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THE CAUSES AND CONSEQUENCES OF INDIVIDUAL VARIATION IN PARENTAL CARE BEHAVIOR

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THE CAUSES AND CONSEQUENCES OF INDIVIDUAL VARIATION IN PARENTAL CARE BEHAVIOR

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Arts and Sciences at the University of Kentucky

By

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Lexington, Kentucky

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ABSTRACT OF DISSERTATION

THE CAUSES AND CONSEQUENCES OF INDIVIDUAL VARIATION IN PARENTAL CARE BEHAVIOR

Behavioral traits can be remarkably flexible depending on the conditions in which they are expressed, yet, in spite of this flexibility, persistent differences between individuals appear to limit the potential expression of behaviors. For example, despite evidence that parents provide variable amounts of parental care in response to changing environmental conditions, they also differ in the overall level of care they provide. I used a behavioral reaction norm approach to study individual variation in parental care behavior in free-living house sparrows (*Passer domesticus*). I investigated the nature of this variation by studying the relationship between different forms of parental care, the biological basis of individual variation in care, and the effect of this variation in care on offspring. First, I found a positive covariance between nestling provisioning and nest defense. Parents that provided high levels of care in one context provided high levels of care in the other context, even after accounting for measures of offspring value. Second, I sought to identify the biological sources that create and maintain consistent individual differences in the level of care a parent provides. I found that the likelihood of feeding nestlings large food items was positively associated with genetic heterozygosity, but did not find evidence that nestling provisioning was influenced by additive genetic variation in this population. Parents hatched from larger eggs provisioned offspring at a higher rate than parents hatched from smaller eggs, but there was no effect of other conditions experienced in the nest on the level of care expressed as an adult. I also tested if differences in problem-solving ability were related to differences in parental care behavior. Although I found that problem-solving parents fledged more offspring than parents that could not solve the problem, parental care was not associated with any measure of problem-solving ability. Finally, I found that individual variation in parental care reaction norms predicted the growth rate, size, and immune response of nestlings, which in turn positively affected offspring survival and recruitment. My findings reveal factors maintaining individual differences in parental care behavior and offer new insights into the causes and consequences of individual variation.

KEYWORDS: house sparrow, parental care, personality, plasticity, reaction norm

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April 8th, 2013

Date

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CHAPTER ONE

Introduction

Variation is ubiquitous in all biological populations. Scientists have been attempting to understand the existence and maintenance of variation among and within species for centuries (e.g., Darwin 1868). Some of the most dramatic examples of variation may exist among species (e.g., body size in field mice versus elephants), but variation at the species level is ultimately a result of natural selection acting on individual phenotypes. Understanding variation at the individual level is therefore critical to our understanding of variation at all levels. Within a species or population, individual variation exists for many morphological, physiological, and behavioral traits. Several of these traits can be fixed at maturity for an individual, for example body size or mating strategy (territorial versus satellite) in ruffs (*Philomachus pugnax*; Lank et al. 1995), but many behavioral traits exhibit considerable plasticity; individuals can vary in the expression of a behavior from day-to-day, hour-to-hour, or minute-to-minute. Plasticity in behavior is often attributed to variation in environmental conditions that influence the expression of a behavior (e.g., longer day-length increases migratory behavior in dark-eyed juncos, *Junco hyemalis*; Nolan et al. 2002). Interestingly, although behavioral traits can be remarkably flexible depending on the conditions in which they are expressed, recent research has found that individuals are consistently different in their expression of a wide variety of behaviors (e.g., activity, aggression, exploration, migration, etc.; see Bell et al. 2009). These between-individual differences in trait expression can persist over long periods of time (within and across years) and across contexts, i.e., between-individual variation cannot be completely explained by differences between individuals in the environment in which they are observed (Dall et al. 2004; Sih et al. 2004). This suggests that in spite of behavioral traits exhibiting considerable flexibility, there exist persistent differences between individuals that appear to limit the potential expression of a behavior. Furthermore, not only do individuals differ in the level of a specific behavior they exhibit, but individuals can also differ in the degree to which they alter that behavior in response to changes in the environment (e.g., exploration behavior; Dingemanse et al. 2012). These consistent individual differences in the level and plasticity of a behavior provide the raw material on which selection can act to shape the patterns of behavior observed within and between populations, despite the considerable flexibility of behavioral traits.

Parental care behavior is a good example of an important behavioral trait that can vary considerably as parents respond to changing environmental conditions (e.g., increasing demand as offspring grow, seasonal food availability). Interestingly, despite evidence that parents respond flexibly to variable conditions, parents have also been found to provide individual-specific levels of care (Freeman-Gallant & Rothstein 1999; MacColl & Hatchwell 2003; Schwagmeyer & Mock 2003; Nakagawa et al. 2007) and respond differently to changes environmental conditions (Westneat et al. 2011). The relationship between the behavioral response of an individual across an environmental gradient describes a reaction norm (Stearns 1989; Pigliucci 2001; Dingemanse et al. 2010). Modeling parental care behavior as a reaction norm allows for the decomposition

of variation in parental care into its key components: variance due to individual differences in the average level of parental care (reaction norm intercepts), variance between individuals in their plasticity of care (reaction norm slopes), covariance between these two components, and residual variance (Dingemanse et al. 2010). The application of the behavioral reaction norm approach to understanding variation in parental care has demonstrated that approximately 20% of the variation in care can be attributed to individual differences in reaction norm intercepts (Westneat et al. 2011). These findings raise a series of interesting questions about the nature of parental care behavior, the biological basis for these consistent individual differences, and the effect of individual variation in reaction norm components on offspring performance.

One question these results raise is: if individuals provide consistently different levels of some types of care, do parents that provide high amounts of care in one context (e.g., offspring provisioning) also provide high amounts of care in other contexts (e.g., defending offspring from predation). Within a species there are several different forms of care a parent may provide (Clutton-Brock 1991), yet it is often unclear how these forms of care are related. Measures of parental care could be negatively correlated if performing each type of care requires use of limited time or resources. Alternatively, some measures of care could be positively correlated because they have reaction norms that are sensitive to the same environmental factors or aspects of the parent's condition. However, because most measures of parental care are phenotypically plastic traits, testing the covariance between forms of care is confounded by environmental factors that are predicted to influence both forms of care. For example, parental investment theory predicts a positive covariance between measures of care because parents should increase their level of care when the benefit of care increases (Trivers 1972; Winkler 1987). If parents can assess offspring value then they should respond flexibly to changes in value, and, according to theory, measures of care should covary due to any variation in the benefit of care. Previous studies that found correlations among measures of care have assumed that variation in offspring value is driving any relationship between measures of care (Greig-Smith 1980; Rytkönen et al. 1995). However, a more relevant and more interesting question is how measures of care are related after accounting for factors predicted to similarly influence them, i.e., are there underlying attributes of individual parents that create covariance between forms of care. In chapter three, I test the nature of the relationship between offspring provisioning rate and nest defense, two common forms of parental care.

A significant portion of the level of care a parent provides appears to be a characteristic of individual parents, yet the attributes of parents that determine the amount of care they provide are poorly understood. Environmental factors can clearly create differences between individuals in the amount of care they provide. Indeed a significant portion of parental care research has focused on attempting to identify environmental factors that create between-individual variation in parental care. For example, parents with more or older offspring typically provide more care than parents with fewer or younger offspring (e.g., Breitwisch et al. 1986; Wright & Cuthill 1990). However, consistent individual differences in parental care behavior could also be created by differences in attributes of individual parents. Differences between parents could be the result of differences in genetic attributes of an individual, or differences in the conditions experienced during development, or even differences in the cognitive ability of an

individual. In chapters four through seven, I test for several of these biological sources of individual variation in parental care behavior.

Genetic variation can create differences among individuals in many life history traits. One way life history traits like parental care can be influenced by genetics is through additive genetic variation. Additive genetic variation has been found to explain a significant portion of the variation in traits like clutch size and egg size (Christians 2002) and even offspring provisioning rate (MacColl & Hatchwell 2003) in some bird populations. Another source of genetic variation that could create individual differences in reproductive traits is non-additive genetic variation, such as genetic heterozygosity. Heterozygosity has been found to have an effect on a wide variety of fitness-related traits, including the number and size of offspring produced by an individual (Chapman et al. 2009), and is thought to reflect the effect of a few loci that have a large effect on these traits or traits that affect over-all condition (e.g., immunity), or reflect a general effect of heterozygosity across many loci as a result of inbreeding depression or overdominance (Chapman et al. 2009; Szulkin et al. 2010). Despite the fact that reproductive traits like clutch size or parental care are phenotypically plastic and exhibit considerable variation depending on the conditions under which they are recorded, most studies fail to account for these sources of variation when estimating genetic effects. Plasticity in these traits can generate within- and between-individual variation that may bias or obscure the effect of genetic variation on reproductive performance. Furthermore, genetic variation could create differences in both the level of reproductive performance (individual reaction norm intercepts) and how individuals respond to varying environmental conditions (individual reaction norm slopes). Few studies have tested how additive and non-additive genetic effects can influence individual-level plasticity in wild populations (Nussey et al. 2007). In chapter four, I test for heritability in the level and plasticity of parental care behavior in a wild bird population. In chapter five, I use a long-term data set to test for additive genetic variance in clutch size and egg size, and test for an effect of heterozygosity on clutch size, egg size, hatching success, and fledging success in the same study population. Chapter six explores the effect of heterozygosity on three measures of offspring provisioning behavior (provisioning rate, variance in the time between feeding events, and the likelihood of provisioning with large food items), testing for an effect of heterozygosity on both the level and plasticity of care provided by individual females.

Factors other than genetic variation, such as the conditions experienced early during development, could create permanent differences between individuals and possibly influence the expression of parental care behaviors later in life. Studies have found that rearing conditions can influence behavioral traits expressed later in life. For example, male song sparrows (*Melospiza melodia*) hatched from large eggs had larger song repertoires as adults than males hatched from small eggs (Zanette et al. 2009). Few studies have attempted to address how parental care behavior could be influenced by environmental variation experienced during development, particularly in wild populations. Variation in rearing conditions should create differences in offspring condition, which in turn could lead to consistent differences in level of parental care an individual provides. In chapter four, I test this idea in a cohort of wild birds that were tracked from eggs through to breeding adults.

Although there are several potential explanations for consistent individual differences in parental care behavior, the idea that these differences could be due to

neurological differences among individuals could be a novel explanation for the existence and maintenance of phenotypic variation in wild populations. Recent research on wild birds has found that individuals differ in their ability to solve foraging problems (Morand-Ferron & Quinn 2011), and individual differences in problem-solving ability are repeatable across time (Cole et al. 2011). Differences between individuals in their foraging and problem-solving abilities likely have a large effect on their ability to provide parental care to rapidly growing, dependent offspring. Parents with better cognitive abilities could forage more efficiently or effectively and thereby provide a higher level of parental care or respond to changing conditions more effectively than parents with poorer cognitive abilities. In chapter seven, I test the ability of parents to complete a problem-solving task in the wild, and I evaluate whether variation in the ability or speed at which parents solved this foraging problem explains between-individual differences in the level care parents provide.

A basic expectation of parental care theory is that offspring should benefit from increased levels of care (Clutton-Brock 1991). However, a fundamental problem with testing the effects of parental care on offspring performance is that both parental care and offspring performance vary in response to many environmental factors (e.g., date in season). Plasticity in response to these environmental conditions could inflate or obscure the covariance between care and performance and must be taken into account when estimating the effect of parental care on offspring. Furthermore, consistent differences in how parents respond to environmental conditions could influence offspring performance. Offspring likely benefit when parents provide a stable supply of food despite a fluctuating environment. Consistency and stability at this level require parents to be plastic at other levels: parents must flexibly respond to changes in the environment and in offspring demand to maintain the food supply required by offspring. In chapter eight, I test how individual differences in components of parental care reaction norms (intercepts and slopes) influence offspring condition and survival.

Finally, in chapter nine, I summarize the findings of my research on variation in parental care behavior, explore the broader implications of this work, and propose avenues of future research to aid in our understanding of this topic.

CHAPTER TWO

Review: Individual variation in parental care in birds

Parental care is a key life history trait for many organisms, and is broadly defined as any parental behavior directed towards offspring that has the potential to increase the fitness of the parent's offspring (Clutton-Brock 1991; Royle et al. 2012; Klug et al. 2012). Parental care can take a number of forms, including building a nest or burrow, caring for eggs, provisioning offspring, defending offspring from predators, and assisting independent offspring. Not surprisingly, considerable variation in the amount of care provided by parents exists both between and within taxa regardless of the type of care (Curio 1988; Clutton-Brock 1991; Eggers et al. 2005). A central tenet of parental care theory is that such variation in parental care is adaptive and reflects environmental differences in the benefits of care to offspring or the costs to the parent (Winkler 1987; Clutton-Brock 1991; Gross 2005). Despite an overall fit to theory, there are a number of details that remain unexplained. One problem is that even when one or more factors affect the level of care, considerable variance in care (in some cases up to 90%) remains unexplained (Winkler 1992; Redmond et al. 2009). For example, the male's share of incubating eggs in northern lapwings (*Vanellus vanellus*) was found to vary among individuals from 0% to 74% (Lislevand et al. 2004). Another puzzle is the extent to which individuals can adjust their care flexibly or exhibit individual specific levels of care; within-species studies of parental care variation have generally not distinguished between the ability to adjust to changing conditions and fixed individual differences. Many experimental studies provide strong evidence that parental care behavior is phenotypically plastic since individuals can adjust their care to environmental changes (e.g., Eggers et al. 2005; Peluc et al. 2008). Yet, in most cases we do not know how much of the variation in parental care arises from plasticity or fixed, individual differences. Because selection acts on individual phenotypes, studying variation in parental care at the individual level is critical to understanding the evolutionary response to selection on care. I suggest that by treating parental care as a phenotypically plastic trait that varies at the individual level, we may be able to better understand the causes and consequences of variation in parental care behavior.

In this review, I seek to explain variation in parental care within a population using a reaction norm approach and assess how and why individuals vary in attributes of the reaction norm. To do this, I first give a brief history of how parental care has been modeled, and explain how a reaction norm approach can be applied to parental care. I then examine some of the more well-studied intrinsic and extrinsic factors that can influence parental care. Finally, I review what is currently known about individual-level variation in care, the potential sources of consistent individual differences in parental care reaction norms, and the effect of this variation on offspring.

Parental care behavior is widespread across many taxa (mammals, birds, reptiles, amphibians, fish, and invertebrates) and is expressed in a wide variety of ways. The approach outlined here can be applied to almost all taxa and applicable to most forms of care. However, because the majority of research on parental care behavior has been performed in avian taxa (Stahlschmidt 2011), for the purposes of this review, I will focus

on parental care in birds, measured as parental food provisioning to offspring. A substantial body of literature has examined this measure of care, and it appears to be a relevant form for studying individual variation in care. This form of care has been shown to have costs to the parents and benefits to the offspring, both of which parents respond to. Provisioning altricial young is likely to be the most energetically demanding form of parental care (Winkler & Wilkinson 1988; Clutton-Brock 1991), and there is evidence that increasing offspring provisioning reduces parental survival (Nur 1984a, 1984b; Schroeder et al. 2012) and future reproductive success (Nur 1988). There is also evidence that increased levels of offspring provisioning enhance nestling survival and recruitment (Nur 1984a, 1984c; Schwagmeyer & Mock 2008). Though this review focuses primarily on only this one measure of parental care, most other forms are care could easily be studied in this framework.

Modeling parental care

The use of models to understand parental care behavior has a long history in evolutionary biology. Early models of parental care, such as those produced by Maynard Smith (1977) and Grafen & Sibly (1978), sought to understand broad-scale patterns of care across species, specifically when mates should desert their partners. These models helped explain why some species exhibit uni-parental care while others have bi-parental care. Additional models of parental care were developed to examine the optimal levels of parental care using economic optimization and dynamic modeling techniques (Chase 1980; Sargent & Gross 1986). These broad-scale models produced testable predictions which researchers used to study smaller-scale phenomena such as the care strategy of a parent given their energy reserves or nesting status (Sargent & Gross 1986). Life history theory has also been used to model how parents should provide care (Winkler 1987; Winkler & Wallin 1987). These models use a more quantitative approach toward parental care, in that they predict population-level responses to a given circumstance. Winkler's 1987 "general model" for parental care was the first attempt to incorporate the multitude of factors that can influence parental care behavior into one model (e.g., parent-offspring relatedness, offspring age and number, partner's effort, time of season). Since that time, empirical research has focused on attempting to uncover and identify the factors that have the largest impact on parental care within a population.

While these early models guided research on parental care for decades and added to our understanding of the ecological and evolutionary processes that have shaped patterns of care, these models generally overlook the fact that population- and species-level differences in parental care are products of individual variation. Individual variation in parental care phenotypes provide the raw material on which natural selection can act to shape the patters of care we observe within a population, but we also predict that individuals should provide different levels of care (e.g., Kisdi et al. 1998; Wilson 1998; Bêty et al. 2003). Each individual has a different genetic background and experienced a unique developmental pathway to arrive at the point where it must decide the amount of care to provide under the current environmental conditions. Therefore, individuals may produce mean levels of parental care that are consistently different from other individuals in the population, and individuals may respond differently from other individuals to changes environmental conditions. These differences can be described as individual variation in parental care reaction norms. I suggest that modeling parental care as a

reaction norm that varies at the individual level is critical to understanding the existence and maintenance of individual differences in this trait, as well as the evolutionary response to selection on parental care.

Parental care meets the reaction norm

The reaction norm has a long history for studying phenotypic variation (Woltereck 1909; Bradshaw 1965; Stearns 1989; Pigliucci 2001). This approach has classically been used for studying variation in a trait expressed once in an individual's lifetime, typically in an experimental setting (Pigliucci 2001; Nussey et al. 2007). More recently, the reaction norm approach has been used in wild populations to model individual phenotypic variation in behavioral traits where individuals are measured multiple times under varying environments (i.e., the natural environment; Nussey et al. 2007). The behavioral reaction norm approach is based on the "phenotypic equation" (Nussey et al. 2007, Dingemanse et al. 2010, Westneat et al. 2011), where any quantitative, behavioral phenotype (y) expressed at observation (i) by an individual (j) can be described by the equation:

$$y_{ij} = \beta_0 + u_{0j} + (\beta_1 + u_{1j})E_{ij} + \varepsilon_{ij} \quad (1)$$

This equation can be used to partition and understand variation in parental care behavior. In this case, y_{ij} represents the number of provisioning trips made to offspring per hour by individual j during observation session i. This equation accounts for the fixed effects of population mean provisioning rate (β_0) in the average environment, and the population mean slope with respect to environmental variable E (β_1). This equation also explains variation due to the random effect of individual identity by modeling individual phenotypic differences from the population mean intercept in the average environment and population mean slope. The jth individual's permanent deviation from the population mean intercept (independent of E) is represented by u_{0j} (individual reaction norm intercept; also referred to as "animal personality"; Dingemanse et al. 2010). The term u_{1j} accounts for the deviation of individual j from the population mean slope with respect to E_{ij} (individual plasticity, or an individual by environment interaction). The environmental variable E is assumed to be zero-centered, such that all intercepts are estimated at the average environment. Residual variance over all individuals and observation sessions is accounted for by the term ε_{ij}.

This notation for the phenotypic equation is commonly used in current literature (e.g., van de Pol & Wright 2009; Dingemanse et al. 2010), but is slightly confusing because intercepts and slopes are represented by similar variables (for example: β_0 represents an intercept and β_1 represents a slope) even though these terms have different units. Perhaps a better way to represent the phenotypic equation is to let μ represent intercepts and β represent slopes, such that:

$$y_{ij} = \overline{\mu} + \Delta\mu_j + (\overline{\beta} + \Delta\beta_j)(E_{ij} - \overline{E}_j) + \varepsilon_{ij} \quad (2)$$

In this equation, let $\Delta\mu_j = \mu_j - \overline{\mu}$, such that $\Delta\mu_j$ represents the deviation of individual j's intercept (μ_j) from the population intercept ($\overline{\mu}$) in the average environment, and $\Delta\beta_j = \beta_j - \overline{\beta}$, such that $\Delta\beta_j$ represents the deviation of individual j's slope (β_j) from the population

slope ($\overline{\beta}$). The best way to interpret this equation may be to visualize what each component means in graphical form (Figure 2.1).

This equation requires further modification because individuals are often non-randomly distributed across environments. These differences in the conditions under which individuals are observed (e.g., number of offspring being provisioned) can lead to biased parameter estimates (Martin & Réale 2008; van de Pol & Wright 2009; Dingemanse et al. 2010; Westneat et al. 2011). This concern can be addressed by separating the dependence on the environment into a between-individual component ($\overline{\beta}_B$) dependent on the average environment experienced by each individual ($\overline{E}_j$), and within-individual components dependent on the variation in environment experienced within-individuals ($E_{ij} - \overline{E}_j$). This equation is now represented as:

$$y_{ij} = (\overline{\mu} + \Delta\mu_j) + \overline{\beta}_B\overline{E}_j + (\overline{\beta}_W + \Delta\beta_{Wj})(E_{ij} - \overline{E}_j) + \varepsilon_{ij} \quad (3)$$

where $\overline{\beta}_W$ represents the population mean slope within-individuals, and $\Delta\beta_{Wj} = \beta_{Wj} - \overline{\beta}_W$, such that $\Delta\beta_{Wj}$ represents the deviation of individual *j*'s within-individual slope (β_{Wj}) from the population mean within-individual slope ($\overline{\beta}_W$). The behavioral reaction norm can be extended to non-linear reaction norm shapes (e.g., quadratic, logistic) using modified or additional parameters in the phenotypic equation (e.g., Gilbert et al. 1998), but for simplicity, this review focuses on the linear reaction norm.

The reaction norm approach allows us to decompose the variation in a complex behavioral trait (V_T), such as parental care, into its key variance components:

$$V_T = V_\mu + V_{\beta E} + 2\mathrm{Cov}(\mu, \beta) + V_R \quad (4)$$

where V_μ represents the variance due to individual differences in the level of parental care in the average environment or reaction norm intercepts. Variance attributed to individual differences in reaction norm slopes is represented by $V_{\beta E}$. The covariance between individual reaction norm intercepts and slopes is represented by $\mathrm{Cov}(\mu, \beta)$, and the residual variance is V_R. The components of this equation can be interpreted visually in Figure 2.2.

To test the significance of each of these components of the reaction norm, we can couple this approach to the statistical framework of the random regression, which allows the estimation and significance testing for both fixed and random effects (Nussey et al. 2007). To test for an effect of individual variation in the level of parental care in the mean environment, we can compare the fit of the model with and without the effect of individual included, using the likelihood ratio test (Crawley 2002; Westneat et al. 2009). The value of two times the difference in log-likelihood is distributed as a chi-square distribution with one degree of freedom for each term estimated, in this case $DF = 1$. This approach allows us to test if a significant amount of variation in parental care is due to individual differences in reaction norm intercepts (i.e., do individuals differ in the level of parental care in the mean environment, V_μ; Figure 2.2). An effect of individual variation in the response to an environmental condition can be tested by adding a random interaction effect between individual identity and a fixed effect, and testing for significant improvement in the log-likelihood score (e.g., Westneat et al. 2011). This allows us to test how much of the variation in parental care is explained by individual differences in

reaction norm slopes (i.e., do individuals respond differently to environmental condition, $V_{\beta E}$; Figure 2.2).

To adequately measure individual variation, researchers must quantify parental care multiple times for each subject across the different environments of interest. Many parental care researchers already collect repeated measures of parental care for each individual across environments (e.g., date in season, offspring age, etc.). Unfortunately, very few of these studies use all of this data they have gathered to examine variation among individuals or repeatability within individuals. In fact, to avoid pseudo-replication, almost all studies that collect repeated parental care measures for an individual reduce the information collected by summing, averaging, or by only retaining one of the repeated measures for analysis. Modeling parental care with the behavioral reaction norm approach uses all measurements that were collected. This approach uses these repeated measurements recorded across environments to account for differences in the conditions under which each individual was observed, and the effect of numerous environmental conditions on the average population response (van de Pol & Wright 2009; Dingemanse et al. 2010). Finally, the application of the behavioral reaction norm allows for the detailed decomposition and accounting of these repeated measures of phenotypic variation in parental care into its key components, variance due to individual differences in the average level of parental care (reaction norm intercepts), variance between individuals in their plasticity of care (reaction norm slopes), covariance between these two components, and residual variance (Dingemanse et al. 2010; Westneat et al. 2011).

Variation in parental care

If we are to study parental care as a trait that is phenotypically plastic, it would help to know which environmental variables are expected to influence parental care. The most commonly studied factors that influence care are the number of offspring, offspring age, parental condition, partner's level of care, time of season, and the parent's relatedness to the offspring. To test for the effect of these factors, investigators usually take the average of their experimental groups and compare the means across the treatments, for example, comparing provisioning rates in birds where mates have been handicapped by weighting their tails. This could result in the finding that on average, birds increase their level of provisioning if their mate decreases provisioning level (Figure 2.3; Wright & Cuthill 1989). Another way to test for an effect of an environmental factor, like nestling age, on parental care is to use a regression. Regressing the level of parental care on the age of the offspring when you performed the observation typically results in the finding that feeding rate increases with nestling age (Figure 2.4; Breitwisch et al. 1986). In both of these analyses, researchers are able to ascertain if an environmental variable has an effect on parental care and how large that effect is. In some cases, the analysis may even tell how much of the variation in the level of parental care is explained by the variable of interest. Unfortunately, performing these analyses often fails to account for the largest portions of the variance in parental care. For example, in Figure 2.4 we observe that while there is a trend for individuals to feed their offspring more when nestling age increases, there is a large amount of variation in this trait not explained by the age of the nestlings. Using a reaction norm to model parental care as a phenotypically plastic trait that varies at the individual level allows us to examine the environmental effects that influence care in a somewhat different light than before.

Previous studies have identified many factors that influence care, but these studies have always identified the population-level response and ignored the source of these effects, i.e., individual-level responses. It is worth reviewing the factors that researchers have identified as important to parental care (at least for the population mean) to understand how these factors may influence individual responses differently.

Factors such as the number of young or the age of the young have been found to influence parental care. As the metabolic demands of offspring increase, either by increasing the number or size (which usually increases rapidly with age) of offspring, parental care models predict that parents should respond to the increased demand with an increased response (Winker 1987). To test the prediction that brood size effects parental effort, Wright & Cuthill (1990) manipulated the brood sizes of European starlings (*Sturnus vulgaris*) by increasing the number of nestlings in some broods just above the typical range of brood sizes and reducing brood size in other broods just below the typical range. As expected, parents of artificially enlarged broods made more feeding trips per hour than control parents and parents of reduced broods made fewer feeding trips per hour (Wright & Cuthill 1990). Other studies that have examined natural variation in brood size as a factor of parental care found slightly different results. A study that measured provisioning as the number of trips per hour per nestling found that parents with smaller broods had increased provisioning rates (Schwagmeyer & Mock 2003), and a study that measured provisioning rate as the dry mass of larvae delivered per hour per nestling found that parents with larger broods had decreased provisioning rates (Grieco 2002). The discrepancy between the experimental and observational studies is likely due to the fact that Schwagmeyer & Mock (2003) and Grieco (2002) measured offspring provisioning per nestling, a common way of measuring provisioning when brood size varies in a population. This suggests that although total parental effort does shift as models predict, parents are unable to provide the same level of care to each nestling when brood size increases and provide more care per nestling when brood sizes decrease (Nur 1984a). This pattern is likely the result of a benefit curve with diminishing returns for increased brood sizes, which is also predicted by parental care models (Winkler 1987).

Most studies on the effect of nestling age on offspring provisioning have been fairly simple observational studies, as it is difficult to experimentally manipulate the age of nestlings or the age parents perceive the nestlings to be. On the whole, researchers have found that increasing nestling age does increase the number of feeding trips per hour (Sanz & Tinbergen 1999), the load size of prey delivered to nestlings (Breitwisch et al. 1986), and the mass of larvae delivered per nestling per hour (Grieco 2002). The level of parental care provided as nestlings age commonly plateaus as the offspring near independence (Barkowska et al. 1995).

The condition of a parent is also likely to have effects on their ability to provide parental care. Parental care models predict that parents, in most circumstances, should increase the level of parental care with increasing condition (Winkler 1987). Many studies have attempted to manipulate the condition of parents by performing manipulations, such as adding weights to a bird's tail (although this method really increases the effort required to provide care rather than decreasing condition). Studies have found that nestling provisioning rates decrease when required parental effort is experimentally increased (Wright & Cuthill 1989, 1990; Schwagmeyer et al. 2002), however the story is a bit more complicated. Schwagmeyer et al. (2002) found that

provisioning rates decreased only in the short-term (the day after weighting), but over the long-term (the remainder of the nestling period) females do not decrease provisioning rate, whereas males decrease provisioning rate and the number of large prey items brought to the nestlings over the long-term. Additionally, in a follow-up to their 1989 study, Wright & Cuthill (1990) found that increasing parental effort by adding tail weights only decreased nestling provisioning rates when brood sizes were large. Parents with experimentally increased effort also brought smaller prey items to the nest when they provisioned (Wright & Cuthill 1989, 1990). This suggests there could be methodological problems with studies of this nature (is the weighting a sufficient strain on individual condition/parental effort?). There could also be discrepancies in our predictions because differences in individual quality or condition (regardless of experimental design) can lead to different optimal levels of care for each individual, emphasizing the need to study factors such as condition using a framework that accounts for these individual differences.

The level of parental care a mate provides may or may not affect the focal parent's level of care. Winkler's model for parental care predicts that in most cases, if an individual's mate increases their level of care, the focal parent should decrease their care, and vice versa (Winkler 1987). However, bi-parental care represents a unique scenario in which both parents wish to obtain the maximum benefit from their partner while they expend the smallest possible effort to successfully raise their offspring. The conflict between parents that results has been modeled extensively, with varying degrees of success and varying results (see Houston et al. 2005 for review). Without delving into the models of bi-parental care, we can summarize the expectations this theory in that parents either "negotiate", where partners are constantly modifying their level of care based on their mate's care, use a "sealed bid", where partners do not change their level of care based on their mate's care, or use some combination of both (Schwagmeyer et al. 2002; Houston et al. 2005). Early studies of the effect of a mate's level of care physically removed the mate of a focal bird for an extended period of time (Sasvari 1986), which is a completely different phenomenon from having a mate that is present not provide its share of care (Wright & Cuthill 1989). Most recent studies that seek to test for an effect of the mate's level of care on a focal bird use tail weights (as above) to decrease the amount of care a mate provides. Some studies have found that focal individuals increase their level of care when their partner cannot provide the same level of care prior to the manipulation, although the compensation for the lost care is not always complete (Figure 2.3; Wright & Cuthill 1989; Markman et al. 1995). Other studies have found no compensation in parental care by the focal individual (Slagsvold & Lifjeld 1988; Schwagmeyer et al. 2002). Although it seems clear that the mate's level of care could impact the focal parent's amount of care, the solution to this question is difficult to address because sexual conflict over care is involved. More theoretical work and empirical work is needed to dissect this issue (see Houston et al. 2005).

Other factors may influence a parent's level of care as well. For example, the availability of food in the environment could change the level of care provided to offspring. To test this, Boland et al. (1997) gave some groups of breeding white-winged choughs (*Corcorax melanorhamphos*; cooperative breeders) shredded cheese once a day. They found that giving birds extra food increased the rate of provisioning to the nestlings, which increased nestling survivorship and fledging success compared to control groups.

In another example, Grieco (2002) supplemented breeding pairs of blue tits (*Cyanistes caeruleus*) with mealworms and waxworms. As expected, Grieco found that supplemented parents brought more total food to the nestlings, but this was mostly due to the increased provisioning of the supplemented food items. The supplemented parents actually decreased the number of natural food items fed to nestlings, made longer foraging trips, and brought fewer large, natural items (Grieco 2002). Interestingly, bringing fewer but larger items worked out to be approximately the same dry weight as bringing many small items (which the un-supplemented groups brought). These studies suggest that resource availability can impact parental care, which may be especially important in territorial species where parents forage within territories that vary in quality.

The availability of more ephemeral resources in the environment may also impact parental care. The potential for additional mating opportunities with other females has been shown to influence parental care. Smith (1995) gave a subset of male European starlings an extra nest box at the beginning of the nesting season (starlings are facultatively polygynous). Males with two nest boxes increased their singing rates and decreased the amount of time they incubated their eggs (Smith 1995). Similarly, Wittingham found that polygynous male red-winged blackbirds (*Agelaius phoeniceus*) decreased their nestling provisioning rate to a focal nest when another mate in his territory was fertile (Wittingham 1994). The opportunity for extra-pair matings could also affect parental care (Magrath & Komdeur 2003). For example, in fairy martins (*Hirundo ariel*), when the proportion of fertile females in the nesting colony increases, males decrease the time they spend incubating their eggs, which increases the incubation period of the clutch (Magrath & Elgar 1997). However, this pattern was not detected in red-winged blackbirds, where it was found that males did not decrease nestling provisioning rates when neighboring females were fertile (Wittingham 1994). In another study, although male indigo buntings (*Passerina cyanea*) did not decrease their level of parental care when neighboring females were fertile, they did increase the number of forays off the territory (Westneat 1988).

How the factors discussed in this section affect parental care have all been studied by examining the response of the population or an experimental group. Individuals behave differently than the population mean, and it is variation at the individual level on which natural selection acts. Yet we still know very little about how and why individual parents might be consistently different in response to these factors. One place to start is to review studies that have measured parental care behavior multiple times within individuals and quantified their repeatability.

Individual variation in parental care

Researchers have noted for years that there is often large variance associated with mean parental care levels in a population (e.g., Lyon & Montgomerie 1985; Winkler & Wilkinson 1988; Winkler 1992; Lislevand et al. 2004). A basic interpretation of all this variation might be that parents are providing a somewhat random or unpredictable level of care at any given time. This seems rather unlikely though, since we know that individuals respond to a variety of environmental factors within and across breeding events (see above). Furthermore, recent research has shown that within an individual, the level of care provided is surprisingly consistent over time (Freeman-Gallant & Rothstein

1999; Hatch 2003; MacColl & Hatchwell 2003; Schwagmeyer & Mock 2003; Nakagawa et al. 2007).

Consistent individual differences in a behavioral trait is not a new concept. For example, song rate in male blackcaps (*Sylvia atricapilla*) decreases steadily throughout the breeding season for all males, but relative to other males, each individual remains consistent (Hoi-Leitner et al. 1993). Individual consistency, or repeatability, is easy to visualize using a reaction norm (Figure 2.2b). Significant individual repeatability suggests that there is individual variation in the average phenotype, or variation in the intercepts of individual reaction norms. In terms of parental care, recent research has also shown that individuals are consistent in their level of care within and across breeding seasons. Schwagmeyer et al. (2002) performed a tail-weighting experiment on house sparrows (*Passer domesticus*) and found that there were substantial individual differences in how the birds reacted to being handicapped by the weights. Although many birds decreased their nestling provisioning rates the day after the tail-weighting as would be expected, some individuals actually increased their feeding rates. Interestingly, the best predictor of individual provisioning level (for males and females) after being handicapped was the individual's provisioning level prior to being handicapped (Schwagmeyer et al. 2002). Further study on this population by Schwagmeyer & Mock (2003) found that parental care was significantly repeatable across broods (within year) for male house sparrows; approximately 44% of the variation in male feeding rate was attributed to consistent individual differences among males (Schwagmeyer & Mock 2003). A recent study on the repeatability of nest defense behaviors, another form of parental care, found that males and females were repeatable in their level of nest defense (Redmond et al. 2009). These results may not be entirely surprising. Individuals could know their level of parental competency or quality in the given breeding season, and/or assess the value or quality of their nestlings at the initiation of the breeding season, then decide their optimal level of parental care to provide to their offspring that season. A more interesting question that relates to how permanent individual differences (due to genetic or lasting environmental effects) might influence parental care is: are individuals repeatable over longer periods of time?

Nakagawa et al. (2007) studied house sparrows in an attempt to address how consistent individuals are across years. Researchers found that there was consistency in nestling feeding rates across years for both males and females. Other research on the long-tailed tit (*Aegithalos caudatus*; MacColl & Hatchwell 2003) and the savannah sparrow (*Passerculus sandwichensis*; Freeman-Gallant & Rothstein 1999) have both shown repeatability of parental care between years. Potti et al. (1999) measured repeatability of parental effort by quantifying daily energy expenditure of parents using doubly labeled water in consecutive breeding seasons of pied flycatchers (*Ficedula hypoleuca*), and found that parental effort of female flycatchers was repeatable. Interestingly, if we examine the patterns of repeatability by sex, almost all studies that measured parental feeding rates between years found males had higher repeatability estimates (but see Potti et al. 1999). These findings raise questions as to why males would be the more repeatable sex. It may be that the variation among males in feeding rate is higher than females because female feeding rate is a more canalized trait (Figure 2.5; Schwagmeyer & Mock 2003). Alternatively, it could be due to males being less variable across time than females (less responsive to changing conditions), or larger

amounts of between-individual variance in males than females (males vary more among individuals than females). The factors that create this divergence between the sexes are unknown, but it is possible that differences in the time devoted to other behaviors by each sex (males could mate-guard or seek EPCs more than females during parental care phase of breeding) or differences in the responsiveness to the needs of the offspring may have driven this result.

These results suggest that individuals do vary in their level of parental care, and that they vary in consistent ways, both within and between breeding seasons. Besides individual variation in parental care being interesting in itself, this variation at best adds noise to studies of parental care, and at worst, may completely obscure patterns in care. Although there are some nuances to this generalization, it is clear that this variation exists and should be examined and accounted for studies of parental care.

Sources of individual variation in parental care

One important question about consistent individual differences in parental care is how this variation arises. While the biological basis for this variation is relatively unstudied, several genetic and environmental factors may create or maintain these consistent individual differences in parental care, which are explored below.

