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Climate and Intraspecific Geographic Variation of Nests and Eggs in the Eastern Kingbird *Tyrannus tyrannus*

By

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ABSTRACT

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The reproductive success of birds is ultimately driven by the traits of nests, eggs, and incubation behavior and the interactions among them. Avian reproduction varies on large geographic scales, most prominently between tropic- vs. temperate-breeding species, but also on finer scales, such as along latitudinal and elevational geographic gradients. However, despite the importance of eggs and nests, how their traits vary geographically in response to differences in regional climate remains understudied.

We studied Eastern Kingbirds (*Tyrannus tyrannus*), which have been previously demonstrated to alter incubation length in response to local climate conditions. Our study sites, Kansas (KS), New York (NY), South Dakota (SD), and Louisiana (LA) were chosen to represent a range of latitudes and climates. Our analysis of study site climate during the breeding season (April - July) showed that NY had the coldest mean temperature and most frequent precipitation, while LA had the warmest mean temperature and least cloud cover. SD and KS represented the climate of the Great Plains with moderately warm temperatures and higher wind speeds.

We collected the nests of kingbirds breeding in NY, SD, and LA to test our predictions that kingbirds breeding in colder, more northern climates would build larger nests with thicker walls, smaller nest cup openings, and greater insulative capacities than kingbirds breeding in warmer, more southern climates. We found that SD nests were the heaviest, with the thickest walls, greatest cup volumes, and smallest relative cup openings, while LA nests had the thinnest walls and largest relative cup openings. NY nests were intermediate. Nests at all sites were composed of mainly fine stems and rootlets, forb stems, and plant down, followed by coarse woody stems, though material composition varied greatly within populations.

Experiments measuring rate of heat loss from nests in environmentally controlled chambers revealed that the insulative capacity of nests only differed across sites when nest cup openings were covered. LA nests were less well insulated than SD and NY nests. We used principal components analysis (PCA) to reduce dimensionality of nest structure and material composition variables to PC axes, which we included in best subsets regressions to model predictors of nest insulative quality. For all sites, nests with the greatest insulative capacity had small nest cup openings relative to nest cup volume and were larger overall, but insulative capacity was not significantly related to material composition of nests. When we considered only SD and NY nests, we found that shallow nests with asymmetrical cup openings and low cup volume were the most insulative, as well as nests composed of plant down, fine stems and rootlets, and coarse woody stems.

We also studied variation of kingbird eggs from KS, NY, SD, and LA to perform the first analysis of intraspecific geographic variation of egg components and egg mass. We test our predictions that birds breeding in colder, more northern climates would lay heavier eggs with greater yolk content than birds breeding in warmer, more southern climates. We also predicted that egg mass would correlate directly with clutch size, reflecting a resource-based response to female reproductive investment, or negatively with clutch size, reflecting a trade-off in reproductive investment. We found that egg mass did not vary across sites, but that dry yolk mass was greater in NY than LA, which we posit supports the longer incubation period of NY kingbirds that has been demonstrated in previous work. Neither egg mass nor egg composition was correlated with clutch size, suggesting that environmental factors, and potentially unaccounted-for temporal effects, drive geographic differences in egg contents. Notably, eggs across sites were composed differently as egg mass increased. In other words, kingbirds build larger eggs differently depending on the population. KS and LA kingbirds increased mainly albumen and yolk, respectively, as egg mass increased, whereas NY and SD kingbirds added albumen and yolk in roughly equal amounts. The basis for these different allocation patterns is unknown and, moreover, it remains to be determined whether these are fixed differences among sites or if they reflect a proximate response to prevailing environmental conditions at the time of laying. While adaptive interpretations of the site differences in egg composition are possible, our results, more than anything, graphically highlight our very limited understanding of egg production in wild birds and that it is unsafe to assume that equally sized eggs provide the same amounts of energy (yolk) and protein (albumen) to embryos.

DEDICATION

To Brittany, my sister, who restores my faith in dreams when I falter.

To my parents, Tammy and Tony, who taught me to never give up, took me to wilderness, and have always entertained my love of birds.

And to Michael Murphy, the “Kingbird Guy”, and my hero.

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Chapter I

Introduction

Uniquely among amniotes, birds lay multi-egg clutches over a series of days (never more than 1 egg/day) that they must then regulate the temperature of and protect to ensure embryonic development to the point of hatching. This they share, but from this point on diversity in reproductive strategy reigns!

Due to their accessibility, presence on every continent, and use of a wide variety of habitats, birds have served as the foundation for the development of much of what has come to be known as life-history theory (Stearns 1976). For instance, in many cases, egg and clutch mass (Sibly et al. 2012), incubation period (Western & Ssemakula 1982), age at first breeding (Ashmole et al. 1971), and lifespan (Lindstedt & Calder 1976) have been demonstrated to scale to differences in body mass even across different taxa or ecological guilds (Western & Ssemakula 1982). However, differences in reproductive traits exist between species that breed in temperate versus tropical climates (Martin 1996), and intraspecific variation has been documented across latitudinal gradients in incubation length (Gillette et al. 2021, Lundblad & Conway 2021), clutch size (Owen 1977), and nest structure (Crossman et al. 2011, Mainwaring et al. 2012, 2014, Rohwer & Law, 2010).

Because the reproductive traits of birds are interrelated and do not evolve independently of one another nor from environmental pressures, a comprehensive understanding of bird reproduction is necessary for an examination of even one facet. For example, there generally appears to be an inverse relationship between clutch size and egg mass (Williams 2001), and incubation length often varies according to clutch size (Moreno & Carlson 1989). Perhaps the most influential of bird reproductive traits, upon which the others rely, is mode of development (Jetz et al. 2008).

DEVELOPMENTAL MODE

Modes of avian development are described along a continuum of precocial to altricial (Nice 1962). In precocial birds, offspring hatch feathered (usually only with down), are capable of moving around on their own shortly after hatching, and are reasonably able to feed themselves. Precociality is the ancestral mode of development, appearing in the most basal groups of birds and likely in the enantiornithines, an extinct sister taxon to subclass Neornithes (O'Connor et al. 2020). Conversely, altricial offspring hatch naked or with varying amounts of sparse down. They are unable to move, thermoregulate, or feed themselves, and require extensive parental care (Winkler 2016). Altriciality appears in the most derived bird species, such as the passerines and parrots. The benefit of altricial development seems to be a reduction of energy required for self-maintenance, for which the pattern of ontogeny requires 30-50% less energy during development than that of a precocial species with a comparable egg size and incubation period (Vleck et al. 1980). Indeed, altricial birds invest less energy in the production of a clutch when compared to precocial species (Sibly et al. 2012), but the increased mobility of precocial young is matched by a reduction of parental care and, therefore, precocial species are able to support larger broods (Ricklefs 1984). Regardless of the location along the developmental continuum, mode of development is highly conserved phylogenetically, and does not vary at the family level (Jetz et al. 2008).

Along the altricial-precocial continuum, a vast diversity of reproductive strategies have evolved. On the extreme end of precocial birds are the Megapodes (family: Megapodidae). Considered “super-precocial”, they have uniquely freed themselves of the burden of parental incubation. Some species incubate their eggs by laying them within a mound of vegetation, which generates heat as the organic materials are decomposed by microbes (Harris et al. 2014) and thus earning them the moniker “mound-builders”. Other megapodes lay their eggs in burrows heated by the sun or volcanic activity (Jones et al. 1995). In either case, offspring dig their way to the surface upon hatching and are entirely self-sufficient—they receive no post-hatching parental care, and within a few hours are capable of flight (Jones et al. 1995).

Many species of fowl and wading birds have a typical precocial mode of development and the chicken (or Jungle Fowl; *Gallus gallus*) has historically been the model of precocial species (Vleck et al. 1980). However, along the precocial-altricial continuum, there are birds that do not fit neatly into either category; rather, they represent some combination of traits from both developmental modes. Semi-precocial hatchlings such as those of gulls, for example, are capable of locomotion but require parent feeding. Semi-altricial offspring, such as found among herons, have more extensive feathering and open eyes, but remain dependent on parents and remain in the nest for a period of time similar to those of altricial birds (Hansell 2000).

VARIATION OF REPRODUCTIVE STRATEGY

Adult mortality is proposed as the primary driver behind the evolution of life history variation (Skutch 1949, Ashmole 1963, Ricklefs 1997) but there is evidence that, in birds, rates of nest predation and the effect of food availability contribute to variation in reproductive rates (Lack 1947, McNamara et al. 2008) are also important, if not more so. Evolutionary and ecological drivers of variation in life-history have resulted in an impressive diversity of reproductive strategies. Reproductive investment per reproductive bout, for example, is highly variable across species. Shorter-lived birds such as passerines that breed as year-round residents of north temperate regions, invest heavily in each reproductive effort through increased clutch sizes or number of broods per year (Jetz et al. 2008, Martin 1996). Though north temperate-breeding songbirds have been the focus of most research, their clutch sizes of an average 4.5 eggs are by no means the norm among birds, for which the overall modal clutch size is 2 (Jetz et al. 2008). For species with longer lifespans, a slower mode of reproduction is favored, with an increased time investment to a smaller clutch. The oldest known wild, reproducing bird is a Laysan Albatross (*Diomedea immutabilis*) named "Wisdom". Often recognized for their longevity (Young et al. 2008), the average individual of this species does not reach sexual maturity until they are 10 years old (Fisher 1975), lay only one egg per year (Fisher 1969), and frequently skip years between reproductive attempts (Young et al. 2009). At the other end of this spectrum are waterfowl with double digit clutch size (Drilling et al. 2020, Bielefeld et al. 2020, Hepp & Bellrose 2020), but even among altricial passerines, Blue Tits (*Cyanistes caeruleus*) stand out with an average clutch size of 12 (Perrins 1965).

Although most birds seemingly pair monogamously (Lack 1968), they display a variety of social mating systems. The Laysan Albatross is the icon of monogamous breeding systems, but female-female pairs have been reported to raise chicks, with both individuals having the opportunity to breed (Young et al. 2008). And among birds that appear monogamous, seeking out other mates is not uncommon. With advancements in molecular biology, birds previously thought to be monogamous are now known to frequently have extra-pair young in their broods, arising from extra-pair copulations between females and males outside of their pair bond. This is especially the case for migratory and north temperate zone breeding passerines (Westneat & Stewart 2003, Rowe et al. 2001). Others, such as the charismatic Greater Sage-grouse (*Centrocercus urophasianus*) and birds of paradise (family: Paradisaeidae), have lek mating systems in which females have their choice of sexually displaying males to mate with but the male has no involvement past copulation. In such groups, sexual selection is driven by extreme among-male variation in fertilization success (Payne 1984).

Other birds breed communally, as is observed in Ostriches (*Struthio spp*.). Ostrich nests may contain the eggs of up to eighteen females, most of which mate with multiple males (Kimwele & Graves, 2003). Females lay 6-11 eggs in a communal nest, which have been documented to contain as many as 67 eggs. However, only about 20 eggs in a nest will receive incubation, thus, the nest's "major" female does her best to ensure that her own eggs receive incubation by arranging them toward the center. It is thought that incubating and hatching the eggs of other females' eggs benefits the major female by a dilution effect, lowering the chances of the predation of her own offspring (Kimwele & Graves 2003). Cooperative breeders are yet another system, in which individuals temporarily forgo the chance to reproduce to support the reproduction of others of their species, who are virtually always close kin. Oftentimes, limitation of habitat prevents individuals from establishing their own territories and helping to raise siblings, which, in this common situation, is the next best option (Cockburn 1998). In the cooperative breeding system of the Florida Scrub Jay (*Aphelocoma coerulescens*), kin of a mated pair live in the family territory, participate in nest defense, and sometimes care for the nestlings (Woolfenden 1975). Beyond limited space, competition for resources is another explanation for the sacrifice of cooperative breeders. In the cooperatively breeding Acorn Woodpecker *(Melanerpes formicivorus*), the opportunity for an individual to breed is constrained by the paucity of trees for acorn storage that support and sustain the group (Koenig et al. 2011).

For all birds, megapodes being the exception, contact incubation is necessary to keep embryo temperatures above physiological zero (24-26°C; Webb 1987). Contact incubation is further facilitated by nests that serve the basic function of supporting the eggs and incubating parent for most species (Collias & Collias 1984)—although some birds, such as the White Tern (*Gygis alba*), lay their eggs directly on a surface (Marshall 1949). While the primary purpose of a nest is to serve as a platform for laying and incubating eggs (Collias & Collias 1984), they also may serve to protect eggs and nestlings from predators (Calvelo et al. 2006, Hansell 2000) or function as a sexual display (Álvarez & Barba 2008, Sergio et al. 2011).

Nest-building appears to have existed in non-avian dinosaurs, suggesting that nest construction and brooding behavior evolved long before the appearance of modern birds (Norell et al. 1995, 2018). Since then, the construction of bird nests has evolved to yield an amazing array of diverse structures. They may use scrapes (a depression dug into the ground, possibly lined with rocks or vegetation), burrows, abandoned cavities, or woven open-cup or pendant nests (Hansell 2000). Burrowing Owls (*Athene cunicularia*), for example, nest in burrows dug and abandoned by rock squirrels (*Spermophilus variega*) or prairie dogs (*Cynomys spp*.) (Martin et al. 2007). Belted Kingfishers (*Megaceryle alcyon*) dig their own burrows into mud banks along streams and rivers (Sullivan et al. 2006). The nests of weaver birds (family Ploceidae) are especially elaborate, often woven into spheres or pendants that hang from trees (Collias & Collias 1964). In some cases, large nests, such as those constructed by Thornbirds (*Phacellodomus spp.*), support multi-species nesting assemblages. Thornbirds build multi-chambered nests that are used by other bird species once the Thornbirds fledge, but are also regularly nested in simultaneously by other bird species (Skutch 1969). These so-called "nest associates" increase thornbird nest success by exploiting the nest defense behavior of other birds (Lindell 1996, Quinn 2008), although associates sometimes prey on the eggs or nestlings of thornbirds (Lindell 1996). Even within nest types there are a variety of sizes and material compositions. The archetypal bird's nest, the open-cup nest, is the most common structure but may starkly contrast from others of its form depending on its construction, which may consist of various parts of plants, arthropod silk, mud, snake skin, fur, feathers, fungi, and man-made materials (Hansell 2000, Price & Griffith 2017, Reynolds et al., 2019).

Nest type is phylogenetically conserved (Jetz et al. 2008) and influences the amount of environmental pressures—both biotic, such as predation (Martin & Li 1992) and arthropod parasites (Collias & Collias 1984) and abiotic, such as ambient temperature (Kendeigh 1961), wind (cited in Collias 1997), and humidity (Deeming 2011)—on the clutch and incubating bird. For example, open-cup nests leave eggs and incubating parents more exposed to precipitation than do burrows or cavity nests. Furthermore, environmental variables differ with breeding habitats on both large and small geographic scales, further affecting the evolution of life history traits.

INTERSPECIFIC GEOGRAPHIC VARIATION

Studies of geographic variation in avian life-histories have contributed heavily to the development of modern life history theory (Ricklefs 2000) mainly because of the existence of pronounced geographical trends. Principal among these is intra- and interspecific variation in reproductive traits along latitudinal gradients (Jetz et al. 2008, Lundblad & Conway 2021, Mainwaring et al. 2012, 2014, Owen 1977). Indeed, disparities often exist between the life-histories of taxonomically closely related bird species that breed in the tropics vs. temperate regions (Barrionuevo et al. 2014, Martin 1996). For example, there are latitudinal differences in nest orientation, with birds that build closed nests at higher latitudes tending to orient nest openings facing northwest and birds at more southern latitudes orienting their nest openings facing northeast (Schaaf et al. 2019). On a larger scale, tropical birds are known to lay fewer eggs (Jetz et al. 2008), have longer incubation periods (Martin 2002; Martin et al. 2007), and live longer (Martin et al. 2015, Ricklefs et al. 2011, Ricklefs & Shea 2007), than birds breeding at higher latitudes.

Clutch size is the primary metric of female investment into a reproductive bout because egg production (Williams 2005), incubation of a larger clutch (Haftorn &

Reinertsen 1985) and raising a larger brood to fledging is energetically costly (Lack 1946). Across species, those breeding in temperate regions lay an average of ~4.5 eggs, while those breeding in the tropics lay ~2.2 (Jetz et al. 2008). According to classic life-history theory, the seasonal pulse of productivity during spring at higher latitudes, combined with greater seasonality, predicts this greater reproductive investment per breeding attempt because adults (1) are assumed to have more resources available to them during the breeding season (Lack 1968) and (2) are less likely to survive to the next year to reproduce again (Martin 2004). Adult survivorship is higher in the tropics (Ricklefs 1992) and it is therefore predicted that parents should invest less in each reproductive attempt, in this case, by laying fewer eggs (Slagsvold 1982, Stearns 1976). Additionally, high nest predation in the tropics favors more nesting attempts per year (Martin 1996), and laying fewer eggs presumably allows parents to fledge offspring and begin the next nest cycle sooner. A smaller brood also requires less frequent provisioning trips (Skutch 1949), lessening the chances of a predator's attention being drawn to the nest (Sargent 1993). However, it is also possible that predation selects against larger broods because they are noisier and more easily found (Perrins 1965, Newton 1964). Ashmole's hypothesis, on the other hand, argues that birds at higher latitudes experience the greatest per capita food availability because non-breeding season mortality is highest outside the tropics. This combined with the spring flush of food supply reduces competition for resources and permits greater reproductive investment (Ashmole 1963).

Incubation is an energetically expensive activity. As noted above, developmental mode is highly conserved within families and is unrelated to environment. Incubation length scales to body size, but for a given body size, the greater maturity of precocial species at hatching is linked directly to longer incubation periods (Ricklefs and Starck 1998). Within altricial species, greater investment per reproductive bout in temperate-breeding birds is also reflected in a shorter incubation period compared to those breeding in the tropics (Martin 2002, Martin et al. 2007). This difference has been tied to both extrinsic and intrinsic factors. Frequent nest predation (an extrinsic factor) favors short incubation periods, but longer incubation may enhance the developing embryo's fitness by trading off rapid development for improved immune function at hatching (an intrinsic factor). The latter may result in increased adult survival (Ricklefs et al. 2017). However, external drivers, such as ambient temperature, but especially variation in nest-attentiveness driven by selection to promote adult survival at the expense of parental care, have been correlated with increased incubation period length (Austin et al. 2019, T. E. Martin et al. 2015, 2018, Matysioková & Remeš 2018).

Adaptations to the abiotic environment have, for many years, been recognized as drivers of variation in reproductive traits of birds but the depth to which it extends continues to be explored. For instance, though the ancestral condition of eggshell color is white (Kilner 2006), eggshells today come in shades of blue, green, and brown (Wisocki et al. 2020) and many have speckles or scrawling. Historically it has been thought that the primary driver affecting eggshell coloration was crypsis from predators; however, some studies have failed to support this hypothesis and an increasing body of evidence points to the thermal properties of these pigments (Cassey et al. 2011). Eggs with darker colored shells heat more quickly under solar radiation than those with lighter colored shells and remain warm for longer (Wisocki et al. 2020). An analysis of the geographic distribution of eggshell color revealed that darker eggshells are positively correlated with latitude (Gómez et al. 2018, Wisocki et al. 2020).

While variations in life history traits are correlated with geographic location, the mechanisms by which geographic location affects the traits of birds is debated. For example, clutch size varies latitudinally (Owen 1977, Jetz et al. 2008), but it also varies temporally across seasons and years, is conserved to some degree within taxonomic groups, and covaries with other traits such as body size, developmental mode, and nest structure (Jetz et al. 2008).

INTRASPECIFIC GEOGRAPHIC VARIATION

The value of interspecific geographic life history studies is often lessened by the difficulty of disentangling the influences of geography from phylogeny. Intraspecific geographic comparisons of life history traits control, to some degree, the potential influence of intrinsic factors associated with "species" that may confound our understanding of the drivers of variation on an interspecific level. Many Nearctic-Neotropical migrant songbirds have expansive breeding ranges, making them an ideal system for documenting geographic variation in traits such as incubation length (Gillette et al. 2021, Lundblad & Conway 2021), clutch size (Slagsvold 1989), nest size (Crossman et al. 2011, Mainwaring et al. 2012, 2014, Rohwer & Law, 2010), and extra-pair paternity (Valcu et al. 2021, but see Brouwer & Griffith 2021). Direct proximate effects of environmental variables such as weather may be responsible for some intraspecific variation, but intrinsic differences may exist among populations of the same species. For instance, a common garden experiment between House Wren (*Troglodytes* *aedon*) populations from Panama and Illinois showed that eggs from the tropical population exhibited longer incubation periods, even when parental attentiveness did not differ (Robinson et al. 2008).

Temperature is one of the most important influences on embryonic survival in species that lay eggs (DuRant et al. 2013). Changes in ambient temperature or adverse weather, such as sever rain or snow storms, affect female nest attentiveness (Morton & Pereyra 1985, Zerba & Morton 1983) and development of the embryos (Ardia et al. 2010, Nord & Nilsson 2011, Olson et al. 2006). Experimental studies have shown that embryos that experience suboptimal temperatures are negatively impacted in terms of body condition, with potential carry-over effects later in life (Ardia et al. 2010, Pérez et al. 2008). Parents can influence the microclimate experienced by developing embryos through nest design, but species that build open-cup nests are probably most impacted by changes in weather due to their direct exposure to the ambient environment.

