Title

Science & Education: Genetic Analysis Of Winter Social Structure And Social Traits In A Migratory Sparrow & Teaching Argumentation In Stem Education

Author

Arnberg, Nina Nowshiravani

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SCIENCE & EDUCATION: GENETIC ANALYSIS OF WINTER SOCIAL STRUCTURE AND SOCIAL TRAITS IN A MIGRATORY SPARROW & TEACHING ARGUMENTATION IN STEM EDUCATION

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Nina N. Arnberg

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The Dissertation of Nina N. Arnberg is approved:

Professor Bruce E. Lyon, Chair

Professor Grant H. Pogson

Professor Jerome M. Shaw

Tyrus Miller
Vice Provost and Dean of Graduate Studies

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Nina N. Arnberg

Stable social organization in a wide variety of organisms has been linked to kinship, which can minimize conflict due to the indirect fitness benefits from cooperating with relatives. In birds, kin selection has been mostly studied in the context of reproduction or in species that are social year round. Many birds however are migratory and the role of kinship in the winter societies of these species is virtually unexplored. A previous study detected striking social complexity and stability in wintering populations of migratory golden-crowned sparrows (*Zonotrichia atricapilla*)—individuals repeatedly form close associations with the same social partners, including across multiple winters.

In chapter one I test the possibility that kinship might be involved in these close and stable social affiliations. I examine the relationship between kinship and social structure for two of the consecutive wintering seasons from the previous study. I found no evidence that social structure was influenced by kinship—relatedness between most pairs of individuals was at most that of first cousins (and mostly far lower) and Mantel tests revealed no relationship between kinship and pairwise interaction frequency. Kinship also failed to predict social structure in more fine-grained analyses, including analyses of each sex separately (in the event that sex-

biased migration might limit kin selection to one sex) and separate analyses for each social community. The complex winter societies of golden-crowned sparrows appear to be based on cooperative benefits unrelated to kin selection. Although the complex social structure detected in wintering golden-crowned sparrows is not predicted by kinship, genetic variation may play a role in variation of winter social traits.

In chapter two, I investigate the genetic causes of variation in fitness-related traits in a winter population of golden-crowned sparrows. Individuals show great variation in morphological and behavioral traits that may play a role in winter dominance and ultimately survival. I found that individuals that were more heterozygous—based on internal relatedness measures reflecting individual genetic variation—were more socially dominant, had larger gold crown patches (which predict social dominance in some contexts) and had stronger social connections within their social networks.

Although the underlying mechanism driving the HFCs detected in this study is unknown, the detection of moderate correlations between an individual's heterozygosity level and social dominance, winter plumage, and sociality is interesting. Theses traits connect in important ways to winter social behavior, suggesting that these HFC analyses detected true relationships. It is therefore feasible that more heterozygous individuals are more socially dominant because they may be, for example, better foragers and have energy to expend on aggressive behaviors. Another reasonable explanation for the relationship between heterozygosity and social dominance could be that more socially dominant birds could be older;

heterozygosity might be linked to longevity. Although a physiological mechanism is unknown in sparrows, it is possible that plumage may reflect the general quality (e.g., better immune function) of the individual. As with social dominance, “core” individuals of a community (i.e., individuals that are more likely to be found flocking with other birds of their community) may also be older or higher quality individuals. “Core” individuals interact and have contact with others in the community and may therefore be at higher risk of encountering disease, parasite, and pathogens. If this measure of heterozygosity reflects immune function heterozygosity, then more heterozygous individuals may be more likely to be “core” individuals.

The recent focus on preparing students to pursue careers in science, technology, engineering, and mathematics (STEM) fields have resulted in educational researchers investigating how best to support this goal. Government and private funding has been directed to STEM internship programs (particularly at the undergraduate level). These internship programs are designed to provide workplace opportunities as well as provide support from the program to acquire skills needed by students to transition from school to the workforce. Most of this support has been developed (by internship programs and researchers) in the sciences. Engineers are in high demand and universities and internship programs are tasked with preparing more engineering students to be professional engineers. Our development of the Solution Articulation Framework (SAF), supports learners’ (interns) acquisition of argumentation skills in engineering; engaging in argumentative practices promotes content knowledge and communication skills in many disciplines but has not been explored deeply in the engineering education literature. We focused our attention on improving learners' engineering argumentation practices with a particular emphasis on articulation of engineering solutions.

In chapter three, I identify a critical component (functional requirements) to effectively articulating a proposed engineering solution and offer an operational definition along with suggestions for implementing these ideas in engineering education. My findings were immediately used to revise our SAF and warrants further research on other components of the SAF. The research in this chapter advances the emerging research field in engineering education by highlighting the importance and difficulties associated with teaching argumentation skills in engineering.

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Introduction

Group living is ubiquitous in animals despite increased competition and exposure to disease (Krause & Ruxton 2002). Individuals may benefit from living in a group, especially when resources are limited. Animal social groups may, for example, huddle together for warmth, cooperate in herding prey, learn about good feeding sites, gain access to mates, or lower individual predation risk (Inman & Krebs 1987). Social groups would be expected in associations where the benefits of grouping outweigh the costs. Since the fitness effects of grouping individuals are context dependent, sociality can change in both space and time (Lehmann et al. 2007; Sueur et al. 2011). A social system can be described in terms of the degree to which it expresses fission-fusion dynamics, the extent of variation in spatial cohesion and individual membership in a group over time (Aureli et al. 2008).

Fission-fusion dynamics have been reported in a variety of species (e.g., guppies *Poecilia reticlata* in Croft et al. 2003, bats *Myotis bechsteinii* in Kerth et al. 2006, and birds *Parus major* in Aplin et al. 2012). Recently, Silk et al. (2014) highlight the importance of using avian systems to further understand social systems and how fission-fusion dynamics may have important implications for the evolution of social behavior, information transfer, and the spread of diseases. In birds, sociality in the breeding context has been shown to range from simple pairs to cooperative breeding groups (Emlen 1982). However, less attention has been paid to understanding social behavior in winter flocks of birds, a frequently observed social organization. Although social interactions are widespread during migration and overwintering even in solitarily breeding species (Ekman 1989), the few studies on the dynamics and structure of winter sociality have mainly focused on year-round resident populations (e.g., western bluebirds *(Sialia mexicana)* in Kraaijeveld & Dickinson 2001; house sparrows *(Passer domesticus)* in Liker et al. 2009; vinous-throated parrotbill *(Paradoxornis webbianus)* in Lee et al. 2010). Migratory birds may show less stability across-years since individuals in a single wintering area may be comprised of individuals from different breeding areas (Ryder et al. 2011; Seavy et al. 2012). Thus, long-term social bonds between individuals would have to withstand breaks in contact between winter seasons.

Shizuka et al. (2014) recently discovered a surprising level of social complexity of a wintering population of a migratory songbird, the golden-crowned sparrow (*Zonotrichia atricapilla*). Although individual membership in short-term winter flocks is dynamic, the use of social network analysis clearly identified three larger social communities. These communities were also found to show unexpected stability across years. This complex social structure could be influenced by social preference of individuals or preference for spatial use (Pinter-Wollman et al. 2014). It is possible that individuals prefer to forage in similar areas independent of benefits of being social (Ramos-Fernandez et al. 2006). Instead, individuals that consistently seek out other individuals may drive the detected social structure. Preference for individuals may be based on previous familiarity or kinship.

IN CHAPTER ONE, I explore the relationship between winter social structure formation in golden-crowned sparrows and kinship. One of the most important, and well documented, genetic drivers that influences associative behavior in animals is the genetic relatedness (kinship) of group members (Hatchwell 2009); individuals that join groups based on kinship may increase their inclusive fitness. However, studies in birds have been mainly conducted in reproductive settings or on organisms that are social year round despite the fact that many species are migratory and/or non-cooperative breeders. Since social interactions increase conflict between individual actors, the presence of related individuals may explain the formation of social groups during the nonbreeding season. Kin selection (Hamilton 1964; Wilson 1975) may increase benefits and decrease costs to individuals who join winter social communities by increasing the inclusive fitness of related group members. Based on kinship detected using 14 microsatellite loci, I found no evidence that the formation of winter social communities is due to underlying kin structure. The data also suggests that social communities are not simply an assortment of individuals into groups originating from the same breeding populations. These findings, along with a few recent studies on wintering flocks of birds, differ significantly from data on cooperative breeding birds, which may form kin-based social communities in the winter. Therefore, social cohesion of wintering flocks may be a result of other factors outside of genetic relatedness.

This wintering population of golden-crowned sparrows has been the focus of a long-term study conducted by Bruce Lyon and colleagues. Over the course of ten years, detailed observational data shows that individuals show tremendous variation in winter behavior and morphology, characteristics that may be important to winter survival. Individuals vary in body size and in the size of two plumage patches, central gold plumage patch flanked by two black stripes. Both plumage patches are more variable in winter than during the breeding season (B.E. Lyon, D. Shizuka & A.S. Chaine, unpublished data). Chaine et al. (2011) found that these plumage traits were correlated with social dominance in encounters between unfamiliar individuals. In addition to morphological variation, there is great variance—independent of sex—in several behavioral traits such as social dominance (Chaine et al 2011), sociality (Shizuka et al. 2014), and winter singing behavior (Lyon et al., unpublished data).

Are these traits, which may be important to overwinter survival, underpinned by genetic variation? One approach taken to understand the genetic causes of variation in fitness in populations is to explore relationships between within-individual variations at genetic marker loci (some measure of heterozygosity) and variation in fitness (or traits that are potentially important to fitness). Collectively, studies that have compared individual genetic diversity with fitness-related traits (measures of life-history, morphological, physiological, and behavioral traits) are referred to as heterozygosity-fitness correlations (HFC) and have been studied and debated for over three decades (reviewed in Coltman & Slate 2003; Kempenaers 2007; Chapman et al. 2009; Szulkin et al. 2010).

Three hypotheses have been proposed to account for HFCs (reviewed in Hansson & Westerberg 2002), all of which rely on the assumption that genetic diversity at a suite of marker loci reflects genetic diversity at loci that affect variation in traits. First, the ‘direct effect hypothesis’ predicts that the loci used in the HFC exhibit heterozygote advantage. This hypothesis potentially explains HFCs detected in allozyme studies (Mitton 1997), but are less informative in noncoding and neutral markers, such as microsatellites (Pogson & Zouros 1994; Thelen & Allendorf 2001). Second, the ‘general effect’ hypothesis predicts that individual heterozygosity reflects genome-wide heterozygosity and such only correlates with fitness if it also correlates with individual inbreeding level. For example, inbred individuals with lower fitness than their ancestors exhibit inbreeding depression; outbred individuals with higher fitness than their ancestors exhibit heterosis (reviewed in Charlesworth & Willis 2009). Both inbreeding and outbreeding simultaneously modify heterozygosity across all loci of a genome; therefore if heterozygosity is correlated among loci markers, then multi-locus heterozygosity may be an informative measure of inbreeding (Balloux et al. 2004). Third, the ‘local effect’ hypothesis predicts that HFCs could be the result of some of the molecular loci used in the study being in physical linkage with genes that influence fitness (Slate et al. 2004).

IN CHAPTER TWO, I explore the relationship between individual genetic variation—heterozygosity calculated using data from 14 microsatellite loci—with variation in traits thought to affect fitness, including both morphological traits (size and color of gold and black crown patches, body size) and behavioral traits (social dominance, singing, and three measures of individual sociality). I included the same individuals that were used in chapter one. I found that multi-locus heterozygosity was significantly correlated with two behavioral traits (social dominance, sociality) and one morphological trait (gold patch size). Since levels of heterozygosity among markers within individuals were not correlated, the findings do not provide evidence for the role of genome-wide heterozygosity. However, I cannot rule out that inbreeding depression did not cause the detected HFCs. Also, no direct evidence for local effects driving detected HFC patterns was found using the procedure outlined in Szulkin et al. (2010) and no homology was found between the markers used in this study and published avian expressed-sequence tags. Although the underlying mechanism driving the HFCs detected in this study is unknown, other examples suggest there is a true pattern that warrants further study.

Since the context of my last chapter deviates from the previous chapters, I describe the experiences that motivated the research. During my tenure as a graduate student, I worked with three programs that focused on preparing undergraduate students for careers in science, technology, engineering, and mathematics (STEM) fields. I held positions as a graduate student research for the University of California's **L**eadership **E**xcellence through **A**dvanced **D**egree**s** (UC LEADS), a fellow for the Institute for Scientist & Engineer Educators (ISEE), and a communication instructor for the Akamai Internship Program (AIP). In each of these roles, I was tasked with supporting students in STEM communication.

Communication itself is a valuable skill for students to develop and is intertwined with thinking and understanding. Early philosophers—Plato, Socrates, and Aristotle—regarded the construction of reasoned arguments as the core of thinking. Toulmin (1958) published a seminal book on the advantages of regarding thinking as argument and developed essential components of practical arguments including claim, data, and warrant (i.e., a statement justifying movement from data to the claim). Based on Toulmin's original framework, science education researchers have developed a claims-evidence-reasoning model of scientific argumentation (McNeill et al. 2006; McNeill & Krajcik 2008; Sampson & Clark 2008). According to this model, scientific argumentation is characterized by attempts to establish warrants for interpretive claims through reasoning about evidence. Berland & Reiser (2009) suggest that scientific explanation and argumentation inform one another, "explanations of scientific phenomena can provide a product around which the argumentation can occur," and "argumentation creates a context in which robust explanations…are valued" (p. 28). Staff and education researchers at ISEE drew from this research as we developed a conceptual framework and educational tool to support students in understanding and developing scientific arguments and articulating these understandings. While this framework and tool was appropriate in the context of science, it was less useful for students whose projects centered on designing an engineering solution.

Scientists study the natural world. They ask questions about the world around us—what is out there, how do things work, and what rules can be deduced to explain the patterns we see. In contrast, engineering is the process of designing the human-made world. Engineers design the world to satisfy humanities needs and wants (Katehi et al. 2009). Although science and engineering inform one another, there are inherent differences between the scientific method and an engineering design. By understanding these differences, we can begin to identify key components of engineering argumentation while drawing from research conducted on scientific argumentation (for review see Sampson & Clark 2008). Although the field of scientific argumentation is well supported in the education literature, research in engineering argumentation is sparse and is mostly found in non-educational contexts (e.g., Stouffer et al. 2004; Robertson & Robertson 2012).

IN CHAPTER THREE, I focused my attention on improving learners engineering argumentation practices with a particular focus on their articulation of engineering solutions. This work builds on the conceptual framework for engineering argumentation, developed concurrently by my colleagues and me (J. Shaw, L. Hunter, & N. Arnberg *in prep*). A review of the current literature (e.g., Weiringa et al. 2006) and interviews we conducted with engineering professionals highlight the importance of engineers' ability to persuasively argue for a particular engineering solution. Our previous work focused on the development of a solution articulation framework [SAF], a tool used throughout the internship program's communication curriculum. The goals of this research were to clarify our preliminary conceptualization of engineering argumentation by (1) operationally defining a key component in the articulation of engineering solutions: <u>requirements</u> and (2) providing engineering educators and mentors with suggestions for practical implications to improve learners articulation of engineering solutions. A qualitative analysis of 12 self-reported AIP intern responses using the SAF and 12 final presentations resulted in the identification of three key challenges interns faced when identifying engineering requirements—a key component of the SAF. Informed by these findings, I identified three key challenges faced by interns when articulating project requirements. Interns identified constraints as requirements, identified non-functional requirements as functional requirements, and did not state functional requirements in a verifiable manner. To address these challenges, I propose an operational definition of requirements in engineering that has three components: functional requirements (what a solution must do), non-functional requirements (qualities that a solution must have), and constraints (limitations on possible solutions). Of the three, functional requirements are most relevant because a viable solution *must* meet all functional requirements. Furthermore, functional requirements must (1) be relevant to the problem/need (2) focus on what the solution must do or accomplish (actions a solution must take) and (3) be verifiable, stated in a manner where action is completed or not completed. I conclude with ideas for implications of our findings in engineering education.

References

Aplin LM, Farine DR, Morand-Ferron J, & Sheldon BC (2012) Social networks predict patch discovery in a wild population of songbirds. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1745), 4199-4205.

Aureli F, Schaffner CM, Boesch C, Bearder SK, Call J, Chapman CA, Van Schaik CP (2008) Fission-Fusion Dynamics. *Current Anthropology*, **49**, 627-654.

Balloux F, Amos W, & Coulson T (2004) Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology*, *13*(10), 3021-3031.

Berland LK, & Reiser BJ (2009). Making sense of argumentation and explanation. *Science Education*, *93*(1), 26-55.

Chaine AS, Tjernell KA, Shizuka D, & Lyon BE (2011) Sparrows use multiple status signals in winter social flocks. *Animal Behaviour*, *81*(2), 447-453.

Chapman JR, Nakagawa S, Coltman DW, Slate J, & Sheldon, BC (2009) A quantitative review of heterozygosity–fitness correlations in animal populations. *Molecular Ecology*, *18*(13), 2746-2765.

Charlesworth D & Willis JH (2009) The genetics of inbreeding depression. *Nature Reviews Genetics*, *10*(11), 783-796.

Coltman DW & Slate J (2003) Microsatellite measures of inbreeding: A meta-analysis. *Evolution*, *57*(5), 971-983.

Croft DP, Arrowsmith BJ, Bielby J, Skinner K, White E, Couzin ID & Krause J (2003) Mechanisms underlying shoal composition in the Trinidadian guppy, Poecilia reticulata. *Oikos*, *100*(3), 429-438.

Ekman J (1989) Ecology of non-breeding social systems of Parus. *The Wilson Bulletin*, 263-288.

Emlen ST (1982) The evolution of helping. II. The role of behavioral conflict. *American Naturalist*, 40-53.

Hamilton, WD (1964) The genetical evolution of social behaviour. I. *Journal of theoretical biology*, **7**, 1-16.

Hansson B & Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology*, *11*(12), 2467-2474.

Hatchwell, BJ (2009) The evolution of cooperative breeding in birds: kinship, dispersal and life history. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 3217-3227.

Inman AJ & Krebs J (1987) Predation and group living. *Trends in Ecology & Evolution*, *2*(2), 31-32.

Katehi L, Pearson G, and Feder M (2009) Committee on K–12. Engineering Education Engineering in K-12 Education: Understanding the status and improving the prospects. http://www.nap.edu/catalog/12635.html

Kempenaers B (2007) Mate choice and genetic quality: a review of the heterozygosity theory. *Advances in the Study of Behavior*, *37*, 189-278.

Kerth G, Ebert C & Schmidtke C (2006) Group decision making in fission–fusion societies: evidence from two-field experiments in Bechstein's bats. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1602), 2785-2790.

Kraaijeveld K, Dickinson JL (2001) Family-based winter territoriality in western bluebirds, Sialia mexicana: the structure and dynamics of winter groups. *Animal Behaviour*, **61**, 109-117.

Krause J, Ruxton GD (2002) *Living in groups*. Oxford University Press.

Lee JW, Simeoni M, Burke T, Hatchwell BJ (2010) The consequences of winter flock demography for genetic structure and inbreeding risk in vinous-throated parrotbills, Paradoxornis webbianus. *Heredity*, **104**, 472-481.

Lehmann J, Korstjens AH & Dunbar RIM (2007) Fission–fusion social systems as a strategy for coping with ecological constraints: a primate case. *Evolutionary Ecology*, *21*(5), 613-634.

Liker A, Bókony V, Kulcsár A, Tóth Z, Szabó K, Kaholek B, Pénzes Z (2009) Genetic relatedness in wintering groups of house sparrows (Passer domesticus). *Molecular ecology*, **18**, 4696-4706.

McNeill, KL, Lizotte DJ, Krajcik J, & Marx RW (2006) Supporting students' construction of scientific explanations by fading scaffolds in instructional materials. *The Journal of the Learning Sciences*, *15*(2), 153-191.

McNeill, KL & Krajcik J (2008) Scientific explanations: Characterizing and evaluating the effects of teachers' instructional practices on student learning. *Journal of Research in Science Teaching*, *45*(1), 53-78.

Mitton JB (1997) *Selection in Natural Populations*, Oxford University Press.

Pinter-Wollman N, Hobson EA, Smith JE, Edelman AJ, Shizuka D, de Silva, S ... & McDonald DB (2014) Response to comments on the dynamics of network dynamics.

Pogson GH & Zouros E (1994) Allozyme and RFLP heterozygosities as correlates of growth rate in the scallop Placopecten magellanicus: a test of the associative overdominance hypothesis. *Genetics*, *137*(1), 221-231.

Ramos-Fernández G, Boyer D & Gómez VP (2006) A complex social structure with fission–fusion properties can emerge from a simple foraging model. *Behavioral ecology and sociobiology*, *60*(4), 536-549.

Robertson S & Robertson J (2012) *Mastering the requirements process: getting requirements right*. Addison-Wesley.

Ryder TB, Fox JW, Marra PP (2011) Estimating migratory connectivity of Gray Catbirds (Dumetella carolinensis) using geolocator and mark-recapture data. *The Auk*, **128**, 448-453.

Sampson V & Clark DB (2008) Assessment of the ways students generate arguments in science education: Current perspectives and recommendations for future directions. *Science Education*, *92*(3), 447-472.

Seavy NE, Humple DL, Cormier RL, Gardali T (2012) Establishing the breeding provenance of a temperate-wintering North American passerine, the Golden-crowned Sparrow, using light-level geolocation. *PloS one*, **7**, e34886.

Shizuka D, Chaine AS, Anderson J, Johnson O, Laursen IM, Lyon BE (2014). Across□year social stability shapes network structure in wintering migrant sparrows. *Ecology Letters*.

Silk MJ, Croft DP, Tregenza T & Bearhop S (2014) The importance of fission–fusion social group dynamics in birds. *Ibis*, *156*(4), 701-715.

Slate J, David P, Dodds KG, Veenvliet BA, Glass BC, Broad TE & McEwan JC (2004) Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity*, *93*(3), 255-265.

Stouffer WB, Russell JS & Oliva MG (2004) Making the strange familiar: Creativity and the future of engineering education. In *Proceedings of the 2004 American Society for Engineering Education Annual Conference & Exposition* (pp. 20-23).

Sueur C, King AJ, Conradt L, Kerth G, Lusseau D, Mettke□Hofmann C ... & Aureli F (2011) Collective decision□making and fission–fusion dynamics: a conceptual framework. *Oikos*, *120*(11), 1608-1617.

Szulkin M, Bierne N & David P (2010) Heterozygosity-Fitness Correlations: A Time for Reappraisal. *Evolution*, *64*(5), 1202-1217.

Thelen GC & Allendorf FW (2001) Heterozygosity-Fitness Correlations in Rainbow Trout: Effects of Allozyme Loci or Associative Overdominance? *Evolution*, 1180-1187.

Toulmin S (1958) The Uses of Argument. Cambridge University Press. *Cambridge, UK*.

Wieringa R, Maiden N, Mead N & Rolland C (2006) Requirements engineering paper classification and evaluation criteria: a proposal and a discussion. *Requirements Engineering*, *11*(1), 102-107.

Wilson DS (1975) A theory of group selection. *Proceedings of the national academy of sciences*, **72**, 143-146.

CHAPTER 1

Complex social structure in wintering golden-crowned sparrows is not predicted by kinship

Introduction

Group living is common in a wide diversity of organisms. Understanding why social groups arise in populations remains an important focus of social evolution research. Kinship is often an important correlate of complex societies (Hamilton 1964, Alexander 1974, Wilson 1975). For example, the stable complex cooperative societies of many birds, mammals, invertebrates, and microbes often involve family groups or extended families (Bourke and Franks 1995, Solomon and French 1996, Koenig and Dickinson 2004, Clutton-Brock 2006, Hatchwell 2010, Strassmann et al. 2011, Kuzdzal-Fick et al. 2011, Kamel and Grosberg 2013). The observation that many of these societies combine high levels of cooperation and even altruism with associations between close kin has made these societies model systems for understanding the relevance of kin-selection to social evolution. However, kin structured populations are also common in non-cooperative animals (reviewed in Hatchwell 2010), and could have much more general effects on social behaviors beyond cooperation.

Social organization and kinship have both been particularly well studied in birds, but the vast majority of this research has focused either on reproduction or societies that remain together year round—typically cooperative breeding species (Emlen 1982, Koenig and Dickinson 2004, Ekman 2006). Many species of birds however are migratory, and for these species we have very rudimentary understanding of social behavior on migration or on the wintering grounds. For example, the most basic pattern of whether individuals are solitary or live in groups (flocks) is well understood, but we know little about the composition or stability of these groups. The conceptual framework of fission-fusion dynamics (Aureli et al. 2008) provides a useful perspective for thinking about the range of possible social patterns that flocking migratory birds could have—from completely dynamic and unstable groupings to social groups with considerable stability. In general fission-fusion societies in non-avian taxa are beginning to reveal that complex and stable societies may be more widespread than previously realized. The societies that are particularly stable and close knit are also the ones where kinship may be most likely to be involved (e.g. Kraaijeveld and Dickinson 2001, Krutzen 2003, Archie et al. 2006, Ostrowski et al. 2008, Portelli et al. 2009, Rollins et al. 2012; see also Silk et al. 2014).