Genetics

The heritability of parental care has been implied for some time, since genetic variation must exist for parental care as a trait to evolve (Winkler 1987), but few have measured the heritability of this trait. Studies that have tested for additive genetic variation in parental care behavior have found it exists in several species, including the burying beetle (*Nicrophorus vespilloides*; Walling et al. 2008), European earwig (*Forficula auricularia*; Meunier & Kölliker 2012), and African striped mouse (*Rhabdomys pumilio*; Rymer & Pillay 2011). Focusing specifically on studies of birds, heritability of parental care (provisioning rate) was studied by Freeman-Gallant & Rothstein (1999) in the savannah sparrow. These researchers found offspring provisioning rates of sons was almost perfectly related to their father's provisioning rate (95% CI for h^2 = 0.88 to 3.1) and there was a trend for a positive relationship between sons and their mother's provisioning rate (95% CI for h^2= -0.09 to 0.89). Female offspring had no significant relationship with their mother or father's provisioning rate (Freeman-Gallant & Rothstein 1999). The authors suggest that there is little evidence of paternal effects, as the number and quality of young was unrelated to male feeding effort. A second study, by MacColl & Hatchwell (2003) examined the heritability of nestling provisioning in the long-tailed tit. These authors found that the level of care sons provided to their offspring was significantly related to the level of care they received from their parents (h^2 = 0.59; MacColl & Hatchwell 2003). Additional evidence of the heritability of care strategies comes from a recent study on cooperative breeding in birds. Charmantier et al. (2007) found that there is a heritable component to the likelihood of breeding cooperatively in a natural population of western bluebirds (*Sialia mexicana*). Natural studies of the heritability of parental care are likely to produce results confounded by the fact that parental care is a maternal (and paternal) effect and can influence offspring quality. More cross-fostering studies are needed to separate out the confounding effects of the heritability of parental care. Interestingly, both of the studies

that have examined the heritability of parental care found that it was only inherited by sons, not daughters. Because of the scarcity of studies on heritability and parental care, we cannot make sweeping generalizations about differences between the sexes, although combining this information with the information about repeatability of parental care does raise some questions about why the sexes may differ.

Most parental care theory assigns the majority of variation in parental care behavior to environmental factors and methodological or statistical noise; however, these studies demonstrate that parental care may have a genetic component (Freeman-Gallant & Rothstein 1999; MacColl & Hatchwell 2003). This suggests that although individuals do, on average, modify their level of parental care to changes in environmental conditions, differences between individuals in their genetic make-up could have a significant effect on the level or plasticity of care they provide. The idea that the level of parental care an individual provides is inherited from its parents is interesting, but not without some puzzles. If the amount of parental care has an effect on fitness and is heritable, then we might assume that stabilizing selection would reduce the variance in care in the population. But we know a large amount of variation exists between individuals. One potential explanation to this problem is that the ability to provide parental care is due to non-additive genetic factors, such as genetic diversity. If this were the case, then one could observe that parental care is heritable because individual genetic diversity is somewhat heritable from parent to offspring (Mitton 1993; Neff & Pitcher 2008). However, because genetic diversity is not a strictly inherited trait, we would not expect the population to be composed entirely of high-caring individuals, and variation between individuals would be maintained. One interesting component of genetic diversity that may contribute to individual variation in parental care is heterozygosity.

There is a long history of studies on the fitness consequences of genetic diversity, with considerable evidence suggesting that individuals with high levels of heterozygosity can experience "hybrid vigor" or heterosis (Darwin 1876; Shull 1952; David 1998; Chapman et al. 2009). There is a potential for heterozygosity at specific loci to have large impacts on traits such as parental care. A classic example of the effects of heterozygosity at specific loci comes from Ward Watt's work with *Colias* butterflies, where individuals that are heterozygotes for the phosphoglucose isomerase (PGI) enzyme are active at a wider temperature range than homozygotes, which could lead to increased reproductive success (Watt et al. 1983). In a recent study, perhaps more relevant to parental care abilities, researchers found that different alleles of the gene *foraging* (*for*) have drastically different impacts on memory in *Drosophila*. Individuals homozygous for one allele (for^R) have better short-term memories but poorer long-term memories, while individuals homozygous for an alternate allele (for^s) have poorer short-term memories but better long-term memories (Mery et al. 2007). Although this study did not examine the effect of heterozygosity on memory, it is not unreasonable to expect that individuals carrying both *for* alleles may have intermediate or better short-term and long-term memories than homozygotes. In terms of parental care, individuals with high levels of genetic diversity may be able to perform tasks in a wider array of environmental conditions, or could be the "jack-of-all-trades" and be able to remember where the best foraging sites are from day-to-day or year-to-year, for example.

Without knowing which specific genes or alleles confer the variation in parental care, an alternative way to examine heterozygosity is to estimate genome-wide

heterozygosity. If genetic diversity is linked, either directly or indirectly, to parental care, then parents with high levels of heterozygosity would provide high levels of care. This link remains unexplored, although there is some suggestion that such a relationship exists, as recent studies have shown an interesting pattern of inter-generational effects of individual heterozygosity on offspring. For example, in the song sparrow (*Melospiza melodia*), nestlings of parents with low genetic diversity have weaker immune responses and lower survival rates (Reid et al. 2003; Marr et al. 2006). Foerster et al. (2003) found that fledging success and subsequent recruitment in blue tits was positively related to the father's heterozygosity. Better evidence of the effect of heterozygosity comes from a cross-fostering study on the Seychelles warbler (*Acrocephalus sechellensis*), where researchers found that nestling survival was positively related to the genetic mother's and the social father's heterozygosity (Richardson et al. 2004). Furthermore, a study by Fossøy et al. (2008) on the bluethroat (*Luscinia s. svecica*) where extra-pair paternity was assessed, found that the immune response of extra-pair offspring was positively related to the heterozygosity of the social father and not the genetic father. These studies suggest that the heterozygosity of not only the genetic parents, but also the social parents (the primary caregivers) may play some role in the survival and performance of offspring. Parental care may be the link between the inter-generational effect of heterozygosity, and heterozygosity may in turn explain individual variation in parental care.

Environmental factors

Another way in which the variation between parents in the level of care could arise is through environmental conditions, especially conditions experienced during development. This section examines one of the ways in which environmental conditions could create consistent individual differences in parental care behavior.

Developmental stress is likely ubiquitous in nature, as most developing organisms at some point face environmental stressors in early life. In addition, organisms experience stress throughout their lives from factors such as food limitation, the presence of predators, inclement weather, and reproductive activities. The most well studied stress response in birds (and most vertebrates) is the increase in circulating glucocorticoid levels, specifically corticosterone (Wingfield 1994). This stress response is highly variable between individuals (Kitaysky et al. 2003; Lendvai et al. 2007). Because the stress response is moderated by the hormone corticosterone, this section focuses on how corticosterone levels (induced by stress or exogenously introduced) effect individual variation in parental care behavior when experienced as an adult or during development.

Stress has the potential to play a large role in individual variation in parental care. In adults facing stress, corticosterone levels vary by resource availability (good versus poor resource habitats), time of season, number of breeding attempts, and clutch size (Romero et al. 1997; Kitaysky et al. 1999; Lendvai et al. 2007). Although few studies have examined natural variation in the level of stress or corticosterone between individuals impacts the level of parental care provided, Lendvai et al. (2007) did find that individuals differ in their corticosterone levels when the value of a brood is manipulated (by changing the number of young in the nest). These researchers found that house sparrows with an increased brood size (increased value of current brood) had a reduced corticosterone levels relative to birds with a decreased brood size (Lendvai et al. 2007). Research on a wild macaroni penguin (*Eudyptes chrysolophus*) population found that

individuals with artificially increased corticosterone levels foraged more and had heavier chicks (Crossin et al. 2012). Individual differences in the average level of or receptiveness to corticosterone could produce consistent differences in the level or plasticity of care individual parents provide.

There are no studies directly linking stress experienced as a nestling with the level of parental care provided as an adult, but work by Kitaysky et al. (2003) demonstrates an interesting mechanism of how these could be linked. Kitaysky et al. (2003) implanted hand-reared black-legged kittiwake (*Rissa tridactyla*) chicks with corticosterone for one month and tested their learning skills compared to control birds. Shortly after the implants were removed, cognitive tests were performed to examine how long exposure to corticosterone influenced learning abilities. The researchers found that treated chicks took longer to learn a trained foraging technique than control chicks (some treated chicks failed to learn altogether), and could not learn to associate a color with the presence of food. Amazingly, the researchers were also able to examine the effect of early corticosterone on cognitive abilities over a longer time period as well. Approximately 9 months after the implants were removed, Kitaysky et al. (2003) again tested the cognitive abilities of chicks and found that treated chicks did significantly worse at solving spatial tasks than control chicks. These results suggest that extended exposure to corticosterone during development can have huge impacts on variation in cognitive abilities, which has obvious implications for adult survival and reproduction. Individuals with poor cognitive skills will likely be poor foragers, be in worse body condition, and could be unable to provide appropriate levels of parental care to offspring at the appropriate times. The conditions experienced during early development and while in the nest could have lasting effects on an individual, and potentially provide an explanation for individual variation in parental care.

Effects of individual variation on offspring

Many studies have examined how parents modify the level of care they provide in response to changes in brood value, but the number of studies that have actually tested the assumption that the level of care a parent provides has a discernible effect on offspring is somewhat limited (Clutton-Brock 1991; Krist 2009). Studies that have tested for an effect of parental care on offspring typically find a positive relationship between the two. For example, Sheldon (2002) demonstrated that paternal feeding rates and the share of feeding performed by the male had a significant positive effect on offspring weight and recruitment in collared flycatchers (*Ficedula albicollis*). Parent tree swallows (*Tachycineta bicolor*) with high provisioning rates produced offspring that grew faster and were heavier just prior to fledging (Ardia 2007). One problem with measuring the benefits of parental care in terms of offspring fitness is that these measures are separated by potentially large time intervals, and often cannot be measured in the same study. Researchers that test for effects of care on offspring fitness typically measure one component of parental care (e.g., nestling provisioning) and test its effect on an estimate of offspring performance (e.g., offspring mass), and if they find a significant effect, imply that parents with high levels of care have offspring with higher fitness. Very few studies have actually quantified how parental care affects long-term offspring survival or recruitment to breeding (MacColl & Hatchwell 2003, 2004; Schwagemeyer & Mock 2008). One relevant study that did follow the effects of parental care on offspring

survival until recruitment found that it was not the provisioning rate (trips per hour) of parents that affected recruitment, but the rate at which parents provisioned offspring with large food items (Schwagemeyer & Mock 2008). Furthermore, these types of analyses miss out on other interesting and potentially important aspects of individual variation in care that could influence offspring performance, specifically, how individual deviation from the population mean response (in the level and slope of care) influences offspring performance and fitness.

A fundamental problem with testing the effects of parental care on offspring performance is that both parental care and offspring performance vary in response to many environmental factors (e.g., date in season, brood size, brood age, etc.). Plasticity in response to these conditions can create an artificially strong covariance between parental care and offspring performance, or obscure a potential causal relationship if both respond plastically in different directions to the same factors. For example, the level of parental care and offspring fitness both typically decline with date in breeding season (Bortolotti et al. 2011), which if unaccounted for, could lead to a strong positive covariance between parental care and offspring fitness. Modeling parental care behavior using the reaction norm approach allows us to estimate individual-specific reaction norm components of care while accounting for variation in care due to environmental factors that influence the population response (Nussey et al. 2007). Individual estimates of both the level of care in the mean environment and plasticity of care across specific environments can then be used to ask if individual variation in components of the parental care reaction norm affect offspring performance or fitness. It would not be surprising to find that individuals with consistently high mean levels of care produce offspring that grow faster, but individual plasticity in care could have large impacts on offspring as well. For example, two parents could provide the same mean level of care in the average environment, but differ in their response to offspring age. The parent with a more positive reaction norm slope across offspring age may produce better (i.e., bigger, healthier, etc.) nestlings than a parent with a flat slope across offspring age. Individual variation in components of the parental care reaction norm could have important and untested consequences for offspring.

Conclusions

Providing parental care to dependent offspring is certainly a strenuous activity, demanding a high metabolic cost to parents (Drent & Daan 1980; Clutton-Brock 1991). Parents are known to flexibly adjust the level of care depending on the costs of care to the parent and benefits to offspring (Winkler 1987; Clutton-Brock 1991), and, like many behavioral traits, parental care behavior can be modified on very short time-scales (day-to-day, hour-to-hour, minute-to-minute). Selection has likely favored the high degree of plasticity in this trait because environment factors that influence the amount of care required by offspring or the level of care that can be provided by a parent can change rapidly. Despite this considerable degree of flexibility, individual parents provide care in consistently different ways than other parents. On the one hand, it seems likely that these consistent individual differences in parental care behavior are intrinsic attributes of an individual, created or maintained by differences in genetics, development, or physiology. But on the other hand, the majority of research has focused on the extrinsic factors that influence population-level responses in parental care.

A new focus of the biological basis of consistent individual differences in behavioral traits like parental care is needed. Specifically, the role of genetics in creating among-individual variance in care is poorly understood but likely important in generating the patterns we observe in populations. For example, in a long-term study of great tits (*Parus major*), Nussey et al. (2005) found significant heritability of plasticity in the timing of breeding which was under selection because of changing environmental conditions (see also Charmantier et al. 2008). In addition to additive genetic variation, non-additive genetic effects could influence the level and degree of plasticity of care a parent provides, which would respond differently to selection on care than additive effects. Variation in the conditions experienced early in life are also predicted to create permanent differences among individuals in phenotypes expressed as an adult, however, the effect of developmental conditions on important behavioral traits, such as parental care, are still poorly understood. There are also several potential mechanistic explanations for consistent individual differences in parental care behavior, such as hormonal (Kempenaers et al. 2008; Angelier & Chastel 2009) or neurological differences (Koolhaas et al. 2010). Modeling parental care as a behavioral reaction norm allows us to test how these factors contribute to individual variation in care while accounting for the contribution of environmental conditions known to impact care.

Individual variation is a prominent feature of all life and life history traits, and it is this individual-level variation on which selection acts. However, in the study of phenotypic traits (especially behavioral traits), researchers often focus on sample or population means without much regard for variation around the mean. I suggest that the "tyranny of the golden mean" (Bennett 1987) must end, and that researchers should focus on examining sources and effects of variation in phenotypically plastic traits (Winkler & Wilkinson 1988; Halama & Reznick 2002). The framework outlined here to study variation has been used successfully by others in quantifying individual level variation (Nussey et al. 2005; Smiseth et al. 2008; Westneat et al. 2009). While most modeling efforts and research of plastic behavioral traits like parental care have focused on understanding population-level phenomena, incorporating and specifically studying individual-level variation in these traits is important for understanding the biological basis of this variation, its effects on offspring, and the evolutionary response to selection on care.

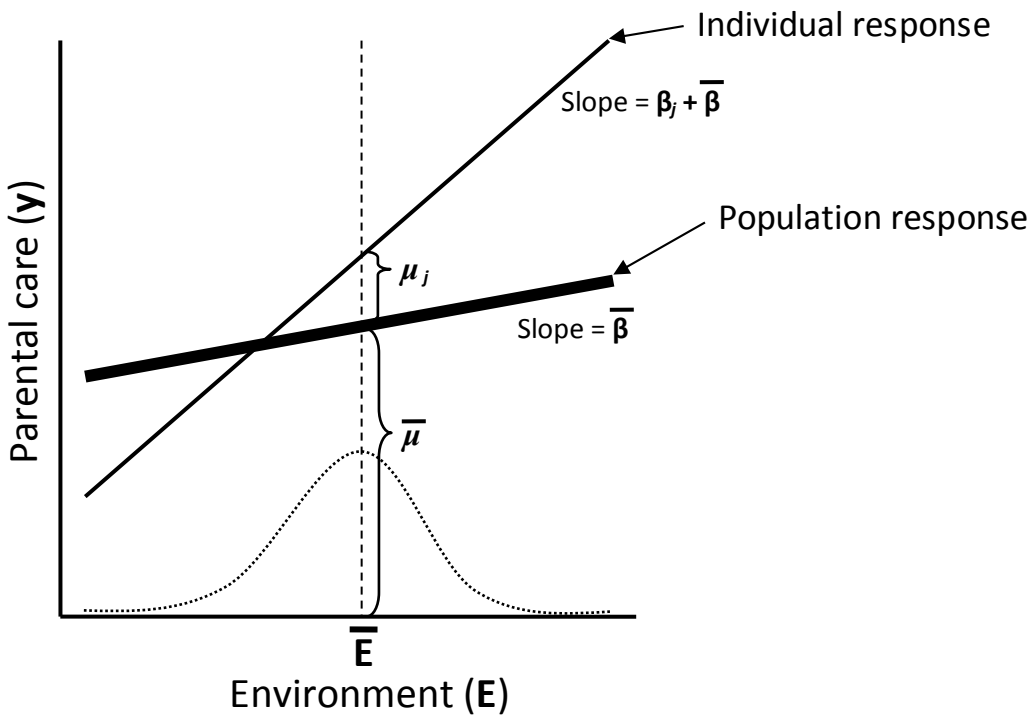

Figure 2.1. A diagrammatic representation of the basic reaction norm model (equation 2), where the phenotype *y* (e.g., amount of parental care) varies in response to an environmental variable (*E*). The variable $\overline{\mu}$ represents the population mean phenotype in the average environment, and $\overline{\beta}$ is the population mean slope across environment *E*. The permanent deviation of individual *j* from the mean population phenotype in the mean environment (individual intercept) is represented by the term μ_j. Individual *j*'s deviation in slope from the population mean slope across environment *E* (individual plasticity) is represented by β_j. The environmental variable *E* is zero-centered, such that all intercepts are estimated in the average environment.

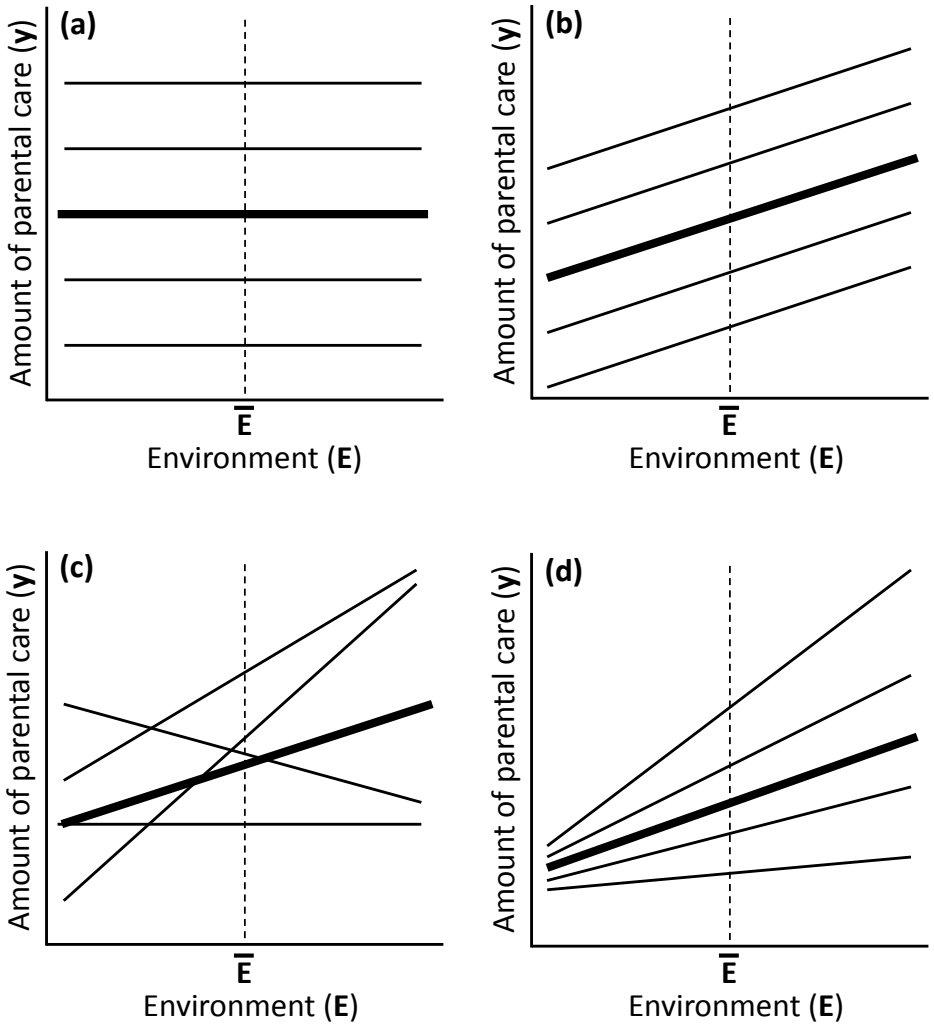

Figure 2.2. Examples of reaction norms with different degrees of variation (equation 4). The thick line in each panel is the mean population response to the environment (E), and each thin line represents an individual's phenotype across the environment. Figure (a) demonstrates a scenario where there is individual variation in the level of the phenotype expressed in the average environment (V_μ; intercepts of the thin lines in the average environment), but that there is no phenotypic plasticity in response to environment variable E; (b) demonstrates individual variation in phenotype (V_μ; intercepts of the thin lines), the phenotype is plastic in response to the environment (slopes of all lines are non-zero), but individuals do not differ in their response to the environment (no difference in slopes of thin lines); (c) demonstrates individual variation in the mean phenotypes (V_μ; intercepts of the thin lines), there is phenotypic plasticity (slopes of the thin lines are non-zero), and individuals differ in their response to the environment ($V_{\beta E}$; slopes of the thin lines are different); finally, (d) demonstrates individual variation in the mean phenotypes (V_μ; intercepts of the thin lines), phenotypic plasticity (slopes of the thin lines are non-zero), individual variation in response to the environment ($V_{\beta E}$; slopes of the thin lines are different), and a covariance between the level of the phenotype and response to the environment (Cov(μ, β); intercepts and slopes of the thin lines covary).

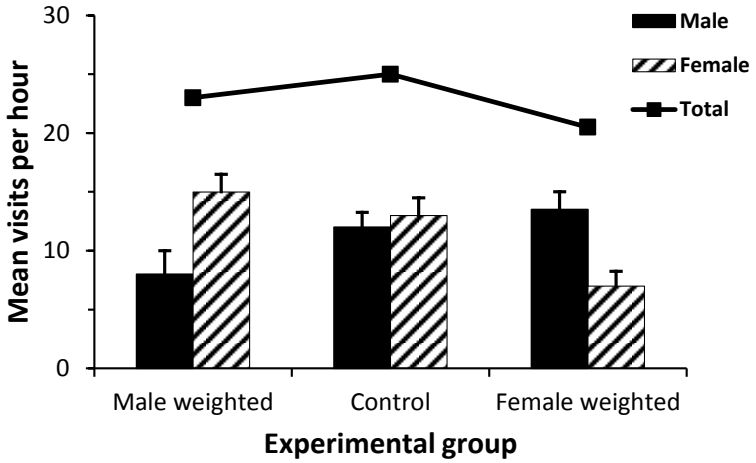

Figure 2.3. Mean feeding visit rate (visits per hour) for nests in a mate compensation study of European starlings (*Sturnus vulgaris*). Females paired with tail-weighted males (Male weighted) increased their level of care compared to controls, and males paired with tail-weighted females (Female weighted) increased their level of care compared to controls. Compensation of the un-weighted partner was incomplete (line). Redrawn from Wright & Cuthill (1989).

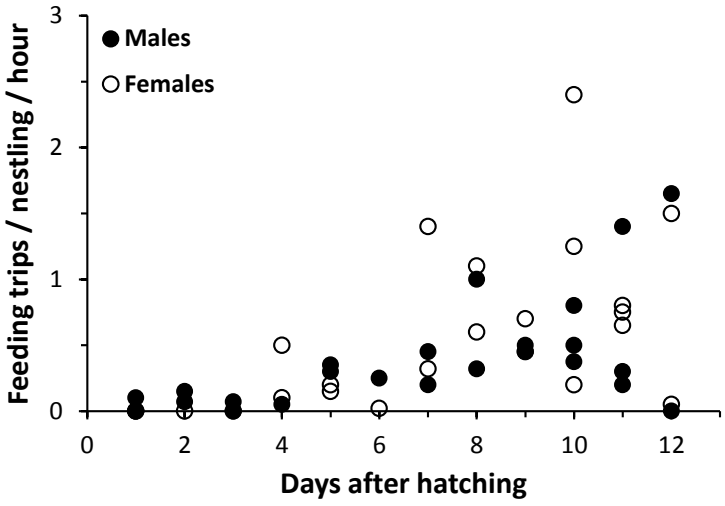

Figure 2.4. The fruit provisioning rate (trips per nestling per hour) of individual male and female northern mockingbirds (*Mimus polyglottos*) at different nestling ages (days after hatching). Redrawn from Breitwisch et al. (1986).

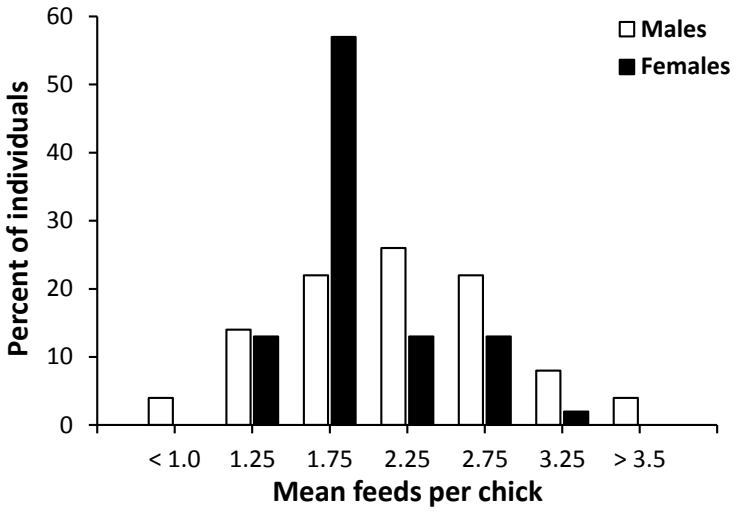

Figure 2.5. Distributions of male and female house sparrow (*Passer domesticus*) provisioning rates averaged across broods. Redrawn from Schwagmeyer & Mock (2003).

CHAPTER THREE

Parental care syndromes in house sparrows: positive covariance between provisioning and defense linked to parent identity

Parental care is a key life history trait for many organisms, and is broadly defined as any parental behavior directed towards offspring that has the potential to increase the fitness of the parent's offspring (Clutton-Brock 1991; Royle et al. 2012; Klug et al. 2012). Forms of parental care include preparing a nest or burrow, production of eggs, brooding, guarding, and provisioning eggs or young, and providing assistance to mature offspring. Patterns of care vary widely both across and within taxa (Clutton-Brock 1991; Gross 2005; Kvarnemo 2010). Within a species there are many different forms of care a parent may provide, yet it is often unclear whether the processes underlying different types of care are similar or different. One might expect different forms of care to trade-off if performing each involves limited energy, resources, or time. For example, time spent performing brood care and fanning directly trades-off with time spent guarding or defending the nest in some fish species (Rangeley & Godin 1992; Lissåker & Kvarnemo 2006). Alternatively, some forms of parental care might reflect common fitness outcomes, and so be positively correlated. For example, high-quality individuals might provide high levels of most types of parental care if higher efficiency in foraging (for self or dependent offspring) allows high-quality parents to provision offspring with more or better food and also permits them more time for nest guarding (Nur 1984d; Watanuki 1992). This would constitute a behavioral syndrome (Sih et al. 2004; Sih 2011) driven by quality differences (e.g., Botero et al. 2010). Another explanation that would create a positive correlation between forms of care is that the types of care are actually independent, but have reaction norms that are sensitive to the same environmental factors. For example, parents with many or more valuable offspring might provide higher levels of all types of care than parents with fewer or low-quality offspring (Griggio et al. 2009). A significant problem with testing how different forms of parental care covary is that many factors could influence all forms of care in similar or different directions; these confounding factors could create or obscure the relationship between forms of care.

In birds, two of the main forms of parental care after the eggs hatch are provisioning nestlings and defending the nest from predators (Clutton-Brock 1991). The vast majority of parental care studies in birds have quantified aspects of nestling provisioning rates. Only a few studies to date have examined both nestling provisioning and nest defense simultaneously, and these have produced mixed results (positively related: Greig-Smith 1980; Rytkönen et al. 1995; not related: Hoi et al. 2003; Kopisch et al. 2005; Duckworth 2006; Hainstock et al. 2010). Measuring multiple aspects of parental care gives us a more complete estimate of the level of care a parent is able to provide, allows us to test how different forms of care are related, and provides insight into a potential behavioral syndrome affecting parental care. Parental investment and nest defense theory predict that parents derive an increased benefit from successfully defending high-value offspring (i.e., both forms of care respond to the same factor – brood value; Trivers 1972; Montgomerie & Weatherhead 1988). In support of this prediction, some studies have found that parents increase their level of defense not only

as the number and age of nestlings increase (e.g., Greig-Smith 1980; Olendorf & Robinson 2000), but also when nestling ornaments are artificially enlarged (Griggio et al. 2009) and when nestlings are food supplemented (Rytkönen 2002). Theory predicts that any trade-off between nestling provisioning and defense is relatively unimportant and that forms of care should positively covary: parents will intensify their level of parental care (provisioning and defense) as the benefit or value of the care increases (e.g., when a parent has more or high-condition offspring).

One problem with appropriately testing such a prediction is the high degree of phenotypic plasticity in behavioral traits like parental care (Westneat et al. 2011). Nestling provisioning can be modified on a short time-scale and therefore often varies widely between observations (e.g., Nur 1984a; Wright et al. 1998). Much of this variation is likely due to environmental and within-individual effects (e.g., date in season, number and age of offspring, age of parent, breeding attempt number, etc.). Despite this plasticity, research has shown that individuals provide significantly different levels of care (i.e., an 'animal personality'; MacColl & Hatchwell 2003; Schwagmeyer & Mock 2003; Nakagawa et al. 2007; Dor & Lotem 2010; Westneat et al. 2011; Low et al. 2012). In an attempt to address these problems, many studies observe parents several times over a breeding cycle and then use mean values to obtain an estimate of the level of care. Unfortunately, this method often fails to account for the largest portions of variance in parental care (up to 90% of variance can remain unexplained; Winkler 1992; Redmond et al. 2009; Westneat et al. 2011). Another problem with testing how nestling provisioning covaries with nest defense is that many factors are predicted to influence both forms of care in similar directions (e.g., time of season, brood value). Such parallel reaction norms could produce covariance where none exists, or possibly mask a relationship when it actually does exist. One solution to both of these issues is to use a behavioral reaction norm approach. This approach simultaneously accounts for the variance due to environmental and within-individual factors as well as variance due to consistent individual differences (Nussey et al. 2007; van de Pol & Wright 2009; Dingemanse et al. 2010).

In this study, I tested the nature of the relationship between nestling provisioning and nest defense in the house sparrow (*Passer domesticus*). First, I used a reaction norm approach to test and account for phenotypic plasticity, variance due to consistent individual differences, and factors that affect both forms of care. Second, I tested how the two forms of parental care were related. I predicted that if there is a trade-off underlying nestling provisioning and nest defense, these forms of parental care would be negatively related. On the other hand, if parental quality or brood value impacts both forms of parental care, I reasoned that nest defense and nestling provisioning should have a positive relationship. Finally, I investigated the hypothesis that variance in brood value mediates the relationship between nestling provisioning and nest defense by accounting for measures of brood value and observing if the relationship between the two forms of parental care changed.

Methods

I conducted this study from March through August of 2008 at the University of Kentucky Agricultural Experiment Station located just north of Lexington, KY (38°06´ N, 84°29´ W). I monitored sparrows breeding in 50 nest boxes placed on the outside

walls of four barns at the site (10 to 20 nest boxes per barn). House sparrows in this population lay an average of five eggs per clutch with a range of one to eight eggs, and each pair attempts one to six clutches per season (Westneat et al. 2009; Wetzel et al. 2012). This species provides bi-parental care; both sexes feed offspring and defend the nest from potential predators and competitors (Lowther & Cink 2006). Adults were trapped early in the season using mist nets and seed-baited cage traps, and banded with a metal USGS band and a unique combination of plastic color bands so they could be identified by sight. Starting in mid-March, I checked nest boxes twice weekly for breeding activity. Active nests were checked at least three times per week through egg-laying, incubation, and nestling stages. Nestlings were weighed approximately every other day; nest checks ended after the nestlings were banded at 10 days of age (mean ± SD nestling age: 9.9 ± 0.8 days). I calculated nestling growth rate for each brood as the slope of the regression of mean nestling weight on nestling age (in days). Growth rates were calculated for nests that were weighed at least three times (mean ± SD: 4.6 ± 0.7 weights, $N = 53$) and for nestlings that survived to banding. The calculated growth rates were highly correlated with the change in mean nestling mass from the first to last weight ($r = 0.97$, $N = 53$, $P < 0.0001$).

Nestling provisioning trips made by parent sparrows were videotaped in two, approximately 2 hour sessions or visually observed in two, approximately 1 hour sessions (mean ± SD session duration: 105 ± 28 min, $N = 151$) when the nestlings were 5 and 7 days old. If the box was videotaped, a video camera was hidden inside a small enclosure located 1 – 5 m from the nest box. If the box was visually observed, the researcher sat in a vehicle 15 – 30 m from the nest and viewed parents with a 45x spotting scope. Observations were typically performed in the morning (mean ± SD start time: 8:50 am ± 1 hr), and the number of nestlings in each nest during an observation ranged from 1 – 6 nestlings (mean ± SD: 3.8 ± 1.3 nestlings). Nestling provisioning data were gathered from 89 breeding attempts over 151 observation sessions (a total of 263 observation hours). Most pairs were recorded in more than one breeding attempt, and a few parents obtained new partners for subsequent attempts. This resulted in a dataset that included 302 nestling provisioning observations from 98 unique individuals (49 birds of each sex). On average, each parent was observed three times (mean ± SD: 3.1 ± 1.6, range: 1 – 8 observations). All videos were scored by one researcher. Provisioning rate was quantified for each parent by measuring their feeding trips per hour during each observation period (observation session duration minus the latency of the first feeding by either parent).

Nest defense behaviors were elicited from parent sparrows when the nestlings were approximately 6 days old (mean ± SD nestling age: 6.7 ± 1.4 days, $N = 64$), and were typically performed at midday (mean ± SD start time: 11:05 am ± 2 hr). On the target day, I placed a mounted European starling (*Sturnis vulgaris*) at the entrance of the nest box for 10 min. Starlings are a common nest site competitor of house sparrows (Weitzel 1988; Kopisch et al. 2005). Although the nest boxes at the study site are too small for starlings to enter, this does not stop them from attempting to usurp boxes from the sparrows, and these attempts can cause damage to sparrow eggs, nestlings, and adults in the process (IRK Stewart, personal communication). Breeding sparrows frequently attack starlings that investigate their nest boxes. To initiate an observation, I pinned the mounted starling to the front of a nest box. The body of the starling was directly below the nest box entrance hole and the head was just in front of, but not completely

obstructing the hole, facing the entrance. The observer retreated to a vehicle 15 – 30 m away from the nest and immediately began observing the nest using binoculars and a 45x spotting scope. For each parent, I recorded the latency of the bird to approach the nest box, number of times it approached the nest box (within 1 m), and if the bird struck the starling. After the starling was knocked from the box by a parent or 10 min had elapsed, the starling was removed and the trial ended. The duration of the defense bout was calculated as the total length of the bout minus the latency until the subject was first observed approaching the nest box. The frequency of approaching the box was divided by the duration of the defense bout to obtain the rate of the behavior per minute. Striking the starling was scored as either "yes" or "no" rather than a rate because the starling sometimes fell off the box after a variable number of strikes. Birds that were not observed in the vicinity of the nest box within the 10 min were not included in the analysis.

Nest defense data were collected on 60 house sparrow breeding attempts; however, data from only 41 attempts (64 unique individuals; 32 birds of each sex) were used. The remaining nests and parents were excluded because neither parent came to the box during the trial or because an individual had been observed more than once. I only included data from the first time nest defense behaviors were elicited from an individual. The provisioning observations described above were typically performed on days before and after the parents were exposed to the nest defense protocol, therefore, I also tested if parents changed their provisioning rate after exposure to the mounted starling on a subset of nests. Approximately 45 minutes immediately prior to and 45 minutes immediately after eliciting nest defense behavior, I performed nestling provisioning observations on a subset of 36 nesting attempts using a spotting scope or video camera. These data were only used to test if parents changed provisioning behavior after exposure to the starling and were not included in the larger data set on nestling provisioning.

Nestling provisioning was measured multiple times for each individual at varying times of season under varying conditions (different observation initiation times, varying help from partner, etc.). These varying conditions are known to affect the rate of nestling provisioning (Nur 1984a; Breitwisch et al. 1986; Hegner & Wingfield 1987; Westneat et al. 2011), so I used a reaction norm approach to analyze provisioning rate with regard to nest defense. This approach is based on the "phenotypic equation" described in Chapter 2 (Nussey et al. 2007; Dingemanse et al. 2010; Westneat et al. 2011).

I used a linear mixed model to test if nestling provisioning rate was affected by any of the three nest defense variables. In this model, nestling provisioning was the dependent variable and bird identity was entered as a random factor. I accounted for two types of fixed effects: between-individual and within-individual factors. First, I took the mean of each individual for parent age, observation start time, and partner provisioning rate and centered each with respect to other individuals to control for between-individual differences in the circumstances under which observations were measured (van de Pol & Wright 2009). None of these factors explained a significant amount of variance between individuals in nestling provisioning, so no between-individual effects were included in the next step. Second, I mean-centered each observation within individuals for each fixed factor (including date, number and age of nestlings, observation start time, and partner provisioning rate) to account for within-individual variation in provisioning rate. All main within-individual factors were added to the model, and then backward elimination was used to generate a best-fit model describing variation in provisioning rate. I

sequentially removed terms that had the smallest *F*-value and a *P*-value greater than 0.1; date in season was the only term that fit this criteria. I then used this model to test if provisioning rate was related to any of the three measures of nest defense by independently adding each measure of defense as a between-individual factor to the model. To evaluate how brood value influenced the relationship between provisioning and nest defense, I added measures of brood value to the best fit model of nestling provisioning that contained the nest defense variable. Measures of brood value I included were the number of nestlings, age of nestlings, date (offspring produced earlier in the season are predicted to be more valuable), and nestling growth rate (residuals of the growth rate by age at banding). Measures of brood value were not correlated (all $P > 0.05$). I then examined the change in the effect size of the defense variable to determine if adding the measures of brood value drastically altered the relationship between provisioning and defense.

I used Proc Mixed in SAS 9.2 (SAS Institute Inc., Cary, NC) to construct and analyze the reaction norm models and JMP 9.0 for correlation and regression analyses (SAS Institute Inc., Cary, NC). Birds used in this study were caught and banded under USGS banding permit #22813, and the experiment was conducted with permission from the University of Kentucky Institutional Animal Care and Use Committee.

Results

Parent house sparrows responded to the starling an average of 3.0 ± 0.3 (SE) minutes after the trial was initiated, approached the starling at an average rate of 2.6 ± 0.7 approaches per minute, and 34% of the parents struck the starling. Nest defense behavior did not differ between the sexes for any of the three measures (Wilcoxon signed-ranks test, Logistic regression: all $P > 0.10$). Nestling sparrows grew at an average rate of 2.2 ± 0.05 grams per day, and mean nestling survival within a nest was 76% ± 4%. Nest defense measures were correlated with date in season and nestling growth rate, but not observation start time, age or tarsus length of the subject, or the number or age of nestlings (Table 3.1). A parent breeding earlier in the season had a higher approach rate (Table 3.1). Parents with nestlings that grew faster approached the starling at a significantly higher rate (Table 3.1) and tended to be more likely to strike the starling (Logistic regression: $X^2_{47} = 3.7$, $P = 0.05$). There was also a trend for parents with more nestlings to be more likely to strike the starling (Logistic regression: $X^2_{64} = 3.3$, $P = 0.07$).

