies vary dramatically from full iteroparity to semelparity (Cole 1954), and any given reproductive bout might produce anywhere from one to thousands of offspring (Lack 1947, Cole 1954, Roff 2002). Many mammals produce less than one offspring per year while some fish species produce thousands or even millions of eggs annually (Pope et al. 2010). Such extreme diversity may seem difficult or impossible to understand and explain. Yet, ecologists eventually began to recognize many general patterns in life-histories that provided a basis to begin to make sense out of patterns of diversity and the basis for formulating and testing hypotheses to explain those patterns.

A new era in life-history thinking began with the recognition that certain life-history traits varied with respect to season and, especially, geography (Ricklefs 2000a). A growing awareness that clutch size (in particular) was positively associated with latitude, both within and across species, culminated in seminal works by Lack (1947, 1948, 1954), Moreau (1944, Lack and Moreau 1965), and Skutch (1949). These and other authors (e.g., Ashmole 1961, 1963) formulated a number of well-known hypotheses to explain latitudinal patterns in clutch size, and those works spawned a vast literature on the topic of clutch size regulation and evolution. Eventually, life-history thinking expanded to consider variation in other traits and the ways those other traits interact and co-vary and interact with clutch size and fecundity (Martin 1995, Ricklefs 2000a, Ricklefs 2000b, Roff 2002, Martin 2004). Hence, recognition of the association between clutch size and latitude inspired an entire field of ecological research that we now know as life-history evolution.

Despite the considerable progress we have made in proposing and testing the mechanisms that drive clutch size and other life-history traits, and in integrating those ideas into our understanding of population regulation (Roff 2002, Ricklefs 2000a, Martin 2004), our understanding of life-history evolution remains limited in several ways. First, the majority of existing studies are still devoted to documenting patterns of life-history variation rather than testing mechanistic hypotheses to explain those patterns. While latitudinal and other biogeographical patterns are intriguing, they are intriguing because they provide tantalizing hints regarding the mechanisms that drive variation in life-history strategies. Studies that explicitly test and reveal the mechanisms underlying those patterns are now needed to understand the dominant selective pressures that shape variation, globally. Second, theoretical and empirical studies have remained focused on a relatively small number of traits including clutch size. However, life-histories are comprised of a full suite of inter-related traits that are all functionally linked through allocation decisions and tradeoffs. Hence, selection on any one of those traits inherently affects evolution of the others. A more complete understanding of the drivers of life-history variation requires studies that consider a larger suite of traits and the complex interactions between them. Patterns of variation in many life-history traits remain to be documented, much less explained. Third, most attention has been on formulating and testing hypotheses driven by biotic factors such as variation in food availability and nest predation risk. Much less theoretical and empirical work has been devoted to the possible abiotic and thermal mechanisms that could explain the same biogeographic patterns in life-history traits. Finally, previous studies have most often examined interspecific trait variation, but the mechanisms that drive interspecific patterns should also generate life-history variation within individual species. Comparative studies are subject to a multitude of possible confounding effects and phylogenetic constraints. Hence, documenting and testing hypotheses to explain life-history variation within individual species is a powerful complementary approach to interspecific comparisons but remains underutilized.

I examined intraspecific patterns and causes of life-history variation within a single widespread species, the Burrowing Owl (*Athene cunicularia*), breeding along a 1400-km latitudinal gradient in western North America from 2015 – 2018. My overall objectives were to: 1) document latitudinal and other patterns of variation in a large suite of life-history traits and breeding behaviors, 2) test mechanistic hypotheses to explain that variation with an emphasis on examining abiotic and thermal mechanisms, and 3) understand the complex trade-offs and interactions among traits, activities, and allocation decisions occurring at different stages of the avian reproductive cycle. We used motion-activated continuous video recording inside of occupied Burrowing Owl nests to gain detailed data on the reproductive behaviors of Burrowing Owls across all stages of their reproductive cycle.

Chapter Two documents latitudinal patterns in a suite of traits related to hatching patterns and hatching asynchrony, tests a thermally-based hypothesis to explain the cause of asynchronous hatching, and considers whether the same mechanism responsible for asynchronous hatching might also explain the latitudinal variation in clutch size. Chapter Three documents latitudinal variation in the most poorly-known stage of the avian nesting cycle (the egg-laying stage), including variation in an overlooked life-history trait (the laying interval between eggs), and tests hypotheses to explain variation in laying interval. Chapter Four documents latitudinal variation in incubation behavior and tests a pair of alternative hypotheses to explain variation in incubation period length. Chapter Five reviews the historical development of “Ashmole’s hypothesis” of clutch size evolution, which is based in a simple abiotic mechanism driven by seasonality of productivity. I summarize the empirical evidence in support and against Ashmole’s hypothesis, and then discuss and clarify numerous sources of confusion in the existing literature surrounding the hypothesis. As a whole, this dissertation documents numerous biogeographic patterns in avian life-histories that have heretofore been unknown or poorly documented, it provides new insights into the drivers of that variation in into the drivers of life-history variation generally, and it identifies mechanisms by which avian reproduction is adapted to the abiotic environment and may therefore respond to climate change.

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Chapter 2: Geographical Patterns and Trade-offs Among Life-history Traits are Driven by Thermal Intolerance of Avian Embryos

Abstract

Variation in life-history strategies is central to our understanding of population dynamics and regulation. Yet, we lack consensus regarding the ecological processes that drive variation in traits related to reproduction and survival. For example, we still do not understand the cause of two widespread patterns: 1) the ubiquitous positive association between avian clutch size and latitude, and 2) asynchronous hatching of eggs within a single clutch. Well-known hypotheses to explain each pattern have largely focused on biotic processes related to food availability and predation risk, but the *egg viability hypothesis* could explain both patterns with a single abiotic mechanism. We measured variation in five functionally-linked life-history traits of Burrowing Owls at five sites spanning a 1400-km latitudinal transect in western North America: the timing of incubation onset, the degree of hatching asynchrony, the frequency of hatching failure, nestling survival between hatching and independence, and clutch size. We found that most traits varied clinally with latitude, but all the traits were more strongly associated with individual nest microclimates than with latitude, and all in the directions predicted by the *egg viability hypothesis*. These results suggest that abiotic conditions in the nest drive an important life-history tradeoff and that thermal gradients are sufficient to account for observed biogeographic and seasonal patterns in life-history strategies. Furthermore, our results reveal a potentially important indirect mechanism by which reproductive success and recruitment could be affected by climate change.

Introduction

The life-history strategies by which animals allocate resources to reproduction, survival, and self-maintenance vary dramatically, both across and within species. Explaining the causes of variation in traits related to reproduction and survival is a tenet of ecology because that variation is inherently linked to how populations are regulated (Lack 1954) and is central to population biology and management of rare or harvested populations. Although hundreds of papers have been published on the topic, empirical tests of alternative mechanisms proposed to drive life-history variation remain rare. For example, the positive correlation between avian clutch size and latitude is well-known, as are several mechanistic hypotheses proposed to explain that ubiquitous pattern (Lack 1947, Skutch 1949, 1985, Ashmole 1961, Ricklefs 1980), but surprisingly few empirical studies have tested those hypotheses (but see Ricklefs 1980, Arcese and Smith 1988, Martin 1995, Martin et al. 2000, Fontaine and Martin 2006, Jetz et al. 2008, Stoleson and Beissinger 1999, Zanette et al. 2011). Geographic and latitudinal patterns in life-history traits, like the latitudinal gradient in clutch size, provided an initial basis for formulating and testing mechanistic hypotheses to explain the causes of variation in life-history traits more generally. Although the association between clutch size and latitude is well-documented, latitudinal variation in many other reproductive traits (and hence life-history strategies in general) remains largely undocumented. Furthermore, many ecological and abiotic processes co-vary along geographic gradients. Hence, testing mechanistic hypotheses proposed to explain observed latitudinal patterns in life-history traits remains a formidable challenge (Hussell 1985).

Another well-known life-history pattern that has generated more speculation and hypotheses than empirical tests and evidence is asynchronous hatching (Lack 1947, 1968, Magrath 1990, Stenning 1996, Stoleson and Beissinger 1999). While all eggs in a single clutch hatch roughly synchronously in many birds, the clutches of other birds hatch over the course of 2 – 14 days (Dunlop 1913, Lack 1947, Beissinger and Waltman 1991). Asynchronous hatching has important repercussions for life-history strategies because it results in substantial variation in size among siblings in a brood, and the last-hatched brood members often compete poorly with their larger siblings and have poor survival (Howe 1978, Amundsen and Slagsvold 1991; Fig 1). Because nestling deaths appear to be maladaptive, the evolution of asynchronous hatching is often described as an evolutionary paradox (Dunlop 1913, Stoleson and Beissinger 1995). Yet, asynchronous hatching has evolved independently in numerous avian lineages, and many causal hypotheses have been proposed which assume that asynchronous hatching is adaptive and is part of an explicit life-history strategy (Lack 1947, reviewed by Magrath 1990, Stoleson and Beissinger 1995).

Well-known explanations for both patterns (asynchronous hatching and the correlation between clutch size and latitude) have largely focused on biotic processes like variation in food availability and predation risk (e.g., Lack 1947, 1968, Ashmole 1961, Ricklefs 1980, Skutch 1985, Magrath 1990). Less attention has been paid to abiotic and thermal mechanisms that could explain these two ubiquitous patterns (but see Cody 1966, Stoleson and Beissinger 1999, Reid et al. 2000, Cooper et al. 2005). The *egg viability hypothesis* proposes that limited thermal tolerance of developing embryos induces some birds to initiate incubation prior to clutch completion which causes eggs to develop and hatch asynchronously (Arnold et al. 1987, Stoleson and Beissinger 1999). Avian embryos develop successfully only within a very narrow range of temperatures (Drent 1975, Webb 1987). The *egg viability hypothesis* suggests that if the ambient nest temperature exceeds “physiological zero” (26°C, the temperature at which embryos begin to develop spontaneously and irregularly, regardless of incubation; Drent 1975, Webb 1987), the female can minimize hatching failure and developmental abnormalities by initiating incubation prior to clutch completion. Early incubation allows the parent(s) to maintain the eggs within the temperature range required for successful development (assumed to be 36 – 40°C in most species; Webb 1987). Otherwise, unincubated eggs exposed to temperatures between 26 – 36°C are at risk of abnormal development, embryo death, and hatching failure (Arnold et al. 1987, Conway and Martin 2000, Beissinger et al. 2005), and those exposed to temperatures $\geq$ 41°C are at risk of immediate embryo death (Webb 1987). Therefore, according to the *egg viability hypothesis*, asynchronous hatching is not adaptive but rather a side-effect of an adaptive incubation strategy. Because the thermal conditions that promote the loss of egg viability are more likely at low latitudes, and because early incubation is costly and might energetically constrain the number of eggs a bird can lay (Ricklefs 1974, Williams 1996, Visser & Lessells 2001), limits to egg viability can also potentially explain the latitudinal pattern in clutch size (Arnold et al. 1987, Stoleson and Beissinger 1999).

By understanding whether and how life-history traits reflect adaptations to climatic variation, we can both gain insights into the evolutionary drivers of these two well-known patterns in avian ecology and anticipate the mechanistic responses of species to climate change. Our objectives were to: 1) document variation in clutch size and a whole suite of life-history traits of Burrowing Owls (*Athene cunicularia*) along a latitudinal gradient in western North America, 2) test numerous predictions of the *egg viability hypothesis* by comparing the relative ability of latitude and individual nest microclimate to explain variation in that same suite of life-history traits, and 3) determine whether the *egg viability hypothesis* can explain both the cause of asynchronous hatching and the latitudinal gradient in clutch size.

Methods and Materials

Predictions Tested and Rationale

Burrowing Owls are an excellent model species for examining causes of intraspecific variation in life-history traits because they have considerable variation in many traits, including clutch size and hatching asynchrony (Conway et al. 2012). Moreover, our study system allowed us to tease apart thermal effects from those based on other ecological processes that also co-vary with latitude because: 1) ambient thermal conditions track latitude imperfectly in western North America, and 2) the microclimate of underground Burrowing Owl nests does not track ambient above-ground thermal conditions as closely as does nest microclimate in other birds. Moreover, Burrowing Owls have a long egg-laying period (>10 days) with substantial intraspecific variation (Conway et al. 2010) which is useful for testing predictions of the egg viability hypothesis. These aspects allowed us to test 20 inter-related predictions of the *egg viability hypothesis* (Table 2.1) regarding variation in a suite of life-history traits along a latitudinally-based thermal gradient and as a function of individual nest microclimates during the egg laying stage. The *egg viability hypothesis* predicts that nest microclimate during egg laying drives variation in the timing of incubation onset and an entire suite of associated traits (Fig. 2.1) including the degree of hatching asynchrony, hatching success, nestling survival, and even clutch size. Although the *egg viability hypothesis* suggests that early incubation is an adaptation to prevent hatching failure, the hypothesis also implies a life-history trade-off between investing in early incubation to maximize egg viability and delaying incubation to maximize nestling survival (Fig. 2.1). Because each strategy is a resolution to a life-history tradeoff that includes potential costs, birds might employ a range of intermediate strategies and the population might suffer both reduced hatching success and reduced nestling survival in warmer locations and warmer burrows (Viñuela and Carrascal 1999, Viñuela 2000). Our study design allowed us to test particularly powerful predictions of the *egg viability hypothesis*; that each trait, including clutch size, should be more strongly associated with individual nest microclimate than with latitude.

Study Species

Burrowing Owls inhabit the western hemisphere from southern Argentina to southern Canada, and nest primarily in burrows constructed by mammals and other burrowing animals (Poulin et al. 2011, Conway 2018). They typically lay large clutches but clutch size varies greatly among nests (3 – 14 eggs/clutch; Todd and Skilnick 2002, Poulin et al. 2011, Conway et al. 2012). Other Burrowing Owl life-history traits, including the degree of hatching asynchrony, also have large intraspecific variation (Olenick 1990, Wellicome 2005, Conway et al. 2012), although geographic patterns in those traits have not been documented. Laying intervals between eggs in a single clutch range from 30 to over 60 hours (Olenick 1990, Wellicome 2005, Lundblad 2020: Chapter Three). Given large clutch sizes and long laying intervals, the earliest-laid eggs could be exposed to ambient conditions for a long time in the absence of early incubation. Females do all of the incubation, often re-nest following nest failure, but rarely raise more than one brood per year (Poulin et al. 2011). Burrowing Owls often experience partial hatching failure, and starvation is a common cause of nestling mortality (Wellicome 2005).

Study Sites

We studied Burrowing Owls at five study sites (Fig. 2.2) including: 1) the Sonny Bono Salton Sea National Wildlife Refuge, California (3°2’N, 115°37’W, elevation -69m), 2) North Las Vegas and Pahrump, Nevada (considered a single site, North Las Vegas at 36°18’N,115°14’W, elevation

670m, Pahrump at 36°18’N, 116°2’W, elevation 790m), 3) Tooele Army Depot South Area, Utah (40°2’N, 112°20’W, elevation 1520m), 4) Morley Nelson Snake River Birds of Prey National Conservation Area, Idaho (43°00’N, 116°00’W, elevation 945m), and 5) Umatilla Army Depot, Oregon (45°5’N, 119°26’W, elevation 152m). At each study site, some Burrowing Owls nested in artificial underground burrows. Artificial burrows followed several widely-used designs meant to approximate the dimensions, depth, thermal, and other properties of natural burrows (Collins and Landry 1977, Smith and Belthoff 2001, Smith and Belthoff 2001, Alexander et al. 2005, Barclay 2008, Johnson et al. 2013). Each artificial burrow included a single entrance tunnel, and the top of each nesting chamber varied in depth from 12 – 65 cm. We used variation in burrow design to our advantage, because different burrow designs and depths increased the variation in individual microclimates among burrows (Nadeau et al. 2015).

Field Methods

We installed one of several models of motion-activated infrared CCTV cameras (Alibi CD700 and CD80B Turret Dome, Observint Technologies, Austin, TX; HT71HG Mini Vandal Turret Dome and HT71TG Mini Turret Dome, Speco Technologies, Amityville, NY) inside the artificial burrows to capture the rear of the burrow chamber, where we anticipated owls would nest. We connected each camera to a mobile DVR recorder (MDVR25HR MPEG-4, Observint Technologies, Austin, TX) placed in a plastic bucket or plastic food storage container external to the burrow. We programmed the DVRs to record a 2.5-minute clip any time there was substantial motion in the burrow (we set DVR sensitivity to medium) and saved video clips to a 32-gb SD memory card that we changed every 5 – 15 days. We powered each camera with a 12-volt deep cycle battery, installed external to the burrow and replaced every 2 – 4 days. We also installed temperature dataloggers (iButton model DS1922L, Maxim Integrated, San Jose, CA and Embedded Data Systems, Lawrenceburg, KY) on the inner wall or ceiling of most burrow chambers and programmed them to record the ambient burrow temperature every 10 – 20 minutes (except in 2015, when we collected no burrow temperature data).

We installed cameras in a total of 150 nests at the five study sites from 2015 – 2018 and collected a total of 1,533,325 2.5-minute video clips accounting for 63,889 hours and 14.0 terabytes of video data. Of the 150 nests, 120 nests produced usable hatching period videos (Table 2.2). We installed 42.2% of the cameras that yielded hatching data prior to nest initiation, 42.2% during the laying stage, and 15.6% during the incubation stage. We temporarily blocked the burrow entrance during equipment installation, allowing the female (if present) to take refuge in the entrance tunnel and preventing her from flushing. We typically installed the equipment in < 5 minutes and kept the burrow covered with the lid or a board as continuously as possible. Opening the burrows for up to 10 minutes did not affect their internal temperature in a previous study at our warmest study site (Nadeau et al. 2015). We kept each camera running, whenever possible, until at least 20 days post-hatching or until the nest failed. Equipment failure and the challenges associated with keeping batteries regularly maintained resulted in partial data losses. Among 120 nests for which we obtained hatching period videos, we obtained complete records of the hatching period for 90.2% of them. For those nests with incomplete hatching period data, we missed an average of 2.6 eggs per nest (range 2-9). On or around the twentieth day after the first egg in each clutch hatched (range nineteenth to twenty-fifth day), we opened the artificial burrow to count the number of surviving nestlings. We also opened and checked nearby artificial burrows, checked any nearby natural burrows for signs of owl use, and trapped at the natural burrows if less than the entire brood was accounted for and we thought the other burrows might contain nestlings that relocated from the focal burrow (rare).

Video Data Processing

We reviewed all of the 2.5-min video segments in their entirety from when the first egg was laid or the camera was installed (for those installed after laying began) until the final viable egg hatched and we recorded the time when each egg hatched. When the female blocked the camera's view of the clutch and prevented us from recording the exact hatching time of an egg, we used the midpoint between when the egg was last seen unhatched and when the newly-hatched nestling was first observed as the hatching time ($\bar{x}$ = 0.74 hours elapsed ± 0.08 SE, range: 0 to 23.9 hours elapsed).

Nest Microclimate Data Processing

Among the 120 nests with hatching period video data, we also had nest microclimate data for 70 of those nests from an average of 89.6% of the laying period (S.E. = 1.89%, range = 33.6-100.0%). We initially calculated two metrics of individual nest microclimate during the laying stage: the mean burrow temperature during the laying stage and the proportion of the day when burrow temperature was between 26° and 36°C (the range in which irregular embryonic development proceeds spontaneously and embryos are at risk of death and hatching failure; Webb 1987). Our results were similar whether we used mean burrow temperature during the laying stage or the proportion of that stage during which the burrow temperature was 26 – 36°C and, hence, we only report results for mean burrow temperature during laying. We averaged all available temperature readings for each burrow from the moment the first egg was laid until the last egg was laid.

Analytical Methods

General Approach—We took multiple approaches to test whether each trait was more strongly correlated with latitude or mean burrow temperature during the laying period. Except where noted, we calculated and compared the univariate Pearson correlation coefficients for each trait with latitude and with burrow temperature, separately, to document the latitudinal patterns with the maximum sample sizes available (we knew the latitude for all nests but obtained nest microclimate data for only 58.3% those with hatching period video data). We then used multiple linear regression to model variation in each trait as a function of latitude, mean burrow temperature during the laying period, and a suite of covariates. We began each modeling exercise by first selecting the optimal statistical distribution for the data (using AICc, based on Burnham and Anderson 2002), except when the best error distribution was determined *a priori* by the structure of the data (i.e., when using logistic regression to model binary data). We next used AICc to select the best random-effects structure (if any) for the data. We then used AICc to identify support for the fixed-effects variables (latitude, mean burrow temperature, and the other covariates). We ran models including all combinations of the fixed-effects variables and then calculated model-averaged parameter estimates (across all models) to account for model uncertainty. The correlation between latitude and mean burrow temperature was moderate (R = -0.67) but was not large enough to seriously degrade precision of estimated effect sizes for these variables (see pg. 309, Fox 2008). We thus felt comfortable including both latitude and burrow temperature in the same models. We performed all analyses in R (R Core Team 2019), fit all models with the glmmTMB package (Brooks et al. 2019), and performed model selection and model averaging with the MuMIn package (Barton 2019). We standardized all the continuous predictors used in each analysis.

Timing of Incubation Onset—All of the eggs present in a nest at the onset of incubation should begin developing at the same time and hatch roughly synchronously. Hence, we used the number of eggs hatched during the first 24 hours of hatching as our metric of the timing of incubation onset, relative to laying order. We assumed less incubation occurred prior to clutch completion (incubation began later) in nests that hatched a greater number of eggs within the first 24 hours of hatching and more incubation occurred prior to clutch completion (incubation began earlier) in nests where a smaller number of eggs hatched within the first 24 hours of hatching. We included clutch size as a potential covariate because we expected that clutch size would vary systematically with latitude. When we modeled the proportion of each clutch (rather than the absolute number of eggs) that hatched within the first 24 hours of hatching, we obtained similar results.

We examined the timing of incubation onset (as estimated by the number of eggs hatching during the first 24 hours of hatching) as a function of mean burrow temperature during the laying stage, latitude, clutch size, hatch date, and the proportion of the clutch that hatched successfully. To model these data, we considered the Gaussian, Poisson, and Conway-Maxwell-Poisson distributions. We considered the Conway-Maxwell-Poisson distribution (with a log link function) because it allows for underdispersion in count data (Conway and Maxwell 1962, Lynch et al. 2014, Brooks et al. 2019).

We then compared global models with no random effects, a random intercept for study site, and both a random intercept and slope for study site that accommodated space-varying relationships between incubation onset and explanatory variables.

Hatch Interval Length—We examined whether the following six variables explained variation in the duration of individual hatch intervals (the time elapsed between the hatching of subsequently hatched eggs in a single clutch, a direct metric of hatching asynchrony): mean burrow temperature during the laying stage, latitude, clutch size, hatch date, the proportion of the clutch that hatched successfully, and egg number within a clutch (i.e., whether it was the interval between first and second-hatched egg, the interval between second and third-hatched egg, etc.). We compared global models that included a Gamma distribution, Gaussian distribution, and a Gaussian distribution after applying a log transformation to the response variable (i.e., a Log-normal model). We then compared global models with all combinations of the following random effects: random intercept for nest ID, random intercept and slope for nest ID, random intercept for study site, and random intercept and slope for study site. We did not consider a model without random effects for nest ID because hatch intervals associated with the same nest are not independent samples (Hurlbert 1984).

Nestling Survival—We used logistic regression (binomial distribution with a logit link function) to examine the variables that influence survival of individual nestlings (after hatching successfully) to the occasion when we opened the boxes to assess survivorship (on or around 20 days after hatching began). We examined nestling survival as a function of mean burrow temperature during the laying stage, latitude, the number of eggs hatched in that clutch (i.e., the initial brood size), date, and the exact number of days elapsed between when the nest began hatching and when we opened the burrow to assess survivorship. We compared a suite of global models that included all combinations of the following random effects: random intercept for nest ID, random intercept and slope for nest ID, random intercept for study site, and random intercept and slope for study site. We did not consider models without a random effect for nest ID because nestlings associated with the same nest are not independent samples (Hurlbert 1984). For consistency with the presentation of other parameters, we also calculated the univariate Pearson correlations between *the proportion of each brood* that survived to day 20 and both latitude and mean burrow temperature during the laying stage.

Hatching Failure—We used logistic regression (binomial distribution with a logit link function) to examine the incidence of hatching failure of individual eggs as a function of mean burrow temperature during the laying stage, latitude, clutch size, and hatch date. We included eggs that were consumed or removed from the nest by the resident female as failed, but excluded those that were depredated, abandoned, or otherwise destroyed. We compared a suite of global models that included all combinations of the following random effects: random intercept for nest ID, random intercept and slope for nest ID, random intercept for study site, and random intercept and slope for study site. We did not consider models without a random effect for nest ID because hatch intervals associated with the same nest are not independent samples (Hurlbert 1984). For consistency with the presentation of other parameters, we also calculated the Pearson correlations between the *proportion of each clutch* that failed to hatch and both latitude and mean burrow temperature during the laying stage.

Survival from Laying to 20 Days Post-hatch —To assess the cumulative effects of laying-period nest microclimate on reproductive success and recruitment, we also examined variation in the combined hatching success and subsequent survival of nestlings (i.e., survival from laying through when we opened boxes to count survivors, ~20 days after the nest began to hatch). We counted eggs that both hatched and produced nestlings that survived to day 20 as successful and those that either failed to hatch or died prior to day 20 as unsuccessful. Because we were specifically interested in the causes of death implied by the *egg viability hypothesis* (embryo death and nestling starvation), we excluded eggs and nestlings depredated at either stage but included (as failed) any eggs or nestlings consumed by the resident female. We used logistic regression (binomial distribution with a logit link function) to model these data as a function of mean burrow temperature, latitude, the number of eggs hatched in that clutch, nest hatch date, and the exact number of days elapsed between when the nest began hatching and when we opened the burrow to assess survivorship. We then compared models including the following random-effects structures: random intercept for nest ID, random intercept and slope for nest ID, random intercept for study site, and random intercept and slope for study site.

Clutch Size—We examined clutch size as a function of mean burrow temperature during the laying stage, latitude, and hatch date. We considered the Gaussian, Poisson, and Conway-Maxwell-Poisson distributions. We then compared global models with no random effects, a random intercept for study site, and both a random intercept and slope for study site.

Results

Nest Box Microclimate—The average proportion of the laying period during which the temperature inside the nest burrows was 26 – 36°C varied among study sites: 85.7% in California, 38.0% in Nevada, 0.1% in Utah, 0.6% in Idaho, and 6.4% in Oregon. The temperature inside the nest burrow did not exceed 36°C during the laying stage at any of the nest boxes that were used by owls. The average temperature of occupied artificial burrows during the laying stage varied among study sites: 29.4°C in California, 24.7°C in Nevada, 16.5°C in Utah, 15.6°C in Idaho, and 21.3°C in Oregon. The mean burrow temperature during the laying stage was correlated with nest hatch date (*R* = 0.17) and declined non-linearly with increasing latitude (Fig. 2.3).

Timing of Incubation Onset—The number of eggs hatched within the first 24 hours of hatching ranged from 1 – 9 ($\bar{x}$ = 4.8 ± 0.19 SE, *n* = 104) across all study sites, which exemplifies the intraspecific variation in hatching asynchrony in Burrowing Owls. The timing of incubation onset increased (became later) with increasing latitude (univariate *R* = 0.44, *n* = 104; Fig. 2.4A), decreased (became earlier) with increasing burrow temperature (univariate *R* = -0.77, *n* = 65; Fig. 2.4B), and was better explained by burrow temperature than by latitude; all predictions of the *egg viability hypothesis*. The Conway-Maxwell model performed substantially better (ΔAICc = 13.3) than models using the next-best error distribution, and the model with only fixed effects performed better (ΔAICc = 2.6) than the next-best random-effects structure. The top model to explain variation in the timing of incubation onset included clutch size, hatching success, and burrow temperature but not latitude. Models including mean burrow temperature received 100.0% of total model weight while those including latitude received only 40.0% of total model weight (Table 2.3). The model-averaged effect of mean burrow temperature on the timing of incubation onset was negative (-0.24 ± 0.04 SE), and the confidence interval excluded zero (Table 2.4). In contrast, the model-averaged effect of latitude was positive (0.02 ± 0.03), but the 95% confidence interval included zero (Table 2.4). The timing of incubation onset was negatively correlated with date; owls began incubation earlier, relative to laying, as the season progressed (univariate *R* = -0.32, *n* = 103; Fig. 2.10a). The effect of date was not in the top model but was included in models receiving > 0% weight (Table 2.3). The model-averaged effect of date was negative, but the 95% confidence interval included zero (Table 2.4).

Hatch Interval Length—The amount of time elapsed between hatching of subsequent eggs in a clutch ranged from 0.02 – 70.67 hours ($\bar{x}$ = 11.05 ± 0.49 SE, *n* = 662). Hatch interval length decreased (became shorter, indicating more synchronous hatching) with increasing latitude (univariate *R* = -0.18, *n* = 685; Fig. 2.5A), increased (became longer, indicating more asynchronous hatching) with increasing burrow temperature (univariate *R* = 0.37, *n* = 393; Fig. 2.5B), and was better explained by burrow temperature than by latitude; predictions that support the *egg viability hypothesis* (Table 2.1). The log-Normal model performed substantially better (ΔAICc = 1201.8) than the Gaussian and Gamma distribution models. The model with random intercept and slope for nest ID failed to converge. The model with a random intercept for nest ID had the lowest AICc among those models that did converge. The top model to explain variation in hatch interval length included burrow temperature, clutch size, egg number, and proportion hatch success. Models including burrow temperature received 99.2% of total model weight. Models including latitude received 9.4% of total model weight (Table 2.5). The model-averaged effect of mean burrow temperature was positive (0.37 ± 0.10 SE), and the confidence interval excluded zero (Table 2.6). In contrast, the model-averaged effect of latitude was slightly negative (-0.01 ± 0.04) and the 95% confidence interval included zero (Table 2.6). Hatch interval length was positively correlated with date (univariate R = 0.20, n = 631; Fig. 2.10B). The effect of date was not in the top model but was included in models receiving > 0% weight (Table 2.5). The model-averaged effect of date was positive, and the 95% confidence interval included zero (Table 2.6). We also repeated this analysis using average hatch interval per nest as the response variable and excluding the random effect for nest ID, and the results were very similar. The univariate correlations between latitude and both mean burrow temperature and *mean hatch interval per nest* (R = -0.35, n = 102 and R = 0.63, n = 65, respectively) were stronger than those with individual hatch intervals.

Nestling Survival—The model with only a random intercept for nest ID performed better (ΔAICc = 2.1) than the second-best random-effects structure (the models including random slope for study site failed to converge). Nestling survival through day 20 varied non-linearly with latitude (n = 697; Fig. 2.6A). Nestling survival decreased with increasing burrow temperature (n = 395; Fig. 2.6B) and was better explained by burrow temperature than by latitude; predictions that support the *egg viability hypothesis* (Table 2.1). The top model to explain nestling survival included effects of burrow temperature, latitude, and the number of eggs hatched. Models including both burrow temperature and latitude received 100.0% of total model weight (Table 2.7). The model-averaged effect of burrow temperature on nestling survival was negative (-1.63 ± 0.37 SE) and the 95% confidence interval excluded zero (Table 2.8). The model-averaged effect of latitude on nestling survival was also negative (-0.93 ± 0.33) and the 95% confidence interval excluded zero (Table 2.8). The effect of date on the survival of individual nestlings was not in the top model (Table 2.7), the model-averaged effect of date was slightly positive (0.01 ± 0.12), and the 95% confidence interval included zero (Table 2.8). We also repeated this analysis using the *proportion of nestlings per nest* that survived to day 20 as the response variable in a weighted logistic regression and excluding the random effect for nest ID, and the results were similar. The proportion of nestlings in a brood that survived to 20 days after hatching ranged from 0 to 1 ($\bar{x}$ = 0.64 ± 0.03 SE) and varied little with latitude (univariate R = -0.08, n = 103), declined with increasing burrow temperature (univariate R = -0.44, n = 61), and was better explained by burrow temperature than by latitude; predictions that support the *egg viability hypothesis* (Table 2.1). The proportion of nestlings per nest that survived to day 20 was only weakly associated with date (univariate R = -0.14, n = 94).

Hatching Failure—The model with only a random intercept for nest ID performed better (ΔAICc = 2.1) than the second-best random-effects structure. Hatching failure varied little with latitude (*n* = 950; Fig. 2.7A), contrary to the prediction of the *egg viability hypothesis*. Hatching failure increased with increasing burrow temperature (*n* = 561; Fig. 2.7B) and was better explained by burrow temperature than by latitude; predictions that support the *egg viability hypothesis* (Table 2.1). The top model to explain variation in hatching success of individual eggs included only burrow temperature. Models including burrow temperature received 97.3% of total model weight, and models including latitude received 28.4% of total model weight (Table 2.9). The model-averaged effect of burrow temperature was positive (0.82 ± 0.30 SE) and the 95% confidence interval excluded zero (Table 2.10). The model-averaged effect of latitude was also positive (0.01 ± 0.18), but the 95% confidence interval included zero (Table 2.10). The effect of date on the frequency of hatching failure for individual eggs was not in the top model but was included in competing models (Table 2.9). The model-averaged effect of date was positive (0.04 ± 0.14) and the 95% confidence interval included zero (Table 2.10). We also repeated this analysis with *proportion of eggs per nest that failed* as the response variable in a weighted logistic regression and excluding the random effect for nest ID, and the results were similar. The proportion of eggs in a clutch that failed to hatch ranged from 0.00 to 0.92 ($\bar{x}$ = 0.14 ± 0.02 SE, *n* = 117). The proportion of a clutch that failed to hatch declined with latitude (univariate *R* = -0.18, based on *n* = 117), increased with increasing burrow temperature (univariate *R* = 0.37, based on *n* = 72; Fig. 2.4B), and was better explained by burrow temperature than by latitude; predictions that support the *egg viability hypothesis* (Table 2.1). The proportion of eggs in a clutch that failed to hatch was only weakly associated with date (univariate correlation *R* = 0.06, *n* = 107).

Survival from Laying to 20 Days Post-hatch—The model with the random intercept for nest ID performed better (ΔAICc = 2.1) than the second-best random-effects structure. Survival from laying to 20 days post-hatch varied non-linearly with latitude (*n* = 467; Fig. 2.8A), decreased with increasing burrow temperature (*n* = 793; Fig. 2.8B), and was better explained by burrow temperature than by latitude. The top model included negative effects of burrow temperature and latitude (Table 2.11). Models including burrow temperature received 96.1% of total model weight, and those including latitude received 89.1% of total model weight (i.e., both latitude and burrow temperature were in most of the competing models; Table 2.11). The model-averaged effect of burrow temperature was negative (-0.94 ± 0.37 SE) and the 95% confidence interval excluded zero (Table 2.12). The model-averaged effect of latitude was also negative (-0.55 ± 0.30) and the 95% confidence interval excluded zero (Table 2.12).

Clutch Size—Clutch size ranged from 3 to 13 eggs ($\bar{x}$ = 8.4 ± 0.16, *n* = 129). Clutch size was positively correlated with latitude (univariate *R* = 0.32, *n* = 130; Fig. 2.9A), negatively correlated with burrow temperature (univariate *R* = -0.59, *n* = 73; Fig. 2.9B), and was better explained by burrow temperature than by latitude; predictions that support the *egg viability hypothesis* (Table 2.1). The model using the Gaussian distribution performed better (ΔAICc = 1.5) than the second-best distribution, and the model with no random effects performed better (ΔAICc = 2.4) than one including a random intercept for nest ID (the model including random intercept and slope failed to converge). The top model to explain variation in clutch size included burrow temperature, latitude, and date, and models including burrow temperature received 94.8% of total model weight (Table 2.13). In contrast, models including latitude received 54.2% of total model weight. The model-averaged effect of mean burrow temperature was negative (-0.64 ± 0.26 SE) and the 95% confidence interval excluded zero, whereas the model-averaged effect of latitude was positive (0.35 ± 0.24) and the 95% confidence interval included zero (Table 2.14). Clutch size was negatively correlated with date (based on the univariate correlation, *R* = -0.42, *n* = 72; Fig. 2.10C). The model-averaged effect of date was negative, and the 95% confidence interval excluded zero (Table 2.14).

Discussion

We found strong, ubiquitous support for the *egg viability hypothesis* and our results suggest that it can explain intraspecific variation in both hatching asynchrony and clutch size. We found that a full suite of functionally-linked life-history traits varied along a latitudinally-based thermal gradient, but that each trait was more strongly associated with individual nest microclimates than with latitude and always in the directions predicted by the *egg viability hypothesis*. These results suggest that climate has a large influence on many important life-history traits in Burrowing Owls and, hence, climate change will affect life-history strategies. Burrowing Owls at lower latitudes and in warmer burrows: 1) began incubation earlier, 2) hatched their clutches more asynchronously, 3) had lower nestling survival, 4) had a higher rate of hatching failure, and 5) laid smaller clutches than their counterparts nesting at higher latitudes and in cooler burrows. Furthermore, seasonal trends in each trait corroborated that variation in each trait closely tracked seasonal and geographic trends in nest microclimate. These results consistently suggest life-history strategies of Burrowing Owls are adapted to thermal variation, and a full suite of life-history traits may be driven by selection on the timing of incubation onset and limits to the thermal tolerance of avian embryos. These results provide compelling evidence for a mechanistic basis for life-history variation, emphasize the potential importance of abiotic conditions in driving the evolution of life-history traits, and suggest that reproductive success and recruitment could be limited by climate change.

Most previous studies of the cause of hatching asynchrony have: 1) assumed variation in hatching asynchrony results from biotic processes like food availability, nest predation risk, and adult predation risk, 2) ascribed some adaptive function directly to asynchronous hatching patterns, and 3) assumed a strict dichotomy between synchronously and asynchronously hatching species (Lack 1954, Hussell 1972, Magrath 1990 and references therein). Variation in individual hatching spans of Burrowing Owls in Alberta, however, was not related to food availability and did not respond to food supplementation during the laying period (Wellicome 2005). Our study builds on several recent studies that show growing evidence that sensitivity of embryos to nest microclimate may instead be responsible for generating asynchronous hatching (Arnold et al. 1987, Stoleson and Beissinger 1999, Wang and Beissinger 2009). Furthermore, our results suggest that asynchronous hatching, rather than being adaptive, may be a side-effect of adaptive incubation patterns that have evolved to maximize hatching success (Arnold et al. 1987). Finally, by considering a full continuous range of incubation strategies and hatching patterns within a single species, our approach is unique and more powerful than those based on interspecific variation in hatching asynchrony, which might be constrained by phylogenetic history.

Our results suggest that asynchronous hatching, instead of being adaptive, might be an unavoidable consequence of limits to the thermal tolerance of avian embryos. According to the *egg viability hypothesis*, birds sometimes initiate incubation prior to clutch completion (the proximate cause of asynchronous hatching) because unincubated eggs exposed to 26-36ºC are at risk of irregular development, embryo death, and hatching failure (Arnold et al. 1987). Previous evidence in support of the *egg viability hypothesis* in wild birds comes from studies that experimentally exposed eggs to ambient conditions before returning them either to natural nests or to artificial incubators and measuring their hatching success. Hatching success declined with the duration of such exposure in *Anas* ducks (Arnold et al. 1987), American Coots (*Fulica americana*; Arnold 1990), House Sparrows (*Passer domesticus*; Veiga 1992, Viega and Viñuela 1993), Green-rumped Parrotlets (*Forpus passerines*; Stoleson and Beissinger 1999), Black Kites (*Milvus migrans*; Viñuela 2000), and Pearly-eyed Thrashers (*Margarops fuscatus*; Beissinger et al. 2005). Across these studies, the rate of viability loss was generally associated with latitude, steepest in the tropics, and shallowest in the subarctic (Stoleson 1999). Hatching success also declined with increasing ambient temperature (Arnold 1993, Viñuela 2000, but see Beissinger et al. 2005) and across the breeding season in temperate (more seasonal) but not tropical (relatively aseasonal) sites (Stoleson 1999, Stoleson and Beissinger 1999).