Here we explore the relationship between social complexity and kinship in wintering communities of a migratory sparrow. Our recent study of winter social organization in the golden-crowned sparrow (*Zonotrichia atricapilla*) revealed unsuspected social complexity and associations between individuals that persist across several years (Shizuka et al. 2014). Golden-crowned sparrows forage in flocks on their wintering grounds, and a key issue was understanding the fission-fusion dynamics of these flocks—is flock membership stable or dynamic, and a what temporal and spatial scales? Using social network analysis, we found that although individual membership in short-term foraging flocks is dynamic, flock members come from larger, more stable social communities (Shizuka et al. 2014). The three communities we detected each year had spatially overlapping home ranges but also had relatively discrete and non-overlapping core areas. The communities were also very stable across years in both membership and home range location (Shizuka et al. 2014). Social affiliations play some role in the persistence of these communities across years—returning sparrows prefer to associate with the same individuals with whom they had associated in the previous years, and analyses show that this pattern is explained more by social preference than shared use of space (Shizuka et al. 2014). This suggests that some individual sparrows form close associations with others that could last their entire lifetimes, at least during the winter portions of their lives. The question we address here is whether kinship plays any role in these tight social affiliations in particular, and community structure more generally.

Determining patterns of kinship in social groups of migratory species is somewhat more challenging than for breeding groups because we lack information on pedigrees and must use indirect approaches to estimate pair-wise relatedness patterns. We used multi-locus genotype data (microsatellite DNA) to estimate patterns of relatedness and quantify the genetic structure of our study population. We used the same individuals used in our social network study (Shizuka et al. 2014), which allows us to determine whether degree of relatedness correlates with social network metrics. We investigated the relationship between kinship and social structure in two different ways. First, we determined whether pairwise relatedness estimates correlate with the strength of the social affiliation between pairs of individuals. Second, we investigated whether relatedness estimates were higher within the social communities we detected than across social communities.

Materials & Methods

Study site and subjects

We studied golden-crowned sparrow flocks at the University of California, Santa Cruz Arboretum. The approximate 7 hectare study area includes a mixture of shrubs, trees and open grassy areas where the birds forage in flocks. We conducted flock observations from October to February during two consecutive non-breeding seasons: fall 2009 through spring 2010 (hereafter 2009) and fall 2010 through spring 2011 (hereafter 2010.) To determine the identities of birds in flocks, we caught birds using Potter style traps baited with millet at regular feeding stations and gave each bird a unique color-band combination. Each captured bird was given a unique combination of colored vinyl leg bands (Hill 1992) and we also collected a blood sample to be used for genetic determination of gender and microsatellite analysis.

Behavior data to determine social structure

We used flock censuses to determine the social structure of the population (further details presented in Shizuka et al 2014). In our study, flocks are short-lived associations of individuals and flock membership can change dramatically over short periods of time. We defined a flock as a group of birds within an approximately 5-meter radius. To minimize the potential effects of concentrated resources on social structure, we include flocks censused on days when we did not seed feeders, or seen away from feeders. For each flock, we recorded as many color-banded individual as could be accurately identified. To ensure that our flocks represent independent observations, we excluded flocks that were seen within 20 minutes of each other and contained two or more of the same individuals.

Using these flock censuses, we built a social network of flock co-membership. Nodes represent individual birds and edges represent the simple ratio association index (Cairns & Schwager 1987). We then applied a network-based community detection process to identify clusters of individuals that are more strongly associated with each other than with others (Newman & Girvan 2004). Specifically, we used the algorithm proposed by Clauset et al. (2004) to find groupings that maximize the proportion of association indices that occur within clusters. . The result is an assignment of individuals into social communities within a network as well as a measure of discreteness of the social clusters (termed *modularity*). Our previous work showed that this population contained three main social communities in both 2009 and 2010 seasons, and these assignments were robust to sampling error (Shizuka et al. 2014).

Genetic data

We collected small blood samples (~20μl) from the brachial vein of each individual and stored it in Queen's lysis buffer (Dawson *et al.* 1998). Samples were then frozen (-20C). DNA extraction was performed with either standard phenol-chloroform procedure, or Qiagen DNeasy Blood & Tissue Kit (QIAGEN). The phenol-chloroform procedure produced higher DNA yields.

Sexing protocol

We used the primers P2 and P8 for sexing individuals (Griffiths *et al.* 1998). PCR amplification was carried out in a total volume of 10 μL. The final reaction conditions were as follows: 0.5 μL of 25 mM MgCl2, 1.5 μL of 2mM dNTPs, 2 μL of 2.5 μM of each primer, 200 ng template DNA, 1 μL of 10X buffer (Qiagen), 1 μL of 1 mg/mL BSA, 0.12 μL of Taq DNA polymerase (Qiagen), 1.38 μL ddH20. PCR was performed on a thermal cycler. An initial denaturing step at 92°C for 2 min followed by 26 cycles of 92°C for 30 sec, 54°C for 45 sec, 72°C for 45 sec. Final extension was 72°C for 5 min. Bands were scored by hand, two bands present for a female and one band present for a male.

Microsatellite protocol

Since there were no published microsatellite primers available for golden-crowned sparrows, we screened potential primers from other closely related species (Primmer et al 1996; Stenzler et al 2004; Poesel et al 2009). Thirty primers were initially screened of which 24 were optimized for PCR reactions. The final 14 primers that were selected for genetic analysis (see below for explanation) were initially optimized for white-crowned sparrows *Zonotrichia leucophrys* (13 primers from Poesel et al 2009) and golden-winged warblers *Vermivora chrysoptera* (1 primer from Stenzler et al 2004.)

PCR reactions were carried out for all individuals. We set up 25-μL PCR reactions containing 10 ng template DNA, 10x *Ex Taq* buffer (Mg^{2+} free), 0.2 mM each dNTP, between 1.0 and 3.5 mM $MgCl_2$, 0.05 U *TaKaRa Ex Taq* polymerase

(Takara Bio Inc.), 0.015 mM forward primer tailed with M13 (Boutin-Ganache et al 2001), 0.2 mM reverse primer, and 0.2 mM M13 labeled with fluorescent dye (Table 1). We used the Dye Set DS-33 (Applied Biosystems Inc.) to fluorescently label primers with 6-FAM, VIC, NED, and PET.

PCR reactions were run under the following conditions: initial denaturation at 94°C for 3 min, then 33 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 40 s (65°C for 40s for GCSPR19 only), extension at 72°C for 40 s, concluding with a final extension at 72°C for 5 min. We verified PCR amplification by visualizing PCR products on 3% agarose gels stained after casting with Gel Red Nucleic Acid Gel Stain, 3X in water (Biotium).

To identify alleles, all amplified PCR products were suspended in formamide and analyzed on an ABI Prism 3100 Genetic Analyzer (Applied Biosytstems Inc.) at the University of California, Berkeley DNA Sequencing Facility using LIZ-labeled 600 internal standard (Applied Biosystems Inc.). The data were analyzed visually using Peak Scanner Software v1.0 (free, Applied Biosystems Inc.). All samples were retyped, 98% were confirmed correct. Scores that differed by 1bp or less were considered to be equal for all tetranucleotide repeats.

Since our study solely relies on microsatellite data to quantify both kinship and population structure measures, we were diligent in our selection of appropriate loci by utilizing a microsatellite screening protocol (Selkoe & Toonen 2006). Loci that were included in our study 1) were accurately scored genotypes (we re-scored a subset of genotypes and calculated an error rate of less than 1%) 2) were void of null alleles and 3) adhered to linkage equilibrium. Although we sought to maximize the number of loci used in our study, we were aware that loci that did not pass this screening process would decrease the accuracy of our genetic estimates. We compared observed and expected heterozygosity and deviations from Hardy-Weinberg (HW) expectations calculated using ML-Relate (Kalinowski et al. 2006) (Table 2). Although GCSPR17 deviated from HW equilibrium, it did not after Bonferroni correction of alpha levels for multiple comparisons, and was included in subsequent analyses. Linkages between the microsatellite loci were investigated using GENEPOP version 4.0.10 (Raymond and Rousset 1995; Rousset 2008). To test for the presence of null alleles, we used Microchecker (Van Oosterhout et al. 2004).

Relatedness estimation

Relatedness coefficients were calculated using the final 14 microsatellite loci. These genotypes were used to generate a pairwise coefficient of relatedness between all pairs of individuals using ML Relate software (Kalinowski et al. 2006) to calculate maximum likelihood estimates. This method is considered to be more accurate than other estimators (Milligan 2003). Pairwise coefficients of relatedness between individuals were calculated separately for 2009 and 2010. Estimation of pairwise genetic relatedness was done by entering the genotypes of all individuals in the program as if the individuals comprised a single population, as no prior reference data were available about this population.

Statistical Analysis

Correlating social associations with genetic relatedness

Our goal was to determine if relatedness and degree of social association were correlated. Our sample included all 31 individuals in the network for 2009, and 40 of 43 individuals in the network for 2010 (3 individuals excluded due to lack of DNA sample). . We conducted three separate analyses to ask different question. First, we tested for correlations between pairwise relatedness coefficients and association indices for all dyads. The second set of analyses used data for sex-specific dyads (females only and then males only) to test for the possibility that relatedness might affect associations in only one of the sexes. Third, we tested whether kinship influenced associations only within social communities, by comparing relatedness and association indices within each of the six communities as determined by social network analysis (3 communities in 2009; 3 communities in 2010). All correlations of pairwise matrices were tested by the Mantel randomization tests with 10,000 permutations using the Ecodist package (Goslee and Urban 2007) using R statistical computing software.

Winter social structure in relation to breeding population structure

One alternative mechanism could influence the genetic structure of winter populations and lead to the appearance of kinship effects. Specifically, individuals within communities might share alleles not due to relatedness but due to shared breeding population with distinctive allele frequencies. This type of connectivity between breeding populations and social communities within wintering grounds could lead to the appearance of kinship. The theoretical false signature of kinship we suggest here is related to the Wahlund effect, whereby population substructure can lead to a reduction in heterozygosity relative to levels expected for a panmictic population (Sinnock 1975). If birds from distinct breeding populations form social communities within our winter population, then this could bias our estimates of kinship. We currently do not have data on what breeding population(s) our wintering study population originates from. However, a study of golden-crowned sparrows in a nearby population found that 4 birds from one winter population all bred in separate breeding populations along the Pacific coast of Alaska (Seavy et al. 2012). In order to rule out the Wahlund effect (where results from combining populations with different allelic frequencies in a single sample could produce false kinship results) we calculated F_{st} values and noted if there was a deficiency of heterozygotes. Pairwise F_{st} values within and between social communities and across all loci were calculated separately for years 2009 and 2010 using the online version of GENEPOP v. 4.2. (Raymond & Rousset 1995). A null distribution of F_{st} values was generated through 1,000 permutations of the haplotypes between populations and the *p* value represents the proportion of permutations leading to an F_{st} value larger than or equal to the observed value.

We then used Structure, a Bayesian, model-based algorithm used for clustering genetic data, to test whether there was population structure within our wintering population (Pritchard et al 2000). Given the number of clusters (*K*) and assuming Hardy–Weinberg and linkage equilibrium within clusters, Structure estimates allele frequencies in each cluster and population memberships for every individual.

Initial structure analysis was run using the admixture model with correlated allele frequencies with no location prior information (all individuals run for 2009 and 2010 separately). In order to help identify more subtle population structure, we then reran the analysis with location prior information (Hubisz et al 2009). Location prior was assigned to individuals based on their membership in one of the three social communities. The number of clusters (K) was determined by plotting the mean of estimated ln probability of data, ln [P(X|K)], of each model as a function of K. To confirm these results we calculated the *ad hoc* statistic delta K suggested by Evanno et al. (2005) and visualized the results using Structure Harvester v.0.6.93 (Earl 2012).

Results

Social association and genetic relatedness

Most dyads show low relatedness based on our estimates of pairwise relatedness values (Figure 1). Nonetheless, a few dyads showed relatedness estimates consistent with first or second-order kinship (Figure 1), suggesting the potential for some kin associations. In 2009, ~6% of relatedness values were $r = 0.25$ or higher while ~85% of values were less than $r = 0.10$. In 2010, ~7% of relatedness values were $r = 0.25$ or higher while ~82% of values were less than $r = 0.10$.

We found no significant correlation between social association and genetic relatedness at the population level, within sexes or within social communities (summarized in Table 3).

Social association and population structure

Across the 14 microsatellite loci, we detected very little genetic structure across social communities (F_{st} range 0 - 0.06 in 2009, 0 - 0.01 in 2010; Table 4). Pairwise F_{st} values between each of the three social groups in both 2009 and 2010 were each 0. The detection of non-positive F_{st} values and a lack of deficiency of heterozygotes suggest that our study does not sample across subpopulations and any detection of kinship would be due to relatedness due to common ancestry.

We sought to determine if there were subsets of the population that comprised distinct clusters based on their genotypes. The results of the analysis with Structure provided no evidence for a difference in allele frequencies between any numbers of subpopulations, the data was first run without location prior and then with location prior (Figure 2). Calculating delta K values for each value of K confirmed this finding. Although the delta K value is highest at K=3 this does not provide evidence for K > 1 since small values in delta K likely reflect small inter-run differences and not population structure. Thus, the data suggest that there is no evidence for population structure in 2009 or 2010 in our overwintering population.

We then ran the Structure analysis using location prior for each social community that was assigned using the social network analyses (Shizuka et al. 2014). The location prior was included in order to detect more subtle population structure (i.e., whether social structure reflected genetically distinct breeding populations.)

Discussion

Our previous work demonstrated unexpectedly strong social structure in this nonbreeding population of golden crowned sparrows, with the population subdivided into relatively discrete social communities within which most flock associations occurred (Shizuka et al. 2014). Here, we tested the hypothesis that this social structure was correlated with kinship. Our study conclusively shows that social structure in our wintering population is not heavily influenced by kinship. The overall average relatedness was low during the nonbreeding season with social interactions occurring mostly between unrelated birds. Only ~6% of all genotyped birds' relationships were considered close kin (pair-wise assignments of $r \geq 0.25$ by maximum likelihood method). In some species of birds the sexes show different migration patterns (Liu et al 2013; Altwegg et al 2000) and migrate different distances (Ketterson &Van Nolan Jr 1983), which potentially could lead to kinship associations being feasible in one sex but not the other. To provide a more direct test of this possibility, we analyzed the data separately for each sex. Again, there was not a detectable relationship between genetic relatedness and social associations within the sexes. Finally, we isolated social associations and genetic relatedness within social communities (as detected by social network analysis) to test if we could detect even subtle patterns found only within a community but did not. Thus according to our analyses, pair-wise genetic relatedness (kinship) does not predict the social associations in this wintering population of golden-crowned sparrows.

Our analyses can also be used to test the hypothesis that social communities arise from assortment of individuals into groups based on shared breeding population. If social communities represent groups that breed together in the same population, we might have observed genetic structure between communities even in the absence of kin-based social cohesion. However, our results do not show any such genetic structure, suggesting that there is unlikely to be any effects of breeding provenance on winter social structure. It is worth noting that this lack of population genetic structure may be attributable to a couple of different causes. Individuals sampled in our wintering population may be arriving from a single breeding location thus while there may be population structure on the breeding grounds, the absence of population structure on the wintering grounds reflects the fact that only one breeding population is present in our study site. However, another plausible explanation is that there is no population genetic structure across the breeding distribution of golden-crowned sparrows. The absence of population structure across the breeding grounds may arise for a variety of reasons including high levels of contemporary gene flow and/or a rapid post-glacial range expansion out of a single refugia. Conclusively ruling out connectivity between social communities and breeding population would require an examination of the genetic structure of breeding populations, or more directly, tracking individual migrations using geolocators. Nevertheless, even if there were social associations that span both the breeding and winter seasons, these associations do not seem to be based on kinship.

Our results align with other recent studies that have also detected low percentages of close kin in winter bird aggregations (Liker et al 2009; Fleskes et al 2010; Lee et al 2010; Liu et al 2013). These stand in stark contrast to cooperative breeding birds which may form kin-based winter groups (e.g., Kraaijeveld & Dickinson 2001). More broadly, the emerging literature on genetic structure of animal social networks suggest that social groups within populations can represent highly cohesive kin-based groups (e.g., African elephants: Archie et al. 2006; spotted hyenas: Holekamp et al. 2012), groups with moderate levels of genetic relatedness due to limited dispersal (e.g., Galápagos sea lion: Wolf & Trillmich 2008; eastern grey kangaroos: Best et al. 2014; vinous-throated parrotbills: Lee et al. 2010), or groups with little to no genetic relatedness (e.g., this study: golden-crowned sparrows; tufted ducks: Liu et al. 2013).

There are several factors that could contribute to social cohesion without kinship, ranging from non-social factors such as shared preferences for location to social factors such as preference for familiar group members. Our previous study in this population suggested that the complex social structure was shaped by across-year fidelity in flockmates—individuals that return to the population across years flock with the same birds as the previous year (Shizuka et al. 2014). The current study provides additional evidence that familiarity need not be related to genetic kinship.

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References

Alexander RD (1974) The Evolution of Social Behavior. *Annual Review of Ecology and Systematics*, **5**, 325–383.

Altwegg R, Ringsby TH, SÆther BE (2000) Phenotypic correlates and consequences of dispersal in a metapopulation of house sparrows Passer domesticus. *Journal of Animal Ecology*, **69**, 762-770.

Archie EA, Moss CJ, Alberts SC (2006) The ties that bind: genetic relatedness predicts the fission and fusion of social groups in wild African elephants. *Proceedings. Biological Sciences / The Royal Society*, **273**, 513–522.

Aureli F, Schaffner CM, Boesch C, Bearder SK, Call J, Chapman CA, Van Schaik CP (2008) Fission-Fusion Dynamics. *Current Anthropology*, **49**, 627-654.

Berthold P (1993) *Bird migration, a general survey*. Oxford, UK:Oxford University Press.

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology & Evolution*, **18**, 503-511.

Bourke AFG, Franks NR (1995) *Social evolution in ants*. Princeton University Press, NJ.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques*, **31**, 24-26.

Cairns SJ, Schwager SJ (1987). A comparison of association indices. *Animal Behaviour*, **35**, 1454-1469.

Clauset A, Newman ME, Moore C (2004) Finding community structure in very large networks. *Physical review E*, **70**, 066111.

Clegg SM, Kelly JF, Kimura M, Smith TB (2003). Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's Warbler (Wilsonia pusilla). *Molecular Ecology*, **12**, 819-830.

Clutton-Brock TH (2006) Cooperative breeding in mammals. In: *Cooperation in Primates and Humans* (eds Kappeler PDPM, Schaik PDCP van), pp. 173–190. Springer Berlin Heidelberg.

Cohn JP (1999) Tracking wildlife: high-tech devices help biologists trace the movements of animals through sky and sea. *Bioscience,* **49**, 12–17.

Crook JH (1970) Social organization and the environment: Aspects of contemporary social ethology. *Animal Behaviour*, **18**, 197-209.

Davies NB, Krebs JR, West SA (2012) *An introduction to behavioural ecology*. John Wiley & Sons.

Dawson MN, Raskoff KA, Jacobs DK (1998) Field preservation of marine invertebrate tissue for DNA analyses. *Molecular marine biology and biotechnology*, **7**, 145-152.

Dickinson JL, Koenig WD (2003) Desperately seeking similarity. *Science*, **300**, 1887–1889.

Earl DA (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359-361.

Ekman J (1989) Ecology of non-breeding social systems of Parus. *The Wilson Bulletin*, 263-288.

Ekman J (2006) Family living among birds. *Journal of Avian Biology*, **37**, 289–298.

Emlen ST (1982) The evolution of helping: an ecological constraints model. *The American Naturalist*, **119**, 29–39.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology*, **14**, 2611-2620.

Fleskes JP, Fowler AC, Casazza ML, Eadie JM (2010) Population structure and relatedness among female Northern Pintails in three California wintering regions. *Waterbirds*, **33**, 1-9.

Ginsberg JR, Young TP (1992) Measuring association between individuals or groups in behavioural studies. *Animal Behaviour*, **44**, 377-379.

Goslee, SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software,* **22**, 1-19.

Gibbs HL, Dawson RJG, Hobson KA (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow.
Molecular Ecology, **9**, 2137-2147.

Greenberg R, Salewski V (2005) Ecological correlates of wintering social systems in New World and Old World migratory passerines. *Birds of Two Worlds: The Ecology and Evolution of Migration (R. Greenberg and PP Marra, Eds.). Johns Hopkins University Press, Baltimore, Maryland*, 336-358.

Griffiths R, Double MC, Orr K, Dawson RJ (1998) A DNA test to sex most birds. *Molecular ecology*, **7**, 1071-1075.

Hamilton, WD (1964) The genetical evolution of social behaviour. I. *Journal of theoretical biology,* **7**, 1-16.

Hatchwell, BJ (2009) The evolution of cooperative breeding in birds: kinship, dispersal and life history. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 3217-3227.

Hatchwell BJ (2010) Cryptic Kin Selection: Kin Structure in Vertebrate Populations and Opportunities for Kin-Directed Cooperation. *Ethology*, **116**, 203–216.

Heckscher CM, Taylor SM, Fox JW, Afanasyev V (2011) Veery (Catharus fuscescens) wintering locations, migratory connectivity, and a revision of its winter range using geolocator technology. *The Auk*, **128**, 531-542.

Heinsohn R, Dunn P, Legge S, Double M (2000) Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **267**, 243-249.

Helbig AJ, Salomon M, Bensch S, Seibold I (2001) Male-biased gene flow across an avian hybrid zone: evidence from mitochondrial and microsatellite DNA. *Journal of Evolutionary Biology*, **14**, 277-287.

Hill GE (1992) An inexpensive source of colored leg bands. *Journal of Field Ornithology*, **63**, 408-410.

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. *Molecular ecology resources*, **9**, 1322-1332.

James R, Croft DP, Krause J (2009) Potential banana skins in animal social network analysis. *Behavioral Ecology and Sociobiology*, **63**, 989-997.

Kalinowski ST, Wagner AP, Taper ML (2006) ml-relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, **6**, 576-579.

Kamel SJ, Grosberg RK (2013) Kinship and the evolution of social behaviours in the sea. *Biology Letters*, **9**, 20130454.

Ketterson ED, Nolan Jr V (1983) The evolution of differential bird migration. In *Current ornithology,* 357-402. Springer US.

Koenig WD, Dickinson JL (2004) *Ecology and Evolution of Cooperative Breeding in Birds*. Cambridge University Press, New York.

Koenig WD, Hooge PN, Stanback MT, Haydock J (2000) Natal dispersal in the cooperatively breeding acorn woodpecker. *The Condor*, **102**, 492-502.

Kraaijeveld K, Dickinson JL (2001) Family-based winter territoriality in western bluebirds, Sialia mexicana: the structure and dynamics of winter groups. *Animal Behaviour*, **61**, 109-117.

Krause J, Ruxton GD (2002) *Living in groups*. Oxford University Press.

Krutzen M, Sherwin WB, Connor RC *et al.* (2003) Contrasting relatedness patterns in bottlenose dolphins (Tursiops sp.) with different alliance strategies. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 497–502.

Kuzdzal-Fick JJ, Fox SA, Strassmann JE, Queller DC (2011) High Relatedness Is Necessary and Sufficient to Maintain Multicellularity in Dictyostelium. *Science*, **334**, 1548–1551.

Lee JW, Simeoni M, Burke T, Hatchwell BJ (2010) The consequences of winter flock demography for genetic structure and inbreeding risk in vinous-throated parrotbills, Paradoxornis webbianus. *Heredity*, **104**, 472-481.

Ligon JD, Stacey PB (1991) The origin and maintenance of helping behavior in birds. *American Naturalist*, 254-258.

Liker A, Bókony V, Kulcsár A, Tóth Z, Szabó K, Kaholek B, Pénzes Z (2009) Genetic relatedness in wintering groups of house sparrows (Passer domesticus). *Molecular ecology*, **18**, 4696-4706.

Liu Y, Keller I, Heckel G (2013) Temporal genetic structure and relatedness in the Tufted Duck Aythya fuligula suggests limited kin association in winter. *Ibis*, **155**, 499-507.

MacDougall-Shackleton EA, MacDougall-Shackleton SA (2001) Cultural and genetic evolution in mountain white-crowned sparrows: Song dialects are associated with population structure. *Evolution*, **55**, 2568-2575.

Milligan BG *(*2003) Maximum-likelihood estimation of relatedness. *Genetics,* **163***,* 1153–1167.

Nesje M, Røed KH, Bell DA, Lindberg P, Lifjeld JT (2000) Microsatellite analysis of population structure and genetic variability in peregrine falcons (Falco peregrinus). *Animal Conservation,* **3**, 267-275.

Newman ME, Girvan M (2004) Finding and evaluating community structure in networks. *Physical review E,* **69**, 026113.

