DOMINANCE AND COMMUNICATION IN A COOPERATIVE BREEDING BIRD

DOMINANCE AND COMMUNICATION IN A COOPERATIVE BREEDING BIRD

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ABSTRACT

Social dominance can influence the allocation of resources in animal groups and has important consequences for individual fitness. In my thesis, I examined the structure, formation, maintenance and consequences of dominance, in the cooperatively breeding pukeko (*Porphyrio melanotus melantous*: Aves). I first describe a quantitative analysis of pukeko dominance networks (Chapter 2), which included one of the first applications of exponential random graph models in behavioural ecology. This study demonstrated that pukeko form highly ordered dominance hierarchies, and that dominance relationships were influenced by both the attributes of individual birds, as well as self-organizational processes such as winner and loser effects. Additionally, I demonstrated that hatching order has an important influence on the formation of dominance relationships, with earlier hatched chicks achieving higher dominance ranks as adults (Chapter 3). To maintain dominance relationships, pukeko use their red frontal shield as a 'status signal', with larger frontal shields indicating more dominant individuals. I showed that sexual dimorphism in frontal shield size is dramatically different in two pukeko populations, probably due to differences in the intensity of intrasexual competition (Chapter 4). Furthermore, by manipulating apparent frontal shield size, I demonstrated that shield phenotype both influences, and is influenced by, social interactions (Chapter 5). This bi-directional relationship between signals and social interactions challenges conventional signalling theory, and has important implications for how honesty is maintained in this signalling system.

Finally, I expanded my findings on pukeko colour traits by exploring interspecific patterns of bill colouration in over 1600 bird species (Chapter 6). This study revealed that colourful bills likely evolved as a signal used in competitive interactions, rather than as a sexual signal. Taken together, my research provides a significant advancement in our understanding of the complex nature of dominance in a wild bird, and provides both a methodological and theoretical basis for future studies on animal social behaviour.

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DECLARATION OF ACADEMIC ACHIEVEMENT

This dissertation is organized in a ‘sandwich thesis’ format as approved by McMaster University. It consists of 7 chapters. Chapter 1 provides a general introduction to social dominance, status signalling and pukeko natural history. Chapters 2-6 are peer-reviewed published papers. Chapter 7 provides a synthetic discussion of the results of Chapters 2-6 including suggestions for future research. Each chapter is formatted to the style requirements of the journal in which it is published. Additionally, I have included 3 published manuscripts as appendices, all of which are based on research I conducted while at McMaster, but are not thematically linked to my main dissertation.

Chapter 1 – General Introduction

Author: Cody J. Dey

Chapter 2 – Individual Attributes and Self-Organizational Processes Affect Dominance Network Structure in Pukeko

Authors: Cody J. Dey and James S. Quinn

Publication: Behavioral Ecology. Volume 25, pages 1402-1408.

Comments: CJD conceived the study, collected the data, completed the analysis and wrote the first draft of the manuscript. CJD and JSQ revised the manuscript. Reprinted with permission.

Chapter 3 – Hatching Order Affects Offspring Growth, Survival and Adult Dominance in the Joint-Laying Pukeko *Porphyrio melanotus melanotus*

Authors: Cody J. Dey, Constance M. O'Connor and James S. Quinn

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Chapter 4 – Reproductive Skew and Female Trait Elaboration in a Cooperatively Breeding Rail

Authors: Cody J. Dey, Ian G. Jamieson and James S. Quinn

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Chapter 5 – Manipulating the Appearance of a Badge of Status Causes Changes in True Badge Expression

Authors: Cody J. Dey, James Dale and James S. Quinn

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Chapter 6 – Carotenoid-Based Bill Coloration Functions as a Social, not Sexual, Signal in Songbirds (Aves: Passeriformes).

Authors: Cody J. Dey, Mihai Valcu, Bart Kempenaers and James Dale

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Chapter 7 – General discussion

Author: Cody J. Dey

Appendix A – Cooperative Males Reduce Incubation in Response to Cues of Female-Female Competition

Authors: Cody J. Dey, Constance M. O'Connor, Sigal Balshine and James S. Quinn

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Appendix B – Network Structure is Related to Social Conflict in a Cooperatively Breeding Fish

Authors: Cody J. Dey*, Adam R. Reddon*, Constance M. O'Connor*, Sigal Balshine. *Authors made equal contributions

Publication: Animal Behaviour. 2013. Volume 85, pages 395-402.

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Appendix C – Dominance Network Structure across Reproductive Contexts in the Cooperatively Breeding Cichlid Fish, *Neolamprologus pulcher*

Authors: Cody J. Dey, Q.Y. Joanne Tan, Constance M. O'Connor, Adam R. Reddon, J. Ryan Caldwell and Sigal Balshine

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Appendix D – Supplementary Information for Chapter 2

Appendix E – Supplementary Information for Chapter 5

Appendix F – Supplementary Information for Chapter 6

Chapter 1: General Introduction

Cody J. Dey

The social environment provides complex challenges to animals and is an important evolutionary force. While there is a long-tradition of research on sexual selection reaching back to Darwin (1871), the importance of non-sexual social interactions wasn't appreciated until much later (Wynne-Edwards 1962, Crook 1972, West-Eberhard 1983). However, evolutionary ecologists now recognize that social interactions (both sexual and non-sexual) can lead to the evolution of traits that would otherwise appear detrimental (Lyon and Montgomerie 2012), can maintain genetic and phenotypic variation (Moore et al. 1997, Wolf et al. 1998), and can influence population level-processes such as disease transmission (Loehle 1995). For these reasons, gaining a comprehensive understanding of the causes and consequences of social interactions is an important goal of behavioural ecology, and this goal forms the core of my dissertation. Specifically, I focused on dominance, which is a type of social relationship that influences the allocation of resources within animal populations and therefore has important implications for individual fitness. In addition, dominance relationships among individuals influence group function and productivity, and may therefore effect group-level selection. While social dominance has been studied in a variety of wild, laboratory and domesticated animals (see Kaufmann 1983, Ellis 1995), there are few systems in which we understand dominance relationships from an integrative perspective. In this thesis, I endeavoured to understand the functional, developmental, and evolutionary factors that influence the formation and maintenance of dominance relationships in a wild bird. In this chapter, I describe the theoretical foundation that guided the research outlined in Chapters 2-7.

1.1 Social Dominance

The concept of social dominance has been an important idea in behavioural ecology ever since Thorleif Schjelderupp-Ebbe (1922) first described ‘peck-order’ in groups of domestic chickens (*Gallus gallus domesticus*). Although many definitions of social dominance have been used in the literature (see Drews 1993 for a review), the most useful are similar in content to Schjelderupp-Ebbe’s original definition of dominance as ‘a repeatable pattern of outcomes during agonistic interactions, whereby one individual consistently wins the interactions, and the other individual yields’. Dominance relationships have been described in most major groups of animals (e.g., insects; Röseler et al. 1986, crustaceans; Winston and Jacobson 1978; fish; Sloman et al. 2000, reptiles; Brattstrom 1974; birds; Baker et al. 1981; mammals; Gese et al. 1996), and their ubiquity suggests that they provide an evolutionary advantage, at least for some species. Indeed, dominance relationships can benefit both dominant and subordinate individuals, in that dominant individuals are able to access resources without the need for energetically costly contests, and subordinate individuals can avoid contests that they would be unlikely to win (Kaufmann 1983). Additionally, dominance relationships can have various group-level benefits by promoting role specialization (King et al. 2009), increasing group productivity (Meese and

Ewbank 1973) and increasing the speed of collective decision-making (Lampkin 1972).

While dominance relationships can reduce the cost of social living for all individuals, they also influence the distribution of limited resources such as food, shelter and mates. Resources that are monopolized by dominant individuals are usually unavailable for subordinate individuals, and as a result, dominance relationships can influence a variety of fitness-linked outcomes including growth (Brockmark and Johnsson 2010), survival (Arcese and Smith 1985), predation risk (Schneider 1984) and access to mating opportunities (Ellis 1995). For this reason, it is important for ecologists to understand how dominance relationships are formed, maintained, and how they change over time, as they can have important consequences for individual fitness.

1.2 Status Signalling

In many animal species, individuals use signals to communicate information about their quality to conspecific receivers (Dale 2006). When signals convey information about fighting ability (also known as resource holding potential), they can be used to form and maintain dominance relationships, and these signals are referred to as ‘status signals’. Dominance relationships do not require status signals to remain stable, as social rank can be determined through other means (i.e. direct interaction) and maintained through individual recognition mechanisms

(Tibbetts and Dale 2007). However, status signals can lower the cost associated with forming and maintaining dominance relationships, and are therefore predicted to be beneficial in species that frequently interact with unfamiliar individuals, or species for which the costs of having ambiguous dominance relationships are high.

The evolutionary stability of status signals has been a source of much debate (e.g. Owens and Hartley 1991, Zahavi 1993, Senar 2006, Számadó 2011), because it seems relatively easy for individuals to dishonestly signal their quality. Theoretical models have shown that dishonest signalling can be advantageous in many contexts and thus we would expect ‘cheaters’ to be common unless there are mechanisms to maintain signal honesty. In general, signal honesty is maintained when the potential cost of cheating exceeds the benefits (Számadó 2011). Such conditions can be met if signal production carries a physiological cost, such that only high quality individuals can afford to produce the signals that indicate strength and dominance (i.e. the ‘handicap hypothesis’; Zahavi 1975, Folstad and Karter 1992). However, in many species signal production has low or negligible costs (e.g. Tibbetts and Dale 2004) and there is theoretical evidence that signals do not require a realized production cost to be honest (Számadó 2011). Instead, honest signalling can be maintained if there are social costs to cheating. For example, in *Polistes* wasps, individuals manipulated to have dishonestly dominant facial markings receive more aggression from competitors than do honest signalers (Tibbetts and Dale 2004). When individuals display incongruent signals (i.e. their markings are not matched by other cues to fighting ability), competitors may be selected to fight them for longer in order to determine their true quality (Tibbetts and Izzo 2010). Thus, social punishment of incongruent signals provides a mechanism by which signal honesty can be maintained.

1.3 Pukeko Natural History

The pukeko (also called the Australian Swamphen or the South-west Pacific Swamphen; *Porphyrio melanotus melanotus*: Figure 1) is a subspecies of the purple swamphen (*Porphyrio melanotus*) found in Australia and New Zealand, and the Chatham and Kermadec Islands. In New Zealand, there has been considerable research on their mating system and social organization, and they have been the focus of behavioural ecology studies since the 1970's. Depending on population and ecological parameters, pukeko will either live in territorial pairs, or in complex social groups of 3-20 individuals (Jamieson 1997). These groups usually contain multiple breeding males, and one or two breeding females that mate without exclusivity (Craig 1980a,b), although there is considerable flexibility in the mating system and other social structures (i.e. polygynous groups) have also been observed (Craig 1980b). Social groups may also contain non-breeding helpers, which are usually offspring from previous broods (Craig 1980a). When multiple female breeders are present within a social group, they will all lay eggs in a single nest, a behaviour known as joint-laying (Vehrencamp 2000). Both breeding males and females contribute to incubation, and all group individuals (including non-breeders) help to raise the offspring. In some populations, pairs and groups will defend their territory throughout the year, while in other populations territoriality will weaken in the winter, and large flocks may form (Fordham 1983).

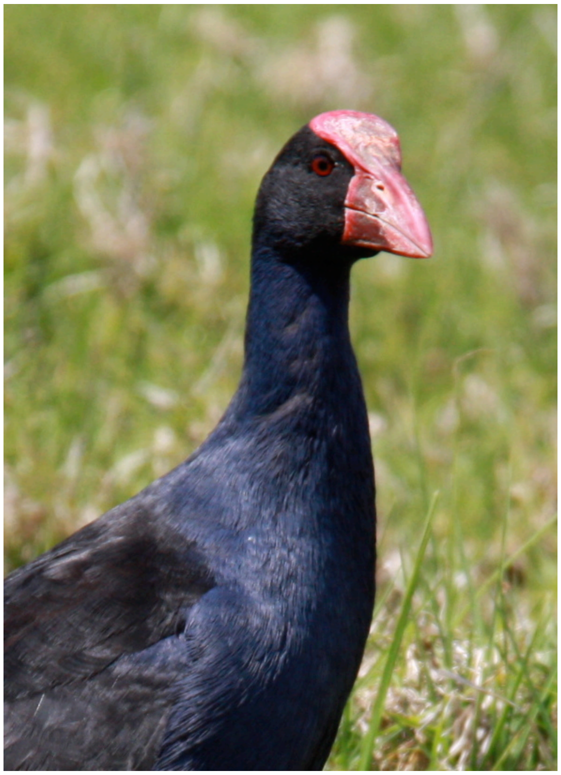

Figure 1. Adult pukeko showing the prominent red frontal shield ornament. Photo provided by CM O'Connor

Due to the complex and flexible social system of the pukeko, individuals in this species must interact with a variety of conspecifics in a variety of contexts. Agonistic interactions over food and space occur between both same-sex and opposite-sex group members, and vary from minor interactions involving posturing, to intense fights with biting and kicking (Craig 1977). Additionally, territorial interactions between individuals from neighbouring groups are common, and may involve large numbers of individuals (*personal observation*). While pukeko are thought to form linear dominance hierarchies within social groups (Jamieson and Craig 1987), the methodology used to ascertain this finding has been recently criticized for becoming statistically biased when pairs of individuals do not interact (Klass and Cords 2011, Shizuka and McDonald 2012). Furthermore, we still do not have a good understanding of the factors that influence the type, intensity and pattern of dominance interactions among individual pukeko. Nonetheless, the diversity of natural interactions in the pukeko provides an excellent system to investigate how conflicts between individuals are resolved, how dominance is related to group structure, and how status signals are involved in the formation and maintenance of dominance relationships.

Most studies of avian status signalling have focused on traits that are expressed in plumage. However, the pukeko's most conspicuous ornament is its fleshy, red frontal shield (Figure 1), and the size of this ornament is also highly variable among individuals. Bare-part ornaments (such as frontal shields) are found in a taxonomically diverse set of avian species and unlike plumage ornaments, they can often respond to acute changes in individual condition because they are vascularized (e.g. Zuk and Johnsen 2000, Gautier et al. 2008). For example, subordinate male red-backed fairy-wrens (*Malurus melanocephalus*) are able to rapidly change their bill colour to reflect an experimental promotion in social status, but are unable to update their plumage colouration until the following molt (Karubian 2008). While a detailed study of pukeko frontal shields had never been performed before this thesis, the frontal shields of other rails show dramatic changes in size with the onset of the breeding season (Gullion 1951) or with pharmacological manipulation (Eens et al. 2000), suggesting that similar traits in close relatives are highly plastic. The potential for flexibility in ornament expression over a short time period provides an interesting system for studies of how the social environment influences the relative costs and benefits of signals, and how social interactions might influence signal expression. Pukeko shields are actively displayed during agonistic interactions and Craig (1977) described an experiment in which he blackened the shield of a single male pukeko and observed increased aggressive challenges directed towards the manipulated individual. Taken together, these studies suggest that the pukeko's frontal shield is a status signal and is likely involved in maintaining dominance relationships in this species.

1.4 Thesis Aims

In my thesis, I examined the causes and consequences of dominance and status signalling on the social life of the pukeko. I used an integrative approach, incorporating descriptive and experimental behavioural studies, morphological and colourimetric studies, and phylogenetic comparative methods. My dissertation first explores the structure of dominance relationships within pukeko social groups, using social network analysis (Chapter 2). Next, I present a study on how early-life experience influences the formation of dominance relationships (Chapter 3). This paper followed individuals from hatching to adulthood to test for an association between hatching order and social rank. In Chapters 4 and 5, I examined how pukeko communicate information about dominance using their red frontal shield ornament. I explored how population differences in the intensity of intrasexual competition lead to differences in shield phenotype (Chapter 4) and how social feedback influences shield expression (Chapter 5). Finally, in Chapter 6, I analyzed patterns of colouration in over 1600 species of birds to test whether bill colour evolved as a signal of dominance or as a sexual signal. Taken together, these papers contribute to our understanding of the complex nature of status signaling and social dominance, and the important influences these factors have on animal ecology.

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Chapter 2: Individual Attributes and Self-Organizational Processes Affect Dominance Network Structure in Pukeko

Cody J. Dey and James S. Quinn

Behavioral Ecology. Volume 25, pages 1402-1408.

2.1 Abstract

Dominance relationships are an important type of social relationship that can influence group dynamics and individual fitness. However, most studies on dominance have been restricted to investigating the orderliness of dominance hierarchies, and how individual traits influence dominance rank. Here, we used a social network approach to investigate the patterns and quality of dominance interactions in the pukeko, a cooperatively breeding bird that lives in stable, mixed-sex social groups. By using a combination of modern statistical techniques, including one of the first application of exponential random graph models in behavioural ecology, we show that pukeko dominance networks emerge from both the attributes of individuals, as well as from endogenous, self-organization of dominance relationships (i.e. structural dependence). Pukeko dominance networks were influenced by sexual differences in dominance interactions, sexual homophily, characteristics of status signals and a tendency to form transitive triad motifs. These factors have differential effects on submissive and aggressive behaviours, but ultimately lead to the formation of orderly and highly asymmetrical dominance hierarchies that are temporally stable. This study demonstrates the utility of multivariate statistical tools for network analysis of animal societies, and provides a rich understanding of the factors that influence dominance interactions in this interesting species.

2.2 Introduction

Dominance relationships are a ubiquitous type of social relationship observed among a taxonomically diverse set of animals. Because dominance relationships influence access to various resources (including mating opportunities), they can be a strong contributor to individual variation in fitness (e.g. Ellis 1995). As a result, understanding both the causes and consequences of dominance has been an intense research focus in behavioural ecology ever since Schjelderup-Ebbe (1922) first described ‘peck order’ in domestic chickens (*Gallus gallus*). Dominance is fundamentally a relationship between a pair of individuals (Drews 1993), however many researchers have been interested in the structure of dominance interactions among groups of actors (hereafter termed dominance networks). In the vast majority of species studied to date, dominance networks are more orderly (i.e. transitive or linear; see below) than expected by random processes (e.g. see meta-analyses by Shizuka and McDonald 2012; McDonald and Shizuka 2013). While orderly dominance networks can be due to differences in individual attributes alone (Piper 1997), there is good evidence that dominance networks are influenced by factors such as winner, loser and bystander effects (Dugatkin and Earley 2003). These processes act independently of the attributes of individuals, and can promote the self-organization of orderly hierarchies (Chase et al. 2002; Chase and Seitz 2011). Regardless of the mechanism, the consistency with which animals form orderly dominance networks suggests that patterns of dominance interactions are an important aspect of group living.

Indeed stable, hierarchical dominance relationships could provide various benefits such as minimizing physical aggression (Meese & Ewbank 1973; Mock & Ploger 1987) and increasing the speed of collective decision making (Lampkin 1972).

Despite general agreement that dominance relationships have consequences for group dynamics and impact individual fitness (Ellis 1995; Barta and Giraldeau 1998), we know very little about variation in dominance network structure beyond measures of orderliness such as linearity (Landau 1951; de Vries 1995) or triangle transitivity (Shizuka and Macdonald 2012). This focus on orderliness is a logical extension of studies that have investigated what factors cause certain individuals to become dominant or subordinate. But while orderliness and rank are certainly important aspects of group living, they do not fully describe the variation in dominance interactions experienced by animals. For example, a dominant animal that is subject to frequent physical challenges from subordinates experiences a much different social environment than one who predominantly receives submissive gestures, despite occupying similar ranks. Likewise, within an orderly hierarchy, individuals may primarily interact with other individuals of similar rank, or may interact equally with other group members regardless of rank. These types of variation in animal dominance networks have been largely unexplored, which may be partially because few dominance studies have considered replicate social groups of their focal species, and it is therefore difficult to test for general underlying factors that contribute to variation in dominance interactions. However, with proper study design and new statistical tools (see below) researchers should be able to explicitly study the causes and consequences of variation in dominance network structure, which will contribute to a much richer understanding of how dominance relationships affect the lives of social animals.

Social network analysis (SNA) is a promising tool for investigating dominance network structure because it provides a quantitative framework for analyzing patterns of interactions among groups of individuals (Croft et al. 2008). In a social network representation of dominance interactions, individual animals are depicted by nodes, whereas dominance interactions are represented by ties (also called edges) that link the nodes. In addition to this relational data, SNA can also integrate aspects of each individual's phenotype (e.g. sex) and the structural dependence between ties (e.g. 'friend of a friend' effects) which allows researchers to analyze the relationships between individual, dyadic, local and group-level phenomena (see Krause et al. 2007; Wey et al. 2008, Sih et al. 2009; Pinter-Wollman et al. 2013 for reviews of SNA in behavioural ecology). To date, the majority of SNA studies in behavioural ecology have used univariate statistical methods (Pinter-Wollman et al. 2013), which do not allow for strong inference on the factors that determine network structure because other potentially important factors have not been accounted for. However, the application of statistical tools that allow for simultaneous evaluation of the influence of multiple variables (e.g. exponential random graph models, ERGMs, Wasserman and Pattison 1996; Snijders et al. 2006) should allow for more rigorous hypothesis testing of the factors that determine network structure in animal groups (Pinter-Wollman et al. 2013). In this paper, we provide one of the first uses of these multivariate methods in the field of animal behaviour.

The study of dominance relationships is especially pertinent for cooperatively breeding animals. In these species, the shared investment in offspring production could have an important influence on dominance network structure, and dominance networks in cooperative breeders may differ significantly from animals that congregate for other reasons (e.g. to exploit food resources). In this study, we explore patterns of dominance interactions in social groups of the cooperative breeding pukeko (*Porphyrio melanotus melanotus*). This ground-dwelling bird has a highly variable social system (Jamieson 1997) and on the North Island of New Zealand (where this study was conducted), most pukeko live in complex social groups that may contain multiple breeding males and females, as well as non-breeding helpers of both sexes. Within these groups, pukeko are thought to form an orderly (i.e. linear) dominance hierarchy (Jamieson and Craig 1987), and socially dominant individuals gain priority access to food and have different social roles than do subordinate individuals (e.g. differences in parental care and territory defence; Craig 1980). Additionally, dominance determines the order of replacement for female breeders (Jamieson and Craig 1987), but whether there is a relationship between dominance and male reproduction is currently unclear due to limited molecular parentage data (Jamieson et al. 1994; Lambert et al. 1994).

While there is some information on the consequences of dominance rank for individual pukeko, we have almost no understanding of the patterns of dominance interactions among members of pukeko social groups. Because this species has a variable social system, it provides an excellent opportunity to test how individual attributes influence the quantity and quality of dominance interactions among group members. In addition to sex, dominance interactions in this species are likely influenced by the frontal shield ornaments of interacting individuals (Craig 1977). These fleshy ornaments extend up from the bill onto the crown, and function as a signal of social status, with larger frontal shields indicating more socially dominant individuals (Dey et al. 2014). In this study, we explored how these individual attributes (sex and shield size) influence the quality and quantity of dominance interactions in replicate social groups of wild, free-living pukeko. Additionally, we asked whether pukeko dominance networks show evidence of self-organization, after controlling for the effects of the individual attributes discussed above.

We considered three particular aspects of network structure in this study: (1) the orderliness of dominance interactions, (2) how individual attributes and structural dependence influence dominance interactions, and (3) the temporal stability of dominance interactions.

First, we investigated two aspects of dominance network orderliness, which collectively describe the degree to which animals form ordered and strong (i.e. directionally consistent) dominance relationships. To measure whether pukeko dominance networks show an ordered structure, we used the triangle transitivity method (Shizuka and McDonald 2012). To measure the strength of dominance relationships, we examined the degree to which dominant pukeko are likely to win a contest over subordinates (i.e. 'hierarchy asymmetry', Dey et al. 2013). Since pukeko are thought to form strong and hierarchical dominance relationships (Jamieson and Craig 1987), we predicted that dominance interactions would be highly transitive and asymmetrical.

Second, we determined how differences in individual attributes (Piper 1997), and structural dependence between ties (i.e. network self-organization, Chase et al. 2002; Chase and Seitz 2011), influence pukeko dominance networks. We performed separate network analyses on subordinate displays and on physical aggression, as the factors we were interested in could have differential effects on these two types of dominance interactions. In particular, we considered how the individual attributes of sex and frontal shield size influence dominance interactions. Same-sex dyads may be more likely to have dominance interactions than opposite-sex dyads because only same-sex individuals compete for reproductive opportunities; therefore we tested whether our networks exhibit sexual homophily (i.e. tendency to interact with others of the same type). Frontal shield size is also likely to influence dominance interactions because it communicates information about fighting ability and social rank. In general, more dominant (i.e. larger shielded) individuals are expected to receive more subordinate displays and are less likely to receive physical aggression, although this need not be the case (Francis 1988). Additionally, the difference in frontal shield size between individuals could influence the likelihood of an interaction. We predicted that subordinate displays would occur more often when dyads had large difference in shield size, as this type of interaction can be used by weaker animals to avoid physical confrontations with much stronger individuals. Conversely, we predicted that physical aggression would occur more often when individuals have similar shield sizes, because when the difference in fighting ability is small subordinates have more motivation to challenge dominants, and dominants should also physically suppress subordinates to maintain their rank. Finally, we analyzed how structural dependence between ties influenced pukeko dominance networks. Specifically, we tested whether pukeko dominance networks show an effect of reciprocity, where interactions from one individual to another influence the likelihood of the reciprocal interaction. We also tested whether these networks exhibit a tendency to form transitive triad motifs, which form the basis of orderly dominance hierarchies (Shizuka & McDonald 2012). Since our analysis controled for differences in individual attributes, a significant effect of transitive triad closure would suggest that the networks have self-organizational features.

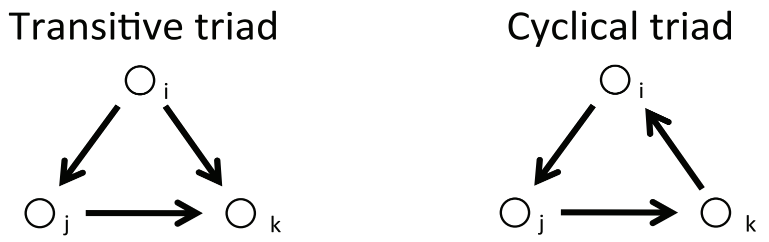

Figure 2.1 Transitive and cyclic triad motifs. Transitive triads can be reordered into a linear hierarchy (here i > j > k) and are therefore thought of as 'orderly'. Cyclical triads do not have this property.

Finally, we evaluated whether pukeko dominance interactions are stable over time. While a previous study showed that dominance rank is stable between years in pukeko (Jamieson & Craig 1987), we do not know if the patterns of dominance interactions are consistent. If dominance interactions are driven by factors that are relatively stable (e.g. individual attributes such as sex or rank), then dyads should have similar patterns of dominance interactions between years. Alternatively, if dominance network structure is primarily influenced by more variable factors (e.g. exogenous ecological conditions), then patterns of dominance interactions may not be consistent between years. Here, we explored this stability by testing whether the frequency of dominance interactions between a dyad is consistent between two successive years.

2.3 Methods

Field methods

This study was conducted at Tawharanui Regional Park (36° 22’ S, 174° 49’ E) on the North Island of New Zealand. At this site, pukeko social groups defend an all-purpose territory throughout the year, and long-term research has resulted in regions with a high percentage of banded individuals (trapping and banding procedures are published in Quinn et al. 2012; Dey et al. 2012). For this study, we selected social groups in which all adult individuals were uniquely banded, which allowed us to record all dominance interactions among members of each group. At the time of trapping, a suite of morphological measurements were taken including measurements of frontal shield size (shield width). When multiple sets of measurements were taken (i.e. an individual was caught multiple times), we used the set of measurements with the greatest temporal proximity to when the behavioural observations (see below) were performed. Sex was determined by measurement as described in Craig et al. (1980).

Dominance observations

Detailed behavioural observations were made in January and February 2012 on 11 social groups (mean group size = 7.5, range = 4-13). This time period is outside the typical breeding season for pukeko at this site (most reproduction occurs from August-October; Dey and Jamieson 2013), and no copulations or courtship behavior was observed during this study. For each group, we performed one 30-minute observation per day for a period of 10 days, resulting in a total of 5 hours of observation (following the methods in Jamieson & Craig 1987; Dey *et al.* 2014). Immediately prior to each period, the observer placed approximately 50 g of dried corn on the territory of the group to increase the frequency of dominance interactions. Pukeko are accustomed to human presence at this site and quickly resumed normal behaviours after this disturbance. The observer then recorded occurrences of two classes of dominance behaviours, subordinate displays (i.e. subordinate postures and displacements; Craig 1977) and aggressive interactions (i.e. pecks, kicks or charges; Craig 1977), that occurred among members of the focal group (average number of interactions over 5 hours of observation = 85.2, range = 5 – 241). Interactions between members of the focal group and members of other social groups occurred very infrequently. These typically consisted of a member of the focal group chasing away an intruder, and they were not recorded or considered in our analysis. The observer was concealed in a camouflaged blind and observed the social group using a spotting scope or binoculars. All observations were conducted between 06.30 and 10.00 hrs.

To determine whether pukeko dominance hierarchies were temporally stable, we performed an additional set of dominance observations on 6 of the same social groups, during March and April 2013 (about one year after the initial observations). At our study site, group membership is highly stable among years (similar to data in Craig and Jamieson 1988). Prior to our observations in 2013, any new adult group members were caught and banded (most of these individuals were offspring from 2012). The group was then subject to behavioural observations on 10 days, identical to those described above.

Statistical analysis

Network analysis was performed in R version 3.0.1 (R Core Team 2013) using the statnet (Handcock et al. 2008; 2014a; 2014b; Hunter et al. 2008) suite of packages and the ergm.count package (Krivitsky 2013). Our analysis of network orderliness and network structure was conducted on the 11 social groups observed in 2012. There were a total of 102 individuals in these social groups; however, 19 individuals were never captured as adults (i.e. they were banded as juveniles) and thus, their sexes and adult shield sizes were unknown. These individuals were removed from all analyses, leaving 83 individuals in our analysis of orderliness and structure. Our analysis of network stability required that known individuals were observed across two years. In the 6 social groups observed in 2012 and 2013, 32 individuals met these criteria, and these individuals were considered for this analysis.

(1) Dominance network orderliness

While one previous study has examined the linearity of pukeko dominance hierarchies (Jamieson and Craig 1987), the authors used methods that become biased when pairs of individuals do not interact or when group size varies (Klass and Cords 2011; Shizuka and McDonald 2012). Accordingly, we tested whether pukeko dominance hierarchies are orderly, using Shizuka and McDonald's (2012) triangle transitivity method. This measure is equivalent to linearity if the relationship among all dyads are known, but does not become biased when dyads do not interact. To perform this test, we first built a weighted, directed network for each of the 11 social groups observed in 2012. These networks were based on all the observed dominance interactions pooled across all 10 observation periods (ties were drawn from the dominant to subordinate in each interaction and tie weight was determined by the total number of dominance interactions). Then, we used these networks to calculate the triangle transitivity, t_{tri} (Shizuka and McDonald 2012), and dominance hierarchy asymmetry (Dey et al. 2013) for each group. Triangle transitivity measures the tendency of groups to form transitive triad substructures as opposed to cyclical triads (see Figure 1), and dominance asymmetry represents the global proportion of interactions in which dominant individual acts as such (i.e. they give aggression or receive submission). To calculate t_{tri}, we converted our weighted network to a binary 'dominant-subordinate' network (i.e. each member of each dyad was designated as the ‘dominant or ‘subordinate’ based on who was dominant in more than 50% of the interactions). Dyads in which each individual acted as dominant in exactly 50% of the interactions were considered unresolved, and were given no tie. Next, we calculated the proportion of transitive triads out of the total number of triads (in null models this proportion is 0.75) and used this value as a test statistic in a permutation test, as described in the supplementary material and corrigendum of Shizuka and McDonald (2012). For dominance asymmetry, we calculated the empirical value for each group by dividing the number of interactions in which the dominant individual in each dyad (as defined in the dominant-subordinate network above) acted dominant (i.e. was the aggressor in an interaction, or received a subordinate display), by the total number of interactions across the group. This value ranges from 0.5 (each individual in a dyad was equally likely to act dominant) to 1 (in each dyad, one individual always acted dominant). Then, for each social group, we permuted tie weight in the weighted network of dominance interactions, while holding the total number of dominance interactions constant and preventing self-loops (i.e. the diagonal in all permuted matrices was set to 0). In both analyses we conducted 2000 permutations per social group and calculated p-values based on two-tailed tests. P-values were then combined using Fisher’s omnibus test (see also Croft et al. 2006; Dey et al. 2013 for a similar approach).

(2) Dominance network structure

We explored how individual attributes influence dominance network structure using two ERGMs (also called p* models, Wasserman & Pattison 1996). These statistical models, which are somewhat analogous to logistic regression, model the presence or absence of ties in an empirical network given various predictor variables (Lusher et al. 2013). Importantly, ERGMs assume that network ties depend on one another and that global network structure is the result of local processes influencing tie formation. Through stochastic model fitting, ERGMs allow researchers to determine whether each predictor variable influences tie formation, while controlling for the other predictor variables in the model. As a result, ERGMs provide a statistically robust method for testing which factors contribute to the complex networks observed in animal societies, and should be a useful tool for SNA studies in behavioural ecology (Pinter-Wollman et al. 2013).

In our analysis, we considered two separate ERGMs using the observed subordinate displays and physical aggressive interactions as our empirical networks. In each network, we included the 11 social groups observed in 2012, and considered only the behavioural data collected in 2012. The individual attributes included in the models were sex, sexual homophily, frontal shield size and the dyadic difference in frontal shield size. Additionally, we investigated structural dependence between ties by considering whether there was a tendency for reciprocity, and transitive triad closure, in each of the networks.

Further details on implementation of ERGMs is provided in the Electronic Supplementary Materials.

(3) Dominance network stability

To analyze temporal stability of dominance networks, we tested if the number of dominance interactions between a dyad in 2012 was correlated with the number of dominance interactions between that same dyad in 2013. For this analysis, we considered two graphs (one from 2012, and one from 2013) composed of individuals that were observed in both years (32 individuals from 6 social groups). These graphs were weighted and undirected, and the tie weight was number of dominance interactions pooled across the 10 observation periods from each year. Dyads who could not possibly interact (i.e. individuals from different social groups) were given ‘NA’ values in the corresponding cell. We tested for a correlation using a Mantel test based on Spearman’s rank correlation (Mantel and Valand 1970; Legendre and Legendre 1998) implemented in the ‘vegan’ package in R (Oksanen et al. 2007). We used 2000 permutations and specified the ‘strata’ argument such that permutations were only done within social groups.