Our study differs from those described above because we measured a full suite of traits including incubation behavior, hatching patterns, and hatching success of unmanipulated eggs at five study sites along a natural thermal gradient spanning > 1400 km of latitude. Only three previous studies, to our knowledge, have tested whether hatching success varies intraspecifically along geographical thermal gradients, and only two have done so with unmanipulated eggs (Beissinger et al. 2005, Cooper et al. 2006, Lundblad and Conway 2019). Hatching success of Pearly-eyed Thrasher eggs declined with increasing pre-incubation exposure, but was lower (not higher, as predicted) in high-elevation cloud forest than at a nearby low-elevation site (Beissinger et al. 2005). Eggs at the cloud forest site, however, were subject to greater rates of trans-shell microbial infections that also reduce hatching success in humid environments (Cook et al. 2003, 2005, Beissinger et al. 2005). Although we did not quantify or address the influence of microbial infections on the hatching success of Burrowing Owl eggs, this mechanism is less likely to be important at our arid study sites. The per-nest and per-egg incidence of hatching failure decreased with increasing latitude among Eastern Bluebirds (*Sialia sialis*; Cooper et al. 2006), but did not vary across a 1000-m elevational gradient among Yellow-eyed Juncos (*Junco phaeonotus*; Lundblad and Conway 2019). Interspecifically, hatching success was positively correlated with latitude among 113 species (Koenig 1982). Our study adds to growing evidence that selection to maintain egg viability drives an entire cascade of events and traits including the early onset of incubation and asynchronous hatching.

The search for causation behind avian life-history variation has primarily focused on clutch size and attempts to explain observed latitudinal and seasonal trends in clutch size (Lack 1947). The positive association between clutch size and latitude has been ascribed to latitudinal variation in food availability (Lack 1947), seasonality of food availability (Ashmole 1961, Ricklefs 1980), nest predation risk (Skutch 1949, 1985), and to optimization of lifetime reproductive success relative to life expectancy and the rate of population turnover (Cody 1966). In contrast, our results suggest that even the latitudinal pattern in clutch size may instead result from limits to the thermal tolerance of avian embryos and resulting selection on the timing of incubation onset. At least three related mechanisms could limit optimal clutch size in warmer burrows, which are generally found at lower latitudes. First, incubation is energetically expensive (Williams 1996, Visser and Lessells 2001, Cresswell et al. 2004), and therefore allocating energy to incubation prior to clutch completion could energetically constrain the number of eggs laid in an individual clutch (Stoleson and Beissinger 1995, 1999, Thomson et al. 1998). Furthermore, each additional egg laid has a lower probability of hatching, and therefore a lower reproductive value, under warmer conditions like that found at lower latitudes (Arnold et al. 1987, Stoleson and Beissinger 1999). Finally, the tactile stimulus provided by early incubation might initiate hormonal feedbacks that inhibit additional egg development and laying (Sharp et al. 1988, Haywood 1993, Sockman et al. 2006). The *egg viability hypothesis* also accounts for the widely-observed seasonal decline in clutch size (Perrins 1970, Murphy 1986, Perrins and McCleery 1989, Hochachka 1990, Decker et al. 2011) because ambient temperature and nest temperature typically increase across the nesting season in temperate systems including our own.

Although the positive association between clutch size and latitude is extremely well-known, latitudinal trends in the timing of incubation onset and hatching asynchrony are generally undocumented in any species. Several studies have quantified variation in the timing of incubation onset or the degree of hatching asynchrony at a single location (e.g., Haftorn 1981, Smith 1988, Kennamer et al. 1990, Bortolotti and Wiebe 1993, Wang and Beissinger 2009, Conway et al. 2012), but only two previous studies have examined how these traits vary geographically in a single species (and each comparing only two study sites at geographical extremes). Tree Swallows (*Tachycineta bicolor*) in Tennessee were significantly more likely to initiate incubation prior to clutch completion (on the penultimate egg) than were conspecifics in Alaska (Ardia et al. 2006). This difference in the timing of incubation onset was associated with the proportion of the laying period that the nest temperature was > 26°C and resulted in differences in the degree of asynchronous hatching between the two populations (Ardia et al. 2006). House Finches (*Haemorhous mexicanus*) at a cold study site in Montana initiated incubation earlier at the start of the breeding season (presumably to prevent the eggs from approaching freezing) and initiated incubation later as the season progressed (Badyaev et al. 2003). House Finches at a warm study site in Alabama initiated incubation earlier as the season progressed and the ambient temperature more frequently exceeded physiological zero (Badyaev et al. 2003). Across 117 non-precocial species, those at lower latitudes began incubation earlier in the laying order, but did not hatch their clutches any more asynchronously, after controlling for clutch size and phylogeny (Viñuela and Carrascal 1999). Seasonal increases in the degree of hatching asynchrony are better documented than geographic trends (e.g., Nisbet & Cohen 1975, Slagsvold & Lifjeld 1989, Arnold 1993, Murphy 1994, Yogev et al. 1996), and are predicted by several of the hypotheses that have been proposed to explain hatching asynchrony (including the *egg viability hypothesis*).

Our data provide some of the strongest evidence yet for a mechanistic basis for observed patterns of life-history variation in birds. Variation in burrow microclimate during the laying period accounted for much of the latitudinal variation in most traits we measured, including timing of incubation onset, hatch interval length, the rate of hatching failure, and clutch size. Latitude explained additional variation, after the effects of burrow microclimate were accounted for, only for variation in nestling survival and overall survival from egg laying to day 20 of the nestling stage. Although most speculation and research into the underlying causes of life-history variation has focused on biotic drivers, our data suggest that natural thermal gradients are sufficient to directly account for the ubiquitous geographic and seasonal patterns observed in life-history traits.

Understanding how wildlife are currently adapted to climate variability is a pre-requisite for anticipating and developing strategies to mitigate the impacts that climate change will likely have on animal populations. The inter-related patterns presented here, and based on cascading effects initiated by limits to the thermal tolerance of avian embryos, suggest a strong constraint on reproductive success and recruitment driven by climate variation. Egg-laying birds faced with an unfavorable nest microclimate face a life-history tradeoff between: 1) initiating incubation prior to clutch completion, hatching their clutch asynchronously, and possibly paying a cost in terms of brood survival, or 2) delaying incubation until clutch completion, hatching synchronously, but paying a cost in terms of hatching failure. Hence, climate change will cause changes in selective pressures on hatching patterns and thereby cause changes in optimal life-history strategies.

Most current efforts to forecast how wildlife populations will respond to climate change are based on over-simplified climate envelope models (Erasmus et al. 2002, Peterson et al. 2002, Hijmans and Graham 2006, Duncan et al. 2009) that assume species' ability to adapt to climate change will depend on their ability to track a shifting climate niche based on the direct thermal tolerance of adults. However, developing embryos and offspring have much narrower thermal tolerance than adults in most animals (Coulombe 1970, Webb 1987) and adult survival is not the only component of fitness that is sensitive to thermal variation. Climate change might impact wildlife populations through a wide range of direct and indirect mechanisms that might not be well captured by projections based on climate envelope models. Our results suggest that climate change may impose strong constraints on reproductive success and recruitment that may be as important as those directly impacting adult survival (also see McCain 2009). Uncovering the specific mechanisms by which life histories are adapted to the biotic and abiotic environment is critical to developing more thorough and mechanistic understanding of how populations will be impacted by, and show resiliency towards, climate change.

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Table 2.1 Predictions of the *egg viability hypothesis* tested among Burrowing Owls in Western North America in this study.

Prediction	**Strength of Support (this study)**
The timing of incubation onset (relative to laying order) should be positively correlated with latitude	Strong
The timing of incubation onset (relative to laying order) should be negatively correlated with initiation date	Moderate
The timing of incubation onset (relative to laying order) should be negatively correlated with nest burrow temperature	Strong
The timing of incubation onset should be more strongly associated with nest microclimate than with latitude	Strong
The degree of hatch asynchrony should be negatively correlated with latitude	Moderate
The degree of hatch asynchrony should be positively correlated with initiation date	Moderate
The degree of hatch asynchrony should be positively correlated with nest microclimate (burrow temperature)	Strong
The degree of hatch asynchrony should be more strongly associated with nest microclimate than with latitude	Strong
Nestling survival between hatching and independence should be positively correlated with latitude	None
Nestling survival between hatching and independence should be negatively correlated with initiation date	Weak
Nestling survival between hatching and independence should be negatively correlated with nest burrow temperature	Strong
Nestling survival should be more strongly associated with nest microclimate than with latitude	Strong
Incidence of hatching failure should be negatively correlated with latitude	Weak
Incidence of hatching failure should be positively correlated with initiation date	None
Incidence of hatching failure should be positively correlated with burrow temperature	Strong
Incidence of hatching failure should be more strongly associated with nest microclimate than with latitude	Strong
Clutch size should be positively correlated with latitude	Strong
Clutch size should be negatively correlated with initiation date	Strong
Clutch Size should be negatively correlated with nest microclimate (burrow temperature)	Strong
Clutch size should be more strongly associated with nest microclimate than with latitude	Strong

Table 2.2. Number of camera-equipped Burrowing Owl nests that produced hatching period video data at five study sites from 2015 – 2018.

Study Site	**2015**	**2016**	**2017**	**2018**	**Total**
California	8	13	11	0	32
Nevada	0	0	3	5	8
Utah	16	14	11	0	41
Idaho	0	0	10	0	10
Oregon	8	10	11	0	29
Total	32	37	46	5	120

Table 2.3. Selection table for models considered to explain variation in the timing of incubation onset (defined as the number of eggs hatched within the first 24 hours of hatching) among Burrowing Owls in western North America. Included are only those models receiving > 0% model weight plus the intercept-only null model. The predictors of greatest *a priori* interest are in bold.

Model	**AICc**	**ΔAICc**	**weight**
~ Burrow Temp[1] **+** Clutch Size **+** Hatch Success	192.7	0.0	0.31
~ Burrow Temp + Clutch Size + Date + Hatch Success	194.0	1.3	0.16
~ Burrow Temp + Latitude + Clutch Size + Hatch Success	194.0	1.9	0.16
~ Burrow Temp + Latitude + Clutch Size + Date + Hatch Success[2]	194.7	2.5	0.12
~ **Burrow Temp** + **Latitude +** Date + Hatch Success	195.3	2.6	0.09
~ Burrow Temp + Date **+** Hatch Success	195.3	3.8	0.09
~ Burrow Temp + Hatch Success	196.5	4.9	0.05
~ Burrow Temp + **Latitude +** Hatch Success	197.6	21.2	0.03
Null model (intercept only)	275.1	82.4	0.00

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

[2] Global model

Table 2.4. Model-averaged parameter estimates for explanatory variables in a model to explain variation in the timing of incubation onset among Burrowing Owls in western North America. Presented are regression coefficients averaged across all candidate models. The predictors of greatest *a priori* interest are in bold.

Parameter	**β**	**SE**	**95% Confidence Interval**
Intercept	1.46	0.03	1.39 – 1.52
Burrow Temp[1]	-0.24	0.04	-0.32 – -0.15
Latitude	0.02	0.03	-0.03 – 0.13
Clutch Size	0.07	0.05	0.01 – 0.17
Hatch Success	0.21	0.04	0.13 – 0.30
Date	-0.02	0.04	-0.13 – 0.02

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

Table 2.5. Top-ranked models to explain hatch interval length (defined as the time elapsed between the hatching of subsequent eggs in the same nest) among Burrowing Owls in western North America. Included are only those models receiving > 0% model weight plus the intercept-only null model. The predictors of greatest *a priori* interest are in bold.

Model	**AICc**	**ΔAICc**	**weight**
~ Burrow Temp[1] **+** Clutch Size + Egg Number + Hatch Success	1189.6	0.0	0.79
~ Burrow Temp + Date **+** Clutch Size + Egg Number + Hatch Success	1194.0	4.4	0.09
~ Burrow Temp + Latitude + Clutch Size + Egg Number + Hatch Success	1194.3	4.7	0.07
~ **Burrow Temp** + Egg Number + Hatch Success	1197.3	7.8	0.02
~ Burrow Temp + Latitude + Date + Clutch Size + Egg Number + Hatch Success[2]	1198.1	8.5	0.01
~ Burrow Temp + Date + Egg Number + Hatch Success	1198.4	8.9	0.01
~ Burrow Temp + Clutch Size **+** Egg Number	1200.0	10.5	<0.01
~ Latitude + Clutch Size + Egg Number + Hatch Success	1200.1	10.5	< 0.01
~ Clutch Size + Egg Number + Hatch Success	1200.9	11.3	< 0.01
~ Clutch Size + Date + Egg Number + Hatch Success	1201.5	11.9	< 0.01
~ Burrow Temp + Latitude + Date + Egg Number + Hatch Success	1201.7	12.1	< 0.01
~ Burrow Temp + Latitude + Egg Number + Hatch Success	1202.8	13.2	< 0.01
Null model (intercept only)	1320.4	130.8	0.00

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle
[2] Global model

Table 2.6. Model-averaged parameter estimates for explanatory variables in model to explain variation in hatch interval length among Burrowing Owls in western North America. Presented are regression coefficients averaged across all candidate models. The predictors of greatest *a priori* interest are in bold.

Parameter	β	SE	95% Confidence Interval
Intercept	1.68	0.07	1.54 – 1.82
Burrow Temp[1]	0.37	0.10	0.19 – 0.56
Latitude	-0.01	0.04	-0.26 – 0.12
Clutch Size	-0.31	0.10	-0.49 – -0.15
Hatch Success	-0.30	0.04	-0.45 – -0.16
Date	0.01	0.04	-0.08 – 0.25
Egg Number	0.69	0.06	0.57 – 0.81

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

Table 2.7. Top-ranked models to explain variation in nestling survival at Burrowing Owl nests in western North America. Included are only those models receiving > 0% model weight plus the intercept-only null model. The predictors of greatest *a priori* interest are in bold.

Model	**AICc**	**ΔAICc**	**weight**
~ Burrow Temp[1]+ Latitude + Number Hatched	435.5	0.0	0.32
~ Burrow Temp + Latitude + Number Hatched + Days Since Hatch	436.3	0.9	0.21
~ Burrow Temp + Latitude	437.5	2.1	0.11
~ Burrow Temp + Latitude + Days Since Hatch	437.6	2.1	0.11
~ Burrow Temp + Latitude + Date + Number Hatched	438.2	2.7	0.08
~ Burrow Temp + Latitude + Date	438.3	2.9	0.08
~ Burrow Temp + Latitude + Date **+** Number Hatched + Days Since Hatch[2]	439.6	4.1	0.04
~ Burrow Temp + Latitude + Date + Days Since Hatch	440.3	4.8	0.03
Null model (intercept only)	452.9	17.4	0.00

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle
[2] Global model

Table 2.8. Model-averaged parameter estimates for explanatory variables in a model to explain variation in nestling survival among Burrowing Owls in western North America. Presented are regression coefficients averaged across all candidate models. The predictors of greatest *a priori* interest are in bold.

Parameter	**β**	**SE**	**95% Confidence Interval**
Intercept	0.78	0.24	0.30 – 1.25
Burrow Temp[1]	-1.63	0.37	-2.34 – -0.91
Latitude	-0.93	0.35	-1.54 – -0.37
Number Hatched	-0.25	0.28	-0.91 – 0.09
Days Since Hatch	0.00	0.10	-0.39 – 0.49
Date	0.01	0.12	-0.34 – 0.55

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

Table 2.9. Top-ranked models to explain variation in the frequency of hatching failure of Burrowing Owl eggs in western North America. Shown are only those models performing as well or better than the intercept-only null model (eight additional models performed worse than the null but received $\geq$ 1% of model weight). The predictors of greatest *a priori* interest are in bold.

Model	**AICc**	**ΔAICc**	**weight**
~ **Burrow Temp**[1]	386.8	0.0	0.37
~ **Burrow Temp** + Date	388.6	1.6	0.16
~ **Burrow Temp + Latitude**	388.8	1.8	0.14
~ **Burrow Temp +** Clutch Size	388.9	2.0	0.13
~ **Burrow Temp** + **Latitude +** Date	390.6	3.6	0.06
~ **Burrow Temp** + Date + Clutch Size	390.6	3.7	0.06
~ **Burrow Temp** + **Latitude** + Clutch Size	392.6	3.8	0.05
~ **Burrow Temp** + **Latitude** + Date + Clutch Size[2]	392.6	5.8	0.02
Null model (intercept only)	397.9	11.1	0.00

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle
[2] Global model

Table 2.10. Model-averaged parameter estimates for explanatory variables from models to explain variation in the frequency of hatching failure among Burrowing Owls in western North America. Presented are regression coefficients averaged across all candidate models. The predictors of greatest *a priori* interest are in bold.

Parameter	**β**	**SE**	**95% Confidence Interval**
Intercept	-2.6	0.29	-3.12 – -1.99
Burrow Temp[1]	0.82	0.30	0.32 – 1.37
Latitude	0.01	0.18	-0.60 – 0.68
Clutch Size	-0.01	0.14	-0.57 – 0.51
Date	0.04	0.14	-0.32 – 0.58

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

Table 2.11. Top-ranked models to explain variation in combined hatching success and subsequent nestling survival of Burrowing Owls through the 20th day after the nest began to hatch in western North America. Included are only those models receiving at least ≥ 1.0% model weight plus the intercept-only null model. The predictors of greatest *a priori* interest are in bold.

Model	**AICc**	**ΔAICc**	**weight**
~ **Burrow Temp**[1] **+ Latitude +** Number Hatched	540.3	0.0	0.31
~ **Burrow Temp + Latitude** + Date + Number Hatched	541.2	0.9	0.20
~ **Burrow Temp + Latitude +** Days Since Hatch + Number Hatched	542.3	2.0	0.11
~ **Burrow Temp + Latitude +** Date + Days Since Hatch **+** Number Hatched[2]	542.5	2.2	0.10
~ **Burrow Temp + Latitude +** Date	543.2	2.9	0.07
~ **Burrow Temp + Latitude +** Days Since Hatch	544.4	4.1	0.04
~ **Burrow Temp** + Date	544.5	4.2	0.04
~ **Burrow Temp + Latitude** + Date + Days Since Hatch	544.9	4.6	0.03
~ Number Hatched	546.4	6.1	0.01
~ **Burrow Temp +** Days Since Hatch + Number Hatched	546.5	6.2	0.01
~ **Burrow Temp**	546.9	6.6	0.01
~ **Burrow Temp** + Date + Number Hatched	546.9	6.6	0.01
~ **Latitude** + Number Hatched	547.0	6.7	0.01
~ Date **+** Number Hatched	548.1	7.8	0.01
~ Days Since Hatch + Number Hatched	548.5	8.2	0.01
~ **Burrow Temp +** Date	548.5	8.2	0.01
Null model (intercept only)	551.6	11.3	0.00

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

[2] Global mode

Table 2.12. Model-averaged parameter estimates for explanatory variables included in models to explain variation in combined hatching success and subsequent nestling survival of Burrowing Owls through day 20 after the nest hatched in western North America. Presented are regression coefficients averaged across all candidate models. Predictors of greatest *a priori* interest are bold.

Parameter	**β**	**SE**	**95% Confidence Interval**
Intercept	0.03	0.20	-0.36 – 0.42
Burrow Temp	-0.94	0.37	-1.62 – -0.34
Latitude	-0.55	0.30	-1.11 – -0.14
Number Hatched	0.34	0.26	0.04 – 0.84
Days Since Hatch	0.01	0.08	-0.26 – 0.32
Date	0.06	0.15	-0.22 – 0.58

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

Table 2.13. Top-ranked models to explain variation in clutch size among Burrowing Owl nests in western North America. Included are only those models receiving > 0% model weight plus the intercept-only null model. The predictors of greatest *a priori* interest are in bold.

Model	**AICc**	**ΔAICc**	**weight**
~ Burrow Temp[1] **+ Latitude** + Date	246.6	0.0	0.50
~ Burrow Temp + Date	246.9	0.3	0.44
~ **Latitude** + Date	251.1	4.5	0.05
~ Burrow Temp	254.0	7.4	0.01
~ Burrow Temp + **Latitude**	255.8	9.2	0.01
Null model (intercept only)	283.2	36.6	0.00

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

Table 2.14. Model-averaged parameter estimates for explanatory variables included in models to explain variation in clutch size among Burrowing Owl nests in western North America. Presented are regression coefficients averaged across all candidate models. The predictors of greatest *a priori* interest are in bold.

Parameter	**β**	**SE**	**95% Confidence Interval**
Intercept	8.20	0.14	7.90 – 8.48
Burrow Temp[1]	-0.64	0.26	-1.12 – -0.24
Latitude	0.35	0.24	-0.09 – 0.78
Date	-0.54	0.18	-0.88 – -0.21

[1] Burrow temp refers to the average burrow temperature during the laying stage of the nest cycle

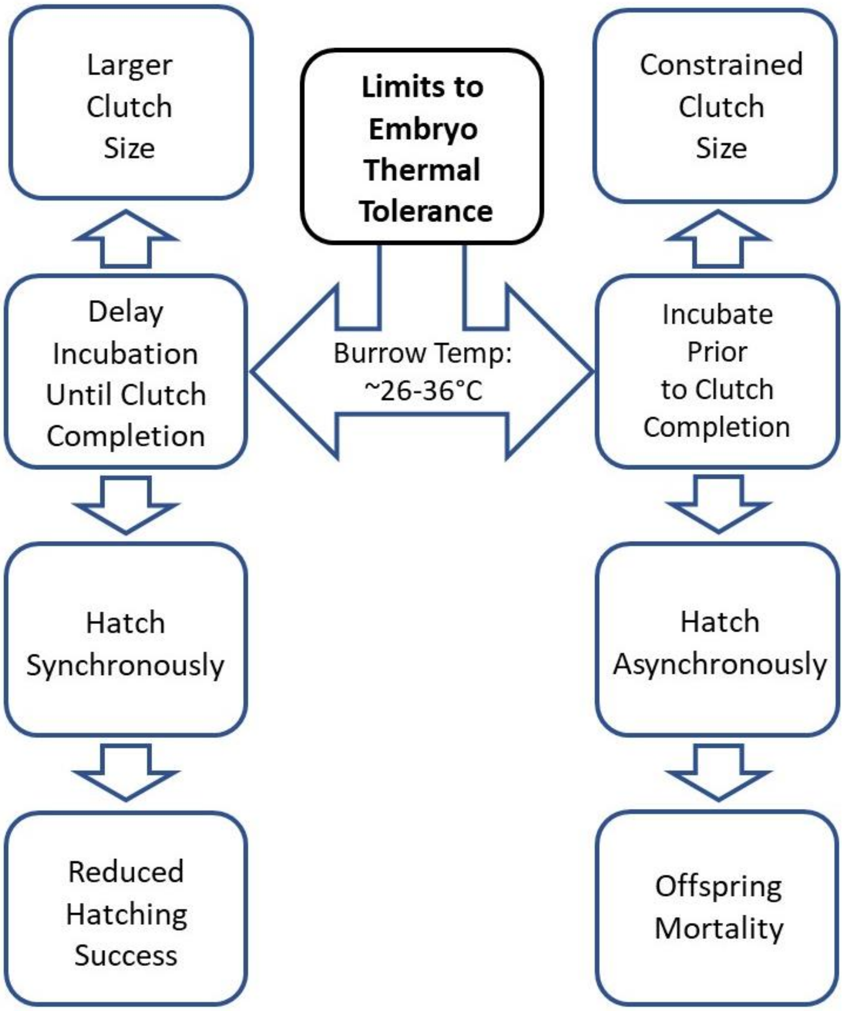

Figure 2.1. Conceptual diagram demonstrating the cascade of events and life-history trade-offs induced by limits to the thermal tolerance of embryos, according to the *egg viability hypothesis* (Arnold et al. 1987, Stoleson and Beissinger 1999).

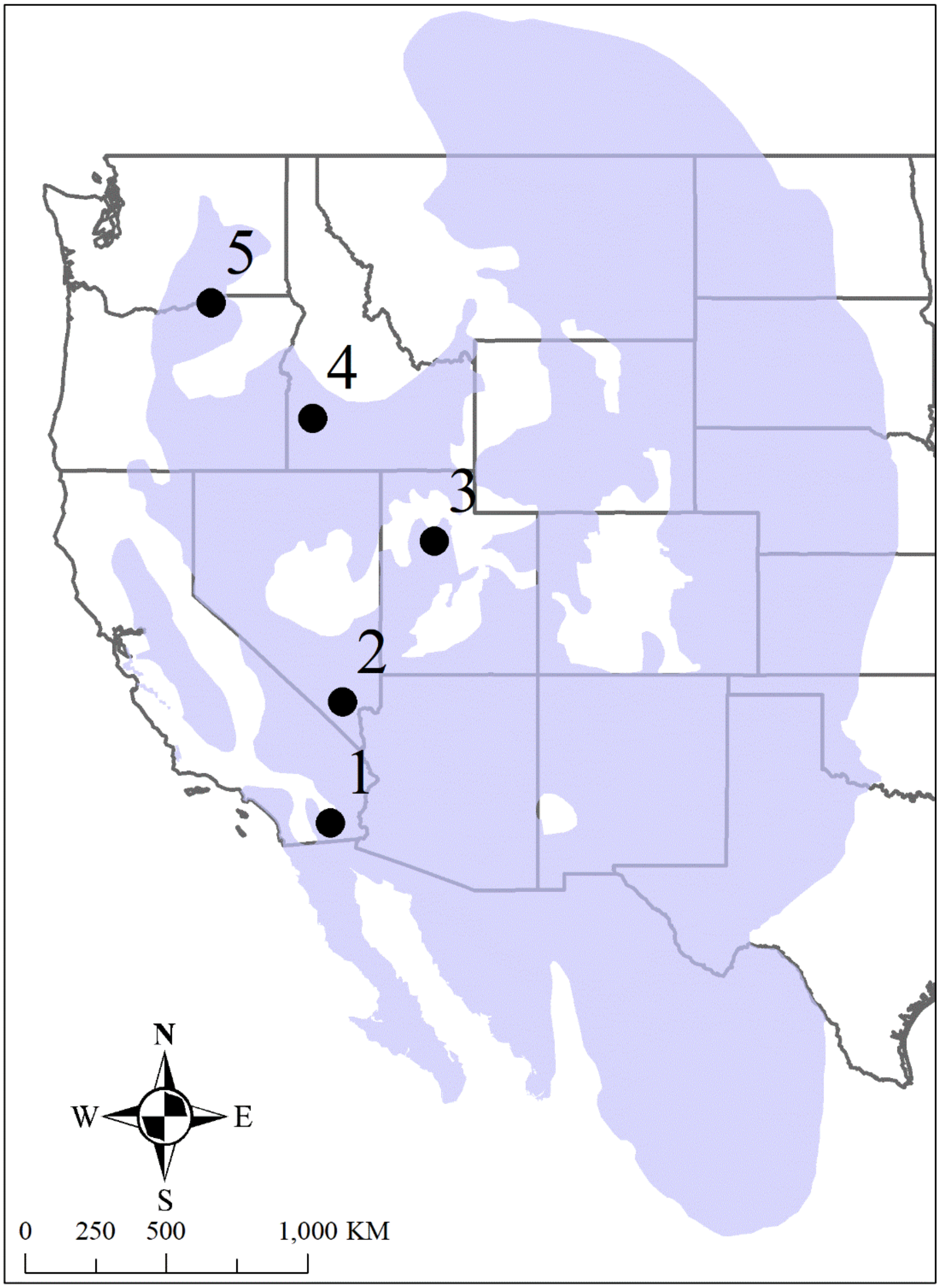

Figure 2.2. Study Sites: 1) Sonny Bono Salton Sea NWR; 2) North Las Vegas and Pahrump, NV; 3) Tooele Army Depot South Unit, Utah; 4) Snake River Birds of Prey NCA, Idaho; 5) Umatilla Army Depot, Oregon. Current Burrowing Owl range in gray (reproduced from Wellicome and Holroyd 2001).

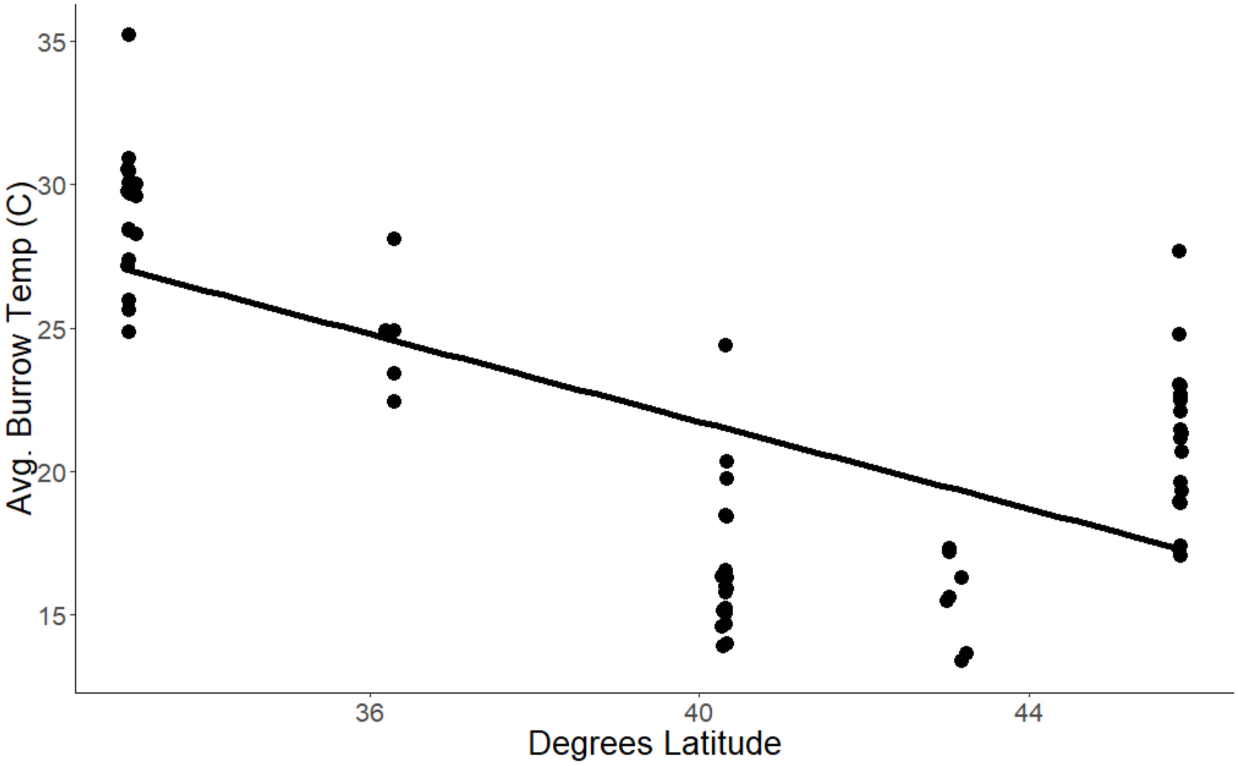

Figure 2.3. The average temperature inside Burrowing Owl artificial nest burrows during the egg-laying stage declined with increasing latitude in western North America, but the decline was non-linear, and burrow temperature did not perfectly track latitude. The linear regression line is included to illustrate both: 1) the negative trend, and 2) that most individual points fall well above or below the regression line.

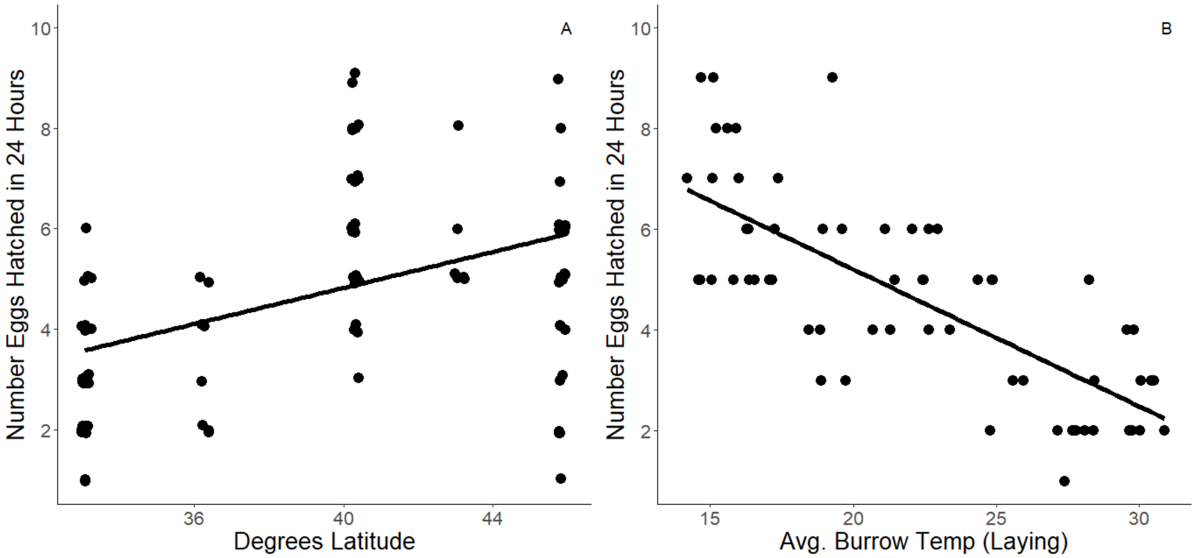

Figure 2.4. The timing of incubation onset: A) was positively correlated (became later) with latitude (*R* = 0.44, *n = 104*), and B) negatively correlated (became earlier) with increasing average burrow temperature (*R* = -0.77, *n* = 65), among Burrowing Owls breeding in western North America. Illustrated are the raw data values and simple univariate linear regression lines. Clutches in which incubation begins earlier, relative to laying, are expected to hatch more asynchronously (fewer eggs during the first 24 hours), and those in which incubation begins later are expected to hatch more synchronously (more eggs during the first 24 hours).

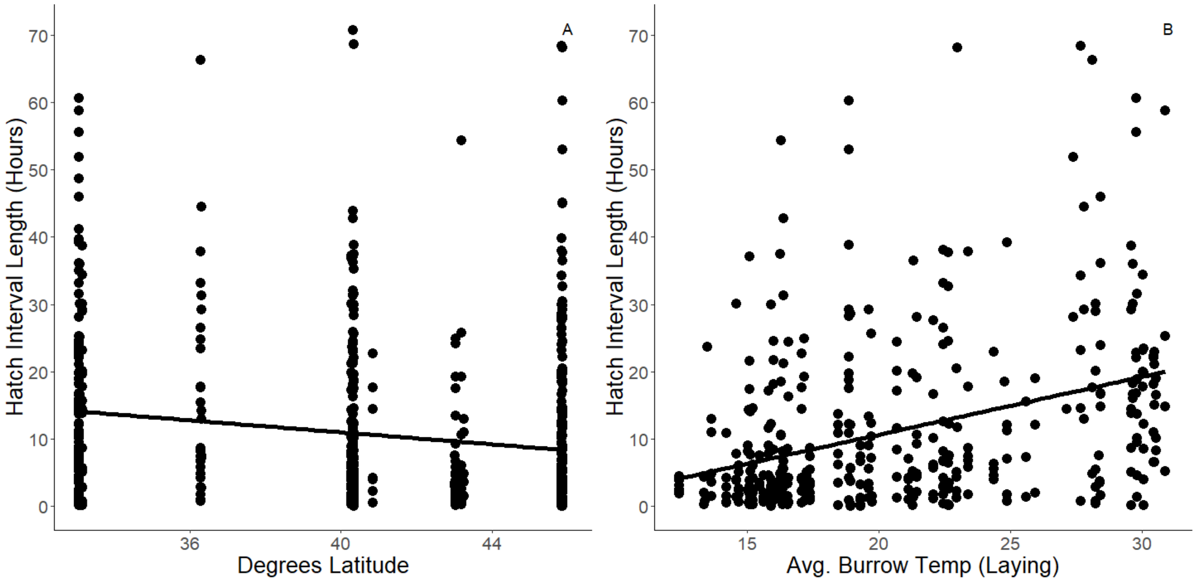

Figure 2.5. Hatch interval length: A) was negatively correlated with latitude (*R* = -0.18, *n* = 685), and B) was positively correlated with average burrow temperature during the laying period (*R* = 0.36, *n* = 391), among Burrowing Owls breeding in western North America. Illustrated are the raw data values and simple univariate linear regression lines.

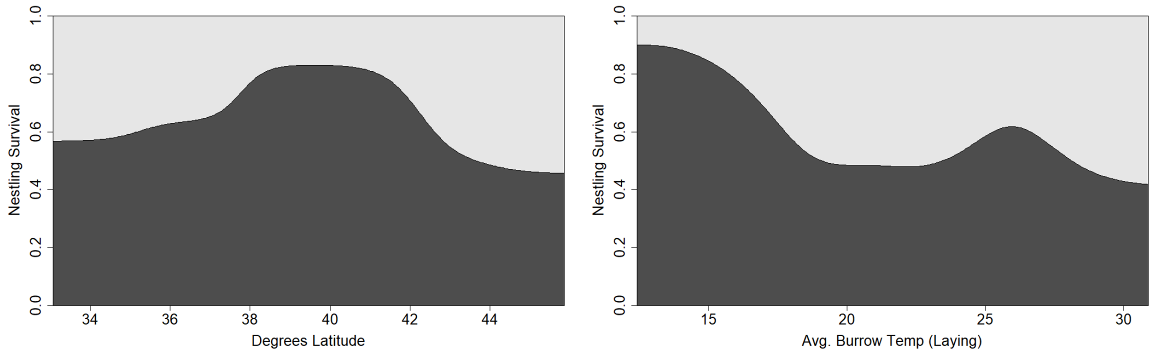

Figure 2.6. The survival of nestlings during the first 20 days after the first egg in the clutch hatched: A) varied non-linearly with latitude (*n* = 697), and B) was negatively correlated with average burrow temperature during the laying period (*n* = 395), among Burrowing Owls breeding in western North America. Surviving young are shown in dark gray and those dying before day 20 are shown in light gray. We excluded those that died from predation and focused on those that are assumed to have died from starvation.