Oh KP, Badyaev AV (2010) Structure of social networks in a passerine bird: consequences for sexual selection and the evolution of mating strategies. *The American Naturalist*, **176**, E80-E89.

Ostrowski EA, Katoh M, Shaulsky G, Queller DC, Strassmann JE (2008) Kin discrimination increases with genetic distance in a social amoeba. *PLoS Biology,* **6**, e287.

Pemberton JM (2008) Wild pedigrees: the way forward. *Proceedings of the Royal Society B: Biological Sciences,* **275**, 613-621.

Pielou EC (1991) *After the Ice Age: the return of life to glaciated North America*. The University of Chicago Press.

Poesel A, Gibbs HL, Nelson DA (2009) Twenty-one novel microsatellite DNA loci isolated from the Puget Sound white-crowned sparrow, Zonotrichia leucophrys pugetensis. *Molecular Ecology Resources*, **9**, 795-798.

Portelli DJ, Barclay H, Russell DJF, Griffith SC, Russell AF (2009) Social organisation and foraging ecology of the cooperatively breeding Chestnut-crowned Babbler (Pomatostomus ruficeps). *Emu*, **109**, 153–162.

Primmer CR, Møller AP, Ellegren H (1996) New microsatellites from the pied flycatcher Ficedula hypoleuca and the swallow Hirundo rustica genomes. *Hereditas*, **124**, 281-284.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.

Raymond M, Rousset F (1995) GENEPOP: population genetics software for exact tests and ecumenicism. *Journal of heredity*, **86**, 248-249.

Rohwer S, Fretwell SD, Tuckfield RC (1976) Distress screams as a measure of kinship in birds. *American Midland Naturalist*, 418-430.

Rollins LA, Browning LE, Holleley CE *et al.* (2012) Building genetic networks using relatedness information: a novel approach for the estimation of dispersal and characterization of group structure in social animals. *Molecular Ecology*, **21**, 1727–1740.

Rousset F (2008) genepop’007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular ecology resources*, **8**, 103-106.

Rubenstein DR, Chamberlain CP, Holmes RT, Ayres MP, Waldbauer JR, Graves GR, Tuross NC (2002) Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science*, **295**, 1062-1065.

Ruegg KC, Smith TB (2002) Not as the crow flies: a historical explanation for circuitous migration in Swainson's thrush (Catharus ustulatus). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **269**, 1375-1381.

Ryder TB, Fox JW, Marra PP (2011) Estimating migratory connectivity of Gray Catbirds (Dumetella carolinensis) using geolocator and mark-recapture data. *The Auk*, **128**, 448-453.

Seavy NE, Humple DL, Cormier RL, Gardali T (2012) Establishing the breeding provenance of a temperate-wintering North American passerine, the Golden-crowned Sparrow, using light-level geolocation. *PloS one*, **7**, e34886.

Seegar WS, Cutchis PN, Fuller MR, Surer JJ, Bhatnagar V, Wall JG (1996) Fifteen years of satellite tracking development and application to wildlife research and conservation. *Johns Hopkins APL Technical Digest*, **17**, 401-411.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology letters*, **9**, 615-629.

Sharp SP, Simeoni M, Hatchwell BJ (2008) Dispersal of sibling coalitions promotes helping among immigrants in a cooperatively breeding bird. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2125-2130.

Shizuka D, Chaine AS, Anderson J, Johnson O, Laursen IM, Lyon BE (2014). Across□year social stability shapes network structure in wintering migrant sparrows. *Ecology Letters*.

Silk MJ, Croft DP, Tregenza T, Bearhop S (2014) The importance of fission–fusion social group dynamics in birds. *Ibis*, **156**, 701–715.

Solomon NG, French JA (1996) *Cooperative Breeding in Mammals*. Cambridge University Press, New York.

Stacey PB, Koenig WD (1990) *Cooperative breeding in birds: long term studies of ecology and behaviour*. Cambridge University Press.

Stenzler LM, Fraser R, Lovette IJ (2004). Isolation and characterization of 12 microsatellite loci from Golden-winged Warblers (Vermivora chrysoptera) with broad cross-taxon utility in emberizine songbirds. *Molecular Ecology Notes*, **4**, 602-604.

Strassmann JE, Gilbert OM, Queller DC (2011) Kin discrimination and cooperation in microbes. *Annual Review of Microbiology*, **65**, 349–367.

van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535-538.

Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: unraveling migratory connectivity. *Trends in Ecology & Evolution*, **17**, 76-83.

Webster MS, Reichart L (2005) Use of microsatellites for parentage and kinship analyses in animals. *Methods in enzymology*, **395**, 222-238.

Wennerberg L (2001) Breeding origin and migration pattern of dunlin (Calidris alpina) revealed by mitochondrial DNA analysis. *Molecular Ecology*, **10**, 1111-1120.

Whitehead H (2008) Precision and power in the analysis of social structure using associations. *Animal Behaviour*, **75**, 1093-1099.

Williams DA, Rabenold KN (2005) Male-biased dispersal, female philopatry, and routes to fitness in a social corvid. *Journal of Animal Ecology*, **74**, 150-159.

Wilson EO (1975) *Sociobiology*. Harvard Univ. Press, Cambridge, MA.

Wolf J, Mawdsley D, Trillmich F, James R (2007). Social structure in a colonial mammal: unravelling hidden structural layers and their foundations by network analysis. *Animal Behaviour*, **74**, 1293–1302.

Woxvold IA, Adcock GJ, Mulder RA (2006) Fine-scale genetic structure and dispersal in cooperatively breeding apostlebirds. *Molecular ecology*, **15**, 3139-3146.

Tables and Figures

Table 1.1 Primer sequences listed by locus. PCR reactions were optimized given the following salt concentrations (MgCl2) and annealing temperatures (TA). The repeat motif for each microsatellite is given in base pairs (bp). NA provides the number of unique alleles for that locus across all samples.

Locus	Primer Sequence (5'-3')	MgCl2	TA	Repeat Motif (bp)	NA	Size Range (bp)
GCSPR1	F: TATCGAGCATTGCCCTCCC R: GCAGAGTATGAGGTTTTCCTTCC	2.5 ul	59C	4	34	300-524
GCSPR2	F: GCAGCCATTTTGCTGTCATTC R: CCATCTGTCTGTCTTTCTGTCTG	3.5 ul	59C	4	11	167-207
GCSPR3	F: ACCCAAAGTGCAAATCCCATC R: ACAAAGTCCCGTTTTCCTTGC	3.5 ul	59C	4	9	252-288
GCSPR5	F: GCCAAACTCAGTGACCTGC R: AGTTCCTGCACGGTTCTTC	2.5 ul	59C	4	12	226-274
GCSPR9	F: CCAGCCTGATTTCCCATGC R: TGTTGAGCATCTCTGGAGG	2.5 ul	59C	4	9	194-243
GCSPR10	F: TGCCAGCAACTCTGCCTC R: TGAGCTTCCAGCCCTTCAG	2.5 ul	59C	4	18	186-279
GCSPR11	F: TCCATGCTTCTGAACTGCC R: ACACCTGCTTTTCCTGACTG	3.5 ul	59C	4	12	171-215
GCSPR12	F: TAGGCAGGGACAGCAAGAC R: ACTACCAGAACCAACTAGGGG	3.5 ul	59C	2	18	221-261
GCSPR14	F: AGAATGCTCTGGAACCGGC R: AGGACCTGTGTGCCAATTAAG	3.5 ul	59C	4	13	188-244
GCSPR15	F: CAGCCTGTTCCATGCATCC R: GCTCGGTTCTTGCTCACAG	2.5 ul	59C	4	10	186-230
GCSPR16	F: AACCAAGCCACCACAATGC R: GACAGGCACTAGGATGGGAG	3.5 ul	59C	4	12	230-278
GCSPR17	F: GCCACGTTACACATCCTGC20 R: CTGGCATTCCAAAGCTGGG	2.5 ul	59C	4	16	200-288
GCSPR19	F: ACTGTTCTTTTCTCCACCCAC R: GGTTGAATCCCAGGTGGAAAC	2.5 ul	65C	4	12	172-216
GCSPR30	F: TGTCCTCCCCCTGTTTGTTTTA R: ATTGTCCCCACTGCATCCTTCA	1.5 ul	59C	3	13	313-352

Table 1.2 Fourteen microsatellite loci that were used to calculate genetic relatedness. *Poesel et al. 2009 □Stenzler et al. 2004

Locus	Size Range	No. of Alleles	Observed heterozygosity (HO)	Expected heterozygosity (HE)	*P* value for Hardy-Weinberg test
GCSPR1*	300-524	34	0.978	0.96	0.5051
GCSPR2*	167-207	11	0.966	0.87	0.903
GCSPR3*	252-288	9	0.903	0.83	0.5908
GCSPR5*	226-274	12	0.915	0.85	0.1453
GCSPR9*	194-243	9	0.919	0.83	0.7627
GCSPR10*	186-280	18	0.9	0.9	0.25
GCSPR11*	171-215	12	0.881	0.89	0.2763
GCSPR12*	221-261	18	0.951	0.87	0.9348
GCSPR14*	188-244	13	0.919	0.87	0.7336
GCSPR15*	186-230	10	0.857	0.83	0.6874
GCSPR16*	230-278	12	0.932	0.86	0.87
GCSPR17*	200-288	16	0.722	0.89	0.0036
GCSPR19*	172-216	12	0.622	0.58	0.1568
GCSPR30⌘	312-352	13	0.812	0.8	0.1568

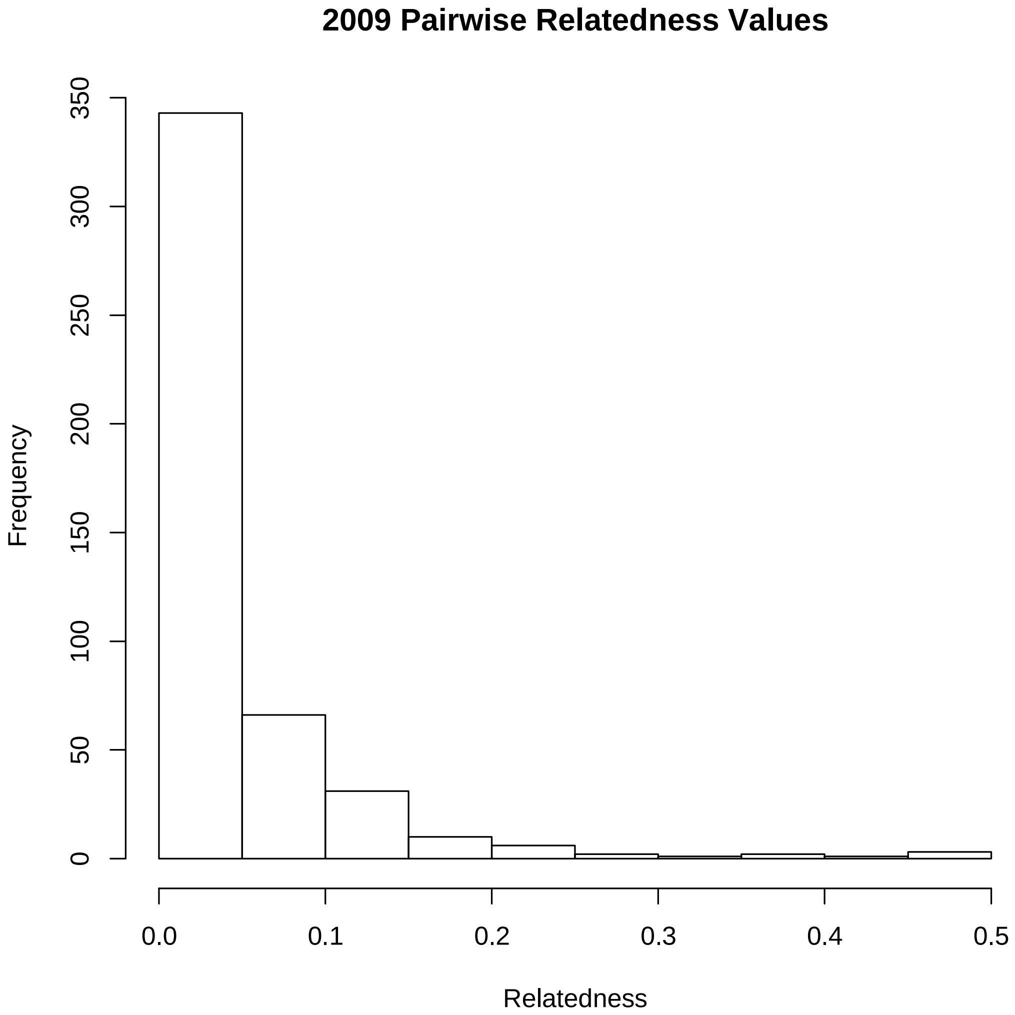
Table 1.3 Correlation between pairwise genetic relatedness and social association (simple ratio) among golden-crowned sparrows. Correlation coefficients (*r*) were calculated by matrix correlation, associated *P* values were calculated by Mantel randomization tests with 10,000 permutations; *n* = number of individuals with number of dyads used in the tests give in parentheses. *P* values are one-tailed, as provided by the software (Ecodist).

Sample	**2009** ***n* (dyads)**	**mantel *r***	***P***	**2010** ***n* (dyads)**	**mantel *r***	***P***
All dyads	31 (930)	-0.003	0.505	40 (1560)	-0.045	0.898
Male-male dyads	8 (56)	-0.188	0.827	10 (90)	-0.307	0.995
Female-female dyads	23 (506)	-0.019	0.593	30 (870)	0.019	0.328
Social Community 1	9 (72)	-0.112	0.729	-	-	-
Social Community 2	17 (272)	-0.058	0.753	-	-	-
Social Community 3	5 (20)	0.426	0.203	-	-	-
Social Community 4	-	-	-	11 (110)	-0.098	0.789
Social Community 5	-	-	-	17 (272)	0.036	0.297
Social Community 6	-	-	-	11 (110)	-0.096	0.713

Table 1.4 Fst values for each loci calculated with GENEPOP.

2009		2010	
Loci	**Fst**	**Loci**	**Fst**
GCSPR 1	0.005	GCSPR 1	0
GCSPR 2	0	GCSPR 2	0
GCSPR 3	0	GCSPR 3	0.009
GCSPR 5	0	GCSPR 5	0.001
GCSPR 9	0.016	GCSPR 9	0.018
GCSPR 10	0	GCSPR 10	0
GCSPR 11	0	GCSPR 11	0
GCSPR 12	0	GCSPR 12	0
GCSPR 14	0	GCSPR 14	0
GCSPR 15	0	GCSPR 15	0
GCSPR 16	0	GCSPR 16	0
GCSPR 17	0	GCSPR 17	0
GCSPR 19	0.061	GCSPR 19	0
GCSPR 30	0	GCSPR 30	0.007

Figure 1.1 Distributions of pairwise relatedness values (*r*) for 2009 (n = 465) and 2010 (n = 781).



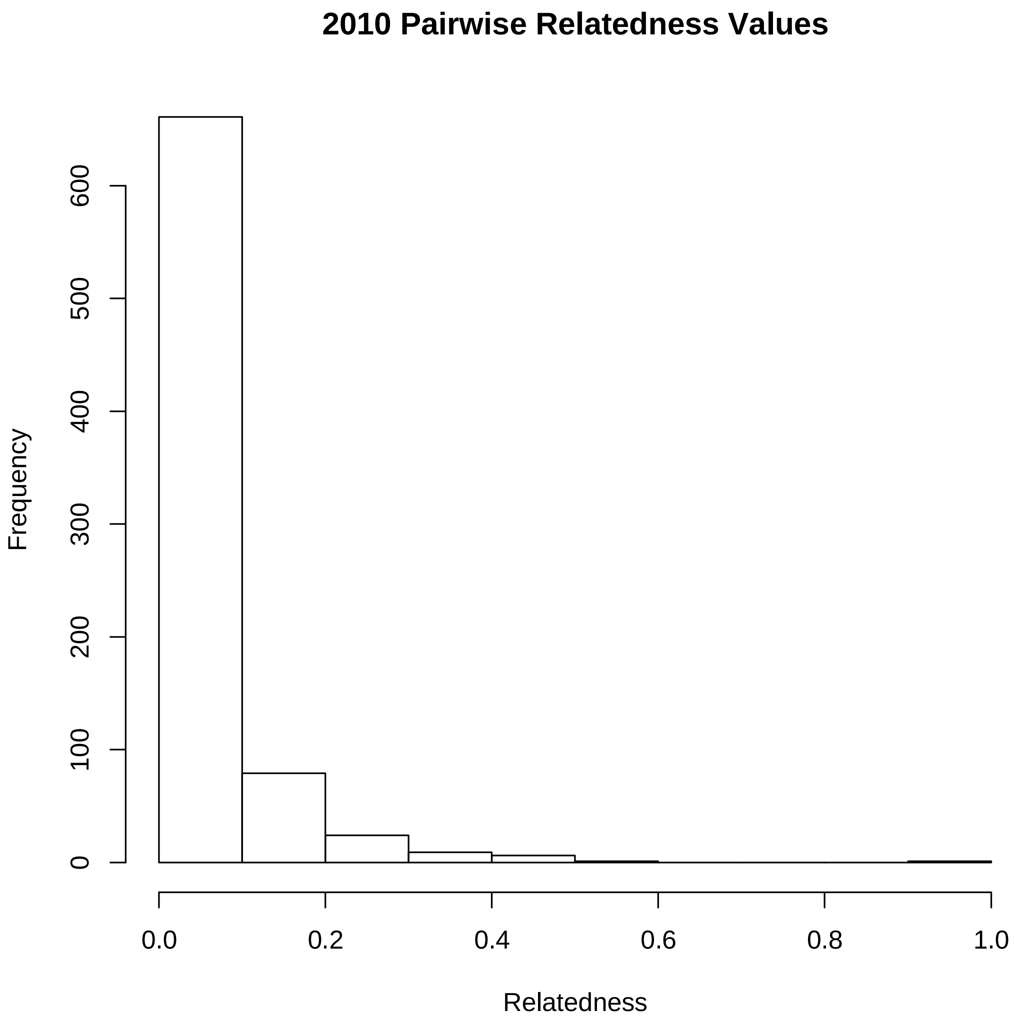
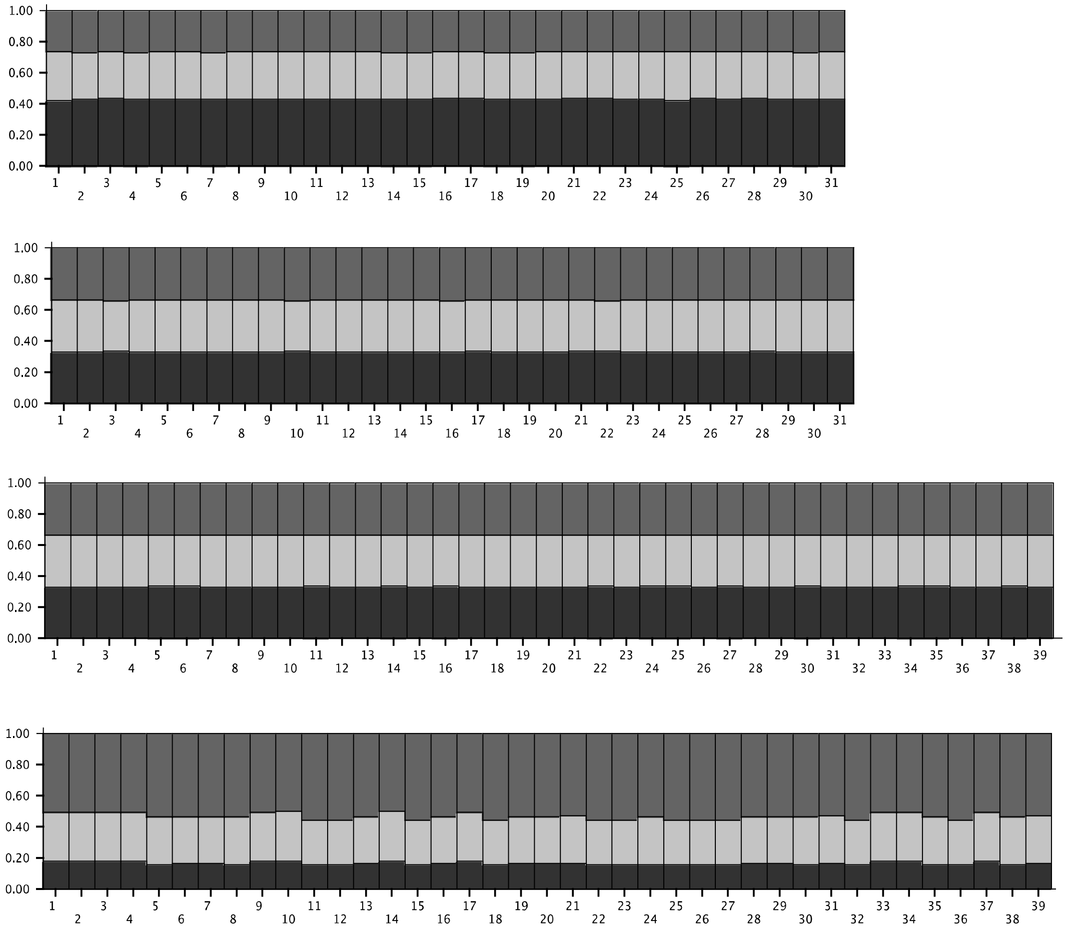

2010 Pairwise Relatedness Values
Frequency
0
100
200
300
400
500
600
0.0
0.2
0.4
0.6
0.8
1.0
Relatedness

Figure 1.2 Results of population structure analysis using STRUCTURE. Individuals are shown in each column with their respective allele frequencies at K = 3.
From top to bottom: A. 2009 without location prior B. 2009 with location prior C. 2010 without location prior D. 2010 with location prior



CHAPTER 2

Variation in behavioral and morphological traits correlates with heterozygosity in a wintering population of a migratory bird

Introduction

Investigating the genetic causes of variation in fitness among individuals in a population informs our understanding of evolution (Merila & Sheldon 1999; Merila & Sheldon 2001; Leinonen et al 2008; Chapman et al 2009) and helps predict the consequences of decreased genetic diversity (Reed & Frankham 2003). A useful approach has been to relate within-individual variation (e.g., measures of heterozygosity using molecular tools) to variation in fitness, or characters that are potentially related to fitness. Associations between heterozygosity and fitness can be complex and have been studied and debated for over three decades. Many studies have found a correlation between individual multi-locus heterozygosity at molecular loci and fitness-related traits [heterozygosity-fitness correlation (HFC)] (reviewed in Coltman & Slate 2003; Kempenaers 2007; Chapman et al 2009). HFC studies rely on a simple assumption; individuals with high heterozygosity levels are expected to have a selective advantage over less heterozygous individuals in a population.

Three biological mechanisms could account for significant positive HFCs (Hansson & Westerberg 2002; Chapman et al 2009). First, the ‘direct effect hypothesis’ predicts that the loci used in the HFC exhibit heterozygote advantage. This hypothesis potentially explains HFCs detected in allozyme studies (Mitton 1997), but are less informative in noncoding and neutral markers, such as microsatellites (Pogson & Zouros 1994; Thelen & Allendorf 2001). Second, the ‘general effect’ hypothesis predicts that individual heterozygosity reflects genome-wide heterozygosity and such only correlates with fitness if it also correlates with individual inbreeding level. For example, inbred individuals with lower fitness than their ancestors exhibit inbreeding depression; outbred individuals with higher fitness than their ancestors exhibit heterosis (reviewed in Charlesworth & Willis 2009). Both inbreeding and outbreeding simultaneously modify heterozygosity across all loci of a genome; therefore if heterozygosity is correlated among loci markers, then multi-locus heterozygosity may be an informative measure of inbreeding (Balloux et al 2004). ‘General effects’ are commonly suggested to explain HFCs in natural populations (reviewed in Chapman 2009) although the extent to which heterozygosity measured by a few marker loci can truly reflect levels of genome-wide heterozygosity is still questioned (Balloux et al 2004; Slate et al 2004; Hansson & Westerberg 2008; Kardos et al 2014). Furthermore, caution should be used when using ‘general effects’ as an explanation for HFCs since cryptic population structure could incorrectly detect a HFC due to variation in heterozygosity levels across subpopulations (Szulkin et al 2010). Third, the ‘local effect’ hypothesis predicts that HFCs could be the result of some of the molecular loci used in the study being in physical linkage with genes that influence fitness (Slate et al 2004). Although several studies have published evidence for ‘local effects,’ (Hansson and Westerberg 2002; Hansson et al. 2004; Da Silva et al. 2006; Lieutenant-Gosselin and Bernatchez 2006; Hansson and Westerberg 2008; Vilhunen et al 2008) Szulkin et al (2010) argue that speculations on local effects have not been supported by relevant and significant statistics. Although the existence of ‘local effects’ is not in contention, methods used to statistically explore whether a large, or detectable, part of the overall HFC is due to local effects are questioned (Szulkin et al 2010).