Strategic nest placement is one method by which parents can mediate the nest microclimate (Collias 1997, Hansell 2000). In a study of two populations of Common Amakihis (*Hemignathus virens virens*) that breed at different elevations on Hawaii, birds breeding at a higher elevation that experienced cooler temperatures placed their nests on the periphery of the forest canopy, thereby increasing exposure to solar radiation (Kern & Van Riper 1984). Similarly, White-crowned Sparrows (*Zonotrichia leucophrys*) build their nests in low vegetation to protect them from wind and rain, but when snow remains into the breeding season, build them higher in shrubs and trees.

Indeed, intraspecific variation of nest size and structure has been observed along latitudinal gradients (Crossman et al. 2011, Mainwaring et al. 2014, Rohwer & Law

2010), for populations breeding at different elevations (Kern & Van Riper 1984) and those otherwise experiencing different ambient temperatures (Britt & Deeming 2011, Mainwaring et al. 2014, McGowan et al. 2004). Changes in nest structure are also sometimes seen across the breeding season (Ricklefs & Hainsworth 1969, Herranz et al. 2004, Britt & Deeming 2011). While nest material composition may also affect the nest microclimate (Hilton et al. 2004, Windsor et al. 2013, Mainwaring et al. 2014), intraspecific geographic variation in the material composition of nests has so far been attributed to the availability of materials near the breeding site (Calvelo et al. 2006, Crossman et al. 2011).

The qualities of eggs themselves have important effects on the future fitness of the developing embryos. Eggs are the fundamental unit of avian reproduction and the health and well-being of the embryo depends upon the resources allotted to the egg at laying. Studies of several bird taxa have shown that larger eggs are positively correlated with offspring growth and survival (reviewed by Williams 1994). This may be especially important when resources are less abundant. A cross-fostering experiment of House Wrens by Styrsky et al. (1999) found that egg mass was positively correlated with nestling growth for a short period for eggs hatched early in the season, but when resources became scarce later in the breeding season, the contribution of egg mass to growth continued for a longer time throughout the nestling period.

Egg size is highly variable within species. The largest egg of a population is usually at least 50% larger than the smallest, but egg size for a single female is highly repeatable and variation of egg size across clutches is generally greater than intra-clutch variation within species (Christians 2002). Most of the variation in egg size within species is not associated with differences among females in age, body size, or energetic condition (Christians 2002). This is not to say that female body mass cannot contribute to egg size variation in some species (Hepp et al. 1987) or, as predicted by life-history theory, vary inversely with clutch size (Martin et al. 2006); however, to date, the evidence for such relationships are weak. Increased food availability tends to promote earlier egg laying, rather than increased egg mass (Meijer & Drent 1999) or larger clutch size (Murphy 1986). But evidence suggests that females complete egg laying prior to emptying their body of endogenous reserves (Murphy 1986, Ward 1996), indicating that neither nutrition nor body composition are major influences on among-female variation in egg mass. Likewise, studies of the potential effects of ambient temperature on egg size have produced both positive and negative correlations, with temperature typically explaining only 10-15% of the variation of egg size (reviewed by Christians 2002).

Surprisingly, few studies of intraspecific geographic variation of egg size exist. Some species of raptors breeding in Australia displayed a positive relationship between egg size and elevation (Olsen & Marples 1993) and a study of eight European wading birds found that egg size decreased in populations at higher latitudes (Väisänen 1977). However, studies of the extent to which perching bird (Order Passeriformes) eggs vary across geographical gradients are few (but see Järvinen & Väisänen 1983, Hõrak et al. 1995, Encabo et al. 2002, Cassey et al. 2005). Though intrapopulation, and even intra-clutch, variation in egg size has been widely documented (Christians 2002), there is only one study that evaluated geographic variation in egg composition in a passerine bird. Ruuskanen et al. (2011) studied variation in egg size and egg composition of the Pied Flycatcher (*Ficedula hypoleuca*) across 16 populations in Europe. Their analysis of egg mass and maternal contributions to the yolk, including androgens, carotenoids, and lysozymes, showed greater variation in egg composition within than among populations. Carotenoid content was the exception, and the authors posited that this was due to differences in the availability of carotenoids in the environment, and thus, the mother's dietary intake.

Although intraspecific geographic variation in incubation length (Gillette et al. 2021, Lundblad & Conway 2021) and nest structure (Crossman et al. 2011, Mainwaring et al. 2014, Rohwer & Law 2010) have drawn increasing interest in recent years, the relationships between geographic location and insulative property of the nest remains understudied, and whether egg size and composition vary geographically is largely unexplored. The main purpose of my proposed work is to elucidate the relationships between these facets of avian reproductive strategy in the context of variable regional climates.

Responses to changes in temperature are especially relevant in the light of climate change, which has affected both migration timing (Both & te Marvelde 2007) and breeding dates (Both et al. 2004, Socolar et al. 2017) of birds. Both et al.'s (2004) analysis of the breeding phenology of 25 populations of Pied Flycatcher (*Ficedula hypoleuca*) revealed that the species' breeding date was significantly correlated with spring temperature across a wide geographic range, indicating that the general advancement of breeding date is likely a result of climate change. Additionally, the phenological mismatch between breeding seasons and food sources resulting from climate change may contribute to declines of bird abundances (Both et al. 2006, Moller et al. 2008). In the face of declining North American bird populations (Rosenberg et al.

2019), it is more important than ever to understand life history traits of birds and their adaptations to different environmental stressors. Studying species that occupy a wide geographic range is one method of examining variation in these traits and identifying their proximate constraints or ultimate basis.

EASTERN KINGBIRDS

Eastern Kingbirds (*Tyrannus tyrannus*; henceforth kingbirds) are a well-studied, Nearctic-Neotropical migrant passerine. Their open-cup nests leave eggs exposed to the ambient environment, which varies widely across their North American breeding range (Fig. 1). Variation in reproductive traits, such as incubation length (Gillette et al. 2021, Murphy 1985), timing of breeding (Murphy 1985), and nestling development (Murphy 1983a), and these traits' associations with weather have been well-documented in this species. Their large breeding range, the vulnerability of their open-cup nests, and demonstrated adaptation of their breeding biology in response to climate make them an excellent system to study intraspecific variation of reproductive strategy along geographic gradients.

***Nest variation* –** The open-cup nests of kingbirds are composed of a main structural layer, constructed often of grasses, woody stems, and forbs, and an inner lining of fine stems, rootlets, and sometimes cattail (*Typha spp.*) fluff. Nests are built often on the distal portions of tree limbs, 2 to over 25 m high, often near and frequently overhanging water (Murphy 1983b). Parents are fierce in their nest defense (Davis 1941), which is their primary strategy of protecting their broods from predation (Murphy et al. 1997, Redmond et al. 2009), although they also select nest sites that are difficult for non-flying predators to reach (Murphy 1983b). The microclimate of the nest site may also have an important effect on kingbird nestling growth, which has been demonstrated to respond both negatively and positively to ambient temperature depending on the stage in the nestling period (Murphy 1985). Thus, if the size and structure of nests have a significant role in controlling the nest microclimate, then nest construction may have important effects on nestling development. In Chapter II, we describe geographic variation of size, material composition, and insulative quality of kingbird nests collected from populations breeding in Louisiana (LA), New York (NY), and South Dakota (SD).

Egg variation - An analysis of kingbird eggs in vertebrate natural history museums across North America shows that egg mass is positively correlated with latitude and negatively correlated with mean ambient temperature during incubation (Murphy unpublished data). Kingbird body size does not vary in a consistent geographic manner (Van Wynsberghe et al. 1992), and thus the larger eggs laid by kingbirds in colder regions is independent of female body size. Detailed population studies of individually marked birds have also shown that egg mass is unrelated to female age, and to whether a clutch is the first of the season or a replacement for a failed nest (Murphy 2004). In Chapter III, we describe egg mass and egg composition of four populations of Eastern Kingbird breeding in Kansas (KS), LA, NY, and SD. We describe how yolk, albumin, and shell scale allometrically with egg mass and compare these relationships across populations.

This thesis is the result of the combined efforts of both my advisor, Dr. Michael T. Murphy, and I, therefore, throughout this work, I use the pronoun “we”. Initially, we proposed to study eight populations breeding in Louisiana, Arkansas, Kansas, Pennsylvania, New York, Oregon (OR), South Dakota, and Saskatchewan (Canada). We selected these sites specifically to span a range of latitudes (30.22° to 52.13°), longitudes (75.06° to 119.05°), and elevations (11 m to 1,264 m above sea level [asl]), in order to sample kingbirds breeding at a variety of geographic locations and experiencing various climates (Table 1). Sampling from so many populations in just three field seasons would have required significant collaboration with five other researchers. In March 2020, as our field season approached, the SARS-CoV-2 (COVID-19) pandemic resulted in widespread community quarantine mandates, closed universities, and restricted access to field sites. We assumed that the severity of the COVID-19 pandemic would diminish in time for June field work to begin, but this did not play out as expected. Our collaborators were unable to participate in 2020 and we were unable to get permission to perform research on site at my proposed 2020 SD and OR locations.

In an effort to find at least one study site from which to collect data in 2020, we obtained permission to study kingbirds in Watauga County, North Carolina. However, as field work began, we found that kingbirds were not breeding in sufficiently high densities for us to collect a meaningful sample size of nests. In the first week of June, as the breeding season began, an opportunity arose for us to stay in Pierre, SD. We received the necessary permits and two days later we arrived in SD. In 2021, the COVID-19 pandemic continued to bring difficulty acquiring permission to use proposed field sites, but we were able to travel to Lafayette, LA, where kingbirds began breeding in April.

Despite our inability to sample our proposed sites, our work spans 12.2° (30.22° to 42.45°), and 20° (75.06° to 95.2°) of latitude and longitude, respectively, and 400 m of elevation (11 m to 417 m asl) (Table 2). More importantly, the long-term climate averages vary substantially among KS, NY, LA, and SD vary substantially, representing a range of mean temperatures, cloud cover, days of precipitation, and mean windspeeds during the breeding season (April - July). LA represents a virtual subtropical climate and has the highest breeding season temperatures and lowest cloud cover of our study sites, while NY is the coldest, has the greatest cloud cover, most days of precipitation, and least wind. KS and SD represent the climate of the Great Plains, with high winds, relatively moderate breeding season temperatures, and few days of rain (Chapter III Fig. 1).

These study sites presented opportunities to examine differences in ecological factors outside of climate as well. SD is notably lacking in corvids (Smith et al. 2020, Verbeek and Caffrey 2021), which are the primary predators of kingbird nests in other regions (Murphy 1986, Blancher & Robertson 1987, Murphy 2001, Murphy et al. 2020). SD was also the only location of our 4 where we observed kingbird use of the nests of heterospecifics. There we witnessed them add lining to, and lay eggs in, an Orchard Oriole (*Icterus spurius*) nest, add structural layers and linings to, and lay eggs in, American Robin (*Turdus migratorius*) nests, and constructing an entire nest and laying eggs within an old Common Grackle (*Quiscalus quiscula*) nest. LA was of particular interest because of its subtropical climate and the early breeding dates compared to other populations of kingbirds (April vs. June in KS, OR, and NY). From LA we were able to collect 10 nests from the coastal prairie and 10 from marsh habitat, allowing us to look for differences that may exist across nests from the same climate, but that were built in different ecological communities.

This work contributes to the body of literature describing intraspecific geographic variation of bird reproductive biology. Open-cup nesting passerines are especially understudied in this respect, and this thesis will lend some understanding of the relationships between geographic location and the qualities of avian eggs and nests.

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Figure legends

Fig. 1. The breeding range of the Eastern Kingbird (*Tyrannus tyrannus*) and our four study sites in Lawrence, KS, Oneonta, NY, Pierre, SD, and Gueydan, LA.

Fig. 1.

BirdLife International and Handbook of the Birds of the World (2021) Bird species distribution maps of the world. Version 2021.1.

Tables

Table 1. List of proposed study sites, comparison of June (breeding season) climate, proposed year of study along with individual(s) primarily responsible for field work.

Site	Latitude	Longitude	Elevation	June temp High/Low (° C)	June precip (mm)	Year	Researcher
Lafayette, LA	30.22	92.02	11 m	32.8/22.8	180.1	2020	Pierce & Murphy
Jonesboro, AR	35.84	90.70	79 m	31.0/18.8	80.0	2020	Boves
Lawrence, KS	38.97	95.23	264 m	28.9/17.2	141.5	2020	Murphy
Schuylkill, PA	40.69	76.18	160 m	19.8/13.2	94.0	2020	Redmond
Oneonta, NY	42,45	75.06	417 m	23.3/10.6	109.0	2019	Murphy
MNWR, OR	43.59	119.05	1,264 m	23.9/5.6	19.0	2020	Murphy & Gillette
Aberdeen, SD	45.46	98.49	397 m	25.0/12.2	94.0	2021	Dolan & Gillette
Saskatoon, SK	52.13	106.67	482 m	24.4/10.0	65.8	2021	Murphy & Gillette

Table 2. List of actual study sites, comparison of June (breeding season) climate, proposed year of study along with individual(s) primarily responsible for field work.

Site	Latitude	Longitude	Elevation	June temp High/Low (° C)	June precip (mm)	Year	Researcher
Lawrence, KS	38.97	95.23	264 m	28.9/17.2	141.5	1983	Murphy
Oneonta, NY	42.45	75.06	417 m	23.3/10.6	109.0	2019	Murphy
Pierre, SD	46.37	100.35	443 m	27.8/15.0	91.0	2020	Gillette & Murphy
Lafayette, LA	30.22	92.02	11 m	32.8/22.8	180.1	2021	Gillette & Murphy

Chapter II

Geographic variation of the morphology and material of nests of the Eastern Kingbird *Tyrannus tyrannus* and their effect on insulative capacity

Abstract.–Nest building is ubiquitous in birds, and although coopted for various purposes, nests serve an essential role in creating a favorable microclimate for eggs and young. Nonetheless, whether nests vary intraspecifically in structure and composition along geographic gradients of climate is understudied. Here we characterize the morphology, material composition, and insulatory capacity of nests from geographically distinct populations of an open-cup nesting Nearctic-Neotropical migrant, the Eastern Kingbird *Tyrannus tyrannus* breeding in Louisiana (LA), New York (NY), and South Dakota (SD). Our study sites differed in mean temperature, days of precipitation, cloud cover, and wind speed during the breeding season. Principal component analysis (PCA) of nest morphology revealed that nests from warmer and sunnier LA were lighter, had thinner walls, and lower nest cup volume than nests from colder and cloudier NY and colder and windier SD. In comparison to NY, SD nests were heavier, had larger outer dimensions, and deeper nest cups than those of NY, despite the lower temperatures and more frequent rainfall of NY. Greater nest mass and cup volume of SD than NY may be related to lower predation pressure, less rain, and/or larger clutch size in SD than NY. Nest material composition did not differ among sites due to high intrapopulation variation in composition, but fine stems and rootlets, forbs stems, and plant down were common materials at all sites. By contrast, NY nests contained more coarse woody stems whereas grasses were common in SD and especially LA. We experimentally determined heat loss coefficients for nests and found significant positive correlations between insulative capacity and overall nest size, greater wall thickness, smaller cup openings, and lower cup volume of nests when covered with insulating foam; however, we found no significant predictors of insulative capacity of uncovered nests. We found no relationships between insulative capacity and material composition when we considered all sites, but when LA nests were removed from the analysis, plant down, woody stems and fine stems and rootlets were predictors of a low rate of heat loss, while grasses and forbs were associated with a higher rate of heat loss. Our results suggest that intraspecific variation in nest morphology and material composition of nests have important impacts on nest insulative capacity that is tied to the breeding season climates of different breeding locations.

Keywords: *Geographic variation, heat loss, insulation, latitude, nest microclimate, thermal conductance, weather*

Introduction

The primary function of bird nests is to serve as a platform upon which contact incubation can occur between eggs and the incubating parent (Collias and Collias 1984, Heenan and Seymour 2011). However, across species, nests serve a variety of other purposes including sheltering eggs, nestlings, and incubating parents (Hansell, 2000), crypsis against predators (Bailey et al. 2015, Calvelo et al. 2006), control of parasites, and sexual signaling (Álvarez & Barba 2008, Sergio et al. 2011). Especially in smaller passerines, the nest plays an essential role in the bird-nest incubation nexus by maintaining a suitable microclimate for embryonic development (Deeming 2013, Heenan 2013). Once incubation has begun, maintenance of embryonic temperatures above egg physiological zero (~26° C) is critical (Webb 1987) because embryos that experience periodic cooling may have a reduced growth rate and experience detrimental effects that carry over into later life (Ardia et al. 2010, DuRant et al. 2013, Nord & Nilsson 2016, Olson et al. 2006, Pérez et al. 2008). Because incubation is energetically expensive (Cresswell 2003), the capacity of a nest to retain heat also potentially effects parental behavior. Nests that are less insulated require greater energy expenditure to warm eggs (Hilton et al. 2004, Kern & Van Riper 1984), which may experience longer incubation periods (Lyon & Montgomerie 1985, 1987). Parents can presumably reduce incubation's energetic cost by building better insulated nests that reduce heat loss.

While building thicker walls may better preserve nest microclimate (Kern & Van Riper 1984; Skowron & Kern 1980) and reduce the duration and energy investment of incubation (Hilton et al. 2004), building a larger nest requires more work and additional flights that may expose parents to predators. Furthermore, larger nests are more likely to be detected by predators (Møller 1990). Thus, nests may represent an evolutionary trade-off between the benefits of an ideal nest microclimate and the risks and cost associated with increased time and energy spent building a larger nest (Mainwaring & Hartley 2009, 2013). We should thus expect that selection favors the smallest nest possible for a given regional climate.

Indeed, size and structure of nests varies with latitude (Crossman et al. 2011, Mainwaring et al. 2014, Rohwer & Law 2010), elevation (Kern & Van Riper 1984), time of year (Ricklefs & Hainsworth 1969, Herranz et al. 2004, Britt & Deeming 2011) , and prevailing temperature (McGowan et al. 2004, Britt & Deeming 2011, Mainwaring et al. 2014). Studies on nest size have focused mainly on cavity-nesters (Briggs & Deeming 2016, Deeming et al. 2012) owing to the ease of study using nest boxes. Open cup nests, however, are more vulnerable to environmental factors, such as temperature and precipitation, and may better demonstrate adaptive nest construction. For instance, Crossman et al. (2011) and Rohwer and Law (2010) found that American Robins (*Turdus migratorius*), *Carduelis* finches and Yellow Warblers (*Setophaga petechia*) built heavier nests with thicker walls at higher latitudes where they experienced colder temperatures. Similarly, Mainwaring et al. (2014) found the insulative capacity of Common Blackbird nests (*Turdus merula*) declined as average spring temperatures increased across a 7^{o} latitudinal gradient (50.05° to 57.10°).

The insulative capacity of nests also varies with the materials used to build them (Hilton et al. 2004, Biddle et al. 2019). Birds are discerning and opportunistic (Bailey et al. 2014, Calvelo et al. 2006) in their choices of material for nest building and said materials may vary on local (Calvelo et al. 2006) and larger geographic scales (Crossman et al. 2011, Deeming & Mainwaring 2015). Calvelo et al. (2006) found that hummingbirds breeding in Patagonia used a decreasing diversity of plant materials as habitats changed from high to extremely low humidity. On a larger scale, Crossman et al. (2011) found that the composition of Yellow Warbler and *Carduelis* finch nests differed between northern and southern breeding populations, while the diversity of materials incorporated into the nests of American Robins and Savannah Sparrows (*Passerculus sandwichensis*) increased proportionately as nest size increased. Although it is generally accepted that material composition may influence the insulative capacity of nests (Hilton et al. 2004, Mainwaring et al. 2014, Windsor et al. 2013, Taberner Cerezo & Deeming 2016), how nest size and material composition are related to insulative capacity and how these features vary on a large geographic scale are not well documented.

Eastern Kingbirds (*Tyrannus tyrannus*) are open-cup nesting, Nearctic-Neotropical migratory passerines that are single sex (female only) intermittent incubators without incubation feeding by males. Their nests are built on the edge of tree canopies (Murphy et al. 1997) such that nest contents are exposed to the elements when females leave the nest. Kingbird incubation length increases with exposure to low temperatures and high precipitation (Gillette et al. 2021),and thus nests are thus potentially critical for maintaining favorable microclimates for eggs or young when females are off the nest. However, regardless of population, 50% or more of nests typically fail due to nest predation (Murphy & Pyle 2018) and thus selection might well favor small nests less easily located by predators. These facts, combined with their high natal philopatry (Murphy et al. 2020) and a breeding range that extends across much of North America, make them an ideal system in which to study large-scale geographic variation in nest properties.