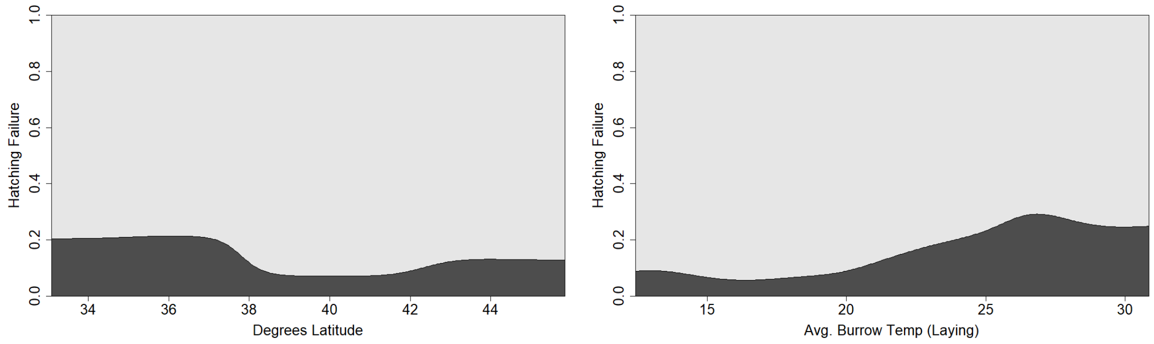

Figure 2.7. The rate of hatching failure of individual eggs: A) varied little with latitude (*n* = 950), and B) was positively correlated with average burrow temperature during the laying period (*n* = 561) among Burrowing Owls breeding in western North America. Successfully hatched eggs are shown in light gray and failed eggs are shown in dark gray.

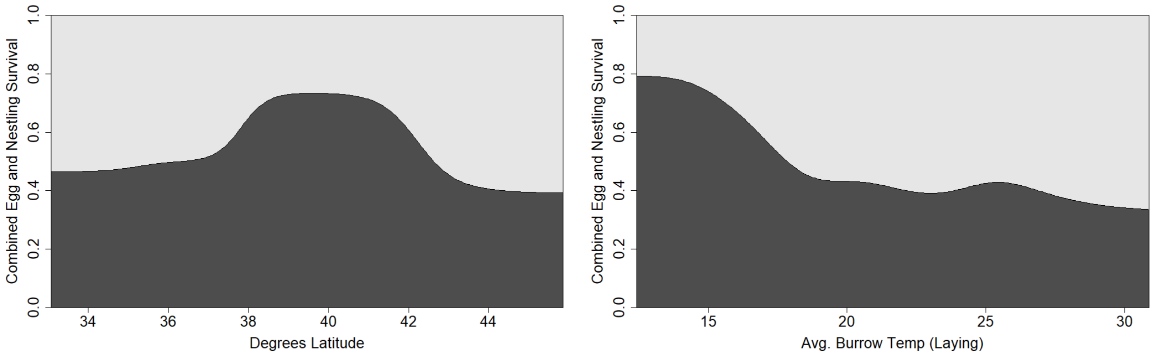

Figure 2.8. The combined hatching success and subsequent nestling survival until 20 days post-hatch: A) varied non-linearly with latitude (*n* = 786), and B) declined with increasing burrow temperature during the laying period (*n* = 470) among Burrowing Owls breeding in western North America. Surviving individuals are shown in dark gray and mortalities are shown in light gray.

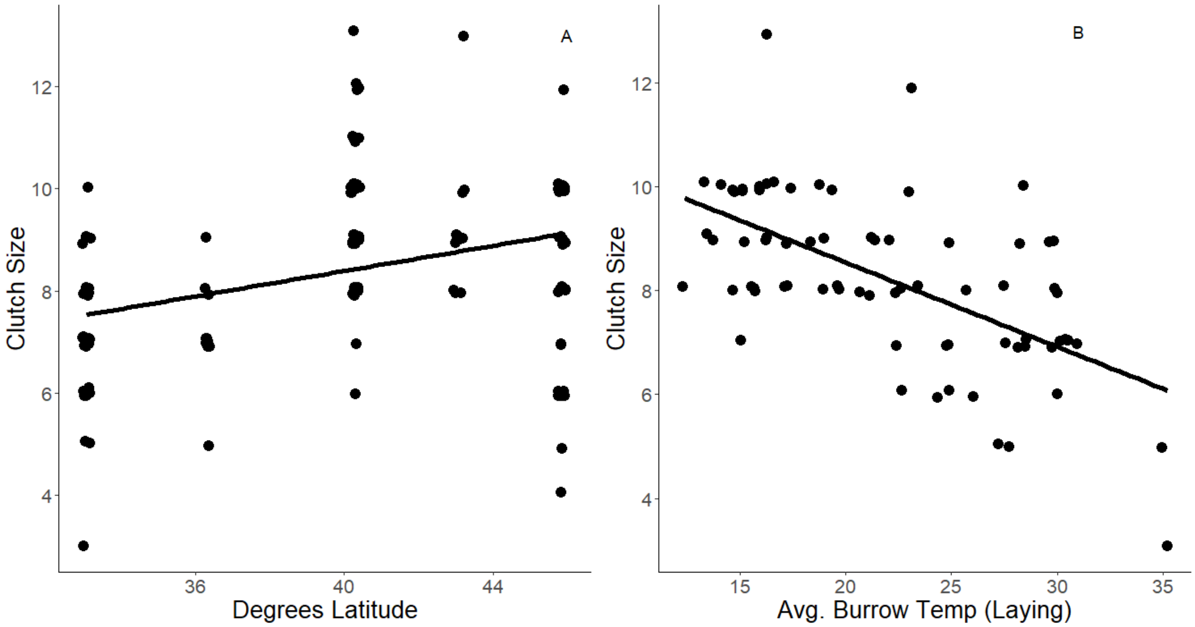

Figure 2.9. Clutch size was: A) positively correlated with latitude (*R* = 0.32, *n* = 130), and B) negatively correlated with average burrow temperature during the laying period (*R* = -0.59, *n* = 73), among Burrowing Owls breeding in western North America. Illustrated are the raw data values and simple univariate linear regression lines.

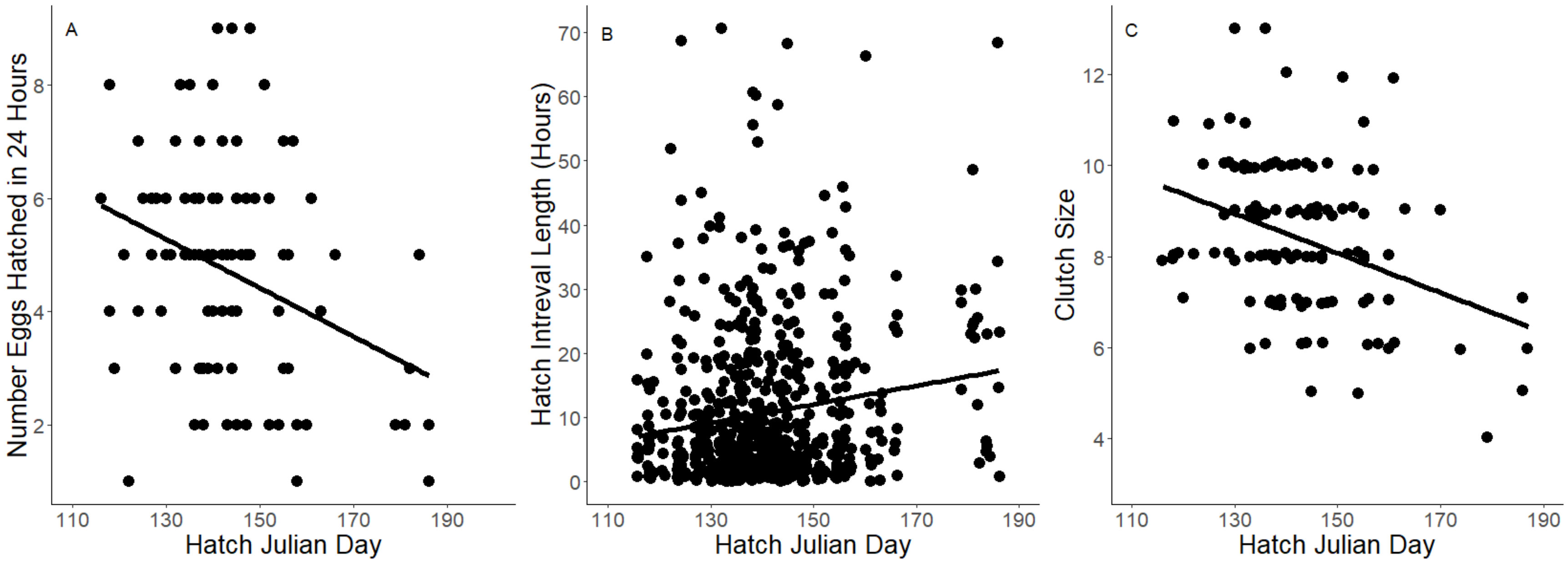

Figure 2.10. Among Burrowing Owls breeding in western North America: A) the timing of incubation onset became earlier, B) average hatch interval length increased, and C) clutch size declined as the breeding season progressed. Illustrated are the raw data values and simple univariate linear regression lines.

Chapter 3: Intraspecific Variation in Egg-laying Behavior Along a Latitudinal Gradient Reveals Tradeoffs with other Life-history Traits

Abstract

Many studies have documented and sought to explain patterns of variation in life-history traits such as the well-known association between clutch size and latitude. However, the egg-laying period remains a poorly known stage of avian reproduction, even though allocation decisions made during the egg-laying stage likely constrain and tradeoff with investment in other traits and activities. We quantified variation in the egg-laying behaviors of Burrowing Owls along a 1400-km latitudinal gradient in western North America. Laying interval length varied dramatically, was negatively correlated with latitude, and was strongly positively correlated with burrow temperature. The strong positive association with burrow temperature indicates that early onset incubation in response to limits to egg viability might energetically constrain the rate of egg production. However, the association between laying interval and burrow temperature became weak once we accounted for the other covariates in a multiple regression model. Laying intervals declined with increasing clutch size and were negatively associated with eventual hatching success of the brood. Laying interval length was positively associated with the degree of hatch asynchrony, indicating that laying interval length is a second mechanism (along with timing of incubation onset) that generates variation in hatching patterns. Burrowing Owls laid eggs at all times of day but laid more eggs in the morning hours, and that tendency was strongest among first eggs in a clutch (i.e., nest initiation times). Our results indicate that allocation decisions during laying are an important part of avian life-histories and that laying intervals may trade-off with other life-history traits. Additional non-invasive studies of the laying stage are required to more fully understand how allocation decisions during the laying stage constrain and interact with other stages of avian reproduction.

Introduction

The evolved strategies by which animals balance reproductive effort with self-maintenance and survival to maximize lifetime fitness (i.e., life-history strategies) have long fascinated ecologists and reflect a central theme of many ecological disciplines. For example, the tradeoff between reproductive effort and survival is central to our understanding of how populations are regulated, and an improved understanding of the causes of variation in those strategies is required to anticipate how populations will respond to global warming and many other environmental changes. Consequently, studies of clutch size, incubation behavior, hatching patterns, nestling growth, and parental care have provided many important insights into how birds navigate these tradeoffs and decide how and when to allocate resources to different activities (e.g., Perrins 1965, Lessells 1991, Stoleson and Beissinger 1999, Martin et al. 2011, Zanette et al. 2011). The egg-laying stage of the avian nesting cycle involves key allocation tradeoffs that influence other life-history traits but has been largely ignored in studies of avian life-history tradeoffs. The egg-laying stage in wild bird populations remains a virtual black box in our understanding of avian reproduction because observing this stage relies on researchers' ability to anticipate when and where birds are going to nest. Even when likely nest sites can be identified prior to egg-laying, quantifying laying patterns typically requires frequent nest checks at a time when many birds are sensitive to human disturbance and prone to nest abandonment (Schroeder and McRae 2018). As a result, we have limited knowledge of egg laying behaviors in wild bird populations including what time of day birds lay their eggs, how much time elapses between the oviposition of consecutive eggs in a clutch, and to what extent these traits vary across and within species and even within individual clutches. Hence, we need to document intraspecific variation in egg-laying behavior and to test whether behaviors associated with egg-laying are key life-history traits that have tradeoffs with other traits. If such tradeoffs exist, this information will help elucidate the drivers of intra- and interspecific variation in clutch size, hatching asynchrony, hatching success, nestling growth, and survival (Schroeder and McRae 2018).

Like many aspects of egg-laying, the diel time of laying is undocumented in most wild birds even though these data could provide insights into the ecological, hormonal, and physiological processes (Birkhead 1988, Watson et al. 1993, Wiebe and Martin 1995) and abiotic constrains (Johnson 2015, Weatherhead et al. 1991) that regulate avian reproductive effort and seasonal phenology of reproduction. For example, the presence of a developing egg in the oviduct could constrain female activity (Schifferli 1979, O'Connor 1984) and, hence, selection would favor laying at a certain time of day. Selection to lay at a certain time of day could in turn affect the rate of egg production (Watson et al. 1993) and therefore the length of the nesting cycle and timing of incubation onset. Birds might also lay at a particular time of day to prevent nest predation (Watson et al. 1993), facilitate brood-parasitism of another bird's nest (Seel 1993, McRae 1995, 1996), or to thwart would-be brood-parasites of their own nests (Feare et al. 1982). Accurately determining the time of laying is also a prerequisite for accurately estimating other reproductive parameters such as incubation period length (Skutch 1952), daily nest survival, and inter-egg laying intervals.

Probably more interesting and consequential than (though directly related to) diel time of laying eggs is how much time elapses between the laying of eggs within a clutch (the laying interval). Laying interval is typically assumed to be a fixed trait in birds (Lack 1968, Astheimer 1985). Birds are assumed to experience strong directional selection to complete laying as quickly as possible (Watson et al. 1993) because minimizing the length of the laying period reduces the time that nests are exposed to predators (Clark and Wilson 1981), increases the potential for renesting after nest failure (Martin 1995, Wiebe and Martin 1995), and may maximize egg viability and hatching success (Arnold et al. 1987, Beissinger and Waltman 1991, Lundblad 2020: Chapter Two). However, physiological, energetic, and nutritional constraints put a lower bound on how quickly female birds can produce eggs (Warren and Scott 1935, Sturkie 1977, Welty 1982, Carey 1986, Johnson 2015, Winkler 2019). Most birds are thought to lay one egg every 24 hours, but many exceptions occur especially among non-passerines (Lack 1968, Astheimer 1985, Carey 1986, Colwell 2006).

Many selective pressures and energetic tradeoffs could potentially mediate variation in laying interval, if laying interval is a plastic trait. For example, intraspecific variation in laying interval could reflect the energetic condition of the female. Laying females often experience substantial energetic constraints during the early part of the nesting season (Perrins 1970, Slagsvold 1986, Nilsson and Svensson 1993a). Moreover, birds generally time their breeding so that peak nestling growth coincides with seasonal peaks in food abundance (Rowan 1926, Lack 1968) which often requires birds in temperate latitudes to initiate laying at a time when food availability may be low and thermoregulatory demands are high. Therefore, cold weather or food limitation at the time of nest initiation could limit the rate of egg production or delay the onset of incubation (O’Connor 1978, Yom-Tov and Hilborn 1981, Slagsvold 1986, Nilsson and Svensson 1993a, Nilsson 1994). Laying schedules could also be constrained by breeding season length (Watson et al. 1993) or seasonal carryover effects on female condition (Runge and Marra 2005) going into the breeding season (especially in “capital breeders”; Drent and Daan 1980, Parker and Holm 1990).

Laying interval durations could also be subject to energetic tradeoffs and thereby affect other traits (Roff 2002, Schubert and Coke 1993). Although short laying intervals might be optimal for minimizing predation risk and maximizing renesting potential, birds might have to sacrifice investment in other traits or activities to achieve them. For example, some species initiate incubation prior to clutch completion, but early incubation onset could limit resources available for egg production because incubation is also energetically intensive (Williams 1966, Stoleson and Beissinger 1999). Conversely, short laying intervals could come at the cost of delayed incubation or egg neglect leading to hatching failure (Arnold et al. 1987). Laying interval could also be constrained by morphological, hormonal, and physiological processes that underlie egg production (Leblanc 1987, Arnold 1991, Watson et al. 1993, Wiebe and Martin 1995). Finally, female birds may facultatively manipulate the length of their laying intervals to achieve optimal hatching patterns (Clark and Wilson 1981, Mead and Morton 1985). Although intraspecific variation in laying interval has been quantified in a handful of species (Skutch 1952, Beissinger and Waltman 1991 Schubert and Cooke 1993, Watson and Cooke 1993, Aparicio 1994, Wiebe and Martin 1995, Oppenheimer et al. 1996, Cooper et al. 2009), most studies of laying behavior have been based on infrequent nest checks or incidental data. Even fewer studies have examined the selective pressures and potential tradeoffs that drive that variation. Furthermore, no previous studies have examined intraspecific variation in laying interval along geographic or environmental gradients. Hence, we sought to document and quantify variation in egg-laying behavior with greater precision and to investigate possible selective pressures that explain that variation.

We were able to overcome the logistical challenges inherent in studies of laying behavior by using motion-activated videos cameras to film Burrowing Owl nests across a 1400-km latitudinal and climatic gradient in western North America. Our objectives were to: 1) document and describe variation in the time of laying by Burrowing Owls (*Athene cunicularia*) across the latitudinal gradient, 2) document and describe variation in laying interval in Burrowing Owls along the latitudinal gradient, and 3) examine what selective factors or tradeoffs might account for variation in laying interval. Burrowing Owls are an excellent model for examining patterns and drivers of variation in laying behavior because they have intraspecific variation in laying interval and exhibit plasticity in other aspects of their reproductive behaviors and life-history strategies (Conway et al. 2012, Wellicome 2005, Lundblad 2020: Chapters One, Three).

Methods and Materials

Study Species

Burrowing Owls inhabit the western hemisphere from southern Argentina to southern Canada. They nest primarily in burrows constructed by mammals and other burrowing animals (Poulin et al. 2011, Conway 2018) and sometimes in artificial burrows constructed by researchers and wildlife managers (Collins and Landry 1977, Olenick 1990, Smith and Belthoff 2001). They range from fully migratory at the highest latitudes to resident in milder climates (Poulin et al. 2011). Their clutch size varies from 3 – 14 eggs/clutch (Todd and Skilnick 2002, Poulin et al. 2011, Conway et al. 2012), and they are “removal indeterminate” and “addition determinate” layers in response to egg removals and additions, respectively (Wade and Belthoff 2016). Females do all the incubation, they often re-nest following nest failure, but they rarely raise more than one brood per year (Poulin et al. 2011). Burrowing Owls often experience partial hatching failure, and starvation is a common cause of nestling mortality (Poulin et al. 2001, Wellicome 2005).

Study Sites

We studied Burrowing Owls at five study sites (Fig. 3.1): 1) Sonny Bono Salton Sea National Wildlife Refuge (33°2’N, 115°37’W, elevation -69m); 2) North Las Vegas and Pahrump, Nevada (considered a single site, North Las Vegas at 36°18’N,115°14’W, elevation 670m, Pahrump at 36°18’N, 116°2’W, elevation 790m); 3) Tooele Army Depot South Area, Utah (40°2’N, 112°20’W, elevation 1520m); 4) Morley Nelson Snake River Birds of Prey National Conservation Area, Idaho (43°00’N, 116°00’W, elevation 945m); and 5) Umatilla Army Depot, Oregon (45°5’N, 119°26’W, elevation 152m). At each study site, some Burrowing Owls nested in artificial underground burrows. Artificial burrow design varied among and within study sites, but followed widely-used designs meant to approximate the dimensions, depth, thermal, and other properties of natural burrows (Collins and Landry 1977, Smith and Belthoff 2001, Alexander et al. 2005, Barclay 2008, Johnson et al. 2013). Each artificial burrow included a single entrance tunnel, and the nesting chambers of artificial burrows varied in depth from 12 – 65 cm (from top of the chamber to ground surface).

Field Methods

We installed one of several models of motion-activated infrared CCTV cameras (Alibi CD700 and CD80B Turret Dome, Observint Technologies, Austin, TX; HT71HG Mini Vandal Turret Dome and HT71TG Mini Turret Dome, Speco Technologies, Amityville, NY) inside the artificial burrows to document laying behavior. We connected each camera to a mobile DVR recorder (MDVR25HR MPEG-4, Observint Technologies, Austin, TX) placed in a plastic bucket or plastic food storage container external to the burrow. We programmed the DVRs to record a 2.5-minute video segment any time there was substantial motion in the burrow (we set motion-detection sensitivity to medium) and saved video clips to a 32-gb SD memory card that we changed every 5 – 15 days. We powered each camera with a 12-volt deep cycle battery, installed external to the burrow and replaced every 2 – 4 days. We also installed temperature data loggers (iButton model DS1922L, Maxim Integrated, San Jose, CA and Embedded Data Systems, Lawrenceburg, KY) on the inner wall or ceiling of most burrow chambers and programmed them to record the ambient burrow temperature every 10 – 20 minutes (except in 2015, when we collected no burrow temperature data). We also placed 1 – 2 iButton temperature loggers in shaded and sheltered outdoor locations at each study site to measure the ambient above-ground temperature at each site and in each year (except in 2015).

We temporarily blocked the burrow entrance during equipment installation, allowing the female (if present) to take refuge in the entrance tunnel without flushing from the burrow. Opening the burrows for <10 minutes did not affect burrow temperature in a previous study at our warmest study site in southeastern California (Nadeau et al. 2015). We kept each camera running, whenever possible, until at least 20 days post-hatching or until the nest failed. Equipment failure and the challenges associated with keeping batteries regularly maintained resulted in partial data losses. We installed cameras in a total of 150 nests at the five study sites from 2015 – 2018 and collected a total of 1,533,325 2.5-minute video clips accounting for 63,889 hours and 14.0 terabytes of video data. We installed 38.3% of the cameras that yielded laying data prior to nest initiation, 15.0% between the laying of the first and second egg, and 47.7% after more than one egg was present. Of the 150 nests with cameras, 107 nests produced usable laying period videos. Among the 107 nests for which we obtained laying data, we observed the laying times for an average of 64.5% (± 0.03 SE) of the eggs in the clutch.

We opened the artificial burrow twice during the nestling stage at each nest to measure and count the number of surviving nestlings: on ~10th day (range ninth to fifteenth) and ~20th day (range nineteenth to twenty-fifth) after the first egg hatched. On both of these days, we measured the wing chord length, tarsus length, and body mass of each nestling. We applied temporary colored rubber bands to their legs for individual identification. We removed the colored rubber bands on the second measurement occasion and replaced them with aluminum USGS numbered leg bands (once their legs were large enough for bands). We also opened and checked nearby artificial burrows, checked any nearby natural burrows for signs of owl use, and trapped at the natural burrows if less than the entire brood was accounted for and we thought the other burrows might contain nestlings that relocated from the focal burrow (rare). We captured most adults prior to nest initiation or after hatching was complete. We took a suite of adult measurements including wing chord length, tarsus length, tail length, and body mass, assessed muscle and fat condition on a standardized scale (DeSante et al. 2013), and applied USGS aluminum bands to any previously-unbanded adult owls.

Video Data Processing

We reviewed all of the 2.5-min video segments in their entirety from when the first egg was laid or the camera was installed (for those installed after laying began) until the final viable egg hatched. We recorded the time when each egg was laid and hatched. When the incubating or brooding female blocked the camera's view of the clutch and prevented us from recording the exact laying or hatching time of an egg, we used the midpoint between when the egg was last seen unlaid/hatched and when the newly-laid/hatched egg/nestling was first observed as the hatching time.

Analytical Methods

We used Rayleigh's *Z*-test (Pewsey et al. 2014) for uniformity of circular data to test whether egg laying times were uniformly distributed throughout the 24-hour day or whether owls laid their eggs disproportionately at a certain time of day. We used a Rayleigh test for all of the data pooled, for each individual study site, and for eggs in each of the first through fourth position in the laying order. We calculated the difference between each laying time and the time of sunrise on that date and location. We calculated sunrise times using the "suncalc" package (Thieurmel and Elmarhraoui 2019) and Rayleigh test statistics using the "circular" package in R (Agostinelli and Lund 2017).

We calculated the duration of individual laying intervals as the amount of time elapsed between the laying of consecutive eggs in the same clutch. We observed a few females removing previously-laid eggs from abandoned nesting attempts, prior to re-laying and, therefore, we considered any eggs that were incubated together to represent the same clutch, even if some of them were laid prior to long laying interruptions (up to 337 hours, see results). We then calculated the univariate Pearson correlations between latitude and: 1) the absolute difference between each laying time and local sunrise, 2) laying interval, and 3) the average laying interval per nest. We used a two-sided *t*-test to examine whether the length of the final laying interval (between the penultimate and final eggs in a clutch) differed from the length of all other laying intervals (as suggested by our casual examination of the data).

We used linear mixed-effects models to examine the effect of variables predicted to either explain variation in, or tradeoff with, laying interval. We applied a square root transformation to the response variable, laying interval, to meet normality assumptions (i.e., reduce right-skewness). We specified a global model that included the following standardized explanatory variables: laying date, egg number (both the linear and quadratic effects, i.e., with respect to laying order), clutch size, latitude, average burrow temperature during the laying stage, percent incubation attentiveness during the laying interval, the proportion of the clutch that hatched successfully, the average hatching interval between eggs in the clutch (a measure of hatching asynchrony associated with the timing of incubation onset; Lundblad 2020: Chapter Two), and an index of female body condition. We replaced any missing covariate values with their means (which became zeros following standardization). We used principal component analysis to generate a multivariate index of Burrowing Owl structural body size that pooled overall variation in wing chord length, tarsus length, and tail length. We then regressed body mass on the resulting first principal component (PC1) and used the residuals as an index of female body condition relative to structural size. We used average burrow temperature during the laying stage as a thermal index because preliminary analyses suggested that it performed better than average burrow temperature during each laying interval and better than the average ambient temperature at the respective study site (from above-ground data loggers) during each laying interval. We also considered a global model that included relative egg number (egg number divided by clutch size) instead of absolute egg number to account for clutch size variation. We used AICc

(Burnham and Anderson 2002) to select among two candidate random-effects structures: a random intercept for nest ID and random intercepts for both nest ID and site-year combination. We did not consider models without a random effect for nest ID because laying intervals associated with the same nest were not independent samples (Hurlbert 1984). We constructed models including each possible combination of the fixed effects and then calculated model-averaged parameter estimates and 95% confidence intervals. Variance inflation factors associated with the predictors in our global model ranged from 1.7 to 3.6 suggesting that excessive multicollinearity was not a problem.

For additional corroboration of the results generated by the multiple regression, we also calculated the univariate regressions between laying interval and each of the predictors in the multiple regression model. We used average per-nest laying interval in each of the correlations, except for covariates measured at the level of the individual laying interval (i.e., egg number and percent nest attentiveness during that interval), for which we calculated correlations with individual laying intervals.

Finally, we used mixed-effects logistic regression to explore what variables were associated with laying interruptions ≥72 hours. We created a binary response variable indicating whether each laying interval was ≥72 hours or not. We collected daily precipitation data from the nearest available weather station to each study site (https://www.wunderground.com/). We used AICc to compare two thermal indices: burrow temperature during each interval and ambient temperature during each interval. We standardized all the predictors and replaced any missing covariate values with their means (which became zeros following standardization). We used AICc to compare global models with two different random-effects structures: 1) a random intercept for nest ID, and 2) a random intercept for nest ID and a random intercept for each site-year combination. Our resulting global model included laying date, egg number, latitude, clutch size, the cumulative amount of precipitation that fell on the day of laying plus the two previous days, and thermal conditions during each laying interval. We then constructed models including all possible combinations of variables and based final inferences on model-averaged parameter estimates.

We performed all analyses in R (R Core Team 2019). We fit all models in the package “glmmTMB” (Brooks et al. 2017) and performed model selection and model averaging in the package “MuMIn” (Barton 2019). We calculated variance inflation factors using the VIF function in the R package “car” (Fox and Weisberg 2019). We based principal component analysis on single value decomposition and implemented with the built-in R function “prcomp”.

Results

We observed the laying times for 675 individual eggs at 111 nests across the five study sites. The average uncertainty surrounding estimated laying times was ± 0.46 hours (± 0.04 SE). Burrowing Owls laid eggs at all times of day and night, but disproportionately laid eggs in the morning (Rayleigh $Z = 0.07$, $n = 675$, $P = 0.03$) with peaks 1 – 3 hours before and 2 – 5 hours after sunrise (Fig. 3.2A). Laying times did not differ from random at 4 of the 5 study sites; the exception was at the Nevada study site (Rayleigh $Z = 0.28$, $n = 40$, $P = 0.05$; Fig. 3.2B – F). The absolute difference between laying times and local sunrise was weakly positively correlated with latitude ($R = 0.09$, $n = 675$, $P = 0.02$). Laying times for the first egg in a clutch (nest initiation times) across all sites were overwhelmingly in the morning (Rayleigh $Z = 0.34$, $n = 51$, $P = 0.003$) with most nests initiated 2 – 4 hours after sunrise (Fig. 3.3A). Laying times became increasingly "out of phase" and random with respect to hour of the day, across the laying order (Fig. 3.3).

We calculated 555 laying intervals at 104 nests. Laying interval ranged from 30.0 – 336.7 hours ($\bar{x} = 46.6$ hours ± 0.95 SE, median = 42.9 hours; Figs. 3.4 – 3.5). Most laying intervals were 40 – 50 hours in duration (Fig. 3.5), 90.7% of laying intervals exceeded the 36 hours mentioned in the Burrowing Owl literature (Olenick 1990, Wellicome 2005, from Idaho and Saskatchewan, respectively), and the average laying interval per nest exceeded 36 hours at 94.9% of nests (range 30.4 – 87.0 hours). Some females interrupted laying for unexpectedly long periods. Interruptions of $\geq$72 hours ($n = 24$, range 75.78 – 336.7 hours) were most abundant at our southern-most study site ($n = 14$) and were less common at the other 4 study sites (1 – 4 instances each).

Laying interval was negatively correlated with latitude ($R = -0.14$, $n = 555$, $P < 0.001$; Fig. 3.6B) as was average laying interval per nest ($R = -0.31$, $n = 97$, $P < 0.002$; Fig. 3.6A). Excluding laying intervals $\geq$72 hours in duration (which generally occurred in the early to middle part of the laying order, see below), final laying intervals (between the penultimate and last egg in each clutch) were significantly longer ($\bar{x} = 45.8$ hours) than all other laying intervals in the clutch ($\bar{x} = 42.1$ hours) ($t = -5.4$, df = 155.1, $P < 0.001$; Appendix A).

The global models to explain variation in laying interval with two different random-effect structures performed within ΔAICc = 0.5 of each other, so we used the simpler model (which included a random intercept for nest ID). The global model including absolute egg number performed better than the global model including relative egg number (ΔAICc = 0.3), so we used absolute egg number. The top model to explain variation in laying interval included clutch size, the linear and quadratic effects of egg number, percent nest attentiveness during that interval, average hatch interval (degree of hatch asynchrony), and hatch success of the entire clutch and received 17% of the total model weight (Table 3.1, Fig. 3.7). The model-averaged effects of clutch size (β = -0.25, SE = 0.08, 95% C.I. = -0.41 – -0.10), egg number (β = -0.74, SE = 0.19, 95% C.I. = -1.11 – -0.37, percent nest attentiveness (β = -0.18, SE = 0.11, 95% C.I. = -0.39 – -0.03), and hatch success (β = -0.19, SE = 0.07, 95% C.I. = -0.32 – -0.07) were negative, the model-averaged effect of average hatching interval was positive (β = 0.09, SE = 0.07, 95% C.I. = 0.01 – 0.23), and each had a 95% confidence interval that excluded zero (Table 3.2).

Based on univariate correlations, average laying interval was negatively associated with the proportion of the clutch that hatched successfully (R = -0.41, n = 89, P < 0.001), clutch size (R = -0.31, n = 96, P = 0.002), nest initiation date (R = -0.10, n = 96, P = 0.36), egg number (R = -0.08, n = 831, P = 0.02), and weakly with female condition (R = -0.06, n = 91, P = 0.59). Individual laying intervals were negatively correlated with percent nest attentiveness during that interval (R = -0.15, n = 605, P < 0.001) and with egg number (R = -0.08, n = 831, P = 0.02), but note that these two tests necessarily involve pseudoreplication. Based on univariate correlations, average laying interval was positively associated with the average hatching interval at that nest (R = 0.50, n = 79, P < 0.001) and with average burrow temperature during the laying stage (R = 0.40, n = 70, P < 0.001).

The global logistic model to explain the probability of laying interruptions (those intervals ≥72 hours) that included a random intercept for only nest ID performed better than the other random-effects structure (ΔAICc = 2.1). The top model to explain the long laying interruptions (≥72 hours) included egg number within the laying order and latitude (Table 3.3). The model-averaged effect of egg number was negative (β = -0.44, SE = 0.35, 95% C.I. = -1.13 - -0.01); long laying interruptions were more common early in the laying sequence. The model-averaged effect of latitude was negative, but the 95% confidence interval included zero (β = -0.37, SE = 0.42, 95% C.I. = -1.37 - 0.15). The model-averaged 95% confidence intervals of all other variables also included zero (Table 3.4).

Discussion

The time elapsed between the laying of consecutive eggs (the laying interval) in a Burrowing Owl clutch varied dramatically, and laying interval was associated with numerous other life-history traits. Correlations with other traits imply that laying intervals may be involved in important life-history tradeoffs that reflect allocation decisions during a time in the annual cycle when many birds face their most critical energetic bottleneck (Perrins 1970, Nilsson and Svensson 1993a, Nilsson 1994). Burrowing Owls were previously reported to have average laying intervals of 36 hours in both

Idaho (Olenick 1990) and Saskatchewan (Wellicome 2005) based on nest-checks ≥2 days apart. Using continuous video recording, we observed laying intervals as short as 30 hours, but most of the laying intervals we observed were longer than the previously reported 36 hours. And we observed no laying intervals shorter than 30 hours (implying that intraspecific egg parasitism and egg dumping were not the cause of the longer intervals we observed). The mean laying interval length in our study also fell short of 48 hours, supporting the hypothesis that selection acts more strongly on laying interval than on the time of day that is optimal for laying (Watson et al. 1993). Lack of selection for laying intervals that are multiples of 24 hours also explains why, although first eggs were generally laid in the morning, successively laid eggs were increasingly out of phase.

The extreme variability we observed in Burrowing Owl laying intervals and the presence of long laying interruptions suggests that laying interval is more variable than previously thought (at least in some birds), is plastic (responsive to environmental influences), and is subject to tradeoffs via allocation with other life-history traits. Most notably, laying interval was negatively associated with clutch size. A negative association between laying interval and clutch size was previously noted in Snow Geese (*Chen caerulescens*; Schubert and Cooke 1993), Common Eiders (*Somateria mollissima*; Watson et al. 1993), and Eurasian Kestrels (*Falco tinnunculus*; Aparicio 1994). Birds laying larger clutches may be under stronger selection to reduce the length of their laying period, and reducing the length of the laying interval may have a lower fitness cost than reducing exposure days by reducing clutch size (Watson et al. 1993, Wiebe and Martin 1995). Indeed, Burrowing Owls at higher latitudes (where clutch sizes are larger) were more prone to lay eggs throughout the day rather than wait for morning. The negative association between laying interval and clutch size could also reflect variation in individual age, quality, or condition whereby individuals who are energetically able to lay larger clutches can also do so more quickly (Watson et al. 1993). For example, the relationship between laying interval and clutch size could be mediated by nutritional state of the female if the duration of the laying period is relatively fixed by hormonal processes (i.e., the ramp up in circulating prolactin that accompanies the onset of incubation; Aparicio 1994). In that case, if females in good condition are able to produce eggs more quickly, they could produce a larger clutch in the time available for laying (Aparicio 1994). However, we found that the length of the Burrowing Owl laying period varied substantially (unpublished data) and laying interval was not associated with female condition. Laying interval was also not associated with female size or condition in White-tailed Ptarmigan (*Lagopus leucurus*; Wiebe and Martin 1995). The negative association between laying interval and clutch size could also reflect variation in egg size if egg size varies inversely with clutch size as might be predicted under an offspring quantity-quality tradeoff (Schubert and Cooke 1993), but we lacked egg size measurements to test this. Regardless of its cause, the negative association between clutch size and laying interval appears to proximately explain the observed negative correlation between laying interval and latitude because clutch size and latitude are positively related (Lack 1947, Lundblad 2020: Chapter Two).

We found that clutches with longer laying intervals experienced reduced hatching success. Unincubated eggs exposed to intermediate temperature ranges (26 – 36° C) experience irregular embryonic development that may lead to embryo death and hatching failure (Arnold 1987, Webb 1987, Beissinger and Waltman 1991, Wang and Beissinger 2009), and the rate of failure increases with the duration of exposure to sub-optimal temperatures (Arnold et al. 1987). Therefore, longer laying intervals might lead to greater erosion of egg viability, especially in species like Burrowing Owls that lay large clutches. Birds exposed to such conditions often initiate incubation before completing their clutches to ensure proper development and maximize hatching success (Beissinger and Waltman 1991, Wang and Beissinger 2009). Burrowing Owls indeed initiated incubation earlier in warmer burrows (Lundblad 2020: Chapter Two), and early incubation might energetically constrain egg production resulting in smaller clutch size or longer laying intervals (Williams 1966, Beissinger and Waltman 1991, Stoleson and Beisinger 1999). We found some evidence suggesting that warmer burrows may be associated with longer laying intervals, as would be expected if early incubation in response to nest microclimate energetically constrained egg production (Stoleson and Beissinger 1995). We observed a strong positive (univariate) correlation between average burrow temperature during the laying period and laying interval, but the effect of burrow temperature was no longer significant once we accounted for the other variables in our multiple regression analysis. Furthermore, we did not find evidence that longer laying intervals were associated with greater nest attentiveness during laying. If anything, the relationship between nest attentiveness and laying interval was negative, although the 95% confidence interval of nest attentiveness overlapped zero. Future studies should more fully explore the complex interplay between nest microclimate, nest attentiveness, and limits to egg viability as a potential driver of variation in laying intervals.

We observed some extremely long laying intervals similar to the laying interruptions documented in several other bird species (Bryant 1975, Dhondt et al. 1983, Nilsson and Svensson 1993b, Yom-Tov and Wright 1993, Westphal and Sealy 2018). Long laying intervals may indicate an interruption of the physiological and hormonal processes that regulate laying or represent abandoned nesting attempts followed by renesting, but female Burrowing Owls typically removed abandoned eggs from previous nesting attempts before laying replacement clutches (hence, we considered all eggs incubated simultaneously to be part of the same clutch). Other than a residual unexplained effect of latitude, we found that only egg number (i.e., position in the laying order) explained significant variation in the probability of a laying interruption $\geq$72 hours. The probability of a laying interruption $\geq$72 hours was greatest following eggs laid early in the laying order and then declined across the laying order rather than being concentrated late in the laying order as would be expected if interruptions reflected a depletion of energetic reserves across the laying stage (Wiebe and Martin 1995). Laying interruptions in Blue Tits (*Parus caeruleus*; Nilsson and Svensson 1993b) and Yellow Warblers (*Setophaga petechia*) also declined across the laying order, and those in White-tailed Ptarmigan were distributed throughout the laying order (Westphal and Sealy 2018, Wiebe and Martin 1995). Previous studies have linked laying interruptions to severe weather during the laying stage (O'Connor 1979, Dhondt et al. 1983, Eeva and Lehikoinen 2010, Westphal and Sealy 2018), but interruptions in our study did not appear to coincide with periods of precipitation or cold weather. Long laying interruptions in the early to mid-portion of the laying order may also represent a physiological mismatch between the development of consecutive ovarian follicles and the release of luteinizing hormone required to cause ovulation (Wiebe and Martin 1995, Oppenheimer et al. 1996).