2.4 Results

(1) Dominance network orderliness

Dominance networks in pukeko social group were found to be significantly more transitive (permutation test: mean $t_{tri} = 0.86$, $f_{24} = 75.92$, $P < 0.001$) and asymmetrical (mean dominance asymmetry = 0.94, f_{24} = 94.53, P < 0.001), than expected by null models.

Table 2.1 Exponential random graph model fit for a network of pukeko subordinate displays. Significant P-values are shown in bold.

Model Term	Estimate	Standard Error	P-value
Sum	-0.73	0.42	0.08
Nonzero	-2.85	0.19	**<0.0001**
Actor effect of sex [male]	-1.04	0.22	**<0.0001**
Sexual homophily			
[male-male]	1.08	0.22	**<0.0001**
[female-female]	-0.10	0.08	0.21
Actor effect of shield size	-0.02	0.01	0.14
Receiver effect of shield size	0.07	0.01	**<0.0001**
Difference in shield size	-0.01	0.01	0.71
Transitivity	0.23	0.07	**0.001**
Reciprocity	-0.24	0.09	**0.007**

Table 2.2 Exponential random graph model fit for a network of physical aggression between pukeko.

Model Term	Estimate	Standard Error	P-value
Sum	-1.64	0.66	**0.01**
Nonzero	-3.57	0.20	**<0.0001**
Actor effect of sex [male]	1.48	0.43	**0.0006**
Sexual homophily			
[male-male]	-0.09	0.10	0.34
[female-female]	1.18	0.44	**0.007**
Actor effect of shield size	0.04	0.02	**0.02**
Receiver effect of shield size	-0.01	0.01	0.62
Difference in shield size	0.00	0.02	0.77
Transitivity	0.37	0.07	**<0.0001**
Reciprocity	-0.15	0.12	0.23

(2) Dominance network structure

Sex and frontal shield size influenced the structure of dominance interactions in pukeko social groups. Male pukeko were less likely to use subordinate displays

(Table 1; actor effect of sex) and more likely to use physical aggression (Table 2; actor effect of sex) than were female pukeko. However, after controlling for these effects, we found that males were more likely to produce subordinate displays to other males (Table 1; male sexual homophily), and that females were more likely to be physically aggressive with other females (Table 2; female sexual homophily). As predicted, individuals with larger frontal shields were more likely to receive subordinate displays (Table 1; receiver effect of shield size), and were more likely to give physical aggression (Table 2; actor effect of shield size), than were individuals with smaller frontal shields. However, we found no significant effect of the difference in shield size on the occurrence of subordinate displays or physical aggression (Table 1 and 2; difference in shield size). After controlling for the individual attributes above, there was still a positive and significant effect of transitive triad closure in both the network of submissive displays and physical aggression (Table 1 and 2, effect transitivity). Additionally, there was a significant, negative effect of reciprocity on the network of subordinate displays (Table 1, effect of reciprocity).

(3) Dominance network stability

The number of dominance interactions between a dyad was highly correlated between years (Mantel test: Spearman's $\rho = 0.57$, $p = 0.005$; Figure 2).

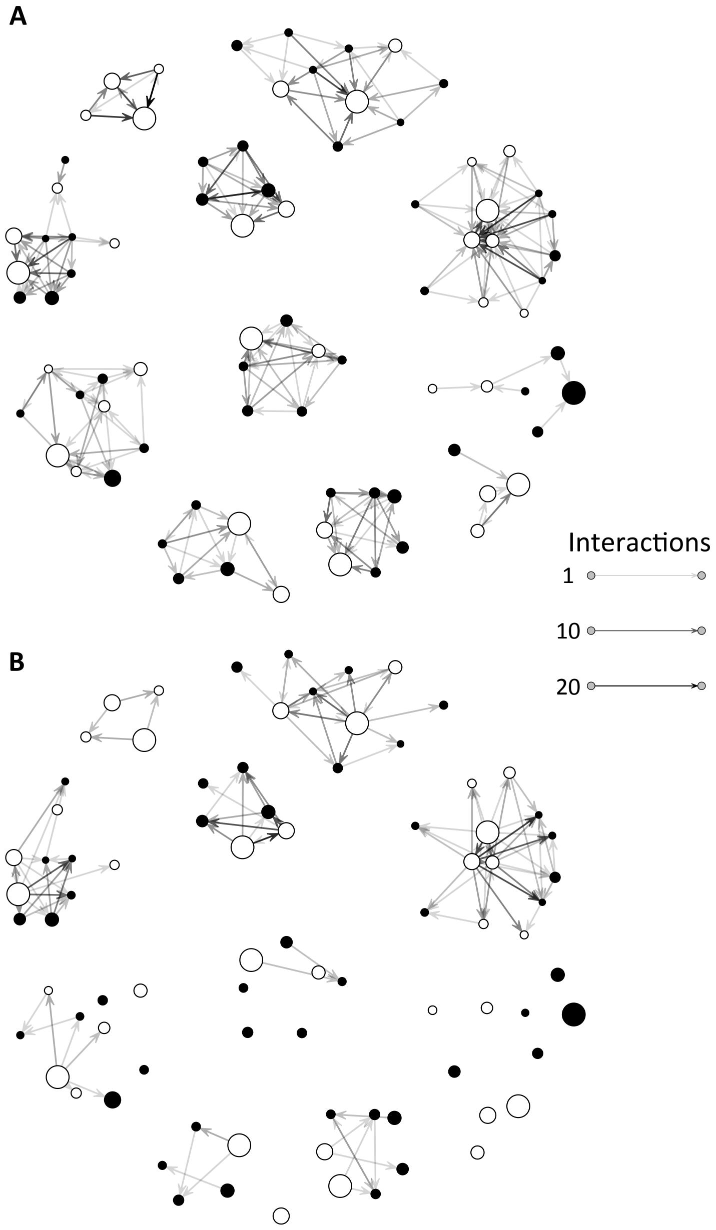

Figure 2.2 Networks of submissive displays (A) and physical aggression (B) in pukeko social groups. Nodal orientation was determined using the Fruchterman-Reingold algorithm and shows individuals clustering into 11 subgraphs representing the 11 social groups. Node size was scaled to frontal shield size, and node colour is used to indicate sex, with white and black filled circles representing males and females respectively. Tie colour indicates the number of interactions, with darker lines indicating more interactions (see legend). Figure created in statnet (Handcock et al. 2008; 2014).

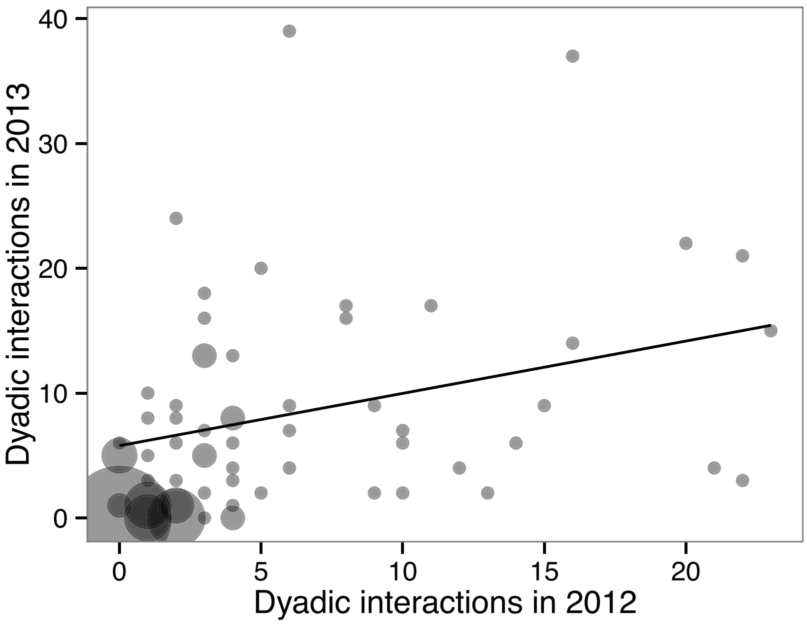

Figure 2.3 Temporal stability of dominance relationships. The total number of dominance interactions (both subordinate displays and physical aggression) is plotted for each dyad observed in both 2012 and 2013 (32 individuals from 6 social groups). Point size is scaled to the number of dyads that share the same coordinates in the plot. Figure created in ggplot2 (Wickham 2009).

2.5 Discussion

Patterns of dominance interactions in cooperatively breeding animals will often have an important influence on group dynamics and individual fitness. In this study, we show that pukeko form highly transitive (i.e. orderly) and strongly directional dominance relationships. Additionally, we show that individual attributes have important influences on patterns of dominance interactions. Specifically, submissive and aggressive interactions were influenced by the sex and shield size of both the actor and the receiver, although these traits may have different effects on different types of dominance interactions. Furthermore, our models show that pukeko tend to form closed transitive triads, which suggests that pukeko dominance networks are not simply due to differences in individual attributes, but are at least partially dependent on structural dependence between ties (i.e. self-organization; Chase and Seitz 2011). Finally, we show that patterns of dominance interactions were stable over a one-year period, as would be expected if dominance interactions are strongly determined by individual attributes and those attributes are relatively stable.

To our knowledge, this study is the first in the field of behavioural ecology to use exponential random graph models to analyze animal social networks. ERGMs have been widely used in the social sciences to analyze relationships among humans (e.g. Goodreau et al. 2009) and their utility in animal studies is readily apparent (Pinter-Wollman et al. 2013). One of the benefits of using ERGMs is that these models can consider how network ties self-organize through local substructures. These local substructures are commonly found in human social networks (Lusher et al. 2013) and are likely to be important factors in the organization of animal networks as well. In this study, we show that pukeko have a tendency to form transitive triad motifs, even after controlling for differences in individual attributes. Additionally, there was a negative effect of reciprocity on the network of subordinate displays, suggesting that pukeko are less likely to produce submissive behaviours towards individuals that have previously submitted to them. These effects could be due to winner, loser or bystander effects (e.g. Dugatkin and Earley 2003) and the models used in this study cannot distinguish among these mechanisms. However, careful combinations of certain structural motifs (e.g. in-stars, out-stars, two-paths and triads) could probably be used to investigate the relative importance of winner, loser and bystander effects at the group level. Unfortunately the use of structural motifs in weighted ERGMs is not as developed as their use in binary ERGMs, and we were unable to perform this type of analysis here.

The influence of individual attributes on dominance networks was largely consistent with our predictions based on expected patterns of conflict in cooperative breeding animals. Network structure was influenced by sexual homophily, which is expected if same sex individuals are competing for breeding positions (Dey et al. 2013). Interestingly, female homophily was more prevalent in the network of physical aggression, which is consistent with data suggesting that females have intense intrasexual competition for breeding positions in this species (Dey et al. 2012). Individual frontal shield size was also related to network structure, being positively correlated with the likelihood of giving aggression and receiving submissive displays. However, contrary to predictions, we found no significant effect of the difference in frontal shield size on these interactions. It is possible that the difference in frontal shield size influences the intensity of dominance interactions, rather than the frequency. For example, aggressive interactions between dyads that have large differences in shield size may be relatively low cost (i.e. pecks), while aggressive interactions that occur between individuals with similar sized shields may be more likely to escalate to more intense interactions (i.e. kicking and biting). Indeed, fights (reciprocal kicking and/or biting in short-time periods) are more likely to occur between individuals who are close in dominance rank than those who have large rank differences (CJ Dey *unpublished data*), which is consistent with this hypothesis. Further examination of the importance of difference in shield size on interaction intensity, as well as the inclusion of other individual attributes not considered in this study (e.g. age) may yet reveal other important influences on the structure of dominance networks in pukeko.

Collecting interaction or association data for network analyses on animals can be challenging and warrants careful consideration (Whitehead 2008). In most cases, researchers are restricted to collecting data in specific contexts (e.g. during daylight hours, or when animals are in a particular habitat type) or taking proxy measures of social relationships (e.g. ‘gambit of the group’), which could result in differences between the data collected and the true social relationships among groups of animals (James et al. 2009). In this study, we used an artificial patch of food to increase the rate of dominance interactions in our focal groups, which is a common approach used to study dominance in both wild and captive animals (e.g. Val-Laillett et al. 2008; Tarvin and Woolfenden 1997). While pukeko do frequently compete over natural food resources (Craig 1977, CJ Dey *personal* observation), these resources are more dispersed than the food piles used in this study and it is possible that the networks produced in this study do not perfectly reflect natural networks of dominance interactions. However, pukeko dominance rank as determined through artificial feeding is correlated with frontal shield size (Dey et al. 2014), with differences in copulations (Jamieson and Craig 1987) and with differences in parental investment (Craig 1980), which together suggest that this method of measuring dominance encompasses at least part of the true dominance relationships among individuals. Nonetheless, it is important to consider the data collection methods when interpreting the findings of social network studies in behavioural ecology, and the results of the current study should be interpreted with an understanding of this caveat.

Dominance is a rich type of social relationship that has important links to individual fitness. While most previous studies have focused on dominance hierarchies and social rank, there is a large scope for social network analysis to greatly improve our understanding of dominance interactions. In this study, we used network analyses to perform a comprehensive exploration of dominance interactions in the cooperatively breeding pukeko. In addition to demonstrating that pukeko dominance relationships are transitive, asymmetrical and temporally stable, we also show that the structure of dominance networks is influenced by both individual attributes and structural dependence between ties. This network-based approach provides a more detailed understanding of the causes and consequences of dominance in this species, and the methods used here should be widely applicable to other studies on dominance in animal societies.

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Chapter 3: Hatching Order Affects Offspring Growth, Survival and Adult Dominance in the Joint-Laying Pukeko *Porphyrio melanotus melanotus*

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3.1 Abstract

In birds with asynchronous hatching, hatching order is an important factor in determining offspring phenotype. Many previous studies have demonstrated that later-hatched offspring show reduced growth and survival during development. However, few studies have followed individuals from hatching to adulthood to test if the effects of hatching order persist into later life. Here, we explore patterns of hatching order and fitness-related traits in the Pukeko *Porphyrio melanotus melanotus*, a cooperatively breeding bird that lives in stable social groups that form linear dominance hierarchies. Pukeko groups sometimes contain two breeding females that lay eggs in the same nest ('joint-laying'). Thus, competition between nest-mates can influence the relative fitness of each laying female. In this study we show that in both single-clutch and joint-clutch nests, earlier-hatched Pukeko chicks grow faster and survive better than later hatched brood-mates. Moreover, earlier-hatched chicks achieve higher dominance ranks as adults, making this study one of the first to find a relationship between hatching order and adult dominance in wild birds. Finally, we show that in groups with two breeding females, the chicks of the primary female hatch earlier than the chicks of the secondary female. As a result, the offspring of the primary female may be at a competitive advantage, which could have important implications for social dynamics in this species.

3.2 Introduction

Hatching asynchrony is widespread among birds (Magrath 1990) and occurs as a result of incubation being initiated before laying of the clutch is complete. In general, hatching asynchrony is thought to be adaptive because the formation of size hierarchies within the brood is beneficial to parents. While the adaptive value of hatching asynchrony is likely to differ between species due to biological and ecological idiosyncrasies (Magrath 1990, Stenning 1996), the most common explanations are that size hierarchies facilitate brood reduction in unfavourable environmental conditions (e.g. Ricklefs 1965), provide insurance against the failure of core offspring (e.g. Mock and Forbes 1995, Forbes *et al.* 1997) and improve the efficiency of raising the brood by decreasing competition among nestlings (e.g. Mock and Ploger 1987). Alternatively, hatching asynchrony may simply be a by-product of selection to initiate incubation prior to clutch completion (e.g. to minimize nest predation; Clark and Wilson 1981), and may not be due to selection on offspring size hierarchies *per se*.

Regardless of the ultimate factors favouring hatching asynchrony, there is strong empirical evidence that hatching order affects offspring phenotype. Typically, later-hatched chicks show reduced growth (Stokland and Amundsen 1988, Rosivall *et al.* 2005) and survival (Magrath 1990, Vinuela 2000) as they are unable to compete with older brood-mates for resources (e.g. Mock 1984, Bryant and Tatner 1990, Legge 2000). Yet, although many studies have investigated the influence of hatching asynchrony on offspring phenotype, we have a limited empirical understanding of whether hatching order effects persist into adulthood, especially in the wild. In captive studies, some species can partially or completely overcome early life challenges (such as late hatching) through compensatory growth or other forms of developmental plasticity (Hector and Nakagawa 2012). Additionally, a series of recent studies on Blue-footed Boobies *Sula nebouxii* have demonstrated that while later-hatched chicks experience increased aggression, food deprivation and chronically increased levels of stress hormones during development, they do not have increased mortality (Drummond *et al.* 2011) or reduced body size (Carmona-Isunza *et al.* 2013), nor do they show different levels of aggression when defending their own nest as adults (Sánchez-Macouzet and Drummond 2011) when compared with earlier-hatched chicks. However, offspring produced in the first three years of life by adults who were raised as junior (later-hatched) chicks were less likely to recruit into the breeding population than offspring of former senior chicks, suggesting that the costs of hatching order are passed on to subsequent generations (Drummond and Rodriguez 2013). It remains unclear how hatching asynchrony affects adult phenotypes in other wild birds, and additional studies are needed to determine if the patterns observed in Blue-footed Boobies are more general.

If hatching order effects persist into adulthood, they could be an important source of inter-individual variation in fitness-related traits such as social dominance.

There is good empirical evidence that dominance influences reproductive success (Ellis 1995) and survival in different taxa (e.g. Kikkawa 1980, Thomas *et al.* 2003, Koenig *et al*. 2011). Dominance relationships usually form as a result of repeated interactions among individuals that vary in their resource-holding potential (i.e. the ability of an animal to win an all out fight), and researchers have found predictable correlations between dominance rank and body size (McElligott *et al.* 2001; McCauley 2010; Reddon *et al.* 2011), body condition (Huang *et al.* 2011) and measures of strength (Sneddon *et al*. 2000, Husak *et al*. 2006). Because these physical traits can be influenced by conditions during growth (e.g. Cooch *et al.* 1991, Searcy *et al.* 2004), it is important to understand how hatching order influences development and adult phenotype, if we are to gain a comprehensive understanding of the causes and consequences of dominance in animal societies.

Cooperatively breeding birds provide an interesting system in which to investigate asynchronous hatching. In many such species offspring show a high degree of natal philopatry, which provides a convenient opportunity to track individuals from hatching to adulthood and to assess the effects of hatching order at multiple time points. Furthermore, in some cooperative breeders, group offspring have mixed parentage, which can increase within-brood competition due to low relatedness among brood members (Briskie *et al.* 1994, Mock and Parker 1998). For example, in some cooperatively breeding birds, multiple females contribute eggs to a single nest and contribute to parental care (termed joint-laying; Vehrencamp 2000, Vehrencamp and Quinn 2004). In many joint-laying species, females employ behavioural strategies such as egg tossing or egg burial (i.e. burying eggs in the nest structure) to avoid having their eggs hatch later than those of other females and to reduce the number of competitors within the group nest (e.g. Mumme *et al.* 1983, Schmaltz *et al.* 2008, Riehl 2010). Similar strategies are observed in communally breeding species from other taxa, which perform infanticide or eviction, or may alter gestation periods to avoid the negative consequences of among-female reproductive asynchrony (Ims 1990, Poikonen *et al.* 2008, Hodge *et al.* 2011). Although these competitive reproductive behaviours have been well-documented, the consequences of hatching asynchrony are poorly understood for almost all joint-laying birds.

Here, we explore the effects of hatching order on offspring growth, survival and competitive ability during adulthood, in the joint-laying Pukeko *Porphyrio melanotus melanotus*. Although mated Pukeko pairs can breed successfully, it is more common for groups of 3-12 individuals to defend a shared territory and raise the group offspring cooperatively (Craig 1980a). Such groups usually contain multiple breeding males and one or two breeding females who mate promiscuously, as well as non-breeding helpers of both sexes. Adult Pukeko form a linear dominance hierarchy (Jamieson and Craig 1987, CJ Dey *unpubl.data*) with breeding males being the highest ranked individuals, breeding females and non-breeding males having intermediate ranks and non-breeding females being the lowest-ranked. When there are multiple breeding females in a group, the dominant female is involved in more copulations, and usually lays more eggs than the subordinate breeding female (Jamieson and Craig 1987, Craig 1980b), and dominance status determines the order of replacement of breeding females by non-breeding helpers (Jamieson and Craig 1987). The dominant breeding male is more likely to perform nocturnal incubation than are other males (Craig 1980a), but it is unclear whether there is a relationship between dominance status and paternity in this species (Jamieson *et al.* 1994; Jamieson 1997).

Incubation of Pukeko nests begins part way through the laying period, and thus hatching is asynchronous (Craig 1980a). Pukeko offspring are nidifugous, leaving the nest after 3-4 days, however they are fed by adults for up to 2 months (Dey and Jamieson 2013). Offspring fledge between two and three months old and groups frequently fledge multiple chicks from a nest. On the North Island of New Zealand (where this study was performed), Pukeko are highly philopatric and offspring of both sexes typically inherit breeding positions within their natal group rather than dispersing to join other groups (Craig and Jamieson 1988; CJ Dey *unpublished data*). As a result, nest-mates will often be in competition for breeding positions when they reach adulthood. We investigated the consequence of hatching order on fitness-related traits by testing three predictions: (1) that earlier-hatched offspring would show increased growth during development relative to later-hatched nest-mates, (2) that earlier-hatched offspring would show reduced mortality during development relative to later-hatched nest-mates and (3) that earlier-hatched offspring would be socially dominant over later nest-mates in adulthood. To control for the possibility that egg size might influence offspring traits we also explored how egg size varied across the hatching period. Finally, we explored patterns of hatching order in joint-clutch nests to begin to understand how hatching asynchrony might affect female reproductive success in this species.

3.3 Methods

Field methods

We conducted this study at Tawharanui Regional Park (36° 22' S, 174° 49' E) on the North Island of New Zealand. During the 2010 breeding season (July-December), we located Pukeko nests by searching suitable nesting habitat (among *Juncus*, *Carex* or *Typha* grasses). While Pukeko groups can breed several times per year, only the first reproductive attempt by each social group was considered in this study. In total, we collected data from 32 social groups (20 single-clutch and 12 joint-clutch nests) for this study. However, not all data were available for each nest and thus sample sizes differ among analyses.

At each nest, we measured length and width of all eggs, marked them with a non-toxic marker and floated them in warm water to estimate the date they were laid

(Hays and LeCroy 1971). In nests with known initiation dates, flotation scores are highly correlated with egg age (Dey *et al.* 2013). We visited nests daily during the laying and hatching periods, and every third day between those periods. During the hatching period, we recorded the hatching date of each chick and fitted each hatchling with a single coloured, plastic leg band and a single metal band (with a unique number) both containing soft foam to allow for leg growth.

To determine whether hatching order affects offspring growth (prediction 1), we recaptured as many chicks as possible between the age of 10 and 59 days old. Chicks were captured by hand or using funnel traps baited with corn. Upon recapture, we measured the length of the each chick’s left tarsus and fitted it with a further three coloured plastic leg bands to create a unique band combination for each individual. In total, 64 chicks from 24 nests were recaptured in this manner.

To determine whether hatching order influences offspring survival (prediction 2), we surveyed the social groups in which we had monitored nests at 60 days after the completion of hatching and again in January 2012 (15-17 months after hatching). At this age, Pukeko are sexually mature but are typically non-breeding helpers within their natal groups. At Tawharanui, Pukeko are highly philopatric (consistent with other North Island sites; e.g. Craig and Jamieson 1988), and no offspring were found in groups other than their natal group. Additionally, long-distance dispersal by Pukeko is extremely rare (Craig and Jamieson 1988). Thus, we assumed that individuals that were not located in their natal group were dead. Groups were surveyed using spotting scopes or binoculars from portable blinds positioned on hilltops. We performed two 30-minute surveys at each time point (i.e. at 60 days and in January 2012), to ensure that all individuals in each group were identified. In total, 30 social groups (with 68 surviving and 53 dead offspring) were observed at 60 days after hatching and 28 social groups (with 34 surviving and 79 dead offspring) were observed in January 2012.

To test whether hatching order influences social dominance in adult Pukeko (prediction 3), we performed detailed behavioural observations on eight social groups that had multiple chicks that hatched in 2010 and that survived until January 2012 (a total of 23 chicks survived in these groups). Observations were made in January and February 2012 and consisted of one 30-minute observation per day for a period of five days (following the methods in Jamieson and Craig 1987 and Dey *et al.* 2014). The observer placed approximately 50 g of dried corn in the territory of the group of interest immediately prior to each observation period in order to increase the frequency of dominance interactions. Pukeko are accustomed to human presence at this site and quickly resumed normal behaviours after this disturbance. The observer then recorded all dominance interactions (physical aggression as well as dominant and submissive postures; Craig 1977) from a camouflaged hide. We only recorded interactions that occurred between the surviving young because not all other group members were banded. Observers were blind to the hatching order of the individuals and all observations were conducted between 06.30 and 10.00 hrs.

To investigate how hatching order might differentially affect the reproductive output of dominant and subordinate female Pukeko, we examined patterns of hatching in joint-clutch nests (12 nests). The eggs of individual female Pukeko are identifiable, as each female lays eggs with a unique colour, shape, size and spotting pattern (Craig 1980a, Jamieson 1997, Quinn *et al.* 2012). We assigned each egg to either the 'A' clutch (i.e. the first egg type laid; assumed to be the dominant female's clutch) or the 'B' clutch (i.e. the clutch that was initiated second) and recorded the hatching date of each egg. Eggs from both the A and B clutch often hatched on the same day, and so while we were able to assess differences in hatching order between the dominant and subordinate female, as well as assess the consequences of hatching order for chicks, we were not always able to determine the maternity of each chick. Thus, we did not perform analyses of the direct relationship between maternal status and growth, survival or dominance

Statistical analysis

All analyses were conducted using R version 3.0.1 (R Core Team, 2013). *A priori*, we restricted all models to include only the variables of interest and essential covariates, as our sample size did not allow for complex models. To determine whether our data met the assumptions of each model, we visually inspected diagnostic plots including residuals vs. fitted and quantile-quantile plots. Generalized linear mixed models (GLMMs) were also checked for overdispersion. To assess the effect of hatching order on various measures of offspring fitness, we calculated a 'hatch order' value for each chick from each nest by ranking the hatching sequence for each nest. When multiple individuals hatched on the same day, they were given equal hatch order values. For example, if three chicks hatched on day 1 they would all receive a hatch order value of 2 (the mean of rank 1, 2 and 3). The next chick to hatch would receive a hatch order value of 4. Hatch order values ranged from 1 to 8 in this study and these data were treated as continuous in all models.

To test whether hatching order influences offspring growth within the first 60 days of hatching we used a linear mixed-effects model (LMM) implemented in the 'nlme' package in R (Pinheiro *et al*. 2013). In this model, tarsus length was used as the response variable, social group was included as a random intercept, and both chick age (days since hatching) and hatch order were included as fixed effects.

To test the effect of hatching order on offspring survival, we performed two separate binomial family GLMMs with logit link functions. In these models we considered whether or not an individual survived to 60 days, or until January

2012, as the response variables. In each model, we included social group as a random intercept and hatch order as the sole fixed effect. Models were fitted with Laplace approximation in the ‘lme4’ package (Bates *et al*. 2013), as suggested by Bolker *et al*. (2009) when the expected number of survivals and deaths are fewer than five for each social group.

To test the effects of hatching order on social dominance, we calculated dominance scores using all of the dominance interactions pooled across the five observation periods. We used the dominance scoring method outlined in David (1987), which incorporates the number of animals that an individual directly dominates, while also considering the dominance status of the focal individual’s opponents. Thus, individuals can receive high scores if they are dominant over many individuals, or if they dominate individuals who are dominant over many other individuals. All types of dominance interactions (physical aggression and dominant/subordinate postures) were included in this analysis and received equal weight. Next, we rank transformed the dominance scores to produce a dominance rank for each individual (with 1 being the highest ranked) and used these ranks as the response variable in a cumulative link mixed model (CLMM), which analyzes ordinal response variables while allowing for random factors. This model was implemented using the ‘ordinal’ package in R (Christensen 2012). We included social group as a random effect and hatch order as the sole fixed effect in this model.

To examine how egg size varied with hatching order we calculated egg volume using the equation volume = 0.51*length*(width^2) (Hoyt 1979). Volume was then used as the response variable in an LMM, which included nest ID as a random intercept and both hatch order and female status (primary or secondary) as fixed effects. This analysis included data from all eggs that hatched in this study (124 eggs from 32 nests).

To test whether the dominant female's offspring hatched earlier than the subordinate female's offspring in joint-clutch nests, we performed an LMM with hatch order as the response variable. In this model, social group was included as a random factor and maternity (i.e. A or B clutch) was included as the sole fixed effect.

3.4 Results

After controlling for age (LMM: n = 64 offspring from 24 nests; estimate = 1.31, 95% CI = (1.15, 1.46), $t_{1,38}$ = 17.21, $p < 0.0001$), hatching order significantly predicted chick size within 60 days of hatching, with earlier-hatched chicks being larger than later-hatched chicks (estimate = -1.34, 95% CI = (-2.60, -0.08), $t_{1,38}$ = -2.16, $p = 0.038$). Additionally, earlier-hatched chicks were more likely to survive to 60 days of age (GLMM: n = 121 offspring from 30 nests; estimate = -0.53, 95% CI = (-0.84, -0.23), z_1 = -3.46, $p < 0.001$; Figure 1) and until January 2012

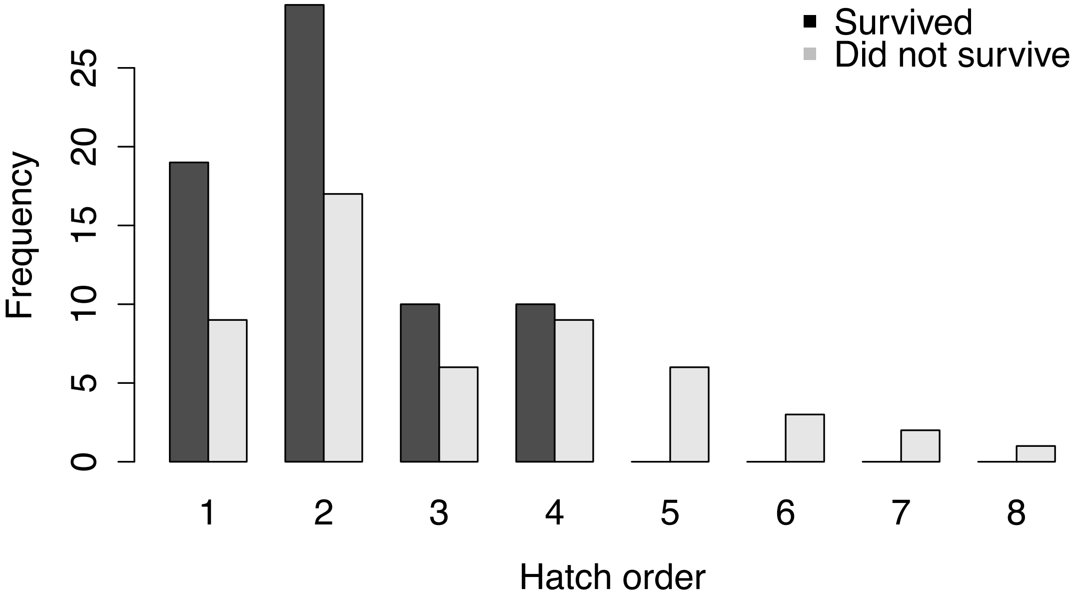

Figure 3.1 Frequency histogram of offspring survival to 60 days after hatching in relation to hatch order. The frequency histogram shows individuals that survived (dark bars) in comparison to individuals that did not survive (light bars) for each hatch order value. Hatch order values are rounded to the nearest integer. See text for details of statistical analysis.

(GLMM: n = 113 offspring from 28 nests; estimate = -0.65, 95% CI = (-1.10, -0.20), z_1 = -2.82, p = 0.005). As adults, individuals that hatched earlier within their broods, were more likely to be socially dominant than were later-hatching individuals (CLMM: n = 23 offspring from 8 nests; estimate = 1.92, 95% CI = (0.72, 3.12), z_3 = 3.129, p = 0.002; Figure 2). These relationships were not due to large hatching spreads in joint-clutch nests, as models conducted with only single clutch nests included yielded qualitatively similar and statistically significant results. Furthermore, these relationships were not explained by differences in egg size, as earlier-hatched eggs were not significantly larger than later-hatched eggs

(LMM: n = 124 eggs from 30 nests; estimate = 87.00, 95% CI = (-162.45, 336.46), $t_{1,92}$ = 0.69, p = 0.49), nor were the eggs of secondary females smaller than the eggs of primary females (estimate[secondary] = -553.93, 95 % CI = (-1557.2, 449.86), $t_{1,92}$ = -1.09, p = 0.28). Finally, while there was considerable overlap in the age of chicks from each female in joint-clutch nests (Figure 3), the primary female's offspring hatched earlier than those of the secondary female (LMM: n = 60 eggs from 12 nests; estimate = 1.27, 95% CI = (0.49, 2.09), $t_{1,47}$ = 3.11, p = 0.003).