Studies reporting correlations between heterozygosity and traits that vary among individuals have used a variety of fitness-related traits. In many study systems, direct measures of fitness traits are not attainable thus traits that correlate with fitness have be used (Merila & Sheldon 1999; Hansson & Westerberg 2002). Three main categories of trait types used in the literature include morphological traits (e.g., size and shape), life-history traits (e.g., survival and breeding success) and physiological traits (e.g., parasite load and immune response) (reviewed in Kempenaers 2007; Chapman et al 2009). More recently, behavioral traits have been explored in HFC studies as some behaviors vary among individuals and may be important for fitness.

Aggression and boldness are the best-studied behavioral traits (Reale et al 2007) and may affect fitness (Smith & Blumstein 2007). Several HFC studies have found links between heterozygosity and a variety of behavioral traits. Individual heterozygosity was positively correlated with social dominance (in brown trout; Tiira et al. 2006), aggressive behavior (in salmon; Tiira et al., 2003), territorial status (in males of the lekking black grouse; Hoglund et al. 2002), territory size and song structure (in a cooperatively breeding bird, the subdesert mesite, *Monias benschi* ; Seddon et al. 2004), and competitive ability (in salmon; Blanchet et al 2009). However, the fitness consequences of behaviors can depend on the context they are exhibited. For example, aggressive individuals may have increased fitness in situations where individuals compete for limited resources but may pay a cost for exhibiting aggression when, for example, avoiding predators (Laine et al 2012).

Studies exploring the evolutionary significance of avian behaviors have primarily centered within the breeding context. However, behaviors that increase reproductive success in a breeding context may differ from behaviors that increase survival in a non-breeding context (Reale et al. 2007). Survival outside the breeding season depends on, for example, habitat selection, foraging, efficiency, and aggression within and between species, as well as physiological and morphological features (Baker and Fox 1978a). Winter survival may be particularly important for migratory species since individuals may encounter harsh conditions during migration (Newton 2006). Individual variation in behavioral, physiological, and morphological traits in wintering populations are well documented but little is known about the influence of genetic diversity on such traits in the non-breeding context (see exceptions: *Junco hyemalis* in Baker & Fox 1978b; *Anas americana* in Rhodes et al. 1993; *Anas platyrhynchos* in Rhodes et al. 1996).

We investigate HFCs in a migratory passerine bird, the golden-crowned sparrow (*Zonotrichia atricapilla*), as individuals show tremendous variation in winter behavior and morphology that may be important to winter survival. Individuals vary in body size and in the size of two plumage patches, central gold plumage patch flanked by two black stripes. Both plumage patches are more variable in winter than during the breeding season (B.E. Lyon, D. Shizuka & A.S. Chaine, unpublished data), after a post-breeding molt in the fall. We previously found that these plumage traits were correlated with social dominance in encounters between unfamiliar individuals (Chaine et al. 2011). In addition to morphological variation, there is great variance—independent of sex—in several behavioral traits such as social dominance (Chaine et al. 2011), sociality (Shizuka et al. 2014), and singing behavior (Lyon et al. unpublished data). We could expect HFCs in wild populations of golden-crowned sparrows as a study on a congener, mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) showed that individuals infected with a blood parasite (parasites reduce fitness) were less heterozygous than uninfected individuals, parasite load decreased with increasing heterozygosity (MacDougall-Shackleton et al. 2005). Also, any HFCs detected in our study population would not be a result of population structure (Balloux et al. 2004). We recently optimized 14 microsatellite markers for golden-crowned sparrows and found no population structure in this wintering population (Arnberg et al. manuscript).

In this study we assess the genetic correlation of individual variation in behaviors and phenotypic traits potentially linked to fitness in a long-distance migratory bird, the golden-crowned sparrow. We first investigate the relationship between genetic variation—heterozygosity calculated using data from 14 microsatellite loci—with variation in traits thought to affect fitness, including both morphological traits (size and color of gold and black crown patches, body size) and behavioral traits (social dominance, singing and three measures of individual sociality) in a winter population over two seasons. We then test whether HFCs in this study are driven by genome-wide heterozygosity under the general effect hypothesis (Hansson & Westerberg 2002) or by a single locus under the local effects hypothesis (Hansson & Westerberg 2002; procedure described in Szulkin et al. 2010). Finally, we explore if any of the microsatellite loci used in this study are homologous with functional genes in the avian genome (Olano-Marin et al. 2010).

Methods:

Study System

We studied a population of migratory golden-crowned sparrows on their wintering grounds at the Arboretum of the University of California, Santa Cruz. Individuals arrive at the study site in October or November and leave for their breeding grounds, likely in different areas along the coast of British Colombia and Alaska (Seavy et al. 2012), in March or April. Individuals included in this study were observed at the study site during one or both of two non-breeding seasons: October 2009–March 2010 (season 1) and October 2010–February 2011 (season 2). Although golden-crowned sparrows are territorial on the breeding grounds, they are social in winter (Norment et al. 1998, Shizuka et al. 2014). They forage in small flocks and, although flock composition is ephemeral, flocks non-randomly comprise subsets of individuals that belong to very stable communities, with different communities using different small areas of the study site (Shizuka et al. 2014). Golden-crowned sparrows are dull-colored birds except for the conspicuous coloring of their crowns; a central gold plumage patch is flanked by two black stripes on either side (starting from the beak and extending towards the back of the head). Previous experiments showed that the crown patches function as badges of status that settle social dominance in some social contexts (Chaine et al. 2011, 2013).

Each season, we captured birds using baited traps. We banded each bird with a metal band and a unique combination of colored bands to allow us to recognize individuals. We took several morphological measurements (plumage crown patch sizes, body size; Chaine et al. 2011) from each bird and collected a small blood sample for genetic analysis.

Genetic Analyses

We used 14 microsatellite primers to quantify heterozygosity in our population. These were initially optimized for white-crowned sparrows Zonotrichia leucophrys (13 primers from Poesel et al. 2009) and golden-winged warblers Vermivora chrysoptera (1 primer from Stenzler et al. 2004.) We genotyped 52 individuals; multi-locus heterozygosity measures were calculated using all microsatellite loci.

We set up 25-μL PCR reactions containing 10 ng template DNA, 10x *Ex Taq* buffer (Mg^{2+} free), 0.2 mM each dNTP, between 1.0 and 3.5 mM $MgCl_2$, 0.05 U *TaKaRa Ex Taq* polymerase (Takara Bio Inc.), 0.015 mM forward primer tailed with M13 (Boutin-Ganache et al. 2001), 0.2 mM reverse primer, and 0.2 mM M13 labeled with fluorescent dye. We used the Dye Set DS-33 (Applied Biosystems Inc.) to fluorescently label primers with 6-FAM, VIC, NED, and PET.

PCR reactions were run under the following conditions: initial denaturation at 94°C for 3 min, then 33 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 40 s (65°C for 40s for GCSPR19 only), extension at 72°C for 40 s, concluding with a final extension at 72°C for 5 min. We verified PCR amplification by visualizing PCR products on 3% agarose gels stained after casting with Gel Red Nucleic Acid Gel Stain, 3X in water (Biotium).

To identify alleles, all amplified PCR products were suspended in formamide and analyzed on an ABI Prism 3100 Genetic Analyzer (Applied Biosytstems Inc.) at the University of California, Berkeley DNA Sequencing Facility using LIZ-labeled 600 internal standard (Applied Biosystems Inc.). The genetic profiles were analyzed visually using Peak Scanner Software v1.0 (Applied Biosystems Inc.).

We compared observed and expected heterozygosity to test for deviations from Hardy-Weinberg (HW) equilibrium expectations calculated using ML-Relate (Kalinowski et al. 2006). Although GCSPR17 was significantly different from HW equilibrium predictions, the contrast was no longer significant after Bonferroni correction of alpha levels for multiple comparisons (Rice 1989), and this locus was included in subsequent analyses. There was no evidence for linkages between the microsatellite loci investigated using GENEPOP version 4.0.10 (Raymond and Rousset 1995; Rousset 2008). Also, based on analysis using Microchecker (Van Oosterhout et al. 2004) there was no evidence for the presence of null alleles.

Multi-locus Heterozygosity

Heterozygosity was estimated using five frequently used measures: the proportion of heterozygous loci in an individual, **H**; the genetic distance between an individual's gametes, **mean d²** (Coulson et al. 1998); the standardized heterozygosity, **H_S** (Coltman et al. 1999); the internal relatedness, **IR** (Amos et al. 2001); and the homozygosity by locus, **HL** (Aparicio et al. 2006).

H is the simplest measure but may be inappropriate when the number of markers used is small, when loci differ in number and frequency of alleles or when the individuals of a sample are not all typed at the same loci (Aparicio et al. 2006). Heterozygosity at a single locus was coded as a binary variable, with "1" representing a heterozygous state and "0" a homozygous one.

$$\mathbf{H} = \frac{\text{number of heterozygous loci}}{\text{number of genotyped loci}}$$

H_S is used to standardize individual heterozygosity to avoid the problem of not all individuals being typed with the same panel of marker loci or if some loci did not amplify for some individuals (Coltman et al. 1999). This method gives equal weight for all loci examined regardless of their allelic frequencies .

$$\mathbf{H_S} = \frac{\text{proportion of heterozygous typed loci}}{\text{mean heterozygosity of typed loci}}$$

IR measures heterozygosity based on allele sharing (an individual has two copies of the same allele at a given locus) where the frequency of each allele counts towards the final score and thus allows the sharing of less common alleles to be weighted more than the sharing of common alleles (Amos et al. 2001). IR measures vary between –1 and 1. Negative values correspond to higher heterozygosity and positive values indicate higher homozygosity.

$$\mathbf{IR} = \frac{(2H - \Sigma f_i)}{(2N - \Sigma f_i)}$$

where H is the number of loci that are homozygous, N is the number of loci and f_i is the frequency of the ith allele contained in the genotype.

HL gives a higher weight to more informative loci (e.g., loci with more alleles which are more evenly distributed). In simulated populations subjected to migration and admixture, HL correlates better with the inbreeding coefficient and with genome-wide heterozygosity than other heterozygosity indexes (Aparicio et al. 2006).

$$\mathbf{HL} = \frac{\Sigma E_h}{\Sigma E_h + \Sigma E_j}$$

where E_h and E_j are the expected heterozygosities of the loci that an individual bears in homozygosis (h) and in heterozygosis (j), respectively (Aparicio et al 2006). This index varies between 0 when all loci are heterozygous and 1 when all loci are homozygous.

Mean d^2 is estimated from the two alleles each individual has at a locus and is a measure of the stepwise difference in microsatellite repeats between the gametes that formed the individual (Coulson et al. 1998). The squared distance (in microsatellite repeat units) between the two alleles within a locus is then averaged over all loci analyzed in an individual. Mean d^2 may detect events (e.g., population admixture) deeper in an individual’s ancestry (Coulson et al. 1998).

$$\textbf{Mean d}^{\mathbf{2}} = \Sigma^{n,i=1} \frac{(i_a - i_b)^2}{n}$$

where i_a and i_b are the lengths in repeat units of alleles *a* and *b* at locus *i*, and *n* is the total number of loci at which an individual was scored.

Measures of **H** and **mean d^2** were calculated by hand. Measures of **H_S**, **IR**, and **HL** were calculated using the R package 'Rhh' (Alho et al. 2010) in R v2.15.1 (R Development Core Team 2012). We calculated five measures of heterozygosity to test the sensitivity of the models to the choice of heterozygosity measure.

To test whether the number of genetic markers used in our study was sufficient to make appropriate inferences on genome-wide heterozygosity, we also calculated heterozygosity–heterozygosity correlation (Balloux et al. 2004) using the 'h_cor' function in the R package 'Rhh' (Alho et al. 2010). This test repeatedly and randomly divides the loci in half and calculates the correlation between them. If neutral markers such as microsatellites carry information about genome-wide levels of heterozygosity, then comparing two random subsets of such markers should yield a positive, significant correlation (Balloux et al. 2004; Alho et al. 2010). To obtain confidence intervals for mean heterozygosity-heterozygosity correlations, we ran 1000 randomizations of the markers for three estimates of heterozygosity—H_S , IR, and HL. If neutral markers such as microsatellites carry information about genome-wide levels of heterozygosity, then comparing two random subsets of such markers should yield a positive, significant correlation (Balloux et al. 2004; Alho et al. 2010). Correlated heterozygosity between loci is referred to as identity disequilibrium (Weir & Cockerham 1973; Szulkin et al. 2010). Tests for ID between pairs of loci throughout the genome can be used to determine whether an observed HFC could be caused by inbreeding depression.

Single-locus Effects

Testing whether a large part of an HFC is due to a single locus effect (e.g. 'local effects') requires appropriate statistical tests. Many studies test each locus separately, exploring whether the slope of the regression of fitness or fitness-related traits on single-locus heterozygosity differs from zero for a given locus (e.g., Hansson et al. 2004; Lieutenant-Gosselin & Bernatchez 2006; Charpentier et al. 2008; Vilhunen et al. 2008). A more appropriate approach to test for local effects is to test whether a multiple regression including individual effects for each locus explains more variance than a simple regression on a multi-locus heterozygosity measure (David 1997).

To test if local effects are driving any of our observed HFCs, we ran a multiple regression following Szulkin et al. (2010), where each locus (N = 14) was included as an individual predictor; all loci for each individual were recorded as 0 (homozygous) or 1 (heterozygous). If this model explains significantly more variance than a basic model where a multi-locus measure of heterozygosity is included as a single predictor, then this lends support for the local effects hypothesis. For each trait, we compared a multiple regression model to its equivalent—where only one multi-locus measure is fitted as a main effect. We used an F-ratio test to determine whether both models explain the same variance or if they differ in the amount of variance explained. To compare the two models, we replaced missing genotypes for individuals with the mean population heterozygosity for that locus (Szulkin et al. 2010; Chapman & Sheldon 2011). Doing so does not add any information on the status of the individual relative to the population average yet it allows the use of the information obtained at other loci and increases the overall power of the test (Szulkin et al. 2010).

Classifying loci as neutral or functional

To explore whether loci used for this study were located near functional genes on chromosomes, we followed the guidelines of Olano-Marin et al. (2010) by assessing the homology of the microsatellite primer sequences to avian expressed-sequence tags (ESTs). Markers with no homology to avian ESTs are considered neutral. We performed a ‘blast’ search of all sequences against the EST database of zebra finch (*Taeniopygia guttata*) using the NCBI blast suite (http://blast.ncbi.nlm.nih.gov/). We queried only the EST database and allowed for both somewhat similar sequences (blastn) and highly similar sequences (megablast).

We accepted matches with an expected (E) value $< 10^{-5}$. The E value is a parameter that describes the number of hits expected by chance when searching a database of a particular size; lower E values represent more significant matches.

Behavioral Traits

Five behavioral traits were used for this study: social dominance, three measures of sociality, and singing. Social dominance was calculated using David's score, a weighted version of the average social dominance index (the average percentage with which an individual wins in interactions with each of its group members) (Gammell et al. 2003). This measure addresses repeated interactions between group members when calculating social dominance hierarchy and the relative success is weighted by the social dominance of the opponent. Sociality measurements were calculated based on a social network analysis on the same population of sparrows used in this study (Shizuka et al. 2014). Three measures extracted from those analyses were node strength, within-community node strength, and betweenness. The nodes in the social network are the individual birds with the links between nodes showing relationships between individuals. *Node strength* is the sum of all association weights connected to an individual. *Within-community node strength* is restricted to the node strengths that occur within a social community (see within-community degree measures in Guimera & Amaral 2005). *Betweenness* is a measure of the centrality of a node in a network. It is calculated as the fraction of shortest paths between node pairs that pass through the node of interest (Freeman 1977; Freeman 1979). Finally, we also observed whether individuals were ever observed singing. A moderate proportion of the wintering population, both males and females, is observed singing each season. Individuals that were seen singing at least once in either of the two seasons were categorized as singers. Winter song is related to social dominance in some species (Kriner and Schwabl 1991, Katti 2001).

Morphological Traits

We examined the relation between heterozygosity and two plumage traits in golden-crowned sparrows— a central gold patch bordered by two black stripes extending from the beak to the back of the head (Norment et al. 1998). We quantified crown patch sizes by taking digital photographs and isolating and calculating the areas (mm^2) of the gold and black portions of each individual's crown (Chaine et al. 2011). We also quantified mass corrected for body size, a potential indicator of condition (Brown 1996), by calculating the residual of mass regressed on a body size principal component [factor loadings: tarsus length = 0.77, beak length = 0.71, and wing chord = 0.67] (details in Chaine et al. 2011).

Statistical Analyses

All response variables (behavioral and morphological traits) were normally distributed with the exception of singing status, which was binary. Some individuals (n = 18) were observed across both seasons. Separate behavioral and morphological trait values were obtained for each of these individuals per season. To pool the data across field seasons we tested for independence of observations across seasons for all heterozygosity-fitness correlations using linear models that included individuals as a fixed effect. Since all P values for individuals as fixed effects were non-significant for all models ($P \geq 0.39$), individual effects are insignificant and were not included in any subsequent analyses.

To test the effects of heterozygosity across loci on golden-crowned sparrow behavior and morphology, we used the 'lm' function in R v2.15.1 (R Development Core Team 2012) to fit linear models for all normal data. Separate analyses were run for each response variable (social dominance, node strength, within-community node strength, betweenness, gold patch area, gold patch intensity, black patch area, black patch intensity, and mass corrected for body size). For each model, we used H, mean d^2, H$_S$, HL, and IR as predictors. Models also included sex (male or female) and season (1 or 2) as an additional fixed effect. We used a stepwise backwards procedure to screen candidate predictors for possible inclusion in the model and retained variables with $P < 0.05$. After running the full model, we removed non-significant single effects in a sequential fashion. This procedure is considered to represent a conservative method for choosing the best model to explain each response variable and is as thorough as modern algorithms (Murtaugh 2009).

To test if singers were more heterozygous than non-singers, we ran a Wilcoxon rank sum test using all individuals (n = 52). Separate tests were run for each estimate of heterozygosity (H, mean d^2, H_S, HL, and IR).

Results

In our study population, all loci were highly polymorphic and the mean number of alleles per locus was 14 (range 9 to 34; Table 1). Mean expected heterozygosity (H_E) was 0.85 ± 0.09, slightly lower than the mean observed heterozygosity (H_O) of 0.88 ± 0.09. There was no evidence for significant deviation from Hardy-Weinberg and none of the markers were in linkage disequilibrium with each other. Thus, all 14 loci were used for subsequent calculations.

Of the five heterozygosity measures calculated, individual H_S, HL, and IR measures were highly correlated with each other (IR/ H_S: $r = -0.97$, $P < 0.001$, $N = 52$; IR/ HL: $r = 0.98$, $P < 0.001$, $N = 52$; HL/ H_S: $r = -0.99$, $P < 0.001$, $N = 52$). Also, there were no significant interactions between heterozygosity measures H or mean d^2 and any behavioral or morphological traits. Therefore, we only present statistics and figures using the IR measure because heterozygosity levels were high and IR weighs sharing of less common alleles more than the sharing of common alleles.

Multi-locus heterozygosity and traits

Multi-locus heterozygosity calculated from all loci was significantly correlated with three behavioral traits (social dominance, node strength, within-community node strength) and one morphological trait (gold patch size). Recall that negative IR values correspond to higher heterozygosity. More heterozygous individuals (based on IR) had higher social dominance measures (Figure 1: $R^2 = 0.084$, $F_{1,51} = 4.71$, $P = 0.034$). IR was also significantly associated with two measures of sociality, with more heterozygous individuals having metrics that denote higher sociality. Node strength was positively influenced by increased heterozygosity

(Figure 2A: $R^2 = 0.082$, $F_{1,68} = 6.04$, $P = 0.017$). This positive relationship was slightly stronger when we looked within social communities (i.e. the analysis only includes estimates based on nodes connecting individuals from the same communities and not all possible dyads; 2B: $R^2 = 0.11$, $F_{1,68} = 8.491$, $P = 0.004$). A single morphological trait, gold patch size, was significantly influenced by heterozygosity. Birds with larger gold patch sizes (mm^2) were also more heterozygous (Figure 3: $R^2 = 0.077$, $F_{1,49} = 4.061$, $P = 0.05$).

A Wilcoxon test showed that multi-locus heterozygosity levels (using any of the five heterozygosity measures) did not differ between singing and non-singing birds. We report the findings using IR as the estimate of heterozygosity (Figure 4: $W = 278$, $P = 0.81$).

No evidence for genome-wide heterozygosity

Multi-locus heterozygosity is not a predictor of genome-wide heterozygosity in this population of golden-crowned sparrows. Following the method outlined in Balloux et al. (2004), we found no significant correlations between randomly assigned subsets of loci for any of the three heterozygosity estimates—H_S, IR, or HL (Table 2).

No evidence for single-locus effects

Since social dominance, node strength, within-community node strength, and gold patch size showed significant associations with overall heterozygosity measures, we also tested these four traits for possible local effects. For all four traits, models run with single measures of multi-locus heterozygosity were better fits than equivalent models using the 14 separate loci. Heterozygosity at two loci had significant effects on node strength (positive effect on Locus 3, $F_{13,56} = 1.85$, $P = 0.011$, Locus 12, negative effect on $F_{13,56} = 1.85$, $P = 0.031$) and within-community node strength (positive effect on Locus 3, $F_{13,56} = 1.66$, $p = 0.036$; Locus 12, negative effect on $F_{13,56} = 1.658$, $p = 0.024$). However, we have no evidence that these significant loci have a stronger effect than others on sociality.

The fourteen loci appear to be neutral

None of the ‘blast’ searches resulted in significant homologies of microsatellite loci to ESTs for zebra finch. Following the guidelines suggested in Olano-Marin et al (2010), we suggest that all of the 14 loci used for this study can be considered neutral.

Discussion

Individuals that were more heterozygous—based on internal relatedness measures reflecting individual genetic variation—were more socially dominant, had larger gold crown patches (which predict social dominance in some contexts) and had stronger social connections within their social networks. We tested for Pearson’s correlations between the four traits that contributed to significant HFCs. As would be expected, the two sociality measures were significantly correlated with each other (node strength vs. within-community node strength $r_{68} = 0.93$ $P = 0.00$). The correlations between behavioral and morphological traits were not significant (social dominance vs. node strength $r_{51} = 0.23$ $P = 0.11$; social dominance vs. gold patch size $r_{41} = 0.09$ $P = 0.57$; node strength vs. gold patch size $r_{49} = 0.04$ $P = 0.77$). The traits we selected to use in our study connect to key attributes of the winter social lives of golden-crowned sparrows and social dominance, in particular, has been shown to be important to fitness.

Interpreting significant results of heterozygosity on fitness-related traits requires considering processes that may have shaped HFCs, which may be species and context specific. Two explanations have been considered. First, inbreeding can decrease fitness of inbred individuals relative to outbred ones, inbreeding depression (Keller & Waller 2002). Inbreeding can lower individual heterozygosity across the entire genome of an individual and increases the expression of recessive deleterious alleles (Charlesworth & Willis 2009). Inbreeding can arise via within and among population processes, which are not mutually exclusive (Keller & Waller 2002). Within-population inbreeding is a result of related individuals mating. Among-population inbreeding is a result of subdivided populations with small, isolated groups undergoing genetic drift. HFC studies have tended to focus on the former explanation despite the importance of the latter in causing inbreeding depression (with some recent exceptions, Von Hardenberg et al. 2007; Garcia-Navas et al. 2009; Hoffman et al. 2010; Luquet et al. 2011; Olano-Marin et al 2011). Second, HFC may result from local effects at single loci and can be detected when neutral markers are linked to functional loci, which can happen through associative overdominance (Frydenberg, 1963; Ohta, 1971). However, this explanation has recently been criticized. Evidence for local effects driving detected HFCs in the absence of inbreeding implies that levels of linkage disequilibrium in the genome is high (Balloux et al. 2004), that many loci show overall heterozygote advantage (Mueller et al. 2010), or that the effects of the linked loci are strong (Szulkin et al 2010). Also, statistical tests used in studies prior to Szulkin et al (2010) may have been inappropriate for testing for local effects. The local effects explanation can be strengthened by understanding the function or location of the marker loci in the genome (Olano-Marin et al 2011), as it would inform biologically relevant explanations for detected HFCs.