Parent sparrows averaged 12.0 ± 0.7 nestling provisioning trips per hour. Males (12.0 ± 0.7 trips per hour) and females (11.8 ± 0.9 trips per hour) provisioned at similar rates (GLMM: $F_{1,80.5} = 0.06$, $P = 0.81$). I found no change in the feeding rate of parents from immediately before (10.4 ± 0.7 trips per hour) to immediately after (9.6 ± 0.7 trips per hour) the nest defense trial in the subset of nests where this information was gathered (Paired *t* test: $t_{71} = -1.2$, $P = 0.24$). I found significant phenotypic plasticity in nestling provisioning rates within individuals with respect to the number of nestlings and the partner's provisioning rate (Table 3.2).

Using the linear mixed model, I found that parents that struck the starling had higher feeding rates than those that did not strike the starling (Figure 3.1a; strike: 14.1 ± 0.8 trips per hour, no strike: 11.2 ± 1.0 trips per hour; GLMM: $F_{1,65.4} = 8.2$, $P = 0.006$). Adding measures of brood value (date, number and age of nestlings, nestling growth rate)

had no discernible effect on the relationship between provisioning rate and the likelihood of striking the starling (Table 3.2). Provisioning rate was significantly positively associated with the number of nestlings and nestling growth rate (Table 3.2); however, the likelihood of striking the starling continued to explain a significant amount of variance in nestling provisioning rate and the magnitude of the effect remained similar (Figure 3.1b; strike: 14.9 ± 0.9 trips per hour, no strike: 11.6 ± 1.1 trips per hour; GLMM: $F_{1,\ 51.7} = 9.9$, $P = 0.003$). I found no relationship between nestling provisioning and the nest defense variables approach rate ($F_{1,82.3} = 1.0$, $p = 0.32$) or latency to approach ($F_{1,61.1} = 0.1$, $p = 0.90$). Adding measures of brood value had no effect on these results.

Discussion

I found no evidence of a trade-off between nestling provisioning and nest defense. Parental investment theory predicts there should be a positive covariance between forms of care because, regardless of the type of care, parents should expend more energy or take increased risk to care for offspring when the benefit or value of care increases (Trivers 1972; Winkler 1987; Montgomerie & Weatherhead 1988). If parents can assess value then they should respond flexibly to changes in value, and, according to theory, all types of care should covary due to any variation in the value of care. My data do not allow me to assess positive covariance within individuals but instead provide evidence for a positive between-individual covariance in these forms of care; parents with high nestling provisioning rates were more likely to strike the starling than parents with low provisioning rates. Others have found a similar, positive relationship between provisioning and defense and have interpreted this as supporting parental investment theory (Greig-Smith 1980; Rytkönen et al. 1995). House sparrows would thus seem to add support to this prediction of parental investment theory.

A positive covariance between different forms of care is not universal. Indeed, I found no relationship between nestling provisioning and two other measures of nest defense (latency to approach the nest box and the rate of approaching the starling). Several studies have found no relationship between defense and provisioning (Hoi et al. 2003; Kopisch et al. 2005; Duckworth 2006; Hainstock et al. 2010). Of these studies, two have been conducted on house sparrows (Hoi et al. 2003; Kopisch et al. 2005). Specifically, Hoi et al. (2003) found there was no relationship between the time male sparrows spent guarding the nest prior to egg laying and his provisioning rate. Kopisch et al. (2005) found that there was no relationship between the level of nest defense against a European starling and nestling provisioning rate. However, Kopisch et al. (2005) did find a significant, positive relationship between time spent incubating eggs and nestling provisioning rate. Results from these previous studies are difficult to compare with ours because each study measured nestling provisioning and nest defense in different ways. Additionally, although both previous studies attempted to take some fixed factors (brood size) and random factors (study year and site) that influence parental feeding rates into account, neither study accounted for plasticity of either form of parental care behavior. A major problem with traditional statistical methods of analyzing parental care (regression and correlation analyses) is the inability to appropriately account for and investigate within- or between-individual covariates that have conceptual significance. This has two consequences: first, some environmental variables may affect both nest defense and

provisioning in ways unrelated to parental investment theory (e.g., an effect of weather condition or time of day). Second, parental investment theory predicts that variables linked to the value of care should affect both forms of care similarly, thereby producing positive covariance.

I used a behavioral reaction norm analysis to control for factors that might affect both types of care and to explicitly test the notion that both forms of care would respond jointly to differences in brood value. This analysis casts doubt on the conclusion that the positive covariance between nest defense and provisioning supports parental investment theory. In some analyses, higher brood value did increase expression of both types of care. For example, both nest defense behavior and provisioning rate were greater in nests with high nestling growth rates. It may be that parent sparrows are modifying the rate at which they provision nestlings based on growth rate; male house sparrows have been found to feed experimentally provisioned offspring at a higher rate (Mock et al. 2005). Alternatively, it is possible that higher nestling provisioning produced higher growth. The increased nest defense with offspring growth rate is nevertheless consistent with theory (Montgomerie & Weatherhead 1988) and two experimental studies in which parents of artificially supplemented nestlings engaged in significantly more and riskier nest defense behavior (Rytkönen 2002; Thünken et al. 2010). A recent study of nest defense in house sparrows found that females did not adjust their level of defense to brood value, though there was a trend for males to defend earlier broods more intensely (Klvaňová et al. 2011). Other measures of brood value produced different effects on each type of care. Provisioning rate was higher for larger broods both between and within individuals, but the effect of brood size on the likelihood of striking the starling was less strong and was not correlated with other measures of nest defense (approach rate or latency). Date had effects on some measures of nest defense, but not on provisioning. Thus the two forms of care appear to correlate differently with various aspects of brood value. The main implication of this result is that variance in brood value did not mediate the positive relationship between nestling provisioning and nest defense – I did not find support for the prediction of parental investment theory. This suggests that each form of parental care has its own reaction norm.

Two important additional findings emerged from this analysis. First, the reaction norm approach revealed that the positive relationship between provisioning and the likelihood of striking the starling persisted after all other variables, including measures of brood value, were added as covariates and statistically controlled. This means that the two forms of care covary due to variables other than those I included. While additional aspects of brood value may exist (e.g., sex ratio), they probably have minor effects and therefore are unlikely to explain the covariance between types of parental care. It is also possible that the way I gathered parental care data influenced the outcome, for example, both forms of care were recorded at the nest box. Parents that visit the nest more often may be more likely to see the model and respond (i.e., decreased latency), though it is unlikely this issue could influence other measures of defense, such as the likelihood of striking the starling once it has been detected by the parent. This leads me to propose that some other underlying variable may be more important in producing the covariance between the forms of care.

The second key finding emerged from the analysis of nestling provisioning. I found significant between-individual variation in provisioning rate (approximately 24%

of the variance in provisioning rate was due to individual identity) even after I controlled for an array of within-individual and between-individual covariates. This repeats a similar finding from a separate dataset on this same population of house sparrows (Westneat et al. 2011). One plausible explanation is that individuals differ in quality, i.e., an underlying attribute of the individual alters either the costs or benefits of all types of care. There are several ways in which quality could produce a positive covariance between provisioning and defense. One, alluded to above, is that efficient foragers spend less time away from the nest gathering food, and so detect threats to the nest more rapidly. Alternatively, parents in good condition could devote more feeding effort to offspring and incur lower costs from attacking starlings. Other studies have found positive correlations between individual quality and reproductive performance. For example, offspring development in blue tits (*Cyanistes caeruleus*) is positively correlated with male plumage coloration, an indicator of foraging ability (Senar et al. 2002). Individual differences in foraging ability were found to predict laying date and clutch size in kestrels (*Falco tinnunculus*; Daan et al. 1990). Handicapping parents by clipping flight feathers or adding weights to a bird has been shown to decrease provisioning rate (Schwagmeyer & Mock 2003; Patterson et al. 2011), but the effect of this treatment varies by the quality of the parent (Schwagmeyer & Mock 2003; Ardia & Clotfelter 2007). These studies lead to the hypothesis that individual quality differences influence both nestling provisioning and nest defense. My results thus provide some support the idea that nest defense and provisioning are part of a behavioral syndrome.

A behavioral syndrome has been defined as correlated behavioral traits exhibiting between-individual consistency across two or more situations, thus constraining plasticity (Sih et al. 2004). Examples include correlations between activity, exploratory behavior and aggressiveness in three-spined sticklebacks (*Gasterosteus aculeatus*; Dingemanse et al. 2007) and risk-taking and activity in house sparrows (Bókony et al. 2012). My results support the existence of a syndrome between forms of parental care, but also demonstrate that patterns of variation in both types of care have a complex mix of effects. I found that parents differ consistently in nestling provisioning rate (i.e., a 'parenting personality'; Dingemanse et al. 2010; Westneat et al. 2011). Although I could not test for it, consistent differences between individuals have also been found for measures of nest defense (Redmond et al. 2009; Kontiainen et. al. 2009). Furthermore, both types of parental care responded plastically in the same direction to some of the same factors, responded plastically in different directions to other factors, and yet after accounting for this plasticity, continued to positively covary. Thus, these data suggest programs for producing nest defense and provisioning that have independent elements and two types of linked elements: joint plasticity to some external factors, and a common linkage to internal attributes I might call quality. The latter could be viewed as a form of plasticity in which both traits respond similarly to the same internal environment (e.g., the parent's condition or other attributes that may change, such as age). Future studies could further document within- and between-individual variance in both types of care and attempt to identify those mechanistic (e.g., hormonal) or developmental (genetic or early environmental) factors that contribute to consistent differences between individuals in their parental care reaction norms.

Table 3.1. Spearman's correlation coefficients comparing two measures of nest defense with factors that could affect defense in parent house sparrows.

	Latency	Approach rate	*N*
Date	0.12	-0.33*	64
Observation start time	0.24	-0.16	64
Tarsus length of subject	-0.11	-0.11	63
Age of subject	0.08	-0.14	64
Nestling age	-0.24	0.16	64
Number of nestlings	-0.15	0.20	64
Nestling growth rate	0.002	0.35*	49

* $P < 0.05$

Table 3.2. Linear mixed model analysis of nestling provisioning rate for 98 house sparrows. All fixed effects are within-individual effects unless noted as "between", in which case they are between-individual effects.

Variable	Effect ± SE	*F* (*DF*)	*P*-value
Individual	8.9 ± 2.8		
Residual	12.8 ± 1.8		
Intercept	14.9 ± 0.9		
Number of nestlings	1.5 ± 0.4	13.6 (114)	0.0003
Age of nestlings	0.5 ± 0.2	4.4 (109)	0.04
Observation start time	-18.0 ± 9.6	3.5 (120)	0.06
Partner feeding rate	0.3 ± 0.09	9.1 (115)	0.003
Date (between)	0.02 ± 0.04	0.3 (67.4)	0.62
Number of nestlings (between)	1.3 ± 0.6	4.3 (49.8)	0.04
Age of nestlings (between)	1.4 ± 0.8	3.4 (58.1)	0.07
Nestling growth rate (between)	4.9 ± 1.7	8.7 (142)	0.004
Strike (no)	**-3.3 ± 1.1**	**9.9 (51.7)**	**0.003**

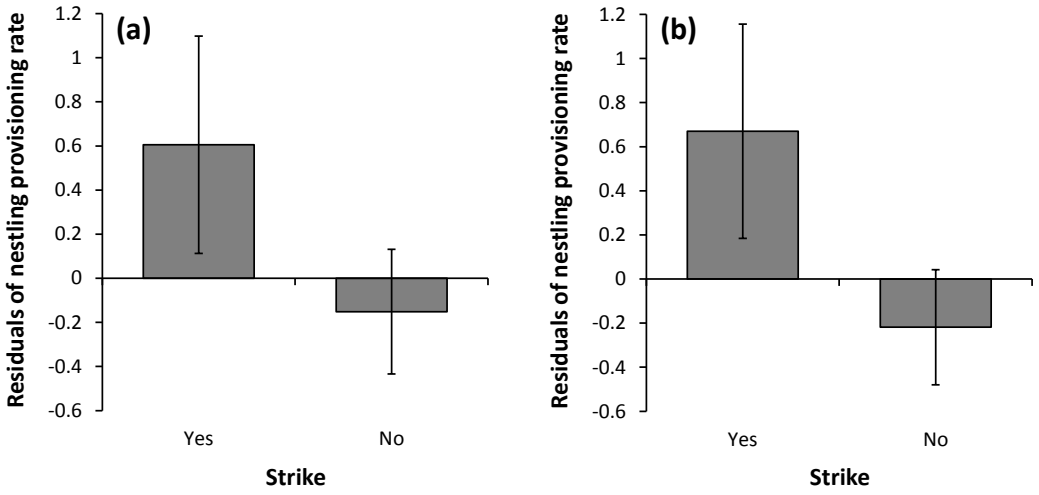

Figure 3.1. The effect of striking the starling on nestling provisioning rate (± SE): (a) parents that struck the starling had a significantly higher nestling provisioning rate than those that did not. Depicted here are the residuals of provisioning rate accounting for the random effect of individual and all fixed effects included in Table 3.2, except the measures of brood value (date, number and age of nestlings, and nestling growth rate) and the variable "strike." (b) After accounting for the effects of brood value, whether or not a parent struck the starling predicted provisioning rate similarly to the effect shown in (a). Data are the residuals of provisioning rate accounting for the random effect of individual and all fixed effects included in Table 3.2 except the variable "strike."

CHAPTER FOUR

Genetic and developmental sources of individual variation in parental care behavior

Parental care is a key life history trait for most organisms (Clutton-Brock 1991). Parental care varies widely among species (e.g., Curio 1988; Clutton-Brock 1991; Eggers et al. 2005) but can also exhibit considerable variation within a species or population. Parental care behavior within a population has been found to vary in two interesting ways: 1) individual parents provide varying amounts of care depending on environmental conditions (e.g., time of season (Ringsby et al. 2009), number of offspring (Breitwisch et al. 1986), or amount of care provided by a partner (Wright & Cuthill 1989)), and 2) despite this phenotypic plasticity, individual parents differ consistently in the level of care they provide to their offspring (Schwagmeyer & Mock 2003; Nakagawa et al. 2007; Dor & Lotem 2010; Low et al. 2012). After accounting for phenotypic plasticity, recent research has found that up to 20% of the variance in parental care can be due individual identity (Westneat et al. 2011). Because selection acts on individual phenotypes, studying the sources of this individual variation is critical to understanding the evolutionary response to selection on parental care. Although there are several potential mechanistic explanations for consistent individual differences in parental care behavior (e.g., hormonal differences, Angelier & Chastel 2009), ultimately, the developmental sources of this variation (e.g., genetic and early environmental effects) are most relevant to understanding the existence and maintenance of individual variation. One problem with testing the influence of additive genetic variation or the natal environment on parental care behavior is properly accounting for the sources of variance in such a labile trait.

Previous studies of several taxa have found additive genetic variation for parental care behavior, including the burying beetle (*Nicrophorus vespilloides*; Walling et al. 2008), European earwig (*Forficula auricularia*; Meunier & Kölliker 2012), African striped mouse (*Rhabdomys pumilio*; Rymer & Pillay 2011), long-tailed tit (*Aegithalos caudatus*; MacColl & Hatchwell 2003), savannah sparrow (*Passerculus sandwichensis*; Freeman-Gallant & Rothstein 1999), and house sparrow (*Passer domesticus*; Dor & Lotem 2010). Although it appears there is significant evidence for the heritability of parental care behavior, these studies have two related problems. First, all but two of the studies were conducted on laboratory populations (all except Freeman-Gallant & Rothstein 1999 and MacColl & Hatchwell 2003). Heritability estimates are typically much larger when measured in a laboratory population than when measured in the wild because of the substantial effects of varying environmental conditions experienced in wild populations (Falconer & Mackay 1996; Dingemanse et al. 2002). Second, despite the fact that behavioral traits like parental care are phenotypically plastic and vary considerably depending on the conditions under which they are recorded, all but one study failed to account for variance due to plasticity or in the environmental conditions when estimating heritability. This is a problem because apparent heritability in parental care behavior could result from different families being recorded under different conditions. For example, if the number of offspring produced is a heritable trait and varies by family, recording parental care behavior without accounting for the number of offspring could lead to consistent differences in parental care among families (i.e.,

apparent heritability). A solution to the latter problem is to use a behavioral reaction norm approach when estimating the effect of additive genetic variation on parental care. This approach simultaneously accounts for variance between individuals in the conditions when they were measured, variance due to phenotypic plasticity, and variance due to consistent individual differences (Nussey et al. 2007; van de Pol & Wright 2009; Dingemanse et al. 2010). Interestingly, phenotypically plastic traits like parental care are heritable, not only in the level of care provided by a parent, but also in the plasticity of care (additive genetic variation for individual by environment (I x E) interactions), though the heritability of plasticity has rarely been examined in wild populations (e.g., Nussey et al. 2005; Charmantier et al. 2008). The reaction norm approach can also be used to estimate the contribution of additive genetic variation to individual plasticity in parental care. Factors other than additive genetic variation, such as the conditions experienced early during development, could create permanent differences between individuals and possibly influence parental care behaviors expressed later in life.

The conditions experienced during early development can immediately influence an individual. However, the more evolutionarily important issue of the long-term consequences of variance in environmental and maternal factors experienced during development is poorly understood, particularly in vertebrates (Lindström 1999; Monaghan 2008). Studies addressing this question in birds have found that rearing conditions can influence life-history traits expressed later in life. For example, individuals reared at a suboptimal time of season, in poor-quality habitats, or during poor environmental conditions, have been found to have lower survival probability, establish breeding territories in low-quality habitat or fail to establish a territory, and have a shorter life-span (Nilsson & Smith 1988; Daan et al. 1990; Metcalfe & Monaghan 2001; van de Pol et al. 2006; Wilkin & Sheldon 2009). In experimental studies, zebra finches (*Taeniopygia guttata*) reared in enlarged broods took longer to begin breeding as adults, allocated less yolk androgen to eggs, hatched and fledged fewer offspring, and produced smaller nestlings (Gil et al. 2004; Alonso-Alvarez et al. 2006; Naguib et al. 2006; Monaghan 2008). Studies that have tested for long-term effects of egg size have found that birds hatched from large eggs became larger adults, and had larger ornaments (larger combs in red junglefowl (*Gallus gallus*) and larger song repertoires in song sparrows (*Melospiza melodia*); Parker 2002; Krist 2009; Zanette et al. 2009; Krist 2011). Nestlings reared with experimentally manipulated high-quality or nutrient-rich diets had increased song performance, performed better on cognitive tests, had a higher likelihood of breeding and reproduced sooner, and had higher survival than nestlings fed a baseline diet (Richner 1992; Birkhead et al. 1999; Nowicki et al. 2002; Blount et al. 2006; Pravosudov & Kitaysky 2006; Bonaparte et al. 2011; MacDougall-Shackleton & Spencer 2012). Finally, and perhaps most striking, laboratory studies in rodents have shown that the amount of parental care received as dependent offspring can influence the level of parental care provided by those offspring once they reach maturity; offspring raised by poor parents become poor parents themselves (Francis et al. 1999; Meaney 2001). These results suggest that conditions experienced early in life could create differences among individuals in phenotypes expressed as an adult. However, the effect of developmental conditions on important behavioral traits, such as parental care, is still poorly understood.

To study the genetic and developmental sources of individual variation in parental care behavior, I used a multi-year, multi-generational data set gathered from free-living

house sparrows. First, I quantified nestling provisioning behavior using a reaction norm approach, which accounts for fixed environmental factors known to influence parental care and the random effect of individual identity. Next, I tested for heritability of nestling provisioning behavior using offspring-parent regression and the reaction norm model to test for heritability in the level and plasticity of care. Finally, I used the reaction norm model to test if parental care behavior expressed as an adult was influenced by environmental and maternal factors experienced as a nestling.

Methods

I monitored and collected parental care data from a population of wild house sparrows at the University of Kentucky's Agricultural Experiment Station, located just north of Lexington, KY (38°06´N, 84°29´W) from 2008 – 2012. The study site consists primarily of agricultural and pastoral fields and multiple barns used for stabling horses and storage. I monitored 10 to 20 house sparrow nest boxes mounted along the outside walls of each of four barns. The number of nest boxes on each barn and the total number of boxed varied from year to year, but the total number of monitored nest boxes ranged from 50 to 60 boxes each year. This house sparrow population has been monitored continuously from 1992 – 2012. Sparrows in this population breed from March through August, with each pair attempting one to six clutches per season. Females in this population lay an average of five eggs per clutch with a range of one to eight eggs (Westneat et al. 2009). This species has bi-parental care – both sexes feed nestlings (primarily insects) throughout the nesting cycle.

Adult sparrows breeding at the study site were trapped with mist nets and seed-baited cage traps. Each bird was banded with a metal USGS band and a unique combination of colored plastic bands so they could be identified by sight. I collected standard body measurements and a 50 µl blood sample from each bird at the time of capture. Starting in March of each year, I checked boxes twice per week for nest building and breeding activity. Active nests were checked at least three times a week to identify the day the first egg was laid, and this nest-checking schedule continued until nestlings were banded with USGS bands at ~10 days after hatching. At the time of banding, for each nestling, I recorded tarsus length with calipers to the nearest 0.1 mm and nestling mass with an electronic balance to the nearest 0.1 g. For each breeding attempt in a monitored nest box, I recorded clutch size, number of eggs that hatched, and the number of nestlings that fledged from the nest. As part of another study (conducted from 2007 – 2009; IRK Stewart, unpublished), the length and width of each egg in every clutch was measured with calipers to the nearest 0.1 mm approximately halfway through incubation. Egg volume was estimated using the equation: egg volume (mm^3) = 0.51 * egg length * egg $width^2$ (Hoyt 1979), and averaged for each clutch. In house sparrows, egg volume is highly correlated with fresh egg mass and is an accurate measure of egg size (R^2 = 0.91; IRK Stewart, unpublished). Sparrows were banded in three age categories: nestlings, juveniles, and adults. Birds banded as nestlings or juveniles that returned to breed at the site could be aged precisely, while birds banded as adults were assigned a minimum age, which assumes they were in their first breeding season (Westneat et al. 2009).

Nestling provisioning trips made by parent house sparrows were video recorded two to three times per brood in ~2 hour blocks. In 2008, 2009, and 2011 each brood was recorded twice, when nestlings were approximately 5 and 7 days old, while in 2010 and

2012 each brood was recorded three times, when nestlings were approximately 3, 6, and 9 days old. Video cameras were concealed in a small enclosure located 1 – 5 m from the nest box, and recordings were performed in the morning (mean ± SD observation start time: 9:00 am ± 1.3 hrs). All videos were scored later by one researcher (DPW). Nestling provisioning rate was quantified for each parent by recording the number of feeding trips per hour during each observation period. An observation period consisted of the length of the recording (typically 2 hours) minus the latency of the first feed by either parent. In addition to provisioning rate, I also quantified the likelihood of bringing large food items to the nest. Any items brought during a feeding trip that could be seen were scored in reference to bill size (house sparrow bills range from ~1.0 – 1.3 cm) as either small (not visible to < 0.2 cm), medium (~0.5 – 1.5 cm), large (> 1.5 cm), or unknown (see also Schwagmeyer & Mock 2008). I was able to score the size of approximately 57% of all food items brought to the nest for the birds used in this study; of the 5,649 food items observed, 8% (438 items) were classified as "large."

To investigate if additive genetics or the conditions experienced during early development influence parental care behavior expressed later in life, I constructed two similar data sets. Both data sets required nestlings that were hatched at the study site, remained at the site and bred in at least one subsequent year, and had nestling provisioning behavior collected from them during one of these breeding attempts. Although I banded approximately 2,900 nestlings from 2007 – 2011, only 67 of these nestlings returned to breed at the site and were recorded feeding young. To test for an effect of conditions experienced in the nest on parental care behavior, I used data from all 67 of these birds. Each of these birds was recorded feeding their nestlings an average of 7 times (mean ± SD: 7.0 ± 5.0, range: 1 – 21 observations), for a total of 469 observation sessions (875 hrs). The data set used to estimate heritability of nestling provisioning behavior required provisioning behavior observations from at least one parent of the nestlings that returned to breed at the study site. These criteria reduced the sample to 58 birds observed over 411 observation sessions.

I estimated heritability of parental care behavior in two ways. First, I used a linear regression to compare mean nestling provisioning rate of return nestlings with the mean provisioning rate of their parents. In addition to nestling provisioning rate (trips per hour), I also tested if the rate at which parents bring large items to the nest was heritable. The rate at which large items were brought was calculated by multiplying the proportion of large items brought to the nest (of all items seen) by the provisioning rate. In the second analysis, I constructed a reaction norm model of provisioning for all parents and offspring that accounted for fixed effects and the random effect of individual identity, and then tested if adding family identity as a random term explained a significant portion of the variation in provisioning (described below). In this analysis, I constructed separate models for nestling provisioning rate and the likelihood of bringing large items (number of large items out of the total number of items seen).

I used Proc Mixed or Proc Glimmix in SAS 9.2 (SAS Institute Inc., Cary, NC) to a generate a linear mixed model for each measure of provisioning (provisioning rate and the likelihood of bringing large food items to the nest). In each model, I accounted for variation in provisioning due to the differing conditions under which each bird was observed, and fixed effects that influence plasticity in provisioning (Nussey et al. 2007; Dingemanse et al. 2010; Westneat et al. 2011). The dependent variable in each model

was the measure of nestling provisioning, and random effect of bird identity was retained in the model throughout the fitting process. First, I examined if the conditions in which parents were observed influenced between-individual variation in provisioning behavior by taking individual means for each fixed factor (age of the bird, sex of the bird (not mean-centered), breeding attempt number, observation date, brood size, brood age, observation start time, and the partner's nestling provisioning rate) and centering them with respect to the mean of the full data set (van de Pol & Wright 2009). All factors were added to the model, then backward elimination was used to generate a best-fit model that described the sources of between-individual variation for each measure of nestling provisioning. Second, I mean-centered each observation within individuals for each fixed factor to account for phenotypic plasticity in provisioning (van de Pol & Wright 2009). I added all main within-individual centered factors and many of their second-order interactions to the previous model, then used backward elimination to generate a best-fit model describing variation in each measure of provisioning. Finally, to test if parental care behavior was heritable, I added a random term indicating the identity of each family (a family consisted of genetically related individuals, including parents and offspring, siblings, half-siblings, and grandparents). If including family identity significantly improved the fit of the model, I interpreted this to mean a significant portion of the variation in parental care behavior was due to additive genetic effects. Bird identity was nested within family identity for this test. I also tested if individual plasticity (I x E) in parental care had a heritable component by adding a random individual (nested within family) by environment term to the best fit model and comparing its fit to a model with and without a random family by environment term.

I tested if conditions experienced early in life influenced nestling provisioning rate or the likelihood of bringing large items to the nest using a reaction norm model constructed in the same way as described above. I only modeled provisioning behavior of the offspring for this analysis and did not include family identity. To each of these best-fit models of provisioning behavior, I sequentially added measures of the conditions experienced in the bird's natal nest (nest initiation date, clutch size, egg size, number of nestlings, proportion of the nestlings that survived, and the mean provisioning rate received), and indicators of the condition of the bird as a nestling (tarsus length and bird mass at 10 days of age) as between-individual factors to test if these variables influenced the variation in provisioning behavior expressed as an adult.

Nestling provisioning rate was not normally distributed nor could it be made normal through transformation; square root transformation brought the data very close to normal so the analysis was performed on the transformed data. Residuals from the transformed data also deviated from normality, but less so than residuals of the untransformed data (Figure 4.1). I used Proc Glimmix with a logit link and a binomial distribution to analyze the likelihood of bringing large items.

Results

Adult house sparrows with nestlings averaged 11.9 ± 0.5 (SE) nestling provisioning trips per hour. Male (12.0 ± 0.7 trips per hour) and female (11.7 ± 1.0 trips per hour) sparrows did not differ in their provisioning rates ($F_{1,52.1} = 0.13$, $P = 0.72$). Results from the mixed model analysis of nestling provisioning reaction norms found that provisioning rate was affected by variation among individuals, and both provisioning rate

and the likelihood of bringing large items exhibited phenotypic plasticity within individuals (Table 4.1). Individual identity explained 14% of the variance in nestling provisioning rate, even after accounting for the fixed effects in Table 4.1 (Likelihood ratio test: -2dLL = -39.1, $DF = 1$, $P < 0.01$). Individual identity also explained a significant portion of the variance in the probability of bringing large items ($\chi^2 = 285$, $DF = 1$, $P < 0.01$).

I did not find any evidence of additive genetic variance in parental care using either method of estimating heritability. There was no relationship between mid-offspring provisioning rate and that of their mid-parent (Table 4.2). In the reaction norm model, family identity did not explain a significant portion of the variance in the level of parental care expressed (nestling provisioning rate: -2dLL = 0.8, $DF = 1$, $P > 0.1$; likelihood of bringing large items: $\chi^2 = 0.0$ $DF = 1$, $P = 0.48$; Table 4.3). Individual parents varied in their response to the environment (significant I x E terms across date in season, brood size, nestling age, and partner feeding rate), but there was no additional effect of family identity on these slopes (Table 4.4)

Out of all the measures of natal nest conditions (nest initiation date, clutch size, egg size, number of nestlings, proportion of the nestlings that survived, and the mean provisioning rate received) only egg size had a significant effect on parental care behavior. House sparrows that hatched from large eggs delivered food at a higher rate to their offspring (Figure 4.2a; effect: 0.0005 ± 0.0003, $F_{1,27.8} = 4.43$, $P = 0.04$). Although these birds had higher provisioning rates, they also were significantly less likely to deliver large food items to their offspring (Figure 4.2b; effect: -0.002 ± 0.0007, $F_{1,26.5} = 5.54$, $P = 0.03$). Egg size predicted the size of the subsequent nestling (nestling tarsus length: $r = 0.43$, $N = 31$, $P = 0.02$; nestling mass: $r = 0.40$, $N = 31$, $p = 0.03$), and the size of the subsequent adult (adult tarsus length: $r = 0.40$, $N = 30$, $P = 0.03$; adult mass: $r = 0.50$, $N = 31$, $P = 0.004$) for those individuals in this dataset. However, neither nestling size nor adult size predicted provisioning rate later in life (nestling tarsus length: $F_{1,43.7} = 0.01$, $P = 0.93$; nestling mass: $F_{1,50.7} = 0.26$, $P = 0.61$; adult tarsus length: $F_{1,53.8} = 0.01$, $P = 0.94$; adult mass: $F_{1,63.5} = 2.47$, $P = 0.12$).

Discussion

In this study, I used five years of parental care data from a wild house sparrow population to test the effect of additive genetic variation and natal nest conditions on parental care behavior expressed as an adult. The parent-offspring regression analysis found no evidence that additive genetic variation underlies individual variation in parental care in this population. Previous studies of parental care behavior in birds that used parent-offspring regressions found that this trait can be influenced by additive genetic variation, though the heritability estimates have substantial variability. For example, in wild bird populations, researchers found nestling provisioning rates were heritable in the long-tailed tit ($h^2 = 0.59$; 95% CI = 0.24, 0.94; $N = 20$ mid-parent to son pairs; MacColl & Hatchwell 2003) and the savannah sparrow ($h^2 = 1.7$; 95% CI = 0.88, 3.1; $N = 12$ father-son pairs; Freeman-Gallant & Rothstein 1999). In an aviary population of house sparrows, Dor & Lotem (2010) found that heritability of parental care was 0.50 (± 0.22 SE; $N = 10$ mid-parent to son pairs). However, this type of analysis can be confounded by the conditions under which individuals were measured and the large degree of phenotypic plasticity in parental care behavior. Using the reaction norm model,

I found that family identity did not explain a significant portion of the variance in parental care (only 2% of the variance in care could be attributed to family identity). Furthermore, there was no evidence that individual variation in response the environment had a heritable component. Thus, additive genetic variation does not appear to contribute to the maintenance of individual variation in parental care in this population.

There are several possible explanations for the lack of significant additive genetic variation in parental care in this population. Primarily, behavioral traits and traits closely associated with fitness typically have low heritability estimates that can only be detected with very large sample sizes (Mousseau & Roff 1987; Houle 1992). In support of this idea, Wetzel et al. (2012) also found no additive genetic variation for two other fitness-related traits, clutch size and egg size, in the same study population (but using a different set of subjects). Given the expectation that it is difficult to detect heritability of fitness-related traits, it is puzzling that other studies, using 50% to 75% smaller sample sizes, found significant heritability of parental care behavior in birds. It could be that the heritability of parental care behavior in this population is much lower than in the other populations tested. Alternatively, it is possible that by not accounting for environmental factors that contribute significant variance to parental care, these studies have biased estimates of heritability. Indeed, when MacColl & Hatchwell (2003) re-analyzed their data while accounting for some fixed effects that influence care, the estimate of heritability decreased from $h^2 = 0.59$ (calculated with parent offspring regression) to $h^2 = 0.43$ (calculated using the animal model), though this estimate of heritability was still significant. A second explanation for the failure to detect any additive genetic variation in parental care in this population is through the influence of non-additive genetic effects. Non-additive genetic variance is predicted to increase residual variation in estimates of heritability (Houle 1992; Falconer & Mackay 1996). Genetic heterozygosity has been found to have significant effects on fitness-related traits in this and other populations (Foerster et al. 2003; Ortego et al. 2009; Tomiuk et al. 2007; Olano-Marin et al. 2011; Wetzel et al. 2012), and has been found to influence some aspects of parental care behavior (García-Navas et al. 2009; Chapter 6). Because heterozygosity is not directly inherited in the traditional sense (but see Mitton et al. 1993; Neff & Pitcher 2008), its effect will confound the estimation of additive genetic variation. Finally, I might have failed to detect heritability of parental care behavior in this population because environmental conditions experienced early in life have a larger influence on phenotypic development than the contribution of additive genetics. Though this is a plausible explanation, it seems unlikely to be the case in this population (see below).

The second major finding of this study was that average egg size of the clutch from which an individual was hatched had a significant effect on the amount of parental care it expressed later in life. This key result provides support for the idea that egg size can have long-term effects on an individual (Parker 2002; Krist 2009; Zanette et al. 2009; Krist 2011), and can help explain why individual variation in parental care persists. However, I must caution that the relationship between egg size and parental care is not necessarily robust. Egg size data were collected from a substantially smaller number of birds than for most of the traits I tested (only 31 birds had egg size data from their natal nest, returned to breed at the study site, and were recorded feeding young). If I corrected for performing multiple tests of natal conditions on parental care, the effect of egg size was no longer significant. Furthermore, it is puzzling exactly how egg size in itself could

influence parental care behavior. Egg size may have a direct effect on offspring size, which in turn is correlated with body size as an adult, but body size at either life stage did not explain variance in parental care in this population. The size of eggs produced by a female could be indicative of other maternal effects, such as concentrations of nutrients or maternal hormones inside the egg (Schwabl 1993; Tobler et al. 2007; Muller & Groothuis 2013). Maternal hormones deposited in eggs have been found to have long-lasting effects on offspring, including sex determination (Bowden et al. 2000), gene expression (Donohue 2009), and some behavioral traits (competitive ability: Strasser & Schwabl 2004; lateralization: Schaafsma & Groothuis 2012). Interestingly, although egg size is not heritable in this population of house sparrows, approximately 60% of the variation in egg size is due to female identity (IRK Stewart, unpublished). If egg size genuinely influences parental care behavior, this suggests adult female condition or quality could indirectly influence the parental care behavior expressed by its offspring.

On the whole, my results indicate that there was little evidence that conditions experienced in the nest influenced parental care behavior expressed as an adult in this population of house sparrows. Previous studies have found significant effects of the natal environment on a range of adult traits (reviewed in the introduction), though most of these studies differ from this study in one of two ways. First, the majority of these studies were conducted using captive study populations (Birkhead et al. 1999; Francis et al. 1999; Nowicki et al. 2002; Parker 2002; Gil et al. 2004; Alonso-Alvarez et al. 2006; Blount et al. 2006; Naguib et al. 2006; Pravosudov & Kitaysky 2006; Bonaparte et al. 2011). Experimental studies such as these are excellent at controlling for environmental sources of variance that could contribute noise or bias to the trait under study but likely fail to observe the full phenotypic variation of a trait if expressed under natural conditions (Pigliucci 2004). Differences in natal conditions experienced in captivity can affect traits expressed in the same environmental conditions (captivity), but it is unclear how generalizable these results are to wild populations. Second, studies of natal effects in wild organisms have the opposite concern: traits of interest that are expressed as adults (e.g., ornament size) are known to exhibit phenotypic plasticity. Failure to account for the environmental differences experienced among individuals, phenotypic plasticity within individuals, or variance due to consistent individual differences could create or obscure covariance between natal conditions and a trait expressed as an adult. Using a reaction norm model, I found that parental care behavior was not explained by the conditions experienced in a bird's natal nest.

The effects of additive genetic and developmental variation on maintaining individual variation in parental care reaction norms is weak in this population. It is unclear if this is true for other important life history traits; nevertheless, the absence of these effects on parental care could be a product of biased sampling and survival. In this population, approximately 3% of all birds banded as nestlings returned to the study site to breed. If we assume that first year survival of house sparrows in this population is similar to the estimate for this species from other populations, approximately 20% (Lowther & Cink 2006), this suggests I am only capturing a very small portion of the variance that could be created by additive genetics and developmental conditions. Additionally, because mortality rates are so large for first-year individuals, selection likely has a disproportionate effect on characters associated with short-term survival, thereby masking the effects of the natal environment on traits expressed later in life (Price &

Schluter 1991; Houle 1992; Kinnard & Westneat 2009; Wilkin & Sheldon 2009). Though egg size potentially provides one explanation for consistent individual differences in parental care behavior, the majority of the sources of this variation remain unexplained. Additional research is needed to test for other genetic, neurological, or physiological components that contribute to consistent differences between individuals in their parental care reaction norms.

Table 4.1. Results of the mixed model analysis of nestling provisioning reaction norms for 67 house sparrow recruits. Bird identity is a random term, and all fixed effects are within-individual effects unless noted as "between," in which case they are between-individual effects.