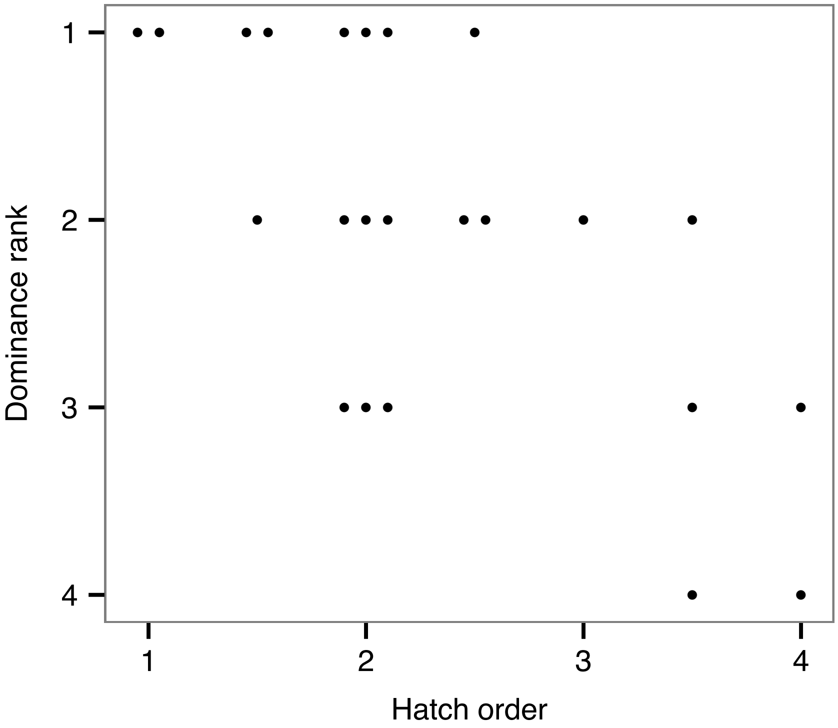

Figure 3.2. Relative adult dominance ranks (1 = most dominant) of brood-mates vs. hatch order (1 = first-hatched) of each individual within their brood. Overlapping data points are offset by 0.05 units on the x-axis. See text for details of statistical analysis. Figure created using ggplot2 package in R (Wickham 2009)

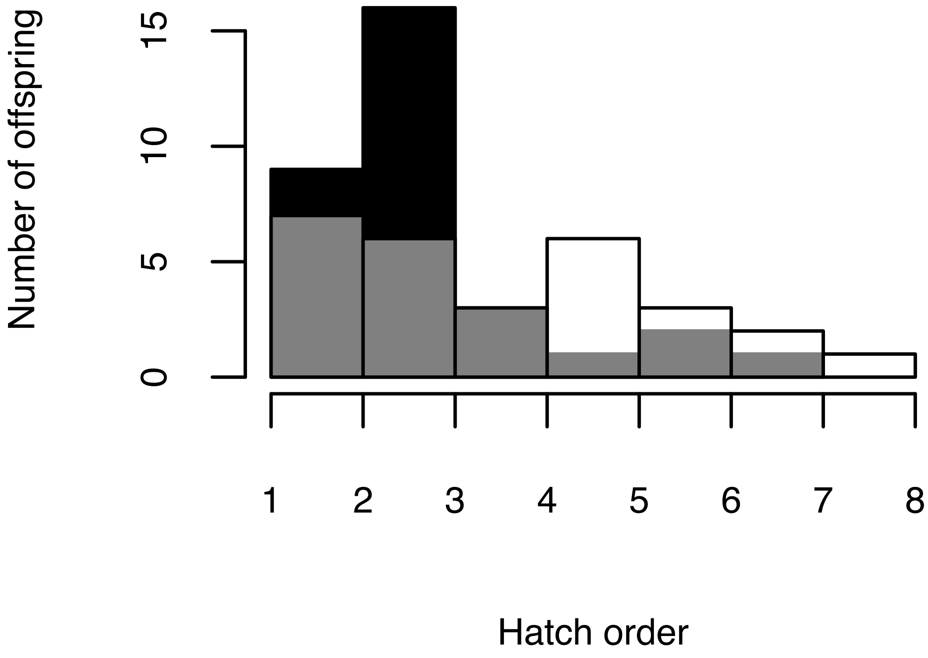

Figure 3.3. Hatching order histogram from joint-clutch nests. Data shown are hatch order values of offspring laid by the primary (black) and secondary (white) females, with grey shading representing the overlap between the histograms from each female type. Hatch order values are rounded to the nearest integer. See text for details of statistical analysis.

3.5 Discussion

In many birds, hatching asynchrony leads to size hierarchies in the developing brood (Mock and Parker 1986, Stokland and Amundsen 1988, Rosivall *et al*. 2005). Here we show that earlier-hatched chicks are larger for their age and have higher survival rates then later-hatched brood-mates. Additionally, we found that hatching order effects persist into adulthood, as earlier-hatched chicks achieve higher adult dominance ranks within their social group. While previous studies have demonstrated that hatching order has effects on offspring phenotype in many birds (e.g. Zach 1982, Love *et al.* 2003), ours is one of the first to follow wild birds from hatching to adulthood to determine how initial differences in competitive ability might influence adult traits. Our results are consistent with laboratory experiments in demonstrating that early life deficits (e.g. dietary restrictions, stress hormone treatments) can alter adult traits such as body size, sexual attractiveness, cognitive performance and dominance (Lindstrom 1999, Royle *et al*. 2005, Fisher *et al.* 2006). However, the manipulations performed in many experimental studies may impose challenges that are more severe than those faced in the wild (Drummond *et al*. 2011), and thus it is important to confirm whether natural early life challenges also lead to these costs during adulthood.

The mechanism by which hatching order affects adult dominance in Pukeko remains unclear. It may be that earlier-hatched individuals achieve larger adult size due to increased food acquisition during growth. Pukeko chicks are led and fed by members of the social group after hatching, and larger chicks appear to receive more food from adult group members (CJ Dey *pers. obs.*). Because adult body size is correlated with dominance in this species (Dey *et al*. 2014), a bias in food acquisition during development could lead to hatch-order mediated dominance hierarchies in adulthood. It is also possible that Pukeko chicks establish dominance hierarchies early in life through direct interactions with their brood-mates, and that these hierarchies persist into adulthood due to winner or loser effects (Dugatkin 1997, Drummond and Osorno 1992, Drummond and Canales 1998). Additionally, this study cannot eliminate the possibility that the relationship between hatching order and social dominance, body size or survival is due to a sex-ratio bias in hatching order in Pukeko nests. Because male Pukeko are generally larger and more dominant than females (Jamieson and Craig 1987, Dey *et al.* 2014), a tendency for males to hatch earlier than females could confound apparent hatching order effects. A sex-ratio bias in hatching order would not be expected in Pukeko, because males and females have about equal variance in lifetime inclusive fitness (Dey *et al.* 2012), and therefore there is no apparent advantage for breeding females to produce either sons or daughters earlier in the hatching sequence (Trivers and Willard 1973). Furthermore, a sex-ratio bias in hatching order would itself suggest that there are some fitness consequences to hatching order *per se*, otherwise there would be no selective pressure to favour a sex-ratio bias. Finally, this study was restricted to observations of the first reproductive event of the year for each social group. The first reproductive event is the most likely to be successful, and is therefore a more important fitness determinant than are later reproductive events (Craig 1980b). However, it is possible that the effects of hatching order may be different for later reproductive events, as there may be dependent offspring from previous nests competing for or providing, (allo)parental care. Therefore, we will require future studies that determine offspring sex, as well as those that observe interactions between nest-mates and manipulate hatching asynchrony, in order to fully understand the casual relationships between hatching order and fitness-related traits in Pukeko.

The long-term effects of hatching asynchrony are particularly interesting in cooperatively breeding species because the group offspring may be of mixed parentage. As a result, there should be strong selection on traits involved in competition between brood-mates. Here, we show that in Pukeko groups with two breeding females, offspring of the dominant female hatch earlier than those of the subordinate female. While we could not directly determine the maternity of each offspring (because multiple offspring often hatched on a single day), it is likely that this systematic difference in hatching order influences the relative competitive ability of the offspring from each breeding female. This could have important implications for social dynamics in this species, because subordinate individuals may be disadvantaged both in breeding opportunities and in the likelihood of success of their surviving offspring. In general, joint-nesting in Pukeko is thought to be disadvantageous for dominant females because a lower percentage of eggs hatch in joint-clutch nests than in single clutch nests (Vehrencamp and Quinn 2004, Quinn *et al.* 2012, Dey *et al*. 2013). However, if the dominant female's offspring are able to out-compete those of the subordinate breeding female, the dominant female may not pay as severe a cost of joint-laying as previously inferred. Hatching asynchrony and the resultant effects on offspring competitive ability have been implicated as an important factor in the social dynamics of other joint-laying birds (e.g. Cariello *et al*. 2006; Schmaltz *et al*. 2008; Riehl 2010), but we are aware of only one other joint-laying species in which this relationship has been directly studied. In the Acorn Woodpecker *Melanerpes formicivorus*, earlier-hatched chicks are larger and more competitive than their later-hatched brood-mates (Stanback 1994, Koenig *et al*. 2011). However, egg destruction by co-breeding females minimizes hatching asynchrony in this species (Mumme *et al*. 1988), and the effects of hatching order therefore probably do not influence the relative fitness of co-breeding females.

In addition to hatching (or birth) order, communally breeding animals could also influence offspring competitive ability by adaptively manipulating the growth environment through so-called ‘maternal effects’ (Russell and Lummaa 2009). For example, several studies have demonstrated that avian parents manipulate the size and content of their eggs to better match their offspring’s phenotype to the environment they will face (e.g. Groothuis *et al*. 2005) or to provide competitive advantages within the brood (Müller *et al*. 2007). Although few studies have investigated this phenomenon in joint-laying birds, in the crotophagine cuckoos, mothers are known to adjust both egg size (Riehl 2010) and yolk testosterone levels (Cariello *et al.* 2006, Schmaltz *et al*. 2008) to improve offspring competitiveness. While our data suggest that variation in egg size is not responsible for the patterns of growth, survival and dominance observed in the current study, it is possible that yolk hormone levels play a role. If females deposit higher levels of androgens in earlier-laid eggs, or if dominant females deposit more androgens than subordinate females, then earlier-hatched offspring may grow faster and achieve higher dominance ranks regardless of any hatching order effect *per se* (Schwabl 1997, Strasser and Schwabl 2004). Future studies investigating how yolk hormone deposition varies with laying order and maternity in Pukeko will be extremely valuable in understanding the complex social dynamics of this species. However, even if females do invest in more in early than late-laid eggs, hatching order will probably still have an important influence on offspring fitness given that hatching order has a stronger effect than yolk androgen levels on offspring fitness in other avian species (Schwabl 1997, Eising *et al*. 2001, Groothuis *et al*. 2005).

In conclusion, Pukeko show persistent effects of hatching order on adult phenotype in wild, free-ranging individuals. Earlier-hatched chicks have early benefits such as increased growth and survival as juveniles, as well as higher social rank as adults. This pattern may increase the fitness of dominant females in joint-nests, as they are responsible for earlier-laid eggs. This research demonstrates that the timing of reproduction has profound impacts on offspring quality, and lends insight into the factors driving intragroup competition in a cooperative breeding system.

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3.6 References

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Chapter 4: Reproductive Skew and Female Trait Elaboration in a Cooperatively Breeding Rail

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4.1 Abstract

Intrasexual competition for reproduction is thought to be an important factor in the evolution of ornaments and weapons in males. However, the evolution of morphologically similar traits in females is often explained through other mechanisms, and the role of intrasexual competition in female trait elaboration has received little research attention. Here, we explore the factors associated with female trait elaboration in the cooperatively breeding Purple Swamphen (*Porphyrio porphyrio melanotus*) by comparing sexual dimorphism in an ornament across two populations. Importantly, the two populations considered differ in several social factors that could affect the degree of female-female competition, and thereby produce differential selection on elaborate female traits. Although recent studies have suggested that high reproductive skew (i.e. monopolization of reproduction by dominants) could influence the intensity of intrasexual competition and select for female elaboration, we found that sexual dimorphism was diminished and Purple Swamphen females had more elaborate ornaments under conditions of low reproductive skew. We discuss alternative factors that could influence the degree of female-female competition, and show that reproductive skew may not always provide an accurate estimator of the scope for intrasexual competition.

4.2 Introduction

Darwin (1871) noted that males often compete intensely for mates; either directly, through physical combat, or indirectly, through attempts to 'excite or charm those of the opposite sex'. Such competitions usually involve secondary sexual characteristics (Hunter 1837), traits that are not involved in reproduction *per se*, but are important in competition for breeding opportunities (e.g. Bergeron *et al.* 2010). Such traits are typically more developed in males than females (Clutton-Brock 2009); however, females of many species have highly developed secondary sexual traits, and the mechanisms underlying their evolution have received less research attention than have similar traits in males. Elaborate female traits are sometimes attributed to genetic correlation between the sexes (Lande 1980), but there has been a recent interest in whether intrasexual competition might be a driving force in the evolution of female secondary sexual characteristics (LeBas 2006, Clutton-Brock 2009, Rubenstein and Lovette 2009, Watson and Simmons 2010).

Cooperatively breeding birds provide an excellent model for investigating how female intrasexual competition influences trait elaboration. In these species direct reproduction is often unequally distributed among females within each breeding attempt or season, a social factor known as high female reproductive skew (Vehrencamp 1983). Thus, in cooperative breeders intrasexual competition can be intense in both males and females (Clutton-Brock *et al.* 2006) because it often pays both sexes to compete for breeding positions. Also, because females often compete for breeding positions in these species, the role of mate choice by males is reduced (Clutton-Brock 2009, Rubenstein and Lovette 2009), and thus, female trait elaboration in cooperative breeders is likely caused by selection on traits that enhance success in intrasexual competition (Clutton-Brock 2009, Rubenstein and Lovette 2009). Indeed, across species of African starlings, cooperative breeders were found to have reduced sexual dimorphism in comparison to non-cooperatively breeding species, and this resulted from increased selection on female traits that enhance access to breeding opportunities in cooperative breeders (Rubenstein and Lovette 2009). To date, no study has examined patterns of female trait elaboration and reproductive skew within a single species, but such studies are necessary to fully understand how social structure influences female trait evolution.

Here, we explore the factors influencing sexual dimorphism in the frontal shield of the cooperatively breeding Purple Swamphen (Pukeko) *Porphyrio porphyrio melanotus*. The fleshy frontal shields of rails are testosterone-dependent characters (Gullion 1951, Eens *et al.* 2000) that are prominently displayed in aggressive interactions. Shield size is correlated with social status in Purple Swamphens (Craig 1974), and social status is important in defining female reproductive success (Jamieson and Craig 1987, Craig and Jamieson 1990). Purple Swamphens show population differences in social structure, providing a rare opportunity to explore the effect of social factors on female trait elaboration within a single species. New Zealand's North Island Purple Swamphens are sedentary and defend territories year-round. The groups are large, composed of related members, and are characterized by high female reproductive skew (Jamieson 1997, Craig and Jamieson 1988, Lambert *et al*. 1994). Conversely, South Island Purple Swamphens only defend territories during the breeding season, after which the group may partially or completely disband. Groups are small, consist of unrelated individuals and have low female reproductive skew (Jamieson *et al.* 1994, Jamieson 1997).

In societies where dominant females monopolize reproduction, competition for social status is expected to be intense as there are substantial reproductive consequences associated with social status (Keller and Reeve 1994, Reeve 2000). High reproductive skew societies typically have high inter-individual variation in lifetime reproductive success (Hauber and Lacey 2005), which increases the scope for selection on traits that enhance success in intrasexual competition (Andersson 1994, Rubenstein and Lovette 2009). As a result, selection on female secondary sexual characteristics is expected to be more intense when reproductive skew is high. However, Purple Swamphens differ from most avian species in that females, as well as males, can be highly philopatric (Craig and Jamieson 1988). Since patterns of philopatry affect the spatial and temporal distribution of related individuals (Clutton-Brock and Parker 1992, Lambin 1994), they may alter the degree of intrasexual competition by influencing the local abundance of kin and non-kin competitors. As a result of female philopatry, the patterns of female intrasexual competition and trait elaboration observed in cooperative breeding African starlings (Rubenstein and Lovette, 2009) may not hold for Purple Swamphens. Thus, our comparison of sexual dimorphism between two populations of Purple Swamphen with differing social structure provides an indication of whether reproductive skew, or other social factors, are more important in driving female trait elaboration in the Purple Swamphen.

4.3 Methods

Field Methods

Purple Swamphen were studied at the Otokia Wildlife Refuge (South Island, 45°56’ S, 170°10’ E) from 1990-95 and at the Tawharanui Open Sanctuary (North Island, 36° 22’ S, 174° 49’ E) from 2008-10. The methods we used to capture, sex and measure birds have been published elsewhere (Craig *et al.* 1980, Jamieson and Craig 1987, Jamieson *et al.* 1994). Briefly, birds were caught using funnel or remote-controlled box traps baited with grain. Both adults and juvenile birds were caught in this manner, but only adults were considered in this study. Upon capture, standardized measurements were taken from each individual, including maximum shield width. Measurements were taken by different researchers at each study site (IGJ at Otokia, JSQ and CJD at Tawharanui).

However, this is a very straightforward measurement that is well illustrated in Craig *et al.* (1980). There is very little subjectivity in this measurement (width of a red structure on a black background) and thus inter- and intra-individual measurement error should be low. Birds were marked with coloured leg bands prior to release, and if individual's were caught more than once, only data from the first capture was considered in this study. Group size and dynamics, as well as territory location, was determined by daily observations during the breeding season at both dawn and dusk. The number of breeders in each group was determined by clutch characteristics (see below) and observation of copulations. Because we did not always capture all individuals in each focal group at Tawharanui, we could not always discriminate the sex of non-breeding helpers. When mating behaviour was observed, suitable nesting habitat within the territory of interest was searched until a nest was located. Philopatry and group composition was tracked by monitoring group membership (via marked individuals) and territory occupancy among years.

Female reproductive skew was estimated by the number of eggs laid by each female in a communal clutch (Jamieson 1997). Nests were checked daily during the laying period, and eggs were separated into clutches laid by individual females based on size, shape, colour and spot patterns. This method of clutch separation has been previously shown to reliably distinguish among eggs laid by different females (Craig 1980, Jamieson and Craig 1987, Jamieson *et al.* 1994, Haselmayer 2000). Reproductive skew was then calculated by the formula:

$$S = (N_T - Q_E)/(N_T - 1),$$

where, N_T is the total number of potential breeders and Q_E is the effective number of breeders (defined as $Q_E = 1/\sum p_i^2$, where p_i is the reproductive contribution of the *i*th breeder, Pamillo and Crozier 1996). Since we could not always determine the number of non-reproductive females in a group (because we did not capture all individuals in each focal group at Tawharanui), we excluded non-reproductive females from our skew calculations. Also, one might expect differences in female reproductive skew based solely on the fact that non-breeding helpers are common at North Island sites and very rare/absent at South Island sites (Jamieson 1997). Thus, by excluding non-breeding helpers we can explore patterns of reproductive partitioning that are not merely driven by the presence of helpers in one population and not the other. By excluding helpers, the measure of reproductive skew used in this study should underestimate the actual difference in reproductive skew between the populations.

Estimates of Lifetime Reproductive Success

Lifetime inclusive fitness estimates of Purple Swamphen breeding on the North and South Island were estimated in terms of offspring equivalents (see Koenig and Mumme 1987). Long-term reproductive data are not available for the

Tawharanui population and thus, we compared data collected at Otokia (South Island, see above) to data collected at Shakespear Regional Park (North Island, 36° 36' S, 174° 49' E, 25.8 km away from Tawharanui, data collected from 1979-85). Since inter-island differences in Purple Swamphen social structure are thought to be based on climatic differences (colder, harsher weather in the South Island), and associated mortality rates and ecological constraints on dispersal (Jamieson 1997, I.G. Jamieson unpubl. data), we would expect the general characteristics of North Island Purple Swamphen populations to be consistent between Tawharanui and Shakespear, as well as through time. Furthermore, the two North Island sites are alike in habitat and have similar Purple Swamphen population densities, group sizes and reproductive skew (Jamieson 1997, this study). Thus, our reproductive success data from Shakespear should be a reasonable proxy for patterns of fitness variation at Tawharanui.

The direct component of fitness for an individual bird was estimated by dividing the annual number of offspring produced by the number of same-sex breeders in the group and totaling across years in which the individual bred. The indirect component was estimated by subtracting the individual's annual number of direct offspring equivalents from the total number of offspring produced in the group, multiplying this number (i.e. the remaining offspring) by an estimated coefficient of relatedness between co-breeders of the same sex and totaling across years of group membership as a breeder. Both males and females at Shakespear are highly philopatric (Craig and Jamieson 1988); thus, for this population, the coefficient of relatedness between group members was estimated at either 0.50 for groups that had no known immigrants (i.e. full siblings) or 0.37 for groups in which one or more birds had joined during the time the individual was breeding. To this total (direct plus indirect component) we added an estimate of the average number of offspring equivalents an individual would have raised as a non-breeding helper, which was based on group reproductive success and estimated relatedness values during seasons in which the individuals did not breed. It was estimated that male helpers gained 0.58 offspring equivalents per year and helped for two years on average while females gained 0.32 offspring equivalents per year and helped for an average of four years (I. G. Jamieson unpubl. data) before becoming a breeder. At Otokia, co-breeders were unrelated and non-breeding helpers were extremely rare (Jamieson *et al.* 1994), and thus only direct components of fitness were calculated.

Statistical Analyses

Statistical analyses were performed with JMP (8.0.2) and R (2.12.1) with a level of significance (α) of 0.05. In all analyses, model residuals were visually examined for normality using a quantile-quantile plot, and transformed when necessary. Population differences in social structure were analyzed with *t*-tests. We used a linear mixed model (LMM) fit with restriction maximum likelihood variance components to investigate population differences in frontal shield dimorphism. In this analysis maximum shield width was square transformed and used as a response variable. This measure is highly correlated with total shield area (C.J. Dey unpubl. data). Performing this analysis with body mass corrected shield size as the response variable did not qualitatively change the results, and thus body mass was not considered in our reported analysis of shield size. Population (North Island or South Island), sex and the interaction of population and sex were included in this model as fixed effects. In these models we used sum-to-zero contrasts, as described in Schielzeth (2010), to allow for the interpretation of main effects in the presence of interactions (Engqvist 2005). Year was included as a random effect to account for the potential variance between field seasons. Following a significant interaction effect in this model, a Holm-Bonferroni multiple comparison test was conducted (Holm 1979) to assess if population differences in males or females were driving population differences in sexual dimorphism. Additionally, we explored patterns of phenotypic variation in shield size. Because variance in a trait tends to increase with mean value of that trait, we instead calculated the coefficient of variation (sample standard deviation divided by sample mean) for each sex within each population, and used the correction for bias suggested by Sokal and Braumann (1980). Then we used two separate tests of heterogeneity of relative variance based on *t*-distributions (described in Sokal and Braumann 1980), to compare variation between males and females in each population. This test is sensitive to departure from normality, and thus we used square transformed shield size as the dependent variable. We were also interested in population and sexual differences in the variation in lifetime reproductive success. However, due to our coarse method of estimating lifetime inclusive fitness, we thought it inappropriate to make inter-population comparisons in this analysis. Instead, we performed intra-population analyses using two separate Levene's tests to compare the variance in estimated reproductive success between males and females. Also, we calculated the opportunity for selection *I,* for each sex within each population. This value is calculated as the variance in reproductive success divided by the mean reproductive success squared, and is an empirical estimate of the maximum change in phenotypic distribution per generation (Crow 1958, Arnold and Wade 1984, Shuster 2009). Unfortunately, we know of no statistical test to compare two *I* values, and therefore these data are presented without statistical analysis.

4.4 Results

Population Demographics

Consistent with previous comparative studies of North and South Island Purple Swamphen populations (Jamieson 1997), our North Island population had larger group size (*t*-test, $t_{80} = 7.56$, $P < 0.0001$, Table 1) and higher female reproductive skew ($t_{33} = 3.00$, $P = 0.005$, Table 1) than our South Island population. In the North Island, both adult and juvenile birds tended to remain on the same territory, with the same group mates across years. However, in the South Island, group membership and territory ownership typically changed between years.

Table 4.1 Comparison of social structure between two populations (Otokia, South Island and Tawharanui, North Island) of Purple Swamphen. Sample size is shown in brackets. Quantitative data on the number of non-breeding helpers and the degree of philopatry were not available, and thus qualitative comparisons are shown. See text for details of statistical analysis.

	South Island	**North Island**
Average Group Size	2.9 ± 0.14 (46)	6.7 ± 0.54 (36)
Female Reproductive Skew	0.04 ± 0.01 (14)	0.19 ± 0.04 (21)
Non-Breeding Helpers	Rare	Common
Natal Philopatry	Rare	Common
Percentage of Breeding Units:		
Pairs	41	0
Polygynous Groups	13	4
Polyandrous Groups	33	30
Polygynandrous Groups	13	67

Dimorphism in Frontal Shields

Across populations, males were found to have larger shields than females (LMM, effect of sex: $F_{1, 329} = 140.26$, $P < 0.0001$, Fig. 1). There was no significant difference in shield size between the two populations ($F_{1,6} = 5.50$, $P = 0.057$), although there was a trend for larger shields overall in the South Island. The degree of sexual shield dimorphism was significantly different between the two

Table 4.2 The opportunity for selection (*I*) in North Island (Shakespear) and South Island (Otokia) Purple Swamphen. Sample sizes used to calculate *I* values are in brackets. See text for details of reproductive success estimates.

	Males	**Females**
North Island	0.17 (16)	0.05 (12)
South Island	0.67 (24)	1.08 (20)

populations (interaction effect $F_{1,\ 329} = 7.53$, $P = 0.006$, Fig. 1). Sexual dimorphism in shield size was lower in the South Island population when compared to the North Island (Fig. 1) and this pattern was driven by population differences in female, but not male, shield size (Holm test, North Female vs. South Female adjusted *P*-value = 0.0006, North Male vs. South Male adjusted *P*-value = 0.45). Furthermore, we observed interesting patterns in phenotypic variation in shield size. In the South Island population, males and females did not differ in shield width variance (Sokal-Braumann test, $t_{191} = 0.14$, $P = 0.89$). However in the North Island population, females had significantly greater variance in shield width than did males ($t_{144} = 5.68$, $P < 0.0001$).

Variation in Lifetime Reproductive Success

In the South Island, females had greater estimated variance in lifetime reproductive success than did males (Levene's test, $F_{1,42} = 51.96$, $P < 0.0001$, Fig. 2). In the North Island, males and females did not differ in estimated variance in lifetime reproductive success ($F_{1,26} = 0.48$, $P = 0.49$, Fig. 2). The calculated values of the ‘opportunity for selection’ were more than three-fold higher for males than females in the North Island population (Table 2), but were only slightly higher for females than males in the South Island population (Table 2).

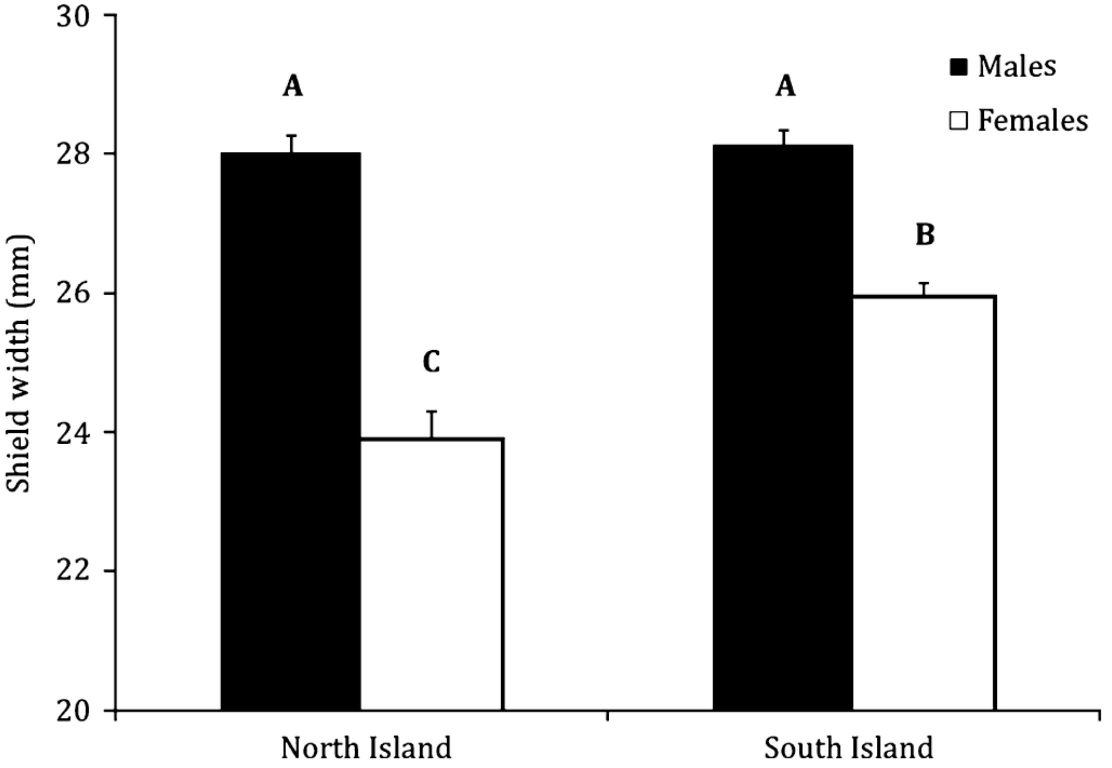

Figure 4.1 Sexual dimorphism in frontal shield size in two populations of Purple Swamphen. Data are untransformed group means (+ SE) for North Island (high reproductive skew; male *n* = 67, female *n* = 79) and South Island (low reproductive skew; male *n* = 105, female *n* = 88) birds. There is significant difference in sexual dimorphism between the two populations (see text for details of statistical analysis). Letters indicate significantly different groups as determined by Holm corrected multiple comparison tests (Holm 1979).

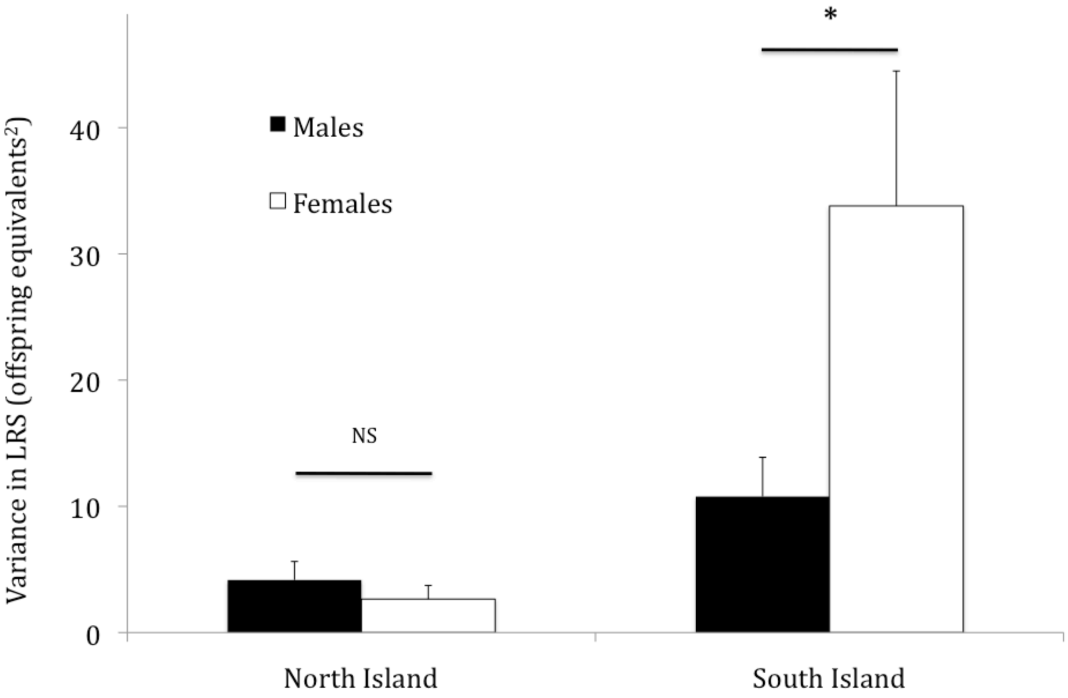

Figure 4.2 Variation in lifetime reproductive success in two Purple Swamphen populations. Data shown are group variances (+ standard error of the variance; Wilks, 1963) for Purple Swamphens sampled in the North Island (high reproductive skew; male *n* = 16, female *n* = 12) and South Island (low reproductive skew, male *n* = 24, female *n* = 20). Asterisks indicate significantly different variances between sexes within a population (see text for details of statistical analysis).

4.5 Discussion

The results of the current study show that female trait elaboration is greater in the South Island population than in the North Island population. South Island Purple Swamphens showed decreased sexual dimorphism in shield size when compared to the North Island population. Our data also suggests that selection on female shields may be more intense on the South Island. Whereas South Island males and females show similar variation in shield size, North Island females had significantly higher variation in shield size than North Island males. Although there are opposing predictions about the magnitude of variation of traits under selection (e.g. traits under selection can have either low variance, Van Homrigh *et al.* 2007 or high variance, Kotiaho *et al.* 2001), under both predictions males and females should have similar variation if they are experiencing similar selective pressure (Rubenstein and Lovette 2009). Thus, our data suggests that South Island females experience similar selection on shield size as males in that population, whereas North Island males and females experience differential selection on shield size. If shield size positively influences reproductive success, as is typical for avian ornaments that signal quality (Dale 2006), then our data suggest that South Island females are also subject to strong selection on shield size, whereas North Island females are not. Certainly, our reproductive success estimates suggest that North Island males have much greater opportunity for selection than do North Island females whereas in the South Island the scope for selection is more equitable between the sexes.

Taken together, these data do not support the hypothesis that high female reproductive skew should enhance selection on traits involved in intrasexual competition and instead suggest that in some situations selection on female ornaments can be more intense in conditions of low reproductive skew (i.e. our

South Island population). We suggest two possible, and non-mutually exclusive explanations for why the observed patterns of shield dimorphism differ from the predicted relationships between reproductive skew and trait elaboration. First, in typical avian societies females disperse from their natal territory and establish territories with unrelated individuals (Greenwood 1980). However, in our North Island Purple Swamphen population, both females and males are highly philopatric (see also Craig and Jamieson 1988). When females are philopatric, they will have long-standing associations with related females in their group, and social status may be more strongly defined by past residence than by direct competition between females (i.e. gerontocratic inheritance of dominance; Bridge and Field 2007). Since the strength of selection on secondary sexual characteristics is directly dependent on the effect that the trait has on reproductive success (Andersson 1994), we would expect there to be relatively weak selection on female secondary sexual characteristics in populations with female natal philopatry if elaborate traits have a reduced role in determining social hierarchies. In South Island groups, females frequently disperse from their natal group and must compete for group membership and dominance status each year (Jamieson *et al.* 1994). Therefore female ornaments are likely to be of increased importance in determining social status and reproductive success in this population.