We found that final laying intervals (the interval between the penultimate and final egg in a clutch) were significantly longer than all other laying intervals (if we ignored those associated with long laying interruptions; Appendix A). That is, most females delayed the development or production of their final egg as has been documented in other species (Nolan 1978, Meijer 1992, Schubert and Cooke 1993, Watson et al. 1993, Cooper et al. 2009). Extended final laying intervals could reflect energetic constraints accrued across the laying order (Lack 1968), but if anything, laying interval decreased across the laying order (up until the final egg) in our study. Extended final intervals recall the tendency of many species (especially passerines) to initiate incubation on the penultimate egg (Howe 1978, Clark and Wilson 1981, 1985, Mead and Morton 1985, Potti 1998) or reduce the size of final eggs (Coulson 1963, Parsons 1970, Cargill 1979, Leblanc 1987, Robertson and Cooke 1993). All three patterns could result from the increase in circulating prolactin, decrease in circulating luteinizing and follicle-stimulating hormones, and inhibition of follicle development that accompany the transition from laying to incubation (Mead and Morton 1984, Leblanc 1987, Arnold 1991, Watson et al. 1993). Some species, however, increase the size or provisioning of the final egg (Gibb 1950, Kendeigh et al. 1956, Pinkowski 1975, Howe 1976, Rydén 1978, Arida et al. 2006), and different patterns of final egg size and production time could reflect context-dependent facultative decisions. For example, adjustment of final laying intervals could be a means by which females facultatively manipulate hatching patterns and asynchrony (Clark and Wilson 1981, Mead and Morton 1985). Extending the final laying interval could therefore be a means to "handicap" the final nestling and enhance or facilitate adaptive brood reduction when resource availability at the time of hatching is unpredictable (Lack 1954, Clark and Wilson 1981). Alternatively, extending the final laying intervals could allow the female to increase the size of the final egg (as suggested by Nolan 1978) to offset the asynchronous hatching imposed by early onset incubation (Clark and Wilson 1981, 1985). Future studies that simultaneously quantify laying interval, egg size, and nest attentiveness across the laying order would help resolve the cause of these patterns.

We found that longer laying intervals were associated with longer average hatching intervals and therefore with greater hatching asynchrony. The degree of hatching asynchrony experienced by a clutch is a function of the timing of incubation onset, relative to laying order, and of the laying intervals of those eggs laid after incubation begins (Mead and Morton 1984, Ricklefs 1993, Stoleson and Beissinger 1995). However, previous studies of hatching asynchrony have focused largely on the first mechanism and ignored the second (Magrath 1990, Stenning 1996). Quantifying the relative importance of the two mechanisms could lend new insights into the causes of variation in hatching patterns (Stoleson and Beissinger 1995). For example, if asynchronous hatching resulted primarily from longer laying intervals that would suggest that limits to the thermal tolerance of embryos is not the ultimate cause of hatching asynchrony (Arnold et al. 1987). Instead, our study suggests that both variation in laying interval and variation in the timing of incubation onset (Lundblad 2020: Chapter Two) contribute to hatching patterns and hatching asynchrony.

One potential confounding factor in studies of laying behavior, laying interval, hatching patterns, and hatching asynchrony is the potential for conspecific brood parasitism or egg-dumping (Yom-Tov et al. 1974, Watson et al. 1993, Lyon and Eadie 2008). Cavity- and burrow-nesting species may be especially prone to brood parasitism because suitable nest sites are limited (Martin 1993, Beauchamp 1997, Wade and Belthoff 2016). Our data suggest that conspecific brood parasitism is rare or absent in Burrowing Owls because we never observed laying intervals <30 hours. If egg-dumping by female Burrowing Owls was frequent, we would have expected a full range of apparent laying intervals shorter than the minimum we observed of 30 hours (Mackie and Buechner 1963, Yom-Tov 1980, Brown 1984). We observed two instances where two owls took turns incubating a single clutch. These may have resulted from disputes among females over a single burrow, possibly cases of polygynous males, or male incubation behavior. Without an individually marked population, we are unable to fully separate these alternatives, although at least one such instance was thought (based on plumage characteristics) to involve fleeting male incubation (unpublished data). Furthermore, because we observed no laying intervals <30 hours, it is unlikely that more than one female laid eggs in these burrows. However, Burrowing Owls sometimes rejected experimentally "parasitized" nests (those where researchers added an egg) by burying or abandoning their clutches in

Idaho (Groves 2014). Such recognition and rejection of foreign eggs suggests an evolved defense against conspecific brood-parasitism (Rothstein 1975).

Burrowing owls tended to lay eggs in the morning, but that tendency decreased across the laying order and with increasing latitude. To our knowledge, this is the first study to quantify variation in laying times for any bird across a large geographic gradient. Previous studies suggest that selection favors laying eggs in the morning (Skutch 1952, Seel 1968, Willis 1972, Nolan 1978, Schifferli 1979, Feare et al. 1982, Muma 1986, Wolf 1991, Scott 1993, 1998, Oppenheimer et al. 1996, McMaster et al. 1999, 2004, Gill 2003, Cooper et al. 2009). The tendency to lay eggs in the morning is particularly common in passerines but is less ubiquitous in waterfowl (Watson et al. 1993, Schubert and Cooke 1993 and references therein, Esler 1999), waterbirds (Mc Allister 1958, Ashmole 1963, Schroeder and McRae 2018), and upland gamebirds (Opel 1966, Wiebe and Martin 1995 and references therein).

Previous authors have suggested that birds lay eggs prior to their most active period (i.e., in the morning for birds that forage during diurnal hours) because carrying a developing egg in the oviduct during active hours might reduce flight efficiency, inhibit foraging and other daily activities, increase predation risk to the female (O'Connor 1984), and increase the risk of damaging the developing egg (Schifferli 1979, Oppenheimer et al. 1996, Schifferli 1979). However, American Robins (*Turdus migratorius*) were not more active immediately after laying an egg than they were immediately prior to laying an egg (Weatherhead et al. 1991), as predicted by the mechanisms proposed by O'Connor (1984) and Schifferli (1979). The hypotheses of Schifferli (1979) and O'Connor (1984) further predict that species with more energetically-intensive and aerial foraging behaviors and those whose eggs are larger relative to adult body mass would be more prone to laying in the early morning and more consistent in their laying times (Oppenheimer et al. 1996). However, the opposite was true in a comparison of the laying times of Dusky Flycatchers (*Empidonax erholseri*) and White-crowned Sparrows (*Zonotrichia leucophrys*). The smaller-egged (Oppenheimer et al. 1996) ground-foraging sparrow laid eggs earlier and more consistently in the morning than the larger-egged aerially-foraging flycatcher (Oppenheimer et al. 1996).

The hypotheses proposed by Schifferli (1979) and O'Connor (1984) also predict that nocturnal birds should lay in the afternoon or evening. Notably, the only nocturnal species (Pauraque) discussed by Skutch (1952) was also the only species observed to lay in the late afternoon (but based on only $n = 2$ eggs). Burrowing Owls forage primarily during nocturnal (Haug and Oliphant 1990, Gleason and Craig 1979) and crepuscular (Thomsen 1971, Poulin et al. 2011, Pezzolesi and Lutz 1994, Poulin and Todd 2006) hours. If Burrowing Owl foraging peaks during crepuscular periods, the

Schifferli-O’Connor hypotheses predict bimodal peaks of egg-laying frequency, in the late afternoon and early morning (Schroeder and McRae 2018). Male Burrowing Owls forage for and feed females during the incubation period, but the extent to which female Burrowing Owls forage for themselves during the laying period is unclear. If females do not forage during laying, then Schifferli and O’Connor’s hypotheses may not be relevant to Burrowing Owls. However, our study provides circumstantial evidence that female Burrowing Owls forage nocturnally during the laying stage (Lundblad 2020: Chapter Three).

Finally, consistent laying times across the eggs in a clutch would require laying intervals that are multiples of 24 hours. However, if selection on laying interval length is stronger than selection on the time of laying, then we would not expect the time of laying to be consistent within or across clutches (Watson et al. 1993). Indeed, we found that Burrowing Owls disproportionately laid first eggs in the morning, but laying times became increasingly random with respect to time of day across the laying order.

The egg-laying period of birds is largely unknown relative to other stages of the avian nesting cycle. We collected one of the largest known data sets for a wild bird that includes details regarding when females lay their eggs and the only data set we are aware of spanning such a large geographic gradient for a single species. Furthermore, most previous studies of the egg-laying stage have typically observed nests no more than once a day (see Weatherhead et al. 1991, Watson et al. 1993 for exceptions), but our use of in-burrow cameras allowed us to record laying times with unprecedented accuracy (usually to the nearest half-hour). We found surprisingly large intraspecific variation in laying times and laying intervals of Burrowing Owls throughout North America which adds to growing evidence that this species is exceptionally plastic in nearly every aspect of their reproductive behavior (Conway et al. 2012, Wellicome 2005, Lundblad 2020: Chapters Two and Three). Burrowing Owl laying behavior varies geographically and across thermal gradients and is correlated with numerous other traits. While other much-discussed reproductive decisions invoke hypothetical life-history tradeoffs that may (or may not) be realized at some point in the future, tradeoffs that occur within the context of a single reproductive attempt are more acute and hence should be more consequential to allocation decisions of nesting females and to the evolution of life-history strategies.

Improved documentation and mechanistic understanding of trait variation along geographic and climatic gradients is urgently needed to anticipate how reproductive strategies of animals may be altered by climate change. Such data can also inform our understanding of how and which populations may be able to adapt to climate change, and what mitigation strategies might be effective to dampen the effects of climate change. We found that a suite of traits related to egg laying behavior track climatic and geographic gradients and in turn impose allocation tradeoffs on other traits. Specifically, we found some evidence that warmer burrows are associated with extended laying intervals, and both warmer burrows and longer laying intervals are associated with reduced hatching success and asynchronous hatching. More studies of the laying stage, in a wide diversity of taxa, are needed to fully understand the myriad tradeoffs and selective pressures that shape avian reproductive strategies. In particular, we need more studies of the abiotic factors that may drive variation in laying interval and other metrics of reproductive effort to help better explain the vast diversity of life-history strategies and the ecological contexts that foster that diversity.

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Table 3.1. Top models to explain variation in laying interval among Burrowing Owls in western North America (2015 – 2018). Models with $\leq$ 2 percent of model weight are listed, plus the global model.

Model	**AIC**	**ΔAICc**	**weight**
~ Hatch Interval + Clutch Size + Egg # + Egg #^{2} + Attentiveness + Hatch Success	1499.0	0.0	0.17
~ Hatch Interval + Body Condition + Clutch Size + Egg # + Egg #^{2} + Attentiveness + Hatch Success	1501.0	1.9	0.07
~ Hatch Interval + Clutch Size + Egg # + Egg #^{2} + Date + Attentiveness + Hatch Success	1501.0	2.0	0.07
~ Hatch Interval + Clutch Size + Egg # + Egg #^{2} + Latitude + Attentiveness + Hatch Success	1501.0	2.0	0.06
~ Hatch Interval + Burrow Temp+ Clutch Size + Egg # + Egg #^{2} + Attentiveness + Hatch Success	1501.1	2.1	0.06
~ Clutch Size + Egg # + Egg #^{2} + Attentiveness + Hatch Success	1501.6	2.6	0.05
~ Hatch Interval + Clutch Size + Egg # + Egg #^{2} + Hatch Success	1502.6	3.6	0.03
~ Hatch Interval + Body Condition + Clutch Size + Egg # + Egg #^{2} + Latitude + Attentiveness + Hatch Success	1502.8	3.8	0.03
~ Hatch Interval + Clutch Size + Egg # + Egg #^{2} + Latitude + Date + Attentiveness + Hatch Success	1503.0	3.9	0.02
~ Hatch Interval + Body Condition + Clutch Size + Egg # + Egg #^{2} + Date + Attentiveness + Hatch Success	1503.0	3.9	0.02
~ Hatch Interval + Burrow Temp + Body Condition + Clutch Size + Egg # + Egg #^{2} + Attentiveness + Hatch Success	1503.0	4.0	0.02
~ Burrow Temp + Clutch Size + Egg # + Egg #^{2} + Attentiveness + Hatch Success	1503.1	4.0	0.02
~ Hatch Interval + Burrow Temp + Clutch Size + Egg # + Egg #^{2} + Date + Attentiveness + Hatch Success	1503.1	4.0	0.02
~ Hatch Interval + Burrow Temp + Clutch Size + Egg # + Egg #^{2} + Latitude + Attentiveness + Hatch Success	1503.4	4.1	0.02
~ Body Condition + Clutch Size + Egg # + Egg #^{2} + Attentiveness + Hatch Success	1503.6	4.4	0.02
~ Clutch Size + Egg # + Egg #^{2} + Date + Attentiveness + Hatch Success	1503.6	4.6	0.02
~ Clutch Size + Egg # + Egg #^{2} + Latitude + Attentiveness + Hatch Success	1504.3	4.6	0.02
~ Hatch Interval + Burrow Temp + Body Condition + Clutch Size + Egg # + Egg #^{2} + Latitude + Attentiveness + Hatch Success[1]	1506.8	7.8	0.00

[1] Global model

Table 3.2 Model-averaged parameter estimates to explain variation in laying interval duration among Burrowing Owls in western North America (2015 – 2018). Hatch success, clutch size, and average hatch interval had parameter estimates whose 95% confidence intervals excluded zero.

Parameter	β	SE	95% Confidence Interval
Intercept	5.69	0.24	5.22 - 6.16
Hatch Success	-0.19	0.07	-0.32 - -0.07
Clutch Size	-0.25	0.08	-0.41 - -0.10
Avg Hatch Interval	0.09	0.07	0.01 - 0.23
Attentiveness	-0.18	0.11	-0.39 - -0.03
Egg Number	-0.74	0.19	-1.11 - -0.37
Egg Number^2	0.04	0.01	0.02 – 0.06
Avg Burrow Temp	0.01	0.04	-0.14 - 0.16
Lay Date	-0.01	0.04	-0.15 - 0.10
Latitude	0.00	0.04	-0.14 - 0.16
Female Condition	0.00	0.00	-0.01 – 0.01

Table 3.3. Top models among candidate models designed to explain the probability of an extended interruption (>72 hours) during the laying period among Burrowing Owls in Western North America (2015 – 2018). Models receiving at least 2% model weight are listed, plus the global and intercept-only (null) model.

Model	AICc	ΔAICc	weight
~Egg Number + Latitude	181.2	0.0	0.10
~Egg Number	181.9	0.7	0.07
~Egg Number + Latitude + Three-day Precip	182.5	1.3	0.05
~Egg Number + Latitude + Lay Date	182.5	1.3	0.05
~Latitude	182.8	1.6	0.04
~Egg Number + Lay Date	182.8	1.7	0.04
~Burrow Temp + Egg Number + Latitude	183.1	2.0	0.04
~Cutch Size + Egg Number + Latitude	183.2	2.0	0.04
Null Model (intercept only)	185.4	4.2	0.01
Global Model	187.4	6.2	0.00

Table 3.4. Model-averaged parameter estimates to explain the probability of an extended laying interruption among Burrowing Owls in western North America (2015 – 2018). Egg number was the only explanatory variable whose 95% confidence interval excluded zero.

Parameter	β	SE	95% Confidence Interval
Intercept	-4.58	0.86	-6.26 - 2.89
Egg Number	-0.44	0.35	-1.13 - -0.01
Latitude	-0.37	0.42	-1.37 - 0.15
Burrow Temperature	0.02	0.14	-0.40 - 0.57
Clutch Size	-0.03	0.24	-0.92 - 0.73
Lay Date	0.08	0.21	-0.38 - 0.85
Three-day Precipitation	0.38	1.05	-1.93 - 4.28

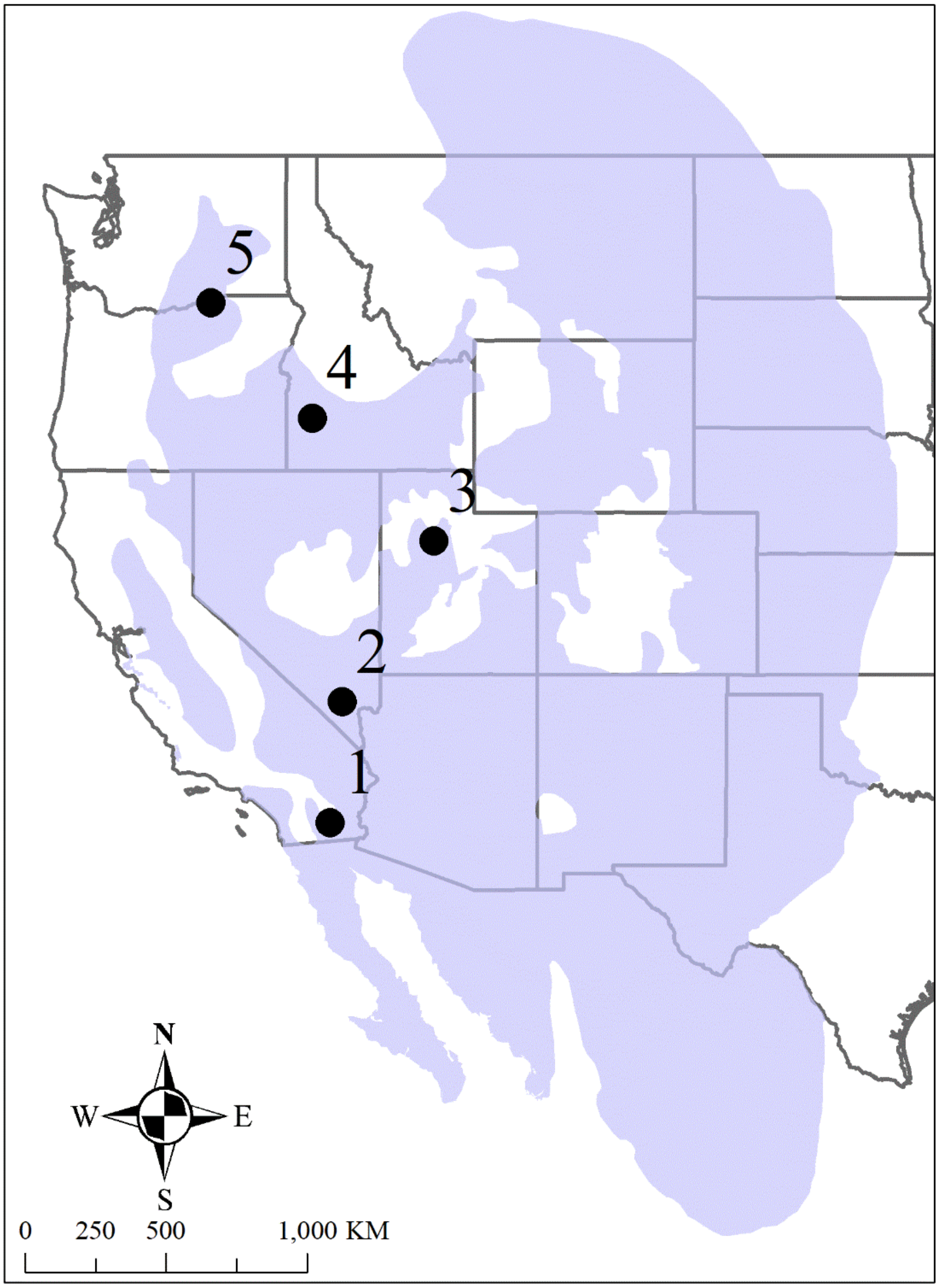

Figure 3.1. Study Sites: 1) Sonny Bono Salton Sea NWR; 2) North Las Vegas and Pahrump, NV; 3) Tooele Army Depot South Unit, Utah; 4) Snake River Birds of Prey NCA, Idaho; 5) Umatilla Army Depot, Oregon. Current Burrowing Owl range in gray (reproduced from Wellicome and Holroyd 2001).

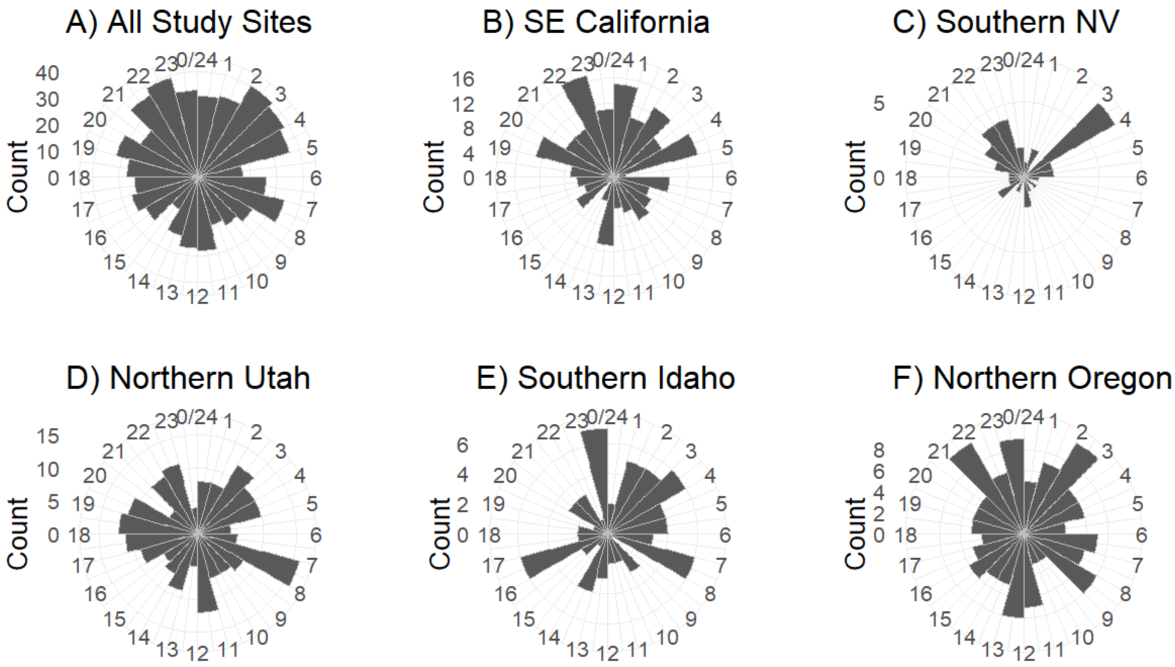

Figure 3.2. Burrowing Owls at five study sites in western North America (2015 – 2018) laid eggs at all times of day but disproportionately laid during the morning hours. A) all study sites combined (*n* = 675), B) southeastern California (*n* = 204), C) southern Nevada (*n* = 40), D) northwestern Utah (*n* = 209), E) southwestern Idaho (*n* = 77), and F) northeastern Oregon (*n* = 145). All laying times are relative to local sunrise which is displayed at the top of each figure (hour zero).

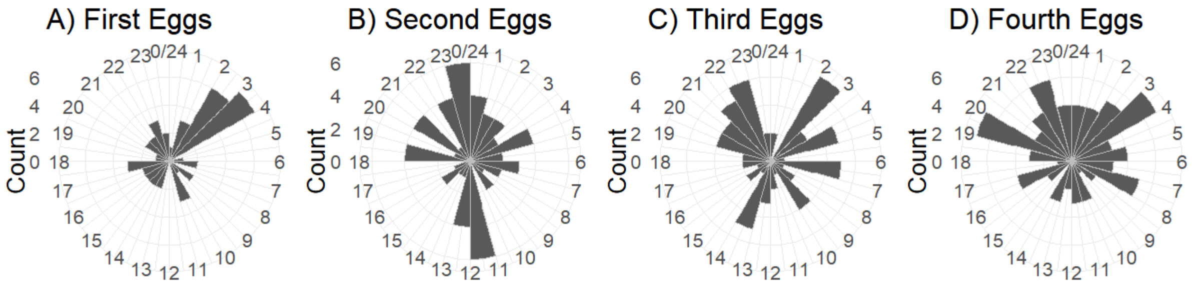

Figure 3.3. Burrowing Owls at five study sites in western North America (2015 – 2018) disproportionately laid first eggs in a clutch during the morning hours, but laying times became less regular across the laying order. Presented here are times for eggs in only the first through fourth position. A) first eggs (*n* = 51), B) second eggs (*n* = 62), C) third eggs (*n* = 71), and D) fourth eggs (*n* = 83). All laying times are relative to local sunrise which is displayed at the top of each figure (hour zero).

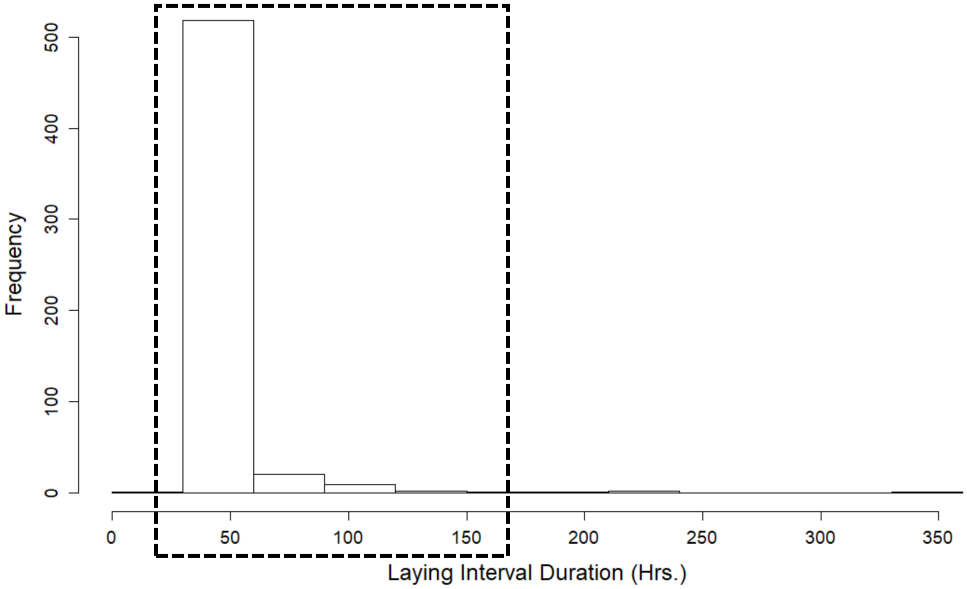

Figure 3.4. Laying interval among Burrowing Owls in western North America (2015 – 2018) ranged from 30.0 to 337 hours but most were less than 60 hours. The data in the dashed box are presented in greater detail in Figure 3.5.

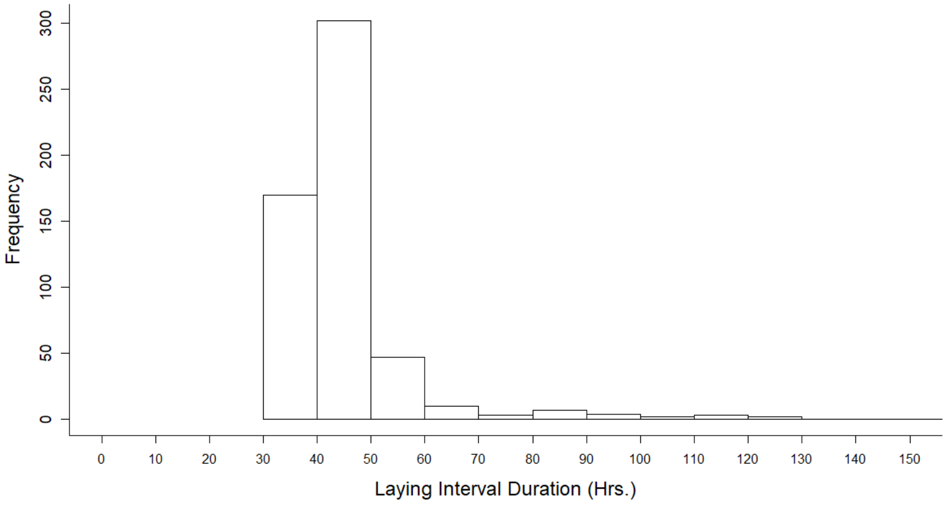

Figure 3.5. Laying intervals from 0 – 150 hours were concentrated between 40 – 50 hours in duration among Burrowing Owls in western North America (2015 – 2018). Those intervals >150 hours are presented in Figure 3.4.

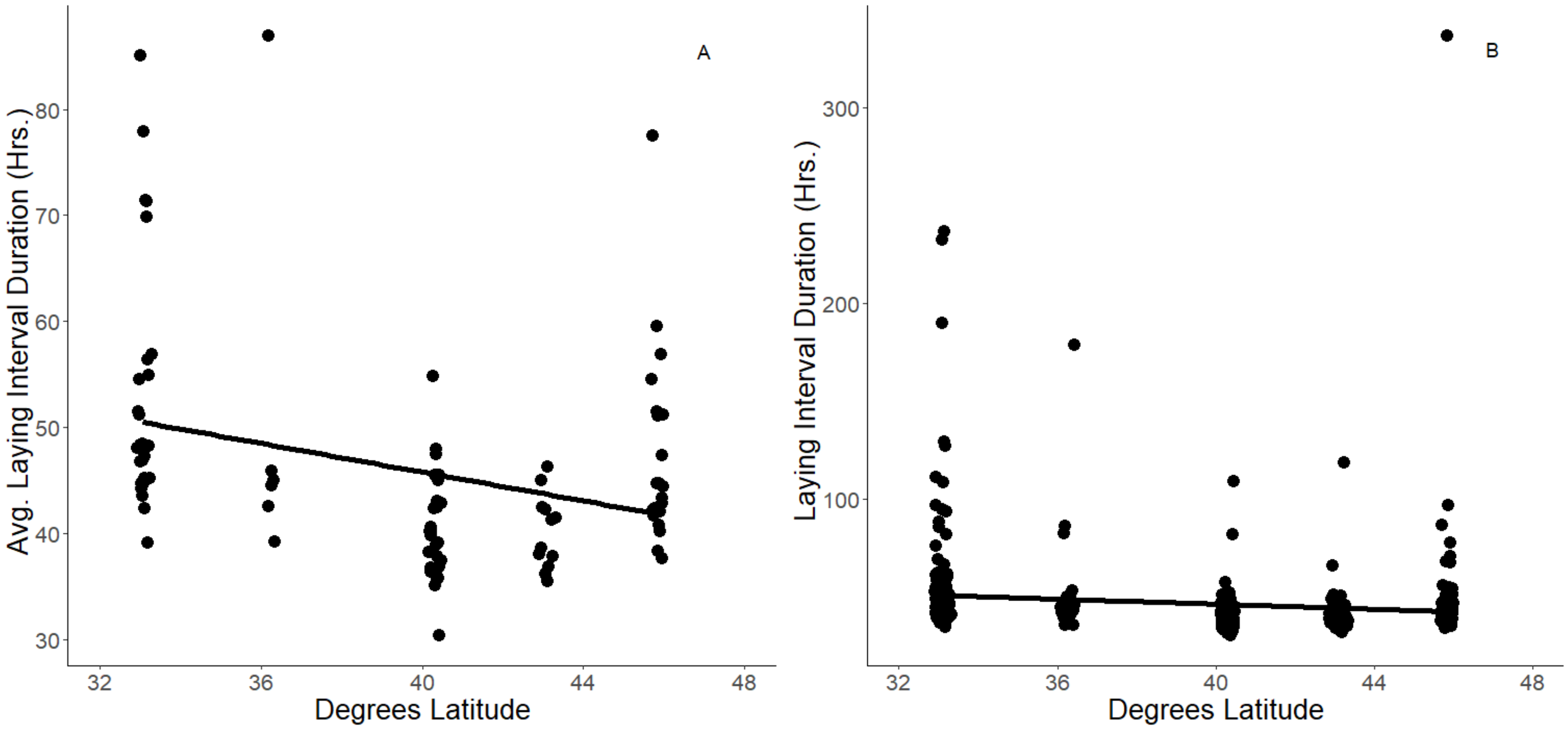

Figure 3.6. Laying interval duration among Burrowing Owls at five study sites in western North America (2015 – 2018) declined with increasing latitude including among: A) the average laying interval per nest (*R* = -0.32, *n* = 97, *P* = 0.002), and B) individual laying intervals (*R* = -0.14, *n* = 555, *P* < 0.001).

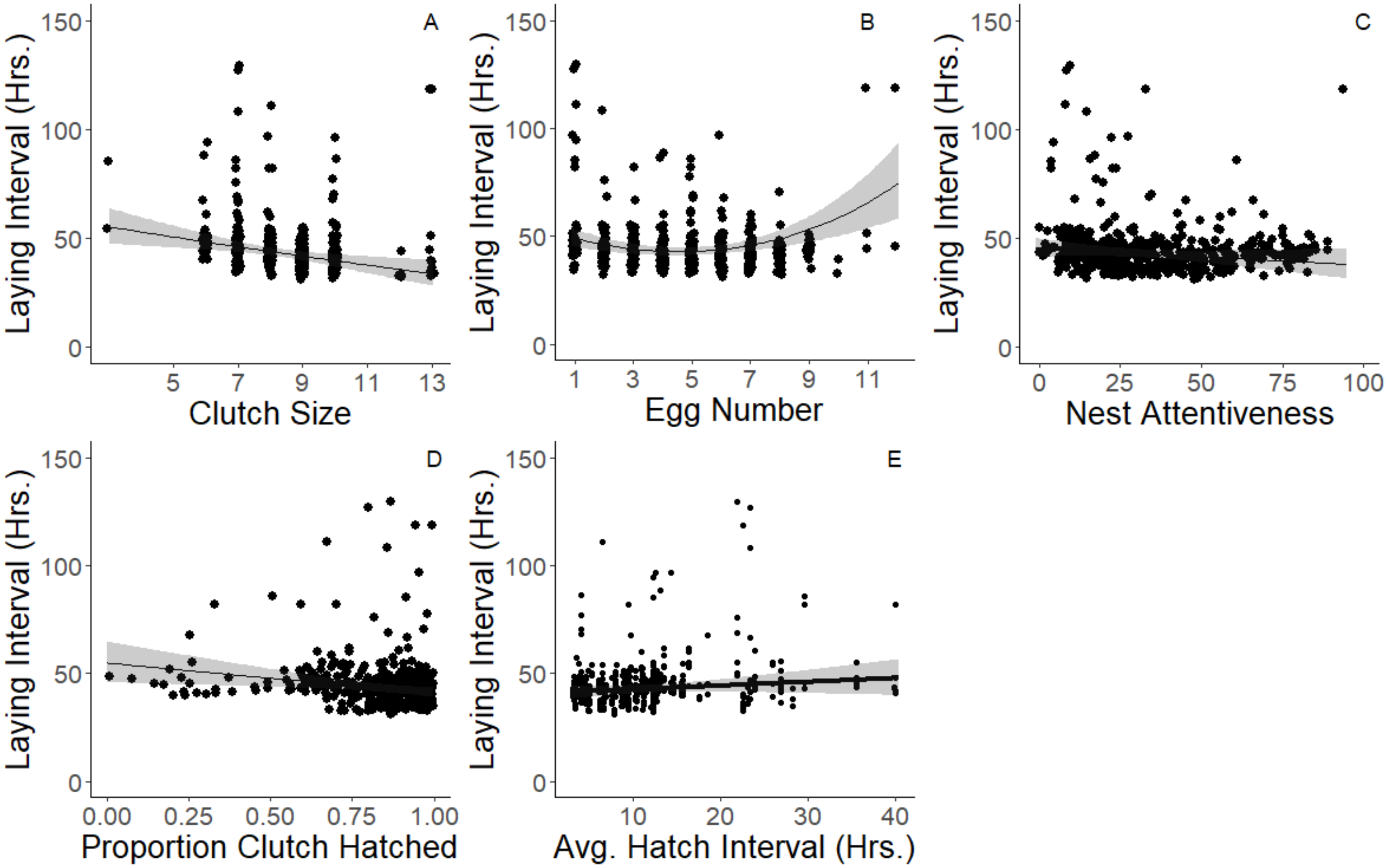

Figure 3.7. Partial effects of five explanatory variables in the top model designed to explain variation in laying interval among Burrowing Owls breeding in western North America (2015 – 2018): A) clutch size, B) egg number, C) percent nest attentiveness during the interval, D) proportion of the clutch that subsequently hatches successfully, and E) average subsequent hatch interval between eggs in the clutch.

Chapter 4: Intraspecific Variation in Incubation Behaviors Along a Latitudinal Gradient are Driven by Nest Microclimate and Selection on Neonate Quality

Abstract

Variation in incubation periods has long puzzled avian ecologists because time-dependent sources of nest mortality (nest predation and weather) should apply strong directional pressure to minimize nest exposure days. Yet, nest predation risk fails to explain variation in incubation periods across species, and the cause of variation in incubation periods remains unresolved. We conducted one of the first studies of intraspecific latitudinal variation in avian incubation behaviors and the first to measure such traits at > 2 study sites across a large portion of a single species' range. We used motion-activated nest cameras inside Burrowing Owl nests at 5 study sites to quantify variation in daily nest attentiveness, cumulative nest attendance, and incubation period duration. We used a hypothetico-deductive approach to test predictions of two previously proposed alterative hypotheses to explain variation in incubation periods: the risk tolerance hypothesis and neonate quality hypothesis. Daily nest attentiveness, cumulative nest attendance, and incubation period duration all increased with increasing latitude, but that variation could be accounted for by predicted life-history tradeoffs. Burrowing Owls reduced their daily attentiveness on days that the average burrow temperature was within the range that is optimal for embryo development. Further, variation in incubation periods was best explained by cumulative nest attendance instead of daily nest attentiveness. These results support the *neonate quality hypothesis;* that longer incubation periods result from stronger selection on neonate quality rather than selection to reduce reproductive effort in response to low extrinsic mortality risk. However, the fact that some owls exhibited less than the maximum possible nest attentiveness supports the general hypothesis that incubation decisions reflect a trade-off between reproduction and self-maintenance, and that the optimal solution to that tradeoff varies systematically with latitude.

Introduction

A current priority for ecologists is to anticipate and mitigate the impacts that climate change will have on plant and animal populations. However, our understanding of how populations will be impacted by changing thermal regimes is limited because we know little about thermal constraints on the demography and behavior of animals. Animals vary, both across and within populations, with respect to how they allocate their limited resources towards reproductive effort across their lifetimes (Cole 1954), but the factors that drive variation in relative allocation to suites of life-history traits remain the subject of much debate. The relevance of understanding the drivers of life-history variation towards anticipating the impacts of climate change is underscored by the fact that variation in life-history traits often tracks biogeographic and climatic gradients (Lack 1947, Ricklefs 1980, Jetz et al. 2008). Variation in life-history strategies between temperate and tropical populations and systematically along climatic gradients provides a basis for formulating and testing hypotheses regarding what ecological processes drive reproductive decisions and life-history variation generally. Studies of geographic variation in life-histories have long focused on patterns in clutch size, fecundity, and survival, but life-histories are comprised of many additional traits that trade-off and interact in complex ways (Martin 2004). An improved understanding of the selective pressures that underlie the vast variation we observe in life-history strategies and how those evolved strategies will interact with environmental change requires consideration of a broader suite of life-history traits spanning all stages of the annual cycle.