	Variable	Effect ± SE	*F* (*DF*)	*P*-value
Provisioning rate (trips/hr)[1]	Bird identity	0.13 ± 0.04		
	Residual	0.52 ± 0.04		
	Intercept	3.4 ± 0.06		
	Brood size (between)	0.50 ± 0.1	24.8 (1, 75.6)	< 0.0001
	Nestling age (between)	0.55 ± 0.1	20.8 (1, 89.4)	< 0.0001
	Partner rate (between)	-0.05 ± 0.02	6.6 (1, 74.3)	0.01
	Date	-0.004 ± 0.001	10.2 (1, 400)	0.002
	Brood size	0.24 ± 0.04	36.8 (1, 400)	< 0.0001
	Nestling age	0.11 ± 0.02	46.4 (1, 400)	< 0.0001
	Partner rate	0.03 ± 0.008	11.4 (1, 402)	0.0008
	Brood size*nestling age	0.06 ± 0.02	11.8 (1, 430)	0.0007
	Brood size*partner rate	-0.02 ± 0.007	4.8 (1, 440)	0.03
Likelihood of bringing large items (logit of large items/seen items) [2]	Bird identity	0.89 ± 0.2		
	Intercept	-2.77 ± 0.2		
	Bird age	-0.32 ± 0.1	8.6 (1, 444)	0.004
	Date	-0.01 ± 0.002	17.1 (1, 444)	< 0.0001
	Brood size	0.23 ± 0.09	7.0 (1, 444)	0.009
	Nestling age	0.17 ± 0.03	33.9 (1, 444)	< 0.0001
	Partner rate	0.005 ± 0.01	0.12 (1, 444)	0.72
	Age*brood size	0.46 ± 0.1	13.2 (1, 444)	0.0003
	Nestling age*nestling age	-0.04 ± 0.01	8.4 (1, 444)	0.004
	Partner rate*date	-0.001 ± 0.0005	5.2 (1, 444)	0.02
	Partner rate*age	-0.11 ± 0.02	21.8 (1, 444)	< 0.0001

[1]Square root transformed

[2]From GLMM model of binomial using logit link and Kenward-Rogers estimation of denominator degrees of freedom

Table 4.2. Results of the offspring-parent regression analysis for heritability of parental care in house sparrows.

Nestling provisioning rate	*N*	h^2	95% CI of β	*P*-value
Mid-daughter by mother	22	0.16	-0.35, 0.51	0.70
Mid-daughter by father	25	0.11	-0.25, 0.35	0.71
Mid-son by mother	20	0.002	-1.3, 1.3	0.99
Mid-son by father	21	-1.2	-1.4, 0.17	0.12
Mid-daughter by mid-parent	22	0.09	-0.39, 0.58	0.70
Mid-son by mid-parent	20	-0.76	-2.0, 0.47	0.21
Mid-offspring by mid-parent	39	-0.26	-0.92, 0.39	0.42
Large item rate				
Mid-daughter by mother	21	-0.41	-0.66, 0.25	0.36
Mid-daughter by father	24	0.59	-0.22, 0.81	0.25
Mid-son by mother	18	-0.008	-0.24, 0.23	0.97
Mid-son by father	19	-0.18	-0.52, 0.35	0.67
Mid-daughter by mid-parent	21	0.05	-0.58, 0.69	0.86
Mid-son by mid-parent	18	-0.09	-0.54, 0.36	0.68
Mid-offspring by mid-parent	36	-0.30	-0.70, 0.10	0.13

Table 4.3. Results of the mixed models testing for an independent effect of family identity on parental care behavior in house sparrows. The fit (-2 residual log likelihood; -2RLL) of each model of parental care was compared two ways: a model that included the random term bird identity nested within family identity, and a model that included the random terms bird identity nested within family identity and family identity.

Parental care measure	Best fit model	-2RLL
Provisioning rate[1]	Bird (Family)	2343.9
	Bird (Family) + Family	2343.1
Likelihood of	Bird (Family)	4261.2[†]
bringing large items[2]	Bird (Family) + Family	4261.7[†]

[1] Square root transformed

[2] From GLMM model of binomial using logit link and Kenward-Rogers estimation of denominator degrees of freedom

[†] Pseudo-likelihood value

Table 4.4. Results of the mixed models testing for an effect of family identity on parental care reaction norm slopes in house sparrows. The fit (-2 residual log likelihood; -2RLL) of each model of parental care was compared with and without the family by environment term.

Parental care measure	Environmental variable	Best fit model[2]	-2RLL
Provisioning rate[1]	Date	I (F) x E	2319.9*
		I (F) x E + F x E	2319.6
	Start time	I (F) x E	2341.4
		I (F) x E + F x E	2338.2
	Brood size	I (F) x E	2319.7*
		I (F) x E + F x E	2316.9
	Nestling age	I (F) x E	2314.5*
		I (F) x E + F x E	2311.7
	Partner feeding rate	I (F) x E	2330.5*
		I (F) x E + F x E	-
Likelihood of bringing large items[3†]	Date	I (F) x E	4165.8
		I (F) x E + F x E	-
	Brood size	I (F) x E	4117.8
		I (F) x E + F x E	4117.7
	Nestling age	I (F) x E	4189.8*
		I (F) x E + F x E	-
	Partner feeding rate	I (F) x E	4172.2
		I (F) x E + F x E	-

[1] Square root transformed
[2] I = individual identity; F = family identity; E = environment
[3] From GLMM model of binomial using logit link and Kenward-Rogers estimation of denominator degrees of freedom
* Significant individual x environment effect
† Pseudo-likelihood values

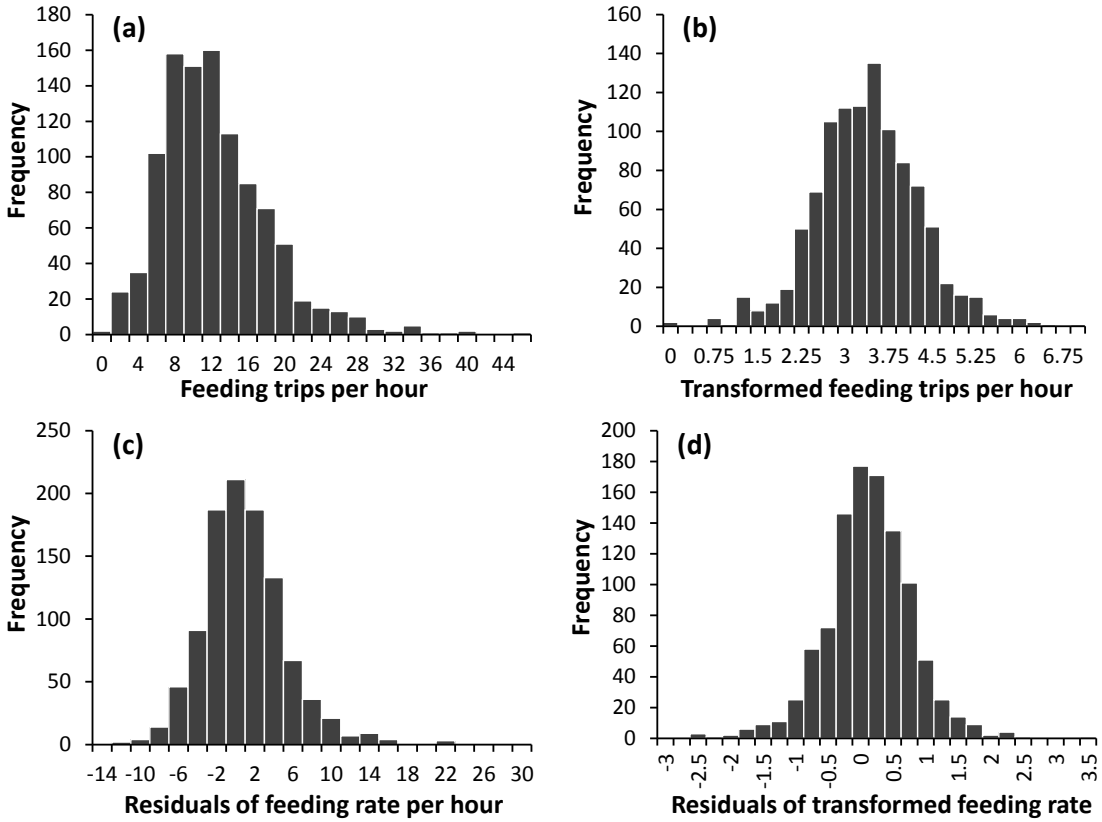

Figure 4.1. Distributions of nestling provisioning rates (feeding trips per hour), transformed provisioning rates, and their residuals for all adult house sparrows with nestlings in the data set. (a) The distribution of nestling provisioning rates, (b) the distribution of the square root transformed nestling provisioning rates, (c) the distribution of the residuals of nestling provisioning rate from the mixed model analysis, and (d) the distribution of the residuals of the square root transformed nestling provisioning rates from the mixed model analysis. Although all distributions differed significantly from the normal distribution, the square root transformed data more closely approximated a normal distribution.

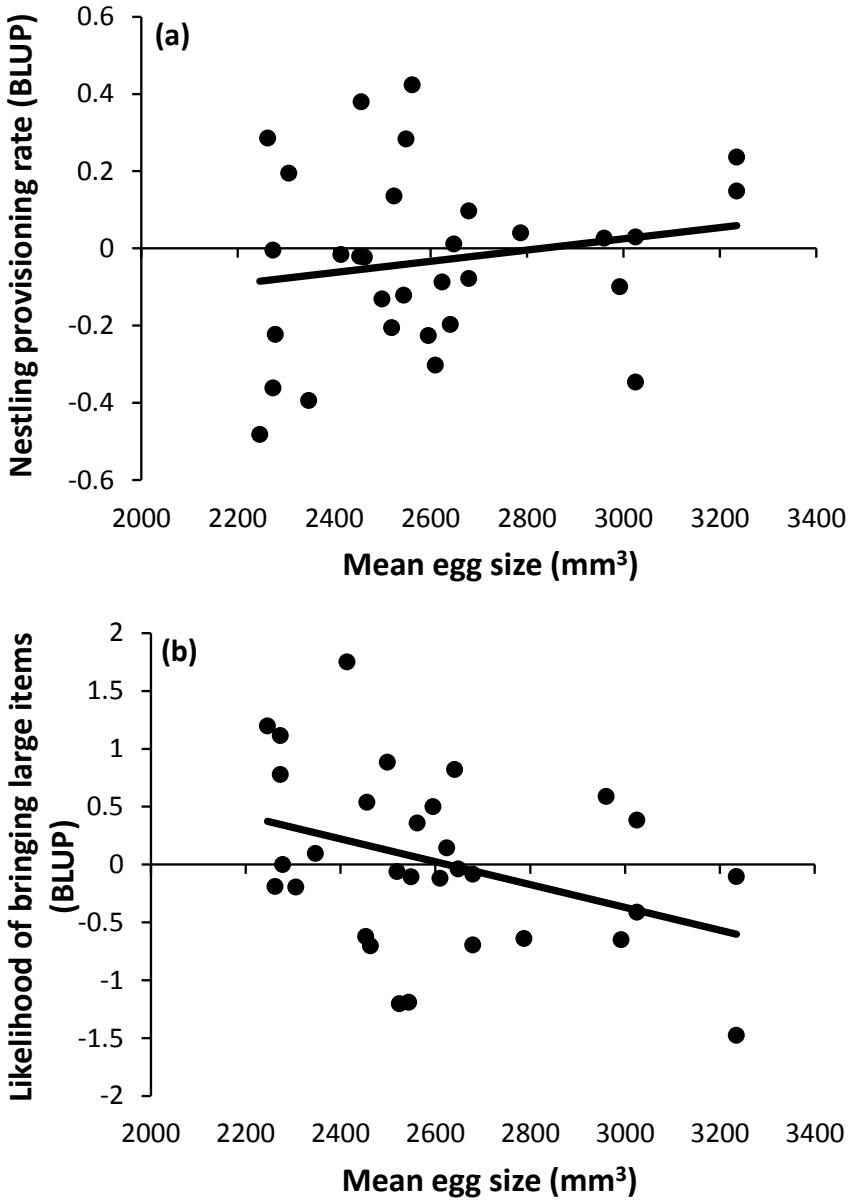

Figure 4.2. The relationship between mean egg size of the natal nest and parental care behavior expressed as an adult for 31 house sparrows. (a) Nestling provisioning rate (trips per hour) was positively associated with mean egg size of the parent's natal nest. (b) The likelihood of bringing large items to the nest was negatively associated with mean egg size of the parent's natal nest. For this figure, nestling provisioning rate (trips per hour) and the likelihood of bringing large food items were independently estimated for each parent as a best linear unbiased prediction (BLUP) from the mixed model analyses of parental care reaction norms. All fixed effects from Table 4.1 were included in the analyses to generate BLUPs for each parent sparrow (see Chapter 8).

CHAPTER FIVE

Heterozygosity predicts clutch and egg size but not plasticity in a house sparrow population with no evidence of inbreeding

The impact of heterozygosity on fitness (a heterozygosity-fitness correlation, hereafter HFC) has a long and somewhat contentious history within evolutionary biology. Darwin (1876) noted that individuals with high levels of heterozygosity can experience "hybrid vigor" or heterosis, and studies since that time have continued to garner support for this idea (e.g., Shull 1952; David 1998; Chapman et al. 2009). However, it is unclear how strong the effect of heterozygosity on fitness may be within natural populations (Chapman et al. 2009; Szulkin et al. 2010).

One major complication is the array of possible mechanisms by which heterozygosity might influence phenotypes and hence fitness. This situation is further confused by the loose usage of terminology and the mix of genetic markers that have been used to assess heterozygosity (reviewed in Szulkin et al. 2010). I distinguish between two broad biological mechanisms that can give rise to positive HFC. First, an HFC could arise through dominance by certain alleles at a locus and some level of inbreeding. If inbreeding results in offspring being homozygous for a recessive and deleterious allele, then their fitness will be reduced compared to either the heterozygote or the homozygote with the dominant allele. An alternative mechanism is that heterozygotes may perform better than either homozygote (overdominance), which can occur in the presence or absence of inbreeding. A classic example of this mechanism comes from work with *Colias* butterflies, where heterozygous individuals of the phosphoglucoseisomerase (PGI) enzyme are active at a wider temperature range than any homozygote (Watt et al. 1983). Generally, however, the overdominance hypothesis has fallen out of favor as an explanation for HFCs (Crow 2008; Charlesworth & Willis 2009; Szulkin et al. 2010).

Overdominance and inbreeding both exert their influence on phenotypes through the action of expressed genes, yet many studies now employ neutral markers to assess heterozygosity. Use of neutral markers requires making inferences about their proximity to expressed genes (Jarne & Lagoda 1996) and assumptions about the mechanisms creating associations between alleles across loci. One possibility is that heterozygosity at many neutral markers throughout the genome is associated with heterozygosity at nearby genes, each of which has small effects on polygenic characters. This idea is termed the "general effect hypothesis" (David 1998; Hansson & Westerberg 2002; Chapman et al. 2009). Heterozygosity at neutral markers may reflect the general effect hypothesis if homozygosity at multiple loci across the genome carries a fitness cost and there is identity disequilibrium across loci, which occurs when there is inbreeding and the observed heterozygote advantage is caused by inbreeding depression (Chapman et al. 2009). Alternatively, such general effects could arise if slight overdominance occurs at many loci, thus having an emergent effect on fitness. There is considerable evidence suggesting that genome-wide heterozygosity can impact fitness, but there is also disagreement regarding the specific mechanism (Foerster et al. 2003; Bean et al. 2004; Ortego et al. 2007; Cohas et al. 2009; but see Balloux et al. 2004; Slate et al. 2004). An

alternative to the general effect hypothesis is that a subset of the neutral markers is closely associated with one or a few loci that have a strong effect on fitness, labeled the "local effects hypothesis" (David 1998; Hansson & Westerberg 2002; Brouwer et al. 2007; Chapman et al. 2009). Recent theoretical research suggests that local effects have a weak signal and have not been appropriately tested in natural situations (reviewed in Szulkin et al. 2010), and so whether they are a common occurrence is not known. Overlaid on these two possibilities is the issue of how alleles at neutral markers become associated with alleles at loci that affect fitness. Such associations could arise through some process of non-random breeding, such as some level of inbreeding or episodes of inbreeding which occur during bottlenecks, or by physical linkage of the neutral markers with fitness loci (Szulkin et al. 2010). A final alternative to the general and local effect hypotheses is the idea that the markers used to estimate heterozygosity have a direct effect on fitness and are under selection (David 1998; Chapman et al. 2009). The "direct effect hypothesis" remains controversial for studies using microsatellite markers because microsatellites are thought to be selectively neutral (Charlesworth et al. 1994; Jarne & Lagoda 1996; Schlötterer 2000), however, some exceptions exist (Li et al. 2002, 2004; Küpper et al. 2010).

Recent reviews of HFCs have noted that although HFCs are widespread, they tend to be weak and inconsistent (e.g., Coltman & Slate 2003; Chapman et al. 2009; Szulkin et al. 2010). Some have suggested this is due to variation in the genetic basis of the key traits (e.g., whether they are polygenic; Houle et al. 1996), or due to variation in the frequency and history of inbreeding in the studied population (Szulkin et al. 2010). Life history traits, such as clutch or brood size, are often polygenic, a genetic architecture that that might favor correlations with heterozygosity at marker loci (Houle et al. 1996; Szulkin et al. 2010). I note that life history and behavioral traits may also be phenotypically plastic (Postma & van Noordwijk 2005). Plasticity can generate within- and between-individual variation that may bias or obscure the impact of heterozygosity, but to my knowledge, no studies have taken this explicitly into account.

Heterozygosity might also affect the expression of phenotypically plastic traits. Although the genetic basis of phenotypic plasticity has been of interest for some time (e.g., Via & Lande 1985; Scheiner 1993), heterozygosity has rarely been considered. One implication from classic studies of HFCs (e.g., Watt et al. 1983; McClelland et al. 2003) is that heterozygotes should have improved homeostasis (e.g., Lerner 1954), which would cause a decrease in the level of phenotypic plasticity (i.e., heterozygotes will maintain a constant phenotype across environments; Marshall & Jain 1968; Gillespie & Turelli 1989). Indeed, this has been found to be the case in several studies where the performance of organisms of differing heterozygosities was tested under a variety of stressful environmental conditions (Pederson 1968; Schlichting & Levin 1984; Yampolsky & Scheiner 1994). However, these studies focus on population- and species-level responses to drastic environmental change where plasticity in the focal variable would decrease fitness (Pigliucci 2001). Because homeostasis in one character might require plasticity at an underlying point in the mechanisms producing that character, heterozygosity might actually contribute to the ability of individuals to respond to normal variation in environmental conditions. Specifically, if plasticity is desirable under normal environmental conditions, highly heterozygous individuals may be better able to respond

(i.e., be more plastic) than less heterozygous individuals. Analyses of the influence of heterozygosity on phenotypically plastic characters have yet to assess this possibility.

Here I examine the effect of heterozygosity on several aspects of reproductive performance in a songbird, the house sparrow (*Passer domesticus*). There is mounting evidence that heterozygosity can affect fecundity in wild bird populations (Foerster et al. 2003; Ortego et al. 2007; Tomiuk et al. 2007; García-Navas et al. 2009; Ortego et al. 2009; Olano-Marin et al. 2011), particularly with regard to clutch size. Clutch size in birds is a well-studied life history trait that is variable among individuals within a population (e.g., Lack & Lack 1951; Postma & van Noorwijk 2005), and this variation exhibits two characteristics. First, individuals lay a different clutch size depending on age (Mauck et al. 2004), latitude (Anderson 2006), time of season (Rowe et al. 1994), and food availability (Nager et al. 1997). Second, despite this phenotypic plasticity, individuals also lay consistently different clutch sizes within a population (Christians 2002; Postma & van Noorwijk 2005; Westneat et al. 2009). Indeed, Westneat et al. (2009) found that female house sparrows exhibit plasticity in response to time of season, female age, and breeding attempt number yet still exhibit individual differences in clutch size, which account for up to 26% of the variation in clutch size.

There are several potential explanations for this individual variation, but the possibility that it could have a genetic component is particularly relevant for evolutionary hypotheses about life history variation. Clutch size has been found to be a heritable trait in wild birds (Flux & Flux 1982; Lessells et al. 1989; Schluter & Gustafsson 1993); however, the estimated heritabilities are low to modest (h^2 = 0.17 to 0.50) and typically have high variance (see Christians 2002). This suggests a considerable influence of environmental or other genetic factors. While there is accumulating evidence that female heterozygosity may affect clutch size, less is known about its effects on other aspects of reproductive performance (e.g., egg size, hatching success, and nestling survival; but see Tomiuk et al. 2007; García-Navas et al. 2009; Küpper et al. 2010; Ortego et al. 2010; Olano-Marin et al. 2011). Moreover, the effects of heterozygosity on plasticity in reproductive performance characters have not been studied.

I tested the impact of heterozygosity on variance in the reproductive performance of free-living female house sparrows. House sparrows are a good candidate for this type of study because they are prolific breeders, producing multiple clutches per year often over several years at the same location (Anderson 2006; Westneat et al. 2009). Thus for many individuals I could collect replicate data on reproductive performance. I used a long-term dataset with some pedigree information to assess inbreeding and test for additive genetic variation in clutch size and egg size, and then typed a subset of well-studied females at an array of microsatellite loci to measure their heterozygosity. I tested for associations between the heterozygosity of these selected females and four measures of reproductive performance (clutch size, egg size, hatching success, and nestling survival) while controlling for possible biases created by plasticity. I also attempted to distinguish between local and general effects. Finally, I tested if heterozygosity influenced plasticity or affected homeostasis.

Methods

Study site and study population

I monitored a population of house sparrows at the University of Kentucky's Agricultural Experiment Station, located just north of Lexington, KY (38°06´N, 84°29´W), from 1992 to 2008. Data from 1992 are not included here as that was the first year birds were caught and no pedigree or reproductive data was collected. This site consists of agricultural and pastoral fields and multiple barns used for storage and stabling horses. I placed nest boxes on the outside walls of several of these barns (10 to 20 nest boxes per barn). The number of barns with nest boxes and the number of nest boxes per barn varied from year to year, but typically consisted of 4 barns with 10 to 20 nest boxes each. House sparrows in this population breed from late March through the middle of August, with each pair attempting one to six clutches per season. Females lay an average of five eggs per clutch with a range of one to eight eggs (Westneat et al. 2009).

Field methods

Starting in mid-March of each year, I checked nest boxes every three days for breeding activity. Active nests were checked more frequently for nesting activity until the clutch was complete. Approximately five days later (halfway through incubation), the length and width of each egg was measured to the nearest 0.1mm with calipers. Egg volume was estimated using the equation: egg volume (mm^3) = 0.51 x egg length x egg $width^2$ (Hoyt 1979). An average egg volume was calculated for each clutch. In house sparrows, egg volume is highly correlated with fresh egg mass and is an accurate measure of egg size (R^2 = 0.91; IRK Stewart, unpublished). I checked nests daily toward the end of incubation to identify the hatching date and the number of nestlings hatching. Nestlings were banded and bled approximately 10 days after hatching. Hatching success and nestling survival were calculated for each female as the proportion of eggs laid that hatched, and the proportion of nestlings that survived to banding.

Adult sparrows breeding in nest boxes provided for them were trapped annually with mist nets or seed-baited cage traps and banded with a USFWS aluminum band and a unique combination of plastic color bands so they could be identified by sight. I collected approximately 50 µl of blood using brachial venipuncture. Blood samples were placed in a tube of 1 x TNE buffer and kept refrigerated in the field until they were returned to the lab and stored at -80 °C. Sparrows were banded as nestlings, juveniles, and adults. Birds banded as nestlings or juveniles that returned to breed could be aged precisely, while birds banded as adults were assigned a minimum age assuming that they were in their first breeding season (Westneat et al. 2009).

Laboratory methods

To incorporate plasticity with respect to date and attempt order into these analyses, I needed females with at least 2 replicate clutches across years for each attempt order (typically 3 but sometimes 4 clutches per season). I chose 42 females that produced at least 7 clutches (mean = 11.6, range = 7-26) because this allowed the polymerase chain reaction (PCR) products from all of the females to be electrophoresed on a single gel together with several ladders. DNA was extracted from 1µl of blood using Chelex® resin (Walsh et al. 1991) and amplified at 21 microsatellite loci (Table 5.1). PCR was

performed in a 20 µl volume containing approximately 5 ng of extracted DNA, 1 X Thermopol buffer (New England Biolabs, Ipswich, MA), 0.5 µM forward and reverse primer, 0.2 µM of each dNTP, and 0.5 units of *Taq* DNA polymerase (New England Biolabs). The PCR profile had an initial denaturing step of 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 sec, the primer-specific annealing temperature (Table 5.1) for 45 sec, then 72 °C for 60 sec, and finished with a 7 min extension step at 72 °C. PCR products were electrophoresed through a 4% polyacrylamide gel together with either 10 or 25 bp ladders, and visualized using silver stain (Bassam et al. 1991; Benbouza et al. 2006). The program Micro-Checker was used to check for genotyping errors (van Oosterhout et al. 2004).

The egg size analyses could be confounded if individual females invested differently in eggs containing embryos of one sex compared to the other. Cordero et al. (2000) sexed embryos from 34 house sparrow clutches collected mid-incubation and found that eggs containing males were significantly larger than those containing females. I therefore sexed embryos from 19 deserted clutches collected at the study site during the course of the study. Genomic DNA was extracted using a proteinase K/ammonium acetate method (Nicholls et al. 2000), amplified using the P2/P8 primer set (Griffiths et al. 1998), and visualized by silver staining of polyacrylamide gels as above.

Analysis

Heritability estimates. Banding and subsequent identification of breeding pairs provided a pedigree that I used to assess inbreeding rates and heritability. I measured heritability of clutch size and egg size in two ways. First, I used a linear regression to compare the average clutch and egg size between all mother-daughter pairs for which data was available. Only banded and known daughters that were reared from banded and known mothers were included in this analysis (68 mother-daughter pairs for the clutch size analysis; 40 mother-daughter pairs for the egg size analysis). Neither clutch nor egg size was normally distributed and neither could be made normal through transformation. However, deviation from normality was small, so I proceeded to use parametric analyses throughout (see also Westneat et al. 2009).

Second, I estimated heritability using an animal model in the program VCE (Neumaier & Groeneveld 1998; Groeneveld et al. 2008) applied to the dataset of all known (i.e. marked) females that bred at least once between 1993 and 2008 (N = 409). Most (72%) of these females had no known pedigree; 115 (28%) were banded as nestlings and had at least one known (marked) parent. The pedigree included 76 offspring (both male and female), 31 grand-offspring, and 13 offspring of additional generations, including 1 in a 5^{th} generation. In only 2 cases were grand-offspring the result of a mating between individuals both of whom also had known parentage. The pedigree also included 19 families of sibs or half sibs. VCE included information on relatedness through either maternal or paternal lines, and assumed no extra-pair paternity. The latter assumption was known to be false as extra-pair paternity accounts for approximately 11% of nestlings in this population (Stewart et al. 2006). However, this is not likely to have major effects on the estimates of heritability (Charmantier & Réale 2005). I used VCE in two ways. First, I averaged the clutch or egg size across all breeding attempts of all females involved in the pedigree and estimated genetic variance (V_g) and environmental variance (V_e) assuming no maternal effects and no non-additive genetic effects. In a second approach, I

used each breeding attempt as a repeated measure, and for analyses of clutch size I included date of first egg plus its quadratic term, nest attempt order, the interaction between these two, year, and female age and its quadratic as fixed effects, since each of these is known to influence clutch size (Westneat et al. 2009). For egg size, I included only year, date, and attempt order as fixed effects (Christians 2002). Female identity was included as a random effect in addition to pedigree information linking relatives.

Heterozygosity. Multi-locus heterozygosity was calculated as the number of loci that were heterozygous divided by the total number of loci typed (21 loci). This method of calculating heterozygosity was chosen because all individuals were typed at all loci, and this calculation was highly correlated ($r > 0.96$) with other commonly used measures of heterozygosity in a subsample of 10 loci (e.g., internal relatedness and homozygosity by locus; Amos et al. 2001; Aparicio et al. 2006). Loci were tested for Hardy-Weinberg (H-W) equilibrium and linkage disequilibrium using Arlequin 3.5.1.2 (Excoffier & Lischer 2010). Linkage tests were run with 10,000 permutations and a significance level of 0.05. H-W equilibrium tests were run with 1,000,000 Markov chain steps with 100,000 dememorization steps. Identity disequilibrium, which is the correlation of heterozygosity or homozygosity across loci, was calculated as g_2 using the program RMES (David et al. 2007). To test if the parameter g_2 was significantly different than zero, genotypes were resampled and tested 1,000 times (David et al. 2007).

Heterozygosity and performance. All four performance variables (clutch and egg size, proportion of eggs hatching and proportion of nestlings surviving to banding) were measured multiple times for each female at varying times of year and at different points in the female's lifetime. I therefore used a reaction norm approach to analyze performance with regard to heterozygosity (e.g., Nussey et al. 2007; Westneat et al. 2009). Each performance trait was assessed for factors affecting both between-individual and within-individual variance. I used linear mixed models in which female identity was entered as a random factor and date, attempt order, and female age as fixed factors. Two types of fixed factors were tested in each model. First I took individual mean attempt, date, and female age and centered those with respect to the full dataset in order control for between-individual differences in the conditions under which each performance variable was measured (van de Pol & Wright 2009). Second, I mean centered each observation within individuals, providing measures of within-individual variation in attempt order, date, and female age for use in measuring phenotypic plasticity. I used backward elimination to generate the best fit description of the reaction norm for each performance variable. Then, I added heterozygosity as a between-individual factor and tested its main effect and the interaction terms between heterozygosity and any variable that contributed to reaction norm shape. I considered the latter a test of the effect of heterozygosity on plasticity. I used Proc Mixed in SAS 9.2 (SAS Institute Inc., Cary, NC) to analyze clutch and egg size, and Proc Glimmix with a logit link and a binomial distribution to analyze hatching success and nestling survival.

I also tested for a relationship between individual heterozygosity and the variance in clutch and egg size (an estimate of homeostasis). For each female, I calculated the standard deviation of clutch and egg size to quantify each female's variance. I tested if heterozygosity was related to variances using linear regression. Additionally, I tested

whether average offspring sex ratio was related to heterozygosity, since for 23 of these females, nestlings from at least one of their broods (mean = 3.8 broods, range = 1-10) had been sexed as part of a previous study (Westneat et al. 2002).

Testing for local effects (single-locus HFCs) by comparing each locus independently with a measure of fitness can produce statistical associations even if the real effect is due to a general effect of multi-locus heterozygosity (Küpper et al. 2010; Szulkin et al. 2010). This is because the general effect is due to a compounded effect of the multiple single loci in the sample, all of which are predicted to differ from zero under the general effect hypothesis (Szulkin et al. 2010). To test for local effects, I compared the fit of a linear mixed model of performance that contained multi-locus heterozygosity with a model of single-locus heterozygosity that contained 21 variables indicating heterozygosity status at each typed locus (both models included all significant fixed effects from Table 5.4; Szulkin et al. 2010). Because these were mixed models, I compared their quality of fit using the difference in Akaike Information Criterion values (dAIC).

For those loci that were found to have significant local effects I compared their sequences to the assembled sequence of the zebra finch (*Taeniopygia guttata*) genome using BLASTN 2.2.22+ and build 1.1 of the zebra finch genome (Altschul et al. 1997; Warren et al. 2010) in order to identify any homology. Specifically, each entire microsatellite sequence was checked for homologs in the zebra finch genome using BLASTN, with a filter on to mask repeated segments and an expected value (-e) set to 0.0001.

Results

Inbreeding

I included data from 2,816 nesting attempts by 409 banded females and 382 banded males over the 16 years included in this study (excluding 1992). The pedigree I constructed revealed no cases of inbreeding at any level. Immigration and emigration were both high; a total of 248 birds hatched in one of the nest boxes bred at a box in a subsequent year (31% of all marked breeders) but these recruits represented only 3.7% of the 6,639 nestlings banded from 1992-2007.

Heritability estimates

I found no evidence for heritability of clutch or egg size in either method of estimation. Parent-offspring regression of mean clutch size on 68 mother-daughter pairs produced an estimate of heritability of 0.64, which was not significant ($\beta = 0.32 \pm 0.45$, $F_{1,66} = 2.01$, $P = 0.16$). For 40 mother-daughter pairs, heritability of mean egg size was estimated as 0.30, which was also not significant ($\beta = 0.15 \pm 0.28$, $F_{1,38} = 1.23$, $P = 0.27$). Analysis of only the first clutches I observed for each trait produced similar results.

The animal model produced two estimates of additive genetic variance for each trait; one based on average clutch or egg size and the other from a mixed model with fixed effects known to influence both traits (Table 5.2). In both approaches for both traits, additive genetic variance was not significantly different from zero; heritability was also low and not significantly different from zero. In the analysis of repeated measures, female identity explained about 14 ± 0.09% of the variance in clutch size and 47 ± 20% of the variance in egg size.

Reproductive performance

The 42 females I selected for genotyping were chosen because they had produced a large number of clutches, and were therefore not a random sample of breeding females. I used a linear mixed model to compare the performance of the 42 females with the other banded females in the population for each measure of reproductive performance. For each dependent variable I included female identity as a random factor in the analysis. The mean clutch size of the selected females was 5.01 ± 0.07 (SE; N = 479 breeding attempts), which was significantly larger than the mean population clutch size of 4.80 ± 0.08 (N = 1108 breeding attempts; $F_{1,144}$ = 6.77, P = 0.01; Table 5.3). The egg size of the selected females (N = 208 breeding attempts) did not differ from the population egg size (N = 576 breeding attempts; $F_{1,247}$ = 0.04, P = 0.85; Table 5.3). The proportion of eggs hatching was not significantly different for the selected females than the rest of the population ($F_{1,175.2}$ = 2.55, P = 0.11; Table 5.3), nor was the proportion of nestlings surviving to banding ($F_{1,187.9}$ = 0.01, P = 0.93; Table 5.3). These results suggest that the 42 females selected for this study differed only slightly from the rest of the females in the population. Indeed, within the dataset of 42 females, neither mean clutch size ($F_{1,41}$ = 0.47, P = 0.50) nor mean egg size ($F_{1,39}$ = 0.83, P = 0.37) was associated with the number of years a female was observed (range 2-6 years). Neither mean clutch size ($F_{1,41}$ = 1.5, P = 0.22) nor the mean egg size ($F_{1,41}$ = 0.96, P = 0.33) were associated with the total number of breeding attempts. I also found no within-clutch difference in the size of eggs containing male embryos and those containing female embryos (t = 0.49, DF = 18, P = 0.63).

Results from the mixed model analysis indicate that all four measures of reproductive performance showed individual variation among the selected females (Table 5.4), with female identity explaining from 5% of total variation in hatching success to 52% of the variation in egg size. Tests of between-individual variation in date of first egg, attempt order, and female age revealed no significant effects on any measure of performance (results not shown; all P-values > 0.1). Clutch size and egg size exhibited significant phenotypic plasticity (associations with within-individual variation). Clutch size declined with date and had a negative response to the quadratic of date (Table 5.4). Egg size increased with attempt order, declined with date, and declined with date more in later attempts (Table 5.4).

Heterozygosity

In the subsample of 42 females, the mean number of alleles per locus was 13.6 and ranged from 7 to 36 (Table 5.1). Mean expected heterozygosity (H_E) was 0.86 ± 0.06, which was higher than the mean observed heterozygosity (H_O) of 0.77 ± 0.10. Four loci (*Fhu2*, *Pdo10*, *Pdo41*, and *Pdo44*) differed significantly from H-W equilibrium after Bonferroni correction. I compared the heterozygosity of the 42 focal females with that of two other sets of females that have been typed from the population. In this comparison I only used those loci that both datasets had in common, and found no differences in heterozygosity (28 females from 1995-1996 (Stewart et al. 2006), 7 loci, H_O = 0.86 ± 0.10, versus 42 focal females, H_O = 0.84 ± 0.13, U = 0.34, P = 0.73; 20 females from 2007 (IRK Stewart, unpublished), 16 loci, H_O = 0.82 ± 0.09, versus 42 focal females, H_O = 0.84 ± 0.13, U = 1.4, P = 0.16). Fourteen of the 210 tests conducted to test for linkage

disequilibrium produced significant results ($P < 0.05$); however, none were significant after correcting for multiple comparisons. Identity disequilibrium was found to be low, and not significantly different than zero ($g_2 = 0.001$, SD = 0.004, $P = 0.32$). Removal of the four loci that were out of H-W equilibrium did not change this result.

Heterozygosity and performance

Multi-locus heterozygosity calculated from the 21 microsatellite loci significantly positively predicted both clutch size (Figure 5.1a; Table 5.4) and egg size (Figure 5.1b; Table 5.4). Removal of the 4 loci not in H-W equilibrium did not alter this result. The relationship was not an artifact of females with higher heterozygosity producing more sons, or sons hatching from larger eggs than daughters, since there was no relationship between heterozygosity and nestling sex ratio ($r_s = 0.06$, $N = 23$, $P = 0.78$), and within a clutch, eggs containing male embryos did not differ in size from those containing female embryos. Heterozygosity did not predict the mean proportion of eggs hatching (Table 5.4) or the mean proportion of nestlings surviving until banding (Table 5.4). Again, this result did not change when the 4 loci not in H-W equilibrium were excluded. Because clutch and egg size showed significant associations with overall heterozygosity, I also tested both traits for possible local effects (single-locus HFCs). I found that for clutch size a model with 21 separate loci was a poorer fit than one with multi-locus heterozygosity (dAIC = +24.4; Table 5.5). Although heterozygosity at two loci had significant positive effects on clutch size (*Pdo25*, $F_{1,19.6}$, = 5.7, $P = 0.03$; *Pdo44*, $F_{1,19.3}$, = 4.8, $P = 0.04$; Table 5.6), I have no evidence that these loci have a stronger effect than the others. In contrast, egg size was better predicted by the single-locus model containing all 21 loci than one with only multi-locus heterozygosity (dAIC = -218.3; Table 5.5). Individuals heterozygous at one locus, *Pdoμ3*, produced significantly larger eggs than those that were homozygous at that locus ($F_{1,17} = 5.9$, $P = 0.03$; Table 5.7). To explore this result further, I re-ran the single-locus model with all loci except locus *Pdoμ3*. This model produced a slightly worse fit than the single-locus model with all 21 loci (dAIC = +16.0), but still predicted egg size better than the multi-locus model (dAIC = -202.3), indicating that multiple loci had divergent effects on egg size. A comparison of homology between the locus sequence and the sequence of the assembled zebra finch genome (Warren et al. 2010) found that the microsatellite *Pdoμ3* could be mapped to the genome, but with a low degree of certainty (e-value = 0.002). This locus was assigned to chromosome 8 of the zebra finch based on sequence homology.