Winter can be a particularly stressful period for migratory birds. For example, variable food availability can lead to starvation especially for small birds (Houston & McNamara 1993). Traits shown to be important to survival include social dominance (in great tit, *Parus major*, Lambrechts & Dhondt 1986; in song sparrows, *Melospiza melodia*, Acrese & Smith 1985; in willow tits, *Parus montanus*, Ekman 1990; in white-throated sparrows, *Zonotrichia albicollis*, Piper & Wiley 1990; in great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*), Røskaft et al 1986; in serins, *Serinus serinus, Figuerola & Senar 2007*), sociality (in Barbary macaques, *Macaca sylvanus*, McFarland & Majolo 2013), plumage used in intraspecific signaling in wintering house sparrows, *Passer domesticus* (Nakagawa et al. 2008), and body mass (in wintering white-crowned sparrows, *Zonotrichia leucophrys*, Ketterson and King 1977). Positive associations between heterozygosity and winter survival have been show in earlier studies in a variety of organisms (summarized in

Allendorf & Leary 1986). For example, a study on dark-eyed juncos experimentally investigated the relationship between heterozygosity (as measured by a peptidase locus) and survival (Baker & Fox 1978). Heterozygous individuals survived longer when exposed to competitive conditions for food in aviaries; this suggests that there may be no effect of heterozygosity on survival when conditions are less stressful. Survivorship of individuals was predicted by heterozygosity levels (21% of variance in survivorship explained by genotype). Interestingly, survivorship was also predicted by social dominance, body weight, and hood coloration. Our findings, along with these studies, suggest that heterozygosity may plan an important role in winter survival of individuals and their overall fitness.

Social dominance. Our results suggest that heterozygosity may be an important factor in predicting the social dominance status of golden-crowned sparrows. These findings are consistent with studies in salmon (*Salmo salar*), where fish with higher heterozygosity behaved more aggressively than fish with lower heterozygosity (Tiira et al. 2003; Tiira et al. 2006). Significant relationships are more likely to be detected during periods of adverse conditions, when individuals are energetically challenged (e.g., Lens et al 2000). Associations detected between metabolic rates and activity, exploration, boldness and aggressiveness (Careau *et al.* 2008, 2011; Biro & Stamps 2010) suggests that individual behavior could be explained by intraspecific variations in energy metabolism (Careau *et al.* 2008). Studies on salmon have found that dominant individuals have a higher standard metabolic rate (Cutts *et al.* 1998, 1999; Lahti *et al.* 2002) and thus have the capacity to engage in costly behavior (Reid et al. 2012). In our population of golden-crowned sparrows it is therefore feasible that more heterozygous individuals are more dominant because they may be, for example, better foragers and have energy to expend on aggressive behaviors. Another reasonable explanation for the relationship between heterozygosity and social dominance could be that more socially dominant birds could be older; heterozygosity might be linked to longevity.

Plumage. We have previously shown that both the gold and black crown patches in golden-crowned sparrows operate as multiple status signals that settle contests over food when unfamiliar individuals meet. The size of the yellow patch and the brightness of the black patch each predict different aspects of the social interaction (Chaine et al. 2011). The relative size of the gold patch, which we have here found to correlate with heterozygosity, predicts whether escalation will occur in a contest—individuals with similar gold crown patch sizes are more likely to escalate, the interaction and engage in aggressive behaviors (Chaine et al. 2011). Gold crown feathers depend on the acquisition of carotenoid molecules from external food sources (Hill & Montgomerie 1994; McGraw 2006a). The positive relationship detected between gold crown patch sizes and increased heterozygosity (Figure 3) may be a result of carotenoid-based plumage coloration being an indicator of nutritional condition during molt (Hill & Montgomerie 1994). Variation in nutritional condition could result from differences among individuals in either their foraging ability or their health (Hill & Montgomerie 1994). The effect size we calculated for this gold patch size and IR (0.077) is much higher than effect sizes previously reported for morphological traits in general (average effect sizes of 0.013 for 29 reported effect sizes reviewed in Chapman et al. 2009; these are based on IR estimates of heterozygosity). Although the physiological mechanism driving the relationship between heterozygosity and plumage in golden-crowned sparrows is unknown, an increasing number of examples suggest there is a true pattern. It is possible that plumage may reflect the general quality of the individual. Dunn et al. (2012) report links between badge size and immune function heterozygosity in common yellowthroats (*Geothlypis trichas*); the size of the melanin based plumage mask correlates with MHC heterozygosity and also with survival and mating success. In three-spined stickleback (*Gasterosteus aculeatus*), MHC heterozygosity correlates with red coloration in males, a carotenoid based signal (Jager et al. 2007).

Sociality. While HFCs with life-history, morphological, and physiological traits are common in the literature (Chapman et al 2009), less is known about the influence of heterozygosity on social behaviors (see exceptions, in social primates, Charpentier et al. 2008; in nine-spined stickleback (*Pungitius pungitius*; Laine et al. 2012). In this study, non-kin individuals (Arnberg et al. manuscript) in a social population of golden-crowned sparrows (Shizuka et al. 2014) showed a positive relationship between heterozygosity and two measures of sociality. Since both measures were highly correlated, we focus on the within-community social measure since this measurement standardizes the node strength measure by community. Within-community node strength is a measure of the degree to which an individual is a "core" individual of a given community. Nodes with high within-community strength are more likely to be found flocking with other birds of the community. Furthermore, the results of social structure in this population is strongly influence by social preference of individuals and not by spatial segregation of individuals (Shizuka et al. 2014). To our knowledge, no study has investigated heterozygosity affects on sociality, as calculated by metrics used to construct social networks. As with social dominance, "core" individuals of a community may also be older or higher quality individuals. "Core" individuals interact and have contact with others in the community and may therefore be at higher risk of encountering disease, parasite, and pathogens. If our measure of heterozygosity reflects immune function heterozygosity, then more heterozygous individuals may be more likely to be "core" individuals.

Whether the underlying mechanism behind associations between behavioral/morphological traits and genetic diversity are driven by general effects or local effects remains a key area of discussion in many studies (Laine et al. 2012; Szulkin et al. 2010). Evidence for genome-wide diversity driving significant patterns include repeated random subsets of markers giving rise to strong heterozygosity-heterozygosity correlations, however this usually does not hold for randomly mating populations (Slate & Pemberton 2002). Since levels of heterozygosity among markers within individuals were not correlated in our study, our findings do not provide evidence for the role of genome-wide heterozygosity underlying relationships with behavioral/morphological traits in golden-crowned sparrows in a nonbreeding context. However, failure to detect genome-wide heterozygosity using HHC should not be interpreted as strong evidence that our HFCs were not caused by inbreeding depression (Kardos et al. 2014).

The underlying mechanism driving the HFCs detected in our study is unknown. Associations between unlinked loci (i.e., identify disequilibrium; Weir & Cockerham 1973) are generated by inbreeding in the population and are detected by calculating heterozygosity-heterozygosity correlations (HHC) under the assumption that heterozygosity at marker loci reflects genome-wide variation. We have no direct evidence for general effects driving HFCs in our study since our HHC were non-significant. However, Kardos et al. (2014) suggest that identity disequilibrium may not be detectable for a large proportion of populations with statistically significant HFCs unless many loci were used (100 microsatellites) (see also Miller & Coltman 2014). They conclude that their results suggest that failing to detect identity disequilibrium in HFC studies should not be taken as evidence that inbreeding depression is absent. Also, the fact that we did not detect identity disequilibrium does not then provide support for a local effects explanation (Kuepper et al. 2010).

We have no direct evidence for local effects driving detected HFC patterns. Although the procedure outlined in Szulkin et al. (2010) allows for statistically appropriate and rigorous testing of local effects, it would be difficult to detect local effects since the multi-locus heterozygosity regressions are already weak (R^2 range from 0.077 to 0.11). Although our data detect weak correlations, they are similar to many studies that report significant but moderate effect sizes (reports of $R^2 < 0.10$ for a variety of traits; Coltman & Slate 2003). The strongest correlations have been published from studies on inbred rabbits *Oryctolagus cuniculus* ($R^2 = 0.31$ using 29 loci; Gage et al 2006) and on common frogs *Rana tempraria* (R^2 values ranged between 0.36 and 0.51 using 4 loci; Lesbarreres et al. 2005). Even with weak correlations, many studies have detected local effects by searching for significant HFCs at a single locus (e.g., Hansson et al. 2004; Van Oosterhout et al. 2004). Earlier papers suggest that significant correlations with fewer loci are likely to be due to local effects through linkage to candidate regions, as opposed to general effects (Hoffman et al. 2010). We therefore tested for marker neutrality and found no homology between the markers we used in this study and published avian ESTs. However, most of these studies were published before Szulkin et al. (2010) and may have used inappropriate tests to detect correlations between individual markers and fitness-related traits by calculating correlations between all individual loci with each of those traits. These results should be interpreted with some caution since this approach increases the probability of Type I statistical errors (Kempenaers 2007). We are confident in our single locus effect results based on our current data since we compared models using multi-locus heterozygosity measures with models using a multiple regression with each individual locus and found that the models that used the multi-locus heterozygosity measure (IR) were better fit models.

A proposed explanation for the presence of HFCs despite lack of evidence for either general or local effects is the possibility of population structure in a study population (Slate & Pemberton 2006). HFC could be detected if there is systematic variation in mean heterozygosity across each subpopulation, and subpopulations vary in fitness-related traits (i.e., variation across environmental quality across geographic areas). Although it is possible that cryptic population structure could drive HFCs detected in our study even in the absence of general and local effects, we have previously shown that there is no genetic structure in this wintering population of golden-crowned sparrows (Arnberg et al. manuscript). It seems unlikely then that population structure explains the HFCs observed in our study.

There are studies that report significant HFCs without clearly establishing the causes behind the HFC (e.g., Foerster et al. 2003), possibly a result of a limited data set. We suggest screening more individuals at more loci to have greater power to detect whether general effects are driving the HFCs in our study. Furthermore, to our knowledge, there have been no published studies that provide evidence for local effects using the procedure outlined in Szulkin et al. (2010).

References

Alho, J. S., Valimaki, K., & Merila, J. (2010). Rhh: an R extension for estimating multilocus heterozygosity and heterozygosity–heterozygosity correlation. *Molecular ecology resources*, *10*(4), 720-722.

Allendorf FW & Leary RF (1986) Heterozygosity and fitness in natural populations of animals. *Conservation biology: the science of scarcity and diversity*, 57-76.

Amos, W., Wilmer, J. W., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D., & Coulson, T. (2001). The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*(1480), 2021-2027.

Aparicio, J. M., Ortego, J., & Cordero, P. J. (2006). What should we weigh to estimate heterozygosity, alleles or loci?. *Molecular Ecology*, *15*(14), 4659-4665.

Arcese, P., & Smith, J. N. M. (1985). Phenotypic correlates and ecological consequences of dominance in song sparrows. *The Journal of Animal Ecology*, 817-830.

Aureli, F., Schaffner, C. M., Boesch, C., Bearder, S. K., Call, J., Chapman, C. A., ... & Van Schaik, C. P. (2008). Fission□fusion dynamics. *Current Anthropology*, *49*(4), 627-654.

Baker, M. C., & Fox, S. F. (1978a). Dominance, survival, and enzyme polymorphism in dark-eyed juncos, Junco hyemalis. *Evolution*, 697-711.

Baker, M. C., & Fox, S. F. (1978b). Differential survival in Common Grackles sprayed with turgitol. *American Naturalist*, 675-682.

Balloux, F., Amos, W., & Coulson, T. (2004). Does heterozygosity estimate inbreeding in real populations?. *Molecular Ecology*, *13*(10), 3021-3031.

Biro PA, Stamps JA *(*2010*)* Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology and Evolution*, 25, 653–659.

Blanchet, S., Bernatchez, L., & Dodson, J. J. (2009). Does interspecific competition influence relationships between heterozygosity and fitness-related behaviors in juvenile Atlantic salmon (Salmo salar)?. *Behavioral ecology and sociobiology*, *63*(4), 605-615.

Boutin-Ganache, I., Raposo, M., Raymond, M., & Deschepper, C. F. (2001). M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques*, *31*(1), 24-6.

Careau V, Thomas D, Humphries MM, Réale D (2008) Energy metabolism and animal personality. *Oikos*, 117, 641–653.

Careau V, Thomas D, Pelletier F et al. (2011) Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (*Peromyscus maniculatus*). *Journal of Evolutionary Biology*, 24, 2153–2163.

Chaine, A. S., Tjernell, K. A., Shizuka, D., & Lyon, B. E. (2011). Sparrows use multiple status signals in winter social flocks. *Animal Behaviour*, *81*(2), 447-453.

Chaine, A. S., Roth, A. M., Shizuka, D., & Lyon, B. E. (2013). Experimental confirmation that avian plumage traits function as multiple status signals in winter contests. *Animal Behaviour*, *86*(2), 409-415.

Chapman, J. R., Nakagawa, S., Coltman, D. W., Slate, J., & Sheldon, B. C. (2009). A quantitative review of heterozygosity–fitness correlations in animal populations. *Molecular Ecology*, *18*(13), 2746-2765.

Chapman, J. R., & Sheldon, B. C. (2011). Heterozygosity is unrelated to adult fitness measures in a large, noninbred population of great tits (Parus major). *Journal of evolutionary biology*, *24*(8), 1715-1726.

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics*, *10*(11), 783-796.

Charpentier, M. J., Williams, C. V., & Drea, C. M. (2008). Inbreeding depression in ring-tailed lemurs (Lemur catta): genetic diversity predicts parasitism, immunocompetence, and survivorship. *Conservation Genetics*, *9*(6), 1605-1615.

Coltman, D. W., Pilkington, J. G., Smith, J. A., & Pemberton, J. M. (1999). Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution*, 1259-1267.

Coltman, D. W., & Slate, J. (2003). Microsatellite measures of inbreeding: A meta□analysis. *Evolution*, *57*(5), 971-983.

Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., ... & Clutton-Brock, T. H. (1998). Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1395), 489-495.

Cutts CJ, Metcalfe NB, Taylor AC (1998) Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard. *Journal of Fish Biology*, 52, 1026–1037.

Cutts CJ, Metcalfe NB, Taylor AC (1999) Competitive asymmetries in territorial juvenile Atlantic salmon, *Salmo salar. Oikos*, 86, 479–486.

Da Silva, A., Luikart, G., Yoccoz, N. G., Cohas, A., & Allaine, D. (2006). Genetic diversity-fitness correlation revealed by microsatellite analyses in European alpine marmots (Marmota marmota). *Conservation Genetics*, *7*(3), 371-382.

David, P. 1997. Modeling the genetic basis of heterosis: tests of alternative hypotheses. Evolution 51: 1049-1057.

Dunn, P. O., Whittingham, L. A., Freeman-Gallant, C. R., & DeCoste, J. (2008). Geographic variation in the function of ornaments in the common yellowthroat Geothlypis trichas. *Journal of Avian Biology*, *39*(1), 66-72.

Ekman, J. (1990). Alliances in winter flocks of willow tits; effects of rank on survival and reproductive success in male-female associations. *Behavioral Ecology and Sociobiology*, *26*(4), 239-245.

Figuerola, J., & Carlos Senar, J. (2007). Serins with intermediate brightness have a higher survival in the wild. *Oikos*, *116*(4), 636-641.

Freeman, L. C., 1977. A set of measures of centrality based up on betweenness. Sociometry 40, 35–41.

Freeman, L. C., 1979. Centrality in social networks: Conceptual clarification. Social Networks 1, 215–239.

Frydenberg, O. (1963). Population studies of a lethal mutant in *Drosophila melanogaster*. *Hereditas*, *50*(1), 89-116.

Gage, M. J., Surridge, A. K., Tomkins, J. L., Green, E., Wiskin, L., Bell, D. J., & Hewitt, G. M. (2006). Reduced Heterozygosity Depresses Sperm Quality in Wild Rabbits, *Oryctolagus cuniculus*. *Current Biology*, *16*(6), 612-617.

Gammell, M. P., de Vries, H., Jennings, D. J., Carlin, C. M., & Hayden, T. J. (2003). David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. *Animal behaviour*, *66*(3), 601-605.

Garcia-Navas, V., Ortego, J., & Sanz, J. J. (2009). Heterozygosity-based assortative mating in blue tits (Cyanistes caeruleus): implications for the evolution of mate choice. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1669), 2931-2940.

Guimera, R., & Amaral, L. A. N. (2005). Functional cartography of complex metabolic networks. *Nature, 433*(7028), 895-900.

Hansson, B., & Westerberg, L. (2002). On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology, 11*(12), 2467-2474.

Hansson, B., Westerdahl, H., Hasselquist, D., Åkesson, M., & Bensch, S. (2004). Does Linkage Disequilibrium Generate Heterozygosity-Fitness Correlations in Great Reed Warblers? *Evolution*, 870-879.

Hansson, B., & Westerberg, L. (2008). Heterozygosity-fitness correlations within inbreeding classes: local or genome-wide effects?. *Conservation Genetics, 9*(1), 73-83.

Hill, G. E. & Montgomerie, R. 1994. Plumage colour signals nutritional condition in the house finch. Proceedings of the Royal Society B, 258, 47-52.

Hoffman, J. I., Hanson, N., Forcada, J., Trathan, P. N., & Amos, W. (2010). Getting long in the tooth: a strong positive correlation between canine size and heterozygosity in Antarctic Fur Seals Arctocephalus gazella. *Journal of Heredity, 101*(5), 527-538.

Hoglund, J., Piertney, S. B., Alatalo, R. V., Lindell, J., Lundberg, A., & Rintamäki, P. T. (2002). Inbreeding depression and male fitness in black grouse. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1492), 711-715.

Jager, I., Eizaguirre, C., Griffiths, S. W., Kalbe, M., Krobbach, C. K., Reusch, T. B. H., ... & Milinski, M. (2007). Individual MHC class I and MHC class IIB diversities are associated with male and female reproductive traits in the three□spined stickleback. *Journal of evolutionary biology*, *20*(5), 2005-2015.

Jawor, J. M. & Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. Auk, 120, 249—265.

Kalinowski, S. T., Wagner, A. P., & Taper, M. L. (2006). ML□RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes, 6*(2), 576-579.

Kardos, M., Allendorf, F. W., & Luikart, G. (2014). Evaluating the role of inbreeding depression in heterozygosity□fitness correlations: how useful are tests for identity disequilibrium? *Molecular ecology resources*, *14*(3), 519-530.

Katti, M. (2001). Vocal communication and territoriality during the non-breeding season in a migrant warbler. *Current Science*, *80*(3), 419-423.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230-241.

Kempenaers, B. (2007). Mate choice and genetic quality: a review of the heterozygosity theory. *Advances in the Study of Behavior*, *37*, 189-278.

Ketterson, E. D., & King, J. R. (1977). Metabolic and behavioral responses to fasting in the white-crowned sparrow (Zonotrichia leucophrys gambelii). *Physiological Zoology*, 115-129.

Kriner, E., & Schwabl, H. (1991). Control of winter song and territorial aggression of female robins (Erithacus rubecula) by testosterone. *Ethology*, *87*(1□2), 37-44.

Kuepper, C., Kosztolanyi, A., Augustin, J., Dawson, D. A., Burke, T., & Szekely, T. (2010). Heterozygosity□fitness correlations of conserved microsatellite markers in Kentish plovers Charadrius alexandrinus. *Molecular ecology*, *19*(23), 5172-5185.

Laine, V. N., Herczeg, G., Shikano, T., & Primmer, C. R. (2012). Heterozygosity–behaviour correlations in nine□spined stickleback (Pungitius pungitius) populations: contrasting effects at random and functional loci. *Molecular ecology*, *21*(19), 4872-4884.

Lahti K, Huuskonen H, Laurila A, Piironen J *(*2002*)* Metabolic rate and aggressiveness between Brown Trout populations. *Functional Ecology,* 16, 167–174.

Lambrechts, M., & Dhondt, A. A. (1986). Male quality, reproduction, and survival in the great tit (Parus major). *Behavioral Ecology and Sociobiology*, *19*(1), 57-63.

Lens L, Van Dongen S, Galbusera P, Schenck T, Matthysen E (2000) Developmental instability and inbreeding in natural bird populations exposed to different levels of habitat disturbance. J Evol Biol 13: 889–896.

Lesbarreres, D., Primmer, C. R., Laurila, A., & Merilae, J. (2005). Environmental and population dependency of genetic variability□fitness correlations in Rana temporaria. *Molecular Ecology*, *14*(1), 311-323.

Lieutenant□Gosselin, M., & Bernatchez, L. (2006). Local heterozygosity□fitness correlations with global positive effects on fitness in threespine stickleback. *Evolution*, *60*(8), 1658-1668.

Luquet et al 2011; Luquet, E., David, P., Lena, J. P., Joly, P., Konecny, L., Dufresnes, C., ... & Plenet, S. (2011). Heterozygosity–fitness correlations among wild populations of European tree frog (*Hyla arborea*) detect fixation load. *Molecular ecology*, *20*(9), 1877-1887.

MacDougall-Shackleton, E. A., Derryberry, E. P., Foufopoulos, J., Dobson, A. P., & Hahn, T. P. (2005). Parasite-mediated heterozygote advantage in an outbred songbird population. *Biology Letters*, *1*(1), 105-107.

McFarland, R., & Majolo, B. (2013). Coping with the cold: predictors of survival in wild Barbary.

McGraw, K. J. 2003. Melanins, metals, and mate quality. Oikos, 102, 402-406.

McGraw, K. J. 2006a. The mechanics of carotenoid coloration in birds. In: Bird Coloration 1: Mechanisms and Measurements (Ed. by G. Hill & K. McGraw), pp. 177-242. Cambridge, Massachusetts: Harvard University Press.

McGraw, K. J. 2006b. Mechanics of melanin-based coloration. In: Bird coloration 1: Mechanisms and Measurements (Ed. by G. Hill & K. McGraw), pp. 243-294. Cambridge, Massachusetts: Harvard University Press.

Merilae, J., & Sheldon, B. C. (1999). Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity*, *83*(2), 103-109.

Miller, J. M., & Coltman, D. W. (2014). Assessment of identity disequilibrium and its relation to empirical heterozygosity fitness correlations: a meta□analysis. *Molecular ecology*, *23*(8), 1899-1909.

Mueller, J. C., Hermisson, J., Olano-Marin, J., Hansson, B., & Kempenaers, B. (2010). Linking genetic mechanisms of heterozygosity-fitness correlations to footprints of selection at single loci. *Evolutionary ecology*, *25*(1), 1-11.

Murtaugh PA (2009) Performance of several variable-selection methods applied to real ecological data. Ecological Letters, 12, 1061–1068.

Nakagawa, S., Lee, J. W., Woodward, B. K., Hatchwell, B. J., & Burke, T. (2008). Differential selection according to the degree of cheating in a status signal. *Biology letters*, *4*(6), 667-669.

Norment, C. J., Hendricks, P. & Santonocito, R. 1998. Golden-crowned sparrows (*Zonotrichia atricapilla*). In: The Birds of North America (Ed. by A. Poole & F. Gill). Philadelphia: The Academy of Natural Sciences/Washington, D.C.: The American Ornithologists' Union.

Ohta, T. (1971). Associative overdominance caused by linked detrimental mutations. *Genetical research*, *18*(03), 277-286.

Olano-Marin, J., Dawson, D. A., Girg, A., Hansson, B., Ljungqvist, M., Kempenaers, B., & Mueller, J. C. (2010). A genome-wide set of 106 microsatellite markers for the blue tit (Cyanistes caeruleus). *Molecular ecology resources*, *10*(3), 516-532.

Olano-Marin, J., Mueller, J. C., & Kempenaers, B. (2011). Heterozygosity and survival in blue tits (Cyanistes caeruleus): contrasting effects of presumably functional and neutral loci. *Molecular ecology*, *20*(19), 4028.

Piper, W. H., & Wiley, R. H. (1990). The relationship between social dominance, subcutaneous fat, and annual survival in wintering white-throated sparrows (Zonotrichia albicollis). *Behavioral Ecology and Sociobiology*, *26*(3), 201-208.

Poesel, A., Gibbs, H. L., & Nelson, D. A. (2009). Twenty□one novel microsatellite DNA loci isolated from the Puget Sound white□crowned sparrow, Zonotrichia leucophrys pugetensis. *Molecular ecology resources*, *9*(3), 795-798.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL http://www.R-project.org/.

Raymond, M., & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of heredity*, *86*(3), 248-249.

Reid, D., Armstrong, J. D., & Metcalfe, N. B. (2012). The performance advantage of a high resting metabolic rate in juvenile salmon is habitat dependent. *Journal of Animal Ecology*, *81*(4), 868-875.

Rousset, F. (2008). genepop'007: a complete re□implementation of the genepop software for Windows and Linux. *Molecular ecology resources*, *8*(1), 103-106.

Reale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological reviews*, *82*(2), 291-318.

Rhodes Jr, O. E., Smith, L. M., & Chesser, R. K. (1993). Temporal components of genetic variation in migrating and wintering American wigeon. *Canadian Journal of Zoology*, *71*(11), 2229-2235.

Rhodes Jr, O. E., Smith, L. M., & Smith, M. H. (1996). Relationships between genetic variation and body size in wintering mallards. *The Auk*, 339-345.

Røskaft, E., Järvi, T., Bakken, M., Bech, C., & Reinertsen, R. E. (1986). The relationship between social status and resting metabolic rate in great tits (Parus major) and pied flycatchers (Ficedula hypoleuca). *Animal Behaviour*, *34*(3), 838-842.

Seavy, N. E., Humple, D. L., Cormier, R. L., & Gardali, T. (2012). Establishing the breeding provenance of a temperate-wintering North American passerine, the Golden-crowned Sparrow, using light-level geolocation. *PloS one*, *7*(4), e34886.