Incubation behaviors are central features of avian reproduction that may constrain other life-history traits. Although the physical mechanics of incubation have been well-studied (e.g., Skutch 1962, Drent 1975, Deeming 2002), less attention has been given to the ecology and evolution of incubation strategies (but see e.g., Ricklefs 1993, Conway and Martin 2000a, Conway and Martin 2000b, Martin 2002). Furthermore, incubation behaviors have received much less attention than traits like clutch size and hatching patterns and yet they may be more plastic and amenable to allocation tradeoffs with other life-history traits. The centrality of incubation to avian life-histories may have been overlooked because incubation was long viewed as a time of relatively low energetic demand (Walsberg and King 1978, Mugaas and King 1981, Walsberg 1983). Incubation is now recognized as an energetically intensive stage of nesting (Williams 1996 and references therein) that has the potential to constrain and trade-off with other traits. Incubation behaviors therefore reflect a balance between the energetic needs of the developing embryos and parental self-maintenance, survival, and future reproduction. Hence, incubation decisions are central to birds’ life-history trade-offs: those between current and future reproduction, between offspring quantity and offspring quality, and intergenerational conflict between parent and offspring.

Two closely coupled aspects of incubation behavior have attracted considerable interest: incubation period duration and nest attentiveness. Time-dependent sources of nest mortality (nest predation and weather-related failure) should impose strong directional selective pressure to minimize the duration of the incubation period (Lack 1968, Case 1978, Ricklefs 1984a, Bosque and Bosque

1995, Martin 1995, Conway and Martin 2000a). Incubation period duration could also have allometric constraints imposed by body size or egg size (Rahn and Ar 1974, Case 1978, Ricklefs and Starck 1998). However, neither nest predation risk or allometry explains the majority of variation in incubation period duration across species (Martin 1996, Martin 2002, Chalfoun and Martin 2007, Martin et al. 2007, Cooney et al. 2019), and many species exhibit surprisingly drawn-out incubation periods (Boersma 1982, Ricklefs 1984b, Martin 1996). Furthermore, interspecific comparisons reveal broad geographic patterns with longer incubation periods at low and southern latitudes compared to the north temperate zone (Ricklefs 1969, Martin 2002, Martin and Schwabl 2008, Martin et al. 2007, Cox and Martin 2009). Hence, the selective pressures responsible for variation in the length of the incubation period remain a topic of debate (Ricklefs et al. 2017, Martin et al. 2018, Cooney et a. 2019).

Debate has recently centered around two alternative (but not mutually exclusive) hypotheses to explain variation in incubation period duration. First, variation in incubation periods could reflect differential selection for neonate quality and resulting intrinsic differences in “developmental programs” among embryos (*neonate quality hypothesis*; Ricklefs 1993, Robinson et al. 2008, Ricklefs et al. 2017). That is, longer incubation periods in some populations are caused by stronger selection on offspring quality that can be enhanced by slower development (Ricklefs 1993). Alternatively, variation in incubation periods could be caused by variation in average egg temperature which, in turn, is caused by variation in the proportion of time that parents spend attending the nest and incubating the eggs (*risk tolerance hypothesis*; Martin 2002, Martin et al. 2015, Martin et al. 2018). Each hypothesis is ultimately driven by variation in the rate of extrinsic mortality (i.e., mortality that is not related to reproductive effort or reproductive decisions) and the rate of population turnover. The *neonate quality hypothesis* suggests that lower extrinsic mortality and slower population turnover impose selection for greater intrinsic offspring quality (MacArthur 1962, Ricklefs 1993). Higher quality offspring are then able to take advantage of the longer life-expectancy afforded by low extrinsic mortality. In contrast, the *risk tolerance hypothesis* posits that reduced extrinsic mortality risk favors reduced parental reproductive effort and lower nest attentiveness (i.e., greater allocation to adult self-maintenance). Lower nest attentiveness is assumed to reduce the energetic costs and risks associated with incubation and thereby preserve residual reproductive value (Williams 1996, Charnov and Schaffer 1973, Martin 2002). Under the *neonate quality hypothesis*, longer incubation periods are intrinsic to the developmental growth program of the embryos, while under the *risk tolerance* , longer incubation periods are extrinsic to the embryo and instead a function of parental behavior.

Nest attentiveness could impose several costs on the incubating parent (Martin 2002). First are the direct energetic costs of incubation that have been shown to increase mortality (Deerenberg et al. 1995, Bryant 1999, Martin 2002, Visser and Lessells 2001). Second, nest attendance may increase predation risk to the adult while sitting on the nest (Magnhagen 1991, Magrath 1998, Stoleson and Beissinger 2001). Finally, nest attendance for cavity- and burrow-nesting species could increase the thermoregulatory demand on the adult if the nest temperature is lower than the ambient temperature and below the adult's thermoneutral zone (Nadeau et al. 2015). Reduced attentiveness, however, could impose costs on the offspring such as increased risk of time-dependent mortality (Clark and Wilson 1981, Boersma 1982, Ricklefs 1993), decreased ability of the incubating parent to directly defend the nest from predation (Blaker 1969, Bollinger et al. 2009), and lower average egg temperature that could compromise embryonic development and reduce the offspring's lifetime fitness (Qualls and Andrews 1999, DuRant et al. 2013).

The *neonate quality hypothesis* and *risk tolerance hypothesis* each generate numerous testable predictions regarding the relationship between incubation period duration and patterns of nest attentiveness (Fig. 4.1). Martin's *risk tolerance hypothesis* assumes that long incubation periods result from lower daily attentiveness and, therefore, predicts a negative association between incubation period duration and percent daily nest attentiveness (e.g., hours per day) (Fig. 4.1A). The *risk tolerance hypothesis* further predicts that the total energetic input required for embryo development is fixed and, therefore, the total cumulative time spent attending a clutch should not vary with incubation period duration (Fig. 4.1B). However, if embryonic development continues during incubation recesses (i.e., because the nest microclimate is above "physiological zero"; Webb 1987), then incubation period duration could also be negatively associated with cumulative hours of nest attendance (Fig. 4.1B dashed line). Finally, the *risk tolerance hypothesis* predicts that neonate quality should either be unrelated to or negatively correlated with (Fig. 4.1C, dashed and solid line, respectively) incubation period duration due to greater egg neglect and lower average egg temperature (Williams 1996, Ghalambor and Martin 2001, Martin et al. 2007, DuRant et al. 2013). The *risk tolerance hypothesis* also predicts that percent daily nest attentiveness should be positively correlated with latitude because the rate of extrinsic risk tolerance increases latitudinally (Rowley and Russell 1991, Martin 1996, Sandercock et al. 2000, Ghalambor and Martin 2001; Fig. 4.1E). Accordingly, incubation period duration should be negatively correlated with latitude if all else, including clutch mass, is constant (Fig. 4.1D).

The *neonate quality hypothesis* (Ricklefs 1993, Ricklefs et al. 2017) predicts that percent daily nest attentiveness should not vary among nests with different incubation periods (Fig. 4.1F).

Instead the *neonate quality hypothesis* predicts a positive association between incubation period duration and the cumulative hours of nest attendance (Fig. 4.1G). The *neonate quality hypothesis* also predicts a positive association between incubation period and indices of nestling quality (Fig. 4.1H). Selection for offspring quality should be greater at lower latitudes (MacArthur 1962) and, therefore, the *neonate quality hypothesis* predicts that percent daily attentiveness should not necessarily vary with latitude (Fig. 4.1I), but incubation period duration should be negatively associated with latitude (Fig. 4.1J).

Nest attentiveness (daily and cumulative) could also reflect trade-offs mediated by nest microclimate relative to thermal optima for embryos and adults. If attentiveness is driven, in part, by the thermal requirements of developing embryos, then nest attentiveness should be lower on days when the average nest temperature is already within the range that is optimal for embryonic development (assumed to be 36 – 40˚C; Webb 1987) than when it is not (Fig. 4.2A), which may be common in burrow- and cavity-nesting birds. If patterns of attentiveness are partially a function of minimizing thermoregulatory demand on the incubating adult, then percent daily nest attentiveness should be lower on days when the average nest temperature is outside the adult thermoneutral zone (assumed to be 25 – 37˚C; Coloumbe 1970) than when it is within the thermoneutral zone (Fig. 4.2B).

We tested these two hypotheses on Burrowing Owls at five study sites along a 1400-km latitudinal gradient in North America. Burrowing Owls provide a good model system for testing these hypotheses because they lay their eggs in underground burrows where nest chambers are ~3 meters below the surface and, hence, their underground nest burrows are sometimes cooler than and other times warmer than above-ground ambient temperature. Moreover, they are assisted incubators and will nest in artificial nest burrows whose nest microclimate can be influenced by the depth of the chamber. Finally, Burrowing Owls lay large clutches (Conway et al. 2012, Poulin et al. 2020), exhibit variable incubation periods (Henny and Blus 1981, Olenick 1990), and latitudinal variation in many reproductive traits (Lundblad 2020: Chapters One and Two, Poulin et al. 2020). Hence, we used artificial nest burrows outfitted with motion-activated cameras to document incubation period duration, daily and cumulative nest attentiveness, and other reproductive traits and behaviors (Chapters One and Two of this dissertation). Our objectives were to: 1) document latitudinal patterns in incubation period duration and daily and cumulative nest attentiveness among Burrowing Owls, 2) identify drivers of variation and latitudinal patterns in incubation period duration and nest attentiveness among Burrowing Owls, 3) test predictions of the risk tolerance hypothesis and the neonate quality hypothesis to explain intraspecific variation in incubation period duration, 4) test whether Burrowing Owls modify their daily or cumulative nest attentiveness in response to nest microclimate, relative to what is optimal for embryonic development and adult thermoregulation.

While geographic variation in nest attentiveness and incubation period duration has been documented across species, only a couple previous studies have examined intraspecific geographic variation in nest attentiveness and incubation period duration (but see Ardia et al. 2006, Rohwer and Purcell 2019, Sofaer et al. 2020), and none have compared more than two different populations breeding near the extremes of a species' range. Furthermore, few past studies have sought to explain the underlying cause(s) of variation in these poorly known life-history traits, neither within nor across species. Examining intraspecific variation across geographic gradients may control for the confounding effects and phylogenetic constraints that are inherent in interspecific comparative studies and is therefore a powerful complementary approach to understanding the drivers of life-history variation (Cooney et al. 2019).

Methods and Materials

Study Species

Burrowing Owls inhabit the western hemisphere from southern Argentina to southern Canada. They nest primarily in burrows constructed by mammals and other burrowing animals (Poulin et al. 2020, Conway 2018) and in artificial burrows (i.e., underground nest boxes) constructed by researchers and wildlife managers (Collins and Landry 1977, Olenick 1990, Smith and Belthoff 2001). They range from fully migratory at the highest latitudes to resident in milder climates (Poulin et al. 2020). Clutch size varies from 3 – 14 eggs and is positively correlated with latitude (Todd and Skilnick 2002, Poulin et al. 2020, Conway et al. 2012, Lundblad 2020: Chapter Two). Burrowing owls exhibit single-sex incubation by females, and males provide all the female's food during incubation (Poulin et al. 2020). A variable amount of incubation often begins prior to clutch completion, and clutches hatch asynchronously over the course of 1 – 8 days (Wellicome 2005, Conway et al. 2012, Poulin et al. 2020, Lundblad 2020: Chapter Two).

Study Sites

We studied Burrowing Owls at five study sites (see Fig. 2.2 from Lundblad 2020: Chapter Two) including the Sonny Bono Salton Sea National Wildlife Refuge, California (33°2'N, 115°37'W, elevation -69m), North Las Vegas and Pahrump, Nevada (considered a single site, North Las Vegas at 36°18'N,115°14'W, elevation 670m, Pahrump at 36°18'N, 116°2'W, elevation 790m), the Tooele Army Depot South Area, Utah (40°2'N, 112°20'W, elevation 1520m), the Morley Nelson Snake

River Birds of Prey National Conservation Area, Idaho (43°00’N, 116°00’W, elevation 945m), and the Umatilla Army Depot, Oregon (45°5’N, 119°26’W, elevation 152m). At each study site, some Burrowing Owls nested in artificial underground burrows. Artificial burrow design varied among and within study sites, but followed widely-used designs meant to approximate the dimensions, depth, and thermal properties of natural burrows (Collins and Landry 1977, Smith and Belthoff 2001, Alexander et al. 2005, Barclay 2008, Johnson et al. 2013). Each artificial burrow included a single entrance tunnel, and the top of each nesting chamber varied in depth from 12 – 65 cm.

Field Methods

We installed one of several models of motion-activated infrared CCTV cameras (Alibi CD700 and CD80B Turret Dome, Observint Technologies, Austin, TX; HT71HG Mini Vandal Turret Dome and HT71TG Mini Turret Dome, Speco Technologies, Amityville, NY) inside the artificial burrows to capture the rear of the burrow chamber, where owls nested. We connected each camera to a mobile DVR recorder (MDVR25HR MPEG-4, Observint Technologies, Austin, TX) placed in a plastic bucket or plastic food storage container external to the burrow. We programmed the DVRs to record a 2.5-minute clip any time there was substantial motion in the burrow (we set motion-detection sensitivity to medium) and saved video clips to a 32-gb SD memory card that we changed every 5 – 15 days. We powered each camera with a 12-volt deep cycle battery installed external to the burrow and replaced every 2 – 4 days. We also installed temperature data loggers (iButton model DS1922L, Maxim Integrated, San Jose, CA and Embedded Data Systems, Lawrenceburg, KY) on the inner wall or ceiling of most burrow chambers and programmed them to record the ambient burrow temperature every 10 – 20 minutes (except in 2015, when we collected no burrow temperature data). We also placed 1 – 2 iButton temperature loggers in shaded and sheltered outdoor locations at each study site to measure the ambient above-ground temperature at each site and in each year.

We temporarily blocked the burrow entrance during equipment installation, allowing the female (if present) to take refuge in the entrance tunnel without flushing and we typically installed the equipment in <5 minutes. Opening the burrows for twice that long (up to 10 minutes) did not affect their internal temperature in southeastern California (Nadeau et al. 2015). We kept each camera running, whenever possible, until at least 20 days post-hatching or until the nest failed. We obtained only partial data on some nests due to equipment failure and the challenges associated with keeping batteries regularly maintained. We installed cameras in a total of 150 nests, at the five study sites from 2015 – 2018, and collected a total of 1,533,325 2.5-minute video clips accounting for 63,889 hours and 14.0 terabytes of video data.

On or around the tenth (range ninth to fifteenth) and twentieth (range nineteenth to twenty-fifth) day after the first egg in each clutch hatched, we opened the artificial burrow to measure and count the number of surviving nestlings. We measured the wing chord length, tarsus length, and body mass of each nestling. We thoroughly inspected each nestling for ectoparasites and recorded their parasite load according to the following classes: (0) none, (1) 1 – 2, (2) 3 – 5, (3) 6 – 10, (4) >10 parasites. The sparse plumage of nestlings made ectoparasites relatively easy to detect and count. We applied temporary colored rubber bands to the nestlings' legs for individual identification, on their first measurement date. We removed the colored rubber bands on the second measurement occasion and replaced them with aluminum USGS numbered leg bands. We also opened and checked nearby artificial burrows, checked any nearby natural burrows for signs of owl use, and trapped at the natural burrows if less than the entire brood was accounted for and we thought the other burrows might contain nestlings that relocated from the focal burrow (rare).

Video Data Processing

We reviewed the 2.5-min video segments from when the first egg was laid, or the camera was installed (for those installed after laying began), until the final viable egg hatched. We reviewed all videos in their entirety during the laying and hatching stages. From the laying of the final egg through the hatching of the first egg, we usually only reviewed four 2-hour periods per day from 00:00:00 – 01:59:59, 6:00:00 – 7:59:59, 12:00:00 – 13:59:59, and 16:00:00 – 17:59:59. If videos were unavailable (due to camera malfunction or battery failure) during the 00:00:00 – 01:59:59 nocturnal period, we substituted the 22:00:00 – 23:59:59 period from the same day (if available) to ensure at least two hours of nocturnal sampling per day. We recorded the start time and stop time of each incubation bout and incubation recess. We did our best to determine when the female was actually making contact between her brood patch and the clutch of eggs. We ignored on and off bouts lasting ≤60 seconds in duration (i.e., if the female transitioned from on to off or vice-versa and then transitioned back within one minute, we treated it as continuous attentiveness). We recorded the time when each egg was laid and hatched. When the incubating or brooding female blocked the camera's view of the clutch and prevented us from recording the exact laying or hatching time of an egg, we used the midpoint between when the egg was last seen unlaid/hatched and when the newly-laid/hatched egg/nestling was first observed as the hatching time (Chapters One and Two of this dissertation).

Analytical Methods

We performed all analyses in R (R Core Team 2019). We fit mixed-effects models in the package "glmmTMB" (Brooks et al. 2017) and performed model selection and model averaging in the package "MuMIn" (Barton 2019). We calculated variance inflation factors using the VIF function in the R package "car" (Fox and Weisberg 2019).

Nest Attentiveness**—**We calculated the average proportion of each day that the female attended the nest and did so for each day of the nesting cycle during diurnal hours, nocturnal hours, and during the entire 24-hour day. For the analysis of nest attentiveness, we defined the incubation stage as the period between the first full day after the last egg was laid until the last full day before the first egg hatched. We used paired *t*-tests to compare mean daily diurnal attentiveness to mean daily nocturnal attentiveness during both the laying and incubation stages. We calculated cumulative nest attendance for 43 nests for which we had either complete daily attentiveness data and those that were missing no more than two consecutive days. For days with missing data, we interpolated daily nest attentiveness (mean = 1.1 missing days per nest, range: 0 – 8) by averaging the daily nest attentiveness values for the previous and following day. If nest attentiveness was unavailable for two consecutive missing days, we filled in both with the same value using the interpolation method. We also calculated cumulative laying period nest attendance from the day of initiation (first egg date) through the day of clutch completion. We then calculated the univariate Pearson correlations between latitude and: 1) 24-hour nest attentiveness, 2) diurnal nest attentiveness, 3) nocturnal nest attentiveness, 4) cumulative hours of nest attendance from initiation through the start of hatching, and 5) cumulative hours of laying period nest attendance prior to clutch completion.

Next, we used multiple regression to examine factors that influence intraspecific variation in daily (24-hour) nest attentiveness during the incubation stage. Visual inspection of the data revealed that percent daily nest attentiveness during the incubation stage followed a strongly left-skewed distribution. We therefore modeled variation in these data using a beta distribution, similar to the approach of Sofaer et al. (2020). First, we applied a transformation to slightly-compress the range of the proportional response variable and eliminate exact zeros and exact ones (following the approach of Smithson and Verkuilen (2006) and Sofaer et al. (2020)). We then modeled daily nest attentiveness as a function of the linear and quadratic effects of clutch size, Julian date, average daily burrow temperature, latitude, and a categorical variable indicating whether the average daily ambient above-ground temperature exceeded the average daily burrow temperature (yes or no). Correlations among explanatory variables were < 60% and variance inflation factors < 2.0. We also included a random intercept for nest, to account for the non-independence of nest days at the same nest, and we standardized each continuous explanatory variable. We then used AICc to compare models including all possible combinations of predictors (Burnham and Anderson 2002). Because our global model included quadratic terms, we did not use model averaging and instead based our inferences on the results of the top model (Cade 2015).

Finally, we used one-sided *t*-tests to test additional *a priori* directional predictions about the relationships between burrow microclimate and nest attentiveness (Fig. 4.2) that would have been redundant with average burrow temperature if included in the multiple regression. First, we tested the directional prediction that nest attentiveness would be lower on days when the average burrow temperature was within the range considered to be optimal for embryonic development (assumed to be 36 – 40.5˚C based on Webb (1987); Fig 3.2B). Second, we tested the prediction that nest attentiveness would be higher on days when the average burrow temperature was within a Burrowing Owl's thermoneutral zone (assumed to be 25 – 37˚C based on Coloumbe (1970); Fig. 4.2A).

***Incubation Period Duration*—**We calculated the duration of the incubation period as the number of days elapsed from when the last egg was laid until the last egg hatched (Nice 1954, Briskie and Sealy 1990, Ricklefs 1993). We censored one nest where the female delayed incubation for a full 10 days after the clutch was complete. To test predictions of the two hypotheses proposed to explain variation in incubation period duration, we examined which of the two metrics of nest attentiveness best explained intraspecific variation in incubation period duration: the percent daily nest attentiveness during the incubation stage or the cumulative number of hours of nest attendance from the day of nest initiation through the day that hatching began (Fig. 4.1). We specified a global model including mean daily nest attentiveness during the incubation stage, cumulative nest attendance from initiation through the start of hatching, clutch size, hatch date (Julian day), and latitude. The response data were normally distributed, so we modeled them using a Gaussian distribution. We compared models (using AICc) including all combinations of predictors and then calculated model-averaged parameter estimates across all candidate models (Burnham and Anderson 2002). Because the number of nests for which we had a full set of covariates reduced our sample size, and for additional corroboration of the results from the multiple regression, we also calculated the univariate Pearson correlations of incubation period duration with both mean daily nest attentiveness during the incubation stage and the cumulative hours of nest attendance from nest initiation through hatching.

Burrowing Owls hatch their clutches asynchronously, and our definition of incubation period duration necessarily does not account for incubation that occurs prior to clutch completion (i.e., partial incubation; Wang and Beissinger 2011). Therefore, a positive relationship between incubation period duration and cumulative hours of nest attendance from the start of laying through the start of hatching could be driven by those hours of nest attendance occurring prior to clutch completion. To test for that possibility, we reran the same multiple regression described above using only the summed hours of nest attendance from initiation through the day of clutch completion and calculated the univariate correlation between incubation period and hours of nest attendance prior to clutch completion.

***Neonate Quality*—**We considered 13 total indices of nestling quality (including indices of growth and immunocompetence): ectoparasite abundance on day 10 and day 20 of the nestling period, nestling body mass on day 10 and day 20, wing chord length on day 10 and day 20, tarsus length on day 10 and day 20, the daily rate of body mass increase between the two measurement occasions, the daily rate of wing growth between the two measurement occasions, the daily rate of tarsus growth between the two measurement occasions, a combined growth index (the summed *z*-score of each of the three growth parameters standardized), and a final multivariate index of nestling growth that pooled variation in the other 10 growth indices (i.e., excluding ectoparasite abundance). To generate the multivariate index of nestling growth, we conducted a principal component analysis that pooled variation in all 10 univariate nestling growth indices and used the first principal component as a final index of nestling growth. We implemented the PCA (based on single value decomposition) with the R function "prcomp". We then used linear mixed-effects models with a random intercept for nest ID to test whether incubation period explained variation in any of the 13 nestling quality indices, as predicted by the *neonate quality hypothesis.*

Results

***Nest Microclimate*—**We calculated average daily burrow temperatures for 2216 nest days at 94 of the 150 Burrowing Owl nests for which we obtained any camera data (Lundblad 2020: Chapter Two). Average daily burrow temperatures during the incubation stage ranged from 11.0 – 39.6˚C (mean = 23.4 ± 0.16 SE).

***Nest Attentiveness*—**We calculated daily 24-hour, diurnal, and nocturnal nest attentiveness for 3510 nest days at 117 Burrowing Owl nests. Mean daily nest attentiveness during the incubation stage (n = 2013 nest days across 111 nests) ranged from 30.7 – 95.2% (mean = 86.0 ± 1.0 SE). Mean diurnal nest attentiveness during the incubation stage ranged from 33.6 – 96.1% (mean = 86.0 ± 1.0 SE), and mean nocturnal nest attentiveness during the incubation stage ranged from 22.4 – 97.8% (mean = 85.9 ± 1.2 SE). Mean daily nest attentiveness during the laying stage was confounded by missing data and variation in what proportion of the clutch had been laid at the time of camera installation. Mean daily nest attentiveness during the laying stage (n = 1156 nest days across 99 nests) was lower than during the incubation stage and ranged from 5.0 – 87.3% (mean = 36.4 ± 1.6 SE) for the 24-hour day, 5.7 – 90.8% (mean = 45.6 ± 1.5 SE) for diurnal hours, and 0.00 – 100.00% (mean = 23.3 ± 2.1 SE) for nocturnal hours. Average daily diurnal attentiveness was significantly greater than average daily nocturnal attentiveness during the laying stage (t_{98} = 14.7, $P < 0.0001$; two-sided paired test), but diurnal and nocturnal attentiveness did not differ during the incubation stage (t_{111} = 0.1, P = 0.95; two-sided paired test). Mean daily attentiveness was positively correlated with latitude: the entire 24-hour period (R = 0.49, n = 110, P < 0.0001; Fig. 4.3a), diurnal hours only (R = 0.49, n = 110, P < 0.0001), and nocturnal hours only (R = 0.50, n = 110, P < 0.0001). Cumulative hours of nest attendance (i.e., from initiation through the first day of hatching) (R = 0.60, n = 43, P < 0.0001, Fig. 4.3b) and cumulative nest attendance during the laying period (R = 0.33, n = 43, P = 0.03; Fig. 4.3c) were both positively associated with latitude.

The subset of observations with a complete set of the predictors in the multiple regression analysis included 1193 nest days from 71 nests. The top model to explain variation in daily nest attentiveness during the incubation stage was the global model (Table 4.1), which received 49% of the total model weight, and the 95% confidence intervals excluded zero for each predictor (Table 3.2). The one closely competing model (ΔAICc = 0.1; Table 4.1) included all the predictors except for whether the average ambient temperature was greater than the average burrow temperature and received 47% of the remaining model weight. All other models had low model weight and ΔAICc > 6 (Table 4.1). Daily nest attentiveness declined with increasing burrow temperature, and that decline was strongest between 30 – 40˚C (Fig. 4.4A). Nest attentiveness was lower on days when average ambient temperature exceeded the ambient burrow temperature, but although that effect was included in the top model, the effect was small (Fig. 4.4A). Daily nest attentiveness increased non-linearly with increasing clutch size (Fig. 4.4B), increased non-linearly with date (Fig. 4.4C), and increased weakly with latitude (Fig. 4.4D) after the other predictors were accounted for. Based on the univariate comparison, daily nest attentiveness during the incubation stage was lower (53.1%) on days when the average burrow temperature was in the range that is ideal for embryonic development than on days when the average burrow temperature was below optimal thermal conditions for development (87.6%), and the difference was significant ($t_{14.5}$ = 26.7, P < 0.0001; one-sided test). Average nest attentiveness was not higher on days when the average burrow temperature was thermoneutral for adult Burrowing Owls (in fact the opposite was true, although the difference was not significant). Average nest attentiveness was 90.1% on days when the average burrow temperature was outside the thermoneutral zone and 82.0% on days when the average burrow temperature was within the thermoneutral zone ($t_{640.2}$ = 12.0, P = 1.0; one-sided test).

Incubation Period Duration**—**We calculated the length of the incubation period at 91 nests. Incubation period duration ranged from 19.0 – 26.4 days (mean = 22.5 ± 0.14 SE). The univariate correlation between incubation period duration and latitude was positive (R = 0.26, n = 91, P = 0.01; Fig. 4.3D). The univariate correlation between incubation period duration and mean daily attentiveness during the incubation stage was positive (*R* = 0.37, *n* = 80, *P* = 0.0008; Fig. 4.5A) as was the univariate correlation between incubation period duration and cumulative hours of nest attendance (*R* = 0.53, *n* = 43, *P* = 0.0003; Fig. 4.5B). Based on the multiple regression analysis, the top model to explain variation in incubation period duration included only cumulative incubation effort from initiation to hatching and received 24.7% of total model weight (Table 4.3). The model-averaged effect of daily mean nest attentiveness on incubation period duration was slightly negative, but the 95% confidence interval included zero (β = -0.14, SE = 0.36, 95% C. I. = -1.34 – 0.61; Table 4.4, Fig. 4.5C). The model-averaged effect of cumulative incubation effort on incubation period duration was positive, and the 95% confidence interval excluded zero (β = 0.70, SE = 0.39, 95% C. I. = 0.03 – 1.46; Table 4.4, Fig. 4.5D).

The association between incubation period duration and cumulative hours of nest attendance was not driven by laying period incubation effort occurring prior to clutch completion. The correlation between incubation period duration and hours of nest attendance prior to clutch completion was extremely weak (*R* = -0.01, *n* = 43, *P* = 0.94). Further, when we ran the multiple regression model using hours of nest attendance prior to clutch completion instead of cumulative hours of nest attendance from initiation through hatching, hours of attendance prior to clutch completion was absent from the top model, received weak support across the set of candidate models, and the model-averaged parameter estimate had a confidence interval that overlapped zero. This result demonstrates that the positive association between cumulative nest attendance and incubation period is not an artifact of our definition of incubation period as beginning when the final egg is laid, even though partial incubation often begins prior to the laying of the final egg.

***Neonate Quality*—** Incubation period duration did not explain variation in the abundance of ectoparasites on nestlings (Table 4.5). Incubation period duration did not explain significant variation in any of the ten univariate indices of nestling size and growth, and the direction of the association between each growth index and incubation period duration varied (positive for 6 univariate growth indices and negative for 4 univariate indices; Table 4.5). The first principal component of nestling growth size, from the principal component analysis accounted for 50.4% of the pooled variation in the ten size and growth indices, but incubation period did not explain significant variation in nestling growth PC1 (β = 0.00, P = 0.99).

Discussion

Burrowing Owl incubation period duration varied among nests by greater than seven days, and that variation tracked latitude. We used a hypothetico-deductive approach to test explicit predictions of two alternative hypotheses to explain intraspecific variation in incubation periods. We found compelling evidence that longer incubation periods are associated with greater cumulative nest attendance rather than a lower daily rate of nest attentiveness. Our results support the *neonate quality hypothesis;* variation in incubation periods reflects variation in intrinsic growth programs of embryos presumably in response to differential strength of selection on neonate quality (Ricklefs 1993, Ricklefs et al. 2017). Embryos at nests with longer incubation periods required more hours of cumulative incubation effort than those at nests with shorter incubation periods. Therefore, parents at nests with longer incubation periods appeared to invest more in their offspring, not less as would be predicted by the *risk tolerance hypothesis*.

Previous tests of the *neonate quality hypothesis* have mostly found positive associations between incubation periods and metrics phenotypic quality including immunocompetence (Ricklefs 1992, Tella et al. 2002, Lee et al. 2008) and an inverse relationship with markers of oxidative stress (Tsunekage 2013, Tsunekage and Ricklefs 2015), but others have failed to detect such relationships (Palacios and Martin 2006). Previous studies testing the *risk tolerance hypothesis* have demonstrated a strong link between egg temperature and incubation period (Deeming 2008, Martin et al. 2007, Robinson et al. 2008, Robinson et al. 2014, Martin et al. 2015, Martin et al. 2018 and references therein). Finally, egg-swapping, warming, and cross-fostering experiments suggest that incubation periods of experimentally-incubated eggs are often intermediate between those predicted by the artificially-induced egg temperature and those predicted if incubation periods result from intrinsic growth programs of embryos (Martin et al. 2007, Robinson et al. 2008, Robinson et al. 2014, Martin et al. 2015, Martin et al. 2018 and references therein). Our study took a different approach by testing novel and opposing predictions regarding the relationships of incubation period with daily nest attentiveness and cumulative nest attendance that are expected under the *neonate quality* and *risk tolerance hypotheses*.

This study is the first to quantify intraspecific variation in a suite of incubation-related traits along a latitudinal gradient that spans a significant portion of a birds' breeding range. We found a surprising degree of variation in incubation behaviors, that variation was correlated with latitude, and the variation could mostly be accounted for by predicted life-history tradeoffs. Our results indicate that incubating female Burrowing Owls modify their incubation effort in ways that balance the needs of their developing embryos with their own energetic condition and their residual reproductive value. Furthermore, the degree to which female owls were willing to incur costs and risks associated with incubation varied along a latitudinal gradient in ways predicted by a view of life-history evolution driven by gradients in extrinsic mortality risk (Cole 1954, Williams 1966, Martin 2002, Martin et al. 2007, Martin et al. 2018).

An implicit assumption in birds is that selection favors maximum nest attentiveness because reduced attentiveness increases the risks of time-dependent nest mortality (Haftorn 1983, Moreno 1989). However, not all female Burrowing Owls in our study maximized their nest attentiveness. Some individuals facultatively reduced their nest attentiveness below the maximum possible, and this result is especially notable given that Burrowing Owls are assisted rather than intermittent incubators (Poulin et al. 2020). Intermittent incubators, those species that must alternate bouts of incubation with foraging, are well-known models for understanding how birds balance the needs of their developing embryos with their own energetic demands (White and Kinney 1974, Williams 1991, Conway and Martin 2000a, Conway and Martin 2000b, Hainsworth and Voss 2002). However, assisted incubators are even better model systems; male Burrowing Owls are thought to provide their mates with all their needed food during the incubation stage (Martin 1973, Poulin et al. 2020), which should allow females to maintain constant incubation. Yet, daily nest attentiveness varied among female Burrowing Owls and was positively correlated with latitude. Life-history theory predicts that populations subject to greater rates of extrinsic mortality should be more willing to incur the added costs and risks associated with individual reproductive attempts. Those populations subject to lower rates of extrinsic mortality are predicted to reduce their exposure to the added risks and costs associated with individual reproductive bouts in order to capitalize on their greater residual reproductive value (Williams 1966, Ghalambor and Martin 2001, Martin 2002). Because extrinsic mortality risk is generally thought to increase with increasing latitude (due to harsher winter weather, greater seasonal food limitation, and the risks associated with migration; Rowley and Russell 1991, Sandercock et al. 2000), our results support the idea that reproductive effort is adjusted to match extrinsic mortality risk.

We found, based on multiple analytical approaches, that Burrowing Owls reduced their nest attentiveness when the burrow microclimate was within or near the range of temperatures that is suitable for embryonic development (Drent 1975, Webb 1987, Lundblad 2020: Chapter Two). Avian embryos develop properly only within a relatively narrow range of temperatures, from about 36 – 40˚C (Webb 1987). Prolonged exposure to temperatures above this range leads to embryo death, and exposure to temperatures of 26 – 36˚C causes abnormal development and an increased risk of hatching failure (Webb 1987). This implies that birds might maximize their nest attentiveness when the nest microclimate is between 26 – 36˚C (or in excess of 40˚C), but that they can reduce their attentiveness between 36 – 40˚C without compromising the embryos (Arnold et al. 1987, Conway and

Martin 2000a). Furthermore, the cost of rewarming the clutch (and therefore the cost imposed by incubation recesses) decreases as the burrow temperature nears the range that is optimal for embryos (Vleck 1981, Biebach 1986, Reid et al. 2000). Indeed, previous studies have found that birds adjust their nest attentiveness and incubation rhythms in response to ambient or egg temperatures (Kendeigh 1952, White and Kinney 1974, Davis et al. 1984, Conway and Martin 2000a, 2000b). Some birds might reduce their nest attentiveness even at the costs of sub-optimal egg temperature and compromised embryonic development because doing so can increase their survival and lifetime fecundity (Martin 2002, Martin et al. 2007, Martin et al 2018). Our finding that nest attentiveness varied latitudinally supports the idea that an individual’s willingness to compromise offspring for their own self-maintenance depends upon their relative risk of extrinsic mortality and residual reproductive value.

Adult thermoregulatory demands could also mediate decisions regarding investment in incubation and the balance between nest attendance and self-maintenance (Bartholomew and Dawson 1979, Nadeau et al. 2015), especially in cavity- and burrow-nesting species. Internal temperatures inside artificial burrows, even at our lowest-latitude study site in the Sonoran Desert, are often below the thermoneutral zone of an adult Burrowing Owl (Coulombe 1970, Nadeau et al. 2015, unpublished personal data). Therefore, incubating adults might either need to invest extra energy in thermoregulation or behaviorally thermoregulate through reduced nest attendance (Haftorn and Reinertsen 1985, Yom-Tov and Wright 1993). Incubating Burrowing Owls reduced their nest attentiveness slightly when the above-ground ambient temperature was warmer than the burrow temperature, but the effect was very small. Furthermore, daily nest attentiveness was not significantly higher on days when the average burrow temperature was within the adult thermoneutral zone compared to days when the average burrow temperature was below the thermoneutral zone. Therefore, while thermal conditions for the developing embryos explained variation in daily nest attentiveness, adult thermoregulation needs did not.

Regardless of thermoregulatory demands, incubation imposes direct energetic costs (Carey 1980, Haftorn and Reinertsen 1985, Williams 1996), and those costs may decrease the survival of the incubating parent (Bryant 1999, Reid et al. 2000, Visser and Lessells 2001, Martin et al. 2018). Nest attendance could also increase the risk of adult predation while sitting on the nest. Adult predation while sitting on a nest is considered rare (Ricklefs et al. 2017 and references therein), and we only observed one such instance during our study, but even small decreases in daily survival probability contribute to the energetic costs of incubation. Hence, the cumulative costs of incubation include direct energetic costs, thermoregulatory costs, and the increased risk of predation and, together, that cost is not trivial and may explain why we observed a latitudinal gradient in daily nest attentiveness.

Incubation period duration in species with asynchronous hatching is often defined (including in this study) as the time elapsed from the laying of the final egg until the hatching of the final egg (Nice 1954, Briskie and Sealy, Ricklefs 1993), and yet such species typically exhibit partial incubation prior to clutch completion (Wang and Beissinger 2011). Therefore, the calculation of incubation period duration could be sensitive to the amount of incubation completed prior to clutch completion (e.g., Ardia et al. 2006). However, cumulative nest attendance during the laying period (prior to clutch completion) did not account for the strong positive association that we observed between incubation period duration and the cumulative hours of nest attendance (i.e., from initiation through the start of hatching). Intraspecific variation in incubation period duration also did not appear to be driven by clutch size variation; the top model to explain intraspecific variation in incubation period did not include clutch size, the model-averaged parameter estimate of clutch size was negative and small, and the 95% confidence interval included zero. And, hence, our finding that incubation periods were associated with cumulative nest attendance was probably not driven by variation in the quantity of nest attendance that occurred prior to clutch completion nor by reduced incubation efficiency of larger clutches.

We found that incubation period duration, daily nest attentiveness, and cumulative nest attendance were all positively correlated with latitude. Several studies have examined interspecific variation in these traits (Ricklefs 1969, Martin 2002, Martin and Schwabl 2008, Chalfoun and Martin 2007, Martin et al. 2007, Cox and Martin 2009), but few previous studies have reported intraspecific variation in incubation period duration across a latitudinal gradient (but see Ardia et al. 2006, Sofaer et al. 2020) and none have compared populations at greater than two study sites. Both Orange-crowned Warblers (*Leiothlypis celata*) and House Wrens (*Troglodytes aedon*) had longer incubation periods at a low-latitude site compared to a higher-latitude site (Dobbs et al. 2006, Tieleman et al. 2006, Sofaer et al. 2020). Similarly, interspecific studies have documented longer incubation periods in tropical species relative to temperate species (Martin 2002, Martin et al. 2007, Martin and Schwabl 2008). Therefore, the positive association between incubation period and latitude that we observed in Burrowing Owls contrasts with previous findings within and across species. Why Burrowing Owl incubation period duration increased with latitude (rather than decreasing with latitude as in other studies) is unclear given that theory generally predicts the opposite (Ricklefs 1993, Martin 2002, Martin et al. 2018). Lower extrinsic mortality risk and slower population turnover at low latitudes is thought to favor greater investment in offspring quality through “k-selected” traits that could result from slower development (MacArthur 1962, Ricklefs 1993). It appears that Burrowing Owls invest more heavily in reproduction at high latitudes, where they lay larger clutches (Lundblad 2020: Chapter Two), exhibit higher daily attentiveness, and higher cumulative nest attentiveness.