Second, the hypothesis that high reproductive skew should lead to increased selection on traits involved in access to breeding opportunities is based on the premise that high reproductive skew leads to high intrasexual variation in lifetime reproductive success (Hauber and Lacey 2005, Rubenstein and Lovette 2009). While this pattern may be generally true, it is important to note that reproductive skew measures are not always accurate predictors of the variance in lifetime reproductive success. Reproductive skew values describe the degree to which direct reproduction is monopolized in a single breeding attempt or season. But if subordinate individuals inherit a dominant breeding position in subsequent breeding seasons (Lucas *et al.* 1997), they may end up having equivalent fitness to the current dominant individuals over their entire lifetime, even if reproductive skew is high within each breeding season. Furthermore, measures of reproductive skew may not capture the variation in inclusive fitness since they do not include indirect fitness benefits. Many cooperatively breeding groups are composed of kin and there may be intersexual or population differences in relatedness that complicate the relationship between reproductive skew and variation in lifetime inclusive fitness (Creel 1990). As such, reproductive skew may not reflect the variation in inclusive fitness on which sexual and social selection can act (Hauber and Lacey 2005). Interestingly, our analysis of lifetime reproductive success provides evidence of such a disconnect between reproductive skew and variance in inclusive fitness in the Purple Swamphen. Despite high reproductive skew, our data show that North Island female Purple Swamphens have low variation in lifetime inclusive fitness (and low opportunity for selection), perhaps due to the kin-based structure and method of dominance inheritance that occurs in North Island groups. In such a situation, high reproductive skew may not necessarily lead to increased intrasexual competition since all individuals expect relatively equal lifetime fitness and thus, there is little incentive to compete for social status. Purple Swamphen may represent a rare situation in this regard, as many cooperative breeding mammals are known to have female biased philopatry and high levels of kinship among group females (Armitage 1987) yet these societies also show high variation in female reproductive success (Hauber and Lacey 2005).

The degree of sexual dimorphism in secondary sexual characteristics is affected by selection on both males and females. In this study, there were no significant population differences in male shield size, and thus it seems that shield dimorphism in Purple Swamphen is largely determined by differences in female shield size (Fig. 1). Shield size is correlated with social dominance in male Purple Swamphens (Craig 1974; C.J. Dey, unpubl. data) and thus we might also have expected to see population differences in male shield size since there are population differences in male (as well as female) philopatry and territoriality (Jamieson 1997). There are several possible reasons why we did not observe such differences. First, previous studies have suggested that social dominance may not predict reproductive success in male Purple Swamphens (Jamieson *et al.* 1994; Lambert *et al.* 1994). If socially dominant males do not have greater reproductive success than socially subordinate males, there may be no basis for strong selection on male shields. Secondly, it is uncertain how variation in male reproductive success differs between the populations. Jamieson (1997) found no significant difference in male reproductive skew between a North and South Island population, albeit this comparison was conducted on a small number of groups. If North and South Island males have similar variation in lifetime reproductive success then the scope for selection on male shield size may be similar between the populations. While we were able to calculate *I* values for males from each population, we did not compare these values because of our coarse methods for estimating reproductive success. Clearly, further research into the functional and adaptive role of male shields is required to fully understand sexual dimorphism in this trait.

To our knowledge, the current study is the first to explore how patterns of female reproductive skew influence trait elaboration and sexual dimorphism within a single species. Because our study is based on only two populations, we present this paper as an exploratory analysis of the social factors associated with female trait elaboration in the Purple Swamphen rather than an explicit and powerful test of the predictions derived from Rubenstein and Lovette (2009). An intraspecific comparison of many populations that differ in terms of reproductive skew, but not other factors that could influence trait expression (e.g. opportunity for dispersal, breeding season length, group size and composition, etc), would be a stronger test of the relationship between reproductive skew and female traits. However, such a situation may be challenging to find as reproductive skew tends to co-vary with many environmental and social parameters (e.g. Hatchwell and Komdeur 2000) and intraspecific variation in reproductive skew is rarely studied and therefore poorly characterized. Also, in comparing patterns of variation in fitness, the current study estimates lifetime reproductive success from group reproduction success and observations of copulations. In our analysis, we estimated equivalent direct reproductive success for same-sex breeders in each group for each year. While this may be appropriate for South Island Purple Swamphens (where reproductive skew is low; Jamieson *et al.* 1994), it is known that North Island groups have a highly unequal distribution of reproduction amongst breeders (Jamieson 1997). Thus, our estimation method could dampen true variation in lifetime reproductive success in the North Island by underestimating variation in fitness between same-sex breeders. However, it is important to note that non-breeding helpers were not estimated to have gained direct reproduction and thus there is still within group variation in fitness considered with our methods. Indeed, variation in direct fitness as a result of non-breeding helpers is likely a large component of total variation in inclusive fitness in many cooperative breeders (Hauber and Lacey 2005) and thus our methods likely retain most of the true variation in lifetime reproductive success. Nonetheless, because North Island within-group variation in reproductive success may be underestimated by our methods, we did not make inter-population comparisons of variation in lifetime reproductive success or the opportunity for selection. Future studies with the ability to determine true variation in lifetime reproductive success (e.g. using parentage analysis) will be valuable in understanding how reproductive skew and variation in lifetime reproductive success influence female trait elaboration.

Both social and mating systems have long been considered important components in defining the dynamics of sexual and social selection in animals (Andersson 1994). But to our knowledge, no previous study has examined how female traits vary between populations of a single species that differ in social structure. Whereas reproductive skew may be important in defining the intensity of female intrasexual competition in some avian societies, it is not always an accurate predictor of the variation in lifetime reproductive success and thus researchers should be cautious about using reproductive skew comparisons to infer patterns of variation in lifetime fitness. Instead, our data suggest that life history characteristics other than reproductive skew (i.e. philopatry, group structure and patterns of dominance inheritance) can also influence the intensity of female intrasexual competition and this may be especially true when females are philopatric. Further research examining the role of these factors on the intensity of female-female competition is necessary for a more complete understanding of female ornamentation. In particular, we suggest that intraspecific studies may be particularly valuable in teasing apart the relative importance of reproductive skew and variation in reproductive success on the evolution of female traits.

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Chapter 5: Manipulating the Appearance of a Badge of Status Causes Changes in True Badge Expression

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5.1 Abstract

Signals of dominance and fighting ability (i.e. status-signals) are found in a wide range of taxa and are used to settle disputes between competitive rivals. Most previous research has considered status-signal phenotype as an attribute of the individual, however it is more likely that signal expression is an emergent property that also incorporates aspects of the social environment. Furthermore, because an individual's signal phenotype is likely to influence its social interactions, the relationships between status signals, social environment and individual quality are probably much more complex than previously appreciated. Here we explore the dynamic relationship between social interactions and signal expression in a previously undescribed status signal, the frontal shield of the pukeko (*Porphyrio porphyrio melanotus:* Aves). We demonstrate that frontal shield size is a strong predictor of dominance status within social groups, even after controlling for potentially confounding variables. Then, we evaluate the relationship between social interactions and signal expression by testing whether manipulating apparent shield size influences: 1) dominance interactions and 2) future signal expression. By showing that decreasing apparent shield size causes both an increase in the amount of aggression received and a decrease in an individuals true shield size, we provide the first evidence of dynamic feedback between signal expression and social interactions. Our study provides important insight into the role of receiver-dependent (i.e. social) costs in maintaining signal

honesty and demonstrates a unique approach to studying status signaling applicable to future studies on dynamic morphological signals

5.2 Introduction

Signals of dominance and fighting ability are used to settle disputes over mates and other resources, and are found in a wide range of taxa [1]. In some species, signals used in a competitive context are inherently related to some aspect of biological quality (e.g. mammalian acoustical formant frequency is intrinsically tied to body size [2,3]) and thus, the production of reliable signals is enforced. However, many animals use convention-based signals of quality (i.e. “arbitrary signals”) that are not obviously physically tied to any aspect of quality. Understanding the factors that maintain a correlation between these signal traits and the unobservable qualities that they reveal has been a major focus of behavioral biology for the last 40 years [4-9].

One prevailing view in animal communication research is that conventional signals are honest because their production is differentially costly to individuals of different quality (the handicap principle [4,5]). Under this model, signal phenotypes are determined by the intrinsic quality of the individual, and signal reliability is maintained by receiver-independent costs. Receivers are selected to attend to these signal traits because they provide reliable information that can be incorporated into deciding whether or not to engage in a physical contest with a rival. However, this model is problematic for two reasons: First, there is considerable evidence that conventional signal expression is influenced by social interactions [10-15]. For example, previous studies have demonstrated that changes in dominance rank *per se*, can cause corresponding changes in status signal phenotypes [12-14] and also that population density can influence signal honesty [15]. Second, the aspects of quality that status signals are purported to correlate with (e.g. health status, androgen levels) are highly dynamic and themselves respond to various social factors [16,17]. Thus, signal expression in many species is probably an emergent property that incorporates both aspects of an individual's intrinsic quality, and also the individual's social environment. As a result, the simplistic model in which status signals influence social interactions in a uni-directional manner is probably unrealistic, and the relationships between signal expression, social interactions and individual quality are likely much more complex than is widely appreciated.

Perhaps the best method of investigating the relationship between signals and social interactions is through cosmetic signal manipulation. While many studies have examined the effect of signal manipulation on receiver behavior [7], we know much less about the feedback effects of signal manipulation on the signaler themselves (but see [18,19]). Because cosmetic signal manipulation does not have a direct effect on the receiver-independent costs of signal production, any observed differences in the cost of bearing signals is likely due to changes in social interactions (i.e. the receiver-dependent costs). If signal expression is influenced by receiver-dependent costs, then manipulation of signal phenotype should cause changes in the social interactions experienced by the signaler. Furthermore, because the social environment can have a strong influence on animal phenotypes [10-15], it is also possible that changes in apparent signal phenotype may feedback on the same processes that control true signal expression. While such a feedback mechanism of signal expression is yet to be demonstrated, recent evidence suggests that the relationship between signal expression and individual quality is more dynamic than previously appreciated [20]. In a study on North American barn swallows (*Hirundo rustica erythrogaster*), Safran and colleagues [19] found that experimentally enhancing male chest plumage reverses a seasonal decline in androgens, likely due to changes in social interactions experienced by the manipulated individuals. Because androgens have been widely implicated in the regulation of status signals, it seems likely that signal expression could be influenced by the very social interactions that are the outcome of the signal itself.

In this study, we explore putative feedback effects between social interactions and signal expression in a hitherto undescribed badge of status, the frontal shield of the pukeko (*Porphyrio porphyrio melanotus*). This cooperatively breeding bird lives in permanent, mixed-sex social groups [21,22] and within each group, individuals have frequent agonistic interactions over access to resources [23,24].

Both male and female pukeko have conspicuous frontal shield ornaments that extend from the bill upwards to cover the front of the crown. These ornaments, which are found in several species in the family *Rallidae*, are testosterone-dependent and have the ability to change size over short time periods [25,26]. Pukeko prominently display their frontal shields during aggressive interactions [23] and population differences in shield dimorphism are thought to be due to variation in the intensity of intrasexual competition [27].

To explore the relationship between dominance and frontal shields, we first investigated the relationship between frontal shield size and dominance status, while controlling for other traits that could be important in determining social rank. Since pukeko prominently display frontal shields in aggressive interactions, we predicted that frontal shield size would be correlated with dominance status. We then explored whether there is a dynamic relationship between signal phenotype and social interactions by testing two key predictions of the model outlined above: 1) that changes in signal expression influence social interactions, and 2) that changes in signal expression cause feedback effects which alter future signal expression. In order to test these predictions, we reduced the apparent size of the frontal shield in two separate experiments and assessed whether the manipulation caused changes in dominance interactions, and true shield size, respectively.

5.3 Methods

(a) *Behavioural Observations*

This study was conducted at the Tawharanui Open Sanctuary, New Zealand (36° 22' S, 174° 49' E). In 2010 and 2012 pukeko were banded as part of a larger study on social behaviour. Upon capture, a suite of morphological measurements were taken including measures of body size (mass) and frontal shield size. Birds were also sexed by measurement according to [28]. Shield area (as determined using digital photography) is highly correlated with field-measured maximum shield width ($R^2 = 0.87$, $n = 50$), thus, we use maximum shield width as our measure of shield size throughout as it is straightforward and highly repeatable (standard error of measurement = 0.13 mm, mean adult shield size = 24.8 mm). Previous research has demonstrated that frontal shield colour does not correlate with dominance status, and thus, we do not consider shield colour in this study (CJ Dey, unpublished data).

In 2012, we performed detailed behavioral observations on 11 social groups in which all group members were banded. Observations were conducted during January and February 2012, which is outside of the peak breeding season for pukeko at this site (breeding typically occurs between August and November at this site). Each group was observed for 30 minutes per day between 0630 and 1000 hrs, for 10 days. Approximately 50 g of dried maize was placed in a small pile on the territory of the focal group immediately prior to each observation period to increase the frequency of interaction between group members [24]. Pukeko are accustomed to human presence at this site and quickly resumed normal behaviours following this disturbance. Observers were concealed in a camouflaged hide and recorded all dominance interactions during the observation period.

(b) *Frontal Shield Manipulations I-Effects on Dominance Behaviour*

From April to June 2013, we performed a shield reduction experiment to test the prediction that changes in signal phenotype would influence dominance interactions involving the focal individual. We randomly selected one adult male out of the banded males in each of 22 social groups (on average 68% of the birds were banded in these groups). Next, we performed a series of five, 30-minute baseline behavioural observations on each of the focal males (one per day for five days). These observations were similar to the group behavioural observations described above, except that the observer followed a single individual (i.e. the focal individual) and recorded the observation period with a video camera (Sony HDR-PJ260, Tokyo, Japan). Following these baseline observations, the focal individual was trapped (n = 6 individuals could not be trapped and were therefore excluded) and alternatively assigned to a shield reduction (n = 8) or a control treatment (n = 8). The shield reduction treatment was conducted by applying a small amount of black paint (Dulux Spraypack Quick Dry, Lower Hutt, New Zealand) to the perimeter of the shield using a small brush, such that the paint made a 6 mm border surrounding the shield. Since the plumage surrounding the shield is also black, this treatment caused the shield of the manipulated individuals to appear smaller. Individuals assigned to the control group had red paint applied in a similar fashion. This treatment did not change the apparent shield size in the control individuals. A pilot study demonstrated that such treatments last for 4-6 days and that the paint used closely matches the reflectance of the plumage (black) and shield (red), respectively (see Electronic Supplementary Material). The paint was allowed to dry for 5 minutes before birds were released back onto their territory. To determine whether manipulation of apparent signal phenotype influenced aggressive interactions, we then conducted a further 5, 30-minute behavioural observations on each focal individual, beginning two days after the manipulation. Videos collected from this experiment were reviewed by two individuals who were blind to the treatments and the study objectives. They observed the videos in a randomized order and recorded all dominance interactions that occurred between the focal individual and other group members.

(c) *Frontal Shield Manipulations II-Effects on Shield Size*

During March and April 2012, we manipulated frontal shield size to test the prediction that changes in apparent shield size would cause changes in true shield size. In 25 social groups we captured a single male pukeko. The first male to enter the trap in each social group was used. Morphometric measurements were taken as described above. Captured individuals were then randomly assigned to a shield reduction treatment (n = 13) or a control treatment (n = 12) by coin-flip, which was identical to the procedure described above. Approximately one week after the treatment (mean = 6.1 days, range = 6-9), we recaptured as many manipulated and control individuals as possible (n = 9 control, n = 7 treatment). Upon capture, individuals were subject to the same set of morphological measurements as were performed prior to the treatment. In most birds, the treatment had worn off by the time of recapture and the measurements were performed blind to the treatment. However, in 4 birds the treatment was visible during this recapture event, and thus analyses were performed both with and without these birds.

(d) *Analysis*

All analysis was conducted using R version 3.0.1 [29]. First we examined the relationship between social status (David's dominance score; [30]) and frontal shield size. Typically, studies of status signalling test for a correlation between signal phenotype and some measure (or proxy measure) of dominance to suggest that the focal signal is informative. We followed these methods, but importantly, we also tested to see if social dominance is predicted by shield size after controlling for confounds. We used two linear mixed-effect models to evaluate the relationship between social dominance and shield size, using normalized David's score as our response variable (see Electronic

Supplementary Material). In our first model we used shield width as the sole fixed effect to estimate the potential information content of frontal shield status signals. In our second model, we included shield width, mass, sex and two-way interactions between shield width and sex, and mass and sex. These interactions were included because the relationship between badge size and dominance, and mass and dominance, could vary between the sexes due to differential selective pressure on competitive traits [31,32]. If shield width is a significant predictor of dominance even after controlling for these covariates it would suggest a strong relationship between signal phenotype and dominance that is independent of body size and sex.

We used a general linear model to test for treatment effects on true shield size. In this analysis, the shield width at recapture was modeled as a function of treatment (reduction/control) and the individual's pre-treatment shield width. Additionally, we used three Poisson family generalized linear mixed models (GLMMs) to analyze how our shield reduction treatment affected the dominance interactions directed at our focal individuals. In each of these models we included two fixed-effects: time (before/after treatment) and treatment (reduction/control), as well as the interaction between these effects. Thus, a significant interaction term would support our prediction that manipulating frontal shield size should cause changes in dominance interactions. The response variables considered in these models were: 1) the number of aggressive challenges received ('upright aggressive' displays [23]), 2) the number of attacks received ('kicks', the primary form of physical aggression, [23]), and 3) the number of 'wings up' displays received (an aggressive display used towards non-group members [23]). Further details on model fitting are described in the Electronic Supplementary Material. Data is available from the Dryad Digital Repository [33]: doi:10.5061/dryad.r6797. All figures were created using the ggplot2 package in R [34].

5.4 Results

(a) *Behavioural Observations*

Dominance rank was highly correlated with frontal shield size (Model 1, Table 1, Figure 1, partial R^2= 0.51), and this finding was conserved even after controlling for body size and sex (Model 2, Table 1).

(b) *Frontal Shield Manipulations I-Effects on Dominance Behaviour*

Overall, focal individuals received less aggression in the post-treatment behavioural observations than during the pre-treatment behavioural observations (significant effect of time, Model 1 & 2, Table 2; Figure 2). However, the shield reduction group received relatively more aggressive displays and physical attacks in the post-treatment period than did the control group (significant interaction

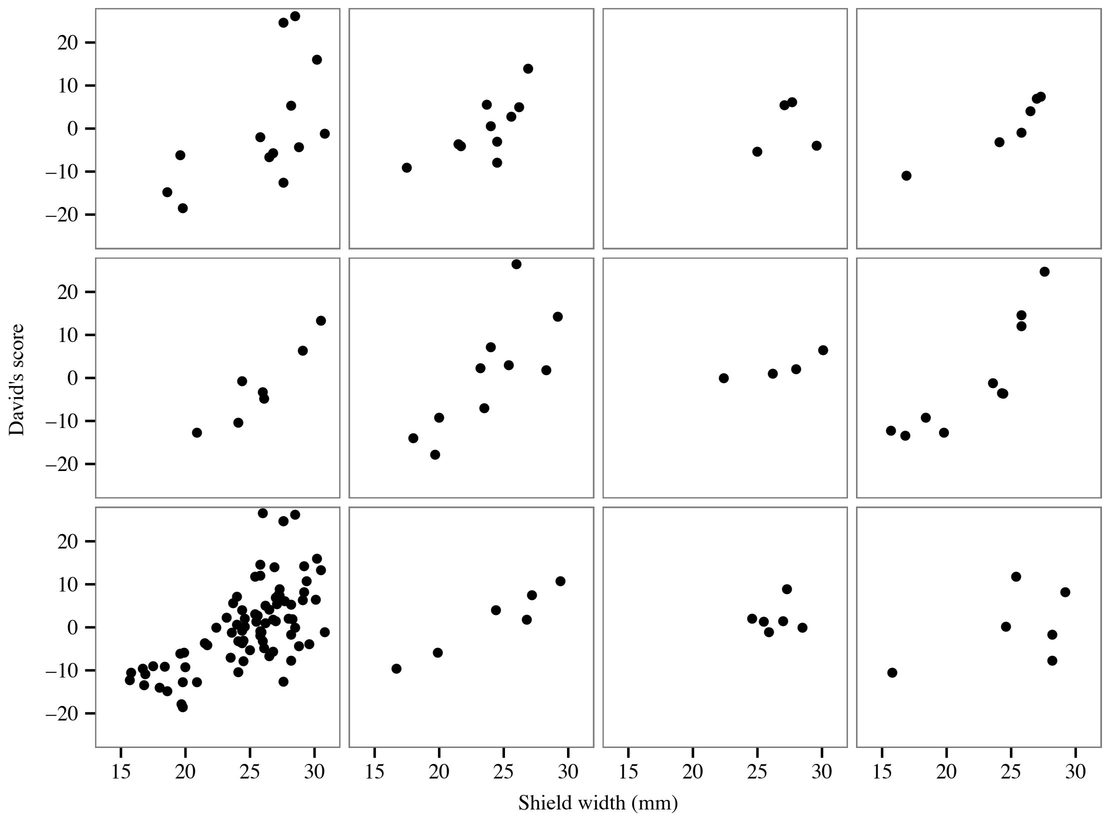

Figure 5.1. The relationship between shield width and David's score (an index of social dominance) is shown for 82 individuals in 11 pukeko social groups (one per panel). Higher David's scores indicate more dominant individuals. The bottom left panel shows all individuals across all social groups combined. See text for details of statistical analysis.

between treatment and time, Model 1 & 2, Table 2; Figure 2). This change in dominance interactions was likely not due to a disruption of individual recognition mechanisms, as there was no significant interaction between time and treatment on the number of wings up displays (Table 2). Furthermore, no manipulated individuals were evicted from their group in this study.

Table 5.1 Linear mixed-effect model of factors predicting social dominance in pukeko. The model allows for a random intercept for each social group and includes data from 82 individuals in 11 groups. Significant *P*-values are shown in bold.

Fixed Effect	Estimate	95 % CI Estimate	*P*-value
Model 1			
Shield Width	0.19	0.15, 0.24	**<0.0001**
Model 2			
Shield Width	0.09	0.03, 0.16	**0.008**
Mass	0.005	0.002, 0.007	**0.0002**
Sex	0.001	-0.27, 0.27	0.99
Shield Width x Sex	0.05	-0.01, 0.11	0.10
Mass x Sex	0.001	-0.002, 0.003	0.62

Prior to treatment, birds assigned to the reduction and control treatments did not differ in shield size (mean shield width ± se, reduction: 27.9 mm ± 0.53, control: 27.6 mm ± 0.43, *P* = 0.40). However, after treatment, birds in the shield reduction group had smaller apparent shield size than those in the control group (reduction: 13.6 mm ± 0.44 mm, control: as above, *P* < 0.0001). True shield size at the time of recapture was significantly predicted by pre-treatment shield size (GLM: estimate = 0.90, 95% CI = (0.77, 1.04), *P* < 0.0001) and was also influenced by treatment, with birds that received the shield reduction treatment having significantly smaller shields than those with the control treatment (estimate = -0.78, 95% CI = (-1.23, -0.32), *P* = 0.003; Figure 3). Qualitatively similar results were found when only birds who were measured blind to the treatment were included (n = 4 individuals removed).

Table 5.2 Poisson family generalized linear mixed models showing the effect of shield size reductions on dominance behaviours directed towards the focal individual. Models include data from 10 behavioural observations for each of 16 focal individuals. Individual ID is included in each model as a random intercept. Significant *P*-values are shown in bold.

Response Variable	Fixed Effect	Estimate	95 % CI Estimate	*P*-value
Challenges	Treatment	0.20	-1.19, 1.61	0.77
	Time	-0.79	-1.56, -0.02	**0.04**
	Treatment*Time	1.77	0.24, 3.30	**0.02**
Attacks	Treatment	-0.93	-3.00, 1.15	0.38
	Time	-1.60	-2.87, -0.34	**0.01**
	Treatment*Time	2.23	0.66, 3.80	**0.005**
Wing's Up Displays	Treatment	-1.62	-3.78, 0.54	0.14
	Time	-0.34	-0.85, 0.17	0.19
	Treatment*Time	-0.12	-1.15, 0.90	0.81

5.5 Discussion

In this study, we found that frontal shield size is strongly correlated with dominance status in pukeko. Furthermore, the relationship between shield size and dominance remained even after controlling for important confounds that could explain the relationship between ornament size and status.

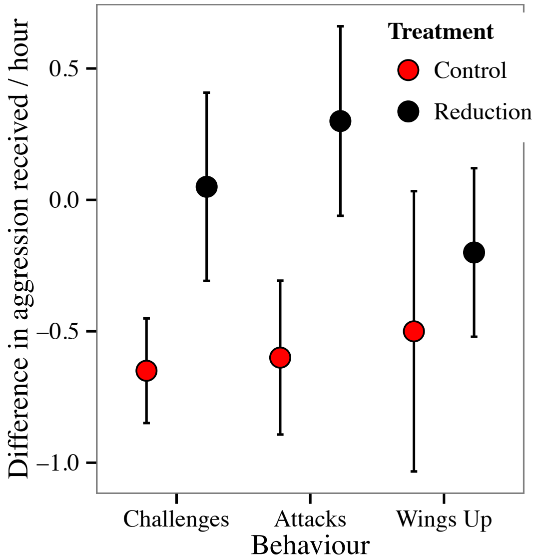

Figure 5.2 Aggressive behaviours directed at focal individuals with natural (control) or decreased (reduction) apparent shield size. Data are the mean differences in the rate each type of behaviour was received (± se) between the pre-treatment and post-treatment observations. See text for details of statistical analysis.

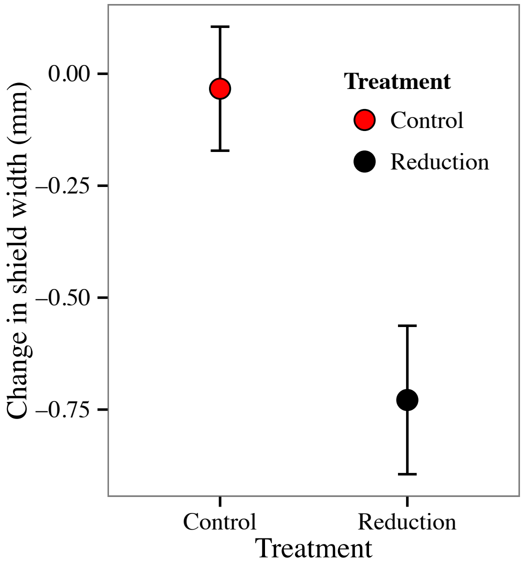

Figure 5.3 The change in true shield width as a result of apparent shield manipulation. Data shown is the mean change (± se) over a one-week period after manipulation for individuals that received the control (n = 9) and shield reduction treatments (n = 7). See text for details of statistical analysis.

These results, in combination with the fact that frontal shields are prominently displayed during aggressive interactions [23], are highly suggestive that the pukeko's frontal shield acts as a status signal. We also found strong evidence for a dynamic relationship between signal expression and social interactions. Pukeko that had the apparent size of their frontal shield decreased received more aggression and also decreased their true shield relative to individuals who did not have their apparent shield size altered. Since our manipulation did not directly affect the receiver-independent costs of signaling, these changes in true shield size must have been mediated by changes in social interactions (see also [19]).

Our results are not surprising, given that previous studies have shown that changes in the social environment can lead to changes in signal expression [10-15], and also that changes in apparent signal expression can cause feedback effects on individual physiology [19]. Proximate control of many avian status signals relies on androgen hormones [7], and androgens are sensitive to social factors [17,19]. It is possible that the increase in aggression received by manipulated individuals lead to a decrease in circulating androgen levels, which could have decreased shield size. While this suggestion is speculative, endocrine physiology can influence both behavioural and morphological traits, and thus is likely to be important to understanding the complex relationships between signals and social environments [20].

There is growing evidence that the social environment has an important influence on status signal expression, and that receiver-dependent costs are important in the enforcement of signal honesty in many species [7,9]. One possible mechanism by which receiver-dependent costs could influence signal expression is through the recognition and punishment of incongruent signals [35-38]. This hypothesis predicts that receivers are sensitive to other cues of quality in addition to badges (e.g. behaviour, body size, individual recognition) and when the various cues to fighting ability are inconsistent, they should challenge signallers to ascertain their true quality. Recognition and punishment of incongruent signalling has been convincingly demonstrated in paper wasps (*Polistes dominulus*). In this species, individuals who had either their facial markings or behaviour experimentally enhanced (i.e. made more dominant) had increased fight costs in paired interactions with unfamiliar individuals [36, 38], while individuals who had both their facial markings and behaviour enhanced (and thus displayed congruent signals) did not incur these increased costs [38]. The data presented in the current study are also consistent with recognition and punishment of incongruent signals. Pukeko whose frontal shields were made more subordinate (and thus incongruent with the other signals it was producing) received more aggressive challenges. However, it is also possible that manipulated individuals were simply perceived as subordinates, and were therefore challenged and attacked when they did not yield to other group members. The current study cannot discriminate between these two mechanisms, and further studies aimed at understanding how receivers integrate signal phenotypes with other dominance cues will be useful in understanding how receiver-dependent costs contribute to signal honesty.

While status signaling has been demonstrated in a wide variety of taxa (e.g., reptiles [39], insects [36], birds [7], fish [13], mammals [3]) the correlation between signal traits and the qualities that they signal is likely to vary among species and with the signal modality involved. Signals that are relatively inflexible could become less informative in environments with high temporal heterogeneity because individual condition may change between the time when the signal was produced and when the signal is used. For example, avian plumage badges are a classic example of a conventional signal of dominance. However, the relationship between plumage badge size or colour and social dominance is often weak, especially after controlling for other confounding variables such as body size, sex and age [7, 40-42]. Feather growth typically occurs during discrete life-history stages and is constrained by energetic and physiological limitations. Thus, plumage traits will typically be representative of the condition of the individual at some past time-point when the feathers were grown (although see [43,44] for examples of the dynamic properties of plumage ornaments). However, birds can also signal quality with non-plumage traits (e.g. shields, legs, eye rings, bills, wattles), which are typically vascularized and are therefore able to respond rapidly to changes in individual condition [11,45,46]. While non-plumage traits have a well-established signalling role in mate-choice [45-47], their role in a competitive context has been underappreciated (but see [11,48-51]) despite their potential to be more informative to rivals than plumage badges. We suggest that future studies on dynamic status signals (e.g. avian bare-parts) will help with understanding the complex relationships between individual quality, social interactions and signal expression because of the increased opportunity to investigate the direct and indirect factors that mediate signal expression.

Social dominance is important in the lives of many animals, and signals of dominance and fighting ability will often be under strong selection because of their role in determining access to mates and other resources. Social factors are ultimately crucial to understanding honest signalling, not only because the behaviour of signal receivers will determine the benefits of producing a certain signal, but also because there may be receiver-dependent costs that make dishonest signalling unprofitable in a range of species. In this study, we identified a hitherto undescribed status signal (the pukeko's frontal shield) that strongly predicts dominance rank, even after controlling for body size and sex. This strong correlation between signal size and dominance status may be due in part to the ability of bare-part ornaments to dynamically respond to short-term changes in individual condition or the social environment. Furthermore, we show that changes in signal phenotype can influence an individual's social interactions, and also feedback on future signal expression. As a result, this study adds to the growing evidence for a dynamic and bi-directional relationship between social interactions and signal phenotype. This study also demonstrates a unique approach to measuring the receiver-dependent costs of signals that could be widely applicable to future studies of dynamic signals.

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Chapter 6: Carotenoid-based Bill Coloration Functions as a Social, Not Sexual, Signal in Songbirds (Aves: Passeriformes)

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6.1 Abstract

Many animals use coloration to communicate with other individuals. While the signalling role of avian plumage colour is relatively well studied, there has been much less research on coloration in avian bare parts. However, bare parts could be highly informative signals as they can show rapid changes in coloration. We measured bill colour (a ubiquitous bare part) in over 1600 passerine species and tested whether interspecific variation in carotenoid-based coloration is consistent with signalling to potential mates or signalling to potential rivals in a competitive context. Our results suggest that carotenoid bill coloration primarily evolved as a signal of dominance, as this type of coloration is more common in species that live in social groups in the non-breeding season, and species that nest in colonies; two socio-ecological conditions that promote frequent agonistic interactions with numerous and/or unfamiliar individuals. Additionally, our study suggests that carotenoid bill coloration is independent of the intensity of past sexual selection, as it is not related to either sexual dichromatism or sexual size dimorphism. These results pose a significant challenge to the conventional view that carotenoid-based avian coloration has evolved as a developmentally costly, condition-dependent sexual signal. We also suggest that bare part ornamentation may often signal different information than plumage ornaments.

6.2 Introduction

Explaining the diverse patterns of bird coloration has been a central focus of evolutionary biology. A large body of literature has examined the signaling function of avian plumage coloration and it is generally accepted that plumage coloration is important in both sexual (Owens and Hartley, 1998; Hill 2006) and non-sexual communication (Senar, 2006). In contrast to plumage traits, comparatively little research has focused on the coloration of avian body parts that are not covered in feathers. These 'bare parts' include legs, eye rings, bills, wattles and crests, which are colourful and conspicuous in many birds. It is likely that bare part coloration is also involved in signaling, as the size and colour of bare parts correlates with aspects of biological quality (Bright *et al*., 2004; Jawor *et al.*, 2004; Peters *et al.*, 2004a; Velando *et al.*, 2006). Additionally, bare part coloration is highly dynamic because bare parts are vascularized and can show phenotypic changes over short time periods (e.g. hours or days; Faivre *et al.*, 2003; Velando *et al.*, 2006; Ardia *et al.*, 2010). As a result, bare part coloration can potentially provide updated information on current quality, and bare parts may therefore be informative signals across a range of contexts.

Carotenoid pigments produce many of the red, orange and yellow colours in birds (Brush 1990). This type of ornamentation is frequently found in avian bills, and represents one of the most conspicuous forms of bare part coloration. Because birds must acquire carotenoids through their diet (they cannot be synthesized *de* *novo*), and because carotenoids are involved in other physiological processes in addition to ornamentation, it has been hypothesized that allocation of these pigments to ornaments represents a developmentally costly trade off that limits carotenoid use elsewhere in the body (Olson and Owens, 1998). As a results, carotenoid ornamentation has received much research attention as an honest indicator of individual quality (see Svensson and Wong, 2011 for a review), and there have been many proposed mechanisms that link carotenoid ornamentation to individual condition, including links with parasites and immunity (Folstad and Karter 1992; Peters *et al.*, 2004a,b; Butler and McGraw 2013), oxidative stress (von Schantz *et al.*, 1999) and foraging ability (Hill 1992; McGraw 2006). If carotenoid ornamentation is costly, then females may gain both direct (e.g. increased offspring provisioning) and indirect (i.e. genetic) benefits from mating with males with more intense carotenoid-based coloration (Griffith and Pryke, 2006).