Seddon N, Amos W, Mulder RA, Tobias JA (2004) Male heterozygosity predicts territory size, song structure and reproductive success in a cooperatively breeding bird. Proc R Soc B 271: 1823–1829.

Shizuka, D., Chaine, A. S., Anderson, J., Johnson, O., Laursen, I. M., & Lyon, B. E. (2014). Across□year social stability shapes network structure in wintering migrant sparrows. *Ecology Letters*.

Slate, J., & Pemberton, J. M. (2002). Comparing molecular measures for detecting inbreeding depression. *Journal of Evolutionary Biology*, *15*(1), 20-31.

Slate, J., & Pemberton, J. (2006). Does reduced heterozygosity depress sperm quality in wild rabbits *Oryctolagus cuniculus*?. *Current Biology*, *16*(18), R790-R791.

Slate, J., David, P., Dodds, K. G., Veenvliet, B. A., Glass, B. C., Broad, T. E., & McEwan, J. C. (2004). Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity*, *93*(3), 255-265.

Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*, *19*(2), 448-455.

Stenzler, L. M., Fraser, R., & Lovette, I. J. (2004). Isolation and characterization of 12 microsatellite loci from Golden□winged Warblers (Vermivora chrysoptera) with broad cross□taxon utility in emberizine songbirds. *Molecular Ecology Notes*, *4*(4), 602-604.

Szulkin, M., Bierne, N., & David, P. (2010). Heterozygosity-fitness correlations: a time for reappraisal. *Evolution*, *64*(5), 1202-1217.

Tiira, K., Laurila, A., Peuhkuri, N., Piironen, J., Ranta, E., & Primmer, C. R. (2003). Aggressiveness is associated with genetic diversity in landlocked salmon (Salmo salar). *Molecular Ecology*, *12*(9), 2399-2407.

Tiira, K., Laurila, A., Enberg, K., Piironen, J., Aikio, S., Ranta, E., & Primmer, C. R. (2006). Do dominants have higher heterozygosity? Social status and genetic variation in brown trout, Salmo trutta. *Behavioral Ecology and Sociobiology*, *59*(5), 657-665.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO□CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535-538.

Vilhunen, S., Tiira, K., Laurila, A., & Hirvonen, H. (2008). The bold and the variable: fish with high heterozygosity act recklessly in the vicinity of predators. *Ethology*, *114*(1), 7-15.

Weir, B. S., & Cockerham, C. C. (1973). Mixed self and random mating at two loci. *Genetical research*, *21*(03), 247-262.

Tables and Figures

Table 2.1 Features of the 14 microsatellite loci used in this study to calculate heterozygosity, including the size range (base pairs), number of alleles, annealing temperature (T), observed heterozygosity (H_O), expected heterozygosity (H_E), and accession number. *Poesel et al. 2009 □Stenzler et al. 2004

Locus	Size Range	# Alleles	T (°C)	H_O	H_E	Accession #
GCSPR1*	300-524	34	59	0.98	0.96	EU410382
GCSPR2*	167-207	11	59	0.97	0.87	EU410383
GCSPR3*	252-288	9	59	0.90	0.83	EU410384
GCSPR5*	226-274	12	59	0.92	0.85	EU410386
GCSPR9*	194-243	9	59	0.92	0.83	EU410390
GCSPR10*	186-280	18	59	0.90	0.90	EU410391
GCSPR11*	171-215	12	59	0.88	0.89	EU410392
GCSPR12*	221-261	18	59	0.95	0.87	EU410393
GCSPR14*	188-244	13	59	0.92	0.87	EU410395
GCSPR15*	186-230	10	59	0.86	0.83	EU410396
GCSPR16*	230-278	12	59	0.93	0.86	EU410397
GCSPR17*§	200-288	16	59	0.72	0.89	EU410398
GCSPR19*	172-216	12	65	0.62	0.58	EU410400
GCSPR30□	312-352	13	59	0.81	0.80	AY542879

§Loci significantly deviated from Hardy-Weinberg equilibrium but not after sequential Bonferroni correction for multiple tests.

Table 2.2 Heterozygosity-heterozygosity correlations (Balloux et al. 2004) for 53 individuals using 14 microsatellite loci. None of the three heterozygosity measures show significant correlations in 1000 randomizations.

Heterozygosity measure	Mean *r*	95% Quantile
H_s	0.013	–0.105 – 0.148
IR	–0.001	–0.128 – 0.123
HL	–0.029	–0.151 – 0.104

Figure 2.1 Relationship between internal relatedness, a multilocus heterozygosity measure, and social dominance in golden-crowned sparrows: $R^2 = 0.084$, $F_{1,51} = 4.709$, $p = 0.034$.

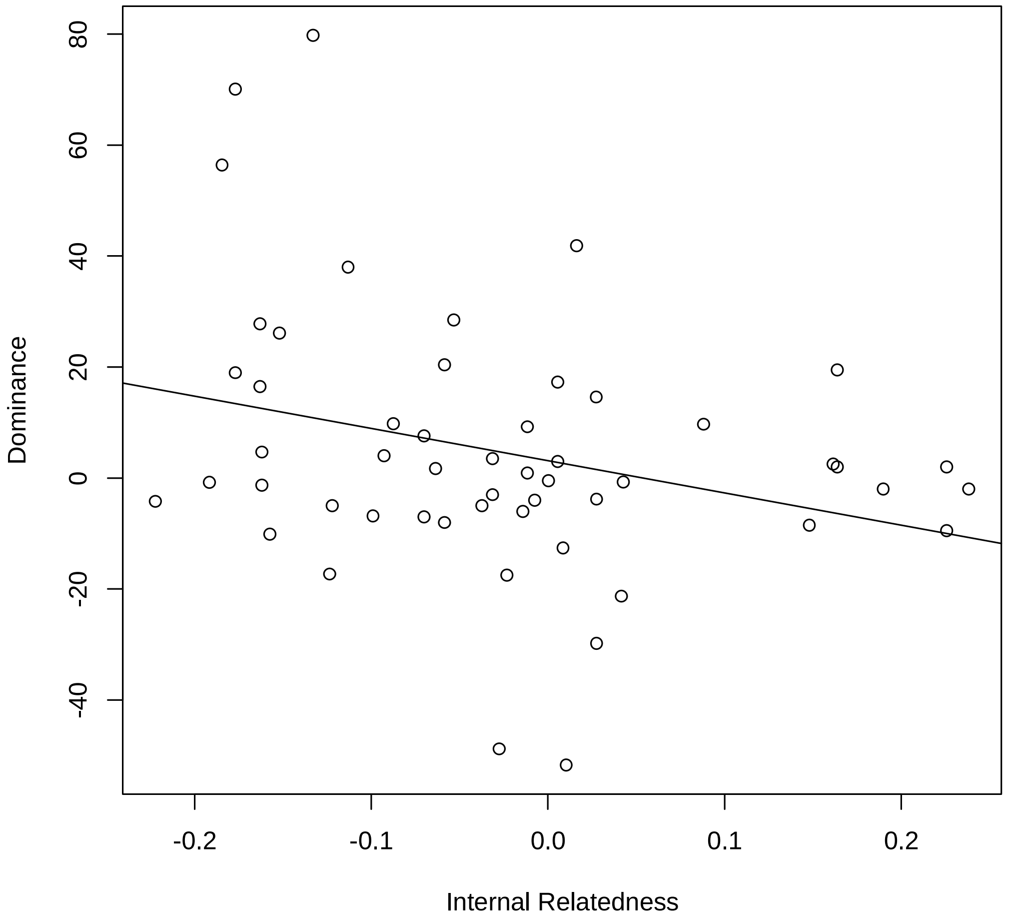
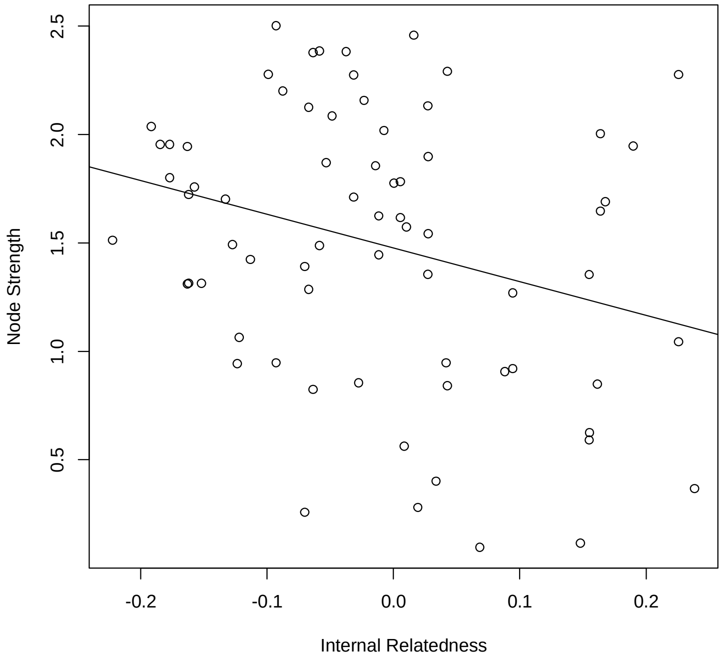


Figure 2.2 Relationships between internal relatedness and two measures of sociality in golden-crowned sparrows.

A: Node strength.



B: Within-community node strength.

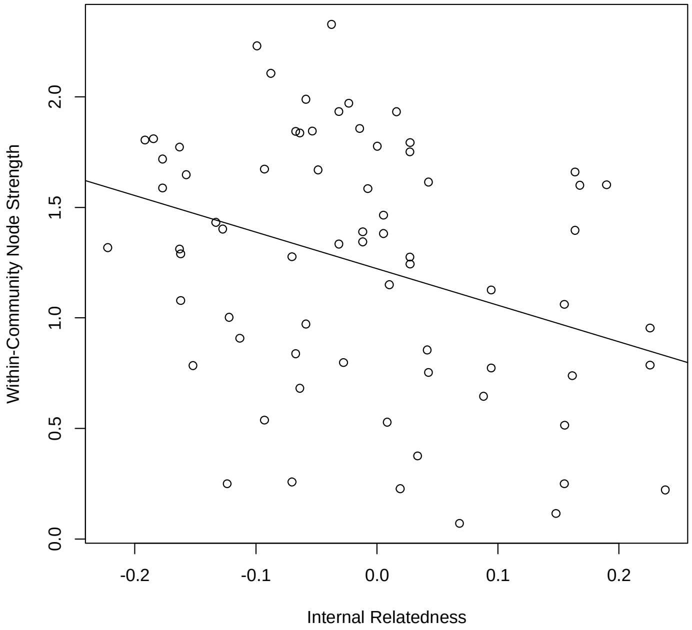


Figure 2.3 Relationship between internal relatedness and plumage (gold patch size) in golden-crowned sparrows.

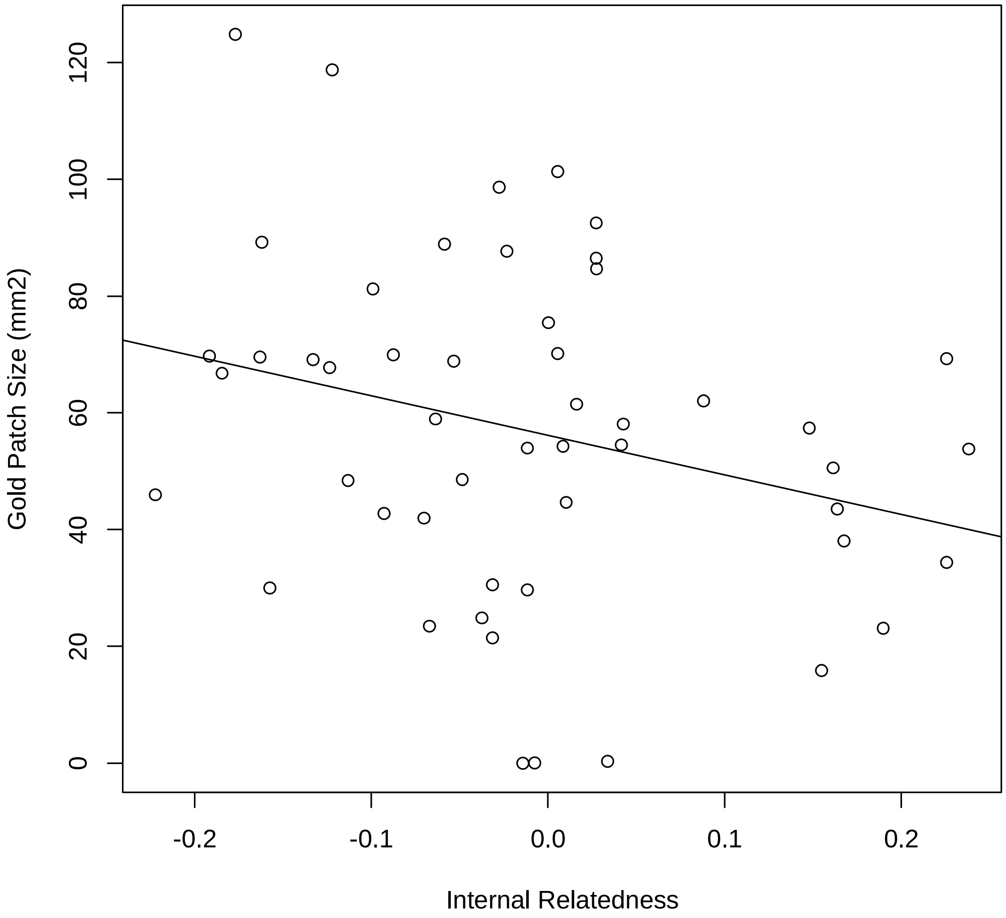
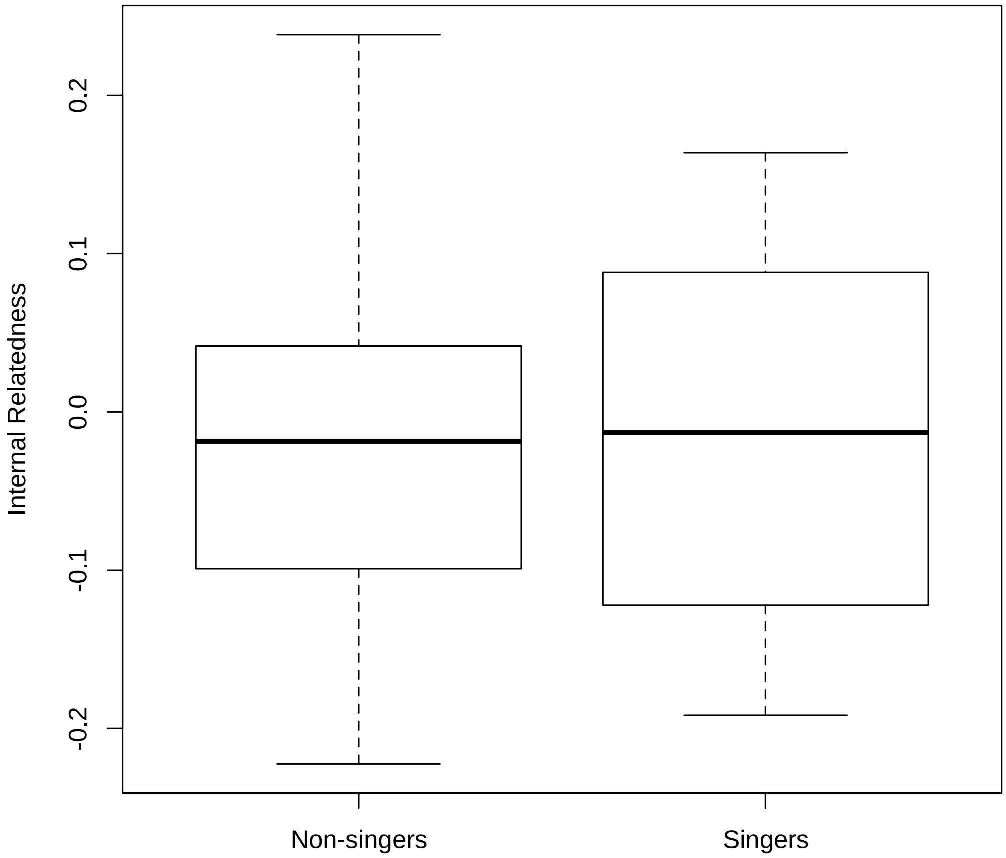


Figure 2.4 Singing status is not associated with heterozygosity. Bar lines at median, boxes outline quartiles, and dashed lines at minimums and maximums.



CHAPTER 3

Supporting the articulation of engineering solutions: An operational definition of engineering requirements

Abstract

Background Engineers solve problems and justify their solutions. Argumentation is the means by which engineers rationally solve problems. Engaging in argumentative practices promotes content knowledge and communication skills in many disciplines but has not been explored deeply in the engineering education literature. We focused our attention on improving learners' engineering argumentation practices with a particular emphasis on articulation of engineering solutions.

Purpose The goals of this research were to clarify our preliminary conceptualization of engineering argumentation by (1) operationally defining a key component in the articulation of engineering solutions: *requirements*, and (2) providing engineering educators and mentors with suggestions for practical implications to improve learners' articulation of engineering solutions.

Method Collected data from self-reported answers from learners (interns) and from their final presentations were analyzed using the constant comparative method. We focused on one critical content component, requirements, for the rest of the study as we found it to be central and challenging to the articulation of an engineering solution.

Results We identified three key challenges faced by interns when articulating project requirements. Interns identified constraints as requirements, identified non-functional

requirements as functional requirements, and did not state functional requirements in a verifiable manner. To address these challenges, we propose an operational definition of requirements in engineering that has three components: functional requirements (what a solution must do), non-functional requirements (qualities that a solution must have), and constraints (limitations on possible solutions). Of the three, functional requirements are most relevant because a viable solution *must* meet all functional requirements. Furthermore, functional requirements must (1) be relevant to the problem/need (2) focus on what the solution must do or accomplish (actions a solution must take) and (3) be verifiable, stated in a manner where action is completed or not completed.

Conclusion We argue that identifying an engineering project's functional requirements is critical to effectively articulating a proposed engineering solution. Our operational definition of requirements, specifically the description and examples of functional requirements, may be used by engineering educators and mentors to support learners' and interns' articulation of solutions. We also offer a number of suggestions for implementing these ideas.

Introduction

Engineers solve problems; engineering practice is problem solving (Sheppard et al. 2009). Engineers seek to generate well-conceived solutions to technological challenges (National Academy of Engineering 2008). In the course of their work, they sift and sort through many variables, settle on potential solutions, and attempt to persuade others regarding the merit of these solutions. In so doing, they make claims that must be supported by evidence. Establishing the validity and viability of such claims entails the ability to clearly state a case, respond to questions, and defend a position; argumentation, in a word.

Argumentation is the means by which we rationally resolve questions, issues, and solve problems (Siegel 1995). Engaging in argumentative practices promotes content knowledge and communication skills in many disciplines. Science education researchers have extensively studied argumentation concluding that it is central to scientific thinking (Driver et al. 2000; Kuhn 1993; Newton et al. 1999). More recently, a few studies have highlighted the importance of argumentation as an essential skill in learning to solve problems (Jonassen 2007, 2010). Finally, argumentation is part of meaningful participation in science or engineering, and has cultural norms that may differ significantly from norms that apprentices are used to. Thus, gaining proficiency with argumentation can be mediated by an apprentice’s sociocultural background. Although it is central and essential to the process of engineering (Rugarcia et al. 2000), engineering argumentation has not been given the attention it merits.

Argumentation has been shown to support learning to solve complex and ill-structured problems. Cho & Jonassen (2002) investigated the production of coherent arguments to justify solutions and actions and found it to be an important skill for solving ill-structured problems. Ill-structured problems are the kinds of problems that are commonly encountered in everyday engineering practice and are characterized as having (a) alternative solutions to problems, (b) vaguely defined or unclear goals and constraints (c) multiple solution paths, and (d) multiple criteria for evaluating solutions (Jonassen 2000). Unlike well-structured problems encountered in formal education, ill-structured problems do not have single solutions and learners are tasked with constructing arguments that justify their proposed solution (Voss and Post 1988; Kuhn 1991; Jonassen 1997; Cho and Jonassen 2002; Shin et al. 2003).

Despite the important role of argumentation in solving complex engineering problems, research in engineering argumentation is sparse and is mostly found in non-educational contexts (e.g., Robertson & Robertson 2012; Stouffer et al. 2004). In the present study we focus on developing further this emerging concept. Our work is informed by research in scientific argumentation (Berland & Reiser 2009; Brewer, Chinn, & Samarapungavan, 1998; Giere, 1988; McNeill, Lizotte, Krajcik, & Marx, 2006; McNeill & Krajcik, 2008; Nagel, 1979; Sampson & Clark, 2008; Toulmin 1958). We recognize that, although there are areas of overlap between scientific and engineering argumentation, there are fundamental areas where the two differ significantly (reviewed in J. Shaw, L. Hunter, N. Arnberg, *in prep*). Recently, the importance of understanding distinctions between science inquiry and engineering design have been highlighted the newly published *Next Generation Science Standards* (NGSS Lead States 2013), which requires that students also learn about engineering design.

One way to appreciate these differences has to do with the use of theories and models. Scientists regularly use theories and interpret models to explain empirical regularities in the world and to provide a principled basis for generalizing to novel cases. Engineers use theories and interpret models to understand the advantages and limitations of design-solutions to problems given specified parameters and criteria (Deek et al. 1999; NRC, 2009). In both cases scientists and engineers develop conceptual understanding as they engage in argumentation. Thus, while a scientist's claim may be analogous to an engineer's solution, the former may be generalizable while the latter is typically suitable for one particular context.

While both processes are non-linear, science depends on the inquiry process, the equivalent for engineering is the design process whose components include "identifying the problem; specifying requirements of the solution; decomposing the system; generating a solution; testing the solution; sketching and visualizing the solution; modeling and analyzing the solution; evaluating alternative solutions, as necessary; and optimizing the final design" (NRC, 2009 p. 120). For example, a major practice of scientists is planning and carrying out systematic investigations, which require identifying data to be recorded as well as dependent and independent variables (NRC, 2013). Engineers use investigations both to gain data essential for specifying design requirements and to test their designs. Like scientists, engineers must identify relevant variables, decide how they will be quantified, and collect data (NRC, 2013) but engineers use this information to justify a solution.

Although engineering solutions are highly context-specific, as with the engineering design process there are ways to conceptualize the articulation of such solutions in a more generalizable manner. Our evolving conceptual framework currently considers the generation of solutions as involving the application of problem solving skills which themselves rely on reasoning, in general, and argumentation, in particular. A review of the current literature and interviews we conducted with engineering professionals both highlight the importance of engineers' ability to persuasively argue for a particular engineering solution (e.g., Weiringa et al. 2006). We refer to the use of argumentation within the context of the engineering design process as "engineering argumentation" (further developed in J. Shaw, L. Hunter, N. Arnberg, *in prep*).

The research team's interest in engineering argumentation stems from our initial work and continued involvement with an internship program aimed at broadening participation in the science, technology, engineering, and mathematics (STEM) workforce. Within this context, we focused our attention on improving the undergraduate interns' engineering argumentation practices with a particular emphasis on the articulation of engineering solutions. This work yielded a tool called the Solution Articulation Framework or SAF, which we describe in more detail below. Using the SAF as an organizing frame, we analyzed intern comments about their projects as well as formal presentations on their projects to identify three key challenges interns faced when identifying engineering requirements—a key component of the SAF. Informed by our findings, we developed an operational definition of engineering requirements to address these specific challenges. We conclude with ideas for implications of our findings in engineering education.

Context for Study

As mentioned above, this study was conducted in the context of an undergraduate STEM workforce development initiative, namely the Akamai Internship Program, and involves a tool known as the Solution Articulation Framework (both of which are further described in this section). The study was conducted by a three person research team, all of whom are based at the Santa Cruz campus of the University of California and have ties to the Akamai Internship Program. Nina Arnberg is a doctoral student in Evolutionary Biology & Education. During her tenure as a graduate student, she has been the lead instructor for communication courses with two internship programs for science and engineering undergraduates, including the Akamai Internship Program. Lisa Hunter directs the Akamai Internship Program. Jerome Shaw is a professor of science education whose research and teaching focuses on improving science teaching and learning for culturally and linguistically diverse students. His involvement with the Akamai Internship Program has included instructing on topics such as scientific explanations and engineering solutions in addition to researching intern’s understanding of and engagement with those processes.

Akamai Internship Program

The Akamai Workforce Initiative (AWI) partners high-tech industry, astronomical observatories, cutting-edge research, and inventive education to meet needs in astronomy, remote sensing, and other technology industries in Hawaiʻi. The AWI provides training for a diverse student population through an innovative, culturally relevant curriculum designed to meet workforce needs. At the heart of the AWI are two internship programs that use a model developed by UC Santa Cruz’s Center for Adaptive Optics to retain and advance students into science and technology careers (Hunter et al. 2008). The Maui Internship Program places students at sites on the island of Maui, primarily high tech companies, for a seven-week research experience. On Hawaiʻi Island, the Akamai Internship Program uses the same model, but places students at astronomical observatories. Through a carefully designed set of programs and activities, the AWI advances students into the technology workforce on Maui, and, more broadly, across Hawaiʻi.