I found no evidence that heterozygosity affected plasticity of any performance variable. I only tested the potential effect on those factors for which there was evidence of plasticity; attempt order and date for egg size, and date for clutch size. None of the interaction terms between heterozygosity and within-individual variation in environment was significant (Table 5.4). Similarly, I found no evidence that heterozygosity contributed to homeostasis of performance traits, since heterozygosity did not significantly predict variance in clutch size ($R^2 = 0.01$, $F_{1,41}= 0.51$, $P = 0.48$) or egg size ($R^2 = 0.05$, $F_{1,37}= 2.1$, $P = 0.16$).

Discussion

I found significant positive effects of heterozygosity at neutral loci on two of four measures of breeding performance in female house sparrows. Both clutch and egg size

increased with heterozygosity, despite the fact that both also exhibit considerable phenotypic plasticity. When I controlled for possible effects of plasticity, I found that heterozygosity explained about 20% of the individual variance in both attributes. Because inbreeding in the focal set of subjects appears to be low, I cannot support the hypothesis that the HFCs I observed are due to inbreeding depression. If correct, these results have implications for prevailing ideas about the genetic basis for reproductive performance traits.

I found that heterozygosity was a more important source of between-individual variance in clutch size than additive genetic variance. I found no evidence that clutch size or egg size were heritable in this population of house sparrows, although sample sizes prevent eliminating the possibility of modest sized heritabilities (~0.3). This was not an entirely surprising result, as other studies have also found these traits to have heritabilities that are low or modest at best (Gibbs 1988; Potti 1993; see Christians 2002 for review). The significant effect of heterozygosity provides a partial explanation for between individual variance in clutch and egg size (Lack 1947; Westneat et al. 2009), even when clutch and egg size may have low heritability. This result has implications also for understanding evolutionary responses to selection; non-additive genetic variance has complex effects on the response to selection. A few empirical studies have found that heterozygosity (or inbreeding coefficient) can be correlated between parents and offspring (Reid et al. 2006; Hoffman et al. 2007), and theoretical studies suggest that some portion of heterozygosity can be inherited (Mitton et al. 1993; Neff & Pitcher 2008). I could not test this possibility in the study, but it is likely that heterozygosity is only weakly heritable. This means that its effect on clutch and egg size likely makes for an inefficient evolutionary response to any selection on these traits, and this may be part of the explanation for why considerable between-individual variance in both traits exists.

The measure of heterozygosity was obtained with 21 neutral microsatellite loci. The results from the analysis of clutch and egg size suggest differences in inferences between the two traits about the genetic structure of the links between neutral marker heterozygosity and expressed loci. In the case of clutch size, multi-locus heterozygosity explained a significant amount of variance; this study thus joins a growing body of evidence that suggests that clutch size is positively related to heterozygosity in wild birds (Foerster et al. 2003; Tomiuk et al. 2007; Ortego et al. 2007; García-Navas et al. 2009; Ortego et al. 2009; Olano-Marin et al. 2011). A test of the independent effects of all 21 loci produced a poorer fit of the model than one that included multi-locus heterozygosity, and so I had no evidence that loci differed in their effects, leaving the general effect hypothesis as the best explanation for an influence of heterozygosity on clutch size.

Egg size was also positively associated with multi-locus heterozygosity. When I tested if egg size was associated differently with individual loci, I found that the single-locus model produced a better fit than either the null model or the one containing multi-locus heterozygosity. This leads me to conclude that heterozygosity has differing effects at different loci. To my knowledge, this is the first study to use the correct method to test between models (as described in Szulkin et al. 2010) and also find evidence of general and local effects (Küpper et al. 2010; Olano-Marin et al. 2011). However, these results raise some questions about tests of local effects. First, the only locus in the single locus model that showed a significant positive effect of heterozygosity on egg size (*Pdoμ3*) is not critical to the better fit of the single locus model. This suggests that other loci also

differ in their effects, but individually do not differ from no effect. The estimated effect sizes of individual loci (Table 5.7) indicate many with negative effects on egg size, and while none are significant, this might occur if multiple loci are exhibiting weak associative dominance, with many other loci exhibiting weak associative overdominance. Because multi-locus heterozygosity has an overall significant positive effect on egg size, I conclude that egg size shows a complex mix of effects that does not fit neatly into either the general effect hypothesis or the local effect hypothesis, but have some elements of both. Current statistical techniques to test between the general and local effect hypotheses seem inadequate to explore these nuances. Further modeling efforts could elucidate ways to better detect and test more complex versions of the general and local effects hypotheses (see also Mueller et al. 2011).

Some authors have suggested that in order for neutral markers to be indicative of HFCs there must be some degree of identity disequilibrium among the markers (Szulkin et al. 2010). I found no evidence of linkage or identity disequilibrium among the microsatellite markers used in this study. However, correlations among pairs of neutral loci are predicted to be less easily detected than an underlying correlation between neutral markers and expressed genes. Szulkin et al. (2010) suggest that even with low g_2, inbreeding can create HFCs. Yet, I detected no evidence of inbreeding in the sample of marked individuals I studied or in the wider study population. Although it is possible that inbreeding may have occurred between lineages produced from the small number of nests that I could not sample or via extra-pair fertilizations, both scenarios are highly unlikely. Given the high degree of immigration into the study population and the small proportion of banded nestlings which remain to breed (< 4%), it is unlikely that hidden inbreeding could explain this results. The female with one of the lowest overall heterozygosity (0.57) was a nestling hatched on the study site but her parents were both apparently immigrants (her father was banded as an adult and her mother was never banded, indicating that both were likely hatched elsewhere). Immigration of individuals with mixed ancestry from other populations into the study population can generate identity disequilibrium (Tsitrone et al. 2001; Szulkin et al. 2010). This would require either inbreeding within source populations or joint immigration of relatives who then breed together at the study site. At the study site and in surveys of outlying sparrow nesting sites I have never observed joint dispersal of relatives (e.g., Foerster et al. 2006) for any of the 6,639 banded nestlings. Population structure created by limited dispersal of one sex has been suggested as a mechanism that can contribute to inbreeding (Olano-Marin et al. 2011), although this does not appear to occur in this population and should have resulted in occasional cases of inbreeding in the pedigree. Given the lack of evidence of any inbreeding in this population, it is difficult to understand how these neutral markers became associated with the genes affecting egg number or size. Other mechanisms besides inbreeding or population structure might exist, but these might require unusual structural domains within the genome. A final possibility is the fact that North American house sparrows went through a brief bottleneck upon their introduction from Europe ~160 years ago. Evidence of this bottleneck remains; North American sparrows have reduced genetic variation compared to ancestral populations (Schrey et al. 2011). This cannot affect variation in expression of homozygous recessive alleles among females within the present study population. However, the inbreeding that may have occurred during colonization might have created associations between alleles across loci that have

persisted until today. Thus I hypothesize that the house sparrow exhibits associative overdominance in either a few specific genes (egg size) or across the entire genome (clutch size) that is detected via neutral markers because of the persistent effect of that bottleneck.

I found that female multi-locus heterozygosity did not predict the proportion of eggs hatching or nestlings surviving, in contrast to the effects I found for clutch and egg size. Clutch and egg size are likely under direct female control (Visser & Lessells 2001; Ortego et al. 2007). The proportion of eggs hatching and nestlings surviving, by contrast, are affected by many other variables outside the realm of female control. These include the offspring's level of heterozygosity and extrinsic factors, some of which may be highly stochastic, such as the fertility of a female's mate, weather conditions, food availability, or nest predation. For example, hatching success has been found to decline with increased breeding density (Koenig 1982), the degree of genetic relatedness between parents (Bensch et al. 1994; Kempenaers et al. 1996; Hansson 2004), and with malarial infection of a parent (Knowles et al. 2010). Hatching success has also been found to be positively correlated with age (Hamer & Furness 1991), although I found no evidence that changes in age within females had any effect in the subset of long-lived females. Similarly, nestling survival has been found to be positively affected by parental age, timing of breeding, mate familiarity (Hatch & Westneat 2007, 2008), and parental provisioning rates (MacColl & Hatchwell 2003, 2004). Female heterozygosity may have little effect on these factors. Other studies have found no effect of multi-locus heterozygosity on hatching success (Tomiuk et al. 2007; Ortego et al. 2010), and mixed evidence for an effect of parent's heterozygosity on nestling survival or fledging success (no relationship: Tomiuk et al. 2007 and García-Navas et al. 2009; positive effect: Ortego et al. 2010 and Olano-Marin et al. 2011; positive and negative effects: Küpper et al. 2010). It is also possible that extra-pair paternity could create a bias in these HFC analyses if females of varying heterozygosity differentially engage in extra-pair copulations, which could lead to differential investment in their offspring. However, in this population there is no evidence that heterozygosity matters for extra-pair paternity (Stewart et al. 2006), and I found that heterozygosity did not affect hatching or fledging success.

An important caveat of these results concerns the biased nature of the dataset. I deliberately selected long-lived females because I was interested in plasticity; this required that I sample females with many replicate clutches. These females did differ from the rest of the females in the population in clutch size. However, there was no difference in heterozygosity between the selected females and two other sets of females that were sampled without any reference to the number of clutches they produced. Nevertheless, even if measures of HFC may be affected by these differences, I think these results still provide interesting insights. First, HFCs measured in a sample without accounting for plasticity may be hidden by unnecessary residual variance (see below) and could also be biased, especially if only one observation is taken from each individual. Second, the 42 females I selected are demographically the most productive breeders in the population during the study period, and so are likely to have a disproportionate effect on subsequent generations. The HFCs revealed by this sample thus could be evolutionarily more important than HFCs revealed by random sampling.

This study was predicated on the idea that at the least, plasticity creates residual variance that reduces the power of any statistical test of HFCs. Indeed, if I randomly select single clutches from the dataset of 42 females, the likelihood of finding a significant relationship between heterozygosity and clutch size is only 15% (resampled 100,000 times). Similarly, the likelihood of heterozygosity remaining a significant predictor of egg size when the data are reduced to a single attempt per female is only 3%. This is not surprising as clutch and, albeit to a lesser extent, egg size, exhibit phenotypic plasticity (Westneat et al. 2009; IRK Stewart, unpublished). This supports my contention that plasticity should be accounted for in studies of HFC. I suggest that studies testing the effect of heterozygosity on plastic traits take repeated measures of individuals and use a mixed model approach for the analysis, and studies only taking single trait measures should interpret non-significant results with caution.

I also hypothesized that heterozygosity might influence plasticity itself. I found no evidence of this: multi-locus heterozygosity had no effect on phenotypic plasticity of individual female sparrows across two environments (breeding attempt number and date in season). Several studies have found a negative relationship between heterozygosity and plasticity (Pederson 1968; Schlichting & Levin 1984; Yampolsky & Scheiner 1994), while others have found a positive relationship (Jain 1978; Weber & Scheiner 1992). However, it is difficult to draw strong connections from the literature to the current study, as most of the research on this topic examines heterozygosity and plasticity across species, not at the individual level. Nevertheless, this study suggests that heterozygosity, as measured using microsatellite markers, does not affect individual plasticity or homeostasis in clutch or egg size.

The conclusion that I have detected associative overdominance in these putatively neutral markers has some important implications for understanding the evolution of clutch and egg size in birds. Overdominance at functional genes has fallen out of favor as an explanation for reservoirs of genetic variation in key traits (e.g., Crow 2008; Szulkin et al. 2010). When directional selection is acting on a trait, overdominance may limit evolutionary change in mean character values, but at the same time, it maintains genetic variation. Such variation could allow a rapid response to selection if a change in environment caused a change in the overdominance. It may not be a coincidence that I have uncovered this effect in the house sparrow, a species that has been highly successful in invading a diverse set of habitats around the world. Further comparative work on the genetic basis of reproductive performance traits within and among populations could provide insights on how invasiveness may be linked to the nature of genetic and environmental variation.

Table 5.1. Description of the 21 microsatellite loci used to calculate heterozygosity, including the number of alleles (*k*), product size range, annealing temperature (T), repeat motif of the locus, expected heterozygosity (H_E), and observed heterozygosity (H_O).

Locus	*k*	Product size (bp)	T(°C)	Repeat motif	H_E	H_O	Locus reference
Ase18	11	193-249	59	$(GT)_{12}$	0.85	0.81	Richardson et al. 2000
Emb112	8	136-156	59	$(GT)_6AT(GT)_{15}$	0.82	0.76	Mayer et al. 2008
FhU2	7	128-148	56	$(CT)_{12}$	0.76	0.52*	Ellegren 1992
Hofi52	14	242-280	56	$(TAGG)_7TATG(TAGA)_{13}$	0.88	0.76	Hawley 2005
Pdoμ1	10	157-201	59	$(G)_6(TG)_{23}$	0.85	0.81	Neumann & Wetton 1996
Pdoμ3	12	118-168	54	$(TCCA)_{18}$	0.88	0.86	Neumann & Wetton 1996
Pdoμ4	36	225-440	55	$(A_nG_n)_n(GAGAGAAA)_{13}(GAAA)_{34}$	0.97	0.88	Neumann & Wetton 1996
Pdoμ5	13	204-264	59	$(CA)_{21}$	0.84	0.79	Griffith et al. 1999
Pdoμ6	33	312-456	59	$(GAAA)_{28}$	0.97	0.90	Griffith et al. 1999
Pdo9	9	377-426	56	$(AAT)_8$	0.82	0.90	Griffith et al. 2007
Pdo10	9	108-144	60	$(CA)_{19}$	0.87	0.74*	Griffith et al. 2007
Pdo17	12	194-246	60	$(CA)_{15}(GA)_1(CA)_3GACG(CA)_2G(CA)_5(TA)_1(CA)_8$	0.83	0.67	Dawson et al. 2012
Pdo22	11	101-133	60	$(CA)_{10}(TA)_4$	0.76	0.76	Dawson et al. 2012
Pdo25	18	81-135	50	$(A)_6(GA)_{28}$	0.90	0.88	Dawson et al. 2012
Pdo33	15	220-266	60	$GA(CA)_7[GA(CA)_3]_3GA(CA)_{18}$	0.90	0.81	Dawson et al. 2012
Pdo34	12	166-194	50	$(GT)_{15}$	0.88	0.81	Dawson et al. 2012
Pdo36	12	186-228	59	$(GT)_{19}$	0.90	0.74	Dawson et al. 2012
Pdo40	11	291-321	50	$(GT)_2GG(CT)_2(GT)_{22}$	0.87	0.81	Dawson et al. 2012
Pdo41	12	178-226	59	$(CA)_{21}$ & $(A/C)_{37}$	0.81	0.60*	Dawson et al. 2012
Pdo44	9	216-260	60	$(CA)_{24}(GA)_7AA$	0.81	0.64*	Dawson et al. 2012
Pdo47	12	170-198	60	$(CA)_{17}$	0.81	0.79	Dawson et al. 2012

* loci that significantly deviate from Hardy-Weinberg equilibrium after sequential Bonferroni correction for multiple tests

Table 5.2. Estimated additive genetic variance (*Va*), residual variance (*Vr*), and heritability for clutch and egg size among female house sparrows using mean phenotypic values or repeated measures with fixed effects included (*N* = 409; VCE analysis, see text).

	Va	*Vr*	Heritability
Mean clutch	0.05 ± 0.09	0.49 ± 0.09	0.16 ± 0.16
Mean egg-size	0.02 ± 0.03	0.09 ± 0.03	0.17 ± 0.24
Mixed: clutch	0.08 ± 0.06	0.54 ± 0.02	0.11 ± 0.09
Mixed: egg-size	0.02 ± 0.02	0.05 ± 0.003	0.16 ± 0.20

Table 5.3. A comparison of selected reproductive parameters from breeding attempts made by the 42 female house sparrows selected for this study and all known females at the study site.

	Selected females			All females		
Breeding variable	Range	Mean	SE	Range	Mean	SE
Clutch size	2 – 7	5.01	0.07	1 – 8	4.80	0.08
Egg size (mm^3)	2110 – 3028	2599	27	1974 – 3235	2593	30
First egg date (Julian)	60 – 201	93	23.0	60 – 220	102	25.3
Proportion hatching	0 – 1	0.66	0.02	0 – 1	0.70	0.02
Proportion surviving	0 – 1	0.68	0.02	0 – 1	0.68	0.03

Table 5.4. Results of linear mixed model analysis of reaction norms and the effect of multi-locus heterozygosity for four reproductive performance traits exhibited by 42 female house sparrows.

Performance attribute	Variable	Effect ± SE	*F* (*DF*)	*P*-value
Egg size	Female ID	16913 ± 4779		
	Residual	15400 ± 1702		
	Intercept	2426 ± 90		
	Year	-	2.0 (8,183)	0.04
	Attempt (within)	55.2 ± 16.8	10.8 (1,180)	0.001
	Date (within)	-2.1 ± 0.5	17.9 (1,180)	0.0001
	Attempt*date	-0.7 ± 0.2	8.8 (1,172)	0.003
	Heterozygosity	551 ± 215	6.6 (1,34.6)	0.01
	Date*heterozygosity	2.8 ± 2.6	1.1 (1,176)	0.29
	Attempt*heterozygosity	24.3 ± 98.2	0.06 (1,176)	0.80
Clutch size	Female ID	0.09 ± 0.03		
	Residual	0.55 ± 0.04		
	Intercept	5.3 ± 0.07		
	Date (within)	-0.006 ± 0.0008	57.9 (1,443)	0.0001
	Date*date	-0.0002 ± 0.00002	46.5 (1,455)	0.0001
	Heterozygosity	1.7 ± 0.6	8.8 (1,41.5)	0.005
	Date*heterozygosity	-0.01 ± 0.01	2.4 (1,444)	0.12
Hatching success (logit of hatch/eggs)[1]	Female ID	0.21 ± 0.11		
	Residual	1.95 ± 0.22		
	Intercept	1.59 ± 0.93		
	Heterozygosity	-0.97 ± 1.19	0.8 (1,42.8)	0.42
Nestling survival (logit of banded/ banded/hatched)[1]	Female ID	0.26 ± 0.16		
	Residual	2.02 ± 0.28		
	Intercept	0.10 ± 1.08		
	Heterozygosity	1.10 ± 1.40	0.6 (1,40.0)	0.43

[1] From GLMM binomial model using logit link and Kenward-Rogers estimation of denominator degrees of freedom

Table 5.5. Comparison of the results of the mixed models testing for general and local effects of heterozygosity on clutch and egg size in female house sparrows.

Performance attribute	Best fit model tested	AIC
Clutch size	No heterozygosity	1171.9
	Multi-locus heterozygosity	1162.9
	Single locus heterozygosity	1187.3
Egg size	No heterozygosity	2648.8
	Multi-locus heterozygosity	2630.1
	Single locus heterozygosity	2411.8

Table 5.6. Comparison of the effects of multi-locus heterozygosity (MLH) and single-locus heterozygosity (SLH) on the clutch size of 42 female house sparrows.

Model	Variable	Effect ± SE	*F* (*DF*)	*P*-value
MLH	Female ID	0.07 ± 0.03		
	Residual	0.55 ± 0.04		
	Intercept	4.0 ± 0.4		
	Date (within)	-0.006 ± 0.0008	58.1 (1,445)	0.0001
	$Date^2$	-0.0002 ± 0.00002	47.1 (1,458)	0.0001
	Multi-locus heterozygosity	1.7 ± 0.6	8.8 (1,41.5)	0.005
SLH	Female ID	0.09 ± 0.04		
	Residual	0.55 ± 0.04		
	Intercept	3.9 ± 0.8		
	Date (within)	-0.006 ± 0.0008	58.2 (1,445)	0.0001
	$Date^2$	-0.0002 ± 0.00002	47.3 (1,451)	0.0001
	Ase18	0.10 ± 0.23	0.2 (1,19.8)	0.67
	Emb112	0.16 ± 0.16	1.0 (1,18.7)	0.34
	Fhu2	-0.12 ± 0.16	0.6 (1,18.9)	0.46
	Hofi52	0.19 ± 0.19	1.0 (1,19.6)	0.32
	Pdoμ1	0.09 ± 0.20	0.2 (1,19.2)	0.66
	Pdoμ3	-0.37 ± 0.23	2.5 (1,20.4)	0.13
	Pdoμ4	-0.15 ± 0.27	0.3 (1,20.4)	0.58
	Pdoμ5	0.19 ± 0.17	1.2 (1,20.2)	0.28
	Pdoμ6	-0.02 ± 0.23	0.01 (1,18.7)	0.92
	Pdo9	-0.09 ± 0.29	0.09 (1,19.4)	0.77
	Pdo10	-0.12 ± 0.18	0.5 (1,20.2)	0.51
	Pdo17	0.10 ± 0.16	0.4 (1,19.9)	0.53
	Pdo22	0.28 ± 0.20	1.9 (1,18.9)	0.18
	Pdo25	0.60 ± 0.25	5.7 (1,19.6)	0.03*
	Pdo33	0.19 ± 0.17	1.1 (1,18.8)	0.30
	Pdo34	0.08 ± 0.21	0.1 (1,19.3)	0.71
	Pdo36	0.08 ± 0.16	0.3 (1,18.7)	0.61
	Pdo40	0.17 ± 0.17	0.9 (1,19.1)	0.35
	Pdo41	0.02 ± 0.14	0.02 (1,17.4)	0.90
	Pdo44	0.36 ± 0.17	4.8 (1,19.3)	0.04*
	Pdo47	0.06 ± 0.21	0.09 (1,17.6)	0.76

* $P < 0.05$

Table 5.7. Comparison of the effects of multi-locus heterozygosity (MLH) and single-locus heterozygosity (SLH) on the egg size of 42 female house sparrows.

Model	Variable	Effect ± SE	*F* (*DF*)	*P*-value
MLH	Female ID	14150 ± 4206		
	Residual	15425 ± 1705		
	Intercept	1994 ± 190		
	Year	-	2.0 (8,181)	0.04
	Attempt (within)	56.5 ± 16.7	11.4 (1,181)	0.0009
	Date (within)	-2.1 ± 0.5	18.5 (1,181)	0.0001
	Attempt*date	-0.7 ± 0.2	9.3 (1,172)	0.003
	Multi-locus heterozygosity	551 ± 215	6.6 (1,34.6)	0.01
SLH	Female ID	16697 ± 6966		
	Residual	15361 ± 1698		
	Intercept	2449 ± 345		
	Year	-	1.6 (8,162)	0.12
	Attempt (within)	52.7 ± 17.1	11.4 (1,172)	0.002
	Date (within)	-2.0 ± 0.5	18.5 (1,172)	0.0001
	Attempt*date	-0.7 ± 0.2	9.3 (1,167)	0.004
	Ase18	-106 ± 93.5	1.3 (1,19.1)	0.27
	Emb112	-39.6 ± 68.5	0.3 (1,20.2)	0.57
	Fhu2	-75.1 ± 65.7	1.3 (1,19.5)	0.27
	Hofi52	100 ± 87.0	1.3 (1,21.1)	0.26
	Pdoμ1	92.1 ± 79.9	1.3 (1,17.7)	0.26
	Pdoμ3	212 ± 87.4	5.9 (1,17)	0.03*
	Pdoμ4	-118 ± 106	1.2 (1,18.8)	0.28
	Pdoμ5	-64.5 ± 70.8	0.8 (1,19.3)	0.37
	Pdoμ6	-8.3 ± 103	0.01 (1,17.4)	0.94
	Pdo9	-37.8 ± 123	0.1 (1,19.9)	0.76
	Pdo10	-24.2 ± 74.2	0.1 (1,19.4)	0.75
	Pdo17	-36.6 ± 66.9	0.3 (1,18.9)	0.59
	Pdo22	-45.2 ± 82.1	0.3 (1,18.9)	0.59
	Pdo25	-59.3 ± 97.7	0.4 (1,18)	0.55
	Pdo33	-13.0 ± 69.3	0.04 (1,16.8)	0.85
	Pdo34	-71.2 ± 93.6	0.6 (1,19.5)	0.46
	Pdo36	-68.3 ± 71.0	0.9 (1,22.7)	0.35
	Pdo40	99.7 ± 68.6	2.1 (1,17.3)	0.16
	Pdo41	71.8 ± 57.7	1.6 (1,17.1)	0.23
	Pdo44	50.8 ± 65.5	0.6 (1,19.3)	0.45
	Pdo47	108 ± 85.3	1.6 (1,17.8)	0.22

* $P < 0.05$

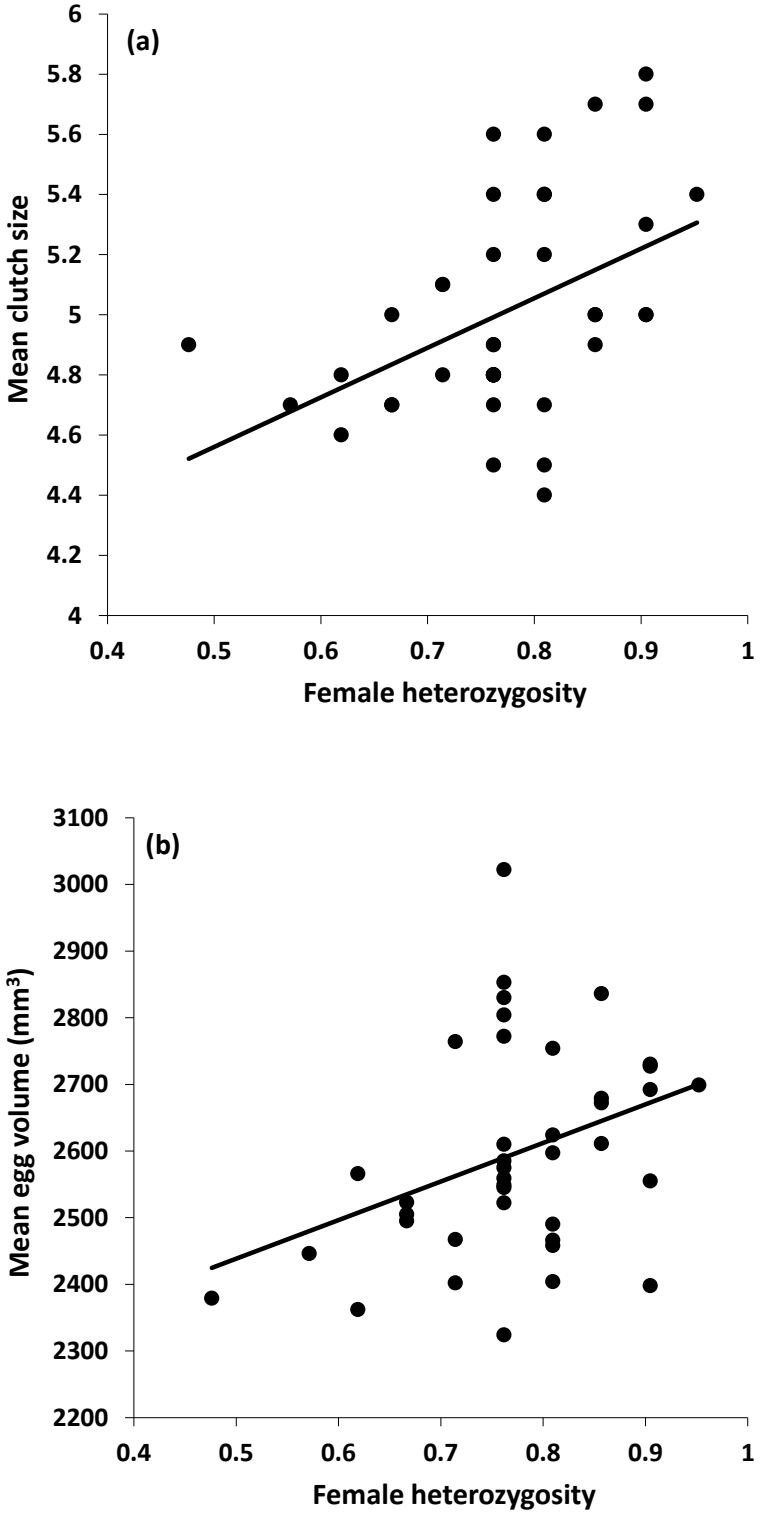

Figure 5.1. The relationship between multi-locus heterozygosity and measures of fecundity among female house sparrows: (a) multi-locus heterozygosity and mean clutch size; (b) multi-locus heterozygosity and mean egg volume (mm^3).

CHAPTER SIX

Heterozygosity predicts individual variation in the level of parental care in house sparrows

Parental care is a key life history trait for most organisms (Clutton-Brock 1991). A central tenet of parental care theory is that variation in the level of care parents provide is adaptive and reflects environmental differences in the benefits of care to offspring or the costs to the parent (Winkler 1987; Clutton-Brock 1991; Gross 2005). In support of such theory, studies have shown that parental care varies widely among species (e.g., Curio 1988; Clutton-Brock 1991; Eggers et al. 2005) but can also vary considerably within a species. Studies on variation within species have found that levels of care relate to the number and value of the offspring to parents (e.g., Breitwisch et al. 1986; Wright & Cuthill 1990), the cost of care (e.g., Ardia et al. 2009), kinship (e.g., Burke et al. 1989; Neff 2003), time of season (e.g., Ringsby et al. 2009), and, in bi-parental-care species, the level of care provided by a partner (e.g., Wright & Cuthill 1989). Despite this evidence that individuals respond plastically to variable conditions, recent research has found that parents provide individual-specific levels of care (Schwagmeyer & Mock 2003; Nakagawa et al. 2007; Dor & Lotem 2010; Westneat et al. 2011; Low et al. 2012). There are several potential explanations for consistent individual variation in parental care, but the idea that it could have a genetic component is particularly interesting and relevant to understanding the evolutionary response to selection on parental care.

Parental care is a heritable trait in some populations, suggesting additive genetic variation can have an effect on individual variation in the level of care (Freeman-Gallant & Rothstein 1999; MacColl & Hatchwell 2003; Walling et al. 2008; Dor & Lotem 2010; Rymer & Pillay 2011; Meunier & Kölliker 2012). However, in other populations, heritability of parental care has been found to be quite low, and fails to explain a significant portion of variation in care (Chapter 4). Factors other than additive genetic variation, such as the conditions experienced early during development, could create permanent differences between individuals and possibly influence parental care behaviors expressed later in life (Meaney 2001; Strasser & Schwabl 2004; Chapter 4). Despite developmental conditions explaining some of the variation among individuals, significant differences between individuals in the level of care they provide persist. This suggests that other factors, such as non-additive genetic effects (e.g., genetic heterozygosity), may also be involved in creating individual differences in parental care behavior. Evidence for the effect of heterozygosity on fitness-related traits has been documented for over a century (Darwin 1876; Shull 1952; David 1998; Chapman et al. 2009). These heterozygosity-fitness correlations (HFCs) have been found in a wide variety of taxa, across a wide variety of traits (see Chapman et al. 2009 for review). There is some evidence to suggest that heterozygosity could affect parental care behavior: in a cross-fostering study and an extra-pair paternity study, researchers found that offspring survival and immunity are positively related to the heterozygosity of the social father, but not the genetic father (Richardson et al. 2004; Fossøy et al. 2008). Parental care could be mediating the trans-generational effect of heterozygosity on non-genetically related offspring.

There are two complications with testing the effect of heterozygosity on parental care. The first has to do with how parental care is quantified. Plasticity in parental care behavior can generate within- and between-individual variation that may bias or obscure the influence of heterozygosity, but many studies fail to account for these sources of variance. Using a behavioral reaction norm approach to quantify parental care can resolve this problem (Westneat et al. 2011), but this approach requires multiple measurements of individuals across environments.

Another complication in testing the effect of heterozygosity on care is the way in which heterozygosity could be influencing this trait. There are three mechanisms typically used to explain the existence of HFCs. First, the marker used to measure heterozygosity could directly affect fitness, the 'direct effect hypothesis' (David 1998; Chapman et al. 2009). The mechanism by which this could work is fairly clear in studies of allozyme heterozygosity (e.g., Watt et al. 1983; Mitton 1993); however, most recent studies have used microsatellite markers to estimate heterozygosity. Microsatellites are typically assumed to be selectively neutral (Jarne & Lagoda 1996), though this is not always the case (e.g., Li et al. 2002, 2004). Second, some markers used to measure heterozygosity could be physically or statistically linked with one or a few functional loci that have a strong effect on fitness, the 'local effect hypothesis' (David 1998; Hansson & Westerberg 2002; Chapman et al. 2009). The mechanism by which markers become associated with functional loci is thought to be through either physical linkage or through nonrandom mating, such as sustained levels or short episodes of inbreeding (Szulkin et al. 2010). Though there have been several studies of HFCs that found local effects (e.g., Hansson et al. 2001; Lieutentant-Gosselin & Bernatchez 2006), theoretical research suggests that local effects will have a weak signal, are difficult to detect, and are often inappropriately tested for (Szulkin et al. 2010). Finally, the 'general effect hypothesis' states that heterozygosity of the markers used to measure heterozygosity could be associated with the heterozygosity of nearby genes, each of which has a small effect on fitness traits (David 1998; Hansson & Westerberg 2002; Chapman et al. 2009). A general effect may arise if there is a small amount of overdominance occurring at many loci across the genome, leading to an emergent effect on fitness. Alternatively, neutral marker heterozygosity could reflect the general effect hypothesis if there is some level of inbreeding producing heterozygote advantage via reduction of inbreeding depression (Chapman et al. 2009).

Life history traits such as parental care are thought to be polygenic, a genetic architecture that could favor relationships with heterozygosity at marker loci (Houle et al. 1996; Szulkin et al. 2010). Not only could heterozygosity influence the mean trait value of parental care, but could also influence phenotypic plasticity in parental care. Plasticity of care could have important fitness consequences beyond just mean level of care because parents must provide the appropriate amount of care as environmental conditions change (e.g., as offspring age). The genetic basis for phenotypic plasticity of traits has been of interest for decades (e.g., Via & Lande 1985; Scheiner 1993), but until recently, few studies have considered the role of genetic heterozygosity. Historically, heterozygosity was predicted to reduce plasticity if highly heterozygous individuals are less sensitive to environmental variation via improved homeostasis or developmental stability (Lerner 1954), and therefore are less phenotypically plastic than individuals with low levels of heterozygosity (Pederson 1968). Most tests of this idea focus on differences

among species or populations under stressful conditions (Pederson 1968; Schlichting & Levin 1984; Yampolsky & Scheiner 1994; Auld & Relyea 2010), and have found mixed or no evidence to support this idea. Few studies have considered how heterozygosity might affect individual-level phenotypic plasticity under natural conditions (Pigliucci 2001; Wetzel et al. 2012). If phenotypic plasticity is beneficial across typical environmental conditions, it is feasible that highly heterozygous individuals will be better able to respond than less heterozygous individuals. Testing this idea for life history traits like parental care will aid in our understanding of the role of non-additive genetics in phenotypically plastic traits.

In this study I examined the effects of heterozygosity on several aspects of nestling provisioning, a common measure of parental care, in the house sparrow (*Passer domesticus*). First, I used a behavioral reaction norm approach to quantify components of nestling provisioning. This approach allowed me to account for the effect of consistent individual differences among parents and patterns of phenotypic plasticity both between and within individuals. House sparrows are a good candidate for this type of study, as they are prolific breeders: they produce multiple clutches each year and typically breed in the same location across several years (Anderson 2006; Westneat et al. 2009). Additionally, recent research on this songbird species found that heterozygosity predicted both clutch and egg size in this population (Wetzel et al. 2012). I used a multi-year parental care data set and selected a subset of females from which I had repeated measures of care, then genotyped those females at an array of microsatellite loci to estimate individual heterozygosity. Next, I tested for any associations between multi-locus heterozygosity and single-locus heterozygosity with the three measures of nestling provisioning (nestling provisioning rate, variance in the interfeeding interval, and likelihood of provisioning with large food items). Finally, I tested for any effects of heterozygosity on individual plasticity in nestling provisioning (individual by environment interactions).

Methods

Study site and population

I monitored a population of wild house sparrows at the University of Kentucky's Agricultural Experiment Station north of Lexington, KY (38°06´N, 84°29´W) from 2008-2011. The study site consists primarily of agricultural and pastoral fields and multiple barns used for stabling horses and storage. I placed 10 to 20 nest boxes along the outside of each of four barns. The number of nest boxes on each barn varied from year to year, but the total number of monitored nest boxes ranged from 50 to 60 boxes per year. House sparrows in this population breed from March through August, with each pair attempting one to six clutches per season. Females in this population lay an average of five eggs per clutch with a range of one to eight eggs (Westneat et al. 2009). Starting in March of each year, I checked nest boxes twice a week for breeding activity. Active nests were checked more frequently until the clutch was complete. Nestlings were banded with a metal USGS band approximately 10 days post-hatching.

Adult sparrows breeding at the study site were trapped annually with mist nets and seed-baited cage traps. Each bird was banded with a metal USGS band and a unique combination of colored plastic bands so they could be identified by sight. I collected standard body measurements and a 50 µl blood sample from each bird at the time of

capture. I refrigerated each blood sample in a tube of 1 X TNE buffer in the field, and then transferred the tubes to a freezer at the end of the day where they were stored at -80 °C. Sparrows were banded in three age categories: nestlings, juveniles, and adults. Birds banded as nestlings or juveniles that returned to breed at the site could be aged precisely, while birds banded as adults were assigned a minimum age, which assumes they were in their first breeding season (Westneat et al. 2009).

Field methods

Nestling provisioning by parent sparrows was video recorded two to three times per brood in ~2 hour blocks. In 2008, 2009, and 2011 each brood was recorded twice, when nestlings were approximately 5 and 7 days old, and in 2010 each brood was recorded three times, when nestlings were approximately 3, 6, and 9 days old. Video recordings were performed during the morning hours (7 am to 12 pm) and the cameras were concealed in a small box attached to the barn 1 to 5 m from each nest box. All videos were scored later by one researcher. Nestling provisioning was quantified for each parent by measuring the feeding trips per hour during each observation period. An observation period consisted of the length of the recording (typically 2 hours) minus the latency of the first feed by either parent. In addition to feeding rate, I also quantified the variance of the interfeeding interval and the likelihood of bringing large food items. Variance in the interfeeding interval was quantified as the standard deviation of the amount of time (in seconds) between each feeding event in an observation period for each individual. All items brought during a feeding trip that could be seen were scored in reference to bill size (house sparrow bills range from ~1.0 – 1.3 cm in length) as either small (not visible to < 0.2 cm in length), medium (~0.5 – 1.5 cm), large (> 1.5 cm), or unknown (see also Schwagmeyer & Mock 2008). I was able to score the size of approximately 38% of all food items brought to the nest by the selected females, of the 3270 food items I scored 11% (377 items) were classified as "large."