Once we accounted for the effects of average burrow temperature, date, and clutch size, the strong univariate correlation that we observed between daily attentiveness and latitude (Fig. 4.3A) was almost fully accounted for (Fig. 4.4). Only two previous studies have measured intraspecific latitudinal variation in nest attentiveness and both of those studies included data only from two study sites (Rohwer and Purcell 2019, Sofaer et al. 2020). Among both Orange-crowned Warblers and Yellow Warblers (*Setophaga petechia*), diurnal nest attentiveness was higher at a high-latitude site than at a low-latitude site, however 24-hour nest attentiveness was similar between the two sites for Orange-crowned Warblers. The difference in Yellow Warbler nest attentiveness between the two study sites was fully accounted for by variation in ambient temperatures and male feeding rates. Under cooler ambient conditions, females increased their attentiveness, and males increased their rate of provisioning food to the female (Rohwer and Purcell 2019). Similarly, average daily burrow temperatures explained a significant proportion of variation in daily nest attentiveness in our study.

Comparative studies across species have also suggested that per-unit-time (e.g., daily) nest attentiveness is higher in the temperate zone than in the tropics (Martin 2002, Chalfoun and Martin 2007, Cox and Martin 2009, Martin et al. 2007, Alvarez and Barba 2014). However, these studies generally only considered diurnal nest attentiveness. Diurnal nest attentiveness may be misleading because latitudinal variation in nest attentiveness during daylight hours can be confounded by latitudinal variation in daylength, and 24-hour nest attentiveness rates are often more comparable (Ricklefs and Brawn 2013, Shaw and Creswell 2014, Sofaer et al. 2020). We reported 24-hour daily nest attentiveness, but percent nest attentiveness during the incubation period did not differ between daylight and nighttime in our study. Hence, latitudinal variation in diurnal nest attentiveness or nocturnal nest attentiveness are not sensitive to the latitudinal gradient in daylength in our system. During the laying stage, nocturnal nest attentiveness was much lower than diurnal nest attentiveness, and most of the nest attendance that occurred prior to clutch completion was diurnal. Burrowing Owls have been described as diurnal (Coulombe 1971, Martin 1973), nocturnal (Haug and Oliphant 1990, Gleason and Craig 1979), and crepuscular (Thomsen 1971, Poulin et al. 2020, Pezzolesi and Lutz 1994, Poulin and Todd 2006), and females may be more diurnal than males (Poulin and Todd 2006). The very low rate of nocturnal nest attentiveness that we observed during the laying stage provides indirect evidence that female Burrowing Owls in our study were hunting nocturnally until they commenced full incubation, typically after clutch completion.

Although our data demonstrate that longer incubation periods are associated with greater cumulative nest attendance, which supports predictions of the *neonate quality hypothesis* (Ricklefs 1993, Ricklefs et al. 2017), we failed to detect a predicted positive association between incubation period duration and nestling quality. Longer incubation periods were not associated with enhanced nestling growth rates or reduced ectoparasite loads. However, variation in nestling quality may be reflected in many morphological and (especially) physiological traits that we did not measure. Although we also quantified variation in ectoparasite load, immunocompetence might be better reflected in blood parasite load or direct measures of immune function (Ricklefs 1992, Tella et al. 2002, Lee et al. 2008). Future studies should examine whether longer incubation periods are associated with a larger suite of physiological metrics of individual quality including immunocompetence, thermoregulatory performance, and rates of oxidative damage. The ideal (albeit logistically challenging) study would examine the association between incubation period duration and offspring longevity and lifetime reproductive performance (Ricklefs 1993).

Ricklefs (1993) speculated that long incubation periods may comprise just one part of a broader “life-history syndrome” driven by variation in adult life-expectancy and characterized by long incubation periods, long laying intervals, and asynchronous hatching. Accordingly, longer life-expectancy selects for long incubation periods that increase neonate quality and longevity, and long laying intervals and asynchronous hatching are adaptations to prevent selection on accelerated development driven by sibling competition (Ricklefs 1993). This broader scenario does not appear to be supported in our study system given that incubation period duration increased with latitude but laying interval length and the degree of hatching asynchrony decreased with latitude (Lundblad 2020:Chapters One and Two).

In conclusion, two alternative hypotheses have been proposed to explain variation in avian incubation period duration. The two hypotheses are not mutually exclusive (Ricklefs et al. 2017, Martin et al. 2018), and we found elements of support for each of them in Burrowing Owls across a 1400-km latitudinal gradient in North America. We found that Burrowing Owls varied in their willingness to incur the risks and costs associated with incubation, and that willingness increased with latitude and with assumed increases in extrinsic mortality risk. These finding lend support to the view that variation in extrinsic mortality risk is a major selective force that could drive variation across a full suite of life-history traits (Martin 2002). We also found compelling support for the hypothesis that longer incubation periods result not from reduced attentiveness but instead from greater total energetic input that presumably reflects selection for higher-quality offspring.

While numerous studies have examined interspecific variation in one or several life-history traits, our study demonstrates the value in documenting intraspecific variation in a larger suite of life-history traits, and testing hypotheses to explain that variation, including variation in overlooked traits related to incubation strategies. Finally, our results emphasize multiple ways that the abiotic environment drives life-history variation within and across species. Variation in nest microclimate explained variation in nest attentiveness, corroborating previous studies in other taxa (Kendeigh 1952, White and Kinney 1974, Davis et al. 1984, Conway and Martin 2000a). Furthermore, inter-annual variability and seasonality vary clinally and the resulting gradient in life-expectancy appears to be a general selective regime that mediates how animals balance their reproductive effort with survival in a way that maximizes lifetime fitness. Our study illustrates that an improved understanding of the many direct and indirect mechanisms by which animals are already adapted to the thermal and abiotic environment is a prerequisite for managing populations on a rapidly changing planet.

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Table 4.1. Models designed to explain intraspecific variation in daily nest attentiveness of Burrowing Owls breeding along a 1400-km latitudinal gradient in the western United States (2015 – 2018). The models that received at least 1% of total model weight plus the intercept-only null model are listed, but we considered models including all combinations of predictors (except those including a quadratic term without the corresponding linear effect). Ambient Temp is the mean daily above-ground ambient temperature, and Burrow Temp is the mean daily burrow temperature.

Model	**ΔAICc**	**weight**
~ Burrow Temp + Burrow Temp^2 + Clutch Size + Clutch Size^2 + Latitude + Latitude^2 + Julian Day + Julian Day^2 + Ambient Temp>Burrow Temp(yes)[1]	0.0	0.49
~ Burrow Temp + Burrow Temp^2 + Clutch Size + Clutch Size^2 + Latitude + Latitude^2 + Julian Day + Julian Day^2	0.1	0.47
~ Burrow Temp + Burrow Temp^2 + Clutch Size + Clutch Size^2 + Latitude + Julian Day + Julian Day^2 + Ambient Temp>Burrow Temp(yes)	6.8	0.02
~ Burrow Temp + Clutch Size + Clutch Size^2 + Latitude + Latitude^2 + Julian Day + Julian Day^2 + Ambient Temp>Burrow Temp(yes)	7.9	0.01
~ Burrow Temp + Burrow Temp^2 + Clutch Size + Clutch Size^2 + Latitude + Julian Day + Julian Day^2	8.4	0.01
~ 1 (null model)	180.4	0.00

[1] Global model

Table 4.2. Model-averaged parameter estimates for predictors of intraspecific variation in daily nest attentiveness among Burrowing Owls breeding along a 1400-km latitudinal gradient in the western United States (2015 – 2018). Parameter estimates are on standardized scale. Ambient Temp is the mean daily above-ground ambient temperature, and Burrow Temp is the mean daily burrow temperature.

Predictor	**β**	**SE**	**95% C.I.**
Intercept	2.08	0.09	1.91 - 2.25
Burrow Temp	-0.42	0.06	-0.53 - -0.30
Burrow Temp^2	-0.08	0.03	-0.13 - -0.03
Clutch Size	0.16	0.05	0.06 - 0.27
Clutch Size^2	-0.11	0.03	-0.16 - -0.05
Julian Date	0.34	0.04	0.26 - 0.43
Julian Date^2	-0.07	0.02	-0.10 - -0.04
Latitude	0.14	0.05	0.05 - 0.24
Latitude^2	0.20	0.07	0.07 - 0.34
Ambient Temp > Burrow Temp(yes)	-0.09	0.06	0.27 - 0.40

Table 4.3. Models designed to explain intraspecific variation in incubation period duration among Burrowing Owls breeding along a 1400-km latitudinal gradient in the western United States (2015 – 2018). The 20 models (of 32 considered) that received at least 1% of total model weight plus the intercept-only null model are listed.

Model	**ΔAICc**	**weight**
Incubation Period ~Cumulative Nest Attendance	0.0	0.25
Incubation Period ~Cumulative Nest Attendance + Daily Nest Attentiveness	1.0	0.15
Incubation Period ~Cumulative Nest Attendance + Clutch Size	1.7	0.10
Incubation Period ~Cumulative Nest Attendance + Latitude	2.4	0.07
Incubation Period ~Cumulative Nest Attendance + Hatch Julian Day	2.4	0.07
Incubation Period ~Cumulative Nest Attendance + Clutch Size + Daily Nest Attentiveness	2.8	0.06
Incubation Period ~Cumulative Nest Attendance + Hatch Julian Day + Daily Nest Attentiveness	3.4	0.04
Incubation Period ~Cumulative Nest Attendance + Latitude + Daily Nest Attentiveness	3.5	0.04
Incubation Period ~Cumulative Nest Attendance + Clutch Size + Hatch Julian Day	4.2	0.03
Incubation Period ~Cumulative Nest Attendance + Clutch Size + Latitude	4.2	0.03
Incubation Period ~Percent Daily Attentiveness	4.9	0.02
Incubation Period ~Cumulative Nest Attendance + Hatch Julian Day + Latitude	5.0	0.02
Incubation Period ~Cumulative Nest Attendance + Clutch Size + Hatch Julian Day + Daily Nest Attentiveness	5.1	0.02
Incubation Period ~Cumulative Nest Attendance + Clutch Size + Latitude + Daily Nest Attentiveness	5.4	0.02
Incubation Period ~Latitude + Percent Daily Attentiveness	6.1	0.01
Incubation Period ~Cumulative Nest Attendance+ Hatch Julian Day + Latitude + Daily Nest Attentiveness	6.7	0.01
Incubation Period ~Cumulative Nest Attendance + Clutch Size + Hatch Julian Day + Latitude	6.8	0.01
Incubation Period ~Clutch Size + Daily Nest Attentiveness	7.3	0.01
Incubation Period ~Hatch Julian Day + Daily Nest Attentiveness	7.3	0.01
Incubation Period ~Cumulative Nest Attendance + Clutch Size + Hatch Julian Day + Latitude + Daily Nest Attentiveness[1]	7.6	0.01
Null model (intercept only)	11.5	0.00

[1] Global model

Table 4.4. Model-averaged parameter estimates for predictors of intraspecific variation in incubation period duration among Burrowing Owls breeding along a 1400-km latitudinal gradient in the western United States (2015 – 2018). Parameter estimates are on standardized scale.

Predictor	**β**	**SE**	**95% C.I.**
Intercept	22.41	0.14	22.12 - 22.69
Cumulative Nest Attendance	0.70	0.39	0.03 - 1.46
Daily Nest Attentiveness	-0.14	0.36	-1.34 - 0.61
Hatch Date (Julian)	-0.01	0.08	-0.36 - 0.30
Clutch Size	-0.04	0.12	-0.52 - 0.23
Latitude	0.01	0.10	-0.35 - 0.44

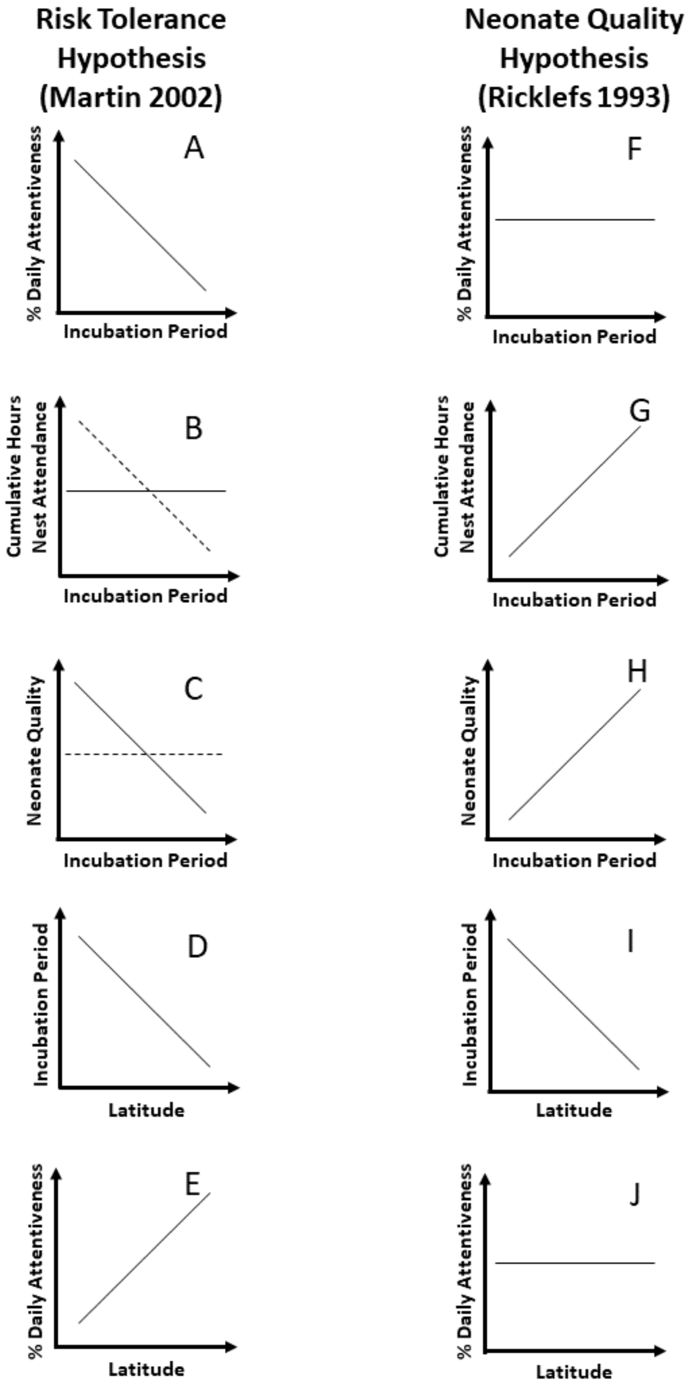

Figure 4.1. Predictions generated by risk tolerance (left column) and neonate quality (right column) hypotheses to explain intraspecific variation in incubation period length. Solid lines illustrate most likely relationships based on predictions of the 2 hypotheses, and dashed lines represent the extreme range of the prediction.

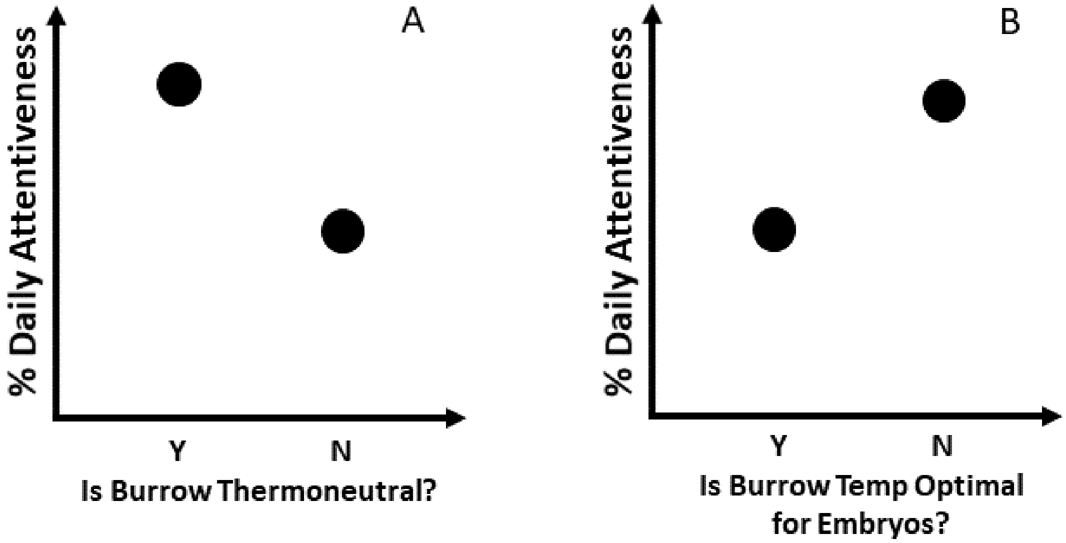

Figure 4.2. Predicted relationships between nest microclimate and nest attentiveness in a cavity-nesting species where internal conditions are buffered from the external environment. (A) Expected relationship if birds modify their attentiveness to minimize their thermoregulatory demand. (B) Expected relationship if birds modify their attentiveness to maximize embryonic development.

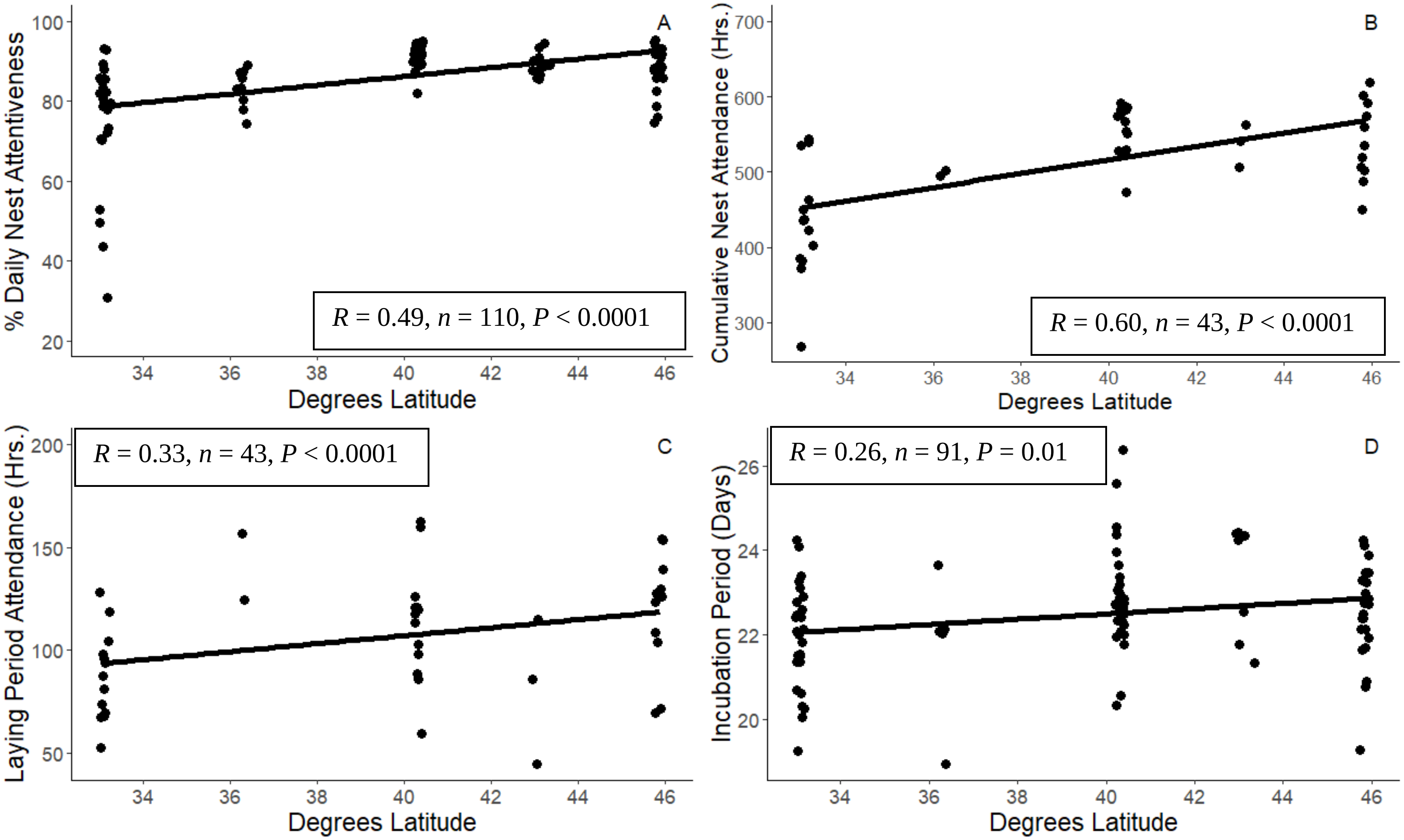

Figure 4.3. Latitudinal patterns in average per-nest: (A) percent daily nest attentiveness during the incubation period, (B) cumulative hours of nest attendance during the laying and incubation periods (from the day of nest initiation through the last full day of incubation), (C) the cumulative hours of nest attendance during the laying period (from the day of initiation through the last day of laying), and (D) incubation period (i.e., number of days from the laying of the final egg until the hatching of the final egg) among Burrowing Owls breeding along a 1400-km latitudinal gradient in the western United States, 2015 – 2018. Solid lines are simple linear regressions and points are raw mean values per nest.

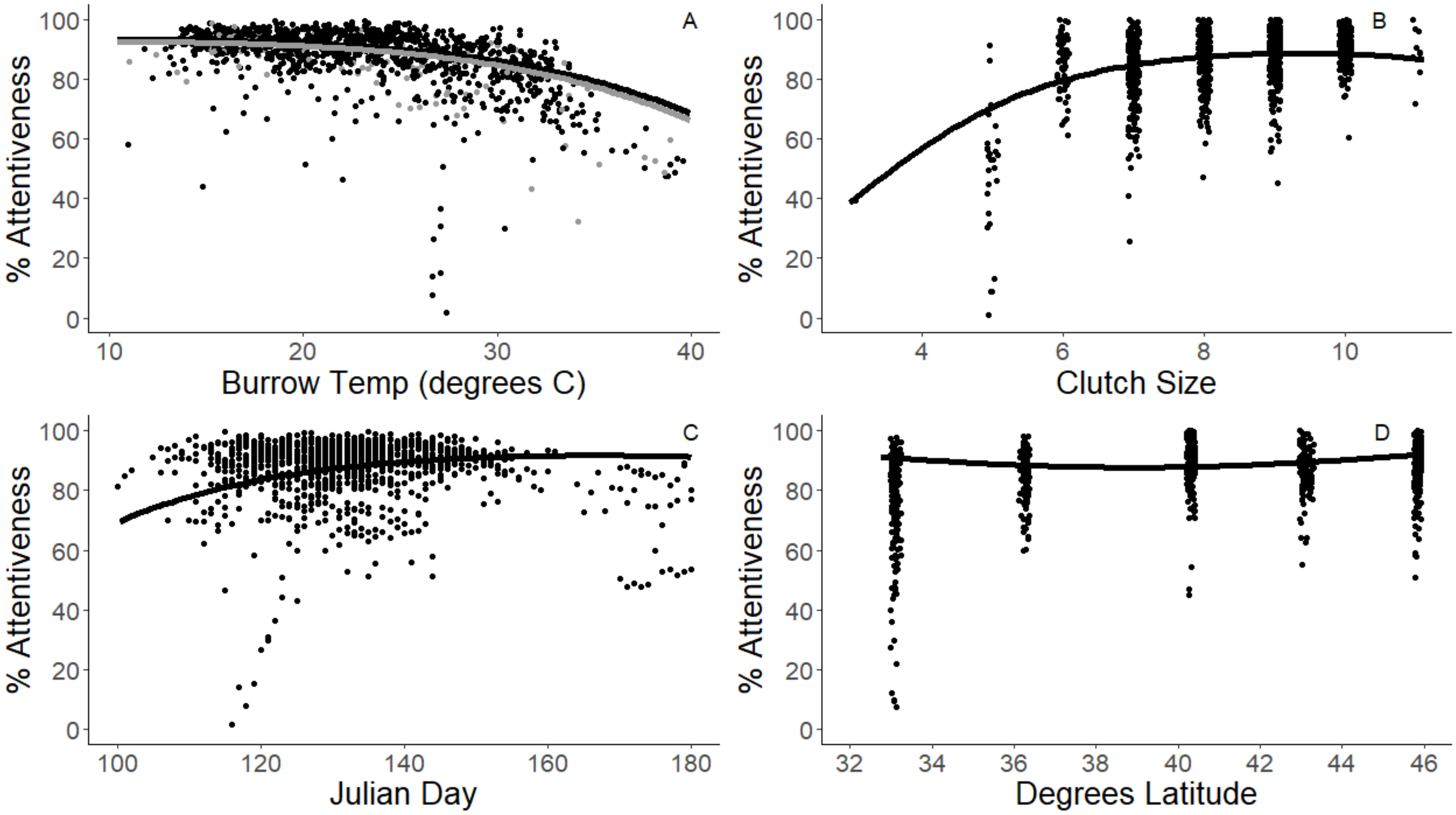

Figure 4.4. Partial effects of explanatory variables (i.e., after the other variables are accounted for) accounting for intraspecific variation in daily nest attentiveness among Burrowing Owls breeding along a 1400-km latitudinal gradient in the western United States, 2015 – 2018. Solid lines are based on model-averaged parameter estimates. Points are raw data points corresponding to individual nest days. In Panel A, black lines and points correspond to days when the average above-ground ambient temperature was less than the average below-ground burrow temperature, and gray lines and points correspond to days when the average above-ground ambient temperature was greater than the average below-ground burrow temperature.

Chapter 5: Ashmole’s Hypothesis and the Latitudinal Gradient in Clutch Size: Confusion, Misinterpretations, and the Path Forward

Abstract

One enduring priority for ecologists has been to understand the cause(s) of variation in reproductive effort and fecundity. Avian clutch size generally increases with increasing latitude, both within and across species, but the mechanism(s) driving that pattern continue to generate hypotheses and debate. In 1961, a Ph.D. student at Oxford University, N. Phillip Ashmole, proposed an influential hypothesis that clutch size varies in direct proportion to the seasonality of resources experienced by a breeding population. *Ashmole’s hypothesis* has been widely cited and discussed in the ecological literature, but misinterpretation and confusion has been common regarding the mechanism that underlies *Ashmole’s hypothesis* and about what testable predictions it generates. We review the development of well-known hypotheses to explain clutch size variation with an emphasis on *Ashmole’s hypothesis*. We then discuss and clarify sources of confusion about *Ashmole’s hypothesis* in the literature, summarize existing evidence in support and refutation of *Ashmole’s hypothesis*, and suggest additional approaches that could be used to test *Ashmole’s hypothesis* and gain an improved understanding of the mechanisms responsible for regulating avian clutch size.

Background and Historical Development

Clutch size variation has interested ecologists for over a century (Moreau 1944 and references therein), and David Lack’s work on the causes of clutch size variation inspired an entire field of research into what we now call life-history evolution (Lack 1947, 1948, 1954). Lack’s two primary contributions were in emphasizing that: 1) traits related to the allocation and timing of reproductive effort are shaped by natural selection, and 2) reproductive effort, recruitment, and hence population growth are constrained and coupled to survival via density-dependent processes (Ricklefs 2000). He effectively married evolutionary and population ecology by linking these two principles, and he also famously emphasized the primacy of food availability as the main determinant of avian clutch size (Lack 1947, 1954). Lack proposed that birds lay the number of eggs corresponding to the largest brood for which the parents can provide food (known as Lack’s day-length hypothesis) and that the latitudinal gradient in clutch size results from latitudinal variation in daylight hours during which breeding birds can provision nestlings (Lack 1947).

In the early 1960s, N. Phillip Ashmole proposed an influential modification to Lack's general food availability hypothesis (an alternative to the day length mechanism). Ashmole (1961, 1963a) suggested that clutch size is a function of per-capita food availability during the breeding season and that per-capita food availability during the breeding season is a function of density-dependent mortality in response to seasonal food limitation during the leanest season (i.e., during winter in most temperate-zone systems). According to Ashmole, the difference between the minimum level of food resources available during the leanest part of the year and the maximum level of food resources during the most productive season is all surplus that can be allocated to reproduction. Seasonality generally increases with increasing latitude, hence Ashmole's hypothesis explains the well-known association between clutch size and latitude and explicitly accounts for the inverse relationship between fecundity and survival with a single elegant mechanism based on feedbacks among density-dependent processes. Ashmole's hypothesis contrasts with other hypotheses (e.g., Lack's daylength hypothesis, also see Owen 1977) that propose that fecundity is a function of absolute food availability or limits to the ability to gather food.

Ashmole's dissertation (Ashmole 1961) and his widely-cited 1963 paper were primarily concerned with only one end of the life-history continuum; accounting for the low reproductive rate of tropical seabirds (which largely lay single egg clutches) while rebutting the group selectionist explanations of Wynne-Edwards (1955, 1962). Ashmole suggested that the lack of seasonality in the tropical ocean kept populations near environmental carrying capacity throughout the year, precluding significant density-dependent mortality and the production of a seasonal food surplus to allocate to reproduction. Ashmole's 1963 paper lays out the basic elements of his seasonality mechanism but makes no direct mention of the latitudinal gradient in clutch size that his hypothesis is now most commonly associated with, except in a couple of passages where he says: "*…competition for food round* [sic] *the breeding colonies…regulate the numbers of most tropical ocean birds, and possibly also those of some species breeding at higher latitudes,"* and, "*while at high latitudes there is generally an enormous flush of marine food in the spring, which must tend to reduce competition for it, in tropical oceans seasonal fluctuations in food abundance are generally much less extreme, and marine food is unlikely to ever be superabundant*". Ashmole's 1961 Ph.D. dissertation is where his model of clutch size regulation is more-fully articulated and linked to latitudinal variation in clutch size: "*I would like to suggest that the tendency for birds to breed only at particular seasons, even in tropical environments where seasonal change is extremely slight, is essentially connected with the well known fact that clutch size is lower in tropical areas than in the temperate zones, and that both are the result of competition for food. As already argued, the main flush of food in most temperate areas comes at a time when there is a relatively very small population of birds. But in tropical land* *environments which show less seasonal variation, it seems likely that the difference between the amount of food available in the most favourable and the least favourable periods will be much less than in temperate regions…..*[where] *the numbers of a species are presumably cut down severely during the hard period between breeding seasons, so that numbers of adults will be low during the more favourable breeding period….*". Ashmole seemed to place little emphasis on the broader biogeographical implications of his proposal, and how his newly proposed mechanism might operate outside of the tropical ocean was essentially an afterthought.

Ashmole's view of clutch size regulation went largely unnoticed during the subsequent two decades except by Lack and Moreau (1965), who performed the first *a priori* test of Ashmole's hypothesis using clutch sizes of African birds. Ricklefs (1980) later recognized the significance of Ashmole's ideas and was the first to formalize them as "Ashmole's hypothesis" where he stated: *"…reproductive rate should depend upon both resource level and population density during the breeding season. If populations were limited by resources during the nonbreeding season, the level of resources available in the breeding season relative to population density would depend upon the seasonality of resources. Clutch size accordingly would increase in direct proportion to seasonality, irrespective of the average resource level*." Ricklefs had previously determined (1977a, 1977b) that the other popular hypotheses that were commonly thought to explain clutch size evolution, including nest predation risk (Skutch 1949) and evolutionary optimization of reproductive effort in relation to life expectancy (Cody 1966, 1971), could account for only a fraction of the observed variation in clutch size. He concluded that: "*…variation in the seasonality of resources experienced by a population is the single most important cause of geographical patterns in clutch size*". Despite Ricklefs opinion regarding the importance of Ashmole's hypothesis for explaining clutch size variation globally, there have been surprisingly few studies that have sought to test Ashmole's hypothesis and an unfortunately large amount of ambiguity regarding the premise of the hypothesis has proliferated in the literature.

Interpretations and Confusion in the Literature

Despite Ricklefs' clear articulation and definition of Ashmole's hypothesis and the mechanism that underlies it, few ecological concepts have been more misinterpreted in the ecological literature. Some authors cite Ashmole (1961, 1963a) or Ricklefs (1980) simply for the latitudinal pattern in clutch size (Court et al. 1988, Meiri et al. 2013, Benharzallah et al. 2015) without reference to Ashmole's proposed explanation of the pattern. Others confuse the hypothesis for the pattern it was formulated to explain; for example describing Ashmole's hypothesis as, "*….clutch size for a bird* *species is larger in northern than in southern latitudes,*" (Guthery 2008). Others invert the pattern and the hypothesis to explain it: "*According to the "Seasonality Hypothesis," Ashmole (1963a) predicted that clutch sizes will be larger at higher latitudes,"* (Liu et al. 2018). Multiple studies associate or blend Ashmole's hypothesis with Lack's daylength hypothesis (Kuitunem and Suhonen 1991, Li and Lu 2012, Zima et al. 2017). Many authors so strongly equate Ashmole's hypothesis simply with "seasonality" that the details of the actual mechanism are overlooked (McNair 1985, Miskelley 1990, Rizi et al. 1999, Berry 2001). Some authors describe only half the mechanism, either suggesting that food availability is greater in more seasonal environments without acknowledging why (breeding resources follow from winter mortality; Matsuoka et al. 1997, Bogner and Baldassarre 2002) or that greater winter mortality is associated with larger clutch size without acknowledging why (winter mortality leads to greater per-capita food availability during the breeding season; Lindstedt and Boyce 1985, Ippi et al. 2012). Although these descriptions are not inaccurate, they contribute to confusion about what Ashmole's hypothesis is and what predictions the hypothesis generates because they only describe part of the mechanism. Goymann et al. (2005) correctly equate Ashmole's hypothesis with variation in seasonality (full mechanism not described) but cite the wrong 1963 paper by Ashmole (Ashmole 1963b). Some have claimed that: "*Ashmole's hypothesis doesn't apply*" in environments of low or no seasonality (Siverio et al. 2013), when in fact Ashmole formulated his hypothesis to explain why tropical seabirds in a non-seasonal environment laid such small clutches. One study states that, "*…Ashmole's hypothesis* [is] *that clutch size of passerine birds increases with the seasonality of resources relative to population density*" (Bruderer and Salewski 2009) instead of the correct interpretation that clutch size increases with resource seasonality *because* seasonality affects the breeding population density and hence the per-capita breeding season resource availability. Some papers incorrectly state that Ashmole's hypothesis applies only to passerines (Bruderer and Salewski 2009, Engen and Saether 2016) perhaps because passerines were the focus of Ricklefs' (1980) original test of the hypothesis. However, seabirds inspired Ashmole's hypothesis, which is relevant to all animals. Statements that: "*…in Ashmole's view the reduction of clutch size and mortality are independent effects of the stable environment,*" (repeated almost verbatim in Crowell and Rothstein 1981, Blondel et al. 1987) fail to recognize how Ashmole's hypothesis directly couples the reproductive rate to mortality via density dependence.

Many papers confuse resource seasonality (intra-annual variation), which underlies Ashmole's mechanism, with other vague phenomena including: inter-annual variability (Gibb 1968) or stochasticity (Møller 1984), productivity (Thomas et al. 1999), absolute food availability (Simmons 1993, Li et al. 2012, Mérő et al. 2015), environmental variability (Engen and Saether 2016), stability, or predictability (Horn 2012). Many of these misinterpretations equate, confuse, or conflate (Gibb 1968, Tarburton 2009, Burski 2011, Gill and Haggerty 2012, Horn 2012, Heming and Marini 2015) Ashmole's mechanism with the mechanism underlying r-K selection theory (MacArthur 1962, Pianka 1970) which was applied to clutch size evolution by Cody (1966, 1971). Ashmole's hypothesis invokes intra-annual variation in per-capita food availability, governed by within-year climate variability, and couples the reproductive rate to mortality through reciprocal density-dependent feedbacks acting in both the winter and the breeding season. In contrast, the ideas of MacArthur and Cody are based on evolutionary optimization of reproductive effort in response to the degree of inter-annual variation, environmental stochasticity, density-independent mortality, and the annual rate of population turnover. Cody's (1966) model of clutch size evolution does not require or invoke density-dependent mortality, whereas Ashmole's hypothesis is explicitly based on density-dependent feedbacks. Seasonality and stochasticity are both likely correlated with latitude, and Cody (1971) considered Ashmole's hypothesis consistent with and "*easily incorporated into*" his own model of clutch size regulation. However, treating them as equivalent only contributes to the large amount of confusion surrounding each concept in the literature. For example, Ashmole's hypothesis does not necessarily suggest or predict an offspring quantity-quality tradeoff or that selection favors K-selected traits in less seasonal environments as implied by Gibb (1968), Gill and Haggerty (2012), and Heming and Marini (2015). Instead, Ashmole's hypothesis predicts that additional available resources that could be invested into K-selected traits would instead be invested into a larger clutch size.

Finally, there are numerous papers that associate Ashmole's hypothesis with and/or cite Ricklefs (1980) in reference to a wide variety of phenomena not mentioned or implied therein including latitudinal variation in annual fecundity (Davanço et al. 2013), body size (Nylin and Svärd 1991, Olsen and Marples 1993), egg size (Olson et al. 2015), species richness (Boone and Krohn 2000, Lawler et al. 2004, Mayal et al. 2009), and even the rate of feather molt and gonadal maturation (Malik et al. 2014). The repetition and perpetuation of these errors and misinterpretations through the literature has contributed to ongoing confusion about what pattern or phenomenon Ashmole's hypothesis addresses, the underlying mechanism the hypothesis invokes, and what the hypothesis predicts empirically. This confusion may be aggravated by the relative inaccessibility of Ashmole's actual 1961 dissertation and over-reliance on citing his 1963 paper in which the hypotheses is only vaguely implied. As a result, the enormous confusion and ambiguity surrounding Ashmole's hypothesis has likely been caused by a proverbial game of telephone whereby misinterpretations in one paper were perpetuated in subsequent ones and accumulated in the literature. Authors citing Ashmole's hypothesis would be better served by securing and reading a copy of Ashmole (1961) or by referencing Ricklefs' (1980) excellent recapitulation of Ashmole's proposed mechanistic hypothesis to explain clutch size variation in birds.

The Role of Migration

One recurring stumbling block in the ability to interpret and test Ashmole's hypothesis involves the role of bird migration, which effectively redistributes bird density on a seasonal basis. Not all species or individuals breed and winter in the same location, yet Ashmole's basic model assumes they do. The ubiquity of seasonal migration has caused previous researchers to question whether the degree of seasonality experienced on the breeding grounds is truly a good predictor of clutch size for migratory species. Some authors have even claimed that Ashmole's hypothesis does not apply to migratory species (Alerstam and Hedenström 1998, Bulluck 2007). Seasonal migration does complicate our ability to formulate quantitative predictions, but we argue that this should not change or negate Ashmole's hypothesis or qualitative predictions of it. Greater seasonality leads to a greater reduction in carrying capacity (K) during the winter (or leanest season), relative to that during the peak of resources during the breeding season. The proportion of the post-breeding population that exceeds winter K starves and perishes among resident species, but migratory species can either stay and risk starvation or migrate to where winter food availability and survival prospects are greater. Migration is also a risky endeavor (Sillett and Holmes 2002, Wikelski et al. 2003, Newton 2008, Strandberg et al. 2010, Klaassen et al. 2014), however, and being forced to migrate in response to seasonal food limitation could simply be thought of as a second mechanism underlying density-dependent mortality of populations breeding in seasonal environments (Yom-Tov 1994). Species with the ability to migrate likely suffer reduced winter mortality compared to resident species in the same seasonal breeding environment (Zúñiga et al. 2017, Dokter et al. 2018, Lundblad and Conway 2020a), but they likely suffer greater winter mortality than an ecologically equivalent resident species in an unseasonal environment that precludes the need to migrate.