In the present study we examine kingbird nests from populations breeding in Louisiana (LA), New York (NY), and South Dakota (SD) to test for (1) the possibility that intraspecific variation of nest size, morphology, material composition, and insulative capacity exist on a wide geographic scale and that (2) variation in nest structure and/or composition influences nest insulative capacity. Although we acknowledge the possibility that nest structure and composition may be influenced by short-term exposure to ambient weather, we assumed that long-term climate averages are the primary drivers of potential geographic differences in nest structure and composition. We thus predicted that the nests of kingbird populations experiencing conditions unfavorable to incubation (i.e., lower ambient temperatures, greater rainfall, stronger winds, frequent overcast skies) would be heavier, have thicker walls, deeper nest cups, smaller nest cup openings, and be composed of materials providing greater insulative value that dry rapidly after being wet (Biddle et al. 2019) than nests from populations that experience the opposite set of weather conditions. We further predicted that thermal conductance (the inverse of insulation) of nests would be lowest among heavy nests with thick walls, deep nest cups and small nest cup openings that were built with materials of high insulative value (Biddle et al. 2019).

Materials and Methods

We studied populations of Eastern Kingbirds in Oneonta, NY (42.45° N, 75.06° W, 339 m above sea level [asl]), Pierre, SD (44.37° N, 100.35° W, 450 m asl), and Gueydan, LA (30.03° N, 92.51° W, 2 m asl) in 2019, 2020, and 2021, respectively. Nests were collected to coincide with the start of the breeding season at all sites so that all nests in our study were initial nests of the season. Comparisons of long-term climate from the 3 sites, based on mean monthly temperature, mean days of rainfall/month, percentage of cloudy days/month, and average monthly windspeed for April through July, summarized using principal component analysis (PCA; Appendix), demonstrate distinct differences (Fig. 1). Compared to SD and LA, kingbirds in NY experience lower temperatures and more cloud cover (described along PC1), and more days of rain but less wind (described along PC2) than the other sites. Taken together, average climatic conditions in NY and

LA are the least and most favorable, respectively, for unattended eggs or nestlings, and therefore we expect the morphology and composition of nests from NY to reflect the poor conditions, whereas nests from LA should exhibit the weakest capacity for heat retention.

NY study sites were centered on Charlotte Creek and surrounding floodplain and uplands habitats in Delaware and Otsego counties. Kingbirds nested in ironwood trees (*Carpinus caroliniana*) in riparian zones but in floodplain and upland sites they used mainly hawthorns (*Crataegus* spp.) or apple (*Malus* spp.) trees. See Murphy (2001) for a full description of the study site. In SD, kingbirds nested in riparian and floodplain habitat of the Missouri river, along the shoreline of Lake Oahe (an impoundment of the Missouri River), and in the draws and valleys of tributary streams of Stanley, Sully and Hughes counties. Kingbirds nested primarily in live Russian olive (*Elaeagnus angustifolia*) trees, as well as Russian olive snags, and Siberian elm (*Ulmus pumila*) trees, but also plains cottonwood (*Populus deltoides*), juniper (*Juniperus spp.*), and ash (*Fraxinus spp.*). In LA, we collected nests of kingbirds in 2 distinctly different habitats; coastal prairie and the marshes of White Lake Wetlands Conservation Area (WLWCA) located in Vermilion Parish. Outside of the WLWCA, coastal prairie kingbird nests were situated in branches overhanging water-filled ditches along roadsides or in canals between agricultural fields. Most LA nests were found in nonnative Chinese tallow (*Triadica sebifera*), but also bald cypress (*Taxodium distichum*), and willow (*Salix spp.*) trees. Nests of kingbirds breeding in the WLWCA were placed mainly in buttonbush (*Cephalanthus occidentalis*), but where embankments supported trees, they also nested in southern live oaks (*Quercus virginiana*) and bald cypress.

Field work in NY and SD began in June 2-3 weeks after kingbirds arrived from spring migration, but because of earlier arrival of migrants in LA, we began our field work there in late April. We located nests by finding kingbird pairs and observing nest building behavior. Nests are typically placed at least 2 m aboveground in a woody structure, but positions varied substantially. We thus categorized nests as being placed on a horizontal limb, in a crotch of a vertical limb, or an intermediate (diagonal) position. We checked nests every 1-2 days using a mirror attached to an extensible pole, and given their height, access to most nests for collection of nests and eggs required that we either climb the nest tree or use ladders. Kingbirds lay one egg per day, with the occasional exception of a skipped day between the laying of the penultimate and the final egg. At all sites, egg-laying was not observed after 10:00. Thus, the egg-laying period was considered to have ended when no new egg had appeared by 10:00 on the second day in the absence of egg-laying. Nests and eggs were collected immediately following the end of egg laying so that activity of parents would not alter nest morphology. We were licensed to collect 20 nests and their clutch of eggs, and therefore our choice of the 20 was simply the first 20 nests that we could access and that survived to the end of egg-laying. Nests were stored in rigid plastic containers with lids for transport back to Portland, Oregon.

Nest morphology

In the laboratory we measured mass of the air-dried nests to the nearest 0.01g using a top loading balance. To measure nest cup volume, we first developed a prediction equation describing the relationship between volume and mass of fine-grained sand by weighing known volumes of sand measured using a graduated cylinder. We then lined each nest cup with cellophane (©) and filled it with fine-grained sand to the rim of the nest cup, weighed the sand, and used sand mass to determine cup volume using the prediction equation. We also measured six nest dimensions: cup depth, wall and floor thickness, minimum and maximum widths of the nest cup opening, and external nest diameter. Cup depth was the mean of three measurements taken from the top of the nest rim to the nest cup floor. Wall thickness was the mean of eight measurements taken at equally-spaced positions around the circumference of the nest. We acquired these values using calipers to measure a length of wire that was passed through the nest to measure distance from the outer to inner wall. Similarly, floor thickness was the mean of three measurements taken by measuring the length of a wire passed through the nest floor. We averaged the 4 measurements of outer nest diameter (by 25 cm ruler) made at equally spaced locations around the nest (N-S, NE-SW, E-W, and SE-NW) to calculate external nest diameter. We calculated cup asymmetry by taking the ratio of cup maximum to minimum width measured at the rim of the nest cup using calipers. We also used minimum (a) and maximum (b) cup widths to calculate area of the nest cup opening using the equation for area of an ellipse (area = $\pi*(a*b)$). In the end we chose to not analyze floor thickness because of large differences among nests that appeared driven by the structure of the actual nest site that seemed to require females to add extra material to fill space when nests were built in tree crotches. In addition, measurement of floor thickness of some nests was compromised because some nests were unintentionally compressed during storage and transport.

Nest Composition

Kingbird nests are composed of an internal lining and an outer “shell” (Murphy & Pyle 2018), the former containing mainly fine stems and rootlets, but also plant down and occasionally grasses. The outer shell consists mainly of coarse woody stems, forb stems, but also grasses and other materials sometimes of anthropogenic origin (e.g., fishing line, landscaping fabric). We did not disassemble nests to quantify the mass of the 6 different materials used to build nests because we wanted to save the nests for potential future use in other experiments. Moreover, given large differences in the density of materials (e.g., plant down vs. coarse woody stems), measurement of mass of different materials would not have been useful in assessing the contribution of each material to the nest’s construction. Instead, we quantified nest composition by individually examining each nest and agreeing upon a visually estimated percentage that each of the six materials contributed to the nest’s total quantify of material. Quantification of nest structure and estimates of composition were made prior to measurement of thermal conductance.

Insulative capacity

Nests were stored at room temperature in sealed containers prior to rate of heat loss experiments. Once experiments began, we stored nests in environmental chambers under experimental conditions for at least 12 hours before recording measurements. We measured insulative capacity of each nest by using iButton temperature data loggers (Maxim: DS1922L and DS1923) under four sets of conditions: at 7°C and 20°C, and for both temperatures, either with the nest opening uncovered or covered. To cover nests, a square 1”-thick piece of polycyanurate insulation foam (Windsor et al., 2013) was placed over the nest to completely cover the nest cup opening (suggested by D. R. Ardia). Experiments were performed in climate-controlled chambers (Environmental Growth Chambers, Chagrin Falls, Ohio) held at constant temperature and 50% relative humidity. To measure the rate of heat loss the iButton was heated to approximately 50°C in a drying oven and then immediately placed at the bottom of the nest cup. iButtons were programmed to record temperature every 20 seconds (McGowan et al., 2004) for the 20 minute period that it took iButtons to reach ambient temperature. Three measurements of each nest were made at all 4 sets of conditions, with iButtons switched among nests for all trials. Heat transfer coefficients were calculated for each trial then averaged to yield average thermal conductance (the inverse of insulative value) of a nest. Thermal conductance was determined from cooling rates obtained for each nest by fitting an exponential curve to the resulting data using Equation 1

$T_{iButton} = T_{ambient} + \left[B^{(-C*time)}\right]$ *Equation 1* (from McGowan et al., 2004)

where B is the gradient between $T_{ambient}$ and $T_{iButton}$, and C (heat transfer coefficient) is a constant that describes the rate of heat loss from the nest cup.

Uncovered nest experiments were intended to determine nest insulative capacity during an incubation off-bout while the female was absent from the nest. Covered nest experiments were intended to measure the insulative capacity of the nest while the female was on the nest covering the nest cup opening. Neither experiment attempted to reproduce the heat supplied by the female during incubation or the heat retained by eggs; rather, experiments were designed to measure the insulative capacity of each nest without other contributing factors except for a covering. Although it was not an explicit part of our experiment, air turbulence was produced in the climate-controlled chambers by circulating air necessary to maintain constant ambient temperature and humidity. Experiments run in 7°C and 20°C should not produce different heat transfer coefficients according to Newton's Law of Cooling (Boyce and DiPrima 1992) and therefore we averaged coefficients generated across trials to produce mean coefficients for each nest.

Data Analysis

Comparisons of nest mass, morphology and composition among sites were made using analysis of variance (ANOVA), followed by a two-way ANOVA with site and nest angle as factors to account for the possibility that nest angle, which differed among sites (see below), was not a contributing factor to among-site differences in nest mass, morphology, or composition. We excluded from all comparisons 4 nests from SD that were built in the nests of other species (twice in American Robin [*Turdus migratorius*) and 1 each in Orchard Oriole [*Icterus spurius*] and Common Grackle [*Quiscalus quiscula*]). For analyses of the relationship between heat transfer coefficients and nest structure and composition, we excluded 6 nests that had holes in their walls or floors that were created by a supporting tree limb that had been integrated into the nest structure, along with 2 other nests with covered heat transfer coefficients that were outliers (i.e., heat transfer coefficients were more than 2 standard deviations away from the mean of their site). We thus retained 18, 18, and 14 nests from LA, NY, and SD, respectively, for our analyses.

Nests are inherently multidimensional structures with potentially high covariation among nest traits. We therefore used PCA to reduce the dimensionality by summarizing the major gradients of variation among nest traits. Separate PCAs were performed on structure and composition but both were based on correlation matrices. Our morphology PCA included nest mass, nest cup depth and volume, wall thickness, area of the nest cup opening, cup asymmetry, and average outer nest width. Our composition PCA included percent composition contributed by fine stems and rootlets, forb stems, grasses, plant down, woody stems, and other. We deemed PC axes worthy of interpretation if their eigenvalues were ≥ 1.0, but we limited this to no more than the first 3 axes given that biological interpretability of axes is questionable beyond the first 3 (McGarigal et al. 2000). We compared the structural and morphological PC scores across sites using one-way analysis of variance (ANOVA).

To evaluate the relationship between heat transfer coefficients of nests and their structure and composition we used best subsets regression analysis with the PC axes that summarized the major gradients of variation among nests as predictor variables instead of individual measures of nest structure and composition. To this we added area of the nest cup opening area divided by nest cup volume as a 7th predictor. This 7th variable, which we refer to as "relative nest cup opening", was intended to represent the ease with which warmed air inside a nest could escape to the outside. Small values for this variable represent a small opening for the area contained within the cup, which should serve to reduce heat loss. PC axes are by definition orthogonal and thus statistically independent of one another. Relative nest cup opening was likewise essentially orthogonal with the structural PC axes as the strongest relationship of relative nest cup opening with the structural PC axes (in this case PC2) was only $r = -0.319$ ($P = 0.075$). We regarded all models within 2.00 $\mathrm{AIC_C}$ units of the top model as competitive and model averaged parameter estimates from competitive models to identify the variables that contributed most to differences in heat transfer coefficients based on whether 85% confidence intervals overlapped zero (Arnold 2010).

All statistical analyses were performed in STATISTIX (Analytical Software v. 9), and we report descriptive statistics as mean ± standard error (SE), and sample size (n). We share the American Statistical Association's view that *P*-values should not be used to establish hard lines between "significant" and "nonsignificant" results, but should instead be viewed as a continuously varying metric of the strength of a result (Wasserstein et al. 2019). Hence, we report and interpret *P*-values for our results.

Results

Nest Placement

Angle of nest placement varied among sites ($X^2 = 11.89$, df = 4, P = 0.018). Most NY nests were on a horizontal limb (58.6%) whereas most SD nests were oriented vertically (60.0%). LA nests were more evenly distributed between a horizontal (38.0%) and vertical (48.0%) placement. The vertical placement of SD nests also included nearly half of those nests being located in a main crotch of the trunk, something never observed at the other sites. Similarly, and as noted above, kingbirds in SD, but not LA or NY, used nests of other species. Modification of the reused heterospecific nests ranged from simply adding an inner lining (Orchard Oriole) to the addition of the structural layer and lining to an old nest (American Robin), or construction of a near complete nest inside an existing larger nest (Common Grackle).

Nest morphology and composition

All variables with the exception of asymmetry of the nest cup opening differed among sites (Table 1). We conducted a second set of analyses with site and nest angle as factors in a 2-way analysis of variance; nest angle never contributed to differences in nest mass or structure (lowest P-value was for nest angle with relative nest cup opening [P = 0.140]). We therefore ignored nest angle in subsequent analyses. SD nests were the heaviest, had the deepest nest cups and greatest cup volumes, thickest nest walls, and the smallest relative nest cup openings (Table 1). LA nests, by contrast, had the largest nest cup openings, thinnest walls, shallowest nest cups, smallest cup volumes, and largest relative nest cup opening. The thin walls of LA nests is consistent with LA nests having the widest inner cup dimensions but smallest outer nest dimensions. NY nests were intermediate in 6 of 9 comparisons, the exceptions being that they had the smallest nest cup openings, widest outer nest dimensions, but narrowest inner dimensions (Table 1). Within each population, nest mass exhibited the greatest variation (based on coefficients of variation; Table 1) of all measured traits, and in paired comparisons of coefficients of variation, NY and LA exhibited similar levels of intrapopulation variation among nest variables (paired t-test = 0.70, df = 8, P = 0.506), but SD nests were more variable in structure than nests from both NY (paired t-test = 6.17, df = 8, P < 0.001) and LA (paired t-test = 4.33, df = 8, P = 0.002).

Intrapopulation variation in nest composition, based on coefficients of variation, was higher than observed for nest structure (Table 2). As a consequence of high intrapopulation variation in nest materials, only 1 of the 6 materials contributed differently among populations; coarse woody stems contributed ~6% of nest materials in

NY but was virtually if not entirely absent from nests in SD and LA (Table 2). Fine stems and rootlets, forb stems, and plant down together contributed between 78% and 90% of nest materials at all sites, with coarse woody stems in NY and grasses in SD and especially LA being the next largest contributors (Table 2). Two-way analyses of variance with site and nest angle as factors never resulted in a contribution of nest angle to variation in nest composition (lowest *P*-value was for nest angle was 0.131 for proportion grass).

Principal component analyses of nest structure and composition

Our PCA of structural variables yielded three factors with eigenvalues greater than 1.00 ($PC1_{morph}$, $PC2_{morph}$, and $PC3_{morph}$) that accounted for 75% of total variance in the dataset (Table 3). The $PC1_{morph}$ was a general descriptor of nest size, with positive scores being indicative of nests that have greater cup volume and outer dimension width, thicker walls, and greater nest mass and nest cup depth. The 3 variables that contributed most to $PC2_{morph}$ indicated that nests with high scores had elongate (i.e., had high cup asymmetry) and shallower nest cups, and lower cup volumes, whereas nests with negative had symmetrical, deeper, and more voluminous nest cups. Nests with high $PC3_{morph}$ scores had smaller nest cup openings, and tended to have thicker nest walls whereas negative scores were associated with nests with wide nest cup openings and thin nest walls (Table 3). $PC1_{morph}$ differed among sites (Table 1) because of the lower scores of LA nests compared to NY and SD. $PC2_{morph}$ did not differ across sites (Table 1), but $PC3_{morph}$ did (Table 1), and post hoc tests (Tukey's test) indicated that the differences were due to higher scores on PC3 in NY than LA (SD intermediate and not different from

NY and LA); compared to LA nests, NY nests had thicker walls and smaller nest cup openings.

The first three axes from the PCA of nest composition explained roughly equal amounts of the variance that summed to 68.3% of the total variance in nest composition (Table 4). Nests with high $PC1_{comp}$ scores had abundant grasses and moderate levels of fine stems and rootlets, but little in the way of forbs stems. Nests with high $PC2_{comp}$ scores had abundant plant down and coarse woody stems, but little grass and few forb stems. $PC3_{comp}$ was a contrast of nests with positive scores that had abundant fine stems and rootlets but little grass and "other" whereas nests with negative scores had little in the way of fine stems and rootlets but abundant grass and "other" (Table 4). None of the PC_{comp} axes differed among sites (Table 2).

Insulative capacity

Mean heat transfer coefficient of uncovered nests across all 3 sites (0.228 ± 0.0054 °C/sec) was higher than that of covered nests (0.203 ± 0.0054 °C/sec; paired t-test, t = 4.56, df = 50, $P < 0.001$). Uncovered heat transfer coefficients did not differ among sites (Table 1), but covered nests from LA exhibited a higher heat transfer coefficient than covered nests from both NY and SD; the latter 2 sites did not differ from one another (Table 1).

Correlation analyses revealed no individual predictors, either morphological or compositional, of uncovered nest heat transfer coefficients with either PCA axes of structure or composition, or of individual nest variables (except possibly for nest wall thickness; $r = -0.260$, $P = 0.068$). By contrast, the correlation analyses of covered nests indicated heat transfer coefficients increased with increasing area of the nest cup opening ($r = 0.400$, $P = 0.004$; Fig. 2a), average inner dimensions of the cup ($r = 0.389$, $P = 0.005$), and as area of the nest cup opening relative to nest cup volume increased ($r = 0.376$, $P = 0.007$), but declined with increasing nest cup asymmetry ($r = -0.275$, $P = 0.053$) and increasing nest mass ($r = -0.272$, $P = 0.056$; Fig. 2b). With respect to the PC_{morph} axes, heat transfer coefficients declined with increasing $PC3_{morph}$ ($r = -0.327$, $P = 0.021$; Fig. 2c) and $PC1_{morph}$ ($r = -0.276$, $P = 0.052$; Fig. 2d). Heat transfer coefficients were independent of all individual measures of nest composition and the 3 PC_{comp} axes that summarized nest composition (strongest correlation with $PC1_{comp}$, $r = 0.211$, $P = 0.133$).

Our best subsets regression analysis of variation in heat transfer coefficients of uncovered nests from all 3 sites in relation to $PC1\text{-}3_{morph}$, $PC1\text{-}3_{comp}$, and relative nest cup opening failed to identify a single model that accounted for more than 1% of the variation. By contrast, the only competitive model from our best subsets regression analyses of covered nests accounted for 27.2% of the variation in heat transfer coefficients ($F = 8.78$, df = 2, 47, $P < 0.001$). Heat transfer coefficients were highest in nests with a large nest cup opening relative to nest cup volume ($\beta = 0.0014 \pm 0.00036$, $P < 0.001$) and in nests with low scores on $PC2_{morph}$ ($\beta = -0.0049 \pm 0.00169$, $P = 0.006$). Based on factor loadings on $PC2_{morph}$, heat transfer coefficients were lowest in nests with shallow and asymmetrical cups with a low nest cup volume.

Given the nearly subtropical climate of LA compared to temperate and highly seasonal NY and SD, and much later arrival and breeding dates of kingbirds in NY and SD, we conducted a separate best subsets regression analysis on heat transfer coefficients from the two latter sites. Four competitive models emerged that accounted for between 26% and 42% of the variation (Table 5). The top 2 competitive models exhibited virtually identical model weights, and differed only in that the 2nd ranked model included relative nest cup opening in addition to the same set of axes from the structural and composition PCAs. The 3rd ranked model included only structural features of nests, while the 4th ranked model included 1 axis each from the structural and composition PCAs (Table 5). Model averaging of variables included in the 4 top ranked models (Table 6) yielded 3 variables whose confidence limits excluded zero, which we use to interpret the results. Heat transfer coefficients of nests declined with increasing $PC2_{morph}$, $PC2_{comp}$, and $PC3_{comp}$. Thus, the lowest heat transfer coefficients of NY and SD nests were detected in nests with a shallow, asymmetrical cup of low volume that was composed principally of abundant plant down, fine stems and rootlets, and coarse woody stems, but few grasses or forb stems. Identical analysis of the LA nests by themselves did not produce models worthy of interpretation.