A neglected alternative hypothesis, however, is that carotenoid bill coloration has evolved as a signal used in social interactions involving competition between rivals over dominance rank or resources. Indeed, competitive interactions can lead to the evolution of the types of traits that have previously been assumed to be associated solely with mating (Lyon and Montgomerie 2012). Many birds use coloured plumage patches to signal fighting ability (Senar 2006), and such ‘status signals’ are thought to reduce the cost associated with agonistic interactions because the outcome can be decided by signal phenotype rather than by escalations in physical aggression. Although such status signals are typically associated with melanin-based coloration, carotenoid-based plumage patches have also been associated with rival competition (e.g., Pryke *et al.*, 2002). In contrast to plumage, however, bills are particularly well suited to serve as status signals because rapid changes in bill coloration could reflect short-term changes in aggressive intent (e.g., changes in reproductive status or changes in physical condition associated with disease). Furthermore, the bill is central and forward facing, and is the main weapon used in fights in many species, and thus competitors likely attend to bill characteristics. Consistent with this hypothesis, several intraspecific studies have found a positive correlation between carotenoid bill coloration and either circulating testosterone (a hormone linked to aggression and dominance) or social status (Ardia *et al.*, 2010; Shawcross and Slater, 1984; Bright and Waas, 2002; Murphy *et al.*, 2009).

While there is comparative (Gray 1996; Badyaev and Hill, 2000) evidence that carotenoid plumage ornamentation is a signal used in mate choice, no comparative studies have investigated the signaling role of carotenoid coloration in avian bills. In this study, we tested whether interspecific patterns of carotenoid bill coloration indicate this ornament has evolved as a sexual signal used during mating interactions (i.e. a sexually selected trait) or as a social signal used in resource competition. Using a comparative analysis of all passerine species from

Africa, Australia and New Zealand, we investigated the relationships between carotenoid bill coloration and socio-ecological traits that are associated with either frequent agonistic interactions or are linked to intense sexual selection.

Predictions

If carotenoid bill colour initially evolved as a trait involved in competitive signaling, we expect a positive relationship between the presence of this type of ornamentation and i) non-breeding sociality, and ii) colonial breeding. This is because species that form aggregations (i.e. flocks) in the non-breeding season experience intense competition for access to food and shelter and must interact with many unknown individuals, which are the conditions under which status signaling should be favoured (West-Eberhard 1983). Similarly, intraspecific aggression increases with breeding density in birds, as there is increased competition for food, nest sites and mates (Møller 1987; Mougeot 2000). Importantly, rates of extra pair fertilization (Westneat and Sherman 1997) and the probability of social polygyny (C.J.D and J.D. *unpublished data*) do not significantly differ between colonial and solitary breeders, suggesting that traits involved in mate choice may experience similar selection in colonial and solitary breeders. In contrast, if carotenoid bill coloration initially evolved as a signal used in mate choice, we expect a positive relationship between this ornamentation and iii) sexual size dimorphism, and iv) plumage dichromatism, as these are both proxy measures of the intensity of sexual selection in birds (Owens and Hartley, 1998; Dale *et al.*, 2007).

6.3 Methods

(a) *Collection of bill colour data*

We collected bill colour data from the 1605 passerine species found in Africa, Australia and New Zealand, as listed in the Handbook of Australian, New Zealand and Antarctic Birds (Higgins *et al.*, 2001), Birds of Africa (Keith *et al.*, 1992) and Robert's Birds of Southern Africa (Hockey *et al.*, 2005). These areas were used because they include a wide taxonomic representation of the passerines (86 families) that live in a diverse array of habitats, and are therefore well representative of passerines as a whole. Additionally, the avifauna from these areas is relatively well studied, which allowed us to collect the relevant life-history data included in our analyses (see below). For consistency, we digitally scanned images of each species from the plates in the Handbook of the Birds of the World (del Hoyo *et al.*, 2003), and used these images to determine whether each species had carotenoid ornamentation in their bill (see below). This approach was necessary because measuring colour from bare parts is not possible using museum specimens (as bare part coloration rapidly fades after death), and measuring live individuals of such a large number of species would not have been feasible. Importantly, our study only required us to classify the presence or absence of carotenoid ornamentation, and therefore should be robust to small differences between plate coloration and true coloration. Nonetheless, previous studies have found that coloration in plates is highly correlated with coloration in museum specimens (as measured by spectrophotometry; Badyaev and Hill 2000), and several previous comparative studies have used coloration in plates to infer the presence of carotenoids (e.g. Gray 1996; Owens and Hartley 1998; Olson and Owens 2005).

To classify the presence or absence of carotenoid ornamentation in bills, we first used the R package 'colorZapper' (Valcu and Dale, 2014) to quantify the RGB values (i.e. the intensity of the red (R), green (G) and blue (B) primary colours) for the upper and lower bill in both sexes of each species. For each measurement, a polygon was subjectively selected that encompassed the typical coloration of the basal part of the bill and that excluded any obvious areas of glare added by the plate artists. colorZapper then calculated the mean values for R, G and B (on scales of 0 to 255) for 400 random chosen pixels within the selected polygon. The above RGB values were then used to quantify bill coloration in the red-green chromaticity space (Martinkauppa and Piettikäinen, 2005), a two-dimensional version of RGB colour space that is normalized by the overall intensity of the colour. Chromaticity is particularly well suited for testing our predictions because high values of red chromaticity (i.e., $r = R/(R+G+B)$) are associated with colours typical of carotenoid pigmentation (see also Gray 1996; Owens and Hartley 1998), with the highest values (i.e. $r \sim 0.55 – 0.85$, Figure 1) indicating red, and slightly lower values being associated with orange, pink and yellow coloration.

Although coloration is an imperfect means of determining whether carotenoids are present (e.g. McGraw et al. 2004), any errors should be unbiased with respect to our hypothesis since no Passerine species are known to produce carotenoid-like coloration in their bills with non-carotenoid pigments. For the analysis described below, we then generated a binary carotenoid bill coloration score for each species. A species was considered to have carotenoid bill coloration if the upper or lower mandible on the male or female had a red-chromaticity score greater than 0.44 (Figure 1). This threshold was chosen by visually examining the distribution of red-chromaticity values in our dataset and selecting a cutoff that best separated colours typical of carotenoid pigmentation (i.e. reds, oranges, yellows) from other colours. Varying this threshold did not qualitatively change the results (see below), and these bill colour scores were strongly correlated with a subjective binary score of carotenoid bill coloration generated by C.J.D. (Yule's coefficient of association = 0.98, n = 1605). We chose this approach (rather than analyzing a continuous response variable) because we were primarily interested in co-evolutionary relationships between life-history factors and the presence or absence of carotenoid bill ornamentation, rather than the degree of 'redness' *per se*. Additionally, using a binary variable eliminates the need to consider the dynamic nature of bare part coloration, since changes in bill coloration typically occur over a small range of colourspace (Faivre *et al.*, 2003; Velando *et al.*, 2006; Ardia *et al.*, 2010) and would not influence our classification of the presence or absence of carotenoid bill ornamentation. Finally, similar analyses conducted with a continuous response variable yielded qualitatively similar results to those presented below (see Table S1).

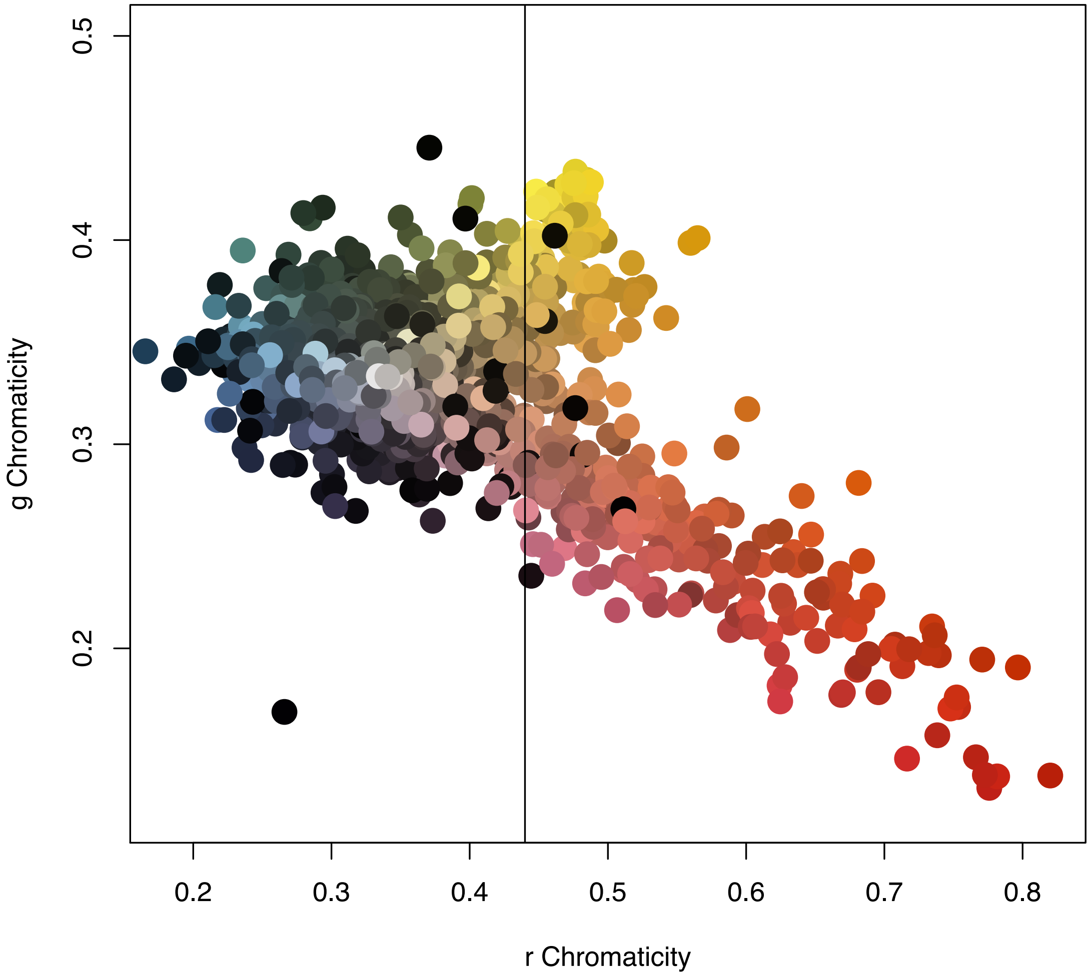

Figure 6.1 Red-green chromaticity colour space of passerine birds. For each species, one point is plotted for each of the upper or lower mandible of the male and female (i.e. 4 points per species). Point colour is determined from tri-stimulus values measured from colour plates in handbooks (see text). In the main analysis, species were classified as having a carotenoid-based bill if their r-chromaticity score was above 0.44 (vertical line).

Because we were also interested in, and wanted to account for, the direction of sexual selection and sexual dichromatism in bill colour, we also generated a second colour score that included only the species with carotenoid bill coloration. This score was similar to the binary score described above, except that species were scored as -1 if only the female had carotenoid bill coloration (on either the upper or lower mandible), 0 if both sexes had carotenoid bill coloration, and 1 if only the male had carotenoid bill coloration (on either the upper or lower mandible).

(b) *Analysis of independent variables*

Ecological and life history data were collected for as many species as possible and summarized in the following five independent variables. (i) Non-breeding sociality (social or solitary) and (ii) breeding dispersion (colonial or dispersed breeders) were binary scores generated by C.J.D. using bird handbooks (Keith *et al.*, 1992; Higgins *et al.*, 2001; del Hoyo *et al.*, 2003; Hockey *et al.*, 2005). These classifications were made blind to bill colour and were based on whether a species formed breeding aggregations and whether they primarily lived in groups during the non-breeding season, respectively. From Dale et al. (2007), we included: (iii) sexual size dimorphism as log(male wing length)-log(female wing length)) and (iv) sexual dichromatism, on a scale from -1 to 4 (where -1 = females slightly more colourful, 0 = monochromatic, 1 = males slightly more colourful, 2 = males clearly more colourful, 3 = males much more colourful or 4 = males much more colourful with additional ornaments). Our dichromatism scores are based on human perception, but several studies have demonstrated that sexual dichromatism estimates based on human vision are a reliable proxy for those based on avian vision (Armenta *et al.*, 2008; Seddon *et al.* 2010), and a subsample of the dichromatism scores used in this study were found to be highly correlated with dichromatism scores from UV-VIS spectrophotometry (see Dale et al. 2007). Finally, we also included (v) body size (mean of male and female wing length). The latter was included, because sexual size dimorphism varies with body size (Dale *et al.*, 2007).

(c) *Statistical analysis*

Statistical analyses were performed in R 3.0.1 (R Core Team, 2013). We based our analyses on avian phylogenetic trees created by Jetz *et al.* (2012), which include all 9993 extant bird species (including all species in our study). These trees are based on molecular data from 6663 species and taxonomic constraints for data-deficient species (using pre-established phylogenetic backbones), and were resolved and dated using relaxed clock methods (see Jetz *et al.* 2012 for details). To account for phylogenetic uncertainty, we performed parallel analyses on a set of 100 trees from the tree pool found at http://birdtree.org (Jetz *et al.* 2012), using the Hackett backbone (Hackett *et al.* 2008). Since trees are represented in the tree pool based on their posterior probability, sampling many trees eliminates the need to weight results by each tree's posterior probability (see also, Logue and Hall, 2014). Here, we report the mean and the most conservative (i.e. the estimate associated with the highest p-value for each term) model estimates from this set of 100 statistical models.

Complete data were available for 1499 species (93.4% of the 1605 species for which bill colour was scored), and these are included in our statistical models. We used the binary bill carotenoid coloration score as the response variable in a set of binomial family generalized estimating equations (GEE) (Paradis and Claude, 2002), with a logit link function. These analyses were conducted in the 'ape' package (Paradis *et al.*, 2004), and used the 'corPagel' covariance structure, which accounts for the phylogenetic covariance between the response and independent variables using Pagel's λ, an estimate of the phylogenetic signal that ranges from 0 (no phylogenetic signal) to 1 (all variation explained by phylogeny; Pagel, 1999). Because the function we used for model fitting (compar.gee) does not optimize λ in the model fitting process, we calculated lambda for each phylogenetic tree prior to running our analyses using the 'phylosig' function in the 'phytools' package (Revell 2012).

All five independent variables described above were included in each model. In these models we were primarily interested in the intensity rather than the direction of sexual selection, therefore we used the absolute value of sexual size dimorphism and sexual dichromatism, rather than the raw values. Additionally, all continuous predictor variables were scaled by twice their standard deviation so that their regression coefficients were directly comparable to one another and to the binary predictor variables (Gelman, 2008).

To determine whether our basic results were robust to varying the red-chromaticity cutoff score used to classify a species' bill colour as carotenoid-based, we performed the GEE analysis with cutoffs ranging from 0.38 (316 of 1499 species (21.1%) scored as having carotenoid-based bills) to 0.46 (94 of 1499 species (6.3%) scored as carotenoid-based) at 0.01 intervals. We analyzed 50 trees for each cutoff. GEE models using cutoffs higher than 0.46 failed to converge, probably due to the conservatively low percentage of species classified as having carotenoid-based bills.

To further explore the influence of past sexual selection on carotenoid bill ornamentation, we ran an additional set of models that explicitly considered sexual dimorphism in carotenoid bill ornamentation and the direction of sexual selection. For these models, we included only the species that had carotenoid bill ornamentation, and used the directional bill coloration score described above (-1 = only females have carotenoid bill ornamentation, 0 = both sexes have carotenoid bill ornamentation, 1 only males have carotenoid bill ornamentation). We used Gaussian family phylogenetic generalized linear models (PGLS) and included sexual size dimorphism and sexual dichromatism as predictor variables.

These predictor variables were similar to those used in the models above, except we did not take the absolute value for either metric, so that negative scores would indicate larger or more colourful females. We ran parallel models on 100 phylogenetic trees and report the mean estimates.

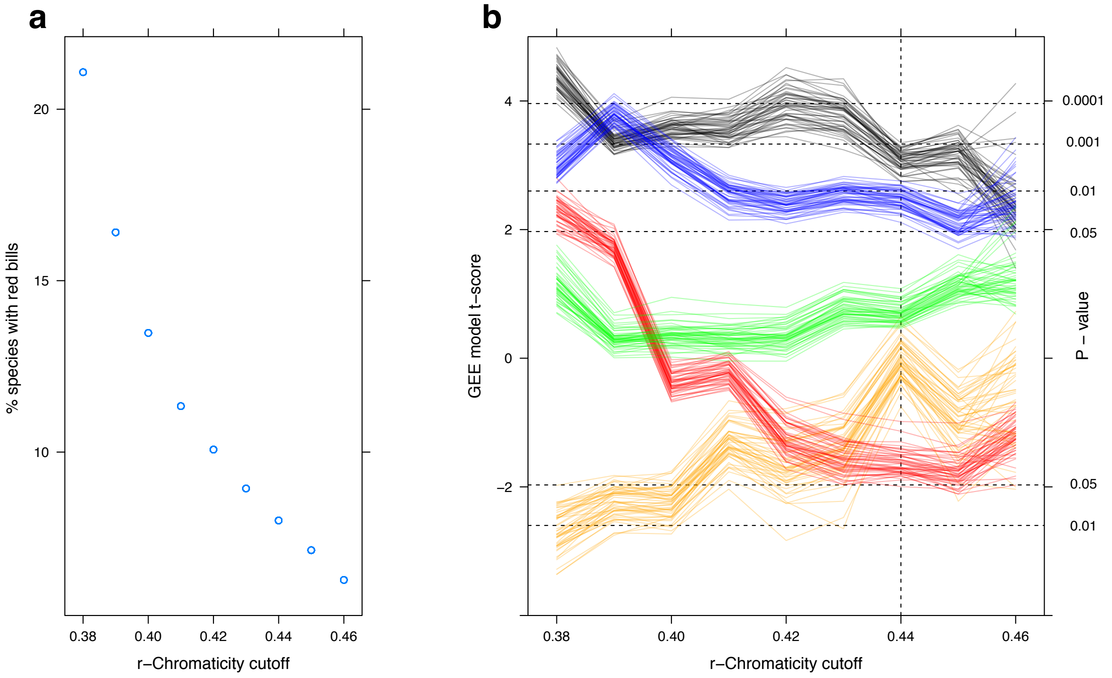

Figure 6.2 Analysis of the effects of varying the value of the r-chromaticity cutoff used to determine whether species is scored as having carotenoid-based bill coloration. (a) the percentage of species scored with carotenoid-based bills goes down with higher r-chromaticity cutoffs. (b) t-scores from multivariate GEE models on 50 different phylogenetic trees (see methods) using different r-chromaticity cutoffs (black = non-breeding sociality, blue = breeding dispersion, green = plumage dichromatism, orange = body size, and red = sexual size dimorphism). P-values associated with t-scores are indicated on the right hand axis, and the vertical line shows the cutoff used in the main analysis of this study. At all cutoffs, both non-breeding sociality and breeding dispersion were significantly associated with carotenoid-based bill coloration.

6.4 Results

In our dataset, 8.0% (120 / 1499) of species had carotenoid ornamented bills (Figure 1). This value varied according to the cut-off used (Figure 2a), but all thresholds of red chromaticity considered (between 0.38 and 0.46) produced qualitatively similar results (Figure 2b). The phylogenetic signal (λ) in carotenoid bill ornamentation ranged between 0.59 and 0.81, with a mean value of 0.69. Phylogenetically controlled generalized estimating equations demonstrated that carotenoid bill coloration is more frequent in species that live in groups in the non-breeding season compared to solitary species, and in those that breed in colonies compared to solitary breeders (Table 1; Figure 3). Carotenoid bill ornamentation was not significantly related to sexual dichromatism, sexual size dimorphism or body size (Table 1; Figure 3). Additionally, sexual dimorphism in carotenoid bill ornamentation was not related to sexual size dimorphism (PGLS: average estimate = 0.01, 95% CI = (-0.34, 0.35), $t_{1,117}$ = 0.01 P = 0.94) or sexual dichromatism (average estimate = 0.04, 95% CI = (-0.17, 0.25), $t_{1,177}$ = 0.33, P = 0.74).

6.5 Discussion

The results of this study suggest that carotenoid bill colour is a signal that primarily evolved for use in competitive interactions. We found a positive co-evolutionary relationship between the occurrence of carotenoid bill coloration and two ecological factors associated with high rates of agonistic encounters: colonial

Table 6.1 Statistical models testing for a signalling function of carotenoid bill coloration. Log-odds estimates, 95% confidence intervals, t-statistics and p-values were averaged across 100 models run on different phylogenetic trees (see Methods). We also present the most conservative estimate for each variable. Models are based on observations from 1499 species and had an average phylogenetic degrees of freedom (Paradis and Claude 2002) of 213.14 Significant *P* – values are shown in italics.

	Average				Most Conservative			
Variable	Estimate	95% CI	*t*	*P*	Estimate	95% CI	*t*	*P*
Non-breeding sociality	0.79	0.27, 1.32	2.89	*0.004*	0.61	0.09, 1.13	2.28	*0.024*
Breeding dispersion	0.53	0.10, 0.97	2.52	*0.018*	0.46	0.03, 0.90	2.09	*0.038*
Sexual dichromatism	0.29	-0.09, 0.67	1.38	0.142	0.20	-0.18, 0.58	1.03	0.302
Sexual size dimorphism	-0.51	-1.09, 0.06	-1.56	0.087	-0.33	-0.91, 0.25	-1.12	0.264
Body size	-0.01	-0.36, 0.36	0.04	0.803	0.00	-0.36, 0.36	0.002	0.998

breeding and non-breeding sociality. Because individuals experience intense competition with many unknown individuals in non-breeding flocks and in breeding colonies, it is likely adaptive to signal dominance or fighting ability in a way that would allow most individuals to settle contests quickly without having to resort to physical combat (West-Eberhard, 1983). Carotenoid bill coloration may serve this function, as bill colour is correlated with aspects of individual quality that influence social dominance and can also change over short time periods in response to changes in circulating hormones (Ardia *et al.*, 2010; Murphy *et al.*, 2009).

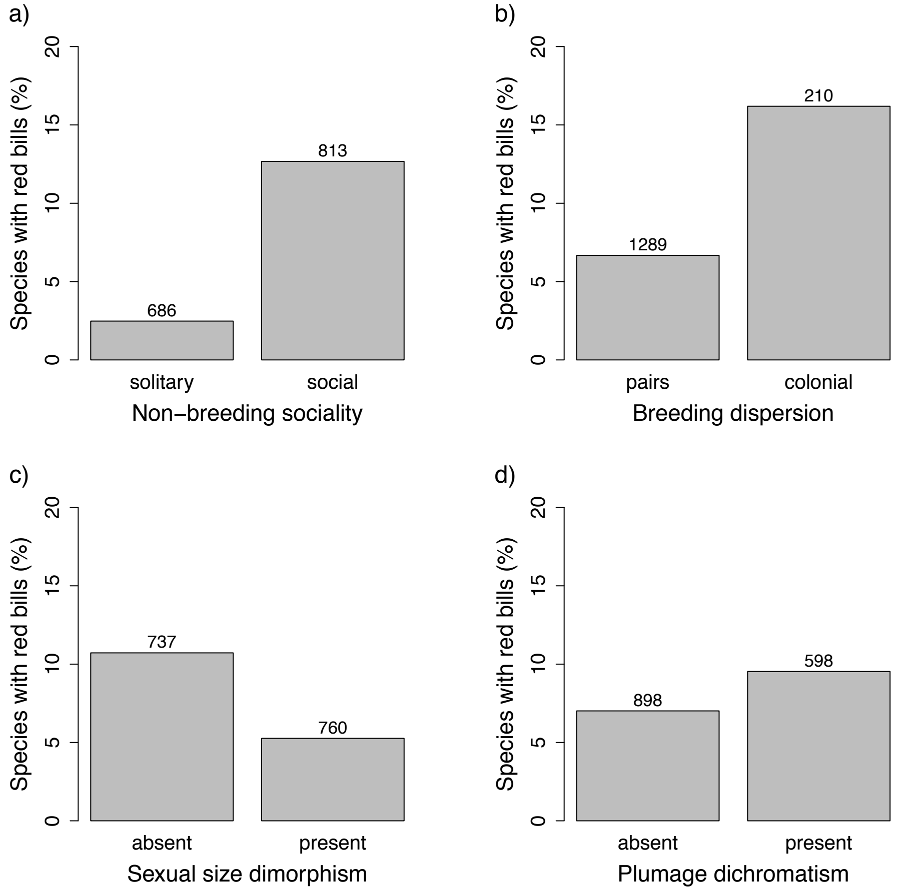

Figure 6.3 Percentage of species with carotenoid bill coloration as a function of (a) sociality in the non-breeding season and (b) breeding dispersion (c) sexual size dimorphism and (d) plumage dichromatism. Numbers above each bar indicate number of species in each category. For graphical purposes only, sexual size dimorphism was considered to be present if there was at least a 4% difference in wing length between males and females. Similarly, sexual dichromatism was scored as present if there was at least a slight difference in coloration between males and females (i.e. a non-zero score for sexual dichromatism).

Interestingly, our study suggests that carotenoid bill coloration likely did not evolve as a signal used in mate choice, as we did not find a relationship between carotenoid bill colour and two indices of the intensity of past sexual selection.

Neither sexual size dimorphism, nor sexual dichromatism were related to the presence of carotenoid bill coloration. Additionally, there were no significant relationships between sexual dimorphism in bill coloration and our measures of sexual selection. These findings conflict with the conventional thought that carotenoid ornamentation in birds is a condition-dependent sexual signal, while other types of ornamentation with lower production costs (i.e. melanin-based) are more typically used to signal social status (Badyaev and Hill, 2000). However, some researchers have questioned this stereotyped view of the signalling roles of different pigment types (Griffith *et al.*, 2006). Indeed, both melanin and structural coloration can be condition-dependent ornaments, and both mates and rivals should be interested in body condition (Griffith and Pryke, 2006; Senar, 2006). Additionally, a meta-analysis by Griffith et al. (2006) suggested that there is no fundamental difference in the signal content of melanin *versus* carotenoid based traits, as the two types of coloration do not show a difference in condition-dependence nor in heritability. The results from the current study suggest that carotenoid bill coloration may have evolved in response to similar selective pressure as the melanin-based chest and throat patches seen in New and Old World sparrows. In these species, patches were also more commonly found in species exhibiting winter sociality and were therefore argued to be status signals (Tibbetts and Safran, 2009).

Our findings are not inconsistent with intraspecific studies showing that carotenoid bill ornamentation is involved in mate choice in some species (e.g. mallard ducks, *Anas platyrhynchos*; Omland, 1996). Indeed, the comparative approach cannot directly infer the current function of traits, however it can identify co-evolutionary patterns that suggest the selective pressures leading to the evolution of certain traits. In this regard, comparative analyses provide a historical perspective on the origin of traits, and can therefore compliment within population studies that directly test trait function. Of course, trait function can change over evolutionary timescales; for example, the ornament-armament hypothesis suggests that traits which initially evolved for use in a competitive context, may be assessed by potential mates if it pays to choose a dominant or aggressive partner (Berglund *et al.*, 1996). Under the ornament-armament model, carotenoid bill ornamentation could be used in mate choice by some species, but should still be strongly associated with ecological conditions that lead to frequent aggressive interactions, as these are the conditions under which the preference for dominance in a mate should be strongest. Additionally, recent research suggests that there is no clear boundary between the sexual and non-sexual components of social selection, and that similar traits may be involved in competition for mating opportunities as in competition for other types of resources (Lyon and Montgomerie, 2012). However, our analyses shows that carotenoid bill ornamentation has a strong co-evolutionary relationship with grouping behaviour, which suggests that it initially evolved for use as a competitive signal, regardless of its current function in each species.

In contrast to the current study, previous comparative research (using similar measures of sexual selection) has indicated that carotenoid plumage ornamentation is positively related to the intensity of sexual selection (Gray, 1996; Badyaev and Hill, 2000). Whether these divergent results underline a general difference in the information content of bare part and plumage traits remains unknown, however there is reason to suggest this could be the case. Plumage traits are established during discrete moult periods, and are therefore indicative of the quality of the individual at the time the feathers were grown (although see (Møller and Erritzøe, 1992; Adamik and Vaňáková, 2011) for examples of the dynamic properties of plumage ornaments). Since current condition is probably more relevant than past condition during a resource contest, bare part ornaments may be more likely used as status signals (see also Laucht *et al.*, 2010). Conversely, potential mates may place relatively more weight on past condition than rivals, since having information on both past and present condition may reveal additional information about an individual's overall genetic constitution and quality. As a result, plumage ornaments may be more likely to be used as sexual signals than as status signals. The results of separate comparative analyses on carotenoid-based bare part and plumage ornamentation in (Olson and Owens, 2005) are consistent with this idea: at the family level, plumage ornamentation was more strongly related to mating system, while bare part ornamentation was more strongly related to coloniality. Olson and Owens

(2005) additionally argued that carotenoid ornamentation in plumage, but not in bare parts, was related to dietary carotenoid content. As a result, carotenoid ornamentation in bare parts may not be limited by the same production costs that limit carotenoid expression in plumage, and the two signal modalities may therefore have different signaling functions.

Avian bare parts are potentially highly informative signals due to their ability to change size and colour over relatively short time periods. In this study, we tested whether there is a co-evolutionary relationship between patterns of carotenoid bill coloration and either the intensity of past sexual selection, or the level of competition between conspecifics. Our results suggest that carotenoid bill coloration co-evolved with social grouping behaviours, and therefore may be generally used as a signal of competitive ability. Whether these findings underlie a fundamental difference in the signaling role of bare part and plumage traits remains unknown, but further studies directly comparing these signal modalities would be useful.

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Chapter 7: General Conclusion

Cody J. Dey

My thesis greatly expands our understanding of the development and function of social dominance in pukeko. While this bird has a rare social system, the methods that I used and developed, as well as the insights gained through my research, are widely applicable to the study of social organization in other animals. Below, I provide a summary of my main thesis findings, as well as my specific contributions to the study of social dominance and status signalling, and some suggestions for future research.

7.1 Thesis summary

In my thesis I aimed to increase our understanding of the role that the social environment has in shaping animal lives. In particular, I focused on social dominance relationships as they can have important implications for individual fitness and are found in a wide range of taxa.

In Chapter 2, I performed a quantitative analysis of the structure of dominance relationships within 11 replicate pukeko social groups. Using social network analyses, including one of the first applications of exponential random graph models in a non-human system, I showed that pukeko form highly orderly dominance relationships that are stable across years. Additionally, pukeko dominance relationships are influenced by individual attributes (i.e. sex and frontal shield size) in complex ways, including differential effects on aggressive versus submissive behaviours, and interactive effects of the attributes of different individuals (i.e., sexual homophily). This study also demonstrated that pukeko dominance relationships are not merely a product of individual attributes, but that pukeko also have a strong tendency to form transitive relationships, even once individual attributes are controlled for. Since transitive relationships form the basis of orderly dominance 'hierarchies', this finding suggests that pukeko dominance relationships self-organize to create orderly hierarchies.

In Chapter 3, I investigated the ontogeny of dominance relationships by following individuals from hatching to adulthood. Through a combination of behavioural observations and recapturing of known individuals, I showed that within-brood hatching order is linked to survival and growth, with earlier hatched chicks having both higher survival rates and higher growth rates as juveniles. Additionally, I showed that earlier hatched individuals achieve significantly higher dominance ranks as adults, suggesting a link between the developmental environment, hatch order, and an individual's social phenotype.

In Chapters 4 and 5, I explored how pukeko use their frontal shield ornament as a signal of social dominance (i.e. a 'status signal'). Previous research had suggested that the frontal shield was actively displayed in agonistic interactions (Craig 1977), however there had been no detailed studies of the relationship between shield phenotype and dominance, nor of sexual dimorphism in shield phenotype or the factors that influence signal honesty. In Chapter 4, I compared sexual dimorphism in frontal shield size across two populations of pukeko that differ in the degree of reproductive skew. Following Rubenstein and Lovette's (2009) seminal paper, I predicted that female ornamentation would be increased, and sexual dimorphism decreased, in the population with higher female reproductive skew. My analysis revealed the opposite pattern, that female ornamentation was increased in the population with lower reproductive skew. This unexpected finding may be due to a disconnect between reproductive skew (which is measured within breeding attempts or seasons), and variation in lifetime reproductive success in pukeko, and suggests that reproductive skew may not always be an appropriate proxy for the intensity of intrasexual competition. Alternatively, there may be decreased intrasexual competition in the population with high reproductive skew because groups are typically composed of kin that have long-term relationships with one another. In Chapter 5, I performed a more direct test of the function of the frontal shield ornament. First, I collected behavioural observations on 11 replicate social groups and showed that there was a strong correlation between dominance rank and frontal shield size, even after controlling for other confounding variables. Then I performed cosmetic manipulations to change the apparent size of the frontal shield ornament in two separate experiments. Through these experiments, I showed that changing the apparent size of an individual's frontal shield causes changes in social interactions; individuals who had their shield size experimentally decreased were aggressively challenged more often. Additionally, individuals who had their shield size cosmetically changed also showed changes in their true shield size, one week later. This experimentally induced change in shield size suggested that the relationship between an individual's phenotype and their social environment is much more complex than previously appreciated.

In Chapter 6, I explored interspecific patterns of bill coloration in over 1600 passerine birds as a way to study the evolution of bare-part (i.e. non-feather based) ornamentation. I found that there is a co-evolutionary relationship between carotenoid based bill ornamentation and social factors that promote high levels of competition. This finding is consistent with other intraspecific studies showing that carotenoid bill ornamentation is correlated with aggressiveness and dominance (e.g. Ardia et al. 2010). Additionally, I found no relationship between bill ornamentation and proxy measures for the strength of sexual selection (i.e. sexual size dimorphism and sexual dichromatism), suggesting that bill coloration has not evolved as a sexual signal. Taken together, these results suggest that bare-part ornaments may have evolved as signals of social status and may communicate fundamentally different information than plumage ornaments.

7.2 Advances in our understanding of social dominance and some outstanding issues

My primary contribution to our understanding of social dominance was through the use of social network analysis to study complex patterns of dominance interactions. While I was not the first to use network analyses to study dominance (see e.g., Shizuka and McDonald 2012), I greatly expanded upon previous methods and provided a much more detailed analysis of dominance networks than previous researchers. Networks analysis is a powerful framework for understanding dominance relationships because dominance interactions are influenced by other dominance relationships (e.g. through winner, loser or bystander effects; Dugatkin and Earley 2003), which are not usually considered in traditional dominance studies. One particularly powerful method for studying the inherent complexity of dominance interactions is through the use of exponential random graph models (ERGM; Wasserman and Pattison 1996, Lusher et al. 2013). These models are useful because they are able to simultaneously test the importance of individual, dyadic, and higher-level processes in determining the structure of networks, and such multivariate approaches to network structure have been lacking in behavioural ecology (Pinter-Wollman et al. 2014). In Chapter 2 I provided one of the first uses of exponential random graph models in a non-human study and the first in a study of social dominance.