To meet the goal of broadening participation in the science, technology, engineering, and mathematics (STEM) workforce, the internship program was designed to increase retention of college students in STEM, in particular those from groups underrepresented in STEM and/or from communities underserved. Like many internship or research experience programs, the core activity for the students is an authentic experience in STEM. However, the program differs from others through an array of program components that provide extensive support for program participants to successfully complete a productive STEM project. The program is interwoven with a professional development program for scientists and engineers that focus on how to teach STEM practices, and has served as a teaching laboratory for more than a decade. For example, each year a team of early career scientists and engineers in the professional development program design and teach a preparatory course for the interns to improve their proficiency with the STEM practices they will be expected to use in their coming project (e.g. supporting an engineering solution). The research team became involved at a time when leaders of these two intertwined programs were grappling with defining argumentation in an engineering context. It had become clear that imposing the structure and components of a scientific explanation on an engineering solution was not adequately supporting learners. The research team identified a need for a framework for engineering solutions to complement existing frameworks for scientific explanations, and became integrated into a mature infrastructure of programs and people engaged in innovative science and engineering education. As will be described below, SAF is a both a heuristic as well as practical tool to help interns and others understand and communicate key components of a solution to an engineering problem.

The Solution Articulation Framework

In order to support interns' understanding of engineering argumentation, Shaw and colleagues (*in prep*) have proposed an engineering Solution Articulation Framework (SAF). Development of the SAF is ongoing; a portion of the process occurred concurrently with the research presented here. The SAF builds on the "claim-evidence-reasoning" model of scientific argumentation (McNeill, Lizotte, Krajcik, & Marx, 2006; McNeill & Krajcik, 2008; Sampson & Clark, 2008), the complementary notion of argumentation and explanation, and the dynamic engineering design process. This process begins with a need/problem and an investigation of that need/problem and includes the articulation of a new solution and a validation process of that solution resulting in an idea, theory, process, or physical product (Stouffer et al. 2004).

The SAF was developed to support learners' understanding of how professional engineers justify proposed engineering solutions by articulating how they arrived at the proposed solution. In its current state, the SAF lists six components considered essential to articulating an engineering solution: context, need, requirements, constraints, solution, and justification. An engineering *solution* is a potential way to address a specific human *need* or problem. To provide *justification* for the solution, evidence must be presented that convinces the audience that the solution meets each *requirement*—need-related specifications, *constraints*, and parameters. The component of *context* was included in the framework to ensure that individuals articulating their solutions provided enough background information for an audience to understand the engineering need that was being addressed.

As described above, an earlier version of the SAF subsumed *constraints* under the component of *requirements*. Discussions between program instructors and interns highlighted the complexity of differentiating between the two, which led to calling out constraints as its own component in subsequent versions. *Requirements* came to be described as "what the solution must do or accomplish" while *constraints* are "limits on tasks and resources used to reach the solution." This separation of constraints from requirements in the SAF sparked our interest in developing a more comprehensive definition for engineering requirements.

WHY FOCUS ON REQUIREMENTS?

Our research team focused on defining requirements based on several lines of evidence pointing to its importance. First, the research team's own experience with the challenging task of defining the requirements component in the Solution Articulation Framework paralleled interns' challenges in defining an engineering project's requirements. Ill-defined or undefined requirements led to unsupported justification for solutions. Second, interviews with professional engineers highlighted the importance of understanding engineering requirements as an early step that is revisited throughout the design process. Third, academic literature in engineering suggests that requirements must be specified before attempting to seek an appropriate solution. Finally, since developing the original SAF, the research team has continued to find the component of defining engineering requirements challenging. Our first-hand experience working alongside interns to support articulation of their engineering projects' solutions peaked our interest in investigating ways in which to support interns' articulation of requirements. We argue that a clear and effective definition of requirements is a crucial step in the iterative process of improving our SAF, in particular, and engineering argumentation, in general.

Subsequently, the research team solicited two sets of interviews, a one-on-one and focus group interview with professional engineers (who were themselves mentors of the engineering interns). Our first interview was conducted with a senior engineer with more than 16 years of experience in engineering projects. The interview was structured around discussing components that should be included in a comprehensive explanation of an engineering solution, particularly what information it should contain. The engineer repeatedly referred to the process of engineering a solution as a life cycle, the first step after identifying an engineering need should be identifying requirements. The engineer goes further by differentiating between different types of requirements: functional, parametric, operational, instrumental, and lifetime requirements.

> "We talk about projects in engineering solution … like a life cycle. You start with … the big picture … why is there a need for it. From there you flow [to] the requirements and between the requirements and solutions there is a huge amount of work there. … There are all different kinds of requirements generally you hear the term functional requirements."

We then conducted an interview with a focus group of four professional engineers centered on gathering feedback for the content and language included in the SAF framework. Some specific recommendations about the framework included focusing on requirements statements and ensuring that interns differentiated between mandatory, recommended, and desired requirements. They also suggested that these statements should be discrete (where an engineering requirement was met or not met by the engineering solution). Since interns work on a variety of projects spanning many disciplines, it is helpful to be explicit about what is included as a requirement.

> "When you deal with people from different backgrounds, different disciplines, they have different words for the same thing. It's like, ok: What does "requirements" mean, because it could mean a lot of things to a lot of people. Not just what is written in black and white on a [presentation] slide."

As the research team's importance on identifying engineering requirements were echoed by interviews with engineering professionals, we then referenced academic literature that could provide additional insight. To the best of our knowledge, there is no research on identifying and defining engineering requirements in the engineering education literature. We therefore relied on resources in the engineering literature. The process of engineering a solution involves creating the solved state while satisfying the solution requirements and constraints (Nickols 2003). Requirements play a major role in engineering of a system; they serve the role of defining the engineering design problem and capturing the key information needed to describe design decisions (Buede 2009). Functional requirements are statements of the specific performance of a design, i.e., what the solution should do. A clear definition of the functions is essential in design because the function is an abstract formulation of the task that the solution must accomplish but it is independent of any particular solution that is used to meet the requirements (Otto & Wood 2000). Requirements do not provide solutions but rather define the problem to be solved. Identifying requirements correctly is a very difficult task; requirements are not static throughout a project and may change as the project proceeds. The software engineering literature has recently focused on how to design solutions given dynamic requirements (see Agile Software Development, e.g., Beck et al. 2001).

Driving Questions

We argue that the support for and articulation of an engineering solution relies on the solution meeting the engineering requirements. With the original SAF as our template, the research presented in this paper was driven by 2 central questions.

1) What difficulties do learners face with defining requirements?

2) How can requirements be operationally defined to support their understanding and articulation by learners and educators?

Methodology

In a recent article in the Journal of Engineering Education, Case & Light (2011) outline emerging methodologies in the field of engineering education highlighting the need for researchers to explicitly justify decisions made when choosing study methodologies. We explicitly discuss why we chose this focus, what questions we were asking, how and why we designed the study and how we ensured our confidence in the data gathered and in our analyses of those data. We also justify the use of qualitative methods for this study by describing the framework within which they are situated.

Grounded Theory & Constant Comparative Method

Grounded theory (Glaser and Strauss 1967) was one of the first methodological positions supporting the use of qualitative data in social research. Corbin & Strauss (1994) describes it as “a general methodology for developing theory that is grounded in data systematically gathered and analyzed”. Olds and colleagues (2005) define qualitative research as characterized by the collection and analysis of textual data (surveys, interviews, focus groups, observation, etc). While numbers are used to summarize qualitative data, answering some research questions requires rich, contextual descriptions of the data, also referred to as "thick" description (Borrego et al. 2009). It is important to differentiate qualitative research (which is rigorous and involves data collection and analysis) with anecdotal information. Anecdotal information is collected haphazardly as it becomes available, while qualitative research involves the careful planning of a research design that considers the research questions and what data to collect (Borrego et al. 2009).

Borrego and colleagues (2009) further argue that the concept of *generalizability* in quantitative studies is replaced by the term *transferability* in qualitative studies. Quantitative research is focused on generalizing to the larger population independent of context, and thus there is a heavy emphasis in the research design on random sampling and statistical significance. In contrast, qualitative research seeks to generalize through thick description of a specific context, allowing the reader to make connections between the study and his or her own situation. In short, quantitative research places the burden of demonstrating generalizability on the researcher, while qualitative research places the burden of identifying appropriate contexts for transferability on the reader. Just as rigorous statistical analysis is essential in quantitative research to ensure reliability and generalizability of the results, so too is rich description of the context and experiences of the participants essential in qualitative research to ensure trustworthiness of the findings to transfer to other contexts.

Central to grounded theory is the idea that theory is generated from gathered data, instead of already existing theory being used to drive data analysis. This is particularly useful in studies such as ours where there are a limited number of studies and theories to guide the specifics of our research. Thus, we kept an open mind in the early phases of the research allowing emerging patterns in the data to drive the direction of the study.

A method that provides a clear outline of a process for analyzing such qualitative data is the constant comparative method. This method generates theory systematically and compares the meaning of indicators with one another to build or define a concept (Glaser 1978; Glaser & Strauss 1967). A recent paper published by Ahn and colleagues (2014) utilized this method to develop an operational definition of leadership, change, and synthesis in engineering. This approach is appropriate for studies, like ours, where there are a limited number of studies and theories to guide the research.

The constant comparative method follows a series of steps. In Phase I, the qualitative data is first transcribed into text then coded according to a phrase or an idea (rather than individual words). In Phase II, researchers descriptively label coded text. This allows for relationships to be identified among labels and categories can be generated. In Phase III, categories are then constantly compared to other categories with the aim to ground the categories in the data. Constantly comparing phrases and ideas for similarities and differences is an iterative process and was guided by questions proposed by Glaser (1978): What is going on here? What category or what property of category does this incident indicate? What is actually happening in the data? Finally, in Phase IV of the analysis, the researchers discuss emerging themes, such as common challenges faced by interns when tasked with identifying engineering requirements.

Data Sources & Collection

The primary source of data for this study was intern responses to a worksheet that asks them to describe the components of their project as outlined on the SAF (i.e., context, need, requirements, constraints, solution, and justification). Referred to as the *Clarifying Your Project* worksheet (provided in Figure 1), 14 interns in the 2012 Akamai program completed this worksheet twice.

Interns attended a Solution Articulation Framework workshop that the research team designed and conducted during the midpoint of the internship. We discussed the various components of the framework. Our goal was to make the interns aware of these important components as they were preparing to produce and present a final presentation on their summer engineering projects. In order to discuss these components as would be relevant to their projects, we presented a previous intern's project using the *Clarifying Your Project* worksheet. We then asked the interns to reflect on their own projects and presentations by filling out a *Clarifying Your Project* worksheet for their own work. Interns also completed a *Clarifying Your Project* worksheet at the end of the internship program, once the final symposium had been held. As a precursor, all Akamai interns were introduced to the SAF as part of the pre-work site placement short course held the week before the start of the internship program.

We also examined PowerPoint presentations submitted by the interns after the final symposium. We considered information included in the presentation notes section. Data from the following responses was pooled: 1) interns' self-reported answers from the *Clarifying Your Project* worksheets, 2) final PowerPoint presentations. The rationale behind pooling the data was that project requirements may not have been reported in the *Clarifying Your Project* worksheets but may have been presented in the PowerPoint. We used both sources of data to assess whether requirements had been articulated for each intern's project.

Data Analysis

Our analysis was divided into four phases. In Table 1, we provide examples for each of these phases for the purpose of clarification. In phase I, we listed all interns' a) self-reported responses from the requirement component of the SAF and b) translated into text requirements that were presented in interns' final presentations. All of the responses were written in response to "The solution must __________." We initially reviewed all 14 of the intern projects. We excluded two projects from further analysis as they were primarily focused on completing tasks rather than justifying a solution. Our original SAF was developed to support interns' justification of a solution to problem or need. Justifying a solution requires that there are alternate solutions to compare to, or some other means of comparison. If a project turned out to be more along the lines of a repair or the completion of a task, it was outside the boundary of what the SAF was designed for (and probably not what one would refer to as an engineering project).

In phase II, we generated descriptive labels for each requirement statement. For example, an intern's requirements statement was "be able to read a specified wavelength coverage of 1.2-1.8 micrometer". We generated labels that described what this statement included (e.g., action solution must take, stated in a manner where action is completed or not completed, measurable, target value presented, relevant to engineering problem/need).

In phase III, we identified relationships among labels and created preliminary categories. Phases II and III informed one another and the research team worked iteratively between these phases to develop appropriate descriptive labels and categories. For example, statements that included "action solution must take" were categorized as "functional requirements". The intern's statement presented in phase II included the following categories: functional requirements, verifiable, measurable, and relevant to engineering problem/need.

Finally, in phase IV, we compared and contrasted categories for overlap. We looked across categories and highlighted the most important relationships among labels that would allow a requirement statement to be justified. For example, the previous statement included functionality and was verifiable. We present the findings of this process as main challenges interns faced when identifying project requirements that could be justified.

In this study, the focus of our analysis was on the quality of the interns' requirement statements. Whether or not an individual intern's ability to articulate a requirement changed over time (e.g., as a result of the mid-point workshop) was beyond the scope of this study.

Findings

Our analysis led to the three main challenges that interns faced when defining their project's requirements: identifying constraints as requirements, identifying non-functional requirements as functional requirements, and not stating requirements in a verifiable manner. Although non-functional requirements and constraints are important to the design process and ultimately in the justification of a proposed solution, the most important component is identifying and articulating the project's functional requirements. We provide empirical examples below to clarify our findings. Informed by these challenges, we propose an operational definition of requirements relevant to engineering education.

Challenge 1: Identifying constraints as requirements.

Given the definitions provided on the *Clarifying Your Project* worksheet, interns were incorrectly stating as requirements (what the solution must do or accomplish) aspects of their project's solution that were actually constraints (limits or restrictions placed on a solution). Robertson & Robertson (2012) suggests that requirements must take an action – check, calculate, record, and retrieve. In other words, the solution must _______ (action the solution must take). We argue that constraints are limits that constrain the way the problem must be solved.

One example of this confusion was a project for which the intern was to design an alternative cooling system for a computer room to reduce power usage and cooling costs. As one of the requirements, the intern listed “keep the room within ASHRAE A2 accepted standard temperatures and humidity”. ASHRAE (American Society of Heating, Refrigerating, and Air-conditioning Engineers) publishes guidelines for temperature and humidity operating rages of IT equipment. A2 refers to the class of equipment that the given conditions are recommended for, e.g., volume servers, storage products, personal computers, and workstations. Justifying and testing for adherence to these guidelines is an important component to arguing for a proposed solution. However, keeping the computer within these standards is a design constraint on any proposed solution. Adherence to ASHRAE standards would be a constraint and not a project requirement.

In this example, the intern fails to communicate a requirement, there is no description of what the solution must do or accomplish. Examples of appropriate functional requirements for this project may include statements identifying what data must be received by the solution or what data must be produced by the solution? Central to the redesign of the computer room is that it must maintain its current functionality (a point that the intern fails to identify). For example a solution that meets ASHRAE standards, but in which the computers are no longer working properly is not a reasonable solution.

Confusing constraints for requirements may hinder interns from identifying any requirements, which brings into question the solution. That is, how did the intern test his solution? Was he satisfied that it met ASHRAE standards and did not consider the functionality of the computers? The lack of justifying that a proposed solution meets requirements (because no requirements were ever identified) weakens the overall argument for such a solution. Although we cannot differentiate between an intern's lack of communication and lack of understanding of their project's requirements, it is important to highlight that requirements statements are essential to the justification of an engineering solution.

Challenge 2: Identifying non-functional requirements as functional requirements.

In their review of the literature, Chung and Leite (2009) describe non-functional requirements as "how well" a solution should function (e.g., usability, flexibility, and security). This distinction proved to be another area that was challenging for interns. Intern responses to functional requirements included many non-functional requirements. Similar to the inclusion of constraints as functional requirements, when interns included non-functional requirements as functional requirements the intern may also fail to identify any functional requirements.

Robertson & Robertson (2012) defines non-functional requirements as properties (characteristics or qualities) that the solution must have. They divide the properties into 8 categories (Table 2). An area of contention for the research team was distinguishing some requirements from constraints. The non-functional requirements described by Robertson & Robertson (2012) allowed us to better define functional requirements by separating the qualities of how well a functional requirement (i.e., non-functional requirements) from the actual functional requirement. We used these non-functional categories to separate intern requirement statements into those that are truly functional requirements and those that are non-functional requirements and include some examples in Table 2.

We present an example of an intern that identified a non-functional requirement and how this may have hindered them from identifying functional requirements. An intern's project was to design a layout for an updated wireless network at an observatory. The intern was to determine locations throughout the building that would optimize placement of wireless access points (WAP). A project requirement identified by the intern for this project was to minimize the security risks from signal bleeding. Stating that a project requirement is to minimize security risks is, informed by the categories posed in Robertson & Robertson (2012), a non-functional requirement. Although a design (solution) that minimizes security risks may be a high priority for the observatory staff that will implement this design, if the design does not meet necessary functional requirements then it is not a viable solution. A possible functional requirement for this project may have been to provide wireless Internet access to all computers currently placed within the building. The non-functional requirement of minimizing security risk could then be used to assess the quality of the proposed solution, but this would only be useful if all the functional requirements are met (i.e., a design solution that minimizes security risk but does not provide wireless Internet to half of the computers is not a viable solution.) Thus, when the intern justifies the proposed solution it is unclear whether the solution meets the project’s functional requirements. In this case, we cannot evaluate the appropriateness of the solution because we are not presented with functional requirements and whether the solution meets them.

For comparison, we also present an example where an intern identified both a non-functional requirement and a functional requirement for a project. The intern’s project was to design an electronic equivalent of paper observing logs used by astronomers at an observatory. Astronomers must track what happens during their observing runs in order to properly utilize their science data later. Each measurement that is taken needs to be logged along with information about the telescope and weather conditions. This data was documented manually on paper logs. Some of the requirements identified by the intern included the following: allow observer comments on everything, generate and email reports, filter data views. The intern’s proposed solution was a viable solution because the functional requirements (i.e., “what” the solution must do) were met. The intern also included non-functional requirements (e.g., be user-friendly and intuitive), which falls under the usability requirement category (Table 2). The non-functional requirements that this intern included as part of their requirements statement did not hinder them from also identifying functional requirements, unlike the previous example. Thus, the separation of requirements into non-functional and functional may focus the interns’ attention to identifying functional requirements.

Although non-functional requirements may improve a given solution, identifying the functional requirements of an engineering project is needed to justify the appropriateness of the solution. Non-functional requirements can be addressed to quantify how well a solution meets the requirements and/or to choose been several possible solutions. However, when interns present non-functional requirements in lieu of functional requirements, the validity of a proposed solution cannot be evaluated.

Challenge 3: Not stating functional requirements in a verifiable manner.

Stating what a solution must do (i.e., its functional requirements) means some action is to be completed (Otto & Wood 2000). An observer should be able to tell whether this action was completed or not, in other words, the requirement should be verifiable. Conveying this aspect of a functional requirement emerged as another characteristic of identifying requirements that interns grappled with.

We initially divided intern's functional requirements statements into two groups, functional requirements that were verifiable (e.g., solution could be tested to show whether functional requirement was met or not met) or verifiable with benchmark values (e.g., solution could be tested to show whether functional requirement met benchmark values). Although some statements could be changed to include benchmark values, for some requirement statements setting a benchmark value was arbitrary. For this reason, we do not suggest imposing benchmark values if they are not appropriate. We then compared verifiable functional requirements to requirements statements that were not stated in a verifiable way.

We provide examples of requirements from intern's projects and describe the importance of stating functional requirements in a verifiable manner. Examples of verifiable and non-verifiable requirements statements are presented in Table 3. Unverifiable requirements that are ambiguous may reflect a requirement that is not correctly understood (Robertson & Robertson 2012) and/or unverified after the solution has been proposed. McConnell (2004) and Weinberg (1997) estimate that as many as 60 percent of failed solutions stem from errors made while requirements were being defined. Also, when interns are articulating a solution, verifiable requirements should be specific so that a solution's adherence to a functional requirement can be verified, for example, through testing. Intern examples of ambiguous and unverifiable requirements include statements that the solution must "have same functionality as current implementation" or "continue operations". The interns have not articulated what the components of functionality are so justification of the solutions' adherence to the requirements is impossible. It is therefore critical to both the intern's understanding and to their articulation of a solution to state requirements in a verifiable manner.

Unverifiable requirements that are less ambiguous may only require rephrasing to be stated in a verifiable manner. For example, an intern identified a requirement as "align the file systems". Although this statement is a functional requirement, justifying that the solution has met this requires rephrasing. Will the file systems be aligned with one another or with an external file system? If more detail is included, then the specific functional requirement can be verified. Similarly, another intern identified a requirement as "filter data views". It is unclear, for example, whether filtered material will be included or excluded from the solution. Overall, when the requirements are less ambiguous and stated in a clear, verifiable manner interns can justify that their solution meets the stated requirements.

To ensure confidence in our data analysis, we concluded by revisiting all requirements statements and categorizing them into one of the three challenges to ensure accuracy of fit. We determined that the three challenges reflected the responses provided. Commonalities of descriptive labels not presented here were left for further analyses. Our goal was to focus on the three challenges that may be addressed in revisions of the SAF. We considered these newly identified challenges when we developed our operational definition of requirements for engineering projects.

Discussion

Operational Definition of Engineering Requirements

We considered these newly identified challenges when we developed our operational definition of requirements for engineering. We propose that an operational definition of requirements in engineering include three components—functional requirements, non-functional requirements, and constraints (Figure 2). Functional requirements are key to articulating engineering solutions because a viable solution must meet all functional requirements.

Functional requirements **must** (1) be relevant to the problem/need (2) focus on what the solution must do or accomplish (actions solution must take) and (3) be verifiable, stated in a manner where action is completed or not completed. Furthermore, *functional requirements* **are not** (1) non-functional requirements (qualities that the solution must have) nor (2) constraints (limitations imposed on possible solutions.)

Implications

Establishing an operational definition of engineering requirements provides a common language around which improvements for the understanding and articulation of engineering solutions can be developed. We suggest several implications of our findings based on the three key challenges learners faced when articulating project requirements identified in this study.

A. Diagnosing difficulties with articulating engineering requirements

We have produced a preliminary rubric to assess interns' articulation of engineering requirements using the language presented in our operational definition. Recently, an article on the development and validation of a rubric for diagnosing students' experimental design knowledge and difficulties was published (Dasgupta et al. 2014) and informs our continued development of a rubric to assess understanding and articulation of engineering requirements. This rubric has great potential for diagnosing articulation of engineering solutions by focusing on identifying and articulating functional requirements, a key finding of this study. The development of this rubric may also guide a discussion within the PDP design group, which could facilitate revisions of the rubric as well as highlight areas for improvement in existing instructional designs. The rubric will also be implemented in the Akamai Internship Program's communication curriculum and could be utilized more broadly by engineering educators in formal and informal settings, by practicing engineers who are mentoring the work of junior engineers or interns, and by learner's themselves as a tool to self-assess their own understanding of engineering requirements.

B. Providing feedback

The language used in our operational definition of engineering requirements will be incorporated throughout the Akamai Internship Program. Interns have varying backgrounds and engage in engineering design projects in a variety of contexts and work sites. Program staff and mentors also vary in their educational and work backgrounds. We believe that providing interns, mentors, and staff with an operational definition of engineering requirements focuses the attention on the essence of what constitutes an engineering requirement (particularly functional requirement) and away from semantics. We develop and facilitate mentor workshops before the program start date; mentors will practice providing feedback to interns by engaging in discussions around previous interns' projects.

C. Learning an expert practice through reflection

While schools and programs have been relatively successful in conveying concepts and facts, less attention is paid to the processes that experts engage in to use or acquire knowledge in carrying out complex and realistic tasks (Collins et al. 1989). One such process skill that engineers engage in, and central to engineering design, is articulating an engineering solution (Clough 2004). In order to make a difference in learners' acquisition of this skill, it is first necessary to understand the nature of expert practice before devising methods appropriate to learning that practice (Collins et al. 1989). In developing an operational definition of engineering requirements, we make tangible a skill that is often invisible. In this study we have identified three key challenges learners face when identifying engineering requirements and provide empirical examples.

Informed by the role of reflection in cognitive apprenticeship (Collins et al. 1989), we will revise the peer-review activity that the interns participate in during the mid-point of the internship. Pairs, or groups, can critique each others' articulation of engineering requirements and self-assess their own progress using a revised *Project Clarification* worksheet that we will produce based on a revised SAF. This opportunity for reflection provides interns with a chance to revise and improve their engineering requirement statements. Engineering educators can integrate activities of reflection around articulating engineering requirements in classroom settings (e.g., students completing capstone projects) where learners articulate solutions to an engineering need or problem.