Discussion

Our understanding of the factors influencing avian nest construction has been hampered by a paucity of data, the challenge of distinguishing between multiple selective pressures shaping nest construction, and potentially, the difficulty of separating ultimate from proximate influences on nest building behavior. We chose to focus on the thermal biology of nests by asking whether kingbirds from 3 different climatic regions built nests that differed in their capacity to oppose heat loss. Our results showed that kingbird nests exhibit considerable geographic variation in size and structure, little consistent geographic variation in material composition, but that differences in nest structure and composition had thermal consequences.

Nest morphology and composition

The more northern NY and SD nests differed from one another only in total mass, nest cup volume, and inner nest dimensions (all > in SD). By contrast, the nests from southern LA were the lightest, had the thinnest walls, shallowest nest cup and smallest nest cup volume, but widest nest cup opening. Among site variation in PC axes of nest morphology reflected these differences by showing that NY and SD never differed on the first 3 axes, but that both differed from LA on $PC1_{morph}$ (and NY from LA on $PC3_{morph}$). Others have shown that birds often build larger nests at high latitude (Britt & Deeming 2011, Crossman et al. 2011, Mainwaring et al. 2014, Rohwer & Law 2010) and high elevation sites (Kern & Van Riper 1984) where temperatures are lower, but few have examined multiple nest features to identify where most of the differences reside. The exception (Mainwaring et al. 2014) found that although volume of Common Blackbird nests did not change with increasing temperature across 7° of latitude, nest diameter and wall thickness declined over the same gradient. In the case of kingbirds, the lower mass of LA nests was attributable mostly to the thinner nest walls, shallower nest cup depth (i.e., lower walls), and narrower outer dimensions compared to NY and SD. These features, plus the wider nest cup opening, especially when viewed relative to the nest cup volume, would do little to impede heat loss from LA nests.

The generally heavier nests, deeper nest cup, and larger (i.e., wider) outer dimensions of NY and especially SD nests compared to LA nests makes thermal sense, but why should SD nests be heavier and have more voluminous nest cups if weather conditions for incubation, if anything, are worse in NY (Fig. 1)? Several possible explanations exist, the first of which is that the difference in nest size is a response to differences in nest predation pressure. Corvids are one of, if not the most common nest predators of kingbirds (Murphy 1986, Blancher and Robertson 1987, Murphy 2001, Murphy et al. 2020). Both Blue Jays (*Cyanocitta cristata*) and American Crows (*Corvus brachyrhynchos*), the most common corvids of SD, are much less abundant there than in NY (Smith et al. 2020, Verbeek and Caffrey 2021), and indeed, we did not observe a single corvid at our SD sites in 2020. Small nest size in response to high threat of nest predation has been documented in the Common Blackbird (Møller 1990), and the possibility exists that kingbirds experienced predator release in SD that was expressed as larger nest size. However, we are dubious because the often "bulky", "large" and "rough and unkempt" appearance of kingbird nests (Bent 1942) suggests they do little to hide them. Rather, the main means by which kingbirds contend with nest predators is nest placement near the edges of tree canopies (Murphy et al. 1997) coupled with aggressive nest defense (Blancher and Robertson 1982, Redmond et al. 2009); placement of nests on the periphery of trees facilitates defense by species with powerful flight abilities (Ricklefs 1977).

Resource limitation, in the form of a limited supply of quality nest sites, may also help explain the heavier SD nests. Nest reuse in kingbirds is rare in most populations and is a response to limited availability of quality nest sites (Cancellieri and Murphy 2013), and nearly 10% of SD nests were built in nests of other species. Another 28% were built in the crotches of the main trunk of the nest tree, a generally uncommon behavior elsewhere (Blancher and Robertson 1985, Murphy 1983, Murphy et al. 1997). Use of suboptimal nest sites in SD may have been the source of the very high variability in nest structure of SD nests (Table 1) and may have forced kingbirds to add extra building material to secure nest placement. SD nests placed in tree crotches tended to be heavier (58.6 ± 6.72 g, n = 6) than other SD nests (47.0 ± 4.21 g, n = 14), but overlap between the 2 groups was nonetheless extensive ($t = 1.50$, $P = 0.152$).

Thermal considerations go beyond temperature, and deeper cups and smaller cup openings could help reduce convective heat loss or excessive heat gain from solar radiation. Both may be important given the typical placement of kingbird nests near the edge of the tree canopy (Murphy et al. 1997). These considerations would predict smaller cup openings and greater cup volumes in windier, high insolation environments such as SD (Fig. 1). However, nest cup depth and nest cup openings did not differ between NY and SD. Precipitation is an additional factor that might play a role in explaining smaller nest size in NY. The amount of water absorbed, and the time taken to dry a wet nest, increases with nest mass (Rohwer and Law 2010, Biddle et al. 2019). Rain falls more often and in greater amounts in NY than SD (Fig. 1), and thus smaller nests in NY may facilitate rapid drying of nests following rain, and reduce energetic demands on incubating or brooding females. Lastly, and perhaps the most parsimonious explanation for the larger cup volume in SD, is that clutch size was larger in SD (3.5 ± 0.13 eggs, n = 30) than NY (3.0 ± 0.10 eggs, n = 26) in the years of our study ($t = 2.67$, $P = 0.010$). NY nests had the narrowest internal dimensions, but NY and SD nests did not differ in nest cup depth. Thus, greater volume of SD nests was attributable to greater lateral rather than vertical expansion, consistent with greater space accommodation for a larger brood.

In contrast to nest morphology, we found no consistent differences in nest composition among sites due to the very high intrapopulation variation in material use. The latter might reflect individual preferences, but we suspect was more a product of limited availability of particular materials at a local scale, as reported for hummingbirds from Patagonia (Calvelo et al. 2006) and Pied Flycatchers (*Ficedula hypoleuca*) in England (Briggs and Deeming 2016). That said, fine stems and rootlets, forb stems, and plant down were the primary nest materials at all sites, suggesting more than passive collection. Feathers, on the other hand, were almost never observed (included in "other"). Grasses, although present in nests at all sites, was an uncommon material in NY but their use approached 20% in LA. Hilton et al.'s (2004) report that dry grasses were of particularly low insulative value possibly explains why grass was little used in NY, but was common in LA where temperatures are higher. However, high grass use in LA is almost certainly a product of local availability as the nests from the WLWCA, where grass dominated the landscape, contained abundant grasses (41.1 ± 10.6%, n = 10) in comparison to nests at other sites in LA (0.4 ± 0.45%, n = 9; $t_{\text{unequal variance}} = 3.83$, $P = 0.005$). Information on the wettability and insulative value of different nest materials indicate that rootlets and small woody stems absorb little water in simulated rain, drain easily and dry rapidly (Biddle et al. 2019), and possibly explain why coarse woody stems were essentially restricted to NY nests (Table 2).

Insulative capacity

We found that heat loss from a nest was lower when covered than not, and that there were no differences in the rate of heat loss from uncovered nests across our study sites. By contrast, covered LA nests lost heat at a higher rate than nests from NY and SD (Table 1). That nest thermal conductance differed among sites for covered, but not uncovered, nests suggests that factors beyond the nest cup opening are the primary influences on thermal conductance. Those “factors” must be structural or compositional features. However, we found no predictors of variation in the rate of heat loss from uncovered nests, suggesting that the true thermal benefits of different nests are not manifest without the physical presence of the female covering the nest contents. Incubation is energetically expensive (Vleck 1981), and an incubating bird may be constrained by a threshold during cold exposure beyond which they may not be able to warm their clutch (Cresswell 2003). We posit that if nest structure has little effect on the nest microclimate during an off-bout (equivalent to an uncovered kingbird nest), the significance of the insulative capacity of the nest structure is to reduce the energetic cost to the incubating/brooding parent and/or nestlings.

Pearson’s correlations of covered nest heat transfer coefficients suggested that heat loss was unrelated to nest composition, but was slowest in nests in a small nest cup opening relative to nest cup volume, in nests with thicker walls and smaller nest cup opening ($PC3_{morph}$), and in larger nests with thick walls and deep nest cups ($PC1_{morph}$). These results essentially distinguish LA nests from NY and SD nests (Table 1). Interestingly, the only competitive model from our multivariate analysis of heat loss included relative area of the nest cup opening and $PC2_{morph.}$ Greater heat loss from nests with large nest cup opening and a small nest cup volume is unsurprising. The inverse relationship between heat loss and $PC2_{morph}$ indicated that heat loss was lower in nests with asymmetrical nest cup openings that had shallow and low volume nest cups.

Although it is unclear what drove the relationship between heat loss and nest cup asymmetry, the lower heat loss from nests with shallow and low volume nest cups makes intuitive sense and may be a product of the warming of the smaller volume of air within the covered nest by the iButton, thus reducing the gradient for heat loss from the iButton. Whether this would have bearing for a nest whose contents were covered by a female is unclear, and might instead reflect an outcome of our experimental design.

Restricting our analysis to the more structurally uniform NY and SD nests led to a somewhat different set of conclusions. Model averaged results from the 4 competitive models indicated that $PC2_{morph}$ contributed (as described above), but that nest composition was a major source of variation in the thermal properties of nests. Lower heat loss occurred in nests in which plant down, coarse woody stems ($PC2_{comp}$), and fine stems and rootlets ($PC3_{comp}$) were abundant and grass and forb stems ($PC2_{comp}$ and $PC3_{comp}$) were uncommon. Grass is a poor insulator whether wet or dry (Hilton et al. 2004), while rootlets and small woody stems do not absorb water easily (Biddle et al. 2019) and can thus provide effective insulation in a wide range of conditions. Hilton et al. (2004) showed that dry down feathers were the most insulative of all materials in their study, and plant down presumably provides similar benefits. Wet down feathers have reduced insulative capacity, but assuming a parent bird covers its nest during periods of rain, inclusion of plant down should add high insulative value to a nest. And, indeed, abundant plant down was associated with low heat loss. Thus, thermal qualities of kingbird nests appear as much a product of the materials used to build the nest as its mass and dimensions.

Conclusion

Although further research is needed to account for most of the observed variation in kingbird nests structure and composition, our results show that kingbirds breeding at sites experiencing substantial differences in breeding season weather build nests that conform well to prevailing conditions. Like Rohwer and Law (2010) and Mainwaring et al. (2014), our intraspecific comparisons showed that the insulative capacity of nests was greater at higher latitude sites where ambient temperatures were typically lower. However, factors other than temperature likely contribute to differences in nest structure, and in particular, the contribution of rainfall to differences in nest design should draw further attention from researchers. Open-cup nesting kingbirds at all sites face the potential threat of excessive solar heat loads that must be mitigated by either selective nest placement or parental shading of nest contents. Kingbirds on the Great Plains face the additional pressure of potentially greater convective heat exchange due to high and persistent winds (Fig. 1). Furthermore, conditions during the later breeding season when nestlings are being fed may dictate building designs to protect against higher temperatures (Schaefer 1980). As suggested by Audubon (cited in Schaefer 1980), thinner nest walls in Baltimore Orioles (*I. galbula*) breeding in LA, as we also found for kingbirds, may provide greater capacity to lose heat from nests that regularly experience high and potentially dangerous temperatures. Finally, an important need is to better integrate analyses of nest structure of open-cup nesting species with nest placement in trees or shrubs to evaluate the extent to which deficiencies in nest construction can be offset by placement that yields thermal benefits for eggs, nestlings, and/or parents.

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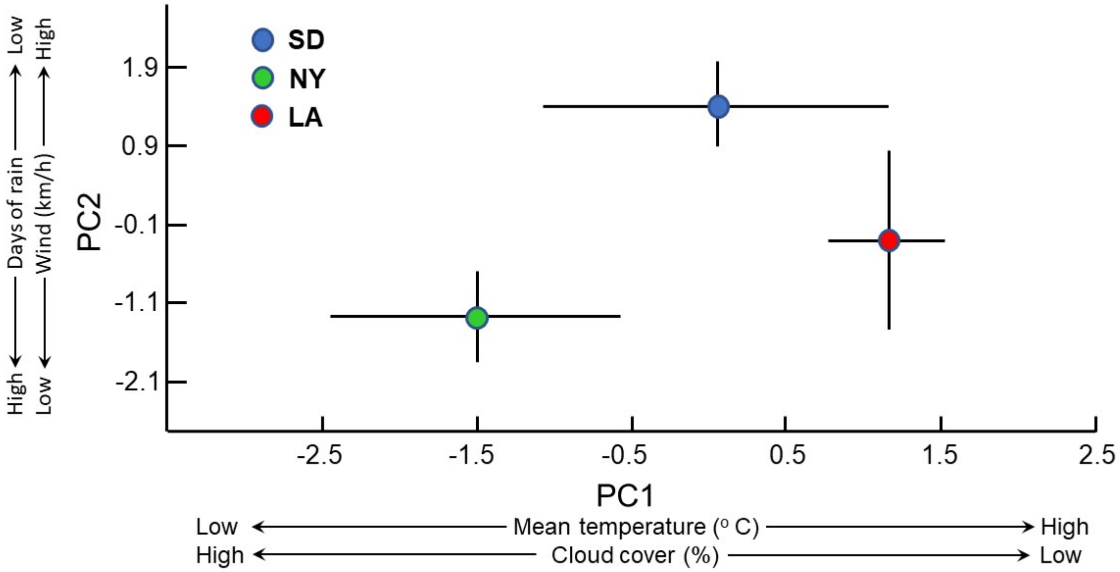
Figure legends

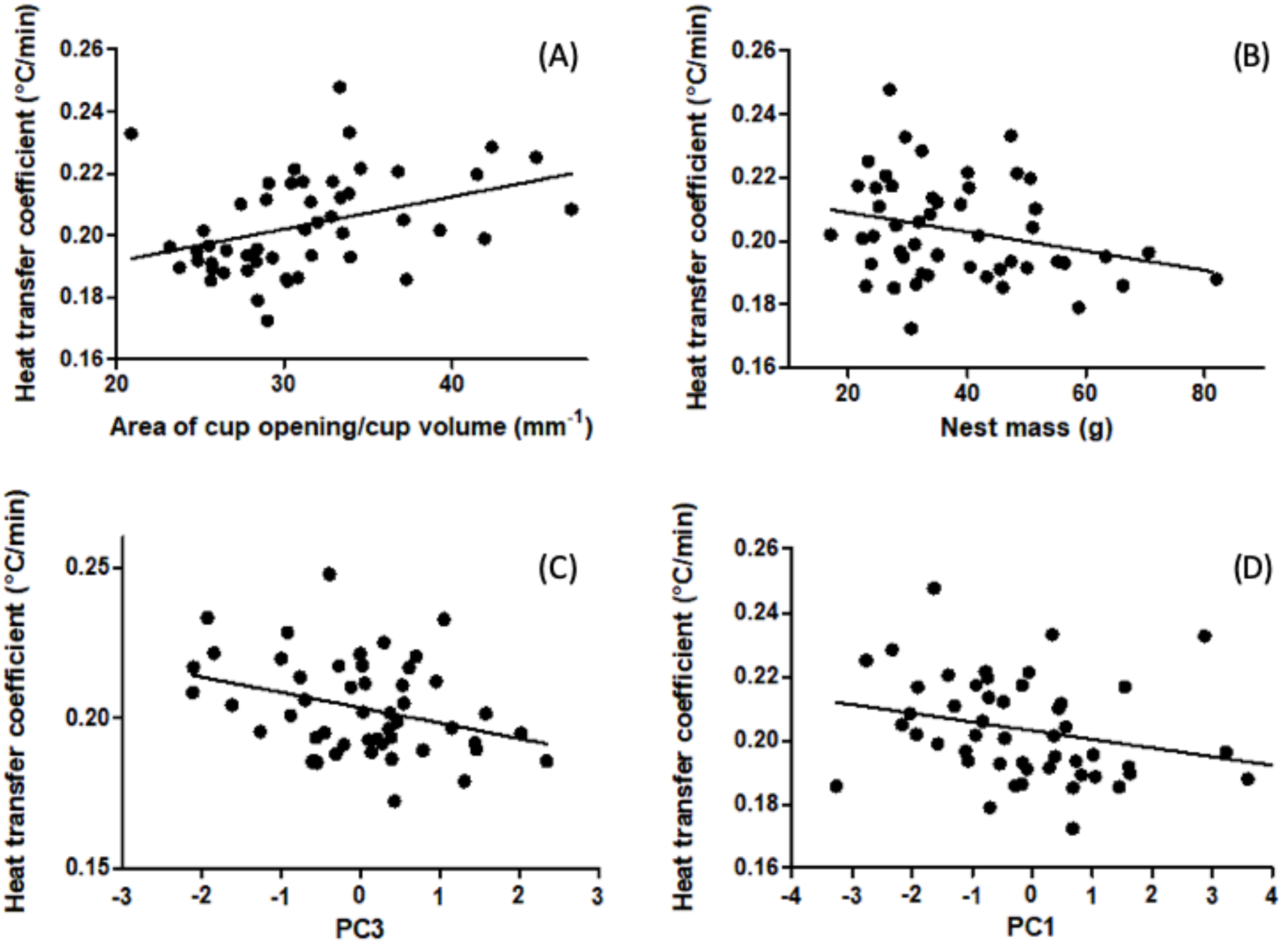
Fig. 1. Comparisons of average climatic conditions over the 4-month period including the month prior to breeding (April) and the remainder of the breeding season of Eastern Kingbirds (May through July) in Kansas, Louisiana, New York, and South Dakota. The four variables in the principal component analysis (PCA) included mean monthly temperature, percentage of days/month that were cloudy, number of rainy days/month, and average monthly wind speed. The 2 axes accounted for 94% of the variation in climate across study sites. Data are presented as mean ± 95% confidence intervals along factor 1 and factor 2 from the PCA.

Fig. 2. Heat transfer coefficient of nests as a function of (A) the ratio of area of the nest cup opening/cup volume, (B) total nest mass, (C) $PC3_{morph}$, for which high values are nests with thick walls and small cup opening area, and (D) $PC1_{morph}$, for which high values are large nests. Rate of heat loss was measured with iButtons (Maxim: DS1922L and DS1923) in a climate-controlled chamber set to 50% relative humidity and 7°C or 20°C. Nests were collected from New York (2019), Louisiana (2021), and South Dakota (2020). (NY n = 18; SD n = 20; LA n = 20).



SD
NY
LA
1.9
0.9
-0.1
-1.1
-2.1
PC2
High ← Days of rain → Low
Low ← Wind (km/h) → High
-2.5
-1.5
-0.5
0.5
1.5
2.5
PC1
Low ← Mean temperature (° C) → High
High ← Cloud cover (%) → Low

Fig. 2.