There is considerable scope to expand the use of social network analyses in studies of social dominance. Indeed, the recent surge in the application of network analysis to other topics in behavioural ecology (e.g. disease transmission, sexual selection, personality, etc.) demonstrates the utility of these analytical tools for studying the organization of animal groups, and they should be equally (or more) useful in the study of dominance (Pinter-Wollman et al. 2014). Many basic questions about the organization of dominance networks have not yet been answered. For example, while we know that most animals form orderly dominance networks (Shizuka and McDonald 2012, McDonald and Shizuka 2013), we don’t know if dominance interactions occur more frequently between similar or differently ranked individuals. Similarly, we don’t know how dominance networks relate to other social networks, such as networks of spatial associations or relatedness, and we have little understanding of how dominance relationships change with ecological and social context (although see appendix C). These gaps in our knowledge limit our understanding of how dominance relationships affect the costs and benefits of living in social groups, and should therefore be a subject of future research for sociobiologists.

One particularly interesting aspect of dominance that could be studied using network approaches is how information about dominance spreads throughout social groups. Some elegant laboratory studies have demonstrated that winner, loser and bystander effects can have important influences on dominance relationships (e.g. Chase et al. 2002, Mennill et al. 2002, Dugatkin and Druen 2004), but their relative importance in natural systems is unclear. For example, modeling studies have shown that loser effects should be stronger for young individuals and winner effects stronger for older individuals (Fawcett and

Johnstone 2010), but this has not yet been supported empirically, nor do we understand how this would influence the structure of dominance networks. Furthermore, in light of the importance of winner, loser and bystander effects, it would be interesting to study how changes in particular dominance relationships influence other dominance relationships within a group. For example, in some cooperatively breeding animals α individuals eventually senesce and are overthrown by β individuals (Woolfenden and Fitzpatrick 1978), but we do not know if there are knock-on effects for dominance relationships between other group members. Such issues could be potentially addressed by using ERGMs to study temporal changes in dominance networks (e.g. using separable temporal exponential random graph models; Krivitsky and Handcock 2014). Such studies would be particularly valuable in fission-fusion societies where individuals must repeatedly establish dominance relationships with new individuals as group membership changes, or in species that show seasonal differences in social organization (i.e. birds that form winter flocks).

Additionally, network analysis provides a useful set of tools to study group-level selection on dominance relationships. Several researchers have suggested that orderly dominance relationships improve group productivity by minimizing conflict, improving role specialization and increasing the speed of collective decision-making (Lampkin 1972, Meese and Ewbank 1973, King et al., 2009). However, I am unaware of any study that has examined the relationship between the organization of dominance relationships and group function for a non-human animal.

In addition to orderliness, many other aspects of dominance network structure (e.g. homophily, assortativity) could influence group productivity, but have not been subject to study. Investigating the relationship between global dominance network structure and how well the group functions would be highly insightful in understanding why some individuals readily accept low dominance ranks and why low ranked individuals are retained in social groups. The potential advantages of forming stable dominance networks may explain some patterns of social partner choice. For example, recent studies have demonstrated that groups composed of a mix of different personalities function better than homogenous groups (Johnstone and Manica 2011, Aplin et al. 2014), and that productivity increases with variation in aggressiveness among group members in an ant species (Modlmeier and Foitzik 2011). These effects could be partially due to stable dominance network formation in groups with a mixture of individual attributes, however this hypothesis requires testing.

7.3 Advances in our understanding of status signalling and some outstanding issues

My thesis has provided a key empirical finding in understanding the complex relationship between signal phenotypes and social interactions. In Chapter 5, I showed for the first time that changes in signal phenotype cause changes in social interactions, which then feedback to modify signal expression. This finding supports a key prediction of the integrative costs model (Tibbetts 2014), and is probably the most direct evidence to date for a dynamic relationship between signals and social interactions. Early models of status signalling based on the physiological costs of signal production assumed that signals influence social interactions in a static and unidirectional manner (e.g. Folstad and Karter 1992). However, recent research has emphasized that social interactions can influence individual physiology (e.g. hormone titers; Adkins-Regan 2005), and so when signal phenotype is influenced by physiology there is the possibility that changes in social interactions will indirectly affect signal expression (Rubenstein and Hauber 2008, Tibbetts 2014). This unappreciated complexity in the relationships between signals, behaviour and individual physiology has lead to the formulation of an integrative model of the social and physiological costs of ornamentation (Tibbetts 2014).

The feedback effects of changes in signal phenotype on future signal expression in Chapter 5 were most likely mediated by androgen titers, as frontal shield size in other rails is known to be testosterone dependent (e.g. Eens et al. 2000) and androgen levels respond to changes in agonistic interactions (e.g. HIrschenhauser et al. 2003). However, the mechanistic link between changes in social interactions and signal phenotype was not directly studied in the course of my thesis work, and it is possible that the observed changes in shield size were due to changes in glucocorticoid levels, carotenoid metabolism or oxidative stress rather than androgens (Tibbetts 2014). Additionally, these physiological mechanisms are known to interact with one another (e.g. Viau 2002), and could have interactive effects on signal expression. Determining the specific physiological mechanisms that are involved in the integrative costs of shield signalling will be difficult, but is worth further study if we are to gain a complete understanding of the factors that maintain signal honesty.

Additionally, it is unclear what the costs of exaggerated (i.e. more ‘dominant-like’) signal expression are in pukeko, as I only studied the costs of signal reductions. Increases in aggressive challenges towards individuals with reduced signals could have happened in two ways: 1) non-focal individuals identify incongruence between signal phenotype and other cues to dominance (i.e. other signals or behaviours) and increase challenges to ascertain the focal individual’s true quality (Tibbetts and Izzo 2010) or 2) non-focal individuals simply behave towards the focal individual in accordance with their new signal size and may challenge them more often because they are advertising low quality. Studying the response to signal exaggeration should lend some insight into which of these mechanisms is in operation. If experimentally exaggerated individuals receive more challenges, then changes in receiver behaviour are likely due to signal incongruence. Alternatively, if experimentally exaggerated individuals receive fewer aggressive challenges, then receivers would appear to be acted in accordance with the characteristics of the modified signal. This test is also critical for understanding how the dynamic relationship between social interactions and signal expression relates to signal honesty. If individuals with dishonestly large signals experience decreased aggressive challenges it would suggest there is a benefit to dishonest signaling, and would suggest that receiver-dependent costs do not maintain signal honesty in this system.

In addition to advancing our understanding of the complex relationship between signals and social interactions, I also provided evidence that bare-part ornaments are underappreciated signals in birds. In general, bare-part ornaments have received much less research attention than have plumage ornaments, despite the fact that many birds have colourful bare-parts and their characteristics makes them good candidates for signalling. In Chapter 6, I showed that there is a strong co-evolutionary relationship between carotenoid bill ornamentation and natural history traits that lead to high levels of intraspecific competition. Additionally, I demonstrated that frontal shields (a bare-part ornament) are used for status signalling in pukeko. Together, these results question the historical focus on studying status signalling in birds by examining plumage ornaments (see Senar 2006 for a review) because bare-part ornaments may be more informative status signals across a range of contexts. Additionally, studies on bare-part ornaments allow for different experimental approaches, as the influence of various social and physiological manipulations on signal expression can be viewed over short time periods (e.g. Zuk and Johnsen) rather than having to waiting for discrete moult periods (e.g. Laucht and Dale 2012). Indeed, studies on dynamic signals may be the best option to examine the interaction between the social and physiological costs of ornamentation (Tibbetts 2014), and could provide new insight into old questions about the evolutionary stability of honest signalling systems.

7.4 Suggestions for future research

In this section I briefly outline a number of studies that could address some of the outstanding issues presented above, and are tractable in the pukeko study system.

7.4.1 How does dominance network structure influence group productivity and function?

A descriptive study examining the relationship between the structure of dominance networks and group productivity would be an important start in understanding how group-level selection has acted on dominance networks in pukeko. To make this study feasible, a small number of social groups would need to be followed across several years. These groups would need to have all members marked for individual identification and dominance networks could be generated for each group each year, using the methodology developed in Chapter 2 and Chapter 5. Variation in dominance network structure (both within and among groups) could then be correlated with measures of group productivity including reproductive success (i.e. laying patterns, chick mortality, offspring growth rates, etc.), territorial defence (to natural or simulated territorial intrusions) and changes in territory size.

7.4.2 Costs of signal exaggeration

In Chapter 5, I demonstrated that reductions in apparent shield size lead to an increase in aggressive challenges and result in a decrease in true shield size. However, the social costs and resultant effects on true shield expression for dishonestly large frontal shield size remains unknown. A similar experiment to that described in Chapter 5 could be conducted but using frontal shield enlargements. Such a manipulation would be logistically more difficult because coloring the plumage surrounding the shield is difficult (it is black and filamentous, which were not issues faced when coloring the frontal shield itself). However, it may be possible to attach a model frontal shield over top of an individual's existing frontal shield using adhesive. I have tried such approaches but have thusfar been unsuccessful in attaching the artificial shields and observing subsequent behaviours. Optimally, both the changes in social interactions caused by the manipulation, as well as the changes in true signal size caused by the manipulation could be measured during a single study, although constraints in the ability to recapture focal individuals may require two separate experiments (as in Chapter 5).

7.4.3 Incongruent signalling and multiple ornaments

Signal incongruence is hypothesized to be a key factor contributing to the social costs of dishonest signalling (Tibbetts and Izzo 2010). In Chapter 5, I demonstrated that dishonest frontal shield size leads to an increase in aggressive challenges directed towards focal pukeko, however the specific behavioural mechanism leading to the change in social interactions was not uncovered. Non-focal group members may have been responding to incongruence between shield signals and the focal individual's behaviour, to incongruence between shield signals and other ornamental traits, or may have been responding to the reduced trait itself without any notice of the incongruence.

Pukeko have a deep blue chest plumage and we have evidence that the chroma of pukeko breast feathers is correlated with dominance status (CJ Dey *unpublished data*). As a result, chest plumage coloration may also be used as a signal or cue to dominance. It would be informative to perform an experiment where both breast plumage coloration and frontal shield size were manipulated, to test the hypothesis that incongruence between different ornaments leads to social costs. Breast feather coloration and shield size could be manipulated in the same direction (i.e. both increased or decreased) and also in opposite directions (i.e. one increased while the other decreased) so that focal individuals either had congruent or incongruent ornamentation. The effects on interactions with non-focal individuals could then be assessed.

7.5 Conclusions

My thesis has improved our understanding of the importance of social dominance in the lives of animals. Using the cooperatively breeding pukeko as a model system, I explored the structure, formation, maintenance and consequences of dominance relationships, and provided both empirical and methodological advances for the field of behavioural ecology. I greatly expanded the application of social network analyses to the study of dominance and demonstrated that dominance relationships are influenced by both the attributes of individuals, as well as self-organizational processes. Additionally, I showed that hatching order has an important influence on the formation of dominance relationships, with earlier hatched chicks achieving higher dominance ranks as adults. I also examined the relationship between social interactions and signal phenotype, focusing on the pukeko's frontal shield. I showed that sexual dimorphism in frontal shield size is dramatically different in two pukeko populations, probably due to differences in the intensity of intrasexual competition, and that shield size is a dynamic trait that is influenced by social interactions. Finally, I expanded my findings on pukeko shield colour by exploring interspecific patterns of bill colouration in over 1600 birds. This study revealed that carotenoid bill ornamentation evolved as a signal used in competitive interactions. Taken together, my research provides a comprehensive demonstration of the importance of social dominance to wild animals, and generates important methodological tools for future studies on animal social behaviour.

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Appendix A: Cooperative Males Reduce Incubation in Response to Cues of Female-Female Competition

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Short communication

Cooperative males reduce incubation in response to cues of female–female competition

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Social groups of the joint-laying Pukeko *Porphyrio porphyrio melanotus* typically contain one or two breeding females. Male Pukeko mated to two females father more offspring and therefore benefit from this mating arrangement; however, primary females should not prefer this system, because fewer eggs hatch per female in the larger joint clutches. Here, we investigated male response to simulated egg destruction, a common female competitive tactic observed in other joint-laying species. In response to egg removal, males reduced the consistency of their incubation and in some cases nests were abandoned. Such decreases in paternal effort could eliminate any putative advantage gained by a female that destroys the eggs of a co-nester. Our study demonstrates facultative adjustments in paternal care in a joint-laying species and suggests that primary females may be limited in their ability to monopolize reproduction.

Keywords: communal breeding, intersexual conflict, joint-laying, ovicide, parental care, reproductive skew.

The ability to fine-tune parental effort in response to changing costs and benefits of parental care has been demonstrated in a number of species. A reduction in care is thought to be adaptive when the benefits of providing care decrease, such as during a reduction in offspring number (Hegner & Wingfield 1987), quality (Clutton-Brock *et al.* 1981, Burley 1986) or relatedness (Neff 2003). Similarly, a number of studies have shown that changes in the costs of providing parental care can affect parental investment. For example, parental investment is known to change with parental age (Velando *et al.* 2006) and resource availability (Townshend & Wootton 1985). Theoretically, such facultative adjustment in care should increase an individual's lifetime reproductive success by optimizing how it will allocate time and energy among all of the offspring it will raise over a lifetime (Velando *et al.* 2006). If the current reproductive effort is particularly costly or the attempt is unlikely to be successful, parents may even abandon young altogether to conserve resources for future breeding events (Székely *et al.* 1996).

While a number of studies have investigated parental effort in socially monogamous species, we know much less about parental care decisions in species that live in complex social groups. We examined how cooperatively breeding male Pukeko *Porphyrio porphyrio melanotus* adjust incubation behaviour in response to experimental brood reduction. Pukeko can breed independently in mated pairs, but it is more common for groups of 3–12 individuals to defend a shared territory and cooperatively raise the group's offspring. Such groups usually contain multiple breeding males, one or two breeding females that mate promiscuously, and sometimes also non-breeding helpers (Jamieson 1997). When there are two breeding females in a group, both will lay in the same nest (termed a joint-nest). The resulting joint-clutches contain more eggs than single clutches, but are less than twice the size of single clutches (Craig 1980a, Quinn *et al.* 2012). Larger Pukeko clutches produce more chicks overall than do smaller clutches, and males therefore generally benefit whenever a joint-clutch is laid (Quinn *et al.* 2012). However, the primary female (who initiates laying and is socially dominant over other females in the group; Craig 1980b) suffers a reproductive cost as a result of joint-nesting, as a lower percentage of eggs hatch in joint-clutch nests (Quinn *et al.* 2012). As a result, joint-nesting primary females are likely to raise fewer offspring than if they had nested without a co-breeding female (Craig 1980a, Quinn *et al.* 2012).

Despite the cost of joint-nesting to primary females, it does not seem that Pukeko destroy, eject or bury the eggs of co-breeders in the nest structure (Quinn *et al.* 2012, C.J. Dey & J.S. Quinn pers. obs.). Such ovicidal behaviour is observed in several other species of joint-laying birds (Vehrencamp & Quinn 2004) and is thought to be a competitive tactic used by females to maximize their reproductive success. Male Pukeko perform the majority of incubation and all nocturnal incubation (Craig 1980b), and it has been suggested that females do not engage in ovicide because it may lead to reductions in paternal care (Quinn *et al.* 2012). We tested this idea by conducting a partial clutch removal experiment to assess whether male Pukeko adjusted

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their incubation behaviour in response to simulated competition between the joint-laying females. We predicted that males would decrease incubation when clutch sizes were decreased by elimination of the secondary female's clutch and that clutch-abandonment would occur more frequently in diminished clutches.

METHODS

Nest success

We first explored the costs of joint-nesting by examining whether it resulted in egg loss during incubation or hatching failure in retained eggs. During the breeding seasons (July–December) of 2008 and 2010, we monitored Pukeko nests at the Tawharanui Open Sanctuary in New Zealand (36°22′S, 174°49′E). Nests were located by searching suitable nesting habitat. Only the first nest of the season for each group was used, and if data were collected from the same social group in both years, we only used that collected in 2008 to avoid pseudoreplication ($n = 2$ cases). Once located, nests were visited daily during laying and hatching periods, and every 3rd day during incubation. If a nest was located after laying had commenced, the clutch initiation date was estimated by floating eggs in warm water and comparing their buoyancy and angle relative to the water's surface with eggs of known age (Hays & LeCroy 1971). In nests with known initiation dates, floatation scores were highly correlated with egg age ($R^2 = 0.84$, $n = 41$ eggs). Nests were monitored for an average of 26 days (range = 10–33 days, $n = 34$), which is most of the total nesting period (*c.* 33 days, C.J. Dey unpubl. data). Eggs that disappeared between visits were classified as 'lost' (12% of eggs in this study). Egg losses were not likely to be due to predation, as mammalian predators are excluded from this site by a pest-proof fence. Furthermore, the only known aerial predator of Pukeko nests, Australasian Harriers *Circus approximans*, leave behind shell fragments and half-eaten eggs in the nest (Haselmayer & Jamieson 2001). We observed no such signs of predation in our study.

Clutch removal

In 2010, 21 joint-nests were alternatively assigned to either a control treatment ($n = 11$) or removal treatment ($n = 10$). Only nests in which females were still laying were used in this experiment (i.e. if a nest was located after laying had ceased, it was not used), and these nests were not included in the analysis described above. In the removal treatment, we experimentally simulated competition between females by removing one female's entire clutch. The eggs of individual female Pukeko are identifiable, as each female lays eggs with a unique colour, shape, size and spotting pattern (Craig 1980a,b, Jamieson 1997, Quinn *et al.* 2012). We removed one egg per day of the secondary female's clutch (the 'B' clutch) until the entire clutch was removed. These removals began on the first day that a 'B' egg appeared or on the day of nest discovery if the secondary female had already begun laying (mean ± se number of 'B' eggs in nest at time of discovery = 1.05 ± 0.34). In control nests, eggs were handled in a similar manner to the treatment nests (i.e. eggs were picked-up and inspected during nest visits) but no eggs were removed from the nest area. We continued this procedure at control nests until laying ceased. All nests were visited every 3rd day after clutch completion (as above), and nests found with cold eggs on three consecutive visits were considered abandoned.

In 15 of the 21 experimental nests ($n = 8$ removal and $n = 7$ control) an iButton thermologger (Embedded Data Systems, Lawrenceburg, KY, USA) was buried approximately 25 mm deep in the centre of the nest bowl. These loggers recorded the nest temperature every 20 min. Because incubation is often inconsistent during egg laying, we standardized our analysis by considering only the temperatures logged over a 10-day period beginning with the day that the last egg was laid. Furthermore, because we were primarily interested in how male Pukeko respond to cues designed to simulate female competition, we restricted our analysis of incubation temperatures to a period between sunset and sunrise (21:00–06:00 h), when only males incubate (Craig 1980b).

We analysed incubation consistency in two ways. First, the variance in nightly incubation temperature was determined, where low variance indicated high constancy of incubation (Fernandez & Reboreda 2007). Secondly, we calculated the proportion of time that eggs were left exposed. Because ambient nocturnal temperatures were always below the normal incubation temperature for Pukeko, we considered the nightly maximum nest temperature as indicative of the presence of an incubating bird. If the nest temperature was 3 °C or more below the nightly maximum nest temperature, and the nest temperature was decreasing or constant, we considered the nest to be exposed. Performing the analysis with a 2 °C threshold yielded qualitatively similar results. We are confident that any detectable differences in nest temperature reflect a true change in male incubation behaviour, as a separate experiment has shown that the clutch size *per se* does not have a significant effect on nest cooling rate (C.J. Dey unpubl. data). We were unable to use the data from two control nests because we failed to recover the thermologger from one nest, and the data logger from another was unreadable due to water damage. Thus, the final incubation analysis was performed on five control and eight removal nests.

Statistical analysis

Statistical analyses were performed using R version 2.15.1 (R Development Core Team 2012). In all statistical tests, model residuals were visually examined to determine whether they met the assumptions of parametric tests (Zuur *et al.* 2009). Welch's *t*-tests were used when comparing the means of two groups, as some data were heteroscedastic. To explore the effect of clutch size on reproductive success, we used two binomial generalized linear models (GLZ) with logit link functions. In model 1, we examined the proportion of eggs that were lost during incubation and model 2 considered the proportion of eggs that successfully hatched. In both models, we included total clutch size and clutch initiation date as fixed effects to test our main variable of interest while controlling for the timing of nesting, which can be an important correlate of avian nesting success (e.g. Hochachka 1990, Chastel *et al.* 2003, Saino *et al.* 2012). We also included the total number of days the nest was monitored as a fixed effect in model 1 to control for an increased probability of detecting egg losses when nests were monitored for longer time periods.

To understand whether our clutch removal affected male incubation behaviour, we used a linear mixed-effects model (LMM) and a binomial generalized linear mixed model (GLMM), with respectively variance in nightly nest temperature (°C^2) and the proportion of time the nest was exposed as the response variables, respectively. In each model, treatment (control or removal) and incubation date (e.g. day 1 of incubation period = 1) were included as fixed effects, and nest ID was included as a random intercept in each model. The residuals of the LMM were not normally distributed, and thus we transformed the response variable using the Box–Cox power transformation procedure (Box & Cox 1964) with $\lambda = 0$ (equivalent to a log transformation). Models were fitted with and without nests that were subsequently abandoned ($n = 3$ nests). As there were no qualitative differences as a result of excluding nests that became abandoned, we report only the results from the models with all nests included. Finally, we used Fisher's exact test to determine whether our egg removal caused differential nest abandonment rates. Means are presented ±1 se.

RESULTS

Nest success

Joint-clutches were significantly larger than single female clutches (Welch's *t*-test: joint-clutch = 8.5 ± 0.49, single clutch = 4.6 ± 0.17; 95% CI for difference between groups = (2.9, 5.0), $t_{18.4} = 7.60$, $P < 0.001$). Joint-clutches had a higher proportion of eggs lost during incubation (Table 1) and also had lower hatching success in retained eggs (Table 1) than did single female clutches.

Clutch removal

The total number of eggs laid in the removal and control nests was similar (Welch's *t*-test, control = 8.7 ± 0.68, removal = 8.0 ± 0.71; 95% CI for difference between groups = (−2.5, 1.6), $t_{17.3} = -0.47$, $P = 0.65$). However, following clutch manipulations, removal nests had significantly fewer eggs than did control nests (control = 8.7 ± 0.68, removal = 4.9 ± 0.31; 95% CI for difference between groups = (−5.4, −2.2), $t_{17.3} = -5.14$, $P < 0.001$). Secondary females that had their eggs removed did not compensate for lost eggs by increasing the total number of eggs laid (mean 'B' clutch size control = 3.2 ± 0.31, removal = 3.1 ± 0.55; 95% CI for difference between groups = (−1.4, 1.2), $t_{12.0} = 0.14$, $P = 0.89$). Removal nests had higher variation in nocturnal incubation temperature (mean nightly variation in nest temperature: control = 0.83 ± 0.14 °C, removal = 3.54 ± 0.93 °C, Table 2) and were exposed for a greater proportion of time (mean nightly proportion of time exposed: control = 0.07 ± 0.02, removal = 0.17 ± 0.02, Table 2) than were control nests. Finally, removal nests tended to be abandoned more frequently than control nests but this pattern did not reach statistical significance (3/10 removal nests vs. 0/11 control nests; Fisher's exact test, $P = 0.09$).

Table 1. Nest success in Pukeko groups. The proportion of eggs not lost during incubation (egg survival) and the proportion of retained eggs that successfully hatched (hatching success) are modelled for 34 Pukeko nests. Parameter estimates are presented with 95% confidence intervals, test statistics and degrees of freedom (df). Significant *P*-values are shown in bold. See text for details of the statistical analysis.

Model	Effect	Estimate (95% CI)	*Z*	df	*P*-value
Egg survival	No. of laying females	−1.10 (−2.29, −0.12)	2.04	1,30	**0.04**
	Clutch initiation date	0.02 (−0.01, 0.04)	1.63	1,30	0.10
	Days monitored	−0.03 (−0.10, 0.04)	−0.88	1,30	0.37
Hatching success	No. of laying females	−0.94 (−1.76, 0.19)	−2.39	1,31	**0.02**
	Clutch initiation date	0.03 (0.01, 0.04)	2.91	1,31	**0.004**

Table 2. Male incubation behaviour in response to simulated egg destruction. Results of statistical models on measures of nocturnal incubation constancy are shown for 10 nights in each of 13 nests. Nest ID is included in these models as a random effect. Parameter estimates and 95% confidence intervals are presented with test statistics (*t*- or *Z*-value, respectively). Significant *P*-values are shown in bold. Treatment effects are shown for the removal group relative to the control group. See text for further details of the statistical analysis.

Response variable	Effect	Estimate (95% CI)	*t*/*Z* Value	df	*P*-value
Nest temperature variation	Treatment	0.74 (0.03, 1.44)	2.31	1,11	**0.04**
	Incubation date	−0.04 (−0.11, 0.04)	−0.96	1,116	0.34
Nest exposure	Treatment	1.07 (0.25, 1.89)	3.97	1,11	**0.01**
	Incubation date	−0.05 (−0.09, −0.02)	2.88	1,116	**0.007**

DISCUSSION

This study extends previous research showing that primary female Pukeko suffer a reproductive cost from joint-laying (Craig 1980a, Quinn *et al.* 2012). We show that joint-clutches have reduced per-egg hatching success due to both egg losses during incubation and increased hatching failure in retained eggs. Egg losses during incubation were likely due to eggs being accidentally cracked and then removed by parents. Eggs crack when nests contain large clutches in which eggs may become stacked on top of one another (Quinn *et al.* 2012; C.J. Dey pers. obs.). Furthermore, many of the 'lost' eggs in this study were observed to be cracked in the nest prior to their disappearance. Given this apparent cost of joint-laying for primary female Pukeko, the lack of overt female–female reproductive competition within Pukeko social groups is puzzling. However, our study showed that male Pukeko decreased incubation constancy in response to partial clutch removal, which may be a cost that prevents female Pukeko from destroying the eggs of co-breeders.

We also observed nest abandonments by some of our clutch removal groups. Quinn *et al.* (2012) also observed a high rate of nest abandonment (32%) when one clutch was removed from a joint-nest. Nest abandonment in response to partial clutch loss may be a male strategy that encourages re-nesting with a larger overall clutch. Indeed, male Wilson's Phalarope *Phalaropus tricolor* are more likely to incubate large clutches and may abandon small clutches early in the breeding season (Delehanty & Oring 1993). Although male Pukeko that decrease parental care or abandon nests suffer an immediate fitness cost in terms of reduced success of their current brood, their lifetime fitness may be increased, as they will not waste time and energy raising a small brood. Such a strategy may be especially beneficial when the breeding season is long (Delehanty & Oring 1993), re-nesting can occur quickly and the parental investment required for offspring to survive to adulthood is high. Pukeko at our study site regularly breed from July to February and are capable of breeding year-round (Dey & Jamieson 2013). Furthermore, in the two groups we monitored following nest abandonment, new clutches were initiated within 10 days. Finally, although Pukeko chicks are nidifugous, they are fed, guarded and brooded by adults for up to 4 months (Dey & Jamieson 2013). Hence, Pukeko fit the profile of a species in which facultative adjustments in parental effort in response to perceived brood value would be beneficial.

To our knowledge, this study is the first to demonstrate facultative adjustments in parental care in a joint-laying species. However, our study could not distinguish the specific cues used by males when decreasing their investment in incubation. Our partial clutch removal may have decreased the total clutch size to a point where the costs of incubation for males are no longer repaid by the benefits of raising the small brood. In Pukeko groups, paternity is usually shared among multiple males and thus each male breeder can only expect to sire a small number of offspring. It is possible that the additional eggs provided by a secondary laying female are required to entice breeding males to provide parental care. Indeed, many animals reduce paternal care in response to a decrease in perceived paternity (e.g. Hegner & Wingfield 1987, Dixon *et al.* 1994, Neff 2003) and such an effect could be responsible for the observed decrease in incubation consistency in the current study. An alternative explanation is that males perceived our egg removals as a predation event and reduced parental care in response to the nest no longer being considered a safe place to raise young. In joint-laying species in which ovicide occurs, males are unlikely to know the cause of missing eggs because egg destruction usually occurs when the male is absent from the nest (Vehrencamp & Quinn 2004). However, even if males could determine eggs lost to reproductive competition from those lost to predation, their response to any decrease in clutch size should be to decrease paternal investment, as both predation and ovicide decrease the benefits of providing parental care relative to the costs. As such, reductions in male care in response to decreases in clutch size may eliminate the potential benefits to females of destroying a co-breeder's clutch.

Although the available evidence suggests that primary female Pukeko would produce more offspring by nesting without a co-breeding female, it appears they have few options to monopolize reproduction. Because male Pukeko perform most of the incubation, females are probably unable to completely exclude co-breeding females from access to the nest. Furthermore, we show that destroying the eggs of a co-breeder may not be beneficial due to corresponding decreases in paternal care. The best option for females may be to evict or aggressively prevent secondary females from joining the group in the first place. Female Pukeko defend their territory from females that are not part of their group (C.J. Dey pers.obs.). However, whether eviction occurs and who performs evictions is not known for this species. Clearly, the fact that Pukeko females tolerate co-breeders suggests there are benefits of joint-nesting that have not yet been identified or that there are constraints preventing females from excluding competitors altogether.

In cooperative breeders, intrasexual competition will not occur if breeders are constrained in their ability to control the reproduction of same-sex rivals (Hodge 2009). While these constraints have typically been considered a property of competition and transactions among same-sex group members (Nonacs & Hager 2012), researchers are becoming increasingly aware that the behaviour of opposite-sex group members can influence the distribution of reproduction (e.g. Cant & Reeve 2002, Hamilton & Heg 2007). In particular, we suggest that more attention should be given to plasticity in parental and alloparental care in plural breeding cooperative breeders, as this may provide a widespread mechanism by which individuals can influence reproductive dynamics of opposite-sex group members.

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Appendix B: Network Structure is Related to Social Conflict in a Cooperatively Breeding Fish

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Network structure is related to social conflict in a cooperatively breeding fish

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The nature of individual social interactions can have a profound influence on group structure and function. Here, we use social network analysis to examine patterns of dominance interactions and spatial associations in 14 captive social groups of the cooperatively breeding cichlid, *Neolamprologus pulcher*. In this cichlid, social groups are composed of a dominant breeding pair and 1–20 nonbreeding subordinate helpers that form size-based queues for breeding positions. In the current study, we performed the first quantitative analysis of *N. pulcher* dominance hierarchies. We found that dominance hierarchies of *N. pulcher* were highly linear and that interactions within dyads were directionally consistent. We also found that dominance interactions were not equally distributed across the network, but instead occurred most frequently at the top of the social hierarchy. Contrary to our predictions, neither body size asymmetry nor sex predicted the observed dominance interactions and patterns of associations. However, breeders were more connected than helpers within the networks, perhaps due to their policing role. This study is one of a small handful to conduct network analysis on replicate social groups, and thus is one of few studies able to make general conclusions on the social structure of its focal species. The patterns uncovered suggest that conflict over breeding position inheritance has a strong impact on relationships among group members in *N. pulcher*.

Many different species live in groups, and the evolution and maintenance of such social structure strongly depends on the nature of the interactions among individuals. Within social groups, behavioural interactions typically occur nonrandomly among group members (Dugatkin & Sih 1995; Krause et al. 2007). Individuals often preferentially interact with particular social partners because variation in individual attributes (e.g. age, resource-holding potential, sex, personality) causes specific social interactions to be costly or beneficial (Krause 1994; Lusseau & Newman 2004; Pike et al. 2008; Schürch et al. 2010). However, even if all group members are functionally similar, nonrandom interactions may be inherently beneficial. For example, in many species, individuals preferentially group with familiar individuals, because associating with known partners leads to lower aggression and higher foraging success (reviewed in: Griffiths 2003; Ward & Hart 2003). Generally, variation in individual social interactions can influence the structure and function of social groups (Flack et al. 2006), which will in turn influence individual fitness (e.g. Ryder et al. 2008; Silk et al. 2009; Oh & Badyaev 2010). More specifically, patterns of social interactions dictate many aspects of social living, such as the allocation of reproduction (e.g. Herrera & Macdonald 1992), the evolution of cooperation (e.g. Ohtsuki et al. 2006; Voelkl & Kasper 2009), and the transmission of information or disease (Krause et al. 2007; Wey et al. 2008; Godfrey et al. 2009).

Social network analysis provides a quantitative framework for analysing patterns of interactions among individuals (Croft et al. 2004). In its basic form, a social network is composed of individuals (represented by nodes) that are connected by their interactions (represented by edges; Whitehead 2008). In addition to these relational data, attributes of individuals can also be laid onto the network (Croft et al. 2008). Therefore, the network approach allows for analysis of behaviour in the context of an individual's social environment, facilitates exploration of the emergence of behavioural phenotypes at the group or population level (Croft et al. 2008), and is a promising tool for understanding the link between individual traits and group- or population-level phenomena.

While social network analysis has been increasingly employed in behavioural biology (reviewed in: Krause et al. 2007; Wey et al. 2008; Sih et al. 2009), few studies have analysed the network structure of multiple independent social groups of a given species. Indeed, Croft et al. (2008, page 146) noted that such replication is 'conspicuously absent in many network studies'. This trend is beginning to change (e.g. see recent studies by Croft et al. 2005;

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Thomas et al. 2008; McCowan et al. 2008; Madden et al. 2009; Schürch et al. 2010). However, more studies that compare network structure among replicate social groups are clearly warranted if we wish to reach general conclusions about the causes and consequences of the structure of animal societies. For animals that readily perform natural behaviour in captivity, analysing the network structure of captive groups provides a feasible means of gathering data on multiple replicate groups under controlled conditions. While studying the social networks of captive animals may have some drawbacks (e.g. these captive social groups may not precisely mimic the composition of natural groups), there are also advantages in that researchers can more easily manipulate or control factors predicted to affect network structure, and can therefore reach robust conclusions.