At the same time that a migratory species might reduce its winter mortality rate by migrating to an environment with more food, resident species in areas experiencing an influx of winter migrants are also affected. A seasonal influx of migrants during the winter increases the local bird density, depresses per-capita food availability, and likely increases density-dependent mortality of the resident species during the winter. Increased winter mortality leads to increased per-capita food availability and would allow increased clutch size for the resident species during the subsequent breeding season (as per Ashmole's hypothesis). Ricklefs (1980) addressed the implications of migration and made these same points: "*…the* [Ashmole] *model…may be elaborated upon in several ways. By storing* *resources, switching to more abundant food, and migrating to regions of greater productivity during the nonbreeding period, populations might achieve larger size, and thereby restrict access of each individual to resources during the non-breeding season. Influx of migrants to an area during the nonbreeding season might reduce populations of residents through interspecific competition and thereby increase the access of resident individuals to resources during the breeding season*". None-the-less, some investigators have formulated and tested inappropriate predictions that quantify the degree of seasonality experienced by a population as the difference between the maximum level of resources available on the breeding grounds and the minimum level of resources available on their assumed wintering grounds (Møller 1984, Young 1994). Such approaches make unrealistic assumptions that: 1) the disjunct breeding and wintering locations have similar densities of resident birds within the same foraging guild, and 2) migratory connectivity is absolute (Webster et al. 2002). Therefore, a more valid approach may be to characterize the seasonality of resources experienced by a given population based on its breeding grounds, even for migratory species. The seasonal difference in resource availability on the breeding grounds should be associated with the proportion of the post-breeding population that *either* perishes under seasonal food limitation *or* which suffers attrition during risky seasonal migrations (Yom-Tov 1994). We can and should take advantage of migratory behavior when formulating testable predictions (see Ricklefs 1980, Yom-Tov 1994, 1995, Yom-Tov and Geffen 2002, and discussion below) instead of being stymied by the complexity that migration introduces into Ashmole's model of clutch size regulation.

Predictions and Evidence

The Association Between Clutch Size and Direct Measures of Seasonality

Perhaps the most obvious prediction and starting point for testing Ashmole's hypothesis is that clutch size should be positively associated with direct measures of seasonality; $CS = aS/W$, where CS is clutch size, S is the level of breeding season resources, W is the level of nonbreeding resources, and a an arbitrary constant (Ricklefs 1980; Table 5.1, prediction 1). Hussell (1985) corrected and reconciled Ricklefs' equation with his own text by clarifying that, under Ashmole's hypothesis, clutch size should be a function of the ratio between the *surplus* of resources generated by seasonal fluctuation (not the absolute maximum level) and the minimum level of resources available in the winter; $CS = a(S-W)/W$. Subsidiary to this prediction are the predictions that clutch size should be inversely associated with W, but not associated with S (Ricklefs 1980, Møller 1984; Table 5.1, predictions 2-3).

Given the inherent difficulties of measuring resource availability (and hence seasonal fluctuations) directly, Ricklefs (1980) used the magnitude of annual variation in actual evapotranspiration (AE; Rosenzweig 1968) as an index of seasonality. Passerine clutch sizes from seven regions spanning a latitudinal gradient were directly associated with AE seasonality and therefore supported predictions 1 – 3 (Table 5.1; Ricklefs 1980). Support for these same predictions has come from *a priori* tests involving Northern Flickers (*Colaptes auratus*; Koenig 1984), 21 species of North American woodpeckers (Koenig 1986), Eurasian Kestrels (*Falco tinnunculus*; Carrillo and González-Dávila 2010), and across all passerine birds (Olalla-Tárraga et al. 2019). These same 3 predictions (Table 5.1, predictions 1 – 3) were not supported in Barn Swallows (*Hirundo rustica*) and House Martins (*Delichon urbica*) nor was the related prediction (Table 5.1, prediction 4) that clutch size should be positively associated with the ratio of breeding season resources to population density (Møller 1984). The same prediction (Table 5.1, prediction 4) was supported in Tree Swallows (*Tachycineta bicolor*; Dunn et al. 2000) and Northern Flickers (Koenig 1984). In contrast to these other studies, the test with Barn Swallows and House Martins used the questionable approach of contrasting summer AE from the breeding grounds with winter AE from the assumed wintering grounds (Møller 1984). Barn Swallow and House Martin may also have been poor subjects for testing Ashmole's hypothesis given that their clutch sizes did not exhibit a latitudinal gradient (Møller 1984). Moreover, nest-site limitation can be severe in secondary cavity-nesting birds and is a process that influences avian clutch size (Martin 1993). Therefore, secondary cavity-nesters may not be ideal species in which to test Ashmole's hypothesis because other processes may be stronger than density-dependent food availability in determining clutch size in those species.

Additional studies have supported versions of this suite of predictions (Table 5.1, predictions 1-4) using other indices of seasonality instead of AE: seasonal variation in temperature (Virgós et al. 2006, Jetz et al. 2008, Evans et al. 2009, Tökölyi et al. 2014, Battistella et al. 2019, Ferreira et al. 2019), precipitation (Lepage and Lloyd 2004, Tökölyi et al. 2014 for mammalian litter sizes, Ferreira et al. 2019), and the normalized difference vegetation index (NDVI or "greenness"; Hořák et al. 2011, 2015). Seasonality (in temperature) was the single most-important predictor of avian clutch size variation, globally, based on a large meta-analysis (Jetz et al. 2008). Finally, colony size of four seabird species was inversely related to the numbers of competitors from other colonies whose foraging ranges overlapped with their own in a system similar to that which originally inspired Ashmole's hypothesis (Table 5.1, prediction 5; Furness 1984).

Ashmole's hypothesis predicts that clutch size should increase in proportion to the seasonal difference in resource availability *relative* to overall resource availability (Hořák et al. 2015). That is, the resource surplus generated by the same degree of seasonality is relatively larger when the environmental carrying capacity is smaller. Therefore, when seasonality is held constant, clutch size should decrease with increasing overall productivity of the system (Table 5.1, prediction 6). Indeed, clutch size increased with increasing seasonality but decreased with increasing productivity, in a system where productivity and seasonality varied independently and mostly along a longitudinal gradient (Hořák et al. 2015).

Comparing Clutch Size among Regions, Landforms, and Vegetation Types that Differ in Seasonality

Many past tests of Ashmole's hypothesis have compared clutch sizes among different geographic areas, landforms, or vegetation communities that are known or assumed to have different degrees of seasonality, often while controlling for latitude. Numerous studies have reported smaller clutches in the southern hemisphere than in the northern hemisphere (Woinarski 1985, Yom-Tov 1987, Rowley and Russell 1991, Yom-Tov 1994, Yom-Tov et al. 1994, Young 1994, Martin et al. 2000) including at least one published prior to Ashmole's papers (Moreau 1944). Larger clutches in the northern hemisphere may be attributable to the larger size and broader shape of the northern hemisphere continents, which lend their interiors greater continental influence and greater seasonality, consistent with Ashmole's hypothesis (Table 5.1, prediction 7a; Yom-Tov 1994, Young 1994). Following similar logic, islands should be less seasonal than continents due to moderating maritime influence, and numerous studies (Crowell and Rothstein 1981, Michelland 1982, Blondel et al. 2006, Rodriguez and Rodriguez 2007, Wang et al. 2009 among Pond Frogs (*Rana nigromaculata)*) have tested the prediction that clutch size should be smaller on islands than on adjacent mainlands (Table 5.1, prediction 7b; a pattern that had also been recognized prior to being placed in the context of Ashmole's hypothesis; Lack 1947, Marchant 1960, Cody 1966). Finally, geographic variation in litter size of dasyurid marsupials has been attributed to general geographic trends in seasonality (Cockburn et al. 1983).

No studies have tested *a priori* predictions of Ashmole's hypothesis relating clutch size to seasonality expressed along elevational gradients. Seasonality is often assumed to increase with increasing elevation, at least in the temperate zone (Boyce 1979, Martin 2001). Under this assumption, Ashmole's hypothesis predicts a positive association between clutch size and elevation. The preponderance of evidence, however, suggests that clutch size usually decreases with increasing elevation in both temperate and tropical species (Krementz and Handford 1984, Badyaev and Ghalambor 2001, Boyce et al. 2015, Dillon and Conway 2015, Hille and Cooper 2015, Boyle et al. 2016). Environmental conditions, however, vary in complex ways along elevational gradients. Although temperature seasonality likely increases with increasing elevation, directional change in seasonality of precipitation and productivity along elevational gradients are less well-established (Boyle et al. 2016). Furthermore, variation in seasonality along elevational gradients might interact with variation in breeding season length, which has been hypothesized to drive life-history variation along elevational gradients (Grzybowski and Pease 2005, LaBarbera and Lacey 2018, Lundblad and Conway 2020b).

Seasonality also varies among vegetative communities, even those in close proximity to each other, which facilitates comparisons that control for latitude (Table 5.1, prediction 7c). The clutch sizes of a suite of passerine families were smaller in tropical African evergreen forests (considered less seasonal) than in African savannas (considered more seasonal) at roughly the same latitude (Lack and Moreau 1965). Lack and Moreau (1965) cite similar data from Skutch (1954, 1960) and Marchant (1960) showing that the clutch size of American finches and flycatchers was correlated with assumed seasonality of vegetative communities in Central and South America. Similarly, Blue Tits (*Parus caeruleus*) and Great Tits (*P. major*) laid larger clutches in more-seasonal deciduous and mixed forest than in less-seasonal evergreen forests, after controlling for latitude (Klomp 1970 and references therein, Fargallo 2004, Blondel et al. 2006).

The introduction of species into new geographical areas with different levels of seasonality than their native ranges provides a natural experiment for testing Ashmole’s hypothesis (Table 5.1, prediction 7d). Among eleven species of passerines introduced from the United Kingdom, nine of them evolved significantly smaller average clutch sizes in similar habitats and at similar (southern) latitudes in their non-native ranges in New Zealand (Evans et al. 2005). Seasonality was lower in New Zealand based on several metrics (annual range in monthly temperatures, breeding season length, and seasonal variation in clutch size), but the magnitude by which clutch sizes were reduced in New Zealand was not associated with the magnitude of seasonality experienced by each species (based on season length and temporal variation in clutch size; Evans et al. 2005). European Blackbirds (*Turdus merula*) and Song Thrushes (*Turdus philomelos*) also had reduced clutch sizes (and higher population densities) in their introduced New Zealand ranges, relative to their native European ranges (Samaš et al. 2013).

Predictions Based on the Influence of Migration

Some studies have formulated and tested predictions that take advantage of either the seasonal redistribution of population densities induced by migration or the ability of some species to reduce their winter mortality via seasonal migration. Resident birds in regions that experience an influx of migrants during the winter face increased competition for resources, suffer greater winter mortality, and survivors have greater per-capita food available for reproduction during the following breeding season. Therefore, clutch size should be larger than predicted only by the location's degree of seasonality (Ricklefs 1980). Conversely, resident birds in those regions from where large numbers of breeding birds migrate in the winter experience reduced competition for winter food, reduced winter mortality, and lay smaller clutch sizes than predicted by seasonality alone (Table 5.1, prediction 8). Yom-Tov and Geffen (2002) incorrectly attribute this prediction directly to Ashmole (1961, 1963a), who never mentions how migration might modify his basic model of clutch size regulation.

Average clutch sizes in Java, Borneo, and the Greater Antilles had positive residuals in Ricklefs' (1980) original regression of clutch size on AE, suggesting clutch sizes were larger than expected based on seasonality, and each of these regions receives influxes of winter migrants (Ricklefs 1980). Clutch sizes on the Lesser Antilles, which receives a greater influx of migrants during the winter, were lower than those on the Greater Antilles, even though each island group experiences similar climate and similar seasonality (Ricklefs 1980). Variation in the ratios of winter migrants and transients to breeding species was also associated with clutch size differences among passerines: a) between the South African Cape Region and Israel, at similar latitudes (Yom-Tov 1994), b) across Australia, southern Africa, and India (Yom-Tov and Geffen 2002), and c) between desert (where few migrants go for the winter) and adjacent mesic areas in five regions, globally (Yom-Tom 1995).

The clutch sizes of four species of Darwin's Finches (*Geospiza* sp.) on Isla Genovesa were larger, not smaller, than those of mainland Ecuadorian finches (Grant and Grant 1980 citing mainland data reported by Marchant 1959, 1960). Grant and Grant (1980) interpreted this finding in support of Ashmole's hypothesis and flipped the original island-mainland prediction (Table 5.1, prediction 7b) on its head by suggesting that (in their system) the mainland species are better able to avoid seasonal food limitation, during the dry season, via altitudinal migration to more productive high elevations. Hence, the mainland species suffer less winter mortality than the island species, for whom altitudinal migration is not an option, and have less per-capita food available during the breeding season. This mechanism also allowed the formulation of another (yet untested) prediction about clutch size variation among Darwin's Finches; that clutch size should be inversely associated with relative height of the Galapagos Islands if higher islands offer greater opportunities to migrate upslope and avoid seasonal food limitation during the dry season (Grant and Grant 1980).

Predictions that Test Assumptions of Ashmole's Hypothesis

Some studies have tested underlying assumptions regarding geographic gradients in per-capita food availability during the breeding season, winter mortality, and carrying capacity that would be expected under Ashmole's hypothesis. Direct evidence that per-capita food availability during the breeding season is greater in temperate areas and more seasonal environments (Table 5.1, prediction 8) is limited, but prey attack rates were greater among a suite of foliage-gleaning insectivores in temperate France than among similar species in tropical Guiana (Thiollay 1988). Hourly Tree Swallow nest provisioning trips were no more frequent in Alaska than in California (Rose and Lyon 2013), however, and instead the hours spent foraging and *daily* provisioning rates were higher at the higher-latitude site (in support of Lack's daylength hypothesis). Nest provisioning rates were similar or higher in the tropics than at temperate zone sites in several additional studies (Martin et al. 2000, Gill and Haggerty 2012, Boyce and Martin 2017). Ashmole's hypothesis probably does not predict greater per-nestling feeding rates in the temperate zone than in the tropics as implied by Gill and Haggerty (2012). Instead, Ashmole's hypothesis would seem to predict that per-nestling feeding rates should be similar in tropical and temperate sites and across gradients of seasonality, because the hypothesis assumes that clutch sizes have evolved to match per-capita food availability. Overall, the preponderance of evidence seems to cast doubt on whether temperate birds have greater access to food during the breeding season, as assumed by Ashmole's hypothesis.

Higher survival rates in the tropics than in the temperate zone (Table 5.1, prediction 9) have long been assumed (Murray 1985, Karr et al. 1990, Martin 1996), mostly based on indirect evidence, but this assumed latitudinal gradient in adult survival has rarely been tested. Empirical evidence that tropical birds live longer than temperate birds is mixed. Multiple studies have found higher annual survival among tropical species than among related or ecologically similar species in the temperate zone (Yom-Tov et al. 1992, Peach et al. 2001, Gill and Haggerty 2012), as predicted by Ashmole's hypothesis. Other studies have found that survival is similar between temperate and tropical species (Karr et al. 1990), or even greater in temperate species (Faaborg and Arendt 1995), and generalizations about relative survival remain elusive. Between two resident species of *Sylvia* warblers breeding in Kenya, the species (*S. boehmi*) in the more seasonal environment did not show lower annual survival than its congener in a less seasonal environment (*S. lugens*) (Schaefer et al. 2006). The authors considered the former habitat to be more seasonal because it had a longer dry season and a larger coefficient of variation in monthly rainfall. Under Ashmole's mechanism, however, the relevant metric of seasonality should simply be the absolute difference between the maximum and minimum resource level throughout the year (not the cumulative effects of sustained food limitation) because the minimum level of resources sets the carrying capacity going into the breeding season.

Empirical evidence largely supports the assumption that breeding populations of at least some species are regulated by food limitation during the non-breeding season. Breeding populations of two species of Darwin's Finches (*Geospiza fortis* and *G. scandens*) closely tracked the availability of seeds during the dry season (Boag and Grant 1984). Breeding population size of several European Tits (*Parus* sp.) and Eurasian Treecreepers (*Certhia familiaris*) was strongly correlated to temperatures during the previous winter (interpreted as a proxy for winter food limitation), providing evidence that breeding populations are regulated by winter mortality in response to weather severity (Svensson 1981). Two ecologically similar species (*Parus palustris* and *Sitta europaea*) known for caching food for winter showed much weaker associations between winter temperatures and the size of their breeding populations in the subsequent year (Svensson 1981). Finally, those species suffering the highest winter mortality (*P. major* and *P. caeruleus*) also laid the largest clutches among the suite of species examined in the same study (Svensson 1981). Experimental food supplementation of Song Sparrows (*Melospiza melodia*) increased the winter survival of sub-adults (but not of adults) and was associated with increased population density, but the authors reported only a slight and non-significant decrease in clutch size during the following breeding season (Smith et al. 2009). Supplemental winter feeding of Carrion Crows (*Corvus corone*) did not increase their breeding population density relative to a control site (Yom-Tov 1974) and among 18 experimental winter food supplementation studies with passerines, 11 of them reported higher population densities during the following breeding season (Newton 1994).

Other Approaches

Edworthy et al. (2011) took advantage of a major mountain pine beetle (*Dendroctonus ponderosae)* outbreak to test a suite of novel predictions of Ashmole's hypothesis based on temporal rather than spatial patterns in population dynamics. Pine beetles represent a seasonally-buffered resource that strong excavators can access year-round (Wiebe et al. 2006). Edworthy et al. (2011) therefore predicted that resident woodpeckers would respond to the outbreak through numerical increases in density rather than through increased fecundity (Table 5.1, prediction 11a) because carrying capacity should increase at all seasons while the difference between winter and summer carrying capacity may not. Furthermore, the authors predicted that if Ashmole's hypothesis was correct, the two migrant woodpecker species in their system *should* increase their fecundity (Table 5.1, prediction 11b) because their access to breeding resources increases independently of their winter mortality. Each of the two predictions was supported, and two resident weak excavator species (Mountain Chickadee (*Poecile gambeli*) and Red-breasted Nuthatch (*Sitta canadensis*)) each responded to the same beetle outbreak by increasing their clutch size (Edworthy et al. 2011), suggesting that bark beetle availability did not reduce their winter food-limitation or mortality (Table 5.1, prediction 11c).

Support for Ashmole's Hypothesis in Non-avian Taxa

Studies of non-avian taxa have also supported Ashmole's hypothesis. Just as in avian clutch size, a positive association has long been noted between latitude and mammalian litter sizes (Lord 1960, Chapman and Lind 1972, Cockburn et al. 1983, Swihart 1984, Tökölyi et al. 2014, Battistella et al. 2019, Virgós et al. 2019). Lord (1960) actually proposed the same general mechanism to explain the relationship between fecundity and latitude that Ashmole did a year later. Citing Christian and Lemunyan's (1958) finding that litter size was inversely related to population density, Lord suggested: *"Possibly the relation reported by other workers between mortality and reproduction is, at least in part, dependent on an inverse relationship between density of breeding adults and litter size. Also the severity of the winter weather in the higher latitudes had probably been a selective force for larger litters."* Lord based his insights largely on qualitative comparisons of which groups of mammals showed the latitudinal pattern in litter size and which did not. He noted that the litters of hibernating and fossorial species did not vary latitudinally and reasoned that hibernation and the fossorial lifestyle buffered such species from the density-dependent winter mortality experienced by non-hibernators. Furthermore, what Lord calls partial hibernators showed intermediate levels of association between litter size and latitude. Additional studies with mammals supported the association between litter size and season length (which the author equates with seasonality; Swihart 1984), temperature seasonality (Battistella et al. 2019, Virgós et al. 2019), and precipitation seasonality (Battistella et al. 2019). Finally, clutch size of Pond Frogs was smaller on (presumed less seasonal) islands than on the mainland (Wang et al. 2009).

Theoretical Support for Ashmole's Hypothesis

Theoretical modeling approaches and simulation studies have also lent support to Ashmole's hypothesis beginning with Boyce (1979). Griebeler and Böhning-Gaese (2004) noted that Ashmole's hypothesis doesn't incorporate the concept of a cost of reproduction on survival, based on functional constraints, which is often invoked in theoretical treatments of life-history evolution (Cody 1966, Stearns 1992) but difficult to demonstrate with field data. They simulated clutch size along a latitudinal gradient using individual-based models and found both seasonality of resources and a cost of reproduction were necessary to generate the observed latitudinal gradient in clutch size. By contrast, a model to predict a full suite of life-history traits suggested that seasonality and density-dependent food availability are sufficient to generate observed latitudinal increases in clutch size (McNamara et al. 2008). A follow-up simulation study (Griebeler et al. 2010) incorporating interactions with brood-size-independent nest predation risk and breeding season length again supported the importance of resource seasonality and demonstrated that Ashmole's hypothesis was supported in both single- and multi-brooded species (also see Liu et al. 2018).

Paths Forward

The preponderance of accumulated evidence lends considerable support to Ashmole's hypothesis, but rigorous tests have been hard to design and implement. Substantial confusion and ambiguity have muddied the waters and thereby limited the impact and attention that Ashmole's hypothesis deserves. Correlative approaches (e.g., predictions 1-4; Table 5.1) are of limited inferential value because so many ecological and abiotic processes covary along geographic and climatic gradients. Clutch size and seasonality both vary systematically with latitude, so an association between clutch size and seasonality could be completely spurious (Hussell 1985). Tests comparing clutch sizes among different landforms and habitat types are often qualitative or have used small sample sizes. For example, comparing an island to its adjacent mainland arguably has only a sample size of $n = 2$, even if multiple species are considered, and studies that compare suites of species have rarely controlled for phylogenetic relatedness (but see Battistella et al. 2019, Virgós et al. 2019). A comprehensive comparative analysis is needed to better test this prediction.

Many past studies have used assumed differences in seasonality among regions, altitudes, or vegetative communities. Direct measurements of local weather variables will help better test those assumptions and allow future studies to directly compare differences in seasonality with clutch size. Ongoing and future climate change could also provide a natural experiment for testing Ashmole's hypothesis. The effects of climate change are expected to be heterogenous with respect to latitude and season (Hurrell 1995, Serreze et al. 2000, IPCC 2007, IPCC 2014, Polyakov et al. 2002). Hence, the degree of seasonality is likely to change along geographical gradients, facilitating predictions about how clutch size might change in time and space. Ashmole's hypothesis predicts that average clutch size will increase in regions where seasonality becomes more pronounced and decrease in regions where seasonality is reduced. Some region- and species-specific predictions have already been formulated regarding how clutch sizes should respond to climate change if Ashmole's hypothesis is correct (Griebeler and Böhning-Gaese 2004). Additional theoretical modeling and simulation approaches are needed to quantify whether and how life-history trade-offs (e.g., between clutch size and the number of breeding attempts in a season) and costs of reproduction might modify Ashmole's hypothesis (Griebeler and Böhning-Gaese 2004, Griebeler et al. 2010). Such theoretical studies would also help to inform and guide future empirical studies.

Most empirical tests of Ashmole's hypothesis have been in passerines, and additional tests among a wider diversity of taxa (including among more non-avian taxa) are required to provide broader support or refutation. Furthermore, comparative approaches have untapped potential for testing Ashmole's and other hypotheses proposed to explain the association between clutch size and latitude. Previous comparative studies of clutch size have generally used a single value to represent the clutch size of each species (Martin 1993, Yom-Tov et al. 1994, Figuerola and Green 2006, Jetz et al. 2008, Hořák et al. 2015, and Virǵos et al. 2006 for lagomorph litter size), and that value may or may not be representative of the entire species depending on what data sources are available. One potentially fruitful approach would be to take full advantage of intraspecific variation, and in a phylogenetic comparative framework. Ashmole's hypothesis (and in fact, all the hypotheses proposed to explain variation in clutch size) make specific predictions regarding which species should show stronger or weaker associations between clutch size and latitude and these predictions have yet to be tested. Several authors have informally or implicitly suggested a similar approach. Ricklefs (1980) first implied the utility of such an approach when he described the various ways that some species can reduce their winter mortality via adaptations like migration, food storage, or food switching (Table 5.2, predictions 1-2). Other adaptations that could reduce winter mortality in response to seasonal fluctuations in the food supply include the use of energy-saving torpor or the ability to exploit seasonally-buffered resources such as invertebrates living underneath tree bark (Table 5.2, prediction 3). Two genera of thrush had similar levels of latitudinal variation in clutch size but not in other related life-history traits (Boyce and Martin 2007). Differences, among different guilds of mammals, in whether litter size was associated with latitude inspired Lord's (1960) formulation of a hypothesis that closely mirrored Ashmole's. Other studies described above (Edworthy et al. 2011, Svensson 1981, and Hořák et al. 2015) have invoked predictions of this type, but a large-scale meta-analysis of intraspecific latitudinal variation in clutch size has not yet occurred.

One consequence of Ashmole's hypothesis is that the effect of seasonality on per-capita food resources during the breeding season, and therefore latitudinal variation in clutch size, should be dampened in taxa possessing adaptations that reduce their density-dependent winter mortality. Therefore, Ashmole's hypothesis predicts that: 1) migrant species should show less latitudinal variation in clutch size than resident species (Table 5.2, prediction 1), 2) species that utilize food caches during winter should show less latitudinal variation in clutch size than those that do not cache food (Table 5.2, prediction 2) and, species that utilize torpor should show less latitudinal variation than those that do not utilize torpor (Table 5.2, prediction 3). Ashmole's hypothesis also predicts that secondary cavity-nesting birds should show less latitudinal variation than open-nesting species, because the breeding populations of secondary cavity nesters are assumed to be limited by nest-site availability (Martin 1993) rather than by food availability (Table 5.2, prediction 4). Ashmole's hypothesis also predicts that primary cavity nesters should show associations between clutch size and latitude that are intermediate between that of secondary cavity nesters and open-nesting species because they are not limited by nest sites, and are able to access prey resources that live underneath thick tree bark and are buffered from seasonal variation (similar to Edworthy et al. 2011; Table 5.2, prediction 5). Finally, Ashmole's hypothesis predicts that the strength of the association between clutch size and latitude should be negatively associated with body size because larger species have greater fasting endurance and should experience reduced mortality during periods of food limitation (Table 5.2, Prediction 6; following from Newman et al. 2011). Similar predictions could be formulated for Lack's daylength hypothesis, Skutch's nest predation hypothesis (Skutch 1949, 1985), and other hypotheses that account for the association between clutch size and latitude (see Table 5.2, predictions 5-9).

Conclusions

Our ability to manage populations and anticipate how they respond to environmental change is limited by an incomplete understanding of what factors drive the birth and death rates that are central to population ecology. Furthermore, an improved understanding of how species are adapted to their abiotic environment is a pre-requisite for anticipating and mitigating the impacts of climate change. Ashmole wondered why some species like tropical seabirds exhibit such modest reproductive rates when selection has favored much greater fecundity in other species. Building on Lack's ideas about food limitation and density dependence, Ashmole identified a simple but elegant abiotic mechanism that could explain why some animals have more food available to invest in reproduction. Ashmole's hypothesis accounted for the observed geographic patterns in clutch size and the relative stability of populations (which motivated Lack's work; Lack 1954) and explicitly explained the inverse relationship between reproduction and survival. However, substantial misinterpretations of Ashmole's hypothesis in the literature has reduced its impact and created ambiguity and confusion regarding what the hypothesis is and the exact mechanism it asserts. Clarifying this ambiguity and identifying explicit predictions of Ashmole's hypothesis, as we have attempted here, will hopefully help future investigators focus more attention on Ashmole's hypothesis.

Although Ashmole's hypothesis has proven difficult to test directly, many different approaches and lines of evidence lend considerable empirical and theoretical support to his model of clutch size evolution. Novel approaches, formulation of new predictions of Ashmole's hypothesis (e.g., Edworthy et al. 2011, Hořák et al. 2015), wider taxonomic coverage, greater use of modeling and simulation approaches, and new comparative analyses and meta-analyses of the type described above are needed to clarify the relative importance of resource seasonality in driving clutch size patterns and life histories, generally. Finally, researchers interested in Ashmole’s hypothesis should review the details of Ashmole’s mechanism (most clearly and succinctly outlined in Ricklefs 1980), think carefully about what predictions the hypothesis generates, and avoid perpetuating errors and misinterpretations that unfortunately have become common in the literature.

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Table 5.1. Summary of previously tested predictions and empirical evidence supporting or refuting Ashmole's Hypothesis (Ashmole 1961, 1963; Ricklefs 1980) to explain the latitudinal gradient in clutch size.

Prediction	Studies that Support	Studies that Refute
1a) Clutch size should be positively correlated with the ratio between summer AE and winter AE (with or without Hussell's (1985) correction)	Passerines[1,2], Northern Flicker[3], Woodpeckers[4], Eurasian Kestrel[5]	Barn Swallow and House Martin[6], House Wren[7]
1b) Clutch size should be positively correlated with the ratio between summer and winter temperature	Landbirds[8], Lagomorphs[9], Mammals (4 Orders)[10], Didelphid Marsupials[13]	Gray-headed Tody-flycatcher[11], Seven Birds[12], Chiropteran Mammals[10]
1c) Clutch size should be positively correlated with the ratio between summer and winter precipitation	Passerines[2], Didelphid Marsupials[13]	Gray-headed Tody-flycatcher[11], Mammals (5 Orders)[10]
1d) Clutch size should be positively correlated with the ratio between summer and winter NDVI ("greenness")	South African Passerines[14,15]	
2) Clutch size should be inversely related to winter AE	Passerines[1], Northern Flicker[3], Woodpeckers[4], Eurasian Kestrel[5]	Barn Swallow and House Martin[6]
3) Clutch size should be unrelated to summer AE (weak prediction, unless coupled with predictions 1 and 2)	Passerines[1], Northern Flicker[3], Woodpeckers[4], Eurasian Kestrel[5], Barn Swallow and House Martin[6], Landbirds[8]	
4) Clutch size should be positively correlated with the ratio of the breeding population density to summer AE	Passerines[1], Northern Flicker[3], Woodpeckers[4], Tree Swallows[16]	Barn Swallow and House Martin[6]
5) In an area of uniform productivity, seabird colony size should be negatively associated with the number of competitors from other colonies foraging in waters shared by the colony	Seabirds[17]	
6) When seasonality is held constant, clutch size should decrease with increasing productivity	South African Passerines[15]	
7a) Clutch size should be positively associated with continental influence	Birds in the Northern and Southern Hemispheres[7,18,19,20,21,22,23,24,25]	
7b) Clutch size should be smaller on islands than on adjacent mainlands, given that island climates are moderated by maritime influence	Various Species[26,27,28,29,30,31,32,33]	

Prediction	Studies that Support	Studies that Refute
7c) Clutch size should be larger in more seasonal vegetation types (for example, deciduous forest and savannah) than in less seasonal vegetation types (for example, evergreen forest) at the same latitude	African Passerines[34], Blue Tit[31,35,36] Great Tit[35], New World Flycatchers and Finches[34,37]	
7d) Populations introduced into a new region or environment should have smaller clutch sizes, compared to those in their native range, if the non-native region is less seasonal. Similarly, introduced populations should have larger clutch sizes if the non-native region is more seasonal than their native range	Passerines[38], European Blackbird and Song Thrush[39]	
8) Clutch size should be larger than predicted by seasonality alone in regions where many of the breeding birds migrate elsewhere for the winter and should be smaller than predicted by seasonality alone in regions that experience an influx of migrants (i.e., that breed elsewhere) during winter	Passerines[1,23,40,41]	
9) Per-capita food availability should increase with increasing seasonality and/or with increasing latitude	Passerines[42]	Tree Swallow[43], Passerines[25], Buff-breasted and Carolina Wren[44], Catharus and Turdus thrushes[45]
10) Annual survival should decrease with increasing seasonality and/or with increasing latitude	Buff-breasted and Carolina Wren[44,] Passerines[46], Reed Bunting[47]	Small Landbirds[48], Passerines[49], Sylvia Warblers[50]
11a) Resident strong-excavator species should respond to a pine-beetle outbreak by increasing in density without increasing their clutch size	Resident Woodpeckers[51]	
11b) Migrant, but not resident, excavator species should respond to a pine-beetle outbreak by increasing their clutch size	Migratory Woodpeckers[51]	
11c) Resident weak-excavator species, but not strong-excavator species, should respond to a pine-beetle outbreak by increasing their clutch size	Red-breasted Nuthatch and Mountain Chickadee (weak excavators)[51]	

[1]Ricklefs 1980, [2]Olalla-Tarraga et al. 2019; [3]Koenig 1984, [4]Koenig 1986, [5]Carillo and Gonzalez-Davila 2010, [6]Møller 1984 (used winter AE from assumed wintering grounds), [7]Young 1994 (used winter AE from assumed wintering grounds), [8]Jetz et al. 2008 (5290 landbirds, globally), [9]Virgós et al 2006 (litter size), [10]Tökölyi et al. 2014 (litter size), [11]Ferreira et al. 2019, [12]Evans et al. 2009, [13]Battistella et al. 2019 (litter size), [14]Hořák et al. 2011, [15]Hořák et al. 2015, [16]Dunn et al. 2000, [17]Furness 1984, [18]Moreau 1944, [19]Cockburn 1983, [20]Woinarski 1985, [21]Yom-Tov 1987, [22]Rowley and Russell 1991, [23]Yom-Tov 1994, [24]Yom-Tov et al. 1994, [25]Martin et al. 2000, [26]Lack 1947, [27]Marchant 1960, [28]Cody 1966, [29]Crowell and Rothstein 1981, [30]Michelland 1982, [31]Blondel et al. 2006, [32]Rodriguez and

Rodriguez 2007, [33]Wang et al. 2009, [34]Lack and Moreau 1965, [35]Klomp 1970, [36]Fargallo 2004, [37]Lack 1968, [38]Evans et al. 2005, [39] Samaš et al. 2013, [40]Yom-Tov and Geffen 2002, [41]Yom-Tov 1995, [42]Thiollay 1988, [43]Rose and Lyon 2013, [44]Gill and Haggerty 2012, [45]Boyce and Martin 2017,[46]Yom-Tov et al. 1992, [47]Peach et al. 2012, [48]Karr et al. 1990, [49]Faaborg and Arendt 1995, [50]Schaefer et al. 2006, [51]Edworthy et al. 2011

Table 5.2. Comparative predictions generated by Ashmole's Hypothesis (Ashmole 1961, 1963; Ricklefs 1980), and the two most-common alternative hypotheses, regarding which species should show the strongest associations between clutch size and latitude.

Prediction	Ashmole's Hypothesis	Lack's Daylength Hypothesis	Skutch's Nest Predation Hypothesis
Latitudinal Gradient in Clutch Size Will be:			
1) Less among migrant species than non-migrant species	+	-	-
2) Less among species that cache food for winter than among non-caching species	+	-	-
3) Less among species that utilize torpor than among species that do not utilize torpor	+	-	-
4) Open-nesting species > Primary cavity-nesting species > Secondary cavity-nesting species	+	-	-
5) Open nesting species > Secondary cavity-nesting species > Primary cavity-nesting species	-	-	+
6) Negatively correlated with average nest-predation rates	-	-	+
7) Less among (or negative in) nocturnal species compared to diurnal species	-	+	+
8) Less among species with biparental incubation than among those with uniparental incubation	-	-	+
9) Less among species with average fledge dates closer to spring equinox than among species with fledge dates closer to summer solstice	-	+	-

Closing Remarks

Our study documented previously unknown or poorly known variation in a full suite of life-history traits but, that variation tracked latitude for nearly every trait considered, and we were able to explain most of that latitudinal variation via tests underlying mechanisms. Our data suggest that limits to the thermal tolerance of embryos may be the cause of variation in a suite of inter-related traits including the timing of incubation onset and hatching patterns that have implications for nestling survival. Owls in warmer burrows began incubation earlier in the laying stage, hatched their clutches more asynchronously, experienced greater rates of hatching failure, and achieved lower offspring survival than those in cooler burrows. Our data suggest that the same mechanism might energetically constrain clutch size and explain why clutch size is correlated with latitude. In fact, variation in each trait in Chapter Two tracked latitude but was even more strongly associated with nest microclimate. Chapter Three suggested that the rate of egg-laying was also associated with nest microclimate and may also be limited by limits to egg viability. Furthermore, Chapter Three demonstrated that laying interval length explained some variation in hatching asynchrony and that longer laying intervals were associated with high frequency of hatching failure. In Chapter Four we found that Burrowing Owls modify their nest attentiveness in response to nest microclimate relative to thermal optima for embryo development but also match their level of reproductive investment to their extrinsic mortality risk (which varies along climatic gradients). These findings demonstrate that abiotic conditions are a major driver of patterns of variation in life-histories and explain the widespread latitudinal patterns observed in many life-history traits. Furthermore, our study emphasizes the need to go beyond documenting patterns and speculating about their causes and demonstrates the value of detailed field studies that test hypotheses to explain that variation.

The new insights provided by this research identify some important indirect mechanisms by which Burrowing Owls and other species may be impacted by climate change and to what extent (and via what mechanisms) they may be able to adapt (or not) to climate change. For example, understanding how species are adapted to the thermal environment, and understanding species' relative thermal sensitivity, can inform our understanding of relative vulnerability and allow us to prioritize species for conservation actions. Vulnerability to climate change might also vary by life stage. The thermal tolerance of developing embryos (Webb 1987) is much narrower than the thermoneutral zone of an adult Burrowing Owl (Coulombe 1970). Therefore, projections or mitigations based only on climate envelope models, that consider only the direct thermal tolerances of adults, might fail to capture the impacts that climate change could have on reproduction and recruitment. These types of data can also lend us needed insights into the importance of microclimates and thermal refugia on the landscape, which may need to be targeted for conservation. Finally, secondary cavity-nesting birds, such as the Burrowing Owl, may be especially vulnerable to the effects of changing nest microclimate on reproductive success. For example, Burrowing Owls nest in abandoned burrows excavated by a wide diversity of small and medium-sized animals (Conway 2018), implying that the thermal properties of each burrow might vary according to the species that excavated it. Therefore, Burrowing Owls that breed in regions with multiple burrowing animals may have a wider diversity of nest-site options and may be more resilient to the impacts of climate change than those in regions with only a single burrowing animal. Furthermore, conserving species like the Burrowing Owl might benefit from preserving those options, i.e., by protecting the entire community of burrowing animals that owls rely on. Finally, for those species for which artificial nest boxes are used as a local conservation or management tool, detailed studies of the association between thermal conditions and reproductive success can allow managers to modify nest box designs and depths in a way that maximizes reproductive success under changing climate regimes.