(A)
Heat transfer coefficient (°C/min)
0.26
0.24
0.22
0.20
0.18
0.16
20
30
40
Area of cup opening/cup volume (mm^{-1})
(B)
Heat transfer coefficient (°C/min)
0.26
0.24
0.22
0.20
0.18
0.16
20
40
60
80
Nest mass (g)
(C)
Heat transfer coefficient (°C/min)
0.25
0.20
0.15
-3
-2
-1
0
1
2
3
PC3
(D)
Heat transfer coefficient (°C/min)
0.26
0.24
0.22
0.20
0.18
0.16
-4
-3
-2
-1
0
1
2
3
4
PC1

Tables

Table 1. Means, standard errors (SE) and coefficient of variation (CV) for nest mass and structure for nests collected from New York (2019), South Dakota (2020) and Louisiana (2021), along with average scores on axes 1 (PC1$_{morph1}$), 2 (PC$_{morph2}$), and 3 (PC$_{morph3}$) from the principal component analysis of structural nest variables. Average heat transfer coefficient measured from nests whose opening was either uncovered or covered are also reported. Superscripts denote homogeneous groups for materials that differ significantly across study sites. (NY, n = 18; SD, n = 20; LA, n = 20) Add

Variables	NY (mean ± SE); CV	SD (mean ± SE); CV	LA (mean ± SE); CV	F (P)
Nest mass (g)	33.27 ± 1.98^{B}; 25.31	50.48 ± 3.68^{A}; 32.62	32.52 ± 2.30$^{B;}$ 31.64	13.36 (< 0.001)*
Area cup opening (mm^{2})	4280.1 ± 82.49^{B}; 8.18	4483.0 ± 123.44^{AB}; 12.31	4756.1 ± 96.27^{A}; 9.05	5.25 (0.008)
Nest cup depth (mm)	46.55 ± 1.06^{AB}; 9.67	49.66 ± 1.63^{B}; 14.66	45.70 ± 0.98^{A}; 9.61	2.76 (0.072)
Cup volume (mL)	145.02 ± 4.26^{B}; 12.48	163.94 ± 5.26^{A}; 14.35	138.82 ± 4.38^{B}; 14.12	8.02 (< 0.001)
Wall thickness (mm)	21.87 ± 0.70^{A}; 13.63	22.39 ± 1.09^{A}; 21.80	17.73 ± 0.51^{B}; 12.86	10.12 (< 0.001)*
Inner dimension width (mm)	73.88 ± 0.71^{A}; 4.07	75.57 ± 1.05^{B}; 6.25	77.80 ± 0.78^{C}; 4.49	5.01 (0.010)
Cup asymmetry	1.10 ± 0.02; 6.32	1.11 ± 0.02; 8.73	1.07 ± 0.01; 4.24	1.64 (0.204)
Outer dimension width (mm)	135.64 ± 1.57^{A}; 4.92	132.13 ± 2.84^{A}; 9.62	124.59 ± 1.39^{B}; 5.00	7.39 (0.001)*
Relative nest cup opening	30.04 ± 1.20^{B}; 16.88	27.80 ± 1.01^{B}; 21.80	34.84 ± 1.21^{A}; 15.54	10.27 (< 0.001)
PC1$_{morph}$	0.117 ± 0.211)^{A}	1.069 ± 0.433^{A}	-1.175 ± 0.228^{B}	12.91 (< 0.001)
PC2$_{morph}$	0.235 ± 0.277	-0.003 ± 0.310	-0.209 ± 0.204	0.68 (0.511)
PC3$_{morph}$	0.564 ± 0.179^{A}	-0.009 ± 0.275^{AB}	-0.498 ± 0.198^{B}	5.50 (0.007)
Uncovered heat transfer (° C/sec)	0.227 ± 0.0056; 10.44	0.220 ± 0.0121; 20.50	0.234 ± 0.0107; 19.42	0.51 (0.604)
Covered heat transfer (° C/sec)	0.196 ± 0.0026^{A}; 5.53	0.196 ± 0.0038^{A}; 7.20	0.217 ± 0.0029^{B}; 5.70	16.81 (< 0.001)

* Unequal variances using Levene's test (P ≤ 0.05)

Table 2. Means, standard errors (SE) and coefficient of variation (CV) for nest composition materials of nests collected from New York (2019), South Dakota (2020), and Louisiana (2021), along with average scores on axes 1 ($PC1_{comp}$), 2 (PC_{comp2}), and 3 (PC_{comp3}) of the principal component analysis of nest composition variables. Superscripts denote homogeneous groups for materials that differ significantly across study sites. (NY n = 18; SD n = 20; LA n = 20)

Variables	NY (mean ± SE); CV	SD (mean ± SE); CV	LA (mean ± SE); CV	*F* (*P*)
Fine stems and rootlets	33.89 ± 5.17; 64.72	37.00 ± 4.97; 60.02	35.50 ± 4.37; 55.01	0.10 (0.903)
Forb stems	35.83 ± 6.63; 78.53	29.25 ± 5.50; 84.16	24.00 ± 5.75; 107.21	0.97 (0.384)
Grasses	4.17 ± 2.92; 297.52	10.75 ± 5.85; 243.45	18.75 ± 6.55; 156.18	1.75 (0.183)
Plant down	20.28 ± 4.19; 87.68	17.75 ± 5.65; 87.68	21.50 ± 4.65; 96.66	0.16 (0.856)
Coarse woody stems	5.56 ± 3.18^{A}; 242.85	0.25 ± 0.25^{B}; 447.21	0.00^{B}	5.14 (< 0.001)[1]
Other	0.28 ± 0.28; 424.26	5.00 ± 4.07; 364.19	0.25 ± 0.25; 447.21	1.27 (0.288)
$PC1_{comp}$	-0.374 ± 0.257	-0.000 ± 0.244	0.337 ± 0.306	1.68 (0.195)
$PC2_{comp}$	0.211 ± 0.315	-0.153 ± 0.268	-0.037 ± 0.228	0.46 (0.633)
$PC3_{comp}$	-0.002 ± 0.248	-0.006 ± 0.309	-0.004 ± 0.210	0.00 (1.000)
[1] Kruskal-Wallis test				

Table 3. Factor loadings, and eigenvalues, and proportion of variance in data set that was accounted for by the first three axes from principal component analysis (PCA) of seven measures of Eastern Kingbird nest morphology from study sites in New York (2019), South Dakota (2020), and Louisiana (2021). (NY, n = 18; SD, n = 20; LA, n = 20).

Factor	PC1	PC2	PC3
Nest mass	**0.378**	0.298	-0.173
Nest cup depth	**0.349**	**-0.613**	0.085
Cup volume	**0.496**	**-0.345**	-0.137
Wall thickness	**0.456**	0.144	**0.329**
Cup asymmetry	0.210	**0.606**	-0.027
Area cup opening	-0.010	-0.046	**-0.908**
Outer dimension width	**0.486**	0.163	-0.101
Eigenvalue	2.786	1.360	1.126
Percent of variance	39.8	19.4	16.1
Cumulative variation	39.8	59.2	75.3

Table 4. Factor loadings, and eigenvalues, and proportion of variance in data set that was accounted for by the first three axes from principal component analysis (PCA) of materials comprising nests of Eastern Kingbirds from study sites in New York (2019), South Dakota (2020), and Louisiana (2021). (NY, n = 18; SD, n = 20; LA, n = 20).

Factor	PC1	PC2	PC3
Fine stems and rootlets	**0.326**	0.002	**0.757**
Forb stems	**-0.698**	**-0.416**	0.049
Grasses	**0.619**	**-0.320**	**-0.410**
Plant down	-0.124	**0.747**	-0.016
Coarse woody stems	-0.010	**0.406**	-0.292
Other	-0.089	-0.043	**-0.414**
Eigenvalue	1.459	1.381	1.259
Percent of variance	24.3	23.0	21.0
Cumulative variation	24.3	47.3	68.3

Table 5. Results of competitive models (ΔAICc ≤ 2.0) from the best subsets analysis of variation in heat transfer coefficients from nests of Eastern Kingbirds from New York and South Dakota. Output includes number of parameters (k), AICc and ΔAICc scores, along with model weights (w_i), the variation explained by each model (R^2), and likelihood of each model in relation to the top model.

Model	k	AICc	ΔAICc	w_i	R^2	Likelihood vs model 1
$PC2_{morph} + PC2_{comp} + PC3_{comp}$	5	-285.16	0.000	0.353	0.358	1.000
$PC2_{morph} + PC2_{comp} + PC3_{comp\,+}$ Area/Cup volume	6	-285.15	0.007	0.352	0.415	0.997
$PC1_{morph} + PC2_{morph}$ + Area/Cup volume	5	-283.55	1.611	0.158	0.325	0.447
$PC2_{morph} + PC1_{comp}$	4	-283.27	1.889	0.137	0.256	0.389

Table 6. Model averaged coefficients (standard error in parentheses) and 85% confidence intervals from analyses of variation in heat transfer coefficients of Eastern Kingbirds nests from New York and South Dakota in relation to structure and composition of nests. Predictor variables with 85% CI (see Arnold 2010) that excluded zero are highlighted in bold font.

Predictor variable	Estimate (SE)	85% CI
$PC2_{morph}$	**-0.0046 (0.00171)**	**-0.00714 to -0.00212**
$PC2_{comp}$	**-0.0028 (0.00137)**	**-0.00486 to -0.00085**
$PC3_{comp}$	**-0.0029 (0.00150)**	**-0.00513 to -0.00066**
Relative nest cup opening	0.0005 (0.00041)	-0.00004 to 0.001044
$PC3_{morph}$	0.0004 (0.00940)	-0.014170 to 0.01340
$PC1_{comp}$	-0.00054 (0.00052)	-0.0013 to 0.0002

Chapter III

How to build an egg: Geographic variation in Eastern Kingbird *Tryannus tyrannus* egg allometry in relation to climate

Keywords *Albumen, allometry, eggs, geographic variation, weather, yolk*

Abstract

Egg mass and composition have important effects on offspring survival and fitness; however, studies of intraspecific geographic variation of egg qualities are sparse. In this work we characterize geographic variation of eggs from Eastern Kingbirds *Tyrannus tyrannus* studied in Kansas (KS), New York (NY), South Dakota (SD), and Louisiana (LA). Our sites were climatically varied, differing with respect to mean temperature, days of precipitation, cloud cover, and wind speed. Egg mass was highly variable, did not differ across sites, and was most strongly (positively) correlated with albumen mass. Wet masses of egg components increased isometrically with egg mass, except for albumen and yolk in KS, which increased hyper- and hypoallometrically, respectively, and shell in SD, which increased hypoallometrically. Dry masses of components increased isometrically with dry egg mass, with the exception of shell mass in SD. There was no relationship between clutch size and egg mass or composition, but we found significant differences in composition across sites. Eggs from colder and rainier NY contained the least water of all sites and had greater yolk to albumen ratio than eggs from warm, sunny and dry LA or warm and windy SD. We posit that the greater yolk mass of NY eggs supports the extended incubation period observed in NY kingbirds compared to those in KS and that the greater water content of KS eggs supports embryos in the warmer and windier climate.

Introduction

Egg mass is a measure of a female's investment in a single offspring that has important fitness consequences for that offspring (Krist 2011). Interspecific differences in egg mass scale allometrically with body mass (Sæther 1987, Deeming 2007), but although egg mass typically exhibits high variability within species, (Bancroft 1984, Hochacka 1993, Bafibura & Zielifiski 1998, Tryjanowksi et al. 2001), female body size accounts for little of the observed variation (reviewed in Christians 2002). Intrapopulation variation has in some cases, been attributed to proximate effects of female body condition (Murphy 1986a) or food availability (Ardia et al., 2006; Murphy, 1986b; Ramsay & Houston, 1997), but environmental effects on egg size appear weak (e.g., Ojanen 1983). Hence, egg size has emerged as the most repeatable of reproductive traits in birds (Christians 2002). Nonetheless, we remain largely ignorant of what maintains high intrapopulation variation in egg mass, and only rarely have egg traits been integrated into geographic analyses of avian life histories (but see Martin et al. 2006, Martin 2008, Stein and Badyaev 2011).

Intraspecific and comparative geographic analyses of egg composition are even rarer than intrapopulation studies despite the importance of the proportions of yolk and albumen for embryos (Deeming 2007, Willems et al. 2014). Egg yolk comprises 20-25% of egg mass in altricial species, is ~55% water (Sotherland & Rahn 1987), and because of its high lipid content (Sotherland and Rahn 1987), is the primary fuel for embryo metabolism. It has long been known that long incubation periods of precocial species are associated with large yolk volumes (Sotherland and Rahn 1987), but even within development modes, the proportion of yolk is greater in species with longer incubation periods (Sotherland & Rahn 1987). Yolk may also contain female-derived vitamins (Royle et al., 1999), hormones (Schwabl, 1993) and immunoglobulins (Rose & Orlans 1981, Hargitai et al. 2006) that enhance embryo growth and development. Moreover, egg yolk that is not metabolized during development remains with the hatchling (Bancroft 1984, Rofstad and Sandvik 1987, Enemar, 2001) and supplies energy and nutrients for young if foraging conditions at hatching limit food deliveries to newly hatched young (O'Connor 1979, Bancroft, 1985). Albumen, by contrast, accounts for 70-75% of egg mass of altricial birds, is ~89% water, with the remainder being mainly protein (10%) and trace carbohydrates, lipids, and inorganic ions (Ward 1992 in Ferrari et al. 2006). Although yolk content has generally garnered more attention than albumen, the latter is critically important because its large volume, especially in altricial species, make it the main source of protein and water for the embryo (Willems et al. 2014).

Shells provide the barrier that separates the embryo from its surroundings, account for 8-10% of fresh egg mass in altricial species (Deeming 2007), and are essential for regulating water loss. On average, eggs lose 15% of their initial mass as water by hatching (Ar and Rahn 1980) through pores, the density and length (~ egg shell thickness) of which balance essential gas exchange with necessary water loss to avoid embryo dehydration. Change in shell pore density and length is generally viewed as the means of regulating water loss from bird eggs exposed to different hydric stresses (Carey et al. 1983, Arad et al. 1988, Stein and Badyaev 2011), but extra albumen may provide additional water to provide for the embryo’s needs (Ar and Rahn 1980). Moreover, assuming large eggs are favored (Krist 2011), enlarging eggs by increasing albumen rather than yolk conserves energy (due to the much higher energy density of yolk;

Bancroft 1984), and may also promote thermal stability of eggs by enabling larger (and thermally more stable) eggs to be constructed using albumen. Moreover, given that albumen is the primary source of protein for growing embryos, larger eggs with more albumen confer the benefits of a larger hatchling (Willems et al. 2014). The latter may also yield advantages in cold or rainy climates where large egg and large hatchling size may help buffer against declines in temperature from cold stress. However, if incubation is regularly extended by unfavorable ambient conditions such as cold and wet weather (Gillette et al. 2021, Lundblad & Conway 2021), laying larger eggs with more yolk provides embryos with the energy reserves to sustain them through a longer incubation period and potentially even carry over as an energy reserve for hatchlings (Bancroft 1984, Rofstad and Sandvik 1987, Enemar, 2001).

Evidence that birds modify egg composition is sparse, but exists. For instance, high food availability has been shown to positively influence yolk mass in White-rumped Swallows (*Tachycineta leucorrhoa*; Barrionuevo et al. 2014), but not in Tree Swallows (*Tachycineta bicolor*; Ardia et al. 2006). Ferrari et al. (2006) also showed that albumen content of eggs increased with laying order in Barn Swallow (*Hirundo rustica*) and that growth and survival of last-hatched nestlings was enhanced as a result. Whether such subtle modifications to egg composition are widespread is largely unknown, but it seems plausible that populations of the same species may modify egg features as a life history response to predictable differences in environments (e.g., Stein and Badyaev 2011).

Eastern Kingbirds (*Tyrannus tyrannus*; hereafter “kingbird”) breed across much of North America (Murphy and Pyle 2018) and experience large differences in climate during the breeding season. They are aerial insectivores and weather has been shown to influence foraging behavior and success (Murphy 1987), while offspring growth and survival (Murphy 1983), fledging success (Murphy et al. in press), and incubation length (Gillette et al. 2021) are all adversely affected by low temperatures in this open-cup nesting, long-distance Nearctic-Neotropical migrant. In this work we examine egg size and composition (shell, yolk, and albumen) of kingbirds breeding at four widely spaced sites in North America that differ in breeding season climate to evaluate whether kingbird egg size and composition vary geographically in a manner reflecting local selective pressures.

Our sites in Kansas (KS), New York (NY), South Dakota (SD), and Louisiana (LA) present a wide range of climates (described more fully below) in which LA and NY are the endpoints along a continuum. The early reproductive season of LA is warm and sunny with moderate amounts of rain whereas the comparable period in NY experiences cloudy and cool conditions with frequent rain. SD contrasts with the other sites for its limited rainfall, dry conditions, and consistent high winds. Given these differences, we predicted that (1) birds breeding at locations experiencing low ambient temperatures would lay larger eggs because large eggs provide a thermal benefit of cooling slowly when unattended (Williams 1994) in addition to producing heavier hatchlings (Murphy 1985). However, egg size may also vary with the number of eggs produced and therefore we tested for (2) a trade-off between number and size of eggs as predicted by life history theory (Smith and Fretwell 1974), and the alternative possibility that, because egg mass and clutch size both increase in response to high food abundance in studies of annual variation of kingbird reproduction (Murphy et al. in press), larger eggs would be associated with larger clutch size. We also test whether egg composition varies with clutch size or geography. With respect to clutch size, we (3) tested whether yolk varied either inversely or directly with clutch size (for reasons described above for egg mass). We further test the possibility that (4) yolk contributes more to egg mass in colder regions to support either longer embryonic development during the weather-induced longer incubation period that has been observed in kingbirds (Gillette et al., 2021), and the alternative that greater yolk reserves may benefit hatchlings in more climatically stressful environments. By contrast, we predict that (5) eggs laid in hotter, drier and/or windier environments would contain proportionately more albumen to supply water to the developing embryo (Willems et al. 2019).

Methods

Study systems and field work

We studied kingbirds breeding in Lawrence, KS (38.57° N, 95.19° W, 264 m above sea level [asl]), Oneonta, NY (42.45° N, 75.06° W, 339 m [asl]), Pierre, SD (44.37° N, 100.35° W, 450 m asl), and Gueydan, LA (30.03° N, 92.51° W, 2 m asl). The KS clutches were collected 1n 1983 as part of another study (Murphy 1986a) that we combine with clutches collected in 2019, 2020, and 2021 in NY, SD, and LA, respectively. Long-term historical weather records for the breeding season (April – July) (Weather Atlas, 2022) indicated that NY experiences the lowest temperatures, receives the most days and total amount of precipitation, is tied for lowest wind speeds, and experiences the fewest days of sun and most cloud cover (Table 1). At the other extreme, LA experiences the warmest temperatures, but receives similar amounts of rainfall, total days of rain, sun and cloud cover as KS and SD, but is less windy than all other sites except NY. KS and SD weather is intermediate in most respects except that total rainfall in KS does not differ from NY, and SD has the highest wind speeds (Table 1). A plot of eigenvectors 1 and 2 from a principal component analysis of four of the weather variables (mean temperature [average of high and low temperatures], days of rain/month, average wind speed, and cloud cover), which accounted for 94% of the differences in weather among the 4 sites, visualizes the strong differences among sites, especially the distinctiveness of NY in comparison to the others (Fig. 1).

We began fieldwork as kingbirds arrived from spring migration in late April in LA, mid-May in KS, and June in NY and SD. We timed our visits in the specific years of our study to coincide with the start of nest-building and egg-laying. Kingbirds in KS nested primarily in mulberry (*Morus* spp.), osage orange (*Macluara pomifera*) or elms (*Ulmus* spp.); see Murphy (1983) for a complete description of the study site. NY study sites were centered on Charlotte Creek and surrounding floodplain and uplands habitats in Delaware and Otsego counties. In riparian zones, kingbirds nested in ironwood trees (*Carpinus caroliniana*) but in other habitats nested in hawthorns (*Crataegus* spp.) or apple (*Malus* spp.). A full description of the NY study site can be found in Murphy (2001). In SD, the floodplains of the Missouri river, shoreline of Lake Oahe (a reservoir of the Missouri River), and draws and valleys of tributary streams of Stanley, Sully and Hughes counties were the primary nesting sites. Kingbirds nested mostly in Russian olive (*Elaeagnus angustifolia*) or their snags, and Siberian elm (*Ulmus pumila*) trees, but also plains cottonwood (*Populus deltoides*), juniper (*Juniperus spp.*), and ash (*Fraxinus spp.*). We conducted our research in LA in two distinctly different habitats, either coastal prairie or the marshes of White Lake Wetlands Conservation Area (WLWCA), both located in

Vermilion Parish. Kingbirds nesting in the coastal prairie placed their nests on branches overhanging water-filled ditches along roadsides or in canals between agricultural fields and used mainly nonnative Chinese tallow (*Triadica sebifera*), but also bald cypress (*Taxodium distichum*) and willow (*Salix spp.*) trees. Kingbirds breeding in WLWCA nested almost exclusively in buttonbush (*Cephalanthus occidentalis*), but also in southern live oaks (*Quercus virginiana*) and bald cypress.

At all sites kingbird nests were located by searching potential nest trees in habitat occupied by kingbirds and by following nest building behavior of females. Given the timing of our visits to each site, all clutches in our analysis were initial nests of the season. Once located, nests were checked every 1-2 days to document egg-laying. At WLWCA, however, nest checks occurred at 3 to 4 days intervals because of limited access. Nests are typically placed at least 2 m aboveground and thus most nest checks were made using a mirror attached to an extensible pole. Although we were able to access some nests from the ground (or boat at WLWCA), most required that we climb the nest tree or use ladders. Kingbirds lay one egg per day, though occasionally there is a skipped day between the laying of the penultimate and the final egg. We did not observe egg-laying after 10:00, thus we considered the egg-laying period completed when no new egg had appeared by 10:00 on the second day of an absence of egg-laying. Nests and eggs were collected immediately following completion of the laying period so that the onset of incubation would not alter egg mass or composition. The KS permit allowed us to collect >20 clutches and we obtained 21. Our other permits allowed us to collect 20 nests and their clutches, and therefore we collected the first 20 clutches that we were able to access and which survived to the completion of laying. Our LA sample included 21 clutches because separate collecting activities by SMG and MTM mistakenly resulted in 1 extra clutch. Clutches were stored in the field in coolers below ambient temperature until processing. Within one day after collection, eggs were weighed to the nearest 0.001 g (top-loading balance), after which each clutch was held in a separate air-tight container at approximately 4° C until further processing within 1 to 10 days after collection.

Quantifying Egg Composition

We used the same methods as Murphy (1986) to quantify kingbird egg composition. A scalpel was used to open each egg over a pre-weighed "weigh boat" that received the albumen and yolk from each egg. We separated yolk from albumen by using a laboratory spatula to slide the yolk into a second pre-weighed weigh boat. Any albumen remaining attached to the yolk or shell was removed by dabbing with tissue paper. The shell was placed into a third pre-weighed weigh boat. In some nests at all sites, the yolk failed to maintain its integrity and "bled" into the albumen in one or more eggs. We suspect this was a result of either early onset of incubation by females or ambient incubation induced by exposure to high temperatures and solar radiation (Gillette et al. 2021). In either case, we removed these eggs from the analysis and the result was that sample size for egg composition analysis were less than the number of eggs laid by some females.