Conclusions

A clear definition of an engineering project's function(s) is essential to the engineering design process. When faced with a problem or need, engineers need to describe in a clear and reproducible manner the relationship between each of the inputs and each of the required outputs. These relationships establish the function of the solution. In essence, the function is a formulation of the task that is to be accomplished and is independent of any particular solution that is used to achieve the required result (Otto & Wood 2000). The required functions to address a need or problem are not static. In software engineering, the fact that requirements change during the design process is embraced and included in the design process in agile software development. Therefore clearly articulated functional requirements are important to both the design process and are key to justifying a viable solution. Once potential solutions have been generated and one solution has been settled on, the validity of that solution can be evaluated once the engineer has justified that it meets all of the final functional requirements. Thus, clearly identifying and articulating functional requirements are important aspects to the design process and is key to justifying a viable solution, particularly when solving ill-structured problems encountered in workplace engineering practice.

This qualitative study identified three key challenges that engineering interns experienced when identifying functional requirements for their internship projects—identifying constraints as functional requirements, identifying non-functional requirements as functional requirements, and not stating functional requirements in a verifiable manner. We propose an operational definition of requirements based on the challenges we discovered in our research. Requirements in engineering include three components—functional requirements, non-functional requirements, and constraints. Functional requirements, what the solution must do or accomplish, are key to articulating engineering solutions because a viable solution must meet all functional requirements. We then translate our findings into implications for engineering education in general, and, in particular, for supporting learners' articulation of engineering solutions in STEM internship programs. We highlight the importance of identifying and articulating functional requirements in engineering argumentation and suggest further research in this area as well as in the other components included in our Solution Articulation Framework.

Insights from this research will be used to revise our original SAF with continued feedback from Akamai staff and engineers. Our operational definition of functional requirements also provides a common language to use throughout our program with staff, educators, interns, and mentors. Here we have generalized our key exploratory findings. Future studies should include testing a revised SAF (Creswell et al. 2004) and generalizing our qualitative results more broadly to include groups in other contexts (Morse 1991).

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References

Ahn, B., Cox, M. F., London, J., Cekic, O., & Zhu, J. (2014). Creating an Instrument to Measure Leadership, Change, and Synthesis in Engineering Undergraduates. *Journal of Engineering Education*, *103*(1), 115-136.

Beck, Kent et al. (2001). "Manifesto for Agile Software Development". Agile Alliance. Accessed 24 August 2014.

Berland, L. K., & Reiser, B. J. (2009). Making sense of argumentation and explanation. *Science Education*, *93*(1), 26-55.

Borrego, M., Douglas, E. P., & Amelink, C. T. (2009). Quantitative, qualitative, and mixed research methods in engineering education. *Journal of Engineering Education*, *98*(1), 53-66.

Brewer, W. F., Chinn, C. A., & Samarapungavan, A. (1998). Explanation in scientists and children. *Minds and Machines*, *8*(1), 119-136.

Buede, D.M. (2009) *The Engineering Design of Systems: Methods and Models.* New York: Wiley.

Case, J. M., & Light, G. (2011). Emerging research methodologies in engineering education research. *Journal of Engineering Education*, *100*(1), 186-210.

Cho, K. L., & Jonassen, D. H. (2002). The effects of argumentation scaffolds on argumentation and problem solving. *Educational Technology Research and Development*, *50*(3), 5-22.

Chung, L., & do Prado Leite, J. C. S. (2009). On non-functional requirements in software engineering. In *Conceptual modeling: Foundations and applications* (pp. 363-379). Springer Berlin Heidelberg.

Clough, G. W. (2004). The engineer of 2020: Visions of engineering in the new century. *National Academy of Engineering, Washington, DC.*

Collins, A., Brown, J. S., & Newman, S. E. (1989). Cognitive apprenticeship: Teaching the crafts of reading, writing, and mathematics. In L. B. Resnick (Ed.), *Knowing, learning, and instruction: Essays in honor of Robert Glaser* (pp. 453-494). Hillsdale, NJ: Lawrence Erlbaum Associates.

Corbin, J., & Strauss, A. (1994). Grounded theory methodology. *Handbook of qualitative research*, 273-285.

Creswell, J. W. (1999). Mixed-method research: Introduction and application. In G. J. Cizek (Ed.), Handbook of educational polity (pp. 455–472). San Diego, CA: Academic Press.

Dasgupta, A. P., Anderson, T. R., & Pelaez, N. (2014). Development and Validation of a Rubric for Diagnosing Students' Experimental Design Knowledge and Difficulties. *CBE-Life Sciences Education*, *13*(2), 265-284.

Deek, F. P., Hiltz, S. R., Kimmel, H., & Rotter, N. (1999). Cognitive assessment of students' problem solving and program development skills. *Journal of Engineering Education*, *88*(3), 317-326.

Driver, R., Newton, P., & Osborne, J. (2000). Establishing the norms of scientific argumentation in classrooms. *Science education*, *84*(3), 287-312.
(2005).

Giere, R. N. (1998). *Explaining science: A cognitive approach*. University of Chicago Press.

Glaser, B. G. (1978). *Theoretical sensitivity: Advances in the methodology of grounded theory* (Vol. 2). Mill Valley, CA: Sociology Press.

Glaser, B., & Strauss, A. (1967). The discovery of grounded theory. 1967. *Weidenfield & Nicolson, London*.

Hunter, L., Metevier, A., Seagroves, S., Porter, J., Raschke, L., Kluger-Bell, B., Brown, C., Jonsson, P., & Ash, D. 2008, Cultivating Scientist- and Engineer-Educators: The CfAO Professional Development Program.
URL http://isee.ucsc.edu/publications/proceedings/2010ASPC__CultivatingScientist-EngineerEducators.pdf

Jonassen, D. H. (1997). Instructional design models for well-structured and III-structured problem-solving learning outcomes. *Educational Technology Research and Development*, *45*(1), 65-94.

Jonassen, D. H. (2007). What makes scientific problems difficult. *Learning to solve complex scientific problems*, 3-23.

Jonassen, D. H., & Kim, B. (2010). Arguing to learn and learning to argue: Design justifications and guidelines. *Educational Technology Research and Development*, *58*(4), 439-457.

Kuhn, D. (1991). *The skills of argument*. Cambridge University Press.

Kuhn, D. (1993). Science as argument: Implications for teaching and learning scientific thinking. *Science Education*, *77*(3), 319-337.

McConnell, S. (2004) Code Complete (second edition). *Microsoft Press*, 2004.

McNeill, K. L., Lizotte, D. J., Krajcik, J., & Marx, R. W. (2006). Supporting students' construction of scientific explanations by fading scaffolds in instructional materials. *The Journal of the Learning Sciences*, *15*(2), 153-191.

McNeill, K. L., & Krajcik, J. (2008). Scientific explanations: Characterizing and evaluating the effects of teachers' instructional practices on student learning. *Journal of Research in Science Teaching*, *45*(1), 53-78.

Morse, J. M. (1991). Approaches to qualitative-quantitative methodological triangulation. Nursing Research, 40(2), 120–123.

Nagel, E. (1979). Teleology revisited and other essays in the philosophy and history of science.

National Academy of Engineering, 2008

National Research Council [NRC]. (2012). A framework for K–12 science education: Practices, crosscutting concepts, and core ideas. Washington, DC: The National Academies Press.

National Research Council [NRC] (2009). National science education standards. Washington,
DC: The National Academies Press.

National Research Council [NRC] (2013). National science education standards. Washington, DC: The National Academies Press.

NGSS Lead States. (2013). Next generation science standards: For states by States. Washington, DC: The National Academies Press.

Nickols, F. (2003) *Solution Engineering in Action: A Really Good Example*
URL http://www.nickols.us/se_in_action.htm

Newton, P., Driver, R., & Osborne, J. (1999). The place of argumentation in the pedagogy of school science. *International Journal of Science Education*, *21*(5), 553-576.

Olds, B. M., Moskal, B. M., & Miller, R. L. (2005). Assessment in engineering education: Evolution, approaches and future collaborations. *Journal of Engineering Education*, *94*(1), 13-25.

Otto, K. N., & Wood, K. L. (2000). Product design: techniques in reverse engineering and new product development.

Robertson, S., & Robertson, J. (2012). *Mastering the requirements process: getting requirements right*. Addison-Wesley.

Rugarcia, A., Felder, R. M., Woods, D. R., & Stice, J. E. (2000). The future of engineering education I. A vision for a new century. *Chemical Engineering Education*, *34*(1), 16-25.

Sampson, V., & Clark, D. B. (2008). Assessment of the ways students generate arguments in science education: Current perspectives and recommendations for future directions. *Science Education, 92*(3), 447-472.

Sheppard, S. D., Macatangay, K., Colby, A., & Sullivan, W. (2009). Educating Engineers. Stanford, California: Jossey-Bass (Wiley).

Shin, N., Jonassen, D. H., & McGee, S. (2003). Predictors of well-structured and ill-structured problem solving in an astronomy simulation. Journal of Research in Science Teaching, 41(3), 6–33.

Siegel, H. (1995). Why should educators care about argumentation? *Informal Logic, 17*(2).

Stouffer, W. B., Russell, J. S., & Oliva, M. G. (2004). Making the strange familiar: Creativity and the future of engineering education. In *Proceedings of the 2004 American Society for Engineering Education Annual Conference & Exposition* (pp. 20-23).

Toulmin, S. (1958). The Uses of Argument. Cambridge University Press. *Cambridge, UK*.

Voss, J. F., & Post, T. A. (1988). On the solving of ill-structured problems. In M. T. H. Chi, R. Glaser, & M. J. Farr (Eds.), The nature of expertise. Hillsdale, NJ: Lawrence Erlbaum Associates.

Weinberg, J. (1997). Quality Software Management Volume 4: Anticipating Change. *Dorset House*.

Wieringa, R., Maiden, N., Mead, N. & Rolland, C. (2006). Requirements engineering paper classification and evaluation criteria: a proposal and a discussion. *Requirements Engineering*, *11*(1), 102-107.

Tables & Figures

Table 3.1 The four phases of data analysis used to explore difficulties of defining requirements with examples. Descriptive labels in phase II correspond to examples written in phase I.

Phases	**Examples**
Phase I: Write down requirements statements gathered from interns' self-reported responses and transcribed from presentations	The solution must ___________. (1) Keep computer room within set standards of temperature and humidity (2) Ensure code is understandable (3) Use faults to create an intelligent response (4) Be able to read a specified wavelength coverage of 1.2-1.8 micrometer
Phase II: Generate descriptive labels for each requirement statement	(1) Limitation on possible solutions, measurable, target value possible, relevant to engineering problem/need (2) Maintainability, quality the solution must have, not stated in a manner where action is completed or not completed, relevant to engineering problem/need (3) Action solution must take, not stated in a manner where action is completed or not completed, relevant to engineering problem/need (4) Action solution must take, stated in a manner where action is completed or not completed, measurable, target value presented, relevant to engineering problem/need

Phase III: Identify relationships among labels and produce preliminary categories	(a) Functional requirements (b) Non-functional requirements (c) Verifiable (d) Measurable (e) Relevant to engineering problem/need (f) Constraints
Phase IV: Categorize findings according to main challenges	**Challenge I:** Identifying constraints as requirements **Challenge II:** Identifying non-functional requirements as functional requirements **Challenge III:** Not stating functional requirements in a verifiable manner

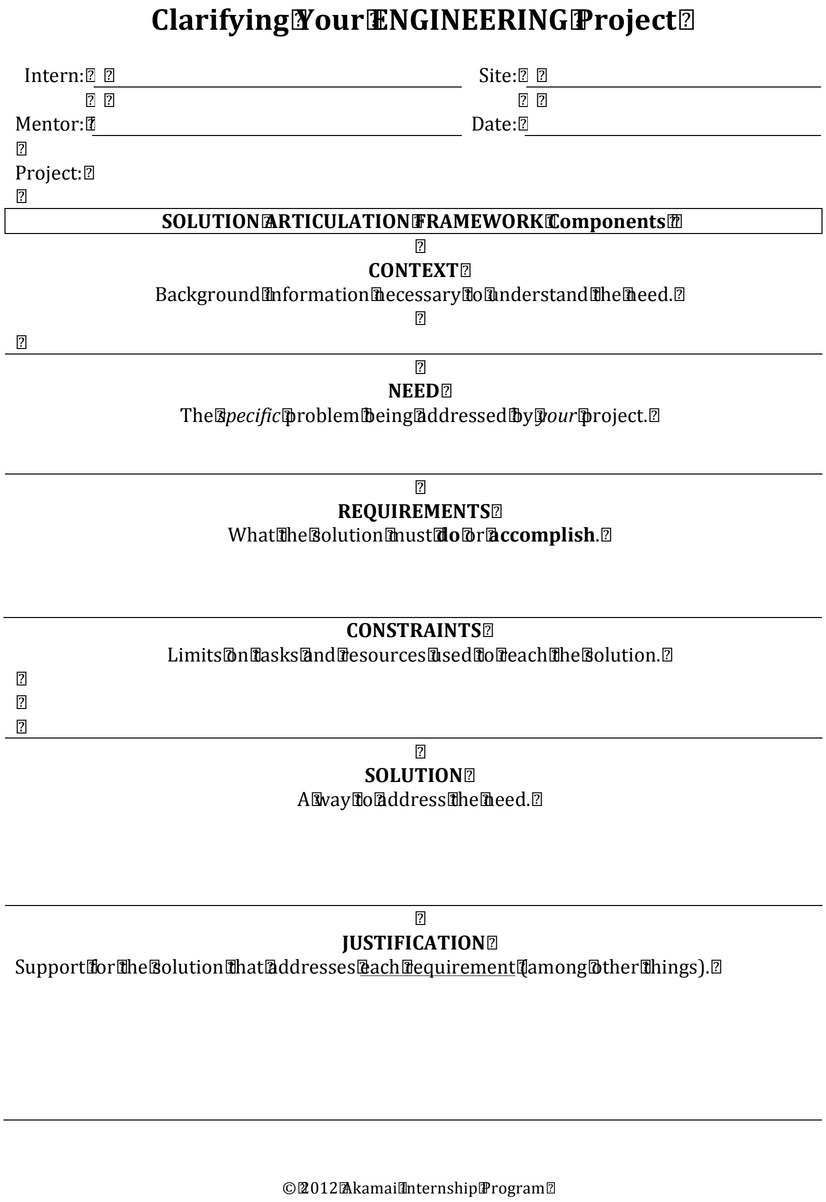
Table 3.2 Non-functional requirement categories (Robertson & Robertson 2012) and examples of intern responses (when available) that we identified as non-functional requirements.

Non-functional requirement	**Description**	**The solution must _____________.**
Look and feel requirements	Solution's appearance	
Usability requirements	Solution's ease of use	*have an easy and collaborative environment for end users *have an extremely intuitive user interface
Performance requirements	How fast, how safe, how many, how accurate the functionality must be	*reduce cooling costs *protect racks from seismic events *reduce power usage
Operational requirements	The operating environment of the solution	*integrate with existing infrastructure *be compatible with Web browser
Maintainability and portability requirements	Expected changes, and time allowed to make them	*address downtime *be accessible for future troubleshooting and maintenance
Security requirements	Security and confidentiality of solution	*minimize security risk
Cultural and political requirements	Special requirements that come about because of the people involved in the solution's development and operation	
Legal requirements	What laws and standards apply to the solution	

Table 3.3 Examples of verifiable requirements statements from interns. We also include examples of how unverifiable requirements statements could be edited to make them verifiable. The solution must ___________. Suggested translation into a verifiable statement presented in italics.

Verifiable Requirements Statements
Pull records from database
Determine whether faults should be reset
Log faults and actions to text file
Use a configuration file to get user input
Get and process 5K bytes of data from the server in real-time
Update displays at a minimum of 15Hz
Non-verifiable Requirements Statements
Have same functionality as current implementation *Display information as images in real time*
Continue operations *Provide employees with continued access to servers*
Align the file systems *Align the file systems with following array vendors best practices*
Filter data views *Filter data views into separate folder*

Figure 3.1 ***Clarifying Your Project*** **worksheet presented to interns that asks them to describe the components of their project as outlined on the SAF (i.e., context, need, requirements, constraints, solution, and justification).**



Clarifying Your ENGINEERING Project

Intern: ____________________ Site: ____________________

Mentor: ____________________ Date: ____________________

Project:

SOLUTION ARTICULATION FRAMEWORK Components

CONTEXT
Background information necessary to understand the need.

NEED
The *specific* problem being addressed by *your* project.

REQUIREMENTS
What the solution must **do** or **accomplish**.

CONSTRAINTS
Limits on tasks and resources used to reach the solution.

SOLUTION
A way to address the need.

JUSTIFICATION
Support for the solution that addresses each requirement (among other things).

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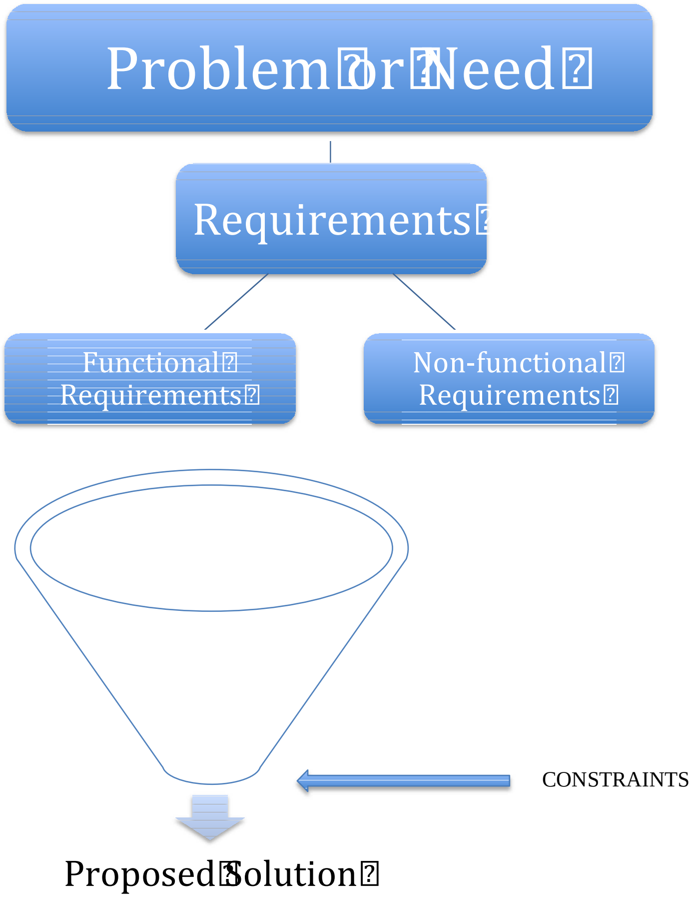
Figure 3.2 In order to argue for a particular engineering solution to a problem or need the solution must meet the requirements. Requirements in general can be divided into 3 subcategories. Functional requirements specify what the solution must do. Non-functional requirements are properties (characteristics or qualities) that the solution must have. A viable solution must meet all functional requirements while considering any limiting constraints. The quality of the solution can be improved by meeting non-functional requirements.



Synthesis:

Motivated by our recent study that detected complex social associations between individuals of wintering golden-crowned sparrows, I investigated the possibility that relatedness plays any role in this complex social structure. The composition of sparrow foraging flocks is dynamic, but is not random. Instead, sparrow flocks are subsets of “communities” of sparrows that show long-term stability in both space and membership. As a result, many individual sparrows appear to form long-term and perhaps even lifelong associations with other sparrows. I tested for kinship in two ways—whether pairwise relatedness estimates correlates with degree of sociality between pairs of individuals (kinship) and whether relatedness estimates were higher within than between social communities (a general assessment of population structure that could pick up more subtle patterns of kinship as well as other factors that could lead to differences in genetic structure among communities). Relationships between kinship and social structure in organisms with stable and cooperative societies have been extensively studied, especially in cooperative breeding birds, mammals and insects. Given evidence that kinship could also shape social evolution in seemingly non-cooperative animals (e.g., in golden-crowned sparrows who display fission-fusion dynamics during migration and winter but are territorial during breeding), it is surprising that relatively few studies explore the relationship between genetic and social structure in this context.

In chapter one, my findings conclusively show that social structure is not affected by kinship in this wintering population. To test for potential differences in migration patterns and distances between males and females, I analyzed the data separately for each sex and found no detectable relationship between genetic relatedness and social associations. I then tested for potentially subtle relationships between genetic relatedness and social associations that may have been detected within each social community (as detected by social network analysis) and again found no detectable relationship. My results consistently show that kinship does not predict the complex social associations in this wintering population of golden-crowned sparrows. Furthermore, I used this data to test for potential genetic structure between communities; social communities may form based on populations of individuals that breed together. My results do not imply any such genetic structure; individuals in this wintering population may be arriving from a single breeding population (although a recent study using geolocators tracked individuals from a nearby wintering site and found individuals migrating to several breeding sites) or there may be recent gene flow and/or rapid expansion from a single glacial refugia such that breeding populations lack genetic structure. Thus, social cohesion without kinship may be a result of other factors, for example, similar preferences for locations or preference for familiar group members. The latter is a particularly interesting explanation for the persistence of stable social communities across years because despite each community losing 30-50% of individuals between years, new members join established social communities. Our understanding of the complex social structure of wintering golden-crowned sparrows could be informed by more detailed studies on their breeding populations and migration patterns between breeding and wintering sites to confirm that genetic structure does not play a role in social organization. It would also be informative to explore other mechanisms that favor cooperation (e.g., reciprocal altruism).

Winter can be a particularly stressful period for migratory birds (e.g., variable food availability can lead to starvation especially for small birds). In chapter two, I investigate the genetic causes of variation in fitness-related traits in this winter population of golden-crowned sparrows. Individuals in this population show great variation in morphological and behavioral traits that may play a role in winter dominance and ultimately survival. I found that individuals that were more heterozygous—based on internal relatedness measures reflecting individual genetic variation—were more socially dominant, had larger gold crown patches (which predict social dominance in some contexts) and had stronger social connections within their social networks. I identified and tested for two processes that may have shaped these significant heterozygosity-fitness correlations (HFC). First, inbreeding depression can lower individual heterozygosity across the entire genome of an individual and decrease fitness of inbred individuals since the expression of recessive deleterious alleles increases. I found no evidence to support this explanation. Second, HFCs may result from local effects at single loci and may be detected when neutral markers are linked to functional loci. I also found no evidence to support this explanation using a statistical test recently developed for this purpose.

Although the underlying mechanism driving the HFCs detected in this study is unknown, the detection of moderate correlations between an individual's heterozygosity level and social dominance, winter plumage, and sociality is interesting. Theses traits connect in important ways to winter social behavior, suggesting that these HFC analyses detected true relationships. In this population of golden-crowned sparrows it is therefore feasible that more heterozygous individuals are more socially dominant because they may be, for example, better foragers and have energy to expend on aggressive behaviors. Another reasonable explanation for the relationship between heterozygosity and social dominance could be that more socially dominant birds could be older; heterozygosity might be linked to longevity. Although a physiological mechanism is unknown in sparrows, it is possible that plumage may reflect the general quality (e.g., better immune function) of the individual. To my knowledge, no previous study has investigated heterozygosity affects on sociality, as calculated by metrics revealed by social network analysis. As with social dominance, “core” individuals of a community (i.e., individuals that are more likely to be found flocking with other birds of their community) may also be older or higher quality individuals. “Core” individuals interact and have contact with others in the community and may therefore be at higher risk of encountering disease, parasite, and pathogens. If our measure of heterozygosity reflects immune function heterozygosity, then more heterozygous individuals may be more likely to be “core” individuals. Future work should focus on directly measuring fitness of individuals in this population and see if, for example, survival correlates with heterozygosity. Also, if these correlations are driven by variation in immune function, genetic data that reflects variation in MHC genes would be informative and/or measurements of parasite or disease loads in individuals.

The recent focus on preparing students to pursue careers in science, technology, engineering, and mathematics (STEM) fields have resulted in educational researchers investigating how best to support this goal. Government and private funding has been directed to STEM internship programs (particularly at the undergraduate level). These internship programs are designed to provide workplace opportunities as well as provide support from the program to acquire skills needed by students to transition from school to the workforce. Most of this support has been developed (by internship programs and researchers) in the sciences. Engineers are in high demand and universities and internship programs are tasked with preparing more engineering students to be professional engineers. Our development of the Solution Articulation Framework (SAF), supports learners' (interns) acquisition of argumentation skills in engineering; engaging in argumentative practices promotes content knowledge and communication skills in many disciplines but has not been explored deeply in the engineering education literature. We focused our attention on improving learners' engineering argumentation practices with a particular emphasis on articulation of engineering solutions.

In chapter three, I identify a critical component (functional requirements) to effectively articulating a proposed engineering solution and offer an operational definition along with suggestions for implementing these ideas in engineering education. My findings were immediately used to revise our SAF and warrants further research on other components of the SAF. The research in this chapter advances the emerging research field in engineering education by highlighting the importance and difficulties associated with teaching argumentation skills in engineering.