Here, we investigate behavioural interactions within social groups in the cooperatively breeding cichlid, *Neolamprologus pulcher*. This species is endemic to Lake Tanganyika, Eastern Africa, and forms permanent socials groups composed of a single dominant breeding pair, and 1–20 male and female subordinate helpers (Taborsky 1984, 1985; Balshine et al. 2001; Heg et al. 2005). The breeding male is always the largest individual, and the breeding female is typically the second-largest individual (Wong & Balshine 2011a), while the nonreproductive helpers form a size-based hierarchy thought to reflect two sex-specific queues for breeding status (Balshine-Earn et al. 1998; Werner et al. 2003; Heg et al. 2004; Hamilton et al. 2005; Fitzpatrick et al. 2008; Mitchell et al. 2009).

To better understand intragroup dynamics in *N. pulcher*, we explored how social conflict influences the structure of social networks. Social conflict may be manifested in aggressive, submissive and/or avoidance behaviours (Balshine-Earn et al. 1998; Aureli & de Waal 2000; Werner et al. 2003; Hamilton et al. 2005; Reddon et al. 2012). Thus, we test five predictions related to social conflict and the structure of dominance and association networks.

(1) There have been widespread claims that *N. pulcher* groups form linear dominance hierarchies (Taborsky & Limberger 1981; Taborsky 1984, 1985; Wong & Balshine 2011a, b; Reddon et al. 2011a, b). However, we are unaware of any specific tests of hierarchy structure in this species. Based on these prior assertions, we predicted that *N. pulcher* dominance hierarchies would be linear, and we performed the first test of this prediction using a quantitative analysis of hierarchy linearity and asymmetry (i.e. directional consistency).

(2) In size-structured groups, conflict is predicted to be highest between individuals of similar size (Enquist et al. 1987; Jennions & Blackwell 1996; Hamilton et al. 2005), either because relative fighting ability is uncertain (Enquist & Leimar 1983) or because subordinates should challenge dominants more frequently if the difference in fighting ability is small (Cant & Johnstone 2000). Therefore, we tested the prediction that dyads with low size asymmetry would be involved in more frequent dominance interactions and would have fewer associations with one another.

(3) Only same-sex individuals are expected to compete for breeding positions. Therefore, we tested the prediction that dominance interactions would occur more frequently and associations would occur less often between same-sex group members.

(4) As the value of a social position rises exponentially with increasing rank, dominance interactions are theoretically expected to most frequently occur towards the top of the dominance hierarchy (Cant et al. 2006). Therefore, we tested this prediction by quantifying the rate of dominance interactions throughout the hierarchy, and explored whether high-ranking group members were involved in more dominance interactions than low-ranking members.

(5) Female *N. pulcher* queue for breeding positions within their natal groups while males more commonly disperse to another group prior to breeding (Stiver et al. 2004, 2006, 2007). We therefore predicted that females would place more value on establishing relationships with group members, and would consequently be more connected within the networks than males. Similarly, we predicted that breeders would be more connected than helpers, because they have a greater interest in the structure of their current group than do the subordinate helpers.

METHODS

Study Animals

Neolamprologus pulcher used in this study were adults from a breeding colony held at McMaster University, Hamilton, ON, Canada. The fish were descendants of breeding pairs caught in Lake Tanganyika, Zambia, and were housed in social groups consisting of a male and female dominant breeding pair with either three or four subordinate helpers of mixed sexes (mean group size $\pm$ SE $= 5.8 \pm 0.1$). This group size and composition is consistent with the structure and size range of wild *N. pulcher* social groups found in Lake Tanganyika (Balshine et al. 2001). The relative size of male and female helpers as well as the ratio of male to female helpers varied among the social groups, but there was always at least one male and one female helper, and there were always both high-ranking and low-ranking helpers within the groups. Each social group inhabited a 189-litre ($92 \times 41 \times 50$ cm) freshwater aquarium outfitted with a heater, thermometer, two filters, about 3 cm of crushed coral sand substrate, and two inverted terracotta flowerpot halves for use as shelters and spawning sites. Social groups were formed approximately 1 month prior to the start of behavioural observations (see below) and each social group had successfully reproduced at least once prior to the start of the study. Fish were fed ad libitum 6 days per week with commercial cichlid flake food.

Study Protocol

In total, 14 social groups were used in this experiment. All fish were captured, weighed, measured, sexed by external examination of their genital papillae, and given a unique fin clip 48 h prior to the first observation, so that each fish could be individually identified. Groups were observed for 15 min twice a week for 2 consecutive weeks, for a total of four observation periods and 60 min of observation per group. During each observation period, a pair of observers simultaneously watched each group from a distance of approximately 1.5 m. Fish were given 5 min to acclimate to the presence of observers prior to the onset of recording, and the fish did not appear disturbed by the presence of human observers. One observer scored associations among individuals, recording the individuals within a single body length of each other at the beginning of each minute. A single body length was chosen since this is the spatial distance that social interactions occur in *N. pulcher* and is a widely used spatial metric in fish behavioural studies. The second observer continuously recorded all dominance-related behaviours among all group members, based on a recent ethogram for this species (Sopinka et al. 2009). Specifically, this observer recorded all aggressive displays and behaviours (aggressive postures, puffed throats, head shakes, rams, chases and bites) and all submissive behaviours (submissive postures and submissive displays) that were both produced and received by each fish in the group.

Social Networks

Using this data, we built two social networks, one was based on dominance interactions, and the other was based on associations, for the individuals in each social group. In each network, the weight of connecting edges was determined by summing the number of interactions or associations for each dyad across the four observation periods (see above). Note that *N. pulcher* social networks were stable over the study time period, as networks built from the first two observation periods were highly positively correlated to networks built from the second two observation periods (Mantel tests followed by Fisher's omnibus test (Louv & Littell 1986; Haccou & Meelis 1992): dominance networks: mean $r_S = 0.55$, $f_{28} = 100.4$, $P < 0.0001$; association networks: mean $r_S = 0.46$, $f_{28} = 75.9$, $P < 0.0001$). The network of dominance interactions was created by combining the sociomatrix of aggressive interactions (a matrix in which columns and rows represent individuals, thus each cell in the matrix represents a dyad) with the transposed sociomatrix of submissive interactions (i.e. the actor and receiver are reversed), for each group. Dominance networks had no maximum edge weight, and could be either directed (when exploring dominance relationships as per prediction 1) or undirected (when we used the total number of dominance interactions as a measure of social conflict; as per predictions 2–4), depending on the analysis employed. The association network was undirected, and each dyad had a maximum edge weight of 60 (i.e. if the dyad was associated during every scan across the four observation periods). Four of the 56 subordinate helpers died during the study period and these individuals were removed from all networks. There were no self-loops in any networks (i.e. the diagonal in all sociomatrices was set to 0) and this feature was conserved during all randomization tests.

Network Analysis

Network analysis was performed in R version 2.14.1 (R Development Core Team 2012) and UCINET version 6 (Borgatti et al. 2006). During randomization tests, we held the total number of interactions or associations constant within each social group. When appropriate, we used Fisher's omnibus test (Louv & Littell 1986; Haccou & Meelis 1992) to combine *P* values obtained from each social group into a single value. We determined the direction of the strongest relationship among groups, and subtracted the contribution of groups with the opposite relationship from the combined test statistic, and the resultant overall *P* value (see also Croft et al. 2006). Networks in Figures 1a, b were created using the 'igraph' package in R (Csárdi & Nepusz 2006).

Dominance hierarchy structure

To test prediction 1, we examined the structure of dominance hierarchies using the triangle transitivity method recently developed by Shizuka & McDonald (2012). This measure of dominance hierarchy structure is equivalent to linearity (sensu Landau 1951; de Vries 1995) when all pairwise dominance relationships are known. However, it is advantageous in that it does not become biased when pairs of individuals have not interacted (see Klass & Cords 2011), and/or when group size varies (Shizuka & McDonald 2012). The directed dominance matrix was reduced to a binary dominant/subordinate matrix ($1 = \text{dominant}$, $0 = \text{subordinate}$) based on which individual in each dyad had a larger value in the dominance matrix (i.e. which individual had 'won' more dominance interactions). If a dyad had not interacted, both members were given a 0. Next, the proportion of transitive triangle motifs (t_{tri}) was determined for this binary network, using the 'statnet' package in R (Handcock et al. 2003). The statistical significance of t_{tri} was determined by comparing the empirical value of t_{tri} with values obtained from 2000 permutations of the dominant/subordinate matrix (see Shizuka & McDonald 2012 for details). We also calculated the global asymmetry in dominance interactions across all dyads in each network. In addition to the structural organization of the dominance hierarchy (i.e. linearity or transitivity), the degree to which dominant individuals are likely to win a contest over subordinate individuals is an important characteristic of dominance relationships (van Hooff & Wensing 1987; de Vries et al. 2006; Whitehead 2008). So, for each dyad, we determined the dominant and subordinate individual (as above). Then, we divided the number of interactions in which the dominant individual behaved as such (i.e. they gave aggression or received submission) by the total number of dominance interactions in the network. The resultant statistic ranges from 0.5 to 1, and describes the global likelihood that a dominant individual would be correctly identified given an observation of a single interaction. To test whether dominance interactions among *N. pulcher* were significantly more asymmetrical than random, we performed 2000 permutations (per social group) of the raw dominance interactions. Then, we compared the dominance asymmetry score for networks built from the randomized data with our empirical values.

Network-level analysis

We tested our predictions about patterns of social conflict (predictions 2–4) using undirected dominance networks and networks of association described above. We first determined whether there was nonrandom structure in our networks (i.e. if individuals preferentially interacted or associated with particular group members). Using R, we performed 2000 permutations (per social group) of the raw dominance interaction data and compared the standard deviations in the weights of all possible edges between our observed networks and networks built from our randomized data. A high standard deviation in edge weight in our empirical networks (relative to the randomized networks) would indicate that individuals preferentially interact or associate with certain partners. Next, we used the multiple regression quadratic assignment procedure (MRQAP) in UCINET (Krackhardt 1988; Borgatti et al. 2006; see also Wey & Blumstein 2010 for a similar analysis) to regress multiple independent matrices on an observed dependent matrix. In this analysis we used three independent matrices.

(1) To test whether patterns of conflict were related to size asymmetry, we created a sociomatrix for each social group in which each element (i.e. cell) was the standard length of the larger individual divided by the standard length of smaller individual. In this size asymmetry sociomatrix, larger values indicate dyads with large differences in body size.

(2) To test whether patterns of conflict were related to sex, we created a sex-similarity sociomatrix in which a dyad was given a 1 if both individuals were the same sex, or a 0 if they were of different sex.

(3) To determine whether conflict was related to social rank, we created a sociomatrix in which each cell was the sum of the size ranks of the dyad. In *N. pulcher*, body size is highly correlated with social rank (Taborsky 1984, 1985), and size hierarchies are maintained by strategic regulation of growth (Heg et al. 2004). Thus, body size is often used to infer rank in this species (e.g. Hamilton et al. 2005). In our analysis, the value for the cell connecting the largest to the second-largest individual would be 3 (size rank 1 + size rank 2), while the value connecting the fifth-largest to the sixth-largest individual would be 11 (size rank 5 + size rank 6). Thus, a negative effect size for this matrix would indicate that dominance interactions or associations occur most often between high-ranked individuals.

We performed separate analyses for each dominance matrix for each social group, using the double Dekker semipartialling method (Dekker et al. 2007) with 2000 permutations per analysis. We performed a similar MRQAP analysis on networks of associations, except in this case we used only two independent matrices; the size asymmetry matrix and the binary sex similarity matrix described above.

Nodal measures

To investigate differences among individuals in their role in social networks (prediction 5) we compared average nodal strength and eigenvector centrality between males and females, and between breeders and helpers. Node strength measures the total weight of all edges connected to a node (Whitehead 2008), while eigenvector centrality measures how well connected an individual is by considering both the direct connections to the focal node and also the connectedness of the focal node's neighbours (Bonacich 1987; Newman 2004). For each network, we calculated an average strength and eigenvector centrality for each class of individuals (i.e. males and females, breeders and helpers). To test whether there was a significant difference between the classes, we performed 2000 permutations of the data and compared the empirical connectivity values with those calculated from networks built using the randomized data.

Ethical Note

Fish were marked with a dorsal fin clip to allow for visual identification. Fin clipping does not adversely affect behaviour (Stiver et al. 2004) and the fish recovered from this procedure immediately. The methods for animal housing, handling and experimental protocols were assessed and approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol Number 10-11-71) and adhere to the guidelines of the Canadian Council for Animal Care and the ABS/ASAB.

RESULTS

Neolamprologus pulcher *Groups Form Linear Dominance Hierarchies (Prediction 1)*

The dominance hierarchies were highly linear (permutation test: mean $t_{\text{tri}} = 0.80$, $f_{28} = 50.68$, $P = 0.005$). Of 133 closed triangle motifs across all 14 social groups, 128 were transitive. We also found that dominance relationships in this species were highly asymmetrical (mean dominance asymmetry $= 0.90$, $f_{28} = 188.44$, $P < 0.001$).

Conflict Is Highest between Group Members of Similar Size (Prediction 2)

Both dominance interactions and associations occurred nonrandomly throughout *N. pulcher* social groups (permutation test: $f_{28} = 189.02$, $P < 0.001$ and $f_{28} = 183.26$, $P < 0.001$, respectively; Fig. 1). However, we found no significant effect of size asymmetry on patterns of dominance interactions or associations (MRQAP: $f_{28} = 1.82$, $P > 0.99$ and $f_{28} = 7.28$, $P > 0.99$, respectively).

Conflict Is Higher between Same-sex Individuals (Prediction 3)

We found no significant effect of sex on the observed patterns of dominance interactions or associations (MRQAP: $f_{28} = 3.75$, $P > 0.99$ and $f_{28} = 9.50$, $P > 0.99$, respectively).

Conflict Is Higher Near the Top of the Social Hierarchy (Prediction 4)

Dominance interactions were significantly more likely to occur between individuals at the top of the hierarchy (MRQAP, effect of rank: $f_{28} = 65.28$, $P < 0.001$; Fig. 2).

Females Are More Connected Than Males and Breeders Are More Connected Than Helpers (Prediction 5)

In dominance networks, breeders had significantly higher strength (permutation test: $f_{28} = 139.66$, $P < 0.001$) and eigenvector centrality ($f_{28} = 133.04$, $P < 0.001$) than did helpers (prediction 5: Fig. 3). There was no difference in strength or eigenvector centrality ($f_{28} = 14.12$, $P = 0.98$ and $f_{28} = 21.74$, $P = 0.79$, respectively) between males and females (Fig. 3). In association networks, breeders had significantly higher strength ($f_{28} = 121.12$, $P < 0.001$) and eigenvector centrality ($f_{28} = 97.21$, $P < 0.001$) than helpers (prediction 5: Fig. 3). Finally, there was no difference in strength or eigenvector centrality ($f_{28} = 6.63$, $P > 0.99$ and $f_{28} = 27.21$, $P = 0.51$, respectively), between males and females in association networks (Fig. 3).

DISCUSSION

In this study, we used social network theory to explore interaction patterns within groups of the cooperatively breeding cichlid, *N. pulcher*. Consistent with our predictions, *N. pulcher* hierarchies were highly linear, with highly asymmetrical and directionally consistent interactions between dyads. While neither dominance interactions nor patterns of associations were directly related to body size asymmetry or sex, we found that dominance interactions were not equally distributed across the network, but instead occurred most frequently at the top of the social hierarchy. Finally, breeders were more connected than helpers within the networks.

We found that *N. pulcher* social groups form highly transitive (and therefore linear) dominance hierarchies with large asymmetries in dyadic dominance interactions (i.e. dominance interactions were strongly directional within dyads). Such a pattern is expected when there is large variation in resource-holding potential among individuals, and dominance ranks should therefore be predetermined by differences in individual attributes (Chase & Seitz 2011). In *N. pulcher*, as in most fishes, resource-holding potential is strongly correlated with body size (Reddon et al. 2011b) and *N. pulcher* social groups are stratified according to body size (Taborsky 1984, 1985). Therefore, there should rarely be multiple individuals with similar resource-holding potential, and as observed, dominance hierarchies should be linear and highly asymmetrical.

Within *N. pulcher* social groups, certain pairs of individuals experienced greater social conflict relative to other dyads. In agreement with our prediction, social conflict was highest towards the top of the size hierarchy. When social groups take the form of reproductive queues, there should be conflict over social status because higher-ranking individuals are more likely to inherit a breeding position. While it is beneficial for all individuals to increase their social rank, the consequences of rank change are greatest for high-ranking individuals (Cant et al. 2006). Thus, high-ranking individuals ought to invest more heavily in deterrent displays towards subordinates and in aggressive tests of dominants compared with lower-ranked individuals. In support of this idea, the aggression levels of several species of social insects (e.g. *Ropalidia marginata*, Chandrashekara & Gadagkar 1992; *Dinoponera quadriceps*, Monnin & Peeters 1999; *Polistes dominulus*, Cant et al. 2006) have been experimentally shown to be influenced by rank, rather than vice versa. Furthermore, aggression rates increase with social rank in several social vertebrates (e.g. *Equus caballus*, Araba & Crowell-Davis 1994; *Pan troglodytes*, Muller & Wrangham 2004), although such tests rarely consider confounding variables such as sex or body size. In the current study, we provide strong support for the influence of social rank on intragroup conflict in *N. pulcher*, by showing a strong correlation between dyad rank and rates of

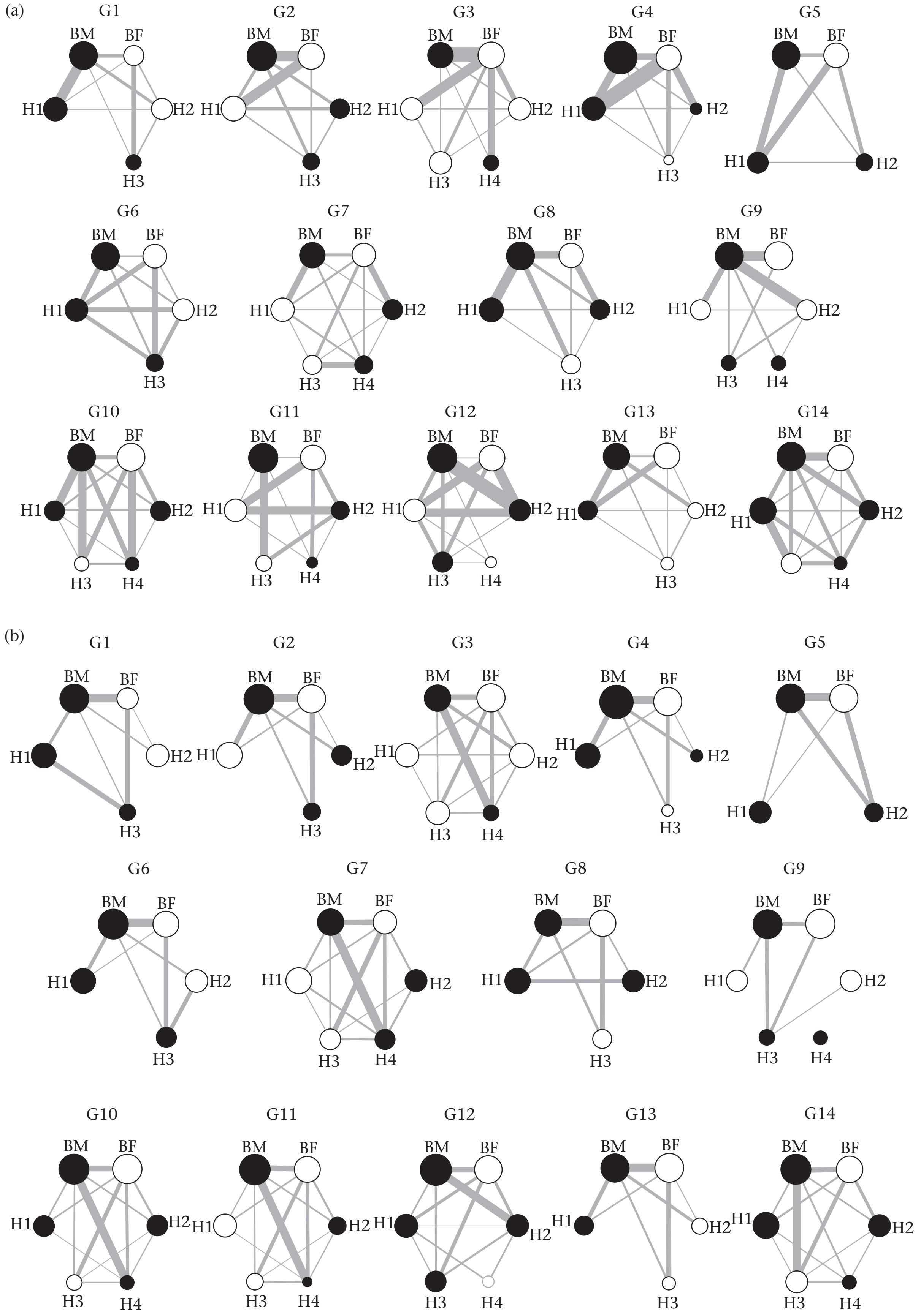

Figure 1. Networks of (a) dominance interactions and (b) associations in *Neolamprologus pulcher*. Nodes (circles) represent individual fish in each of 14 captive social groups; node labels indicate the breeding male (BM), breeding female (BF) and nonbreeding helpers in order of standard length (with H1 being the largest helper); node colour indicates sex (black = male, white = female); node size is scaled to individual body size. The thickness of connecting edges is scaled to the number of dominance interactions and the number of associations that occurred between each dyad across all observation periods and is comparable both within and among groups.

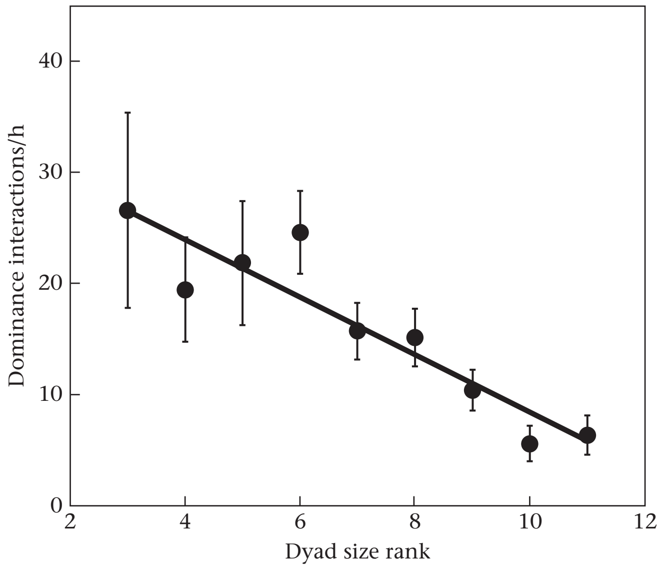

Figure 2. Mean ± SE dyadic dominance interaction rate as a function of the sum of the size rank of the members of the dyad (e.g. size rank 1 + size rank 2 = 3) in *Neolamprologus pulcher* groups. Values are pooled across all 14 social groups. The black line indicates a regression through the average values at each dyad size rank.

dominance interactions while controlling for several alternative hypotheses.

While higher-ranked individuals had more conflict with one another compared with lower-ranked individuals, neither sex nor size asymmetry were related to aggression. We had predicted increased conflict between same-sex and similarly sized individuals, since these individuals should pose the greatest threat to each other within the reproductive queue (Hamilton et al. 2005; Mitchell et al. 2009). When body size asymmetry is low, subordinates ought to challenge dominants more readily because they have a higher chance of being successful (Cant & Johnstone 2000). However, we found no relationship between sex or size asymmetry and the level of conflict. This may be because the relative value of winning a conflict is low for subordinates far down the reproductive queue. Thus, conflicts may be rare among low-ranking individuals, even when those individuals are the same sex and similar in size. As a result there may only be a weak (and in this study, nonsignificant) effect of sex and size asymmetry on the overall patterns of social conflict in a group. It is also likely that individual *N. pulcher* do not always have perfect knowledge of the sex of all other group members, especially among sexually suppressed subordinates, and thus sex-specific dominance hierarchies may simply not form. Finally, note that patterns of aggression are complex, and may be dependent on external ecological factors as well as the characteristics of the individuals involved (Reeve 2000). Furthermore, the current models used to predict patterns of aggression within social groups (i.e. reproductive skew models) consider only direct reproduction as the resource over which individuals compete, which is not appropriate for predicting aggression patterns among nonbreeding subordinates. We argue that models that incorporate future reproductive prospects (e.g. social rank) and resources not directly linked to reproduction (e.g. shelter) may be more appropriate and better predict patterns of conflict in *N. pulcher*.

In the current study, we observed that breeders were more connected than helpers in dominance and association networks. In addition to investing heavily in deterrent signals directed at large helpers to maintain their social status (see above), breeders may also be more connected than helpers if they use dominance interactions to police the behaviours of, and interactions among, helpers. Pay-to-stay models of cooperative breeding predict that breeders will punish helpers who provide insufficient help (Gaston

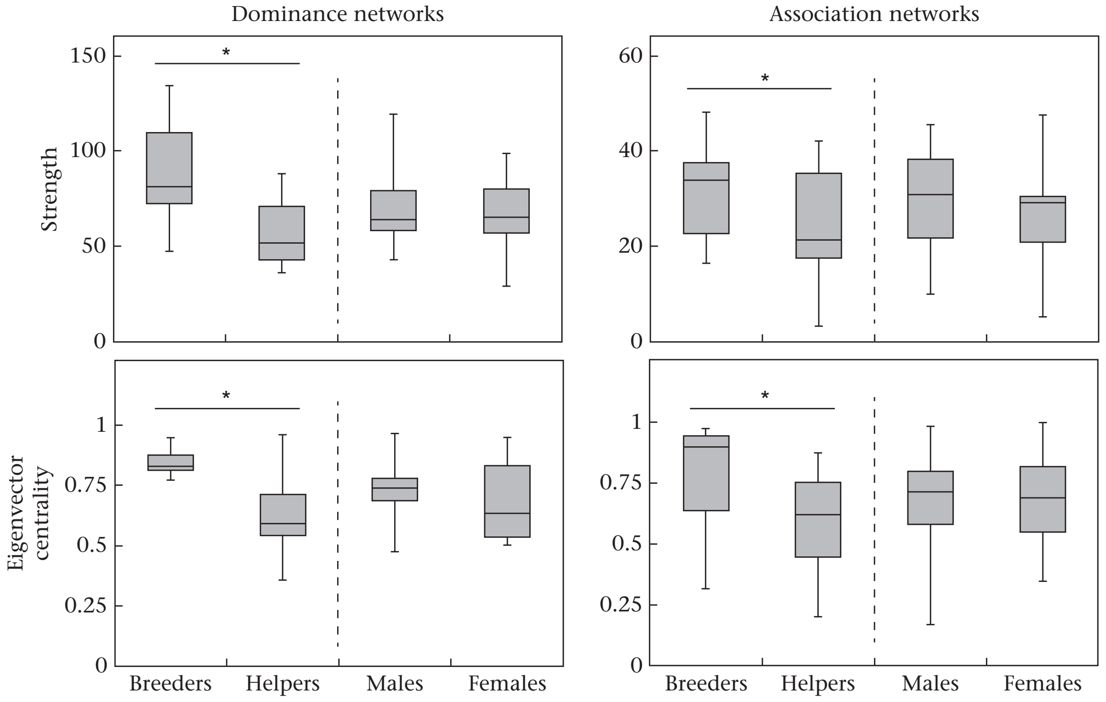

Figure 3. Connectivity in *Neolamprologus pulcher* social networks. Shown are the strength and eigenvector centrality of classes of individuals for networks of dominance interactions and association in 14 captive social groups. Box plots show the 75th percentile, median and 25th percentile, as well as the minimum and maximum values (whiskers). *$P < 0.05$.

1978; Kokko et al. 2002). The evidence for such dominant policing of subordinate helping in *N. pulcher* is weak (Wong & Balshine 2011a). However, it is possible that breeders do police helpers in terms of their shelter or space use. Furthermore, breeders may be more connected if they police interactions among helpers to increase group stability and/or productivity. Third-party policing is thought to be most common in societies with high interindividual variance in power, because policing by high-ranking individuals will be more effective and less costly in this situation (Flack et al. 2005). Size-structured hierarchies in social fish fit these criteria, and there were several observations of breeders interfering in helper–helper conflicts in this study (C. M. O'Connor & A. R. Reddon, personal observation) and in previous field studies (S. Balshine, personal observation). A further comprehensive study of policing in *N. pulcher* social groups is probably warranted.

Finally, we show that patterns of association between *N. pulcher* group members are nonrandom. These patterns were not explained by sex or size asymmetry of group members. One possible explanation is that both in captivity and in the wild, individual *N. pulcher* establish small subterritories within the larger group territory (Werner et al. 2003). Such segregation of space could lead to nonrandom association patterns, because individuals will predominantly associate with neighbours. Subterritoriality was widely observed in this study, however, it seems that the arrangement of subterritories was not dependent on the sex or body size of the individuals in neighbouring subterritories. While captivity will always constrain animals in their ability to move throughout their environment (Estévez & Christman 2006), the aquaria used in this study were a similar size to the median territory size reported for wild *N. pulcher* (Balshine et al. 2001). Thus, the patterns of association observed in this study are unlikely to have been generated by the captive environment alone and may be similar to those in wild populations.

In conclusion, this study is one of only a few to examine network structure in multiple replicate social groups of a given species. This approach facilitates making general conclusions about the social structure in this cooperatively breeding cichlid. We provide the first explicit analysis of dominance hierarchy structure in *N. pulcher*, and confirm the assumption that hierarchies are strongly linear and dominance interactions are directionally consistent. Furthermore, we provide evidence that while dominance interactions and associations occur nonrandomly, they are not related to body size asymmetry or sex. Thus, there is little support for sex-specific dominance hierarchies in *N. pulcher*. Our results do show, however, that there is increased social conflict at the top of the size hierarchy, which is consistent with theoretical predictions based on intensified conflict as the probability of inheriting a breeding position increases. Finally, we demonstrate that breeders are more connected than helpers, which suggests interesting avenues for future research on policing in this species. Taken together our results provide valuable information on the structure of social groups in a model cooperative breeding species. Future research examining how network structure influences reproductive success, growth, survival as well other aspects of group function (e.g. predator defence) will be valuable in gaining a more complete understanding of sociality in this intriguing fish.

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Appendix C: Dominance Network Structure Across Reproductive Contexts in the Cooperatively Breeding Cichlid Fish, *Neolamprologus pulcher*

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Dominance network structure across reproductive contexts in the cooperatively breeding cichlid fish *Neolamprologus pulcher*

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Abstract While a large number of studies have described animal social networks, we have a poor understanding of how these networks vary with ecological and social conditions. For example, reproductive periods are an important life-history stage that may involve changes in dominance relationships among individuals, yet no study to date has compared social networks of dominance interactions (i.e. dominance networks) across reproductive contexts. We first analyzed a long-term dataset on captive social groups of the cooperatively breeding cichlid *Neolamprologus pulcher*, and found that eviction events were significantly more common around reproduction than expected by chance. Next, we compared the structure of dominance networks during early parental care and non-reproductive periods, using one of the first applications of exponential random graph models in behavioral biology. Contrary to our predictions, we found that dominance networks showed few changes between early parental care and non-reproductive periods. We found no evidence that dominance interactions became more skewed towards larger individuals, became more frequent between similar-sized individuals, or became more biased towards a particular sex during brood care. However, we did find that there were relatively more dominance interactions between opposite-sex dyads in the early parental care period, which may be a by-product of increased sexual interactions during this time. This is the first study in behavioral ecology to compare social networks using exponential random graph modeling, and demonstrates a powerful analytical framework for future studies in the field [*Current Zoology* 61 (1): 45–54, 2015].

Keywords Social network, Aggression, Submissive, Hierarchy, Parental care, P* model

Dominance is an important type of social relationship that is characterized by consistency in the outcome of agonistic interactions between individuals (Drews, 1993). In many species, dominance is a key contributor to individual variation in resource acquisition, and can therefore influence a diverse array of fitness-linked outcomes, including growth (e.g. Brockmark and Johnsson, 2010), survival (e.g. Arcese and Smith, 1985), predation risk (e.g. Schneider, 1984), parasite exposure (e.g. Courchamp et al., 1998) and access to mating opportunities (e.g. Ellis, 1995). While dominance is fundamentally a relationship between a pair of individuals (Drews, 1993), there is a strong interest in understanding how dominance relationships are structured at the group level. In most cases, animals form orderly (e.g. linear) dominance hierarchies (Shizuka and McDonald 2012), and there are predictable associations between social rank and traits related to size and strength (Francis, 1988; Mesterton-Gibbons and Dugatkin, 1995).

While past studies on dominance hierarchies have revealed important causes and consequences of social rank, there is a dearth of knowledge in understanding patterns of dominance interactions *per se*. In order to explicitly study variation in dominance interactions within social groups, several authors have recommended the use of social network analysis (Shizuka and McDonald, 2012; McDonald and Shizuka, 2012; Pinter-Wollman et al., 2014). In this analytical framework, individuals are represented as nodes, while the dominance interactions that occur among individuals are represented as the edges in the network. Analyzing the structure of these 'dominance networks' provides a complementary approach to the traditional study of dominance hierarchies, in that it allows for tests of complex factors (e.g. indi-

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vidual and dyadic attributes, higher-order processes) that contribute to variation in dominance interactions among dyads, rather than focusing on the association between individual traits and social rank. For example, Dey and Quinn (2014) recently analyzed dominance networks in a cooperatively breeding bird and found that males and females differ in their tendency to use physical aggression and submissive displays, and also that interactions were more frequent among same-sex dyads than among opposite-sexed dyads. One area in which social network analysis may be particularly useful is in understanding how dominance relationships are influenced by social or ecological context (e.g. population density, resource abundance) as these analytical techniques can be used to evaluate changes in the quantity and quality of dominance relationships even when individuals do not change in social rank.

Dominance relationships are expected to change across life-history stages, since the costs and benefits of social status will also vary across theses stages. In many group-living species, reproduction is partially or completely monopolized by socially dominant individuals. As a result, reproductive periods are an important life-history stage in which individuals might challenge others to increase their social rank and access reproductive opportunities. Similarly, dominant individuals may be selected to suppress subordinate reproductive attempts, and such suppression is often done through physical aggression (e.g. Young et al., 2006). In addition, changes in dominance relationships during reproductive periods could be a by-product of the physiological changes that are required for reproduction. For example, many of the hormones that are involved in reproductive physiology have pleiotropic effects, including effects on dominance-related behaviours (Lincoln et al., 1972). While several studies have described differences in aggressive and submissive behaviours during reproduction (e.g. Garrett and Campbell, 1980; Southern and Southern, 1982), no study to our knowledge has evaluated the change in patterns of dominance relationships across reproductive contexts using a social network approach.