Wet mass of the shell (and shell membranes) and yolk were obtained by subtracting the mass of the weigh boat from that of the weigh boat and its contents. Albumen wet mass was calculated as the difference between egg mass measured on the day of collection and the wet mass of the shell and yolk. Shell, yolk, and albumen were then dried in a dehydrating oven (~50° C) for at least 48 hours and until samples did not change mass across 12 hours of drying. We then calculated total dry egg mass of each egg by adding the dry masses of the three egg components. The proportional water content of albumen and yolk was calculated as 1 minus the quotient of dry mass divided by wet mass of each component. Total water weight was calculated as the sum of albumen and yolk water content. Wet and dry mass of egg components were weighed to the nearest 0.001g.

Statistical Analysis

To avoid violating assumptions of statistical independence of data points, we averaged data for all eggs laid by a single female to generate a single observation/female, which we then compared across sites using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). Post-hoc comparisons of means among sites were made using Tukey's Honest Significant Difference (HSD) test when site differences were $P \leq 0.05$. For statistical differences at $0.05 < P < 0.10$, we used the least significant difference (LSD) test for post-hoc comparisons. We $\log_{10}$ transformed total wet and dry egg mass and components to describe allometric relationships. We defined allometric relationships as isometric (slope [β] = 1), hypoallometric ($\beta < 1$), or hyperallometric ($\beta > 1$). To test for effects of geographic location on the relationship between the mass of each component and total egg mass, we used analysis of covariance of either wet or dry egg mass as the covariate with site and clutch size as fixed effects. This eliminated seven nests for which clutch size was uncertain. Once we established that clutch size did not affect egg properties (see below) we reexamined site differences using all nests. As an alternative approach we also analyzed all individual eggs using general linear mixed models with female identity included as a random effect. Results of the analyses using female averages and individual eggs gave qualitatively identical results and therefore we only report the results from our analyses using female averages.

Statistical analyses were performed in either STATISTIX (Analytical Software v. 9) or JMP (Statistical Analysis System, v. 12). Statistics are reported as mean ± standard error (SE), and sample size (n). We agree with the American Statistical Association's view that *P*-values should not be used to establish hard lines between significant and nonsignificant results. Instead we interpret *P*-values as a continuously varying metric of the strength of results (Wasserstein et al. 2019).

Results

General patterns

Across sites, wet egg mass averaged 4.07 g (± 0.043 g), of which 72.7% was albumen (2.96 ± 0.034 g), 21.6% was yolk (0.88 ± 0.012 g), and 5.6% was shell (0.23 ± 0.002 g, n = 78 clutches). Our observed values for albumen and yolk compared favorably to the allometrically predicted values (2.83 g and 0.88 g, respectively), but shell was only 72% of that predicted allometrically (0.32 g; Deeming 2007). Total egg mass was most strongly correlated with albumen mass ($r = 0.966$, $P < 0.001$) and least correlated with yolk mass ($r = 0.698$, $P < 0.001$), with the relationship with shell being intermediate ($r = 0.785$, $P < 0.001$; n = 78 for all). Water content of eggs was 77.1% of total fresh egg mass and egg water content and egg mass were highly correlated ($r = 0.993$, $P < 0.001$). Albumen and yolk increased in tandem, but the strength of their relationship was not high ($r = 0.492$, $P < 0.001$; Fig. 2A) because of the high variability shown by each and the inescapable trade-off between the proportion of yolk and albumen in eggs (Fig. 2B). Given that increasing egg mass was driven mainly by increasing albumen mass, and the general inverse relationship that exists between surface area and volume of spherical objects, the inverse relationship between the proportions of egg that was shell and albumen was expected (Fig. 2C). On the other hand, the proportion of egg that was shell tended to increase with the proportion that was yolk (Fig. 2D, $r = 322$, $P < 0.001$).

Interpopulation variation in egg mass and composition

Wet egg mass varied widely within all sites (KS: 3.13 g to 4.80 g; LA: 3.37 g to 4.83 g; NY: 3.64 g to 4.78 g; SD: 3.30 g to 4.87 g), but did not differ among them (Table 2). Given that the proportion of albumen that was water (0.896 ± 0.0007) did not differ among sites (ANCOVA: $F = 1.46$, df = 3, 74, $P = 0.233$), it follows that sites did not differ in either wet or dry albumen mass (Table 2). Shell mass likewise did not differ among sites (Table 2). All sites exhibited similar wet yolk mass, but dry yolk mass was higher in NY than at all other sites except for SD (Table 2). This is consistent with our finding that the proportion of yolk that was water (0.558 ± 0.002) differed among sites ($F = 3.16$, df = 3, 74, $P = 0.030$) because water made up a smaller proportion of yolk in NY (0.548 ± 0.0046) than KS (0.566 ± 0.0034; Tukey's post-hoc test, $P < 0.05$). Total water content, the sum of albumen and yolk water, was significantly lower in NY than all other sites (ANCOVA: $F = 5.64$, df = 3,73, $P = 0.002$).

Allometric analyses

Results of our ANCOVA indicated the wet and dry albumen and yolk increased isometrically with egg mass (i.e., log-log slope [β] = 1.0), clutch size had no influence on either wet or dry egg composition (Table 3), but that among-site differences existed for wet albumen and wet yolk mass, but not dry mass (Table 3). The suggestive relationships between shell mass and clutch size were due to a single nest (see Table 3). Given the absence of an influence of clutch size on egg contents, we removed clutch size from the ANCOVA and reanalyzed egg composition using all nests including those in which final clutch size was unknown. Wet and dry albumen and yolk masses again increased isometrically with egg mass, but shell increased hypo-allometrically with wet egg mass, but isometrically with dry egg mass. Among-site differences existed for both wet and dry albumen and yolk (Table 4) that we explore further below using site-specific allometric analyses.

Separate allometric analyses within each site showed that most of the site differences were due to contrasts between NY and LA, but also that the way by which increasingly larger eggs were produced at each site differed. Wet albumen mass increased isometrically with egg mass at all sites, except for a hyper-allometric (i.e., $\beta > 1.0$) increase in KS (Table 5; Fig. 3a). βs describing the allometric relationship between wet yolk and wet egg mass tended to be lower than those for albumen mass (Table 5), but yolk βs also varied isometrically with wet egg mass at all sites, again except for KS where yolk mass increased hypo-allometrically (i.e., slope < 1.0; Table 5; Fig. 3b). With respect to shell mass, however, SD was the odd site in that shell mass increased hypo-allometrically with increasing wet egg mass whereas at all other sites the increase in shell mass was isometric (Table 5; Fig. 3c). Identical allometric analyses of the mass of dry egg components were all isometric with the exception of the hypo-allometric increase in shell mass with egg mass in SD (Table 5; Fig. 4c).

To further explore differences in egg composition among sites, and to describe the means by which yolk and albumen were added to build increasingly larger eggs at all sites, we compared the contribution of yolk relative to albumen by dividing the allometric equation for yolk content by the allometric equation for albumen content. The coefficient for the resulting equation, obtained as the quotient of the ratio of the coefficients for yolk to albumen, represents the elevation of yolk to albumen in a log-log plot. The exponent (β) in the prediction equation is the difference obtained by subtracting the exponent for albumen from the exponents for yolk; positive and negative values for the latter indicate that yolk's contribution to egg mass was either increasing or decreasing, respectively, relative to albumen as total egg mass grew. Separate calculations were made for wet and dry egg contents.

With respect to wet egg mass, eggs were built differently among sites as they increased in size. In KS, the sharp decline in the contribution of yolk as egg mass increased (Fig. 5A) clearly indicated that larger eggs were built mainly by adding albumen. The declining contribution of yolk in larger eggs was also evident in NY and LA, but the decrease in yolk as egg mass increased was less severe than in KS. In SD, the similarity of the exponents for yolk (1.060) and albumen (1.008), and thus the small difference between them (0.052), indicated that as egg mass increased the amount of yolk and albumen were added at equivalent rates. The overall upward displacement of the NY line relative to the other sites (Fig. 5A) suggests that NY eggs contained more yolk relative to albumen than other sites. ANOVA on the predicted values of yolk to albumen for the 6 equally-spaced egg masses (3.2 g, 3.5 g, 3.8 g, 4.1 g, 4.4 g, 4.7 g) indicated that scores of NY eggs were displaced above LA and SD (*F* = 8.39, df = 3, 20, *P* = 0.001); average yolk:albumen scores of NY eggs was 8-10% higher than LA and SD eggs, respectively (Fig. 5A inset). In addition, KS eggs also contained relatively more yolk relative to albumen than eggs from SD, but KS and LA eggs were similar (Fig. 5A).

Examination of dry egg contents yielded similar, but not identical, conclusions. KS eggs again showed a proportional decrease in yolk as egg mass increased, indicating a growing importance of albumen. Exponents (from Table 5) describing the relationship between change in yolk and albumen as egg mass increased differed little in NY (0.985 – 0.959 = 0.026) and SD (1.105 – 1.051 = 0.054). Positive values for the difference suggested a slight increase in dry yolk content compared to dry albumen as egg mass increased, but values close to zero indicated that dry yolk and dry albumen contributed about equally to increasing dry egg mass. LA differed in that the proportional contribution of yolk increased relative to albumen; large eggs in LA contained proportionately large yolks. The upward displacement of NY compared to the other sites again suggested greater relative investment in yolk in NY eggs (Fig. 5B), which we confirmed with ANOVA. Comparison of predicted yolk:albumen scores at the 6 equally-spaced dry egg masses (0.74 g., 0.82 g, 0.89 g, 0.96 g, 1.04 g, 1.12 g) were different (*F* = 4.83, df =3, 20, *P* = 0.011) because of a 10% higher yolk:albumen score in NY (1.33) compared to LA (1.21; Fig. 5B inset)

Discussion

Eggs, parental care, and nest design are the ultimate foundation of avian reproduction. Studies of parental care are copious (Clutton-Brock 1991), and increasing attention is being given to nests and nest building behavior (Hansell 2000, Rohwer & Law 2010), but our understanding of how egg size and composition fit into the larger picture of avian reproduction lags far behind (Deeming 2007). Limited interspecific geographic comparisons suggest that egg size appears to be larger in tropical than temperate-zone breeding species (Martin et al. 2006, Martin 2008), but few intraspecific geographic comparisons of egg size exist, let alone analyses of egg composition. Indeed, to our knowledge, ours is only the second study of intraspecific geographic comparison of egg composition in birds (see Ruuskanen et al. 2011), despite the potential importance of egg size and composition for egg hatchability (Sanchez-Lafuente 2004, Çağlayan 2009), parental nest attentiveness during incubation (Turner 2002), and growth and survival of nestlings (Murphy 1985, O'Connor 1979, Rhymer 1988, Magrath 1992, Williams 1994, Blomqvist et al. 1997, Krist 2011).

We used the large climatic differences among our sites to test several hypotheses related to the potential thermal benefits of laying eggs of different size and/or composition. Weather has important influences on kingbird reproduction (Murphy 1983, Murphy in press), and most germane here is the fact that low temperatures and high precipitation, both of which cool eggs, lengthen incubation (Gillette et al. 2021). Large eggs cool more slowly than small eggs (Williams 1984), and thus, our first hypothesis was that large eggs would be selectively favored to impede heat loss in environments as conditions unfavorable to incubation increased. This seemed likely for kingbirds because, as single sex incubators without male incubation feeding, the contents of their open-cup nests are directly exposed to the elements when females are off the nest feeding. However, our results led to a rejection of our first hypothesis; egg mass did not differ among populations. A possible explanation is that body size of kingbirds varies geographically and that without accounting for adult body size comparisons of egg size among populations are questionable. However, van Wynsberghe et al. (1992) showed that Eastern Kingbird body size does not exhibit large scale geographic variation. Moreover, intraspecific studies indicate that most egg size variation exists independently of female body size (Christians 2002), a conclusion confirmed for kingbirds by Murphy (1986a). A confounding factor that we cannot account for is that any one of our 4 sites may have experienced particularly good or poor conditions for breeding during the year of study and that average population egg mass was affected in a way to obscure real geographic differences. We can, however, reject the two alternative hypotheses that clutch size was an influence on egg size. Egg mass neither declined with increasing clutch size, as predicted by life history theory (Smith & Fretwell 1984), nor increased with clutch size, as expected if females invest more in reproduction under favorable conditions (Murphy et al. in press).

Similarly, we rejected the hypothesis that egg composition was related to clutch size; neither albumen, yolk, nor shell varied with clutch size when egg size and site were accounted for by the ANCOVA. However, our fourth hypothesis, that egg composition would vary with climatic differences associated with geographic location was supported. Analyses of both wet and dry egg mass yielded similar conclusions, and indicated that the primary site differences in overall egg composition were due to contrasts between NY and LA eggs. For wet egg content, the yolk:albumen content was higher for NY eggs than for both LA and SD, while for dry egg contents the site differences were reduced to that between NY and LA. Albumen content of LA eggs relative to yolk consistently exceeded those of NY eggs.

Lipids compose the majority of the yolks of kingbirds (55% of dry yolk mass; Murphy 1986), and birds generally (58%; Sotherland and Rahn 1987), and serve as the energy source for the growing embryo. We assumed that the composition of the dry yolk is consistent across sites based on the sole study to test for geographic differences in yolk composition (Ruuskanen et al. 2011). Given that albumen is the primary constituent of eggs of altricial species (~73% in kingbirds), and that albumen is the primary protein source for avian embryos (~90%; Sotherland & Rahn 1987), the large volume of albumen establishes that albumen is the major protein source for growing embryos. In addition, albumen supplies nearly all of the water needed to support the embryo's metabolism during development. Based on documented climate differences across our study sites (Fig. 1), we predicted that NY would present the most challenges to an incubating female because of low temperatures, frequent rainfall, and high cloud cover. Indeed, kingbirds have a longer average incubation period in NY (15.1 days) than KS (14.6 days) that is the result of exposure to colder and rainier conditions in NY (Gillette 2021). Interestingly, the quotient obtained by dividing dry yolk mass by average incubation length for NY and KS eggs, an approximation of daily energy needs during incubation, were nearly identical (NY = 0.0262, KS = 0.0265). Hence, the additional yolk in NY appears just sufficient to support roughly one more day of incubation for embryos compared to the other sites.

Embryo needs for water are also important and losing more than 15% of initial egg mass through excessive water loss can threaten embryos (Ar & Rahn 1980, but see Carey 1986). Additional water is likely most necessary to offset water loss in warmer, sunnier, or windier environments and in NY, which stands out as the site least likely to experience these conditions (Fig. 1), eggs contained the least water. Although we have no knowledge of how proportional egg composition translates into future growth and survival of kingbird nestlings, the proportional differences observed in albumen and yolk between NY and LA are in the same range as the experimental alterations of Barn Swallow eggs by Ferrari et al. (2006). The latter authors showed that a 6% decline in albumen did not affect hatchability, but resulted in diminished offspring survival.

Given space constraints within eggs, and our finding that egg mass did not differ across study sites, it may be that differences in yolk and albumen content represent a trade-off yielding different optimal yolk-to-albumen ratio for eggs across climates. Dry egg composition of eggs from KS, LA, and SD eggs did not differ but had more water (~1.2% more) and less dry yolk mass (~4% less) than NY eggs. The warmer climate of KS, LA, and SD (Fig. 1) present more favorable ambient conditions for females that would presumably infrequently lead to extended incubation periods that would require more yolk to serve as fuel for embryos. On the other hand, warmer temperatures, less cloud cover, and higher wind speeds would potentially increase water loss from eggs from KS, LA, and SD. The greater albumen (water) content of eggs from the 3 latter sites may therefore buffer eggs against excessive rates of water loss.

An important finding was that production of larger eggs by the addition of the major egg components, albumen and yolk, differed considerably among sites for unknown reasons. KS eggs were enlarged principally by the addition of albumen while increasingly larger LA eggs were produced by mainly adding to the dry yolk component. NY and SD eggs experienced more nearly equal additions of yolk and albumen than eggs from either other site. It is impossible to determine whether these reflect consistent site differences, or possibly more likely, responses to ambient conditions experienced by females during egg-laying. For instance, Barrionuevo et al. (2014) found differences in the yolk mass of White-rumped Swallows was unrelated to ambient temperature, but increased with food availability. Kingbird breeding was delayed by poor weather in 1983 in KS compared to the 3 previous years, but neither egg mass nor clutch size differed from the 1980-1982 averages (Murphy 1986b). Laying date in NY in 2019 was very close to the long-term average, but clutch size was low in our sample (M. T. M. unpubl. data), and temperature and precipitation were average to well-above average, respectively, in NY in the month preceding egg-laying in 2019 (M. T. M. unpubl data). There were thus indications that the eggs laid in KS (1983) and NY (2019) were produced during at least mildly stressful conditions, but the pattern of egg composition differed between these sites (Fig. 5). Clearly, more intensive sampling is needed to determine if annual variation in weather or possibly food result in different patterns of egg composition among years at a single site, or that regardless of environmental exigencies, the same decisions are made regarding apportionment of yolk and albumen to eggs.

Conclusion

Geographic variation in egg composition is a vastly understudied realm of the reproductive ecology of birds. The longer apparent incubation periods of tropical birds compared to temperate species is driven by both behavioral (incubation) differences and disparities intrinsic to the eggs themselves (Martin et al. 2018), but whether similar adjustments occur among geographically separated populations of the same species is unknown. Our study, which appears to be the first geographic comparison of albumen and yolk composition in birds, suggests that clutch size had no influence on egg size or composition, but that subtle variation among sites in the proportion of eggs that are yolk and albumen are possible. Egg composition in NY appeared to prioritize additional yolk over albumen, which is consistent with the relatively longer incubation periods of eggs from NY. Females from the other sites all experience distinctly warmer and mostly drier conditions than found in NY, and they produced eggs with greater water content, which we hypothesize helps to offset high water loss caused by higher ambient temperatures. Additional work is needed to establish the degree of year-to-year consistency in egg composition at each site, to evaluate whether greater yolk content yields larger yolk reserves in hatchlings, and whether adjustments in pore number and structure of eggs might contribute to the balance of water loss/production by embryos in response to highly different hydric environments.

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Figure legends

Fig. 1. Comparisons of average climate conditions over the 4-month period including the breeding seasons for Eastern Kingbirds nesting in Kansas, Louisiana, New York, and South Dakota (April-July). The principal component analysis (PCA) resulted in 2 major axes accounting for a combined total of 94% of the variance in climate across study sites and represented mean monthly temperature, percentage of days/month that were cloudy, number of days of rain/month, and average monthly windspeed. Data are presented as mean ± 95% confidence intervals along factor 1 and factor 2 of the PCA.

Fig. 2. Relationships between (A) the mass of yolk to mass of albumen, (B) proportion egg that is yolk to proportion that is albumen, (C) proportion that is shell to proportion that is albumen and (D) proportion that is shell to proportion that is yolk of Eastern Kingbirds eggs across all study sites. Data points represent the average of all of the eggs belonging to a clutch. Clutches were collected from Kansas (n = 21 females), Louisiana (n = 20 females), New York (n = 20 females), and South Dakota (n = 21 females).

Fig. 3. Allometric relationships between initial egg mass and (A) albumen mass, (B) yolk mass, and (C) shell mass of eggs collected from Eastern Kingbirds in Kansas (n = 21 females), Louisiana (n = 20 females), New York (n = 20 females), and South Dakota (n = 21 females). Relationships are plotted on $\log_{10}$ scales and data points represent the mean values of all eggs belonging to a single clutch.

Fig. 4. Allometric relationships between dry egg mass and (A) dry albumen mass, (B) dry yolk mass, and (C) shell mass of clutches of eggs collected from Eastern Kingbirds in Kansas (n = 21 females), Louisiana (n = 20 females), New York (n = 20 females), and South Dakota (n = 21 females). Relationships are plotted on $\log_{10}$ scales and data points represent the mean values of all eggs belonging to a single clutch.

Fig. 5. Relationship between (A) ratio of yolk to albumen with fresh egg mass and (B) ratio of dry yolk to dry albumen with dry egg mass of Eastern Kingbirds in Kansas (n = 21 females), Louisiana (n = 20 females), New York (n = 20 females), and South Dakota (n = 21 females). Insets show mean values for ratios of (A) wet yolk to albumen and (B) dry yolk to albumen by state. Error bars represent 95% confidence intervals. Letters denote homogeneous groups.