To investigate how heterozygosity might affect plasticity, I needed parents with repeated measures of provisioning across years. I chose a total of 46 female house sparrows; 36 sparrows were selected for being observed the most and for having observations in multiple years. The other 10 sparrows were selected with different criteria as part of another study, but were included in this study to reduce the bias toward long-lived birds in this sample (each of these 10 birds had 2 – 4 observations recorded in only one year). The 46 selected sparrows were each observed an average of 10 times (median number of observations: 9, range: 2 – 25), for a total of 855 observation hours over 465 observation sessions. Because these females were not a random sample, I used linear mixed models to compare parental care data of the 46 females with the other known females in the population from which parental care data were collected. In this analysis, for each dependent variable, I included female identity in the model as a random factor and a variable indicating whether the bird was selected for genotyping as the only fixed factor.

Laboratory methods

DNA was extracted from each blood sample using a modified ammonium acetate method (Nicholls et al. 2000) and amplified at 18 microsatellite loci (Table 6.1). Polymerase chain reaction (PCR) and simultaneous fluorescent labeling was carried out

in a 20 µl volume using the method from Schuelke (2000). Briefly, I added a modified M13 tail to each of the forward primers and to a universal fluorescently labeled primer (FAM or HEX). Each PCR reaction contained approximately 25 ng of extracted DNA, 1 X Thermopol buffer (New England Biolabs), 0.1 µM forward primer, 0.4 µM reverse and fluorescently labeled primer, 0.2 µM of each dNTP, and 0.5 units of Taq DNA polymerase (New England Biolabs). The PCR profile had an initial denaturing step of 95 ℃ for 5 min, followed by 35 cycles of 95 ℃ for 45 sec, the primer-specific annealing temperature (Table 6.1) for 45 sec, then 72 ℃ for 60 sec, followed by 8 cycles of 95 ℃ for 45 sec, 53 ℃ for 45 sec, then 72 ℃ for 60 sec, and finished with a 10 min extension step at 72 ℃. PCR products were run on the ABI 3730 sequencer using GeneScan 600 LIZ size standard (Applied Biosystems) and visualized using Peak Scanner version 1.0 (Applied Biosystems). The program Micro-Checker was used to check for genotyping errors (van Oosterhout et al. 2004).

Analysis

Multi-locus heterozygosity (MLH) was calculated as the number of loci that were heterozygous divided by the total number of loci typed. Because not all individuals could be typed at all loci, I also calculated two other measures of heterozygosity: internal relatedness (IR; Amos et al. 2001) and homozygosity by locus (HL; Aparicio et al. 2006), using the Excel macro IRmacroN4 (W. Amos, http://www.zoo.cam.ac.uk/zoostaff/meg/software/IRmacroN4.xls). All measures of heterozygosity were highly correlated (all $P < 0.0001$) and all produced similar results, so I only present analyses using MLH. All 18 loci were tested for Hardy-Weinberg equilibrium (HWE) using Arlequin 3.5.1.2 (Excoffier & Lischer 2010) and linkage disequilibrium using Genepop 4.1.0 (Rousset 2008). Identity disequilibrium (the correlation of heterozygosity or homozygosity across loci) was calculated as the parameter g_2 using the program RMES (David et al. 2007). I tested if g_2 was significantly different than zero using resampled genotypes iterated 1,000 times (David et al. 2007).

Nestling provisioning was observed multiple times for each female under different environmental situations. To analyze these repeated measurements with regard to heterozygosity, I used a reaction norm approach (e.g., Nussey et al. 2007; Dingemanse et al. 2010; Westneat et al. 2011). Each measure of provisioning (feeding rate, variance in the interfeeding interval, and likelihood of provisioning with large items) was assessed for fixed factors that might affect both within- and between-individual variance. I used Proc Mixed or Proc Glimmix in SAS 9.2 (SAS Institute Inc., Cary, NC) to generate mixed models in which female identity was entered as a random factor, and year, attempt number, date, female age, number and age of nestlings, start time, and partner feeding rate were entered as fixed factors. For each measure of provisioning I constructed a model using two types of fixed factors. First, I took individual means for each fixed factor and centered them with respect to the mean of the full dataset to control for between-individual differences in the conditions in which each female was observed. All factors were added to the model and backward elimination was used to generate the best fit model describing between-individual variation for each of the three measures of nestling provisioning. Second, I mean centered each observation within each individual to produce a measure of within-individual variation (van de Pol & Wright 2009). I added all the main within-individual factors and many of their second-order interactions to the

previous best fit model, then used backward elimination to generate the complete, best fit model describing variation in each measure of provisioning. Next, for all significant within-individual factors, I tested for individual by environment (I x E) interactions. Individually, I x E terms were added to the model and tested to see if they improved the fit of the model (likelihood ratio test; Crawley 2002). All I x E terms that significantly improved the fit of the model were retained. Finally, I added heterozygosity to the model as a between-individual fixed factor and tested its main effect on provisioning. I also tested if heterozygosity explained any of the individual variation in reaction norm slopes by including an interaction between heterozygosity and any term that had a significant I x E effect.

Feeding rate was not normally distributed and no transformation improved the fit, so I analyzed the untransformed data (Westneat et al. 2011). Residuals approximated the normal distribution, but deviated from it significantly due to the large sample size. Interfeeding interval variance was not normally distributed nor could it be made normal through transformation, log-transformation brought the variable very close to normal so the analysis was performed on the log-transformed data. I used Proc Glimmix with a logit link and a binomial distribution to analyze the likelihood of bringing large items.

To test for local effects of heterozygosity on nestling provisioning, I compared the fit of the a best fit linear mixed model (from above) containing MLH with a model of single-locus heterozygosity where I replaced MLH with 18 variables indicating the heterozygosity at each typed locus (Szulkin et al. 2010). I compared the quality of fit between the two models using the difference in Akaike Information Criterion values (dAIC).

Results

The majority of the 46 female house sparrows used in this study were selected because I had collected the most nestling provisioning data from them, and therefore they did not represent a random sample of female sparrows. However, no measure of parental care differed significantly between the selected females and the other known females at the study site (Table 6.2). The mean rate of nestling provisioning for selected females was 10.4 ± 0.5 (± SE; N = 465 provisioning observations of 46 females), which was not different from the mean provisioning rate of the other females in the population 10.9 ± 0.6 (N = 342 provisioning observations of 95 females). No measure of provisioning was associated with the number of years over which females were observed or the number of observations performed (all $P > 0.10$).

Some measures of nestling provisioning were biologically, and therefore, statistically related. For example, as nestling feeding rate increased, the variance in the time between feeding trips typically decreased. Indeed, while only controlling for female identity, I found that interfeeding interval variance decreased significantly as feeding rate increased ($F_{1,400} = 375$, $P < 0.0001$). The likelihood of bringing large items was positively associated with feeding rate ($F_{1,425} = 6.6$, $P < 0.01$), but was not associated with the variance in the interfeeding interval ($F_{1,418} = 2.4$, $P < 0.13$).

Results from the mixed model analysis of nestling provisioning indicate that there was individual variation among females in nestling feeding rate, the variance in the interfeeding interval, and the likelihood of bringing large food items. In the preliminary model that included the random effect of female identity and no fixed effects, female

identity explained 15.6% of the total variation in feeding rate (Likelihood ratio test: -2dLL = 35.7, $DF = 1$, $P < 0.01$), 3.0% of interfeeding interval variance (-2dLL = 1.9, $DF = 1$, $P > 0.1$), and a significant amount of variance in the probability of bringing large food items ($\chi^2 = 109$, $DF = 1$, $P < 0.0001$).

Only feeding rate was affected by variation between-individuals. Females with more nestlings had higher feeding rates than females with fewer nestlings ($F_{1,54.6} = 4.9$, $P = 0.03$), though this effect was not significant in the full model (Table 6.3). All measures of nestling provisioning exhibited significant phenotypic plasticity in response to environmental variables that varied within subjects (Table 6.3). In addition, I found that individual females responded differently across nestling ages and partner feeding rates, and there was significant covariance between individual intercepts and slopes for some measures (Table 6.3). In the best fit model, which included the fixed and random terms in Table 6.3, female identity explained 18.5% of the variance in feeding rate and 5.3% in interfeeding interval variance.

Mean expected multi-locus heterozygosity (H_E) for the 46 female house sparrows was 0.83 ± 0.12, which was larger than the mean observed heterozygosity (H_O) of 0.77 ± 0.14 (Table 6.1). The average number of alleles per locus was 13.7 (range: 3 – 34). Three of the 18 loci (*Fhu2*, *Myc4*, and *Pdo9*) were not in HWE after Bonferroni correction. Excluding these loci from the analysis had no effect on the results. Linkage disequilibrium was detected in 16 out of the 153 tests, though only one pair (*Pdo5* and *Myc4*) remained significantly linked after correcting for multiple tests. Identity disequilibrium among loci was not significantly different than zero ($g_2 = 0.004$, SD = 0.006, $P = 0.13$).

Adding MLH to the best fit models of nestling provisioning reduced the variance due to female identity from 18.5% to 16.9% for feeding rate and 5.3% to 5.0% for interfeeding interval variance, suggesting that ~1.6% of the variation in feeding rate and ~0.3% of the variation in interfeeding interval variance could be attributed to MLH. However, this was not a significant portion of the variation, as there was no significant main effect of multi-locus heterozygosity (MLH) on nestling feeding rate or on the variance in the interfeeding interval (Table 6.3). There was an effect of MLH on the likelihood of provisioning nestlings with large food items (Table 6.3). More heterozygous females were more likely to bring large food items to the nest than less heterozygous females (Figure 6.1). MLH did not have an effect on individual variation in plasticity for any of the three measures of nestling provisioning. The interaction terms heterozygosity by nestling age and heterozygosity by partner feeding rate did not explain significant amounts of variation in individual reaction norm slopes (data not shown).

I was only able to test for local effects of heterozygosity on one measure of nestling provisioning. I found that for nestling feeding rate, a single-locus model with 18 separate loci was a better fit than a model with MLH (dAIC = -1011.2). None of the loci in the single-locus model had an independent, significant effect on feeding rate. Neither single-locus models for variance in the interfeeding interval or the likelihood of bringing large items to the nest would converge, so I could not compare the fit of those models with the MLH model.

Discussion

I found that large portions (up to 18%) of the variation in measures of parental care behavior can be explained by the identity of the parent. While it remains unclear exactly what the sources of consistent individual differences in parental care are, heterozygosity may provide one reason for variation among individuals. Parental care in female house sparrows was correlated with individual genetic heterozygosity, but not as predicted. I found that more heterozygous parents were more likely to bring large food items to the nest than less heterozygous parents, but there was no effect of heterozygosity on nestling feeding rate or the variance in the interfeeding interval. This is only the second study to assess how parental care correlates with individual genetic heterozygosity. García-Navas et al. (2009) found that nestling feeding rates of male blue tits (*Cyanistes caeruleus*) was positively associated with heterozygosity, though no relationship was found for females. I predicted a positive relationship between feeding rate and heterozygosity based on this and other similar studies of the effect of heterozygosity on reproductive performance (Wetzel et al. 2012; see Chapman et al. 2009 for review). Had I only measured nestling feeding rates of these parents, I would have concluded that heterozygosity had no effect on parental care behavior. However, I was able to determine that while heterozygosity does not appear to directly influence nestling feeding rate, when more heterozygous females bring food to the nest, they are more likely to bring large food items. This is significant because the rate at which large food items are brought to the nest has been found to predict both fledgling mass and recruitment in house sparrows (Schwagmeyer & Mock 2008).

Why all parents do not bring large food items to the nest, and how this behavior is mediated by heterozygosity is unclear. After controlling for the fixed and random effects that influenced the likelihood of bringing large food items (Table 6.3), bringing large items was negatively correlated with nestling feeding rate (effect: -0.04 ± 0.02; $F_{1,417} = 4.3$, $p = 0.04$). This suggests there could be a trade-off between food item size and feeding rate, as large food items could be difficult to detect, capture, or handle. Less heterozygous females may remain in close proximity to their nest box if these females experience higher rates of infanticide from nest box usurping conspecifics (a common occurrence in this population; DP Wetzel, personal observation), and are therefore unable to find large prey items (e.g., Grieco 2002). Heterozygosity is predicted to influence numerous fitness-related traits which could influence parental care ability. For example, genetic heterozygosity has been found to negatively correlate with susceptibility to parasites (Coltman et al. 1999; Hawley et al. 2005). More heterozygous female house sparrows may be in better condition and can therefore spend more time foraging for or provisioning nestlings. Furthermore, if immune response trades off with reproductive effort (Bonneaud et al. 2004; Knowles et al. 2010), then more heterozygous individuals may be better able to mediate the costs associated with reproduction. In support of this idea, Agudo et al. (2012) found that heterozygosity at microsatellite loci was positively correlated with MHC diversity and breeding success in the Egyptian vulture (*Neophron percnopterus*). Alternatively, heterozygosity may influence other traits that correlate with parental care behavior. For example, more heterozygous females may be better competitors for access to high-quality food sources (e.g., Seddon et al. 2004). Although untested, heterozygosity could mediate individual differences in cognitive ability (e.g., Cole & Quinn 2012), foraging style or ability (e.g., Barnard & Sibly 1981; Lecomte et al.

2010; Lescroël et al. 2010), or variance sensitivity (e.g., Mathot et al. 2012), all of which could affect a parent's ability to forage for large food items.

Heterozygosity explained a portion of the between-individual variation in parental care behavior, but the portion of variation explained was not very large compared to the amount of variation that was still explained by parent identity. For example, adding heterozygosity to the model of nestling provisioning rate reduced the amount of variation explained by female identity 1.6%, but female identity still explained 16.9% of the variation in provisioning rate. Thus, despite exhibiting considerable phenotypic plasticity and some variation being explained by heterozygosity, individual females still significantly differed in the level (reaction norm intercepts) and plasticity (reaction norm slopes) of care they provided. This suggests that sources other than heterozygosity are creating and maintaining individual differences among parents. Other genetic explanations for consistent individual variation in parental care behavior include additive genetic effects (Freeman-Gallant & Rothstein 1999; MacColl & Hatchwell 2003; Dor & Lotem 2010). However, previous research has found little evidence that parental care behavior is heritable in this study population (Chapter 4). It is also possible that the conditions experienced in the natal nest could create permanent differences among individuals that lead to differences in the parental care phenotype expressed later in life (e.g., Meaney 2001), though previous work on this sparrow population suggests there is little evidence that conditions experienced in the nest influenced parental care behavior expressed as an adult (Chapter 4). One possible exception is that parents hatched from large eggs had higher provisioning rates and were less likely to provision with large food items than parents hatched from small eggs (Chapter 4). Alternatively, individual differences in foraging or cognitive ability (e.g., Cole et al. 2011), as well as differences in the social environment (e.g., Kölliker et al. 2000), could also create individual variation in parental care. Ultimately, a more comprehensive approach may be required to better partition the sources of individual variation in parental care behavior.

I found that individuals differed in the amount of parental care they provided as nestlings aged and as the feeding rate of their mate fluctuated, but genetic heterozygosity did not explain these individual by environment effects. While it is known that genetic variation for phenotypic plasticity exists and can respond to selection (Scheiner 1993; Nussey et al. 2005), the effect of non-additive genetics on plasticity is relatively unexplored. Recent studies have shown that the level of plasticity in different populations can be influenced by inbreeding (Auld & Relyea 2010; Murren & Dudash 2012), but this is one of the first studies to test the relationship between heterozygosity and individual-level phenotypic plasticity. Heterozygosity did not appear to create the individual differences observed in parental care reaction norm slopes. Previous research on this population found no influence of additive genetic variation on reaction norm slopes either (Chapter 4). Thus, the genetic contribution to individual differences in the response to the environment appears to be low in this house sparrow population. It is possible that the sampling design of this study (~40 individuals observed ~10 times each) may have resulted in low power to detect an effect of heterozygosity on individual plasticity. Although I was able to detect significant I x E terms, including these terms in the models of parental care behavior likely reduced statistical power to detect additional effects related to these terms (Martin et al. 2011; van de Pol 2012). Larger sample sizes might be required to detect effects of heterozygosity on individual-level plasticity.

The exact mechanism by which heterozygosity influences parental care or how neutral microsatellite markers became linked to genes influencing care is unknown. Although my results suggest the local effects model was a better fit for nestling feeding rate, no locus had an independent effect on feeding rate, and I was unable to test for local effects in the remaining provisioning traits. I only used 18 microsatellite markers to estimate genetic heterozygosity in this study, but recent work has found that a small panel of microsatellites (10 to 20 markers) can capture a significant portion of genetic variance in laboratory and natural populations (Agudo et al. 2012; Forstmeier et al. 2012). Furthermore, there was no evidence of linkage or identity disequilibrium among the microsatellite markers used in this study, nor is there evidence of inbreeding occurring in the study population (Wetzel et al. 2012). One way in which these neutral microsatellite markers could become indicative of HFCs is through previous periods of inbreeding, which was experienced by ancestral house sparrow populations when introduced to North America (Schrey et al. 2011; Wetzel et al. 2012). I suggest that house sparrows in this population could be exhibiting associative overdominance at specific genes or across the genome that was detected using neutral markers because of this historical bottleneck event. This has implications for our understanding of how selection acts on parental care. Evolutionary change in mean trait values could be limited when directional selection is acting on a component of parental care behavior. This could partially explain the lack of additive genetic variation for fitness-related traits found previously in this population (Chapter 4; Wetzel et al. 2012). However, the influence of genetic heterozygosity can also maintain genetic variation in a population, which could allow a rapid response to major episodes of selection (e.g., translocation of a population to a new continent). Though my results indicate that heterozygosity may be one source of the consistent individual differences observed in parental care behavior, the effect is relatively weak compared to the amount of variation due to individual identity. Because our understanding of the biological basis heterozygosity-fitness correlations remains lacking, additional research on the genetic and epigenetic basis of reproductive traits like parental care is required to understand how genes and environment interact to produce consistent differences among individuals.

Table 6.1. Description of the 18 microsatellite loci used to calculate heterozygosity, including the number of alleles (*k*), product size range, annealing temperature (T), repeat motif of the locus, expected heterozygosity (H_E), and observed heterozygosity (H_O), for 46 female house sparrows.

Locus	*k*	Product size (bp)	T(°C)	Repeat motif	H_E	H_O	Locus reference
Dpu16	3	140-144	TD†	$(AC)_{12}(GC)_4ACGCAC(GC)_2$	0.41	0.35	Dawson et al. 1997
FhU2	12	126-164	56	$(CT)_{12}$	0.83	0.86*	Ellegren 1992
INDIGO41	14	295-335	54	$(TAGA)_{11}$	0.77	0.65	Sefc et al. 2001
Myc4	10	175-199	60	$(GT)_{26}AT(GT)_3$	0.88	0.60*	Double et al. 1997
Pamo12	11	235-257	55	$(GT)_{20}$	0.80	0.83	Izumi et al. 2009
Pdoμ3	13	120-176	54	$(TCCA)_{18}$	0.88	0.85	Neumann & Wetton 1996
Pdoμ5	13	218-278	59	$(CA)_{21}$	0.85	0.85	Griffith et al. 1999
Pdoμ6	34	315-481	59	$(GAAA)_{28}$	0.96	0.82	Griffith et al. 1999
Pdo9	17	382-460	56	$(AAT)_8$	0.88	0.60*	Griffith et al. 2007
Pdo10	14	113-153	60	$(CA)_{19}$	0.90	0.85	Griffith et al. 2007
Pdo17	14	208-256	60	$(CA)_{15}(GA)_1(CA)_3GACG(CA)_2G(CA)_5(TA)_1(CA)_8$	0.89	0.80	Dawson et al. 2012
Pdo22	11	117-141	60	$(CA)_{10}(TA)_4$	0.73	0.76	Dawson et al. 2012
Pdo33	19	235-291	60	$GA(CA)_7[GA(CA)_3]_3GA(CA)_{18}$	0.92	0.78	Dawson et al. 2012
Pdo34	12	183-211	50	$(GT)_{15}$	0.85	0.76	Dawson et al. 2012
Pdo40	14	306-346	50	$(GT)_2GG(CT)_2(GT)_{22}$	0.89	0.93	Dawson et al. 2012
Pdo44	16	226-268	60	$(CA)_{24}\ (GA)_7AA$	0.87	0.87	Dawson et al. 2012
Pdo47	12	182-212	60	$(CA)_{17}$	0.82	0.78	Dawson et al. 2012
PdoF09	7	135-155	53	$(GT)_{10}$	0.80	0.83	Garnier et al. 2009

* loci that significantly deviate from Hardy-Weinberg equilibrium after sequential Bonferroni correction for multiple tests

† touchdown profile where annealing temperature dropped from 62 to 50 °C by 1 °C per cycle, followed by 50 °C for 30 cycles

Table 6.2. A comparison of nestling provisioning measures from all observations performed on the 46 female house sparrows selected for the current study and all other breeding females at the study site (n = 95) at the study site. Feeding rate is measured in trips per hour, variance in interfeeding interval is measured as the standard deviation of all interfeeding intervals (measured in seconds) during an observation, and large item rate is measured as the proportion of large items to all known items brought back to the nest multiplied by the overall feeding rate.

	Selected females			All females			*P*-value
Nestling provisioning	Range	Mean	SE	Range	Mean	SE	
Feeding rate	0 – 38.7	10.4	0.5	0 – 29.5	10.9	0.6	0.4
Interfeeding interval variance	0 – 2387	276	12.5	0 – 2530	266	17.8	0.5
Large item rate	0 – 19.4	3.3	0.3	0 – 15.9	3.03	0.4	0.4

Table 6.3. Results of the mixed model analysis of nestling provisioning reaction norms for 46 female house sparrows. Female identity and individual by environment interactions were included in all models as a random effects. Estimated variances in individual slopes across selected environments are reported as "slope" and covariance between slope and intercept terms are reported as "cov". All fixed effects are within-individual effects unless noted as "between," in which case they are between-individual effects. Heterozygosity was included as a between-individual effect to test its effect on each measure of provisioning.

Measure of provisioning	Variable	Effect ± SE	*F* (*DF*)	*P*-value
Feeding rate (trips/hr)	Female ID	5.3 ± 1.5		
	Cov Female ID*nestling age	0.82 ± 0.3 [a]		
	Slope Female ID*nestling age	0.17 ± 0.1		
	Cov Female ID*partner rate	0.20 ± 0.1		
	Cov Partner rate*nestling age	-0.02 ± 0.04		
	Slope Female ID*partner rate	0.05 ± 0.02 [a]		
	Residual	15.0 ± 1.1		
	Intercept	14.6 ± 2.9		
	Nestlings (between)	1.0 ± 0.7	2.3 (1, 52)	0.14
	Attempt	1.5 ± 0.5	8.0 (1, 392)	0.005
	Date	-0.06 ± 0.02	13.5 (1, 396)	0.0003
	Nestlings	0.99 ± 0.2	22.6 (1, 406)	< 0.0001
	Nestling age	1.1 ± 0.1	87.9 (1, 42.7)	< 0.0001
	Female age	-0.83 ± 0.3	5.9 (1, 396)	0.02
	Partner feeding rate	0.09 ± 0.5	2.9 (1, 40.8)	0.09
	Partner rate*date	-0.005 ± 0.001	12.9 (1, 434)	0.0004
	Partner rate*nestlings	-0.07 ± 0.03	4.6 (1, 375)	0.03
	Partner rate*female age	-0.23 ± 0.05	17.7 (1, 425)	< 0.0001
	Heterozygosity	**-5.4 ± 3.6**	**2.2 (1, 39.8)**	**0.15**
Variance in interfeeding interval (standard deviations in seconds)[1]	Female ID	0.01 ± 0.009		
	Cov Female ID*nestling age	0.002 ± 0.003		
	Slope Female ID*nestling age	0.002 ± 0.002 [a]		
	Cov Female ID*partner rate	0.002 ± 0.001 [a]		
	Cov Partner rate*nestling age	0.00004 ± 0.0005		
	Slope Female ID*partner rate	0.0002 ± 0.0002		
	Residual	0.23 ± 0.02		
	Intercept	5.2 ± 0.2		
	Attempt	-0.13 ± 0.06	4.4 (1, 384)	0.04
	Date	0.003 ± 0.002	3.9 (1, 378)	0.05
	Nestlings	-0.11 ± 0.02	19.7 (1, 388)	< 0.0001
	Nestling age	-0.05 ± 0.01	12.7 (1, 40.4)	0.001
	Partner feeding rate	-0.02 ± 0.005	11.3 (1, 32.1)	0.002
	Heterozygosity	**0.31 ± 0.3**	**1.1 (1, 34)**	**0.31**

(continued)

Table 6.3. (continued)

Measure of provisioning	Variable	Effect ± SE	*F* (*DF*)	*P*-value
Likelihood of bringing large items (logit of large items/seen items)[2]	Female ID	0.17 ± 0.2		
	Cov Female ID*partner rate	-0.01 ± 0.02		
	Slope Female ID*partner rate	0.005 ± 0.003 [b]		
	Year (random)	0.40 ± 0.2 [b]		
	Intercept	-5.8 ± 1.1		
	Date	0.007 ± 0.002	10.1 (1, 418)	0.002
	Nestlings	0.08 ± 0.07	1.2 (1, 418)	0.27
	Nestling age	0.16 ± 0.03	22.8 (1, 418)	< 0.0001
	Partner rate	0.09 ± 0.02	17.8 (1, 30.0)	0.0002
	Partner rate*date	-0.001 ± 0.0004	11.6 (1, 418)	0.0007
	Partner rate*nestlings	-0.04 ± 0.01	8.8 (1, 418)	0.003
	Partner rate*nestling age	-0.02 ± 0.006	15.3 (1, 418)	0.0001
	Heterozygosity	**4.4 ± 1.4**	**10.0 (1, 26.5)**	**0.004**

[1] Log transformed

[2] From GLMM model of binomial using logit link and Kenward-Rogers estimation of denominator degrees of freedom

[a] Significant random term tested with likelihood ratio test

[b] Significant random term tested with covariance test in GLIMMIX

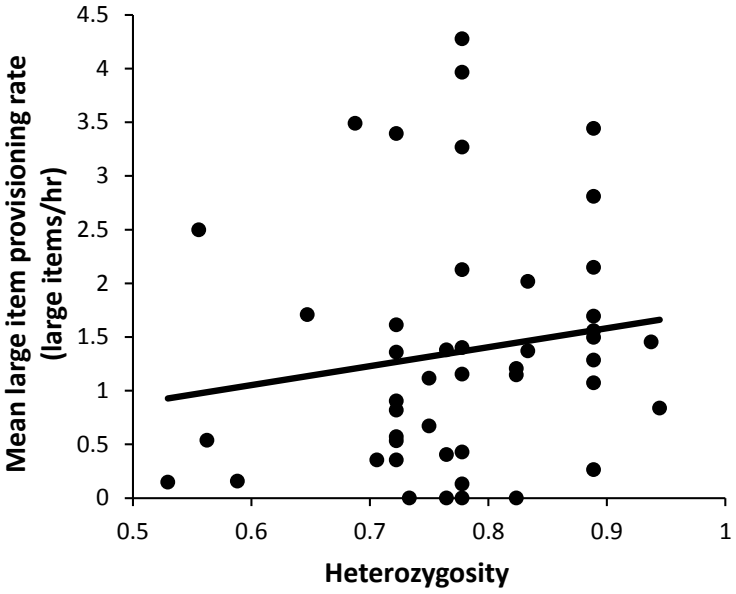

Figure 6.1. The relationship between rate of feeding trips by 46 female house sparrows in which they brought large items and genetic heterozygosity.

CHAPTER SEVEN

Are smart birds better parents? Problem-solving ability and parental care behavior in free-living house sparrows

Foraging can be a complicated task that requires cognitive skills (information acquisition, processing, and storage) and behavioral plasticity (Stephens et al. 2007; Shettleworth 2010). Foragers must find resources that are scarce, cryptic, and variable in space and time. Individual variation in foraging could arise from a wide variety of attributes, such as foraging style or efficiency, or the ability to cope with uncertainty. Indeed individual foragers are known to differ in foraging style (Barnard & Sibly 1981) and foraging efficiency (Lescroël et al. 2010). The way individuals cope with uncertainty also varies – they vary in the sampling rate of the environment (Shettleworth et al. 1988), sensitivity to variance in the environment (Ratikainen et al. 2010), and the way they process information (Mathot et al. 2012). Individual variation in these components of foraging must have a neurological or cognitive basis, though this remains untested. For example, variation in foraging could be due to variation in the ability to learn the cues of food or to find innovative solutions to obtain and handle cryptic or difficult food items. Thus, an individual with better cognitive abilities will pay lower foraging costs and reap higher benefits when attempting to find resources than those with poorer cognitive abilities. This could be especially important when parents are providing food for rapidly growing, dependent offspring under variable environments (Ydenberg 2007).

Parental care behavior exhibits considerable variation within a population, where it is known to be influenced by environmental variables, such as the time of season (Ringsby et al. 2009), number or age of offspring (Breitwisch et al. 1986), or the contribution of a partner (Wright & Cuthill 1989). Despite evidence that individuals respond plastically to variable conditions, recent research has found that parents provide individual-specific levels of care (Schwagmeyer & Mock 2003; Nakagawa et al. 2007; Dor & Lotem 2010; Westneat et al. 2011). After accounting for many of the environmental variables that influence care, individuals still differ significantly in both their level and plasticity of care (20% of the variation in care can be due to individual identity; Westneat et al. 2011; Chapter 8). Because selection acts on individual phenotypes, specifically studying individual variation is critical to understanding the evolutionary response to selection on phenotypically plastic traits like parental care. Although there are several potential explanations for consistent individual differences in parental care behavior (e.g., hormonal differences, Angelier & Chastel 2009), the idea that it could be due to cognitive differences among individuals is potentially important and represents a novel explanation for the existence and maintenance of phenotypic variation at the individual level.

Support for the idea that cognitive ability may influence variation in parental care can be found in comparative studies of feeding innovation, which is defined as the frequency of new and unusual feeding behaviors, including frequent changes in foraging techniques or diet, eating a surprisingly large range of foods, and/or using new food handling behaviors in novel situations (Sol et al. 2002). These studies have found that feeding innovation is correlated with brain size in primates and birds (Lefebvre et al.

1997; Timmermans et al. 2000; Reader & Laland 2002; Lefebvre et al. 2004; Sol et al. 2005). In carnivores, the extent of parental investment by females is correlated with brain size (Gittleman 1994), and a comparative study in birds uncovered a relationship between brain size and the degree of pair bonding and biparental care (Shultz & Dunbar 2010). Differences in cognitive ability at the individual level may have driven these interesting between-taxa patterns.

Lab studies have found that there is a neurological basis for individual differences in some behaviors (e.g., coping style; Koolhaas et al. 2010), and individuals with different coping styles vary in their learning ability (Bolhuis et al. 2004). Few studies have attempted to examine the cognitive abilities of organisms in the wild, but of those that have, individual variation has been found to exist (Cowie et al. 1981; Healy & Hurly 1995; Morand-Ferron & Quinn 2011). I tested the hypothesis that individual variation in the ability to solve a foraging task can explain variation in components of the parental care reaction norm in free-living house sparrows (*Passer domesticus*). There is reason to suspect individual variation in foraging skill may be linked to cognitive ability in house sparrows. The house sparrow's success as an invasive species has been attributed to their remarkable behavioral flexibility, especially in regards to feeding and foraging (Sol et al. 2002; Martin & Fitzgerald 2005). For example, wild house sparrows have learned to open automatic doors to obtain food (Breitwisch & Breitwisch 1991) and forage on the grills of cars for insects (Simmons 1984). Sparrows are also known to steal food from other bird species (Barrows 1889), pierce flowers to eat nectar (Stidolph 1974), and pry bark off trees to find insects (Lowther & Cink 2006). Because this species performs such a wide variety of innovative foraging techniques, it appears that house sparrows are ideally suited for testing how cognitive ability in a foraging context is related to parental care.

I tested the ability of parent house sparrows to complete a goal-oriented, problem-solving task in the wild. This task required parents to employ specific foraging methods to obtain food items. Next, I used a behavioral reaction norm approach to independently quantify two measures of parental care (nestling provisioning rate and the likelihood of provisioning large food items) collected from parents that participated in the foraging trials. Finally, I tested if variance in cognitive foraging ability contributed to consistent differences between individuals in the level care they provided to offspring. Variation in parental care behavior, driven by differences in cognitive ability, may lead to differences in reproductive success among parents. Therefore, I also tested if individuals with better problem-solving abilities had higher nestling survival rates.

Methods

Study site and population

This study was conducted on a nest box population of house sparrows in 2011 and 2012 at the University of Kentucky's Agricultural Experiment Station, located just north of Lexington, KY (38°06´N, 84°29´W). The study site consists of agricultural and pastoral fields, and multiple barns used for stabling horses and storage. I monitored a total of 60 house sparrow nest boxes located on the outside walls of four barns (10 to 20 nest boxes per barn). House sparrows in this study population breed continuously from March through August of each year, with each pair attempting one to six clutches per season. Females in this population lay an average of five eggs per clutch with a range of one to eight eggs (Westneat et al. 2009). Eggs hatch after approximately 10 days of

incubation, and altricial nestlings remain in the nest and are fed by the parents for 14 to 17 days. This species has bi-parental care; both sexes forage and provide food (primarily insects) to dependent nestlings throughout the nesting cycle, and both sexes defend a small territory (1 – 2 m^3) centered around the nest from conspecific intruders (Lowther & Cink 2006). Adult sparrows weigh approximately 28 g at maturity (Lowther & Cink 2006).

Adult sparrows breeding in the boxes provided for them at the study site were trapped with mist nest and seed-baited cage traps. Each bird was banded with a numbered, metal USGS band and a unique combination of colored plastic bands so they could be identified by sight. I collected standard body measurements from each bird at the time of capture. Birds banded as nestlings or juveniles that returned to breed at the site could be aged precisely, while birds banded as adults were assigned a minimum age, which assumes they were in their first breeding season. Beginning in March of each year, I checked each nest box twice a week for nest building and breeding activity. Active nests were checked at least three times a week to identify the day the first egg was laid and the day the eggs hatched. I continued to check active nests at least three times per week until nestlings were banded with a metal USGS band approximately 10 days after hatching. Nest survival was calculated for each nest as the proportion of nestlings that fledged from the nest out of the number of nestlings hatching.

Foraging trials

I tested the ability of parent sparrows to perform a goal-oriented, problem-solving task using test platforms attached to the side of the barn within 0.5 m of each nest box. The test platform consisted of a 23 x 23 cm board with nine, 3.5 cm diameter holes cut into the flat side of the board. The holes were arranged in a 3 x 3 pattern and each contained a 1.5 oz plastic cup secured to the board with clear tape (Figure 7.1a). The platform was protected from the elements by a 25.5 x 25.5 cm vinyl roof attached ~26 cm above the platform. These platforms were erected at approximately 40 nest boxes in February of each year (in 2011 and 2012) before breeding began and removed each August.

I trained parent sparrows to use this test platform as a location to find food in two steps. First, approximately four days after the first egg was laid in a clutch, I began adding white millet to the test platform and the platform roof (the roof was more visible than the platform from the nest box). In 2011, a small amount of millet mixed with sand was added daily to each cup in the platform, while in 2012, each cup was filled daily with only millet. This feeding regimen continued for approximately 10 days. Second, approximately two days prior to the eggs hatching, I emptied all cups in the test platform and began adding two to three mealworms (*Tenebrio molitor*) and/or wax worms (*Galleria mellonella*) to each cup twice daily (once in the morning and once in the afternoon). At this time, I also added nine, ~20 g, 4 cm diameter, metal washers around the edge of the platform to acclimate the birds to their presence (Figure 7.1a). This daily feeding routine continued for four to five days.

When the nestlings were approximately three days old, I created a problem-solving task at the first food replenishment time. Two mealworms or wax worms were placed in each cup on the platform, and then the metal washers were moved to cover each hole (Figure 7.1b). The sparrows were able to see the insect food through the central hole

in the washer, but the hole was small enough that the bird could not reach the food. To access the food, the bird had to figure out how to move the washer and gain access to the cup beneath. The motor skills used to solve this problem, such as pushing, pulling, or flipping objects, are unlikely to be innovative skills for house sparrows, but the application of these techniques to a series of large metal washers covering cups containing food is probably novel (Webster & Lefebvre 2001; Roth et al. 2010). After the washers were placed on the cups, a small amount of millet (~20 seeds) was scattered across the test platform to encourage the parents to investigate the platform. I performed these trials in the morning (mean ± SD observation start time: 9:50 am ± 1.3 hrs), and video recorded the activity at each platform for 1 hour. Video cameras were concealed in a small enclosure located 1 to 5 m from the nest box; all videos were scored later. After 1 hour, all washers were removed from the cups and replaced around the edge of the test platform. I replenished insect food on the platform at the second feeding time and the birds were allowed to feed freely. The problem-solving task was repeated following this same process on the next day.

These trials were conducted on a total of 49 breeding attempts in 2011 and 33 attempts in 2012, and included 110 unique parents. Parental care data were analyzed for all 110 birds that underwent this protocol (described below), but foraging test data could only be analyzed for the 77 birds that participated in the trial (birds that landed on the test platform during the trial). A preliminary viewing of the recorded trials was performed to identify trials in which birds came to the platform and trials in which birds solved the task. Although individuals experienced multiple trials of the foraging task, I restricted the analysis presented here to only include the first trial where an individual solved the problem for those birds that were solvers, or the first trial an individual participated in for those birds that were not solvers. In each of these trials, I recorded which individual solved the problem by removing a washer to obtain the food. For those individuals that solved the problem, I recorded the latency to solve for one washer within that trial (the length of time from first landing on the platform to first removing a washer) and the total amount of time spent on the platform before solving within the trial. Additionally, for individuals that opened more than one washer within a trial, I recorded the amount of time spent attempting to open each washer in the trial to produce a learning curve (i.e., the slope of the best-fit line of time spent trying to open each washer over the number of washers opened; Figure 7.2a).

Parental care

Parental care data was collected from most house sparrows in this study population from 2008 to 2012. To increase the number of repeated parental care observations for each of the 110 birds in the current data set, I used all observations performed on an individual from 2008 – 2012, not just the observations performed in 2011 and 2012. Observations of nestling provisioning trips made by parents were video recorded two to three times per brood in ~2 hour blocks. In 2008, 2009, and 2011 each brood was recorded twice, when nestlings were approximately 5 and 7 days old, while in 2010 and 2012 each brood was recorded three times, when nestlings were approximately 3, 6, and 9 days old. Recordings were performed in the morning (mean ± SD observation start time: 8:45 am ± 1.2 hours) and always completed prior to providing food to the parents on the test platform. Prior to initiating the parental care recording, any food on

the focal parent's test platform was removed. All parental care videos were scored later by one researcher. Each parent was observed an average of eight times (mean ± SD: 8.3 ± 7, range: 1 – 31, $N = 110$), for a total of 910 observation sessions (1725 hours). Nestling provisioning rate was quantified for each parent by recording the number of feeding trips per hour during each observation period. An observation period consisted of the length of the recording (typically 2 hours) minus the latency of the first feed by either parent. In addition to provisioning rate, I also quantified the likelihood of bringing large food items to the nest. Any items brought during a feeding trip that could be seen were scored in reference to bill size (house sparrow bills range from ~1.0 – 1.3 cm) as either small (not visible to <0.2 cm), medium, (~0.5 – 1.5 cm) large (>1.5 cm), or unknown (see also Schwagmeyer & Mock 2008). I was able to score the size of approximately 56% of all food items brought to the nest for the birds used in this study; of the 10,635 food items I scored, 9% (947 items) were classified as "large."