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Webb, D. R. 1987. Thermal tolerance of avian embryos: a review. The Condor 89:874 – 898

Appendix A - Individual Laying Intervals of Burrowing Owls at 113 Nests Spanning Five Study Sites Along a 1400-km Latitudinal Gradient

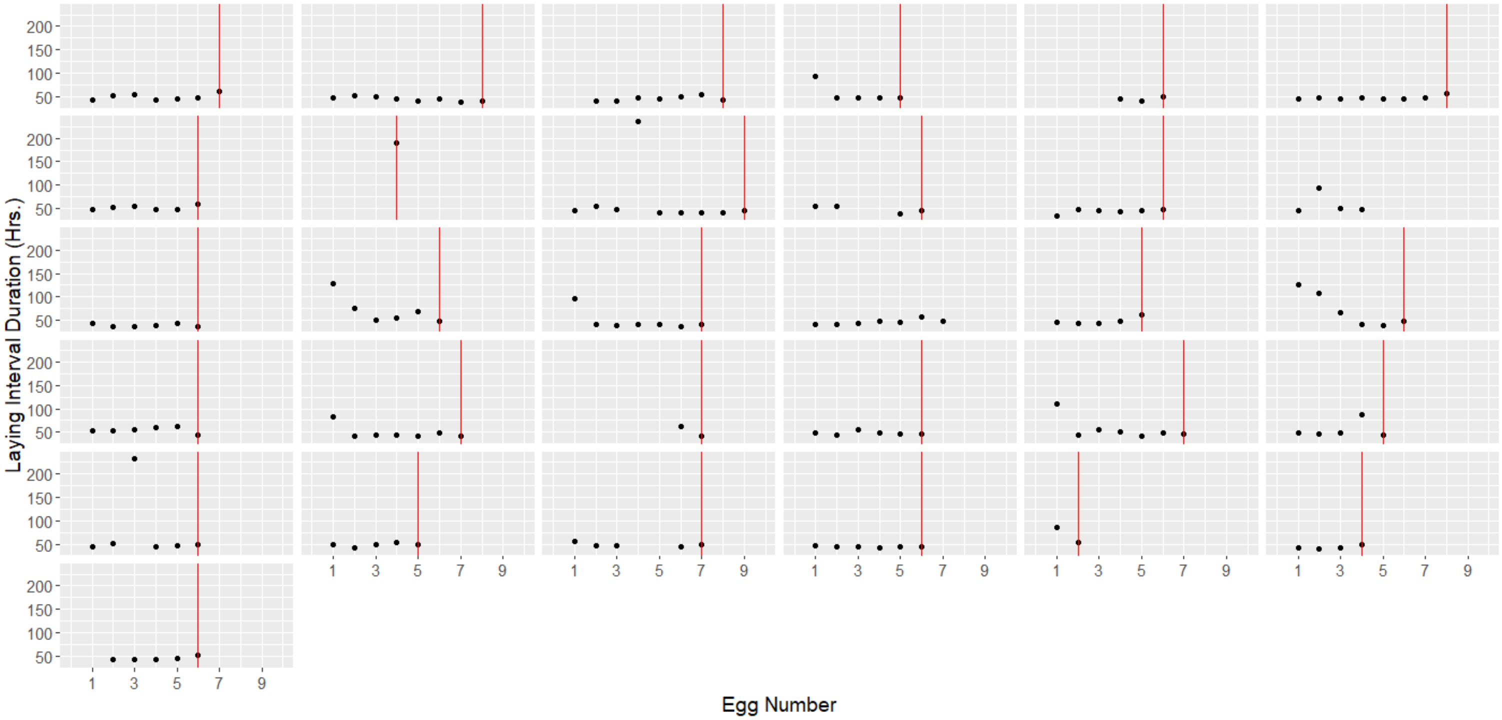

Figure A1. Laying intervals for 31 Burrowing Owl nests at the Salton Sea National Wildlife Refuge, California with each panel representing a single clutch and eggs numbered in the order they were laid. Dots represent laying interval duration between two consecutive eggs in that clutch (not all laying intervals were captured by our nest burrow cameras and so dots do not always represent all laying intervals in the clutch). Vertical lines indicate the final laying interval in each clutch, for those nests where the exact clutch size was known. Panels lacking a vertical red line indicate nests where the clutch size was unknown.

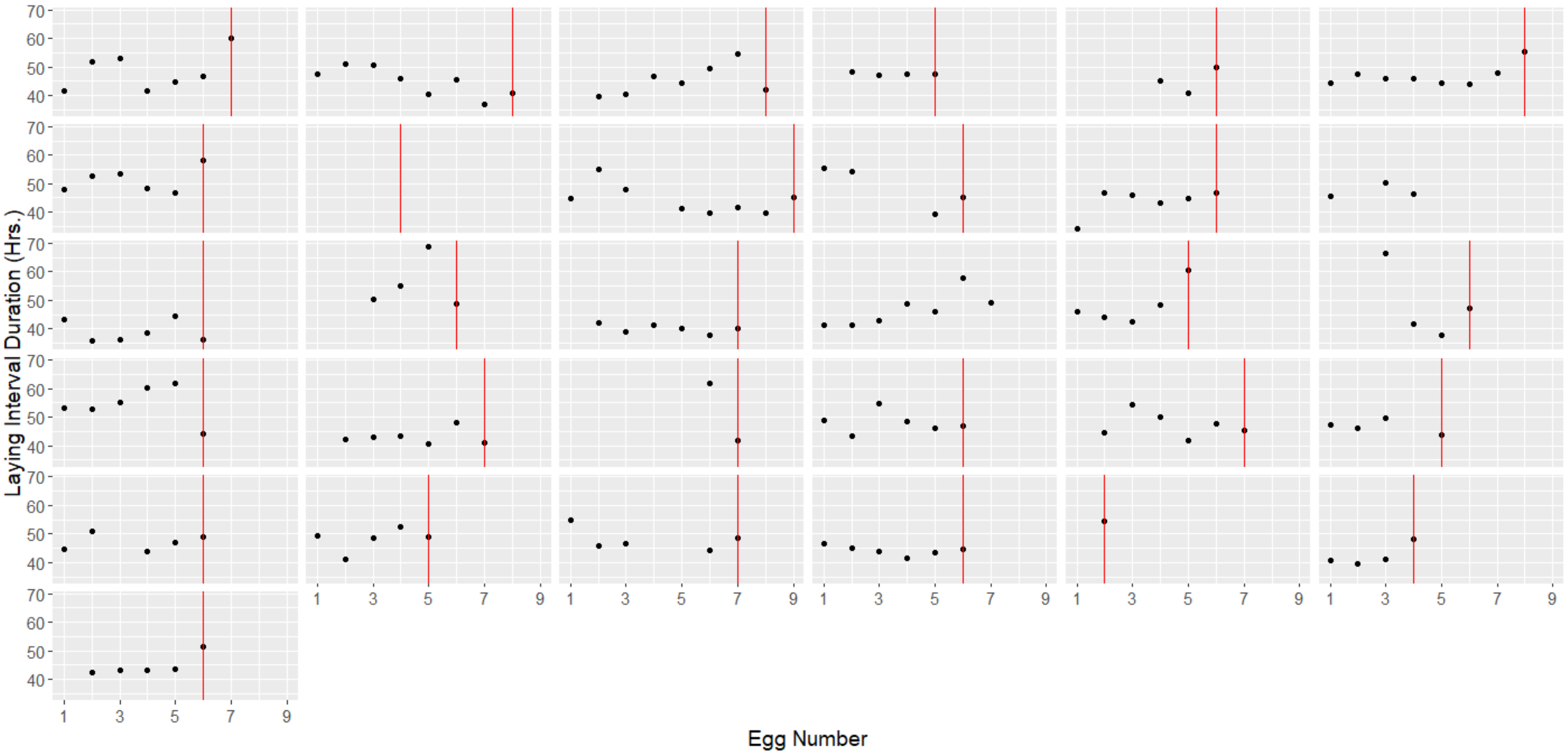

Figure A2. Laying intervals ≤ 72 hours for 31 Burrowing Owl nests at the Salton Sea National Wildlife Refuge, California, with each panel representing a single clutch and eggs numbered in the order they were laid. Intervals >72 hours omitted for better visibility of overall patterns.

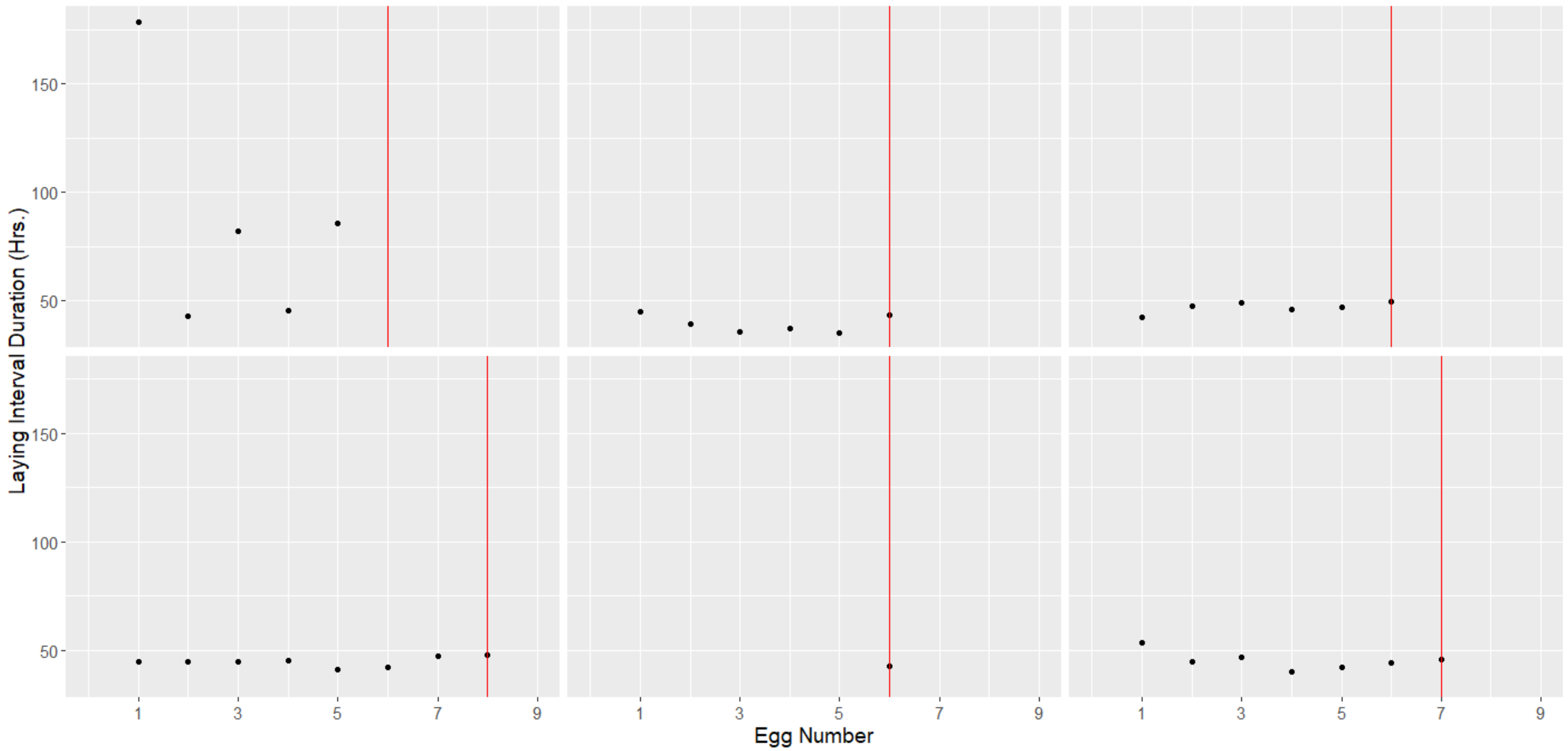

Figure A3. Laying intervals for 6 Burrowing Owl nests in southern Nevada, with each panel representing a single clutch and eggs numbered in the order they were laid. Dots represent laying interval duration between two consecutive eggs in that clutch (not all laying intervals were captured by our nest burrow cameras and so dots do not always represent all laying intervals in the clutch). Vertical lines indicate the final laying interval in each clutch, for those nests where the exact clutch size was known. Panels lacking a vertical red line indicate nests where the clutch size was unknown.

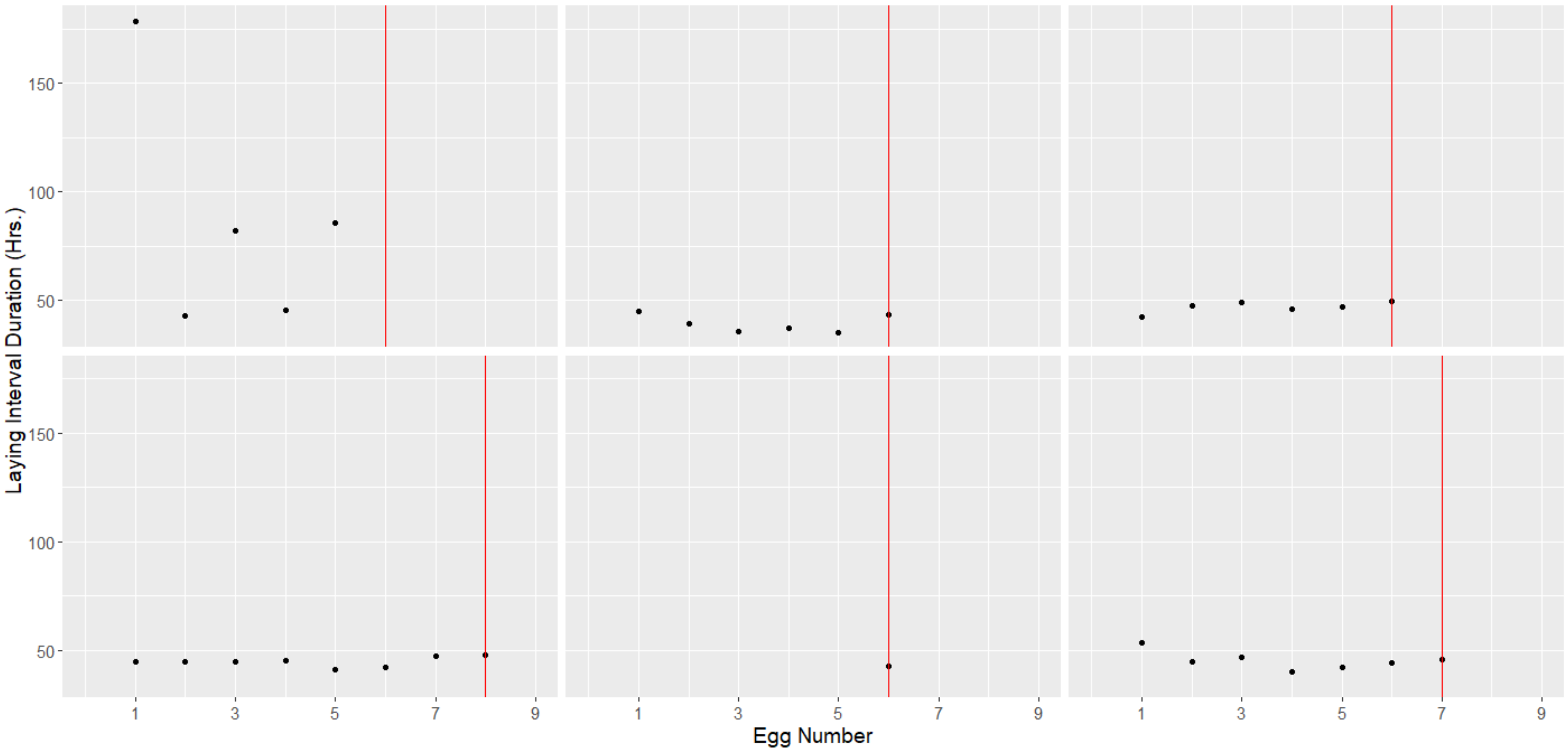

Figure A4. Laying intervals < 72 hours for 6 Burrowing Owl nests in southern Nevada, with each panel representing a single clutch and eggs numbered in the order they were laid. Intervals >72 hours omitted for better visibility of overall patterns. Vertical red lines indicate the final laying position in each clutch.

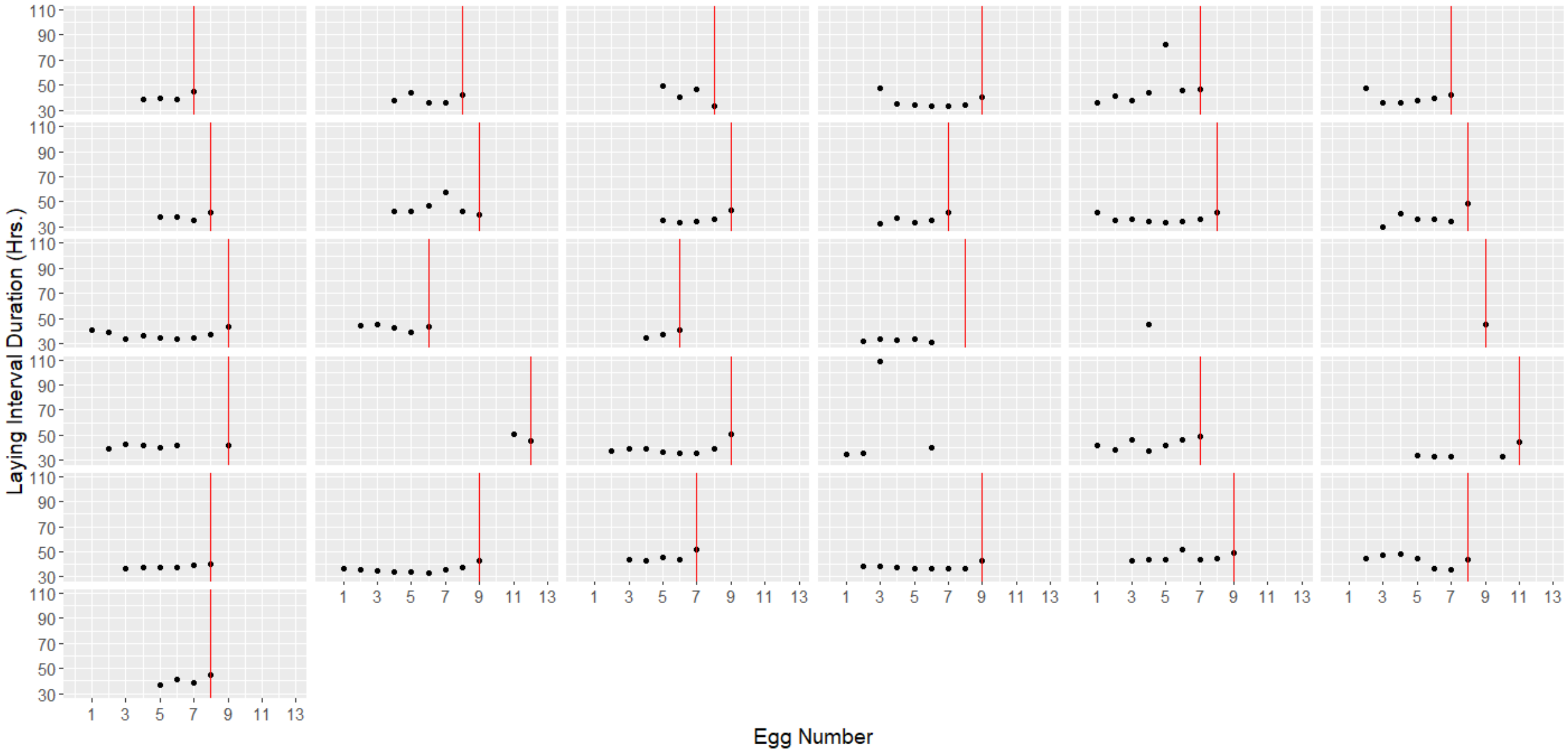

Figure A5. Laying intervals for 31 Burrowing Owl nests at the Tooele Army Depot, Utah, with each panel representing a single clutch and eggs numbered in the order they were laid. Dots represent laying interval duration between two consecutive eggs in that clutch (not all laying intervals were captured by our nest burrow cameras and so dots do not always represent all laying intervals in the clutch). Vertical lines indicate the final laying position in each clutch, for those nests where the exact clutch size was known. Panels lacking a vertical red line indicate nests where the clutch size was unknown.

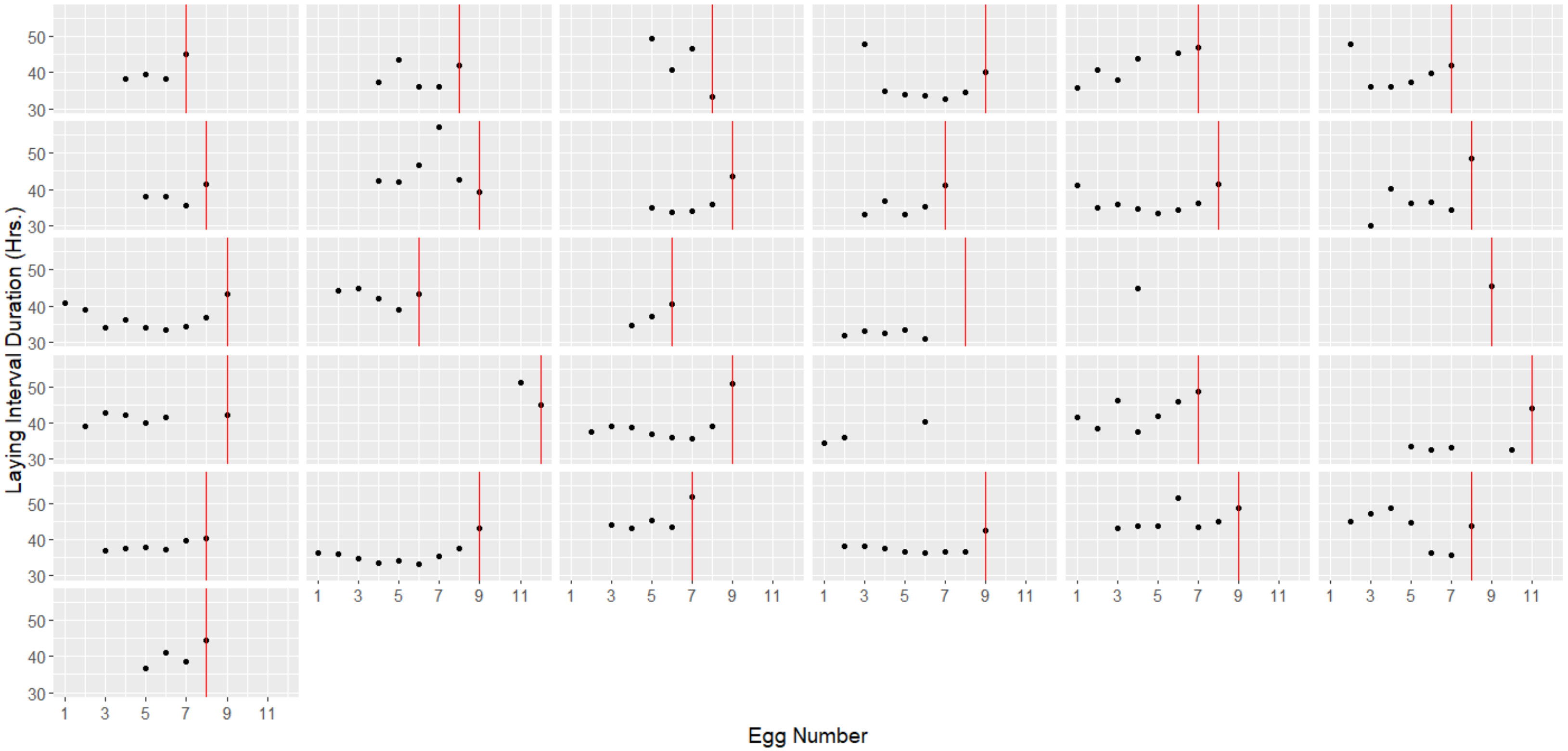

Figure A6 Laying intervals ≤72 hours for 31 Burrowing Owl nests at the Tooele Army Depot, Utah, with each panel representing a single clutch and eggs numbered in the order they were laid. Intervals >72 hours omitted for better visibility of overall patterns. Vertical red lines indicate the final laying position in each clutch.

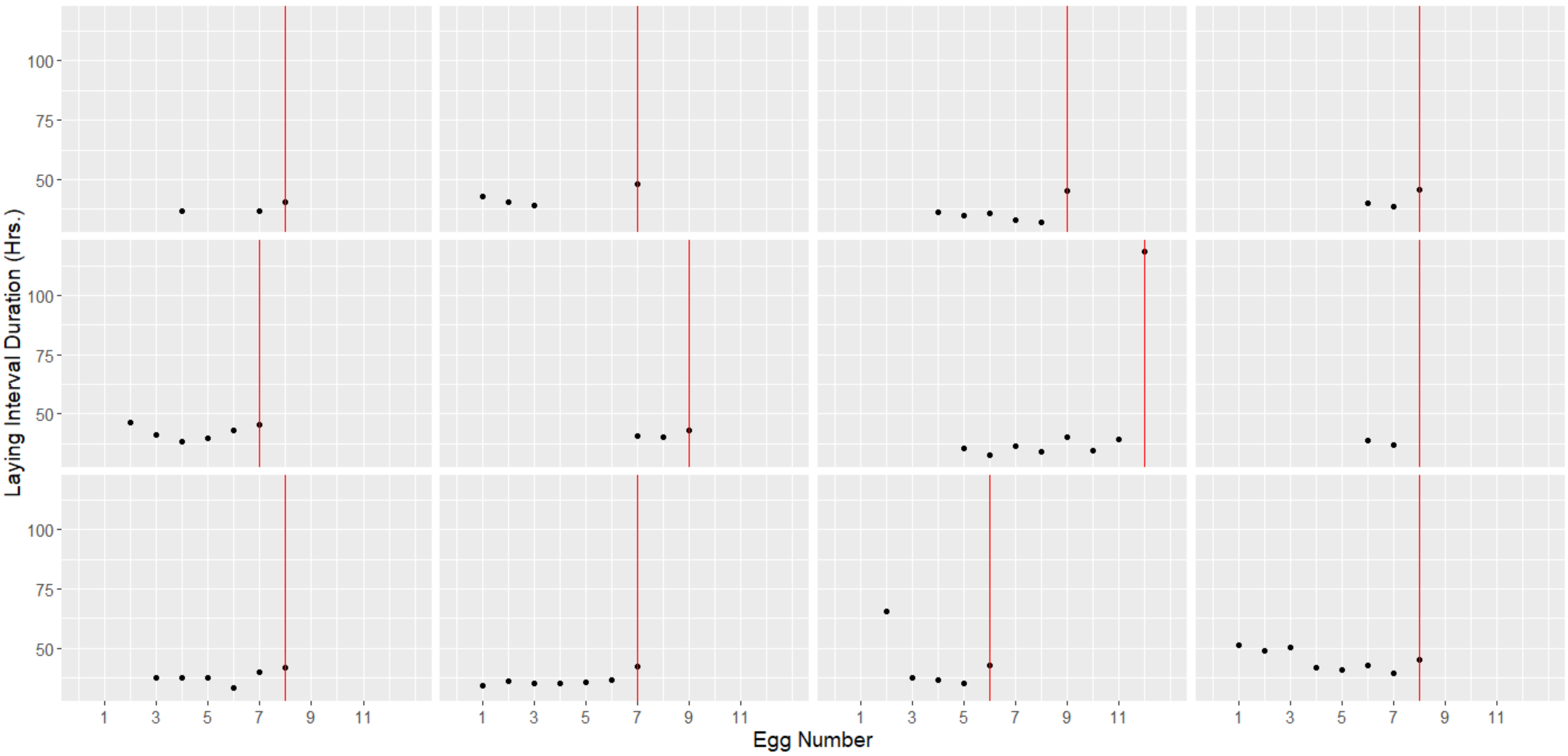

Figure A7. Laying intervals for 12 Burrowing Owl nests at Snake River Birds of Prey Area, Idaho, with each panel representing a single clutch and eggs numbered in the order they were laid. Vertical red lines indicate the final laying position in each clutch.

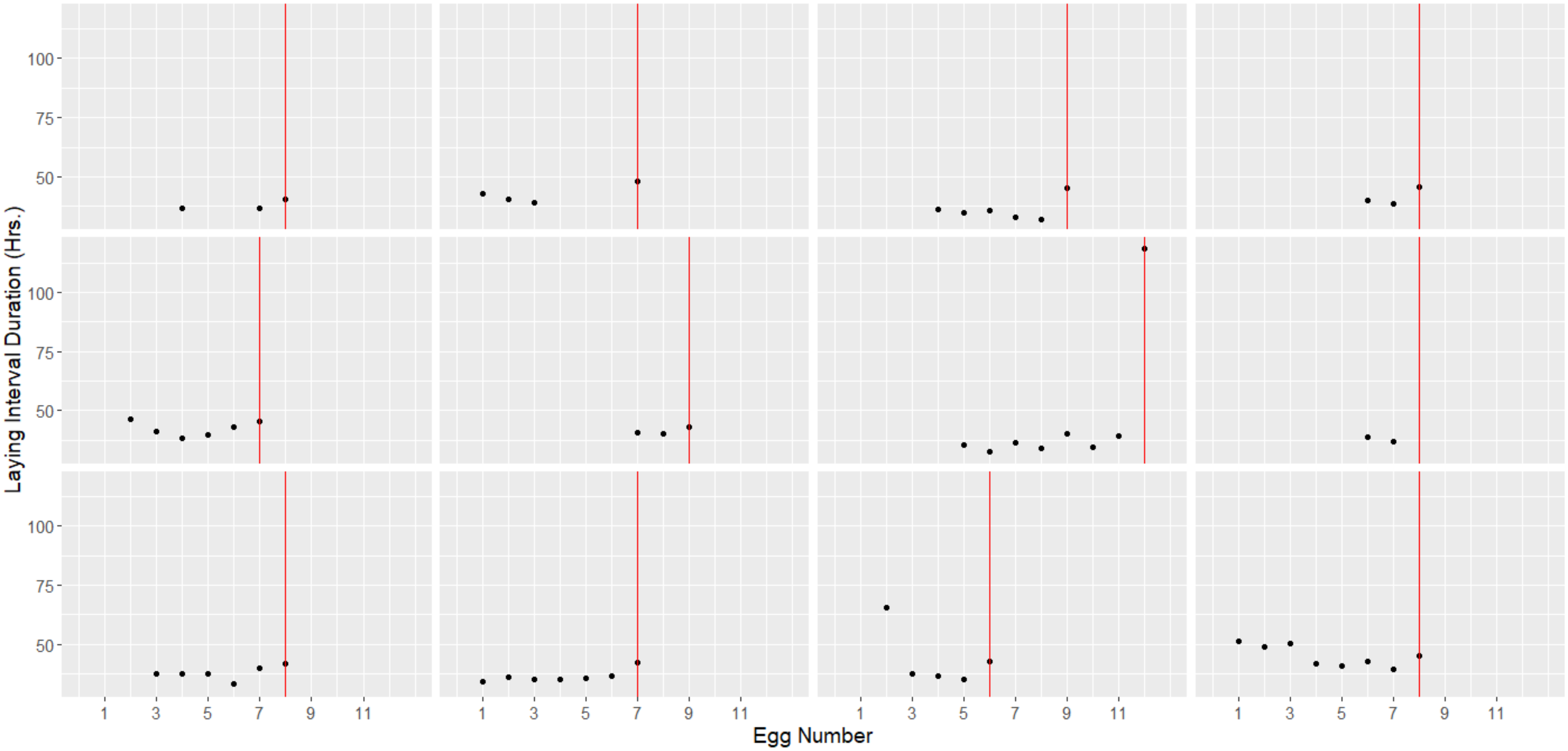

Figure A8. Laying intervals < 72 hours for 12 Burrowing Owl nests at Snake River Birds of Prey Area, Idaho, with each panel representing a single clutch and eggs numbered in the order they were laid. Vertical red lines indicate the final laying position in each clutch.

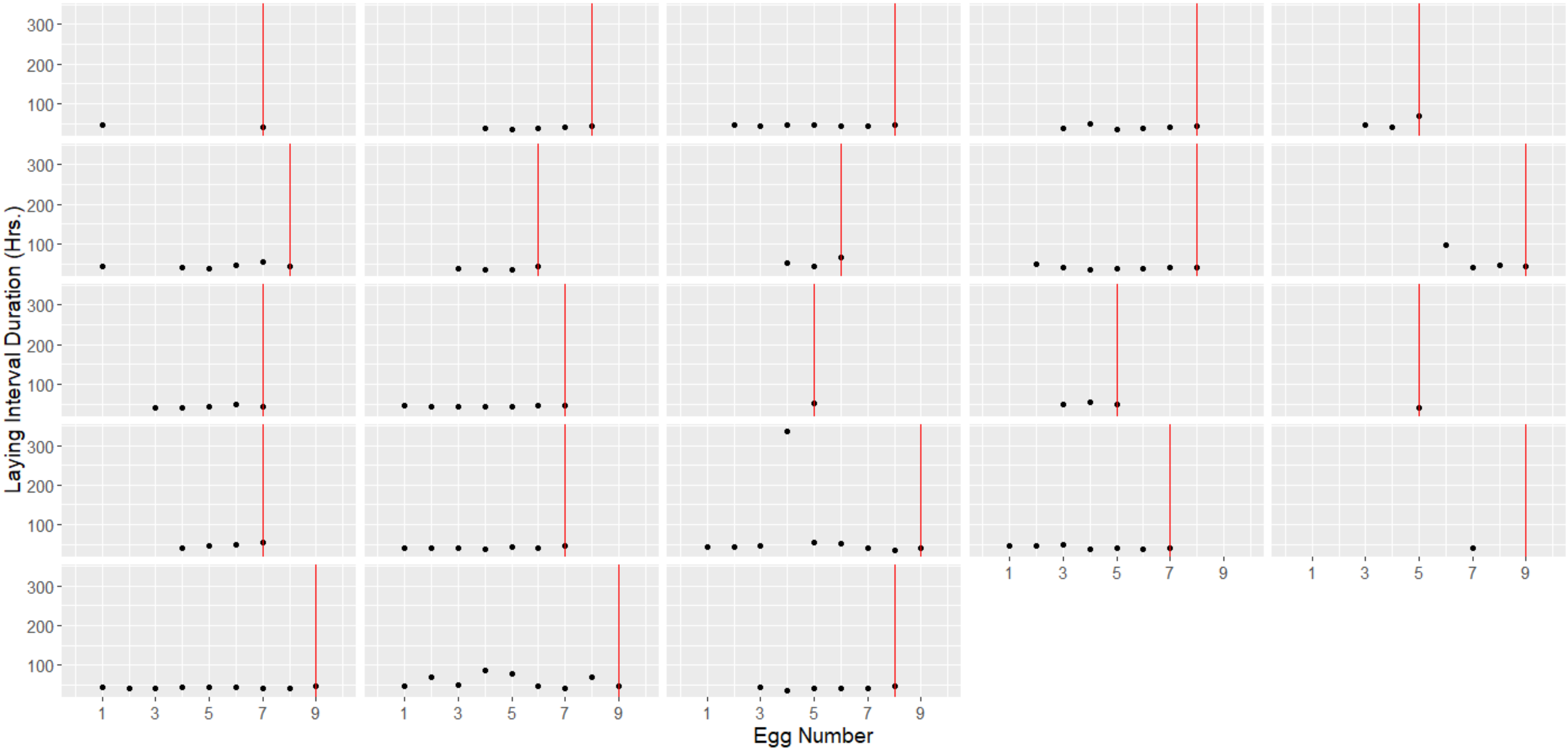

Figure A9. Laying intervals for 23 Burrowing Owl nests at the Umatilla Army Depot, Oregon, with each panel representing a single clutch and eggs numbered in the order they were laid. Dots represent laying interval duration between two consecutive eggs in that clutch (not all laying intervals were captured by our nest burrow cameras and so dots do not always represent all laying intervals in the clutch). Vertical lines indicate the final laying position in each clutch, for those nests where the exact clutch size was known. Panels lacking a vertical red line indicate nests where the clutch size was unknown.

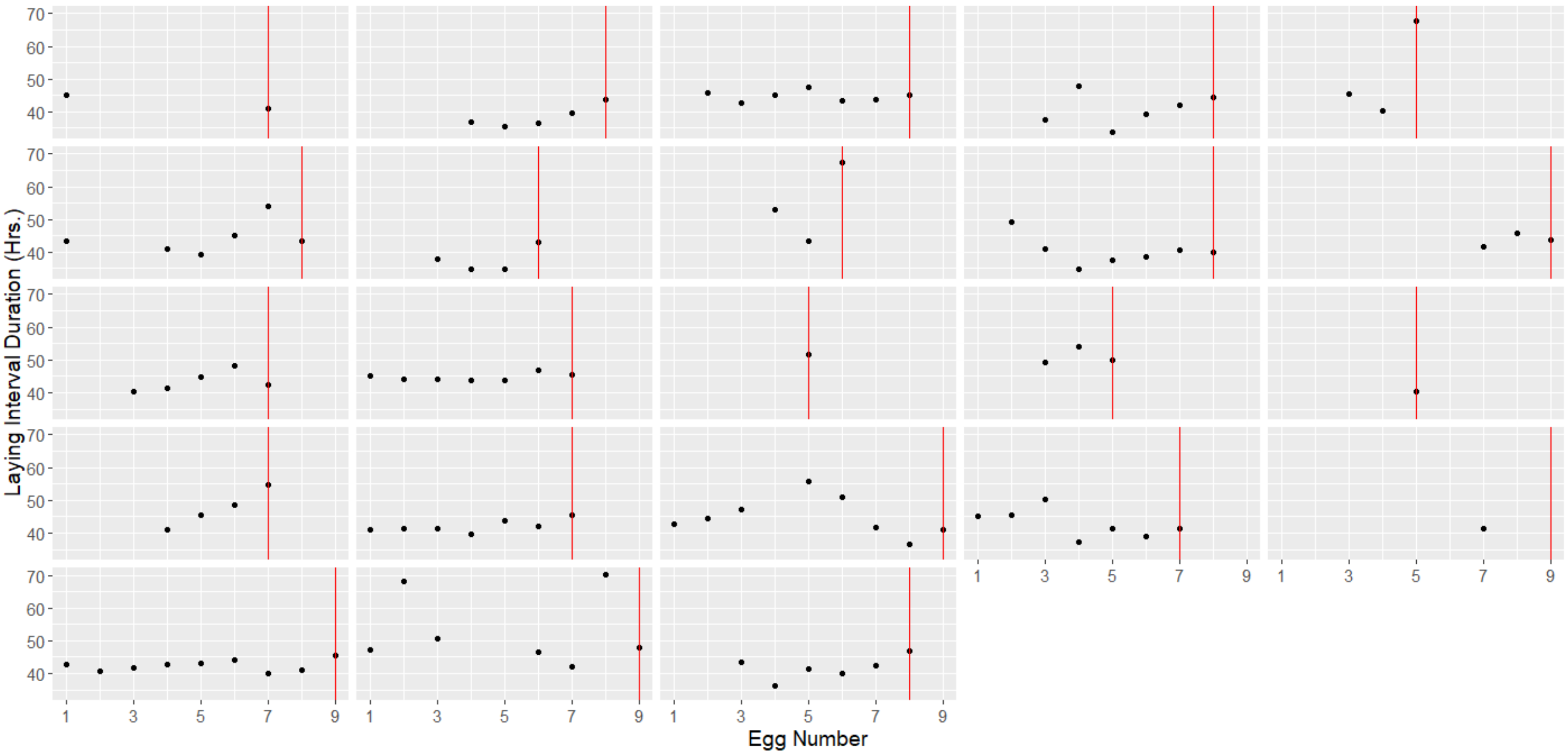

Figure A10. Laying intervals ≤72 hours for 23 Burrowing Owl nests at the Umatilla Army Depot, Oregon, with each panel representing a single clutch and eggs numbered in the order they were laid. Intervals >72 hours omitted for better visibility of overall patterns. Vertical red lines indicate the final laying position 1in each clutch.