Fig. 1

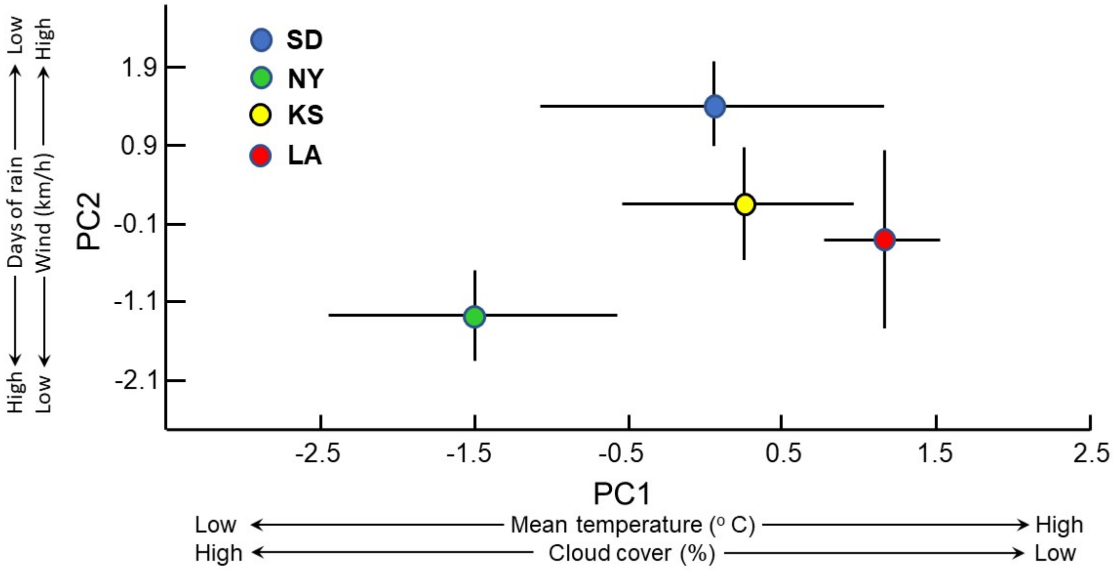
SD
NY
KS
LA
PC2
PC1
1.9
0.9
-0.1
-1.1
-2.1
-2.5
-1.5
-0.5
0.5
1.5
2.5
High ← Days of rain → Low
Low ← Wind (km/h) → High
Low ← Mean temperature (° C) → High
High ← Cloud cover (%) → Low

Fig. 2

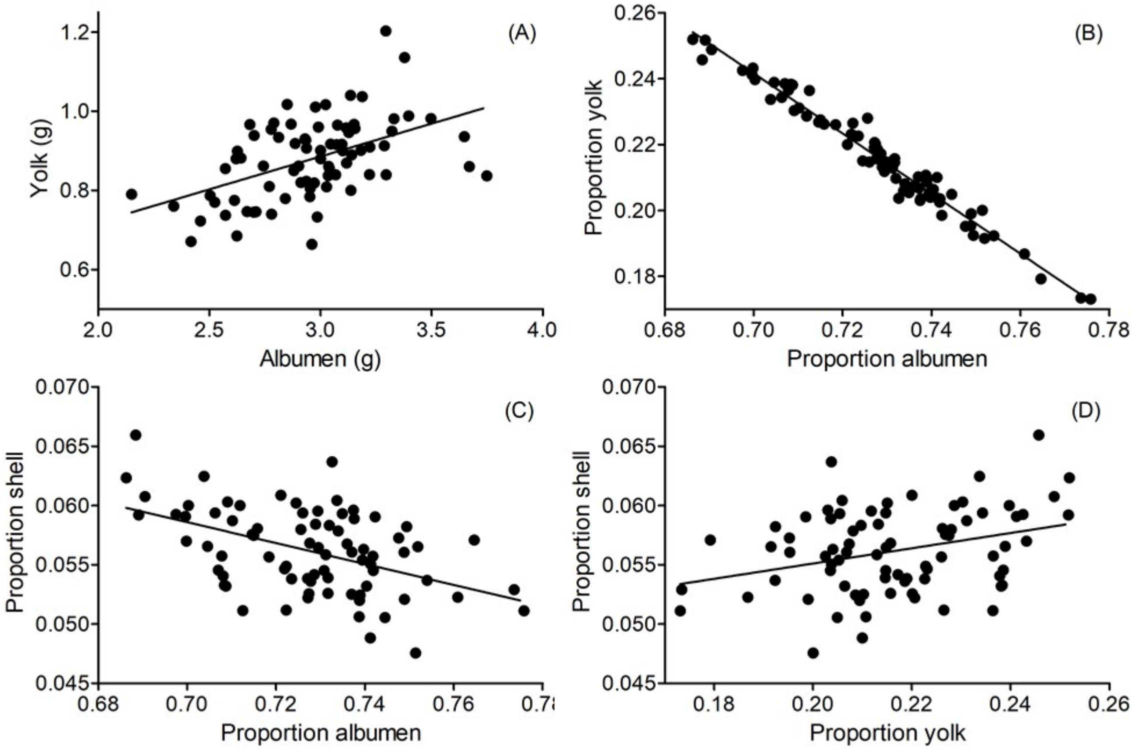
(A)
Yolk (g)
1.2
1.0
0.8
0.6
Albumen (g)
2.0
2.5
3.0
3.5
4.0
(B)
Proportion yolk
0.26
0.24
0.22
0.20
0.18
Proportion albumen
0.68
0.70
0.72
0.74
0.76
0.78
(C)
Proportion shell
0.070
0.065
0.060
0.055
0.050
0.045
Proportion albumen
0.68
0.70
0.72
0.74
0.76
0.78
(D)
Proportion shell
0.070
0.065
0.060
0.055
0.050
0.045
Proportion yolk
0.18
0.20
0.22
0.24
0.26

Fig. 3

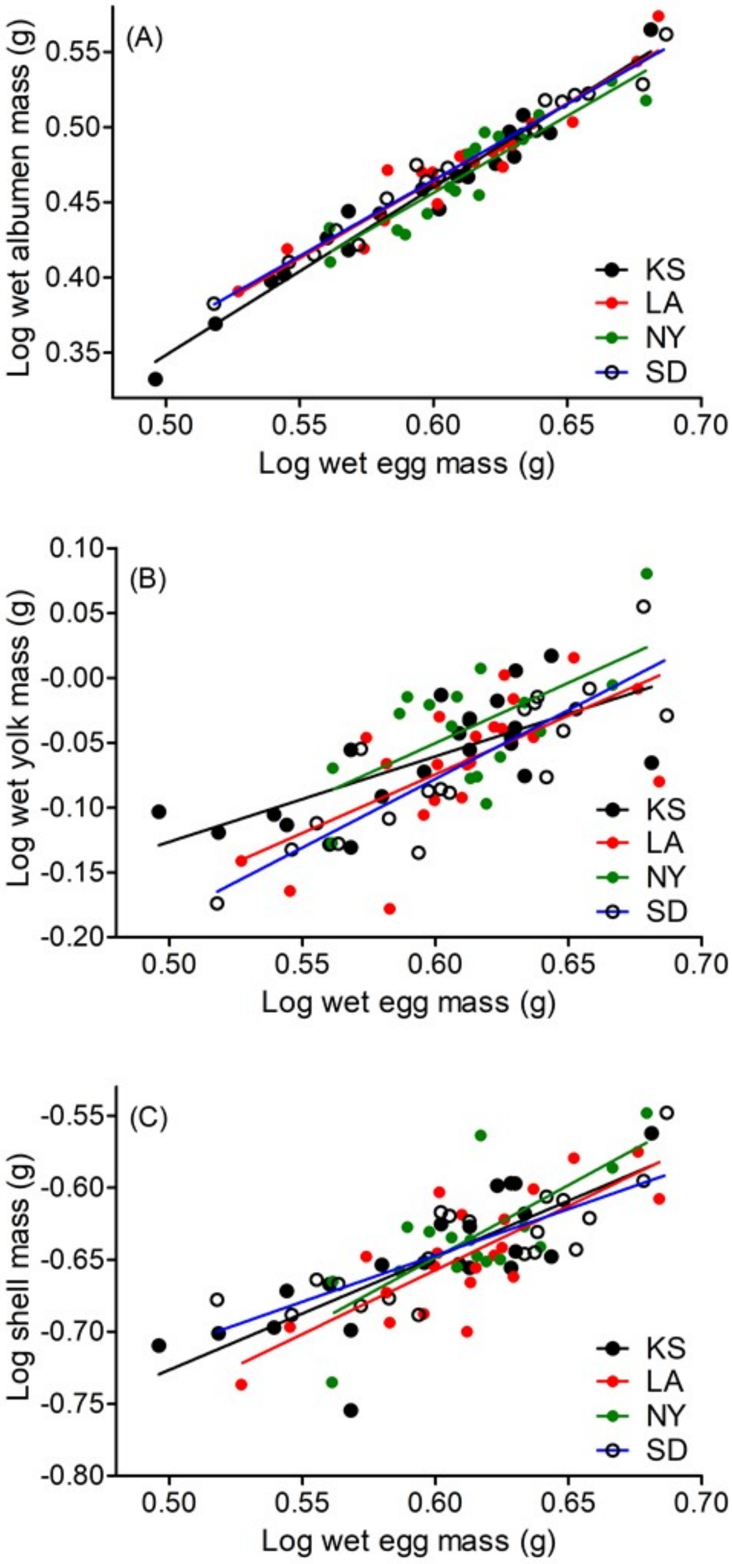
(A)
Log wet albumen mass (g)
0.55
0.50
0.45
0.40
0.35
0.50
0.55
0.60
0.65
0.70
Log wet egg mass (g)
KS
LA
NY
SD
(B)
Log wet yolk mass (g)
0.10
0.05
-0.00
-0.05
-0.10
-0.15
-0.20
0.50
0.55
0.60
0.65
0.70
Log wet egg mass (g)
KS
LA
NY
SD
(C)
Log shell mass (g)
-0.55
-0.60
-0.65
-0.70
-0.75
-0.80
0.50
0.55
0.60
0.65
0.70
Log wet egg mass (g)
KS
LA
NY
SD

Fig. 4

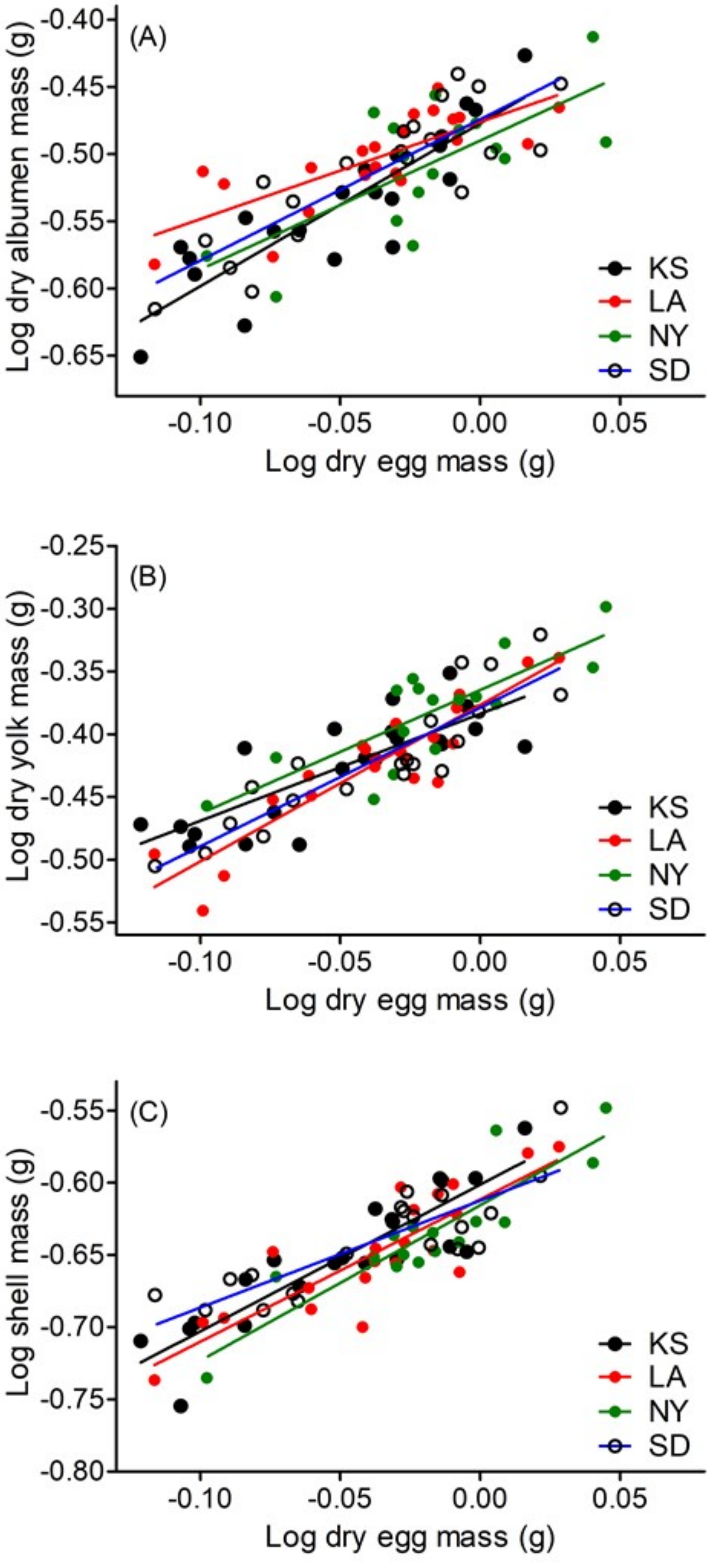
(A)
Log dry albumen mass (g)
-0.40
-0.45
-0.50
-0.55
-0.60
-0.65
-0.10
-0.05
0.00
0.05
Log dry egg mass (g)
KS
LA
NY
SD
(B)
Log dry yolk mass (g)
-0.25
-0.30
-0.35
-0.40
-0.45
-0.50
-0.55
-0.10
-0.05
0.00
0.05
Log dry egg mass (g)
KS
LA
NY
SD
(C)
Log shell mass (g)
-0.55
-0.60
-0.65
-0.70
-0.75
-0.80
-0.10
-0.05
0.00
0.05
Log dry egg mass (g)
KS
LA
NY
SD

Fig. 5

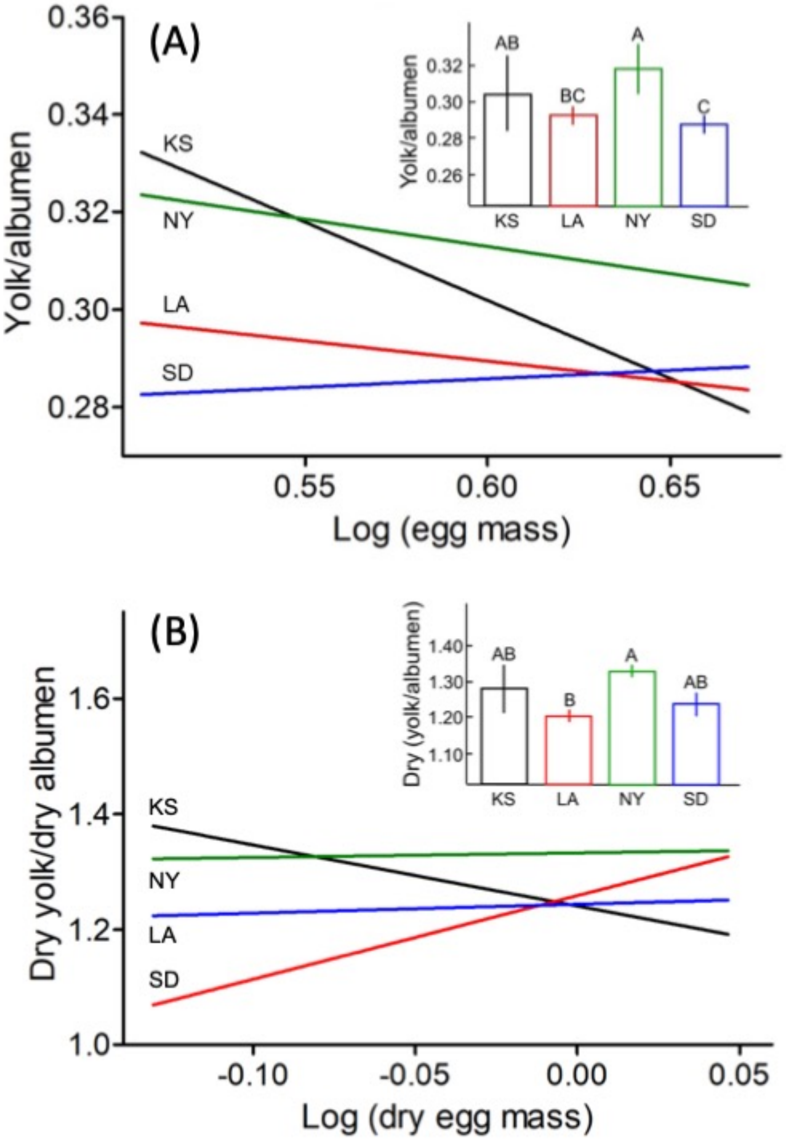
(A)
Yolk/albumen
0.36
0.34
0.32
0.30
0.28
0.55
0.60
0.65
Log (egg mass)
KS
NY
LA
SD
Yolk/albumen
0.32
0.30
0.28
0.26
AB
BC
A
C
KS
LA
NY
SD
(B)
Dry yolk/dry albumen
1.6
1.4
1.2
1.0
-0.10
-0.05
0.00
0.05
Log (dry egg mass)
KS
NY
LA
SD
Dry (yolk/albumen)
1.40
1.30
1.20
1.10
AB
B
A
AB
KS
LA
NY
SD

Table 1. A comparison of average monthly weather for April through July for study sites at Lawrence, KS, Gueydan, LA, Cooperstown, NY, and Pierre, SD. Data are monthly means ± SE. Statistical comparisons were made using analysis of variance (*F*) of the residuals of all variables after accounting for the expected phenological changes using regression analyses of monthly values against month (i.e., April =1, May = 2, June = 3, July = 4). Shared superscripts denote statistically homogenous groups based on Tukey's HSD (honestly significant difference) test at $P < 0.05$.

Variable	Kansas	Louisiana	New York	South Dakota	*F* (*P*)
High temperature (° C)	25.0 (2.94)^{B}	29.3 (1.61)^{A}	19.2 (3.14)^{C}	23.0 (3.97)^{BC}	17.63 (<0.001)
Low temperature (° C)	15.1 (2.93)^{B}	21.2 (1.81)^{A}	8.2 (2.70)^{C}	12.3 (3.55)^{B}	45.33 (<0.001)
Total days of rain	13.8 (0.78)^{B}	13.4 (1.78)^{B}	17.8 (0.56)^{A}	12.7 (0.75)^{B}	6.01 (0.010)
Total rainfall (mm)	64.8 (6.57)^{AB}	50.2 (8.73)^{B}	82.2 (2.29)^{A}	49.0 (4.97)^{B}	7.98 (0.003)
Sunny days (%)	14.6 (0.44)^{A}	14.6 (1.86)^{A}	9.9 (0.57)^{B}	14.6 (0.37)^{A}	5.82 (0.011)
Cloud cover (%)	33.0 (4.04)^{B}	29.8 (1.55)^{B}	45.8 (3.82)^{A}	30.2 (4.85)^{B}	16.21 (<0.001)
Wind speed (km/hr)	13.7 (1.10)^{B}	11.9 (1.16)^{C}	11.8 (1.02)^{C}	17.5 (0.64)^{A}	86.52 (<0.001)

Table 2. Comparison of Eastern Kingbird egg mass and mass of their components (albumen, yolk, and shell) collected from Kansas (1983), New York (2019), South Dakota (2020) and Louisiana (2021) using analysis of variance. Shared superscripts across sites for a given variable denote homogeneous groups.

State	Wet/dry	KS (n = 21 ♀♀) mean ± SE	LA (n = 21 ♀♀) mean ±SE	NY (n = 17 ♀♀) mean± SE	SD (n = 20 ♀♀) mean± SE	*F* (*P*)
Egg mass	wet	3.961 ± 0.089	4.089 ± 0.076	4.092 ± 0.078	4.108 ± 0.095	0.66 (0.579)
	dry	0.896 ± 0.018	0.921± 0.019	0.963 ± 0.020	0.922 ± 0.019	2.13 (0.103)
Albumen mass	wet	2.870 ± 0.073	2.995 ± 0.061	2.942 ± 0.062	3.011 ± 0.071	0.83 (0.484)
	dry	0.293 ± 0.008	0.315 ± 0.005	0.309 ± 0.009	0.309 ± 0.008	1.39 (0.252)
Yolk mass	wet	0.869 ± 0.019	0.867 ± 0.022	0.919 ± 0.025	0.867 ± 0.025	1.17 (0.327)
	dry	0.377 ± 0.007^{B}	0.380 ± 0.009^{B}	0.416 ± 0.010^{A}	0.383 ± 0.010^{AB}	3.44 (0.021)
Shell mass	dry	0.224 ± 0.005	0.225±0.005	0.232 ± 0.006	0.230 ± 0.004	0.53 (0.663)

Table 3. Results of analysis of covariance for differences in wet and dry mass of egg components among sites and clutch size categories while controlling for the allometric relationships between egg components and whole egg mass. Slopes (β) for each allometric relationship are given with standard errors (SE). Sample size is 71 clutches.

	Wet mass				Dry mass		
	Egg mass	Site	Clutch size		Egg mass	Site	Clutch size
	β ± SE	*F* (*P*)	*F* (*P*)		β ± SE	*F* (*P*)	*F* (*P*)
Albumen	1.048 ± 0.033	2.76 (0.050)	1.71 (0.175)		1.081 ± 0.100	1.94 (0.132)	0.97 (0.413)
Yolk	0.914 ± 0.103	2.85 (0.044)	1.83 (0.151)		1.037 ± 0.087	1.82 (0.153)	0.18 (0.912)
Shell	0.701 ± 0.080	0.61 (0.612)	2.11 (0.108)[1]		0.849 ± 0.072	1.71 (0.174)	2.34 (0.081)[1]

[1] P-value for shell mass relationship with clutch size increased to 0.405 and 0.224 for wet and dry mass when the single 5-egg clutch in the analysis was removed.