Analysis

Nestling provisioning data were recorded multiple times for each parent under different environmental situations. I used a reaction norm approach to analyze these repeated measurements and account for phenotypic plasticity within individuals (Nussey et al. 2007; Dingemanse et al. 2010). The two measures of parental care, nestling provisioning rate and the likelihood of bringing large food items, were analyzed using Proc Mixed or Proc Glimmix in SAS 9.2 (SAS Institute Inc., Cary, NC) in three stages. The dependent variable was the measure of nestling provisioning, and in all stages of the analysis bird identity was included in the each model as a random effect. First, I tested if the conditions under which each parent was observed could have created differences between individuals. The factors I tested included the sex, age, and breeding attempt number of the parent, date and time of the observation, number and age of the nestlings during the observation, and the partner's nestling provisioning rate. Each of these fixed effects (except sex) was averaged for each individual and centered with respect to the full data set (van de Pol & Wright 2009). All factors were added to the model, then backward elimination was used to generate a best-fit model that described the sources of between-individual variation in nestling provisioning. Second, I tested for phenotypic plasticity in nestling provisioning by mean-centering each observation within an individual for each fixed effect. I added all main within-individual centered factors and many of their second-order interactions to the previous model, then used backward elimination to generate a best-fit model describing variation in nestling provisioning. Finally, I sequentially added each measure of cognitive foraging ability (a variable indicating if the bird solved the problem, latency to solve, the amount of time on the platform prior to solving, and the slope of the learning curve) as a between-individual factor and tested its main effect on parental care behavior. Nestling provisioning rate was not normally distributed nor could it be made normal through transformation; square root transformation brought the data very close to normal so the analysis was performed on the transformed data. The likelihood of bringing large food items to the nest (number of large items out of the total number of items seen) was analyzed in Proc Glimmix with a logit link and a binomial distribution.

Nestling survival was modeled as the number of nestlings that survived out of the number of eggs that hatched using logistic regression with a binomial distribution and

logit link (Proc Glimmix in SAS 9.2). For this analysis, I included all nests known to have been produced by any individual involved in the foraging trials. To account for repeated measures, the year and the identity of both the male and female parents were included as random terms the model of nestling survival. I also controlled for fixed factors that influence nestling survival (date in season and clutch size) by including them in the model (see Chapter 8). I then tested if nestling survival was related to the ability to solve the foraging task by separately adding categorical variables indicating if the male or female parent solved the task.

Results

Of the 110 birds that were targeted for the problem-solving task, 77 birds participated by landing on the test platform in at least one trial. In 2011, nine of the 36 birds that participated in the trials solved the foraging task, while in 2012, 21 of the 41 participants solved the task, a significant difference between years ($\chi^2 = 5.7$, $DF = 1$, $P = 0.02$). Birds that participated in the trials in 2011 had a longer initial latency to land on the test platform than participants in 2012 (log transformed latency: $t_{73} = 2.2$, $P = 0.03$), and birds that solved the task in either year had a shorter latency to the platform than birds that did not solve the task (log transformed latency: $t_{71} = 3.0$, $P = 0.004$). On average, birds that solved the problem took 16.8 ± 5 (SE) minutes from the time they first landed on the test platform until they first solved the problem. Birds often came to and left the platform several times before solving; these birds spent an average of 1.7 ± 0.3 minutes physically on the platform before solving (within the trial where they first solved the task). The amount of time spent on the board prior to solving positively covaried with the latency to solve the task ($r_s = 0.76$, $N = 29$, $P < 0.0001$). The mean learning curve slope was 0.55 ± 0.7 seconds per washer, indicating that on average, it took birds one additional second to open each successive washer. None of these three measures of problem-solving were correlated with the latency to land on the platform, but there was a trend for birds with a longer latency to the board to have a longer latency to solve (latency to solve: $r_s = 0.35$, $N = 29$, $P = 0.06$). The latency to solve the task and the amount of time on the platform before solving did not differ by year (log transformed latency: $t_{27} = 0.41$, $P = 0.69$; log transformed time on platform: $t_{27} = 0.30$, $P = 0.77$), or sex (log transformed latency: $t_{27} = 0.07$, $P = 0.94$; log transformed time on platform: $t_{27} = 0.14$, $P = 0.89$). The slope of the learning curve did not differ by year ($t_{17} = 0.16$, $P = 0.87$), but there was a trend for males to have more negative slopes than females; males appeared to be faster at opening each successive washer while females were slower (Figure 7.2b; $t_{17} = 2.0$, $P = 0.06$). The latency to land on the board, ability to solve the task, latency to solve the task, the amount of time on the platform prior to solving, and the slope of the learning curve were each unrelated to the date in season, current brood size or age, or the time the trial started (data not shown). Older birds had a shorter latency to solving the task (Figure 7.3; $R^2 = 0.15$, $F_{1,26} = 4.8$, $P = 0.04$).

Parent house sparrows in the study population averaged 11.7 ± 0.3 nestling provisioning trips per hour (note that provisioning behavior was always observed when no food was available on the test platform). Male (11.7 ± 0.5 trips per hour, $N = 52$) and female (11.7 ± 0.7 trips per hour, $N = 58$) sparrows did not differ in their provisioning rates ($F_{1,91.5} = 0.01$, $P = 0.92$), but did differ in the likelihood of bringing large food items (Table 7.1). Females brought large food items at a faster rate (calculated as the proportion

of large items out of all items seen multiplied by feeding rate) than males (females: 1.2 ± 0.2 large items per hour; males: 0.69 ± 0.09 large items per hour). Nestling provisioning reaction norms were influenced by variation in the conditions that individuals were observed and exhibited phenotypic plasticity within individuals (Table 7.1). In addition, individual identity explained a significant portion of the variation in reaction norm intercepts for both provisioning rate and the likelihood of bringing large items (provisioning rate: -2dLL = -62.1, $DF = 1$, $P < 0.01$; probability of bringing large items: $\chi^2 = 319$, $DF = 1$, $P < 0.01$).

Sparrows that participated in the cognitive foraging task had higher provisioning rates (12.2 ± 0.4 trips per hour) than those that did not (10.2 ± 0.7 trips per hour; $F_{1,96.3} = 6.4$, $P = 0.01$), but did not differ in the likelihood of bringing large food items ($F_{1,116.7} = 0.5$, $P = 0.48$). Birds that solved the task did not feed their nestlings at a higher rate ($F_{1,59.7} = 0.1$, $P = 0.74$) nor were they more likely to bring large food items to the nest than non-solvers ($F_{1,66.5} = 0.2$, $P = 0.64$). For those birds that solved the task, there was a tendency for the latency from the time they landed on the platform to the first solve to negatively covary with nestling provisioning rate (effect: -0.0001 ± 0.00007, $F_{1,21.4} = 4.2$, $P = 0.05$). However, this relationship may have been unduly influenced by one individual with an extremely long latency to solve (Figure 7.4). Re-running the analysis with the extreme individual removed or using log transformed latencies resulted in no relationship between nestling provisioning and latency to solve (individual removed: $F_{1,21} = 1.7$, $P = 0.21$; log transformed latency: $F_{1,22.1} = 0.34$, $P = 0.57$). There was no relationship between latency to solve and the likelihood of bringing large food items ($F_{1,34.7} = 0.5$, $P = 0.50$), nor did the total time on the board covary with provisioning rate ($F_{1,25.8} = 0.4$, $P = 0.56$) or the likelihood of bringing large items ($F_{1,23.5} = 2.8$, $P = 0.11$). Neither measure of nestling provisioning was associated with the slope of the learning curve (provisioning rate: $F_{1,16.6} = 0.78$, $P = 0.39$; likelihood of provisioning with large items: $F_{1,12.7} = 0.57$, $P = 0.46$). Nestling survival did not differ by the problem-solving ability of the female parent ($F_{1,62.8} = 0.6$, $P = 0.45$). However, nests of male problem-solvers had a higher probability of fledging nestlings than those of males that did not solve the foraging task (Figure 7.5; intercept: 1.4 ± 0.2, effect of non-solver: -0.88 ± 0.3, $F_{1,119.5} = 8.3$, $P = 0.005$). Including average nestling provisioning rates provided by the male and female at the nest did not substantially alter this result.

Discussion

I predicted that house sparrow parents with better cognitive foraging abilities would provide higher levels of parental care. On the whole, I found that parents that solved a goal-oriented foraging task did not differ in either nestling provisioning rate or the likelihood of provisioning with large food items from parents that did not solve the task. There was some evidence to suggest that of those parents that solved the task, faster solvers had higher rates of parental care; however, this result was driven by one individual with an exceptionally long latency. There was no indication of an effect of problem-solving latency on parental care once I corrected for the influence of this individual. Interestingly, I found that nests of male sparrows that solved the foraging task had a higher probability of nestling survival. Adding measures of nestling provisioning behavior to the model of offspring survival did not change this result. Only two other studies have tested if reproductive performance in the wild was related to problem-

solving ability (Cole et al. 2012; Cauchard et al. 2013). These studies found problem-solving female great tits (*Parus major*) fledged more young than non-solvers (Cole et al. 2012), and nests of problem-solving great tit parents had higher nestling survival than nests of non-solvers (Cauchard et al. 2013). Although both studies implicated differences in parental care behavior between solving and non-solving parents as the likely reason for the differences in reproductive performance, only Cole et al. (2012) were able to test this idea. They found that the ability to solve a foraging problem (conducted in an aviary) did not predict provisioning rate or the amount of quality food items brought to the nest, but solvers did have significantly smaller home ranges when foraging for offspring (Cole et al. 2012). Although I could not test if foraging range size differed among house sparrows, my results support previous findings: problem-solving ability covaries with reproductive success, but this relationship is not mediated by differences in nestling provisioning rate or the size of food items brought to the nest.

Problem-solving males had nests with higher fledging success that appeared to be a result of some factor other than increased nestling provisioning. One possible explanation for this result is that solving males may have had higher nest attentiveness than non-solvers. If solving males spent more time near the nest or had smaller foraging ranges (e.g., Cole et al. 2012), these males could have been more vigilant to changes in nestling demand, intrusions at the nest by other house sparrows, or predators near the nest. Although solving ability did not predict the provisioning rate or likelihood of large item provisioning, solving males may have been more vigilant to nestling demand, and responded to nestling begging calls more than non-solvers. Nestlings forced to beg more than other nestlings for the same amount of food have been found to have decreased growth and immune response in several species, including house sparrows (Kilner 2001; Rodriguez-Girones et al. 2001; Moreno-Rueda 2010; Moreno-Rueda & Redondo 2012). If males that solved the task were more responsive to nestling begging, nestlings of non-solving males may have lower survival because of physiological costs associated with increased begging. In addition, because competition for nest sites and mates is high in my study population, it is likely that a significant portion of nestling mortality is due to infanticide committed by birds attempting to usurp nest boxes or mates. Infanticide in house sparrows is not an uncommon occurrence and is committed by intruding birds of both sexes (Veiga 2003; Veiga 2004; DP Wetzel, personal observation). Solving males could be more vigilant or aggressive toward conspecific intruders at the nest, thereby reducing nestling mortality due to infanticide. Alternatively, males that differ in problem-solving ability could have different behavioral strategies that result in differences in offspring survival. For example, solving males could have been better competitors for high-quality nest sites or food sources, though there is some evidence to suggest this may not be the case (Cole & Quinn 2012). Whatever the reason, these data suggest that selection could be acting on cognitive ability in the wild.

A second important finding of this study was that older birds had shorter problem-solving latencies. This indicates that there was an effect of individual experience on problem-solving speed. Most studies that tested for an effect of age on problem-solving ability found the two are not related (Keagy et al. 2009; Mateos-Gonzales et al. 2011; Benson-Amram & Holekamp 2012), however, Cole et al. (2011) found that juvenile great tits were more likely to complete a problem-solving task than adults, though this effect was only present in one of three study seasons. While there is evidence that suggests

juvenile birds are typically poorer foragers than adults (Sullivan 1989; Yoerg 1998), it is less clear how foraging proficiency changes as adult birds age (but see Desrochers 1992). Because subjects used in this study varied considerably in age (1 to 6 years old), it is possible I was able to detect an effect of age on cognitive foraging ability that other studies with more limited age distributions could not. Older individuals could be more proficient at solving foraging related tasks because annual survival depends, in part, on the ability to solve foraging problems during harsh conditions (e.g., over-winter survival). Over time, individuals with poor problem-solving abilities should be removed from the population by selection. Alternatively, long-lived individuals that have had more foraging experience have probably learned more foraging skills and therefore can quickly solve a novel foraging challenge. In either case, it appears that differences in experience among individuals provide a potential explanation for variation in cognitive ability within a population.

This study was conducted on free-living organisms, which created methodological biases in the data. For example, I had no way of forcing parents to participate in the trials as is done in many studies of cognition (i.e., food deprivation; Roth et al. 2010; Boogert et al. 2011; Cole et al. 2012). Participation in the trials I conducted was probably influenced by the motivation of the bird to come to the platform for food but also could have been due to individual differences in response to novel objects (the test platform and its contents) near the nest box (e.g., Ensminger & Westneat 2012). I did find birds that solved the foraging task had a shorter latency to the test platform than parents that did not solve the task. This suggests motivation for food could have driven some individuals to come to the platform faster and were therefore more likely to solve the problem. However, it is also plausible that solving birds learned my presence at the test platform (during twice daily food replenishments) indicated a good food source was available and came to the platform faster, i.e., birds that were good associative learners were also good problem solvers. A related methodological concern is that parents participating in the cognitive trials had significantly higher provisioning rates and thus were more likely to come to the nest box during the hour-long cognitive trial. In essence, the tests of cognitive ability and its relation to parental care behavior only included individuals with high provisioning rates. However, the average difference in provisioning rate between participating and non-participating birds was only two feedings per hour; parents that did not participate in the problem-solving task still came to the nest box (and consequently came near the test platform) an average of 10 times during a foraging trial. It is unclear why some birds participated in the trials while others did not, but this only allowed me to test the relationship between problem-solving and parental care in a subset of possible subjects. Differences also existed in the likelihood of solving the foraging task by the year; parents were more likely to solve the task in 2012 than 2011. This was probably because of differences in methodology and environmental conditions between years. The 2011 study season (March 1 through August 31) had exceptionally high precipitation (36 inches of precipitation, 10 inches above average), which impacted my ability to maintain a constant food supply on the test platforms when training parents to come to the platform for food. These methodological issues could have made it difficult to detect relationships between cognitive foraging ability and parental care if one actually did exist. One solution to these problems would be to observe behaviors of wild subjects, then capture them and conduct cognitive tests in an aviary (e.g., Boogert et al. 2011; Cole

et al. 2012). However, because individuals respond differently to captivity, measures of cognitive ability recorded in a captive setting are unlikely to predict measures of cognitive ability recorded in the wild.

In summary, I found that the age of a bird influenced variation between individuals in the speed in which they solved a foraging task, and nests of problem-solving males had higher nestling survival rates. Although there is evidence that problem-solving ability is repeatable in other populations (e.g., Cole et al. 2011), I only observed subjects one time in this analysis, and so within-individual variation could not be estimated. Future work should include repeated measurements of individuals in order to determine if differences in problem-solving attributes between individuals are consistent. Problem-solving ability may also be linked to other important behavioral traits, such as neophobia, which influenced the results obtained here. Further research into behaviors correlated with problem-solving ability in the wild could reveal behavioral syndromes that integrate with multiple life history traits. Finally, I found no evidence that variation in problem-solving ability provides an explanation for the existence and maintenance of consistent individual differences in the level of parental care a parent provides. Although house sparrows live in a rapidly-changing, human-modified habitat which probably requires occasional use of problem-solving skills, problem-solving ability may be a poor measure of foraging efficiency and therefore parenting ability. Other measures of cognitive ability, such as associative learning (e.g., Kitaysky et al. 2003) or social learning (e.g., Reader & Biro 2010) could be more important indicators of foraging ability and efficiency than problem-solving ability. Additional investigation of the influence of different cognitive skills on foraging behavior in the wild are required to understand if individual variation in cognitive ability can create differences in parental care behavior.

Table 7.1. Results of the mixed model analysis of nestling provisioning reaction norms for 110 house sparrows. Bird identity is a random term, and all fixed effects are within-individual effects unless noted as "between," in which case they are between-individual effects.

	Variable	Effect ± SE	*F* (df)	*P*-value
Provisioning rate (trips/hr)[1]	Bird identity	0.18 ± 0.04		
	Residual	0.54 ± 0.03		
	Intercept	3.3 ± 0.05		
	Brood size (between)	0.34 ± 0.07	25.7 (1, 115)	< 0.0001
	Date	-0.003 ± 0.0009	8.1 (1, 789)	0.005
	Brood size	0.30 ± 0.03	110 (1, 788)	< 0.0001
	Nestling age	0.13 ± 0.01	127 (1, 790)	< 0.0001
	Observation start time	-1.4 ± 0.6	5.8 (1, 788)	0.02
	Partner rate	0.005 ± 0.005	0.78 (1, 798)	0.38
	Brood size*nestling age	0.05 ± 0.01	24.5 (1, 811)	< 0.0001
	Nestling age*partner rate	-0.005 ± 0.002	7.3 (1, 859)	0.007
Likelihood of bringing large items (logit of large items/seen items) [2]	Bird identity	0.73 ± 0.1		
	Intercept	-2.71 ± 0.1		
	Sex (female)	0.62 ± 0.2	9.7 (1, 94.9)	0.002
	Brood size	0.23 ± 0.05	19.3 (1, 862)	< 0.0001
	Nestling age	0.21 ± 0.02	104 (1, 862)	< 0.0001
	Partner rate	-0.008 ± 0.008	0.97 (1, 862)	0.33
	Nestling age*nestling age	-0.05 ± 0.008	44.0 (1, 862)	< 0.0001
	Brood size*partner rate	-0.03 ± 0.009	11.3 (1, 862)	0.0008

[1] Square root transformed

[2] From GLMM model of binomial using logit link and Kenward-Rogers estimation of denominator degrees of freedom

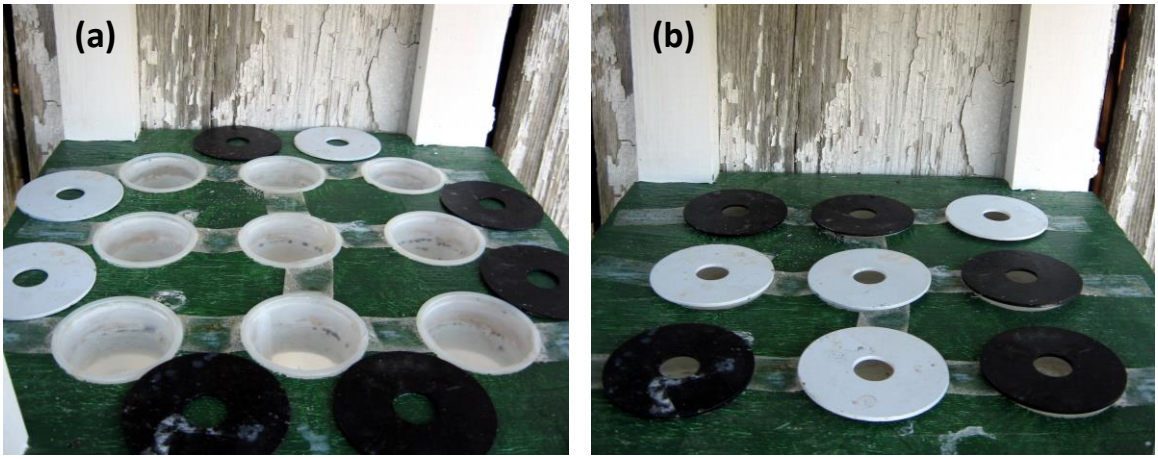

Figure 7.1. Photograph of the test platform used to perform the goal-oriented problem-solving task. (a) The test platform consisted of nine 1.5 oz cups mounted flush on a 23 x 23 cm board. (b) Food was placed in the cups and each cup was covered with a metal washer to create the problem-solving task.

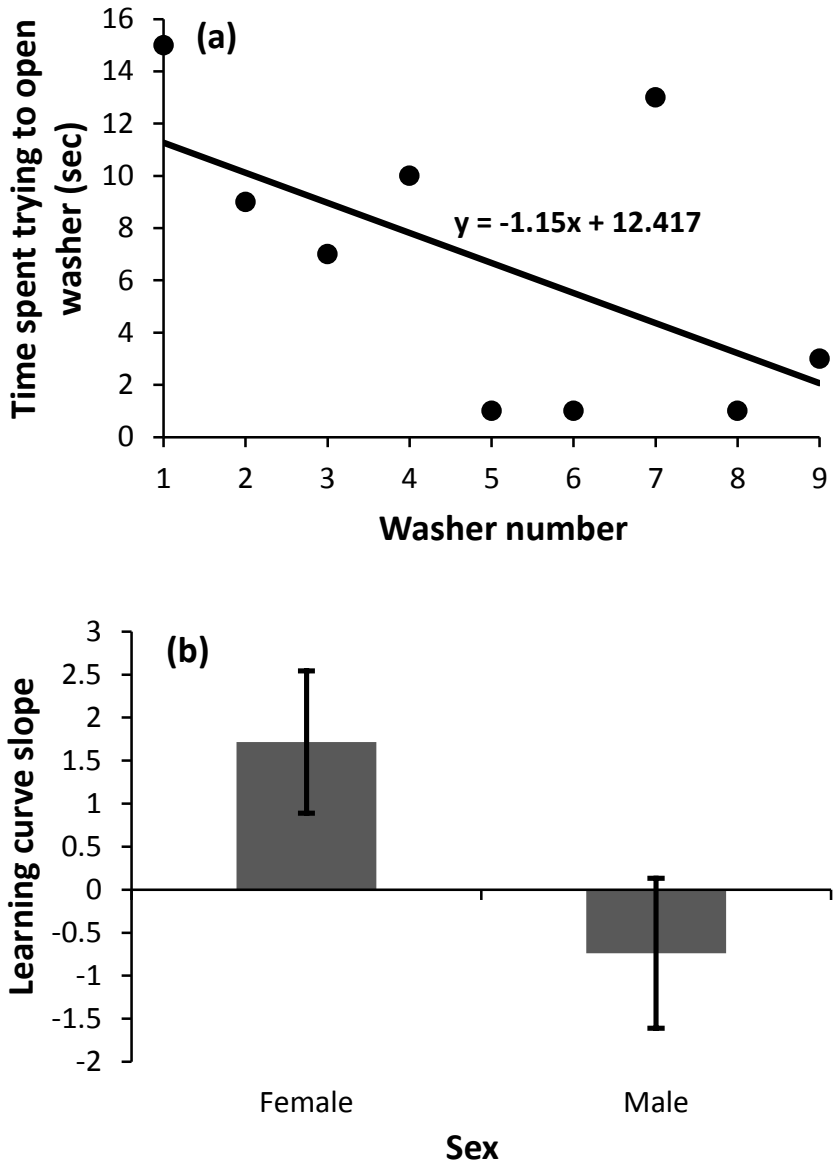

Figure 7.2. A learning curve was calculated for each parent that solved the problem and opened more than one washer within a trial. (a) An example of a learning curve from one male house sparrow. The learning curve was estimated from the slope of the best-fit line of the amount of time (seconds) spent attempting to open each successive washer. (b) There was a tendency for the learning curves of parent house sparrows to differ by sex (*N* = 10 females and 9 males).

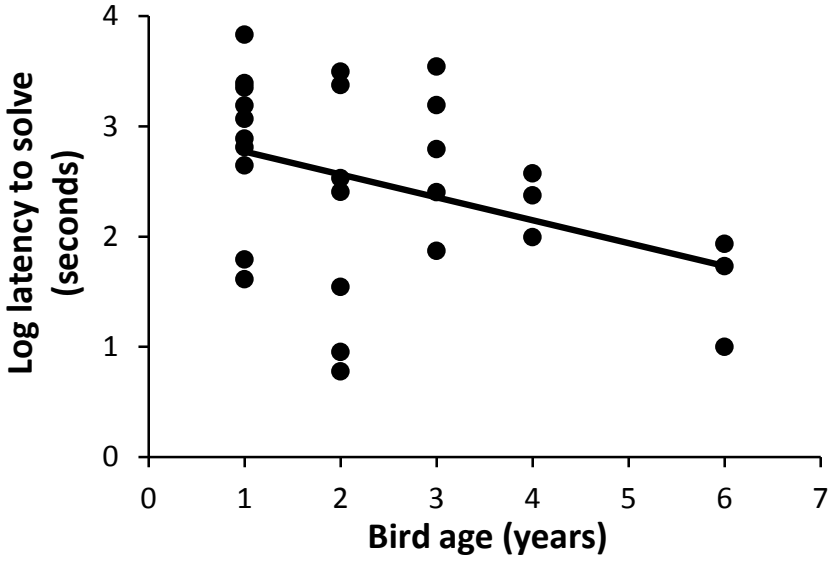

Figure 7.3. The relationship between latency to solve a foraging task (log_{10} of the time between first stepping on the test platform and first solving the problem) and subject age (in years) for 28 parent house sparrows.

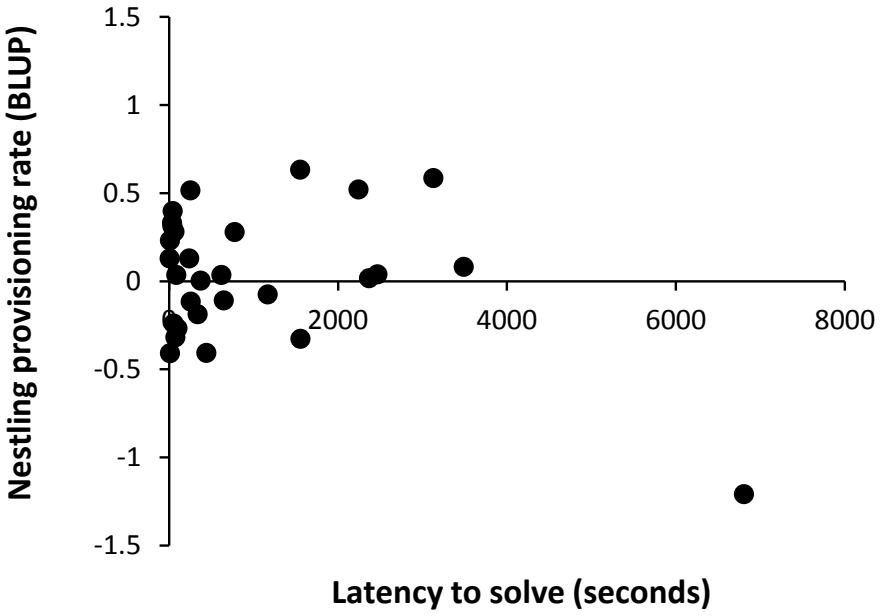

Figure 7.4. The relationship between nestling provisioning rate and latency to solve a foraging task (time between first stepping on the test platform and first solving the problem) for 28 house sparrows. For this figure, nestling provisioning rate (trips per hour) was estimated for each individual as a best linear unbiased prediction (BLUP) from the linear mixed model analysis of parental care reaction norms. All fixed effects from Table 7.1 were included in the analysis to generate BLUPs for each parent sparrow (see Chapter 8).

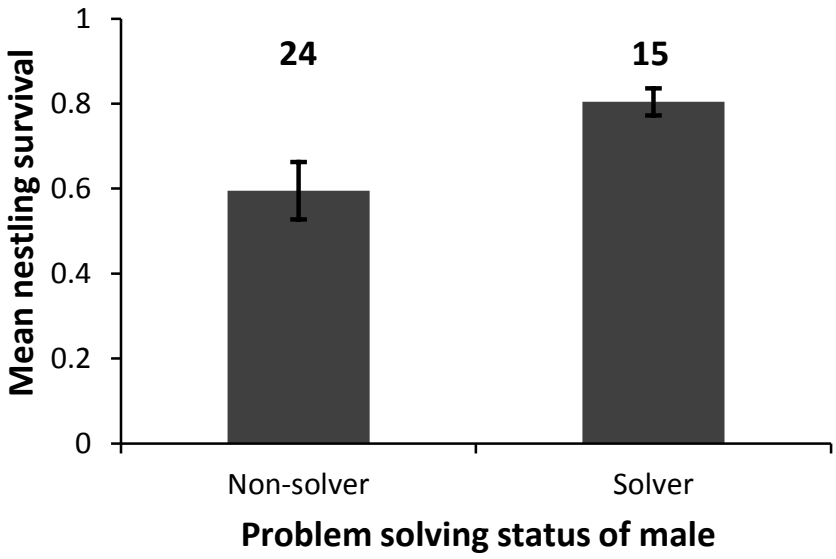

Figure 7.5. Nestling survival to fledging depending on the problem-solving status of the male parent. This analysis included all nests from 2008 – 2012 known to have been produced by a male that participated in the foraging trials. Numbers above the bars indicate the number of male house sparrows in each category.

CHAPTER EIGHT

The effect of individual variation in parental care on offspring

Parental care is a key life history trait that varies both within and among taxa (Clutton-Brock 1991). A central tenet of parental care theory is that such variation is adaptive and reflects environmental differences in the benefits of care to offspring or the costs to the parent (Winkler 1987; Clutton-Brock 1991; Gross 2005). In support of such theory, variation in parental care between species has been related to such variables as lifespan (Curio 1988), predation risk (Ghalambor & Martin 2002; Eggers et al. 2005), and ecology (Clutton-Brock 1991; Russell et al. 2004). Though it is clear parental care varies among species, considerable variation also exists within a species or population. Studies on variation within species have found that levels of care relate to the number and value of the offspring to parents (e.g., Breitwisch et al. 1986), the cost of care (e.g., Ardia et al. 2009), kinship (e.g., Burke et al. 1989; Neff 2003) and, in species with bi-parental care, the level of care provided by a partner (e.g., Wright & Cuthill 1989). What has emerged is broad support for parental care as a generally adaptive trait that has been shaped by selection, where parents adjust their level of care in relation to its benefits to offspring and its costs to themselves (Winkler 1987; Gross 2005; Kvarnemo 2010).

Despite an overall fit to theory, there are a number of details in theory about parental care that remain untested. For example, although many studies have examined how parents modify the level of care they provide (often in terms of provisioning) in response to changes in brood value, the number of studies that have actually tested the assumption that the level of care a parent provides has a discernible effect on offspring is small (Clutton-Brock 1991; Krist 2009). Of the studies that have directly tested this assumption in birds, most find the expected positive relationship between provisioning and offspring performance (Eggert et al. 1998; Ardia 2007; Schwagmeyer & Mock 2008; Ringsby et al. 2009), though some have found no effect of care (Krist 2009; Ringsby et al. 2009; García-Navas & Sanz 2012), and one found a negative relationship (Nur 1984a). The findings of these latter studies suggest that measurement conditions of parental care behavior and offspring performance can have major impacts on their apparent relationship. Furthermore, the measurement of the benefits of parental care, in terms of offspring fitness, is almost always performed in what amounts to a two-step approach. In a first step, behavioral studies test for effects of parental provisioning on offspring size, number, or short-term survival, during the period of care (Nur 1984a; Eggert et al. 1998; Sheldon 2002; Ardia 2007). Unfortunately this type of study misses the transitional phase from juvenile to adult, a period typically associated with high mortality (e.g., Naef-Daenzer et al. 2001; Hegyi et al. 2011). Second, often in other studies, the relationship between short-term estimates of offspring fitness (e.g., offspring size) and long-term estimates of fitness (e.g., offspring survival to recruitment) is tested (Magrath 1991; Schwagmeyer & Mock 2008; Hegyi et al. 2011). These studies cannot separate any direct effects of parental care on offspring fitness from the delayed indirect effects of parental care on offspring traits. For example, provisioning rate likely influences nestling mass (e.g., Ardia 2007) and nestling mass can influence survival to recruitment (e.g., Naef-Daenzer et al. 2001), but this type of study cannot test if parental provisioning has an

independent effect on offspring fitness. Our understanding of the effect of parental care on offspring performance, and therefore offspring fitness, is poorly studied and often reconstructed from multiple studies. The benefits of parental care should be tested directly, in one study.

A fundamental problem with testing the effects of parental care on offspring performance is that both parental care and offspring performance may vary in response to a multitude of environmental factors (e.g., date in season, brood size, brood age, etc.). Plasticity of care in response to these environmental conditions could create a strong covariance between parental care and offspring performance when the real relationship is relatively weak, or obscure a strong relationship if both are sensitive in different directions to the same factors. For example, the level of parental care and offspring fitness both typically decline with date in breeding season (Bortolotti et al. 2011; Westneat et al. 2011), which if unaccounted for, could lead to a strong positive covariance between parental care and offspring fitness. The confounding influence of joint environmental effects must be accounted for to correctly test for the benefits of parental care. Interestingly, despite the fact that parental care is phenotypically plastic, recent research has found that individual parents are consistently different in the level of care they provide to their offspring; in some cases individual identity accounts for 20% of the variance in parental care (Schwagmeyer & Mock 2003; Nakagawa et al. 2007; Dor & Lotem 2010; Westneat et al. 2011; Low et al. 2012). In addition to providing consistently differently levels of care, individual parents can also vary in their plasticity of care by responding differently across an environmental condition (individual by environment (I x E) interaction). One solution to both of these issues is to use a behavioral reaction norm approach. This approach simultaneously accounts for variation in care that can be attributed to between-individual differences in the environmental conditions experienced, within-individual phenotypic plasticity across environments, as well as variation from consistent individual differences (Nussey et al. 2007; van de Pol & Wright 2009; Dingemanse et al. 2010). The reaction norm model can then be used to estimate individual-specific predictor values (best linear unbiased predictions; BLUPs) of the level (individual reaction norm intercepts) and plasticity (individual reaction norm slopes) of care a parent provides, after accounting for environmental effects. These predictors can be used as quantitative characters to test how individual differences in the level and plasticity of parental care provided influence offspring performance and fitness (Nussey et al. 2007).

In this study, I tested how parental care behavior exhibited by adult house sparrows (*Passer domesticus*) affected offspring performance and fitness. First, I used a reaction norm approach to model nestling provisioning rates of parent sparrows. This analysis accounted for differences among and plasticity within individual parents, and from it, I generated predictor values of provisioning rate for each parent (reaction norm intercepts) and each parent's response (reaction norm slopes; individual plasticity) to brood size and brood age. Next, for each measure of offspring performance, I assessed and accounted for environmental factors that could independently influence performance (year, date in season, etc.). Finally, I tested if differences in the level and plasticity of nestling provisioning rates provided by parents influenced offspring performance.

Methods

Study site and population

This study was conducted on a nest box population of house sparrows from 2008 to 2012 at the University of Kentucky's Agricultural Experiment Station, located just north of Lexington, KY (38°06´N, 84°29´W). The study site consists of agricultural and pastoral fields, and multiple barns used for stabling horses and storage. House sparrow nest boxes were installed on the outside walls of four of these barns. The number of nest boxes per barn (10 to 20 boxes per barn) and the total number of boxes available ranged from 50 to 60 boxes in each year. House sparrows in this study population breed continuously from March through August, with each pair attempting one to six clutches per season. Females in this population lay an average of five eggs per clutch with a range of one to eight eggs (Westneat et al. 2009). Eggs hatch after approximately 10 days of incubation, and altricial nestlings remain in the nest and are fed by the parents for 14–17 days. This species has bi-parental care; both sexes provide food to dependent nestlings throughout the nesting cycle.

Adult sparrows breeding in nest boxes at the study site were trapped with mist nest and seed-baited cage traps. Each bird was banded with a numbered, metal USGS band and a unique combination of colored plastic bands so they could be identified by sight. Birds banded as nestlings or juveniles that returned to breed at the site could be aged precisely, while birds banded as adults were assigned a minimum age, which assumes they were in their first breeding season. Beginning in March of each year, I checked each nest box twice a week for nest building and breeding activity. Active nests were checked at least three times a week to identify the day the first egg was laid and the day the eggs hatched. I continued to check active nests at least three times per week until nestlings were banded with a metal USGS band approximately 10 days after hatching (mean ± SD age at banding: 10.1 ± 0.8 days, $N = 363$). At the time of banding I recorded each nestling's tarsus length with calipers to the nearest 0.1 mm, and mass with an electronic balance to the nearest 0.1 g. I collected a 50 µl blood sample from each nestling via brachial venipuncture and refrigerated the samples in the field until they could be returned to the laboratory at the end of the day and stored at -80 °C. For each nesting attempt, I recorded the clutch size, number of eggs that hatched (brood size), and number of nestlings that fledged from the nest.

Offspring performance

Offspring performance variables were collected throughout the nesting cycle of each year for 534 breeding attempts (1790 nestlings). Tarsus length was measured for all nestlings in all nests when the nestlings were banded and averaged for each nest. Nestling growth rates were collected in three years of this study (2008 – 2010). Typically, I weighed each nestling four times when they were between the ages of 1 – 10 days old, though the number of measurements varied between nests and years (mean ± SD number of weights: 2008, 4.5 ± 0.7, $N = 51$; 2009, 4.9 ± 1.9, $N = 38$; 2010, 3.6 ± 2.1, $N = 48$). To estimate the growth rate for each nest, I regressed mean nestling weight on nestling age (in days) and calculated the slope of the regression. These estimates of growth rate were highly correlated with mean nestling change in mass from day 1 to day 10 ($r = 0.98$, $N = 110$, $P < 0.0001$). Nestling immune response was collected from 60 nests in one year (2008) using a bacterial killing assay (Matson et al. 2006; see below). Nest survival was

calculated for each nest as the proportion of nestlings that fledged from the nest out of the number of nestlings hatching. Recruitment was calculated for each nest as the proportion of fledglings that returned to breed at the study site in a subsequent year.

To conduct the bacterial killing assay developed by Matson et al. (2006), blood samples from 215 nestlings in 2008 were collected in heparinized capillary tubes and immediately transferred to a sterile 1.5 ml microcentrifuge tube. These samples were stored on ice in the field and returned to the laboratory at the end of each day. Each blood sample was then centrifuged for 10 minutes at 13,000 RPM and the plasma was pipetted into a new, sterile 1.5 ml microcentrifuge tube. All plasma samples were stored at -80 °C in the laboratory until the assay was conducted. Plasma samples were grouped into batches and each batch was assayed at a different time between October and November of 2008. To initiate the bacterial killing assay, 10 µl of plasma from each sample was diluted with 190 µl of growth media (CO_2-independent media (Invitrogen #18045-088), 4mM l-glutamine (Invitrogen #25030-149), and 5% heat-inactivated fetal calf serum (Invitrogen #10082-139)). To each diluted plasma sample, I added 20 µl of an *E. coli* culture (~600 colony forming units (CFUs)), which was prepared from a lyophilized *E. coli* pellet (5.2 x 10^7 CFUs per pellet; MicroBioLogics, Inc. 0483E7 *Escherichia coli* ATCC® 8739™ Epower). Control samples were created by adding 20 µl of the *E. coli* culture to growth media without any plasma. All samples containing *E. coli* were incubated at 41 °C for 30 minutes, allowing the bacteria time to divide and grow and the immune components of the plasma to interact with and kill the bacteria. After incubation, a 50 µl aliquot of each sample was pipetted and spread evenly on a tryptic-soy agar plate. Agar plates were allowed to dry for 20 minute in a sterile environment, covered, inverted, and incubated overnight at 35 °C. The following day, I counted the number of visible colonies on each plate and determined the proportion of bacteria colonies killed using control plates. Each nestling received a score for the proportion of bacteria colonies killed by their plasma, which was then arcsine square root transformed, centered by batch, and averaged for each nest.

Parental care

Parental care data was collected from almost all nesting attempts made by house sparrows breeding in the nest boxes provided for them. Each nest was video recorded two to three times in ~2 hour blocks. In 2008, 2009, and 2011 each brood was recorded twice, when nestlings were approximately 5 and 7 days old, while in 2010 and 2012 each brood was recorded three times, when nestlings were approximately 3, 6, and 9 days old. Video recordings were performed during the morning hours (7 am – 12 pm) and the cameras (Hi-8 or digital) were concealed in a small box attached to the barn 1 to 5 m from each nest box. Nestling provisioning was quantified for each parent by measuring the feeding trips per hour during each observation period. An observation period consisted of the length of the recording (typically 2 hours) minus the latency of the first feed by either parent. I performed parental care observations on 407 breeding attempts over the 5 years of this study. These observations included data from 298 individual sparrows, each observed an average of 6.5 times (SD: 5, range: 1 – 31) over an average of 2.7 breeding attempts (SD: 2, range: 1 – 13), for a total of 1921 observation sessions (3538 hours).

Analysis

Nestling provisioning behavior was recorded multiple times for each parent under different environmental conditions. I used a reaction norm approach to analyze these repeated measurements, account for phenotypic plasticity within individuals, and obtain estimates of the level of care and individual plasticity of care for each parent (Nussey et al. 2007; Dingemanse et al. 2010; Westneat et al. 2011). Nestling provisioning rate was not normally distributed nor could it be made normal through transformation; square root transformation brought the data very close to normal so the analysis was performed on the transformed data. This analysis was conducted using Proc Mixed in SAS 9.2 (SAS Institute Inc., Cary, NC) in three stages. In all stages of the analysis bird identity was included in the mixed model as a random effect. First, I tested if the conditions under which each parent was observed could have created differences between individuals. The factors I tested included the sex, age, and breeding attempt number of the parent, date and time of the observation, number and age of the nestlings during the observation, and the partner's nestling provisioning rate. Each of these fixed effects (except sex) was averaged for each individual and centered with respect to the full data set (van de Pol & Wright 2009). All factors were added to the model, then backward elimination was used to generate a best-fit model that described the sources of between-individual variation in nestling provisioning. Second, I tested for phenotypic plasticity in nestling provisioning by mean-centering each observation within each individual for each fixed effect. I added all main within-individual centered factors and many of their second-order interactions to the previous model, then used backward elimination to generate a best-fit model describing variation in nestling provisioning. Finally, I accounted for individual by environment (I x E) interactions across brood size and nestling age by adding these two factors as random interaction terms. From this final model, I obtained best linear unbiased predictions (BLUPs) of nestling provisioning for each of the three random terms (individual identity (reaction norm intercept), individual identity by brood size, and individual identity by nestling age), for each parent.

To test for an effect of parental care on each measure of offspring performance, I first accounted for environmental factors that could have affected offspring performance. The performance variables of tarsus length, growth rate, and immune response were each modeled independently using linear mixed models (Proc Mixed in SAS 9.2), while survival was modeled as the number of nestlings that survived out of the number of eggs that hatched using logistic regression with a binomial distribution and logit link (Proc Glimmix in SAS 9.2). Offspring recruitment was modeled using a logistic regression with a Poisson distribution and a log link (Proc Glimmix in SAS 9.2). In all models, I included the year the nest was initiated and the identities of both the male and female parents of each nest as random terms. The model of immune response did not include a year effect since the bacterial killing assay was only performed on nestlings from 2008. I tested if date in season, clutch size, age at banding, or maximum brood size influenced any measure of offspring performance and retained the significant factors in each model. To each best-fit model of performance, I then simultaneously added the BLUP of each parent's nestling provisioning reaction norm intercept. This allowed me to test if offspring performance was affected by variation in the reaction norm intercepts of the parents, and which parental sex had the largest effect on each component of performance.

The effect of plasticity in nestling provisioning on offspring performance was tested by adding the BLUPs of individual by brood size and individual by nest age for each parent.

Results

Performance of nestling house sparrows was influenced by several environmental factors. Mean nestling tarsus length was 18.2 ± 0.06 (SE) mm and was positively affected by date in season and the age at which nestlings were banded (Table 8.1). The rate at which nestlings grew averaged 2.0 ± 0.04 grams per day and was negatively associated with the age nestlings were banded (Table 8.1). The mean proportion of nestlings that survived from hatching to fledging was 0.71 ± 0.02 and was negatively influenced by the date in season and clutch size of the nest (Table 8.1). The mean proportion of fledglings returning to breed at the study site in a subsequent year was 0.035 ± 0.006 and decreased with the date their natal nest was initiated (Table 8.1). The mean proportion of bacterial colonies killed by plasma collected from nestling sparrows was 0.68 ± 0.01 and varied by nest (repeatability = 0.29; $F_{60,154} = 2.4$, $P < 0.0001$), but did not correlate with any of the environmental factors tested (all $P > 0.10$). Mean tarsus length of the nest positively covaried with the nest's mean growth rate and the proportion of nestlings that survived (Table 8.2). The proportion of nestlings surviving positively covaried with the proportion of fledglings that recruited to the study population (Table 8.2).

Parent house sparrows averaged 11.3 ± 0.2 nestling provisioning trips per hour. There was no difference between sexes in provisioning rates (males: 11.5 ± 0.3, females: 11.3 ± 0.4; $F_{1,243} = 0.38$, $P = 0.54$). Provisioning rate was not influenced by the number of observations performed on a parent ($F_{1,194} = 1.35$, $P = 0.25$). Results from the mixed model analysis of nestling provisioning reaction norms found that provisioning rate was affected by variation in the conditions in which individuals were observed (brood size, nestling age, and partner provisioning rate; Table 8.3). Provisioning rates of parent sparrows exhibited phenotypic plasticity across several environmental variables, including date in season, brood size, nestling age, observation start time, and their partner's provisioning rate (Table 8.3). Furthermore, individual parents significantly differed in their response to changes in brood size and nestling age (Table 8.4). After accounting for all significant fixed and random effects in Table 8.3, individual identity explained 20.2% of the variation in parental care (Likelihood ratio test: -2dLL = 145, $DF = 1$, $P < 0.01$).

Offspring performance traits were positively influenced by parental care. Male sparrows with higher nestling provisioning rates (higher BLUPs) produced offspring that had larger tarsi (effect = 0.36 ± 0.17, $F_{1,128} = 4.4$, $P = 0.04$; Figure 8.1b) and tended to be more likely to survive to fledging (effect = 0.55 ± 0.3, $F_{1,109.6} = 2.9$, $P = 0.09$). Female sparrows with higher provisioning rates produced offspring that grew faster (effect = 0.23 ± 0.1, $F_{1,104} = 5.1$, $P = 0.03$; Figure 8.1c), had a better immune response (effect = 0.14 ± 0.06, $F_{1,27} = 4.7$, $P = 0.04$; Figure 8.1e), and tended to recruit more offspring to the breeding population in a subsequent year (effect = 0.91 ± 0.5, $F_{1,86.9} = 2.8$, $P = 0.09$). Individual plasticity with respect to brood size or nestling age had no effect on most of the offspring performance traits (data not shown).

In an effort to disentangle the direct and indirect effects of provisioning rate on offspring survival, I added mean nestling tarsus length as a covariate in the models of nestling survival and recruitment. Mean nestling tarsus length had a strong, positive

effect on both the likelihood of survival within the nest (effect = 0.31 ± 0.06, $F_{1,352} = 24$, $P < 0.0001$), and recruitment to the breeding population (effect = 0.42 ± 0.2, $F_{1,298} = 5.3$, $P = 0.02$), and its addition to these models caused the effect of provisioning rate to become clearly non-significant (male BLUP on nestling survival: $F_{1,83.7} = 1.8$, $P = 0.18$; female BLUP on recruitment: $F_{1,75.7} = 1.3$, $P = 0.26$).

Discussion

As predicted by parental care theory, I found that the level of parental care provided by house sparrows had positive effects on offspring performance after controlling for potential confounding effects of common environmental conditions. Parents that provided higher amounts of parental care produced offspring that were larger, in better condition, and appeared to have a greater chance of surviving to fledging and to adulthood. On the surface, this result is not entirely surprising, as a basic expectation of parental care theory is that offspring should benefit from increased levels of care. My results thus support the results of previous studies that have found positive covariance between parental care and offspring performance or fitness (Drent & Daan 1980; Eggert et al. 1998; Zink 2003; Ardia 2007; Schwagmeyer & Mock 2008; Ringsby et al. 2009; Schroeder et al. 2012). However, my study is different from previous work because it was predicated on the idea that attributes of individual parental care reaction norms (individual intercepts and slopes) can influence offspring performance, not that the total amount of care provided to or received by specific offspring influences their condition. Although there was no effect of individual variation in reaction norm slopes on offspring, I found that individual variation in reaction norm intercepts positively influenced offspring performance in the study population. Examining the consequences of individual variation in parental care reaction norms provides a different perspective on the benefits of care.

The major finding of this study was that offspring produced by parents with high nestling provisioning reaction norm intercepts in the mean environment grew faster, were larger, and had better immune responses, which increased the likelihood to survive in the nest and recruitment to the study site the following year. The most logical explanation for these findings is that the rate at which offspring are fed directly influences their growth, health, and probability of survival, i.e., offspring benefit directly from the amount of care they receive (Clutton-Brock 1991). While this is most likely the case, it is important to remember that in this analysis parental care reaction norms were estimated for parents observed across multiple broods and multiple years, not on a per-nest basis. In essence, this means that I was not estimating the amount of care received by offspring in a particular nest, but asking what are the benefits of being in the nest of a parent that typically has high provisioning rates (estimated in the mean environment across all broods they produced). There could be additional benefits of being in the nest of a high-provisioning parent that are not captured by this study. For example, previous research has found that other components of parental care (e.g., nest defense, time spent incubating) positively correlate with provisioning rate in some house sparrow populations (Chapter 3; Kopisch et al. 2005). Parents that typically have high provisioning rates may also bring large or high-quality items to the nest more frequently (e.g., Arnold et al. 2007; Schwagmeyer & Mock 2008; but see Chapter 6). Other factors such as egg size or the concentration of nutrients and hormones deposited in the egg probably also influence

offspring condition and survival (Groothuis et al. 2005; Krist 2011), and may positively covary with provisioning behavior. Alternatively, it is possible that parents that provision at a high rate also pass along beneficial genetic traits to their offspring (e.g., Schroeder et al. 2012). However, because this study did not cross-foster offspring, genetic effects could not be disentangled from the actual effect of provisioning rate. In any case, individuals that typically have high provisioning rates produce high-condition offspring that have higher fitness, and are therefore likely favored by selection.

Interestingly, while I did not detect a difference in the provisioning rates provided by male and female parents, male and female provisioning affected different components of offspring performance. Female parents that consistently provided high nestling provisioning rates produced offspring that grew faster, had a better immune response, and appeared to have a greater probability of recruiting to the breeding population in a subsequent year. Male sparrows that consistently provided high provisioning rates produced nestlings that were larger and appeared to have a greater chance of surviving to fledging. This finding is similar to a study of the effects of parental care in tree swallows (*Tachycineta bicolor*) where researchers found that nestling growth was positively related to female provisioning rate while nestling mass was positively related to male provisioning rate (Ardia 2007). However, unlike my study, there were differences in the feeding rates provided by male and female tree swallows. The different impacts of parental sex on components of offspring performance could be due to differences in the types of food items fed to nestlings. Parents have been shown to differ in the size of food items they bring to the nest in this sparrow population and in bird populations (Chapter 7; Bańbura et al. 2001; Mitrus et al. 2010). This could be a result of males spending more time foraging near the nest box to be more vigilant toward potential nestling predators or nest box usurping conspecifics (e.g., Veiga 2003), while females spend time foraging in higher-quality locations. Although it is often difficult to determine exactly where parents are foraging at my study site, it is uncommon to observe both parents foraging in the same location (DP Wetzel, personal observation). Differences by males and females in the quality or type of food items provided to offspring could influence different components of offspring development (e.g., Arnold et al. 2007; García-Navas & Sanz 2011). Alternatively, male and female parents may be responding differently to cues of offspring condition. For example, one sex may feed smaller or poor condition offspring preferentially (e.g., Leonard & Horn 1996; Tanner & Richner 2008) or bias provisioning toward offspring of a particular sex (Mainwaring et al. 2011). Parents may also respond differently to signals of offspring need. In a captive canary population (*Serinus canaria*), males responded to and fed nestlings with the tallest begging display, while females responded to both the height and intensity of begging displays (Kilner 2002). Offspring may also influence parental feeding decisions differently for male and female parents by begging differently depending on which parent was present with food (e.g., Bell 2008). If male and female sparrows differed in how or where they forage, or in how they respond to offspring demand or condition, this suggests that selection has favored divergent sex-roles for provisioning parents, and that these roles can have effects on different components of offspring performance.

There are important conceptual issues with studying the effect of parental care on offspring performance and fitness. First, parental care behavior and offspring performance vary with respect to changes in the environment. If this phenotypic plasticity

is not accounted for, environmental conditions could create or obscure a relationship between parental care and offspring performance. For example, I found that nestling provisioning rate and nestling survival both decrease with date in season. If provisioning rate and nestling survival both were independently responding to time of season, failure to include date in the analysis would have resulted in an inflated relationship between provisioning rate and survival (Verhulst & Nilsson 2008; Bortolotti et al. 2011). Although the approach used in this study accounted for many factors that could influence both parental care and offspring performance, additional variables I did not account for could have influenced to these results. This issue is potentially a larger concern for this study because I generated BLUPs to estimate each parent's provisioning reaction norm intercept. While BLUP has been used for decades to predict random effects (such as an individual's reaction norm intercept; e.g., Nussey et al. 2005), using an incomplete model to generate BLUPs can bias their estimation (Postma 2006; Hadfield et al. 2010). These issues could be alleviated by using multivariate mixed models, where the covariance between provisioning rate and measures of offspring performance can be calculated directly while accounting for fixed and random effects (Hadfield et al. 2010; Dingemanse & Dochtermann 2013). A second conceptual issue is that most studies in species where multiple offspring are produced in a single breeding attempt, including this one, test if differences in offspring performance between nests are influenced by variation in parental care received, and ignore within-nest variation. Considerable variation in offspring performance can exist among offspring within a nest (Hegyi et al. 2011), particularly in house sparrows (Kinnard & Westneat 2009). While the primary source of this variation is probably asynchronous hatching, parents can moderate variation among nestlings through parental care. This means that individual variation in provisioning rate can have an effect on mean offspring performance, as shown here, and also influence the amount of variation exhibited among offspring. Variation among offspring within a brood could have persistent impacts on individual performance after leaving the nest and therefore offspring fitness (Hegyi et al. 2011).

Finally, it is often difficult to separate the direct effects of parental care on offspring fitness from the delayed indirect effects of care on other traits. For example, survival to adulthood may be influenced directly by body size or mass, but both fitness and traits affecting fitness could be affected by parental care. My results suggest that parental care behavior influenced offspring survival and recruitment indirectly through offspring size. High provisioning parents produced larger offspring, and offspring size predicted the likelihood of survival within the nest and recruitment to the breeding population in a subsequent year. This finding has two main implications. First, studies that test how offspring traits are affected by parental care behavior may not need to follow offspring through to the following year to estimate the effect of care on fitness. However, interactions between offspring condition and environmental variables (e.g., date in season) could have a larger effect on offspring fitness than just offspring condition, which would be overlooked in these types of studies (Hegyi et al. 2011). On the other hand, this also suggests that the handful of studies that have tracked the effect of parental care through to offspring recruitment (Schwagmeyer & Mock 2008; Ringsby et al. 2009; Krist 2009; Schroeder et al. 2012) have neglected to test the mechanisms by which parental care behavior influences offspring fitness. Second, these results suggest that a more detailed accounting of the factors that are affected by parental care and their

direct and indirect impacts on fitness would yield a better understanding of evolutionary forces acting on individual variation in parental care.

Table 8.1. Results from the analysis of performance traits of nestling house sparrow from 2008 – 2012. Tarsus length and growth rate were analyzed with linear mixed models. Survival was modeled as the number of nestlings that fledged from the number of eggs that hatched and analyzed using a logistic model of the binomial with a logit link. Recruitment was modeled as the number of offspring that returned to breed at the study site using a logistic model of the Poisson with a log link. Year, father identity, and mother identity were entered in all models as random terms. The variable "nestling age" is the age (in days) at which the nestlings were banded.

Offspring performance trait	Variable	Effect ± SE	*F* (df)	*P*-value
Tarsus length	Year	0.05 ± 0.05		
	Father identity	0.04 ± 0.08		
	Mother identity	0.02 ± 0.07		
	Residual	1.1 ± 0.1		
	Intercept	18.2 ± 0.1		
	Date in season	0.008 ± 0.002	19.4 (1, 329)	< 0.0001
	Clutch size	0.09 ± 0.08	1.3 (1, 311)	0.25
	Nestling age	0.23 ± 0.08	9.1 (1, 349)	0.003
	Brood size	-0.14 ± 0.07	3.8 (1, 349)	0.06
Growth rate	Year	0.02 ± 0.02		
	Father identity	0		
	Mother identity	0		
	Residual	0.15 ± 0.02		
	Intercept	2.0 ± 0.1		
	Date in season	0.0003 ± 0.002	0.04 (1, 105)	0.84
	Clutch size	0.03 ± 0.06	0.2 (1, 104)	0.67
	Nestling age	-0.24 ± 0.06	19.5 (1, 104)	< 0.0001
	Brood size	-0.08 ± 0.05	2.9 (1, 105)	0.09
Immune response	Father identity	0.004 ± 0.007		
	Mother identity	0.008 ± 0.007		
	Residual	0.02 ± 0.007		
	Intercept	-0.02 ± 0.03		
	Date in season	0.0004 ± 0.0009	0.2 (1, 39.5)	0.66
	Clutch size	-0.01 ± 0.03	0.2 (1, 59.3)	0.70
	Nestling age	0.005 ± 0.03	0.03 (1, 51.5)	0.86
	Brood size	0.02 ± 0.02	0.6 (1, 40.5)	0.45
Survival	Year	0.003 ± 0.04		
	Father identity	0.62 ± 0.2		
	Mother identity	0.66 ± 0.2		
	Intercept	0.94 ± 0.1		
	Date in season	-0.01 ± 0.002	19.7 (1, 303.6)	< 0.0001
	Clutch size	-0.21 ± 0.09	5.7 (1, 404)	0.02
Recruitment	Year	0.26 ± 0.4		
	Father identity	0		
	Mother identity	0.26 ± 0.3		
	Intercept	-2.4 ± 0.3		
	Date in season	-0.02 ± 0.006	7.5 (1, 341)	0.007
	Clutch size	-0.29 ± 0.3	1.2 (1, 341)	0.27
	Brood size	0.25 ± 0.2	1.3 (1, 341)	0.25

Table 8.2. Spearman's rank correlation coefficients for mean measures of offspring performance for 360 house sparrow nests. Survival was calculated as the proportion of nestlings fledging, and recruitment was calculated as the proportion of fledglings that bred at the study site in a subsequent year.

Trait	Growth rate		Immune response		Survival		Recruitment	
	r_s	N	r_s	N	r_s	N	r_s	N
Tarsus length	0.55**	109	0.06	60	0.21**	360	0.11†	293
Growth rate			0.05	51	0.18†	110	0.14	105
Immune response					-0.11	60	-0.03	56
Survival							0.14*	296

† $P < 0.07$
* $P < 0.05$
** $P < 0.0001$

Table 8.3. Results of the mixed model analysis of nestling provisioning rate for 298 house sparrows. All fixed effects are within-individual mean-centered variables unless noted as "between," in which case they are between-individual mean-centered variables. Nestling provisioning rate (trips per hour) was square root transformed in this analysis.

Variable	Effect ± SE	*F* (*DF*)	*P*-value
Bird ID	0.17 ± 0.02		
Slope Bird ID*brood size	0.03 ± 0.009		
Covariance (intercept, slope of brood size)	-0.004 ± 0.01		
Slope Bird ID*nestling age	0.008 ± 0.002		
Covariance (intercept, slope of nestling age)	0.03 ± 0.005		
Residual	0.44 ± 0.02		
Intercept	3.3 ± 0.03		
Brood size (between)	0.26 ± 0.04	48.9 (1, 361)	< 0.0001
Nestling age (between)	0.22 ± 0.04	24.8 (1, 640)	< 0.0001
Partner feeding rate (between)	-0.01 ± 0.008	1.8 (1, 347)	0.18
Date	-0.002 ± 0.0006	7.8 (1, 1507)	0.005
Brood size	0.29 ± 0.03	133 (1, 147)	< 0.0001
Nestling age	0.12 ± 0.01	138 (1, 200)	< 0.0001
Observation start time	-1.0 ± 0.3	12.4 (1, 1541)	0.0005
Partner feeding rate	0.01 ± 0.004	10.1 (1, 1631)	0.002
Brood size*nestling age	0.05 ± 0.007	41.2 (1, 1661)	< 0.0001
Partner rate*nestling age	-0.006 ± 0.001	15.2 (1, 1736)	< 0.0001

Table 8.4. Results of the linear mixed model testing for an effect of individual by environment interactions in house sparrows. Reported are the estimated variances in individual slopes and covariances between slope and intercept in nestling provisioning rate (trips per hour, square root transformed) across two environmental variables (brood size and nestling age). Significant variance or covariance components are indicated in bold (likelihood ratio test, *DF* = 2).

Environmental variable	Variance in slope	Covariance between slope and intercept	-2dLL
Brood size	**0.03 ± 0.009**	-0.004 ± 0.01	37.8
Nestling age	**0.008 ± 0.002**	**0.03 ± 0.005**	68.4

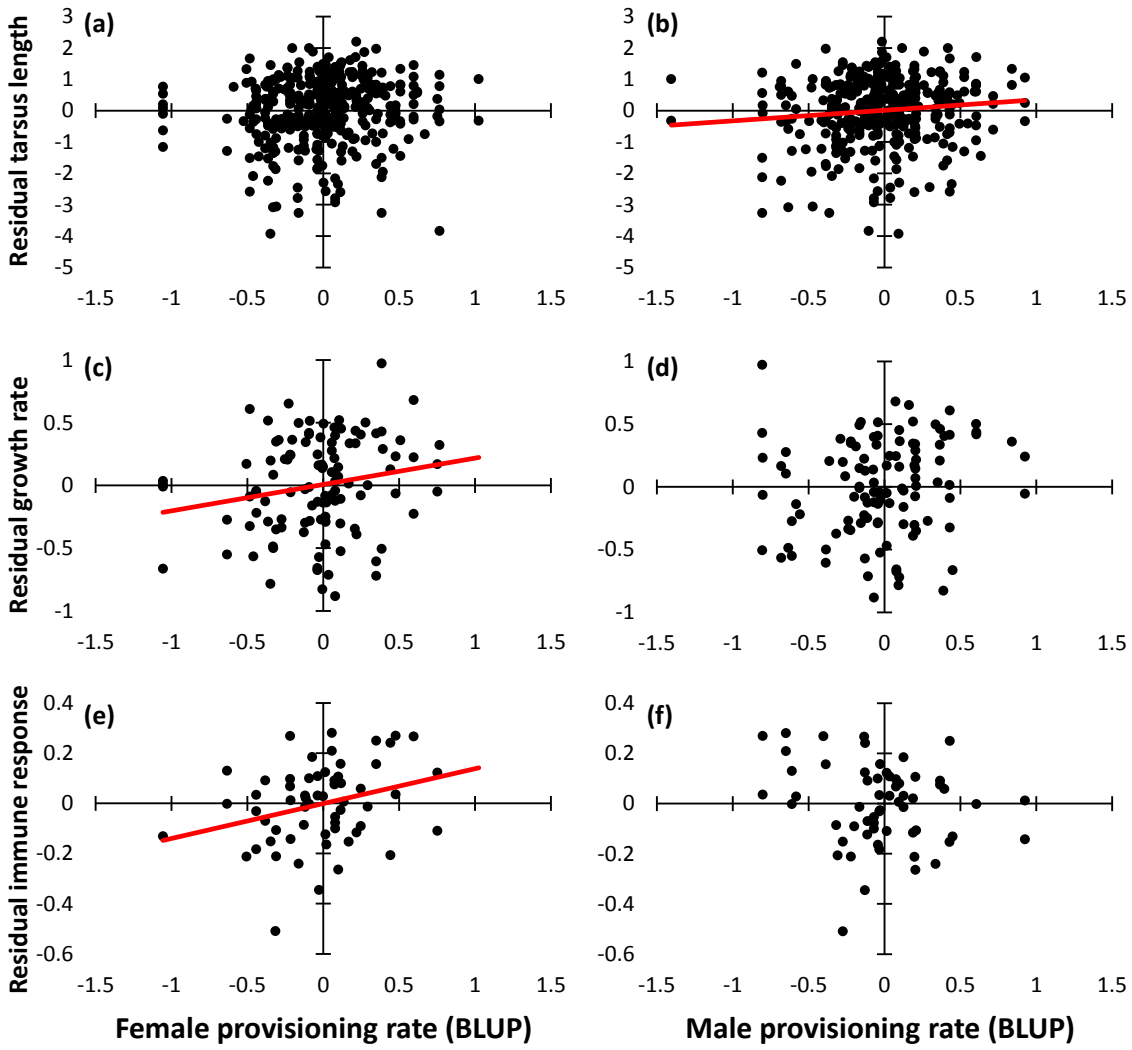

Figure 8.1. Offspring performance was influenced by components of the parents' nestling provisioning reaction norms in house sparrows. For these analyses, the reaction norm intercept of nestling provisioning rate (trips per hour) was estimated for each individual parent as a best linear unbiased prediction (BLUP) after accounting for fixed effects in Table 8.3. The BLUPs of each nests' parents (both sexes) were simultaneously added to each model of offspring performance (see text) to test if offspring performance was influenced by variation in the reaction norm intercepts of the parents, and which parental sex had the largest impact on each component of performance. Growth rate and immune response of nestlings was positively associated with female parent's provisioning rate (c and e), while nestling tarsus length was positively associated with male parent's provisioning rate (b).

CHAPTER NINE

Conclusions and future directions

Behavioral traits like parental care can exhibit considerable flexibility. Despite evidence that parents respond plastically to variable conditions, parents also provide individual-specific levels of care and respond differently to changes environmental conditions (Westneat et al. 2011). I used a behavioral reaction norm approach to study parental care behavior exhibited by house sparrows (*Passer domesticus*) in a free-living population. I found a positive covariance between nestling provisioning and nest defense; parents that provided high levels of one type of care typically provided high levels of the other type of care. While parental care theory predicts a positive relationship between measures of care should be driven by the value of the offspring, I found these two measures positively covaried even after accounting for several components of brood value. Despite finding that a large amount of variation in parental care behavior was attributed to individual parent identity, I was unable to identify the biological basis for the majority of this variation. I found no evidence that reproductive performance (nestling provisioning, clutch size, and egg size) in this population was influenced by additive genetic variation, though these traits were affected by non-additive genetic variation. Female sparrows with high levels of genetic heterozygosity laid more and larger eggs and were more likely to bring large food items to nestlings than females with low heterozygosity. There was little evidence that the conditions experienced during development or the condition of an individual as a nestling created individual differences in parental care behavior expressed later in life. I found some indication that the size of the egg an individual was hatched from created differences in parental care behavior, but this result was not particularly robust. Individual differences in offspring provisioning did not appear to be due to differences in problem-solving ability either. Although neither the ability nor speed of completing a problem-solving task predicted measures of nestling provisioning, male parents that solved the task did fledge more nestlings from their nests. Finally, I found that individual variation in parental care reaction norm intercepts had significant, positive effects on offspring growth, size, and immune response, and that offspring survival to adulthood was influenced by their condition in the nest.

Throughout my research, I have been focused on understanding how and why individuals consistently differ in the parental care behaviors they express. A large amount of recent research suggests that individuals are consistently different in a number of behavioral traits (e.g., aggression, exploration, and antipredator behaviors; reviewed in Bell et al. 2009), including parental care (Freeman-Gallant & Rothstein 1999; MacColl & Hatchwell 2003; Schwagmeyer & Mock 2003; Nakagawa et al. 2007). One important issue that has received very little attention in the rush to identify all these repeatable behaviors is if consistent differences between individuals (animal personality) are genuine characteristics of individuals or an artifact of our measurements (pseudo-personality; Westneat et al. 2011). Pseudo-personality is a product of individuals being measured under different, unaccounted for conditions and results in the appearance of consistent differences among individuals (Martin & Réale 2008; van de Pol & Wright 2009). This has consequences for my research on parental care behavior. I attempted to

determine if individual differences in genetic or cognitive components explained a portion of parental care personality, however, this effort may have been largely unsuccessful if the majority of between-individual variation was a product of differences in measurement conditions. In each of the analyses presented in this dissertation, I tested and accounted for many of the environmental factors that differed between individuals and are predicted to strongly influence parental care behavior (e.g., number and age of offspring, age and sex of the parent, effort of the partner, etc.; Winkler 1987; Westneat et al. 2011). Nevertheless, there may have been other factors that differed between individuals, such as the aggressiveness of neighboring males or the number of predatory birds nesting nearby, that created the parenting "personality" I was attempting to explain. It is worthwhile to note that many parent sparrows observed in this study were recorded multiple times within and between years, reducing the chance that unaccounted for, short-term environmental conditions could have created a bias. Although no study can account for all the environmental factors that can influence an organism's behavior, it is still valuable to try to identify the characteristics of an individual that produce consistent differences in behavioral traits.

A related issue is that although significant progress has been made in the empirical study of individual variation in many behaviors and our understanding of biological sources of this variation is progressing, the ultimate question of why some individuals behave differently than others still remains. Specifically, it is unclear why consistent differences exist, how they are maintained within populations, and if this variation is adaptive. Surprisingly, for a field typically driven by strong theoretical traditions, the majority of research on animal personality in the field of behavioral ecology has been primarily empirical (Réale et al. 2010). Current theoretical modeling of adaptive personality differences focus on how individual differences in an animal's morphological, physiological, or environmental state can create consistent differences between individuals (Dingemanse & Wolf 2010; Wolf & Weissing 2010). However, these models typically fail to explain how variation in an individual's state is created or maintained (Réale et al. 2010; Wolf & Weissing 2010). In my research, I found little evidence that the conditions experienced during early development (e.g., egg size, provisioning rate of parents, etc.) influenced parental care behavior expressed later in life. Although this is a logical place to search for sources of permanent differences between individuals in their condition or state, it is also possible that other experiences contribute to differences in individual condition. For example, in house sparrows, early social experience in the nest (e.g., Kinnard & Westneat 2009) or during the first year of life (e.g., dominance status in a winter flock; McGraw et al. 2003) may play a larger role in creating individual differences than the within-the-nest conditions I tested. Between-individual variation in an event like initial dominance interactions could create a feedback mechanism by which an individual's condition or state is maintained through the expression of condition-dependent behaviors (Wolf et al. 2007; Dingemanse & Wolf 2010). In house sparrows, being a subordinate in the winter flock could lead to reduced resource acquisition and other physiological costs (e.g., impaired immune response; Lindström et al. 2005; Steiger et al. 2012). The effect of these costs imposed by the social environment can modify an individual's condition and could influence current reproductive performance (e.g., Eggert et al. 2008) or future fitness expectations (e.g., Nicolaus et al. 2012). Unfortunately, almost all current models have focused on

personality in aspects of aggressiveness or boldness, and, while it is clear that consistent differences between individuals exist in many other traits (e.g., parental care), it is unclear if personality in these other traits is driven by the same mechanisms. Further conceptual advances and extensions will create empirically testable hypotheses that will help us understand why individuals differ in consistent ways and emphasize the importance of studying this level of variation.

I found that between-individual variation in parental care was influenced by variation in the environment (brood size, offspring age, etc.), and by variation in some components of the underlying characteristics of an individual. However, the majority of the consistent between-individual variation in parental care behavior remains unexplained. Additional reasons for individual variation abound, but the effect of the social environment may play a large, unexplored role in variation in parental care reaction norms. The social environment is composed of the interactions between individuals (interacting phenotypes; Moore et al. 1997), and can influence the phenotype of an individual (McGlothlin et al. 2010; Wolf & Moore 2010). Because traits expressed by other individuals in the social environment have a genetic basis, the social environment has heritable components and can modify the response to selection (Moore et al. 1997). Thus, to more completely understand the sources of individual variation in parental care one must not only assess characteristics of individual parents, but also characteristics of each individual's social environment, such as the effect of the partner's level of care and the effect of offspring solicitation. For example, parents typically increase the supply of care in response to offspring begging or solicitation (Kilner & Johnstone 1997; Wright & Leonard 2002), and, like other behavioral traits, offspring adjust the level of solicitation in response to a number of factors (e.g., brood size; Leonard et al. 2000). While most research has focused on the phenotypic responses of parents to offspring begging, offspring solicitation behavior is likely heritable and the result of coevolution between offspring begging and parental response (Kölliker et al. 2000; Agrawal et al. 2001; Lock et al. 2004). This suggests that parental care behaviors are sensitive to offspring solicitation and that the response rules between parents and offspring also have a genetic basis (Kölliker et al. 2000; Smiseth et al. 2008). A reaction norm approach can be used to decompose parental care and offspring begging into their key components (reaction norm intercepts and slopes) and test exactly which components of offspring begging influence individual-level variation in care. Modeling both the behavior of the focal parent and the behavior of the social partner or offspring as reaction norms would allow us to test how individual parental care reaction norms change and interact with reaction norms of the social environment, and test for the effect of genetic covariance between parental response reaction norms and offspring begging reaction norms (Kölliker et al. 2000; Smiseth et al. 2008).

Finally, I was unable to detect any relationship between problem-solving ability and parental care behavior in wild house sparrows. One major issue of attempting to test cognitive abilities in wild organisms is the motivation of the animals to participate in the trials. This could be a problem because, in my study, individuals that did not participate in the trials had significantly different nestling provisioning rates than participants. In this type of study, it is possible that non-participating individuals are less flexible in foraging strategy or ability, more neophobic, or simply unmotivated to participate in an environment where food is abundant. One solution to this problem is to integrate the

problem into an activity the animals are already highly motivated to perform, for example, entering the burrow or nest site to care for offspring. A recent study did this by creating a problem-solving task at the entrance of the nest box for great tit (*Parus major*) parents with nestlings near peak food demand (Cauchard et al. 2013). Though all individuals participated in this task and researchers were able to measure the solving ability of all subjects, this creates an addition problem because the results are confounded with the motivation to feed offspring. Perhaps a more important issue that must be addressed is understanding which cognitive skills are important for foraging parents or foraging ability in general in the wild. I tested the ability of individuals to complete a problem-solving task because I predicted that better problem-solvers would be better foragers (a prediction mirrored in similar studies; e.g., Cole et al. 2012; Cauchard et al. 2013). Although problem-solving may be one component of foraging ability, especially for house sparrows that live in rapidly-changing, human-modified habitats, better problem-solving skills may not be a good predictor of foraging efficiency or parenting ability. Better problem-solvers should be better at accessing novel sources of food, but the ability to associate an indicator with the presence of food (e.g., Kitaysky et al. 2003) or learn foraging techniques from conspecifics (Reader & Biro 2010) could be more important for foraging parents than problem-solving ability. Further study of the influence of different cognitive skills on foraging behavior in the wild are needed before we can understand if cognitive differences between individuals can produce consistent differences in parental care behavior.

Final conclusions

My research demonstrates that individual parents consistently differ in the amount of parental care they provide to offspring in the mean environment (reaction norm intercepts) and in their response to several environmental variables (reaction norm slopes). Although I tested for several possible biological sources of this variation, the majority of the variation remains unexplained. Consistent individual differences in the level and plasticity of parental care behavior are important because they provide the raw material on which selection can act to shape the patterns of care we observe in a population. Further study of the causes and consequences of these consistent individual differences in behavioral traits is necessary for our understanding of the maintenance and evolution of behavior.

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2006 Fish lab-stock caretaker, Georgia Southern University
2004 Assistant zookeeper, Scovill Zoo, Decatur, IL
2003 Field assistant, Illinois State University
1998-2002 Assistant zookeeper, Scovill Zoo, Decatur, IL

FELLOWSHIPS AND AWARDS

2013 Kentucky Opportunity Fellowship, University of Kentucky
2012 Association of Emeriti Faculty Endowed Fellowship, University of Kentucky
2011 Dissertation Year Fellowship, University of Kentucky
2011 Certificate for Outstanding Teaching, University of Kentucky
2010 Gertrude Flora Ribble Graduate Fellowship, University of Kentucky
2007 Gertrude Flora Ribble Research Fellowship, University of Kentucky
2003 Chemistry Teaching Assistant of the Year, Illinois Wesleyan University

RESEARCH GRANTS

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2010 University of Kentucky, Ribble Research Fund ($500)
2009 American Ornithologists' Union, Research Award ($2,125)
2009 University of Kentucky, Kuehne Research Fund ($100)
2009 University of Kentucky, Ribble Research Fund ($500)
2008 Kentucky Ornithological Society, Avian Research Fund ($1,000)
2007 Sigma Xi, Grant in Aid of Research ($1,000)
2007 University of Kentucky, Ribble Research Fund ($500)
2006 Georgia Southern University, Professional Development Fund ($700)
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PUBLICATIONS

Wetzel, D. P. & Krupa, J. J. 2013. Where are the bluebirds of the bluegrass? Eastern bluebird decline in central Kentucky. *American Midland Naturalist*, **169**, 396-406.

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