Table 4. Results of analysis of covariance comparing sites for differences in wet and dry mass of egg components while controlling for the allometric relationships between egg components and whole egg mass. Slopes (β) for each allometric relationship are given with standard errors (SE). Sample size is 78 clutches.

	Wet mass			Dry mass	
	Egg mass	Site		Egg mass	Site
Component	β ± SE	*F* (*P*)		β ± SE	*F* (*P*)
Albumen	1.050 ± 0.031	2.37 (0.077)		1.000 ± 0.086	2.89 (0.041)
Yolk	0.875 ± 0.099	2.33 (0.082)		1.047 ± 0.073	2.45 (0.071)
Shell	0.790 ± 0.075	0.58 (0.627)		0.938 ± 0.064	1.59 (0.200)

Table 5. Results of allometric analyses of variation in egg components for wet and dry egg mass for Kansas (KS; n = 21 nests), Louisiana (LA; n = 21 nests), New York (NY; n = 16 nests), and South Dakota (SD (n = 20 nests). Coefficient of determination (r^2) given for each analysis along with the intercept and slope (β) of the prediction equation.

		Wet mass (g)				Dry mass (g)		
Component	Site	Intercept	β ± SE	r^2		Intercept	β ± SE	r^2
Albumen	KS	0.619	1.114 ± 0.049	0.965		0.333	1.211 ± 0.153	0.767
	LA	0.703	1.029 ± 0.072	0.915		0.334	0.722 ± 0.137	0.593
	NY	0.680	1.039 ± 0.102	0.875		0.324	0.959 ± 0.262	0.489
	SD	0.724	1.008 ± 0.041	0.972		0.336	1.051 ± 0.155	0.717
Yolk	KS	0.350	0.659 ± 0.155	0.488		0.413	0.853 ± 0.147	0.639
	LA	0.241	0.906 ± 0.236	0.436		0.420	1.246 ± 0.130	0.832
	NY	0.263	0.885 ± 0.174	0.394		0.432	0.985 ± 0.190	0.656
	SD	0.193	1.060 ± 0.145	0.749		0.418	1.105 ± 0.127	0.807
Shell	KS	0.076	0.782 ± 0.138	0.630		0.250	1.015 ± 0.120	0.791
	LA	0.064	0.889 ± 0.159	0.623		0.245	0.986 ± 0.137	0.732
	NY	0.061	0.944 ± 0.214	0.564		0.242	1.076 ± 0.142	0.804
	SD	0.092	0.664 ± 0.109	0.661		0.244	0.739 ± 0.113	0.707

Chapter VI

Conclusion

Bird eggs, nests, and incubation behavior are inextricably linked in their effects on offspring (Fig.1), but are each influenced by pressures of the environment. The size of nests, for example, is dictated by a trade-off between predation pressure – favoring a smaller, less detectable nest– and climate, which may favor a nest with thicker walls to retain heat or thinner walls for heat dissipation, depending on ambient temperature. However, nests must be simultaneously adapted to serve their primary purpose of supporting eggs and nestlings, and therefore, their cups must be large enough to accommodate a clutch of eggs and, eventually, a brood of growing young. Additionally, as our work showed, the influence of nest structure and material composition seems to be most significant when the female is on the nest, suggesting that nest design is not separable from incubation behavior. Incubation duration, which is a product of phylogeny, individual species effects, weather, and individual female behavior, dictates the amount of yolk necessary to support the embryo during development. Prolonged incubation behavior, often attributable to regular periods of "parental neglect" during periods of food shortage (Boersma 1982, O'Connor 1979), require embryonic tolerance of low temperature stress and enlarged yolk stores. Similarly, prevailing dry, warm, or windy weather conditions determine the amount of water that must be contributed to the egg via albumen content. Despite the fascinating interactions that occur between these facets of bird biology, avian reproduction remains a poorly understood realm.

We studied geographic variation of reproductive biology of Eastern Kingbirds *Tyrannus tyrannus* breeding in Kansas (KS), New York (NY), South Dakota (SD), and Louisiana (LA) with the goal to better understand whether, and if so how kingbirds adjust nest construction and egg composition to climate at different breeding locations. Our analysis of breeding season (April-July) climate at our study sites showed that our NY site stood apart from the others as the coldest, with high cloud cover and the most days of rain. LA was the warmest and least cloudy while KS and SD experienced intermediate temperature. SD was also the driest and windiest site. We found that nests and eggs did indeed differ across sites in alignment with our expectations that kingbirds would respond to predictable climatic differences across sites.

Our study of geographic variation of the size, structure, and material composition of Eastern Kingbird nests did not include nests from KS because the historical data on egg composition (Murphy 1986) were not accompanied by information on nest size and composition. Lack of nest information from KS is probably least consequential because, climatologically speaking, KS occupies an intermediate position along our climate gradient (Chapter III, Fig. 1). Among nests from the other 3 sites, SD nests were heavier, with the greatest cup volumes, thickest walls, and smallest relative cup openings. LA nests had the thinnest walls and largest relative cup openings, while NY was intermediate in most respects, but had the smallest nest cup openings and inner dimensions, and the widest outer dimensions. Notably, variation in nest composition was greater within than among sites, but in all populations, fine stems and rootlets, forb stems, and plant down were the largest contributors, followed by coarse woody stems.

To evaluate the insulative capacity of different nests we placed prewarmed iButtons in nests and recorded rate of temperature decline to measure the rate of heat loss from all nests. We ran experiments with nests both uncovered, to simulate conditions with the female off the nest, and covered, to simulate conditions experienced by eggs when the female was on the nest (but not applying the brood patch to heat eggs). The insulative value of nests did not differ across populations when nest cups were uncovered; however, when we measured the insulative value of nests when covered, LA nests were poorly insulated compared to nests from NY and SD, as we had predicted based on the typically warm conditions experienced by kingbirds in LA. This suggests that the importance of insulative capacity of nests is greater during an incubation on-bout than an off-bout, raising the possibility that the nest has important thermal benefits for not only eggs, but also for heat conservation of incubating female. Our results showed that nests with the greatest insulative capacity were those with small nest cup openings, small nest cup opening:cup volume ratios, and nests that were larger overall. When we considered only the higher-latitude NY and SD sites, the most well insulated nests had shallow, asymmetrical nest cups with low volume and were composed of plant down, fine stems and rootlets and coarse woody stems. Grasses and forbs stems, known to be poor insulative materials (Hilton et al. 2004), generally contributed less to the composition of kingbird nests.

While nests serve as a first-line defense for eggs against environmental stressors, we chose to further examine the possibility that females can adjust egg composition itself to better match environmental conditions that could affect incubation length. We thus quantified geographic variation in total egg mass and the contribution of the egg's basic components, albumen, yolk, and shell, to mass of kingbird eggs from KS, NY, SD, and LA. We predicted that egg mass would be larger in colder, more northern sites, but found instead that egg mass was similar across sites. Moreover, egg mass did not exhibit an inverse relationship with clutch size as is predicted by life-history theory (Smith and Fretwell 1974) nor was egg mass larger in large clutches as predicted by the hypothesis that high food abundance should lead to increases in all aspects of reproduction. We also found that egg composition did not vary with clutch size, but our findings supported our prediction that eggs laid in colder climates would have greater yolk mass. Greater yolk content in NY is likely necessary to support the longer incubation period that appears induced by the colder temperatures and more frequent precipitation of NY (Chapter III, Fig. 1). We also found that water content was greatest in LA eggs, which we posit is needed in the warmer climate where the rate of water loss across the shell is likely to be higher.

An examination of the conceptual map (Fig. 1) emphasizes the highly interconnected nature of a bird’s potential responses to solving the ultimate measure of success, production of the greatest number of recruits. Given the many responses possible, and the multitude of bird species, are there an equally great number of solutions to the ultimate goal or does selection narrow the range of suitable options such that particular “syndromes” emerge as successful combinations of traits? Life-history theory is built on this question and the accumulating evidence indicates that, indeed, particular combinations of traits do seem more likely to appear than others. Most notably, large scale geographic comparisons across the world have shown that the “slow-fast” continuum of life histories can largely define the differences between tropical and temperate-zone breeding birds, but that southern and northern hemisphere temperate-zone breeders are not identical (Martin 1996, Lloyd & Martin 2016). Clutch size, lifespan, offspring growth rates, and length of parental care vary predictably with geographic location (Table 1). Nonetheless, many questions remain regarding finer-scale geographic variation of reproductive traits in birds. For example, while we showed that nest structure and egg composition differed among sites, we did not address aspects of parental behavior that might vary in association with both traits. Incubation behavior is as important as any reproductive trait, yet we did not study the frequency and duration of on- and off-bouts, and whether they might covary with nest design and egg composition on a geographic scale in response to differing climates. Given the interactions between the nest, egg, and incubating female, the thermal benefits of a nest may differ in relation to different egg and clutch size or under different on/off bout durations. Our study did not capture any of the interactions that may occur between the incubating female, clutch, and nest that may influence nest structure or material selection.

Nest placement is another method by which a female can influence nest microclimate and, while we demonstrated differences in the angle of the branch that nests were built on across populations, further work on variation of the height, orientation, and type of plant (tree vs. shrub) would contribute to our understanding of how birds contend with environmental pressures through nest construction. It would be critically important to quantify nest microclimate as an integrated unit in which temperature, wind, solar radiation, and wetting due to precipitation combined to affect nest insulation, egg temperatures, and water loss from eggs.

Measurement of the insulative value of nests in the field over the wide range of nest sites chosen by kingbirds, combined with documentation of female incubation behavior, is the next step needed to fully comprehend the outcomes arising from the nexus of nest, eggs, and female behavior. An important question, especially given that nest predation is the primary cause of nest failure in kingbirds, is the role of predator detection in influencing nest placement and design. The smaller nests of NY compared to SD (Chapter II), for instance, suggests a potential role of nest predation in affecting nest construction. Additional studies of the relationship between nest placement, nest size, and predation are thus needed.

We have focused on geographic variation of egg composition; however, a limitation of our study was that we only sampled each population for one breeding season. Years with weather favoring greater food abundance correspond with greater female investment in reproduction. Previous work has shown that kingbirds lay larger eggs earlier in the season during years of greater food availability (Murphy 1986b, Murphy et al. in press) and White-rumped Swallows (*Tachycineta leucorrha*) respond similarly by increasing yolk mass when conditions are favorable (Barrionuevo et al. 2014). It is possible that any of our study populations experienced a particularly good or poor year with regards to prey abundance, but we were unable to account for this in our analyses. Further study, over multiple years, is needed to tease out the contributions of temporal vs. geographic effects on reproductive variation.

We attributed the need for the additional dry yolk mass present in NY eggs, compared to other study sites, to an extended incubation period in the colder, wetter NY climate. But yolk that is not utilized by the embryo remains on the hatchling's body and can be used early on in the hatchling stage as sustenance when foraging is poor. A study of geographic analysis of hatchling body composition would reveal to what extent eggs with relatively greater yolk content are supportive of extended incubation periods or the neonate post-hatching. Additionally, the importance of albumen as the primary source of protein and water to the developing embryo has been a largely overlooked component of eggs. While yolk serves as the main source of energy, fat-soluble vitamins and hormones, and immunoglobulins, the ratio of yolk to albumen in eggs may be restricted in part by need for more water in climates favoring water loss. Further research of how egg composition differs between regions with varying temperatures and relative humidity may shine a light on how environmental pressures constrain egg composition.

There are still vast gaps in our understanding of the effects of climate on avian reproduction, but with this work we contribute the first combined characterization of geographic variation in nest design and insulative quality, as well as the first study of geographic variation of egg composition. To review, we have demonstrated that kingbird nests and eggs vary geographically across sites experiencing different climatic conditions. We found that kingbird nest design is more thermally impactful in the presence of an incubating female and that egg composition is related to incubation period length. Given the inextricable relationships between incubation behavior, egg composition and nest design, future work is needed to understand the interactions between each facet and the extrinsic pressures acting upon them. Recent steep declines of North American birds (Rosenberg et al. 2019) are an urgent call for forward thinking conservation decisions based on geographic variation of avian life-history. Given the shifts in bird breeding range and phenology, as well as migration timing (Both et al.

2004, Both & te Marvelde 2007) further studies of how birds adapt to their environment are essential.

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Figure legends

Fig. 1. Interactions between the traits of nests, eggs, and incubation and the external pressures acting upon them.

Fig. 1

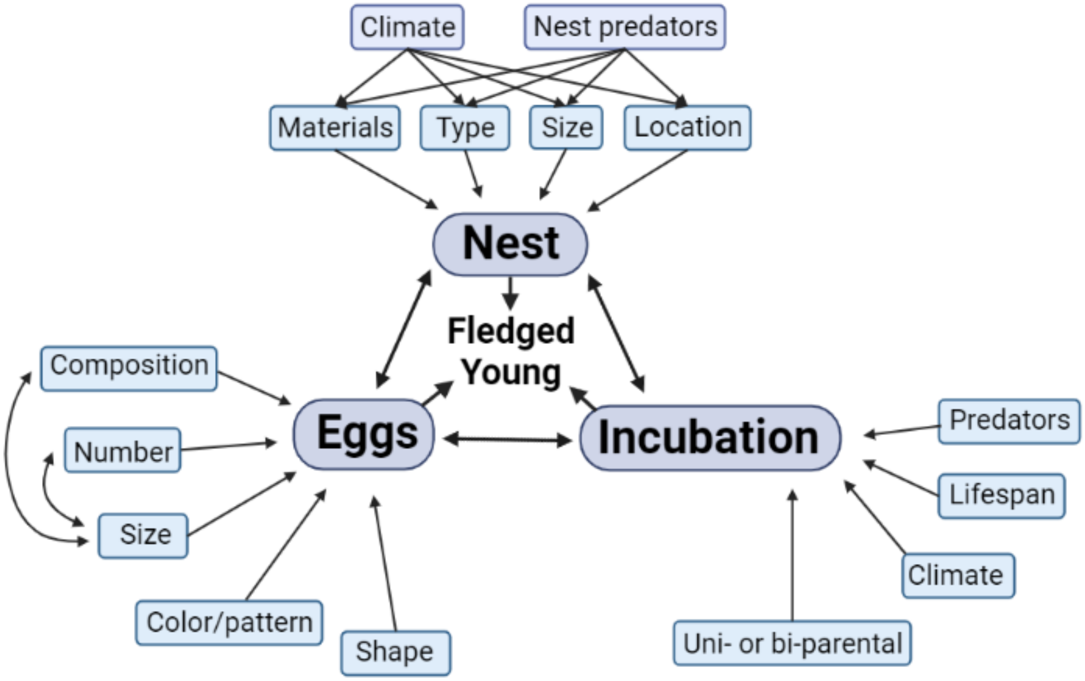
Climate
Nest predators
Materials
Type
Size
Location
Nest
Fledged Young
Composition
Number
Size
Eggs
Incubation
Predators
Lifespan
Climate
Color/pattern
Shape
Uni- or bi-parental

Table 1. Comparison of life-history traits between north temperate breeding birds with tropical and south temperate species. Question marks following descriptions of traits between regions are indicative of less well-established regional differences.

Reproductive trait	North temperate	Tropical/southern hemisphere
Breeding season length	shorter?[1]	longer?[1]
Broods per year	fewer?[1]	more?[1]
Clutch size	larger[1,3]	smaller[1,3]
Egg mass	smaller?[2]	larger?[2]
Incubation length	shorter[1,3]	longer[1,3]
Nestling growth rate	faster[1,3]	slower[1,3]
Nestling period	shorter[1,3]	longer[1,3]
Duration of post-fledging care	shorter?[1,3]	longer?[1,3]
Adult survival	lower[1,3]	higher[1,3]
Juvenile survival	same?[1]	same?[1]

1. Martin 1996
2. Martin et al. 2006
3. Lloyd & Martin 2016

Supplementary materials: Appendix A

We obtained climate data for our field sites in Oneonta, NY (42.45° N, 75.06° W, 339 m above sea level [asl]), Pierre, SD (44.37° N, 100.35° W, 450 m asl), Gueydan, LA (30.03° N, 92.51° W, 2 m asl), and Lawrence, KS (38.97° N, 95.23° W, 267 m asl) from Weather Atlas (Weather Atlas 2022; https://www.weather-atlas.com/en). Oneonta and Gueydan were not available within Weather Atlas, so we substituted Cooperstown, NY (42.70° N, 74.92° W, 374 m asl) and Abbeville, LA (29.97° N, 92.13° W, 5 m asl) for them because they were the locations closest to our field sites with available climate data. We included Lawrence, KS in this analysis because of the availability of egg composition data from a previous study (Murphy 1986a). Kingbirds begin to arrive in LA by April but typically in May at the other sites (although April dates can occur in early years). To characterize weather relevant to our kingbird study we used average weather conditions for the 4-month period including April through July. Data that we extracted included mean daily values for high and low temperature (°C), wind speed (km/h), rainfall (mm), along with number of days/month with precipitation ≥ 0.25 mm, number of sunny days/month, and percentage of days with cloud cover (Table A1). We computed average daily temperature as the mean of daily low and high temperature. We used principal components analysis to generate axes to describe the major gradients of variation in weather among the 4 sites for the 4 months of the breeding season. Most correlations among variables were below an absolute value of 0.600, and we eliminated 1 of the 2 variables in pairwise comparisons if the correlation coefficient exceeded 0.600 for use in our PCA, but with one exception. Mean temperature and percentage of days with cloud cover were correlated ($r = 0.874$, $P < 0.001$) but we included both because both variables have been shown to independently influence foraging rates of Eastern Kingbirds (Murphy 1987).

Raw data upon which our PCA was based are provided in Table S1. The PCA yielded two axes that together accounted 94.0% of the variation in climate (Table A2). High PC1 scores, represented most strongly by LA, represented sites with high temperatures and little cloud cover while low scores, typified by NY, represented sites of low temperature and high cloud cover. PC1 scores for NY ($\bar{x} = -1.50 \pm 0.61$) were lower than LA's ($\bar{x} = 1.16 \pm 0.23$), while SD and KS intermediate ($\bar{x} = 0.070 \pm 0.72$) and not different from either NY or LA. PC2 represented a contrast of sites with frequent days of rain but low wind (negative scores), principally NY, with sites in which average daily wind speeds were high but number of days of rain/month was low (positive scores), primarily SD. Mean PC2 scores for SD ($\bar{x} = 1.40 \pm 0.32$) were substantially higher than those of NY ($\bar{x} = -1.26 \pm 0.34$), with LA and KS falling in between and not different from either other site ($\bar{x} = -0.29 \pm 0.72$).

Table A1. Average monthly climate for Pierre, SD, Cooperstown, NY, Lawrence, KS, and Abbevile, LA for April through July.

State	Month	High temperature (^{o} C)	Low temperature (^{o} C)	Mean temperature (^{o} C)	Mean wind speed (km/h)	Total rainfall (mm)	# of days of rain	Mean cloud Cover (%)	Sunny Days (%)
SD	Apr	13.4	3.6	8.5	19.1	35	10.8	41	14.3
SD	May	20	9.8	14.9	17.8	57	13.6	35	15.3
SD	June	27	16.1	21.55	17.0	55	14.2	26	13.7
SD	July	31.5	19.7	25.6	16.1	49	12.3	19	15.1
NY	Apr	10.8	1.2	6.0	14.6	80	16.6	55	8.6
NY	May	18.4	7	12.7	12.1	87	19.3	48	10.2
NY	June	22.2	10.6	16.4	10.8	85	17.8	43	9.5
NY	July	25.4	13.8	19.6	9.9	77	17.5	37	11.3
KS	Apr	17.9	7.7	12.8	16.5	50	12.6	40	15.4
KS	May	22.7	13.1	17.9	13.7	82	15.8	40	13.3
KS	June	28.8	19	23.9	13.5	63	12.5	26	14.7
KS	July	30.8	20.5	25.65	11.1	64	14.1	26	14.8
LA	Apr	25	16.3	20.65	14.7	42	10.6	34	17.3
LA	May	28.8	20.6	24.7	12.8	35	10.2	28	18.1
LA	June	31.4	23.5	27.45	10.8	49	15.2	27	12.7
LA	July	32.1	24.3	28.2	9.4	75	17.5	30	10.3

Table A2. Results of the principal component analysis of variation in breeding season weather (months of April through July) of Eastern Kingbirds breeding in central NY, eastern KS, central South Dakota, and southern Louisiana.

Variable	Eigenvector 1	Eigenvector 2
Mean temperature	0.670	-0.196
Number of days of rain	-0.257	-0.667
% cloudy days	-0.667	-0.144
Mean wind speed	-0.194	0.704
Eigenvalues	2.065	1.696
% of variance	51.6	42.4
Cumulative % variance	51.6	94.0