In this study, we analyzed patterns of dominance across reproductive contexts, in the cooperatively breeding cichlid *Neolamprologus pulcher*. This fish is endemic to Lake Tanganyika in Eastern Africa, where it forms permanent social groups of 2–20 individuals. Within these social groups, reproduction is highly skewed towards a dominant breeding pair, which is composed of the largest male and female fish (Wong and Balshine, 2011a). Other group members form a size-based queue for the dominant breeding positions, cooperate in raising the group's offspring (Balshine et al., 2001; Heg et al., 2005; Wong and Balshine, 2011a), and may also achieve some reproductive success (Heg and Hamilton, 2008; Heg et al., 2009; Hellmann et al., *in review*). In a previous study, we demonstrated that *N. pulcher* groups have highly orderly dominance networks (i.e. high triangle transitivity; Shizuka and McDonald, 2012) and that interactions within dyads were directionally consistent (Dey et al., 2013). Additionally, we found that dominance interactions were not equally distributed throughout the network, but instead occurred more often among high-ranking individuals (Dey et al., 2013).

To quantify how dominance interactions change with reproductive context, we first analyzed a long-term dataset on captive *N. pulcher* social groups, and tested whether subordinate group members were more likely to be evicted from the group around reproductive events compared to non-reproductive periods. Theory suggests that dominants should evict subordinates who do not contribute sufficiently to the group to offset their residency costs (i.e. 'pay-to-stay', Gaston, 1978), or those subordinates that compete for reproduction with dominant individuals (Johnstone and Cant, 1999). Empirical evidence shows that subordinates in cooperative ing groups are often evicted as punishment for reproducing, or to prevent reproduction in individuals that are likely to reproduce (e.g. Clutton-Brock et al., 1998, Cant et al., 2010). As a result, evictions are predicted to occur most frequently during reproductive periods, and may represent an extreme endpoint of increases in gression that arise from increased intra-group conflict during reproduction. A recent study on *N. pulcher* onstrated that when subordinates were prevented from providing help, they were more likely to receive aggression and be evicted from their group, although this effect was only evident in small groups (Fischer et al., 2014). However, no study on this species has tested whether the timing of evictions is related to reproduction, which if true, would suggest that reproductive conflict is a primary driver of eviction in this species.

In addition to changes in the likelihood of eviction, conflict during reproductive periods could influence changes in dominance networks. To test this hypothesis, we explored how patterns of dominance interactions change between the early parental care period (just after eggs had been laid) and non-reproductive periods. Recent genetic evidence from wild *N. pulcher* suggests that subordinate male and female group members commonly reproduce (Hellman et al., *in review*). In many species where socially subordinate individuals attempt to gain some parentage, they reproduce just after dominant individuals to avoid detection (and subsequent punishment) or infanticide (e.g. Poikonen et al., 2008; Hodge et al., 2011). Indeed, a study in captive *N. pulcher* social groups showed that when subordinate females reproduce, they also do so shortly after the dominant female in their group has laid (Heg et al., 2009). As a result, conflict over subordinate reproduction during the early parental care period could contribute to changes in the structure of dominance networks. This conflict could be dependent on individual body size and sex, since subordinate reproduction (at least for females) is dependent on body size (Heg et al., 2009), and subordinate reproduction will primarily influence the fitness of same-sex group members. Additionally, changes in dominance network structure during the early parental care period could be due to conflict over the provision of parental and alloparental care. There is evidence that 'lazy' *N. pulcher* receive aggressive punishment from other group members, and increase their helpfulness after enforced idleness (Fischer et al., 2014). Such conflict over cooperative behaviour may be most acute during early parental care periods since eggs require frequent tending to develop. We tested for changes in dominance network structure using exponential random graph models (described below), and specifically focused on how sex and body size differentially influenced the structure of networks during each time period.

1 Materials and Methods

1.1 Methods

1.1.1 Study animals and housing conditions

This study was conducted on laboratory-reared *N. pulcher,* housed at McMaster University, that were descendants of wild caught fish from Lake Tanganyika, Zambia. Each social group was housed in a 189-litre aquarium lined with 3 cm of coral sand substrate that included two inverted terracotta flowerpot halves and six black PVC tubes for use as shelters and spawning sites. Water temperature was maintained at 26 ± 2°C and the housing facility was kept on a 13:11 hour light: dark cycle. All fish were fed commercial cichlid flakes *ad libitum*, six days a week. The methods for animal housing, handling, and study protocols (described below) were assessed and approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol Number 10-11-71) and adhered to the guidelines of the Canadian Council for Animal Care.

1.1.2 Evictions and reproduction

Long-term monitoring data on laboratory social groups were used to test for a temporal correlation between reproductive and eviction events. We considered records over a six year period (January 1, 2007 to December 31, 2012) from 14 aquaria that were continuously occupied. Each aquarium contained a group consisting of a breeder male and female, as well as a variable number of subordinate helpers of each sex. The records for each group included the dates of reproductive events (indicated by eggs in the brood chamber or newly emerged fry) and of eviction events (i.e. a fish being aggressively displaced to the upper part of the aquaria). Fish that were 'evicted' were subsequently removed from the aquaria to avoid further physical aggression. As breeding individuals senesced, and juveniles matured and ascended to breeding positions, the entire social group was occasionally replaced to avoid inbreeding and to ensure that each tank consistently contained both a breeder-sized male and female.

1.1.3 Behavioural observations

To test our predictions related to differential social network structure during the early parental care and non-reproductive periods, we selected 14 social groups for behavioral observations. Social groups selected for this study contained 4 to 8 individuals (mean group size ± *SD* = 5.2 ± 1.2). On August 16, 2013, all fish from these 14 social groups were weighed, measured (standard length), and sexed by examination of their genital papillae. Additionally, each fish was given a unique elastomer tag and/or dorsal fin clip to facilitate individual identification. Neither form of marking had apparent affects on individual behaviour (see also Stiver et al., 2004), and the fish recovered from the procedure immediately. Fish were also assigned a size rank, based on their relative size (in standard length, ties were broken by differences in body mass) within their social group (with rank = 1 indicating the largest individual). In *N. pulcher* groups, dominance rank is highly dependent on body size (Taborsky, 1984, 1985; Wong and Balshine, 2011a).

Three days following fish marking, we began carefully monitoring each group for reproduction by checking possible spawning locations for eggs on a daily basis. After spawning, *N. pulcher* provide intense parental care in the form of brood care (i.e. aerating eggs, micronipping) and defense, while the eggs and larvae are still attached to the breeding substrate (0–7 days after spawning; Taborsky, 1984). When eggs were first detected in each group, we performed a behavioral observation (see below for details) on that group the following day. A second behavioral observation was performed 24 hours after the first observation, such that for each social group we performed two behavioral observations during the early parental care period (i.e. 0–3 days after spawning, and always during the egg stage). We performed two additional behavioral observations during the non-reproductive period. These occurred on the 14th and 15th day following the first behavioral observation (i.e. 14–17 days after spawning). At this stage, the young are free swimming, have absorbed their yolk sac and are obtaining their own food independently. While adult *N. pulcher* will defend their territory year-round (and indirectly defend any young in the territory), there is no direct parental care occurring at this stage.

All behavioral observations were conducted between 13: 00 and 16: 00 h by the same observer (Q.Y.J.T.). The observer sat approximately 1.5 m from the focal aquarium, and allowed the fish to acclimate for 5 minutes prior to the start of each observation period. The observer then recorded all dominance-related interactions that occurred among group members for 15 minutes. These behaviours were grouped into two main categories: aggressive interactions (i.e. aggressive posture, frontal display, chase, ram, bite, mouth fights) and submissive interactions (submissive posture, submissive display, displacement, flee), based on a recent ethogram for this species (Sopinka et al., 2009; Hick et al., 2014). For each interaction, the observer also recorded the identification of both the actor and the receiver.

1.2 Statistical analysis

All analyses were performed in the R statistical package, version 3.0.1 (R Core Team 2013). We used a permutation approach to test for a temporal correlation between reproductive events and eviction events. For each eviction event in each aquarium (n = 582 events), we determined the number of days to the nearest reproductive event (n = 394) in that aquarium. We then summed these difference values across all eviction events in all aquaria, to generate an observed test statistic representing the total difference in time between eviction and the nearest reproductive event. We compared this test statistic to similar values generated in 1,000 permuted data sets. For each permutation, we randomized when the eviction events occurred in each aquarium by randomly sampling the dates in the study period (i.e. January 1, 2007 to December 31, 2012) without replacement, and conserving the number of evictions that occurred in each aquarium. Then, we calculated the total difference in the time between these randomized eviction events and the nearest reproductive events, for each permuted dataset. The distribution of values generated from the 1,000 permutations was compared to the observed test statistic, to generate a one-tailed P-value related to whether the observed eviction events were more likely to occur near reproductive events than expected by random. It is possible that our data set is biased towards under recording reproductive events, which are more difficult to observe than evictions and occasionally go undetected within our laboratory (e.g. if eggs are laid in a an atypical spot and are not detected). However, because our analysis considers the closest reproductive event to each eviction event, any missing reproductive events should increase the test statistic and therefore make our analysis more conservative.

Our analysis of dominance network structure was conducted using the *statnet* (Handcock et al., 2008; Handcock et al., 2014a), *ergm* (Hunter et al., 2008; Handcock et al., 2014b) and *ergm.count* (Krivitsky, 2013) packages in R (R Core Team, 2013). For each social group, we built a weighted dominance network for the early parental care period and for the non-reproductive period. Given that this study represents an exploratory analysis of how network structure varies across reproductive contexts, and that directional networks based on aggressive interactions were highly correlated with transposed networks based on submissive interactions (i.e. the actor and receiver were flipped) (Mantel test: 5000 permutations, r_s = 0.34, P = 0.0004 and r_s = 0.43, P = 0.0002 for the early parental care and non-reproductive period respectively), we chose to combine all aggressive and submissive interactions into a single, undirected dominance network for each time period. While analyzing directed networks could have allowed us to test more specific hypotheses, this study focused on broad-scale changes in social conflict between time periods. Therefore, we analyzed undirected networks similar to those previously used to explore social conflict in this species (Dey et al., 2013). The weight of the edges in these networks was the total number of dominance interactions that occurred between the dyad, pooled across the two behavioral observations for each time period. Separate networks generated for each of the two observation periods within each time period were highly correlated (Mantel test: 5000 permutations, r_s = 0.79, P = 0.0002 and r_s = 0.85, P = 0.0002 for the early parental care and non-reproductive period respectively), suggesting that the networks were stable across observations and that our sampling period provided a robust estimate of true network structure. In one social group a 'budding' event occurred, where a subordinate female established a distinct territory within the aquarium and laid her own clutch 1 week after the dominant breeding female had laid (see also Limberger, 1983). This group was excluded from all analyses, and thus the resultant analyses were conducted on the remaining 13 social groups.

We analyzed network structure using exponential random graph models (ERGMs) (Wasserman and Pattison, 1996; Snijders et al., 2006). Similar to logistic regression, these models predict the presence (or weight) of edges based on various independent variables. Because ERGMs can simultaneously consider multiple independent variables, they allow for powerful tests of the factors that contribute to network structure. For this reason they have been widely used in the study of human social networks (Lusher et al., 2012), although their application to non-human animal networks has been restricted to only three previous studies that we are aware of (Ilany et al., 2013; Dey and Quinn, 2014; Edelman and McDonald, 2014). As a first step in exploring the changes in network structure between early parental care and non-reproductive periods, we created separate models for each of these time periods. In each model, we used a supernetwork composed of all 13 social groups as the observed (i.e. response) network and restricted the edges in our models to only occur within groups to retain this structure. We considered 5 independent variables in each model: (1) the 'sum' term, which is similar to an intercept in linear modeling, (2) individual effect of sex, which tests whether males or females are more likely to be involved in dominance interactions, (3) individual effect of size rank, which tests whether an individual's relative size influences their likelihood to be involved in dominance interactions, (4) sexual homophily, which tests whether there is an increased likelihood of interactions between same-sex dyads and (5) dyadic effect of the difference in log (standard length), which tests whether interactions are more likely to occur among dyads that have similar body size.

In addition to considering separate models of the early parental care and non-reproductive periods (above), we also examined the factors that contribute to changes in network structure by performing a similar analysis on a 'difference network'. This difference network was composed of the same set of nodes as the supernetworks above, but each edge weight was the difference in the number of interactions between the early parental care and non-reproductive periods. To create a graph where all edge weights were non-negative numbers, we then added the absolute value of the minimum edge weight (i.e. -29) to all within-group edge weights. The resulting graph ranged in edge weight from 0 to 59, where low edge weights were indicative of fewer interactions during the early parental care period (relative to the non-reproductive period), and high edge weights were indicative of more interactions during the early parental care period. Again, the model restricted ties to only occur within groups, and was fitted with the same 5 independent terms described above. In this case however, the model estimates indicated the effect that each variable had on the change in dominance interactions between the two time periods. All three models were fitted according to the recommendations in Goodreau et al. (2009), Krivitsky (2012) and Lusher et al. (2012). Models required the use of a Markov-chain Monte Carlo (MCMC) estimation technique to approximate the maximum likelihood (Lusher et al., 2012) and we specified a chain length of ten million, a sampling interval of five thousand and a burn-in of fifty thousand proposals. We used Poisson reference graphs for each model. The difference network is based on differences in counts, which are known to be Skellam distributed (Skellam, 1946). However Skellam reference graphs are not implemented in *ergm. count* and the Poisson model fit the data well. Additionally, adjusting the reference graph to a Conway-Maxwell-Poisson distribution did not improve the model fit (Krivitsky, 2012). Visual analysis of MCMC sample statistics, as well as networks simulated from the fitted models, did not show any evidence of degeneracy. Additionally, models were checked for goodness of fit by examining the distributions of nodal strength (i.e. weighted degree) from 100 simulated networks from each model, and comparing those distributions to the observed networks (see also Goodreau et al., 2009).

2 Results

Eviction events in *N. pulcher* social groups were significantly more likely to occur near reproduction than expected by chance (permutation test: mean difference in days between evictions and nearest reproductive event = 40.2, mean difference in permuted data sets = 45.8, *P* = 0.003, Fig. 1). Evictions were most likely to occur 1 day prior to when reproduction was recorded (i.e. median = -1), although there was a large degree of variation (Fig. 1: 25th percentile = -27 days, 75th percentile = +18 days).

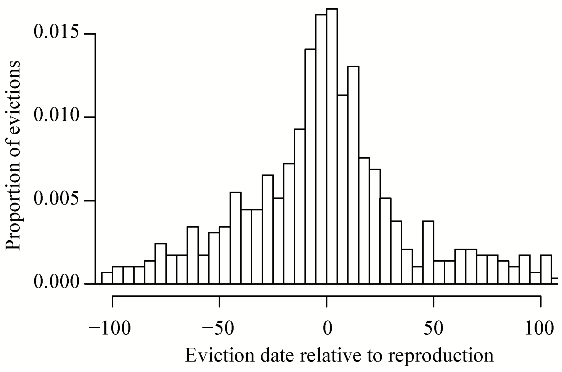

Fig. 1 Histogram of the date of eviction events (n = 582 events) relative to the nearest reproductive event pooled across 14 *N. pulcher* social groups

Evictions were more likely to occur near reproductive events than expected by chance (see text for details).

Separate ERGMs on the early parental care and non-reproductive periods networks showed similar estimates for all independent variables (Table 1, Fig. 2). Both models showed that dominance interactions were more likely to involve high-ranking individuals (negative effect of rank: Table 1) and were more likely to occur among dyads with a small difference in body size (negative effect of size difference: Table 1). Neither model demonstrated a difference in dominance interactions depending on individual sex (non-significant effect of sex: Table 1), however there was an increased likelihood for dominance interactions to occur among opposite-sex dyads relative to same-sex dyads (negative effect of sexual homophily: Table 1).

The difference network (Fig. 3) demonstrated limited changes in network structure between early parental care and non-reproductive periods. We found no significant effect of sex, rank or size difference on the change in dominance interactions (Table 2). We did find a significant, negative effect of sexual homophily in this network (Table 2; effect of homophily), suggesting that dominance interactions became less sexually homophilic during the early parental care period.

3 Discussion

Patterns of behavioral interactions among members of a social group can be influenced by a variety of social and ecological factors. In this study, we explored how patterns of dominance interactions vary with reproductive context in a highly social, cooperatively breeding fish. We found that aggressive eviction events are more likely to occur near reproduction than expected by chance, suggesting that reproductive conflict is an important contributor to eviction in this species. However, the structure of dominance networks showed relatively little change between the early parental care and non-reproductive periods. Dominance interactions did not become more concentrated among individuals of large or small body size, nor did they differ according to the size difference among individuals. Additionally, there was no change in the relative frequency of dominance interactions involving each sex. We did observe a significant decrease in sexual homophily (i.e. relatively more interactions between opposite-sexed dyads) during the early parental care period, however this finding was opposite our prediction of increased reproductive conflict during this life-history stage. Taken together, these results suggest that dominance networks in *N. pulcher* may be relatively stable across reproductive contexts.

In a previous study (Dey et al., 2013), we also analyzed the structure of dominance networks in this species. Consistent with the current study, and with studies on social insects (Chandrashekara and Gadagkar, 1992; Monnin and Peeters, 1999; Cant et al., 2006), our previous study also found that dominance interactions were more likely to occur among high-ranking individuals (Dey et al., 2013). In high reproductive skew societies (such as *N. pulcher*) the value of social position rises exponentially with increasing rank. As a result, the cost of changing rank is higher for higher-ranked individuals,

Table 1 Exponential random graph model results for *N. pulcher* dominance networks during reproductive and non-reproductive periods

Model term	Reproductive period			Non-reproductive period		
	Estimate	*SE*	*P* - value	Estimate	*SE*	*P* - value
Sum	3.29	0.10	< 0.0001	3.42	0.10	< 0.0001
Sex [male]	-0.006	0.06	0.92	0.02	0.05	0.64
Rank	-0.15	0.01	< 0.0001	-0.19	0.01	< 0.0001
Sexual homophily	-0.48	0.07	< 0.0001	-0.17	0.06	0.006
Size difference	-0.60	0.16	0.0001	-0.46	0.15	0.002

Significant *P*-values are shown in italics.

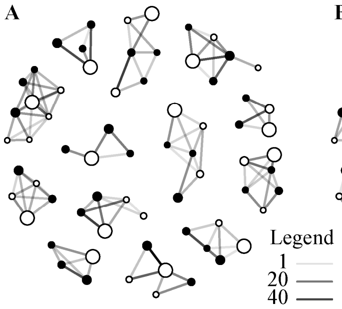

Fig. 2 Dominance network structure of *N. pulcher* social groups during early parental care (A) and non-reproductive periods (B)

Node orientation was determined using the Fruchterman-Reingold algorithm and shows individuals clustering in 13 social groups. Node size was scaled to social rank (with largest nodes indicating highest ranking individuals), while node colour indicates sex (black = female, white = male). Edge colour indicates the number of dominance interactions that occurred between each dyad, with darker lines indicating more interactions (see legend).

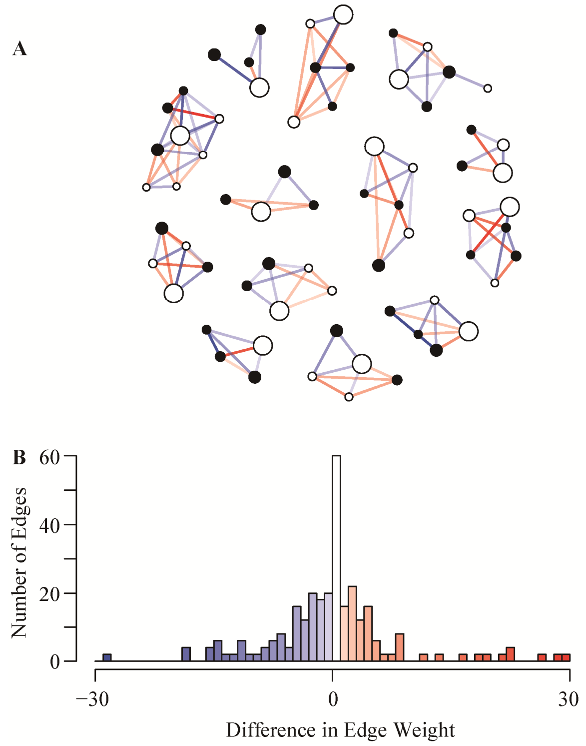

Fig. 3 (A) Social network of the difference in dominance interactions between early parental care and non-reproductive periods. Edge colour (red = more interactions during early parental care, blue = more interactions during the non-reproductive period) and darkness (darker lines = greater disparity between reproductive and non-reproductive period) indicate the differences in number of interactions. Node orientation is identical to that in Fig. 2. (B) Density histogram of edge weight from this network, excluding structural zeros (i.e. inter-group edge weights). Bar colour matches the edge colour from panel A.

Table 2 Exponential random graph model results for the change in dominance interactions between reproductive and non-reproductive periods

Model term	Estimate	*SE*	*P*-value
Sum	3.32	0.05	< 0.0001
Sex [male]	-0.01	0.02	0.64
Rank	0.01	0.006	0.10
Sexual homophily	-0.07	0.03	0.03
Size difference	-0.002	0.07	0.97

Positive estimates indicate an increase in the influence of the variable during the reproductive period, relative to the non-reproductive period. Significant *P*-values are shown in italics.

and establishing dominance relationships may be more important for these individuals (see also Wong and Balshine, 2011b).

The current study, which used a new statistical technique (as well as new behavioral data), revealed additional structure in *N. pulcher* dominance networks that was not observed in our previous study. We found that dominance interactions are more likely to occur among similar-sized individuals, even after controlling for each individual's size rank. This finding is consistent with theoretical predictions about conflict in size-structured groups, since relative fighting ability may be uncertain in similar-sized dyads (Enquist and Leimar, 1983), and because subordinates should challenge dominants more frequently if the difference in fighting ability is small (Cant and Johnstone, 2000). Indeed, Reddon et al. (2011) found that staged contests between *N. pulcher* were almost always won by the larger fish if the size asymmetry was greater than 5%, but when contestants had a size asymmetry of less than 5% contest outcome was not predicted by relative size. A previous experimental study on *N. pulcher* also showed that a small size difference between the breeder male and the largest male helper led to increased conflict, although this conflict was displayed as increased submissive displays rather than increased aggression (Hamilton et al., 2005, see also Heg et al., 2004). Interestingly, there is good evidence that relative size does not have a strong influence on conflict among females (Hamilton and Heg, 2008; Heg, 2010), and so there may be a complex interaction between sex and relative size on patterns of dominance interactions.

In addition to a strong effect of relative size, we also found that dominance interactions were more likely to occur among opposite-sexed pairs. This finding was opposite our predictions, since only same-sex individuals need to compete for breeding positions. However, frequent intersexual dominance interactions during the early parental care period may be a by-product of intersexual interactions in general (e.g. for courtship and reproduction). This does not explain why intersexual dominance interactions are also relatively common during non-reproductive periods, and further study will be needed to understand this phenomenon.

Although we found that eviction events were more likely to occur close to spawning events, our network analysis did not reveal major changes in dominance networks between the early parental care and non-reproductive periods. It may be the case that changes in dominance interactions during reproduction only occur in certain situations (e.g. in unstable groups where dominance hierarchies are not fully established), but that these changes are manifested in a dramatic increase in aggression and eviction. In stable social groups (including all 13 groups considered in this study) there may be few changes in dominance network structure across reproductive contexts, especially if subordinates do not directly compete for dominant breeding positions and if subordinate reproduction does not significantly decrease the reproductive success of dominants. Additionally, the apparent discrepancy between eviction rates and dominance network structure in our study might be the result of the timing of our behavioral observations. Dominance interactions may show the largest changes just prior to reproduction when changes in dominance rank could lead to changes in reproductive success. This idea is consistent with our findings from our long-term data set, which suggest that eviction events are most likely to occur 1 day prior to reproduction. Unfortunately, there are no obvious reliable cues of impending reproduction in *N. pulcher* that would have allowed us to perform our observations prior to spawning. It is possible that dominance networks may have quickly returned to non-reproductive baselines after spawning and that we may have missed a transient change in dominance networks structure due to our observation timing. As a result, future studies that are able to observe *N. pulcher* dominance interactions just prior to reproduction would be highly valuable, although they may be difficult to conduct.

There may also be differences in dominance networks between wild and captive *N. pulcher* that could influence the findings in this study. Although our captive groups simulated the natural composition and territory size of wild *N. pulcher* groups (Balshine et al., 2001), the captive environment lacks much of the complexity found in nature. In particular, captive subordinate *N. pulcher* do not have any option to disperse to another group to breed, and such outside options are key theoretical factors that influence aggressive interactions among members of social groups (Cant and Johnstone, 2009; Nonacs and Hager, 2011). It may be the case that captive *N. pulcher* are more reluctant to challenge dominants for breeding positions during reproductive periods, because they cannot leave the group if they fail to usurp. Alternatively, captive subordinates may be more likely to challenge dominants within their group because it is their only chance to achieve breeding status. Since it is difficult to predict which aspects of network structure are influenced by the captive environment, empirical studies that directly compare social networks between captive and wild *N. pulcher* would be extremely valuable.

By performing behavioral observations on replicate social groups we provide a robust comparison of dominance network structure during two life-history stages in a well studied cooperatively breeding fish species. Our study revealed novel factors influencing dominance network structure in *N. pulcher*, and suggests that dominance networks are relatively stable across reproductive contexts, at least in captive social groups. We also demonstrate the utility of exponential random graph models for testing multivariate hypotheses in behavioral ecology. This analytical technique that has been used in only a small number of studies on animal social networks, but has widespread applicability in this field and will likely increase in popularity in the future.

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Appendix D: Supplementary Material for Chapter 2

Electronic Supplementary Material

Implementation of exponential random graph models

ERGMs were implemented in the ergm (Handcock et al. 2014b) and ergm.count (Krivitsky 2013) packages. Because we were interested in interactions within social groups (and only recorded interactions among members of the same group), we restricted interactions in our ERGMs to occur within social groups using the 'offset' and 'nodemix' arguments. Both of our empirical networks were directed, with ties drawn from the actor (i.e. the subordinate displayer or physical aggressor) to the receiver. The weight of ties in our empirical networks was the total number of subordinate displays or physical aggressive interactions pooled across the 10 observation periods, and we specified a Poisson reference graph in both our models.

In both of our ERGMs we used the same set of 9 terms (i.e. predictor variables) to test which factors influence dominance network structure in pukeko. These terms were specified a priori according to our predictions outlined in the introduction. The terms, along with the statnet code used, were as follows: (1) the 'sum' term, which is a statistic representing the sum of the weight of all ties across the network (analogous to an intercept term in a regression model), (2) the 'nonzero' term, which represents the number of ties with a value greater than zero. A negative estimate for this term indicates and accounts for zero-inflation in the network (i.e. the tendency for empirical networks to be sparse), (3) actor effect of sex [i.e., nodeofactor (“Sex”, base = 1)], which tests whether one sex is more likely to initiate dominance interactions, (4) differential sexual homophily [i.e., nodematch (“Sex”, diff = TRUE)], which tests whether interactions are more likely to occur between same-sex dyads and estimates different values for male-male dyads compared to female-female dyads, (5) and (6) actor and receiver effects of shield size [i.e., nodeicov ("Shield Size") + nodeocov ("Shield Size")], which tests whether frontal shield size influences the probability of initiating or receiving dominance interactions respectively. We used frontal shield width (mean = 24.6 mm, range = 13.6 – 30.8 mm) as our measure of shield size, as it strongly correlates with total area (R2 = 0.87, Dey et al. 2014) and is a highly repeatable field measurement. (7) Dyadic difference in frontal shield size effect [i.e., absdiff (“Rank”)], which tests whether the magnitude of the difference in shield size between members of a dyad influences the likelihood of interaction. We also considered two aspects of structural dependence between ties, (8) transitivity, [i.e., transitiveweights (twopath = ”geomean”, combine = ”sum”, affect = ”min”)], which tests whether individuals tend to form closed, transitive triads if two-path motifs are present and, (9) reciprocity [i.e. mutual (form = “nabsdiff”)], which tests whether the weight of a tie from one individual to another influences the weight of the reciprocal tie. Models were fitted according to the recommendations in Goodreau et al. (2009), Krivitsky (2012) and Lusher et al. (2013). Both models were checked for degeneracy and goodness of fit using the mcmc.diagnostics function in statnet.

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Appendix E: Supplementary Material for Chapter 5

(a) Spectrophotometry

Because birds can see into UV wavelengths of light, invisible to humans, we tested whether the paint manipulations would appear similar to the red shields and the black plumage surrounding the shields respectively by collecting UV-VIS reflectance values of the dried paint on an actual pukeko shield and comparing them to reflectance values of the shield and plumage. We used an Ocean Optics (Dunedin, FL, USA) USB2000 spectrophotometer with a PX-2 pulsed xenon light source to collect reflectance for wavelengths between 300 and 700 nm (integration time, 100ms; 5 readings averaged per recording; boxcar 3). The red and black paints exhibited very similar reflectance values to the shield and black plumage respectively (Figure S1), indicating that our manipulations would appear natural to pukeko.

(b) Further Analysis Methodology

We examined the relationship between social status and frontal shield size using David's scores [1]. This dominance index incorporates both the direct dominance interactions involving the focal individual and also the dominance status of its opponents. Thus, individuals receive high scores if they are dominant over other individuals that also have high dominance scores. Here, we calculated David's scores for each group based on the pooled dominance interactions across the 10 observation periods. Because variance in David's scores increases with group size, we normalized the David's scores by the procedure outlined in [2]. We built two linear mixed-effect models (using the nlme package in R [3]) to evaluate the

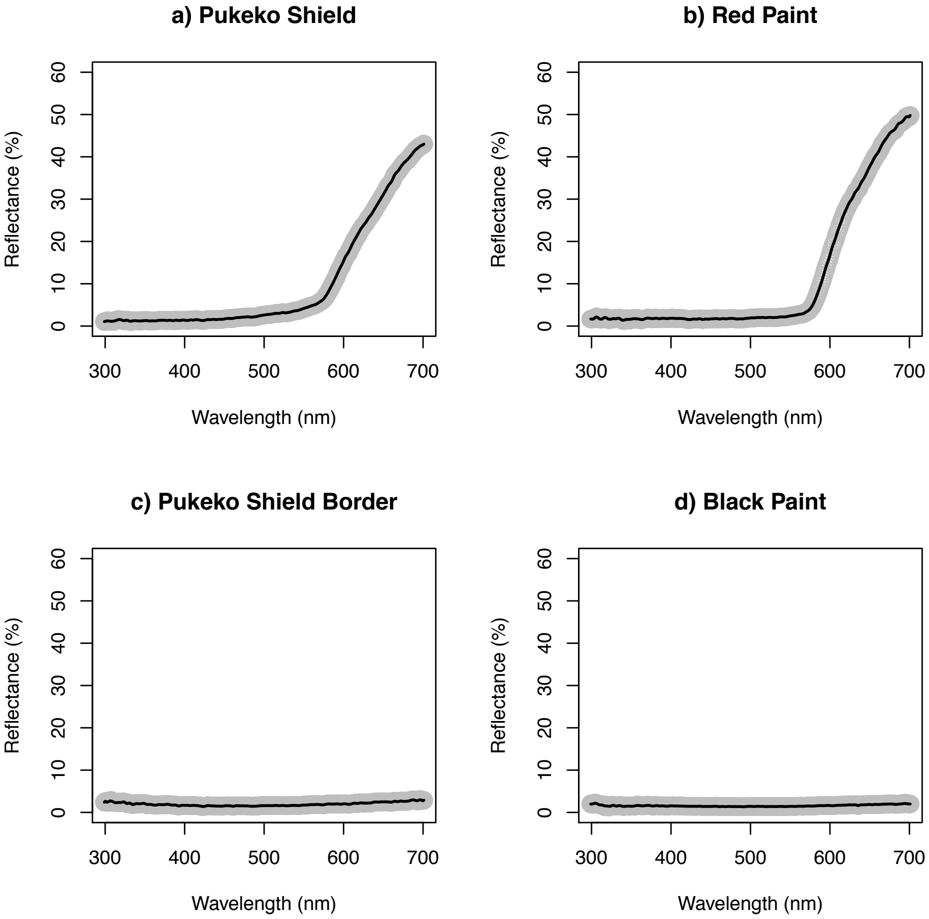

Figure D1 UV-VIS reflectance curves for the pukeko's frontal shield (a) and the black plumage surrounding the shield (c). These reflectance curves are highly similar to the red (b) and black (d) paint used to manipulate apparent shield size in this study

relationship between social dominance and shield size (described in the main text). Group ID was included in both models as a random effect, and the optimal random effect structure was determined using Akaike's Information Criterion, following the recommendations in [4]. In the 11 social groups considered in this analysis, there were a total of 102 individuals; however, 20 individuals were banded as juveniles and thus, their adult mass and shield size was unknown. As a result, these individuals were removed from the analysis. Furthermore, to verify that the relationship between shield size and dominance was not affected by the date of capture, we visually examined the model residuals with respect to capture date and confirmed that there was no difference in variation or means between individuals banded in 2010 and 2012.

To analyze the effects of shield manipulation on dominance behaviours directed towards the focal individuals, we used three Poisson family generalized linear mixed models (described in the main text). In each model, focal individual ID was included as a random effect and the fixed effects were centered (as above). These models were fitted with Laplace approximation in the lme4 package [5], as suggested in [6] when the mean response value is less than 5.

In all models, the fixed effects were centered to allow for the interpretation of main effects in the presence of interactions [7]. Model validation was conducted by visual inspection of diagnostic plots including residuals vs. fitted, quantile-quantile and scale location plots. Additionally, all generalized linear mixed models were checked for overdispersion [6].

Additional References

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Appendix F: Supplementary Material for Chapter 6

Table E1. Results of bill colour analysis using a continuous, rather than binary score for bill coloration. We ran 3 phylogenetic generalized least squares models using a randomly selected tree from the tree pool described in the main text. The response variables used were as follows: for ‘species’ the maximum red chromaticity value for each species was used. For ‘female’ and ‘male’ the maximum value (out of the upper/lower mandible, see main text for details) from the female and male measurements was used. Response variables were power transformed to improve model fit. Predictor variables were identical to those described in the main text and the phylogenetic signal λ was estimated during model fitting (with values of 0.81, 0.82 and 0.77 respectively).

Model	Variable	Estimate	95% CI	*t*	*P*
Species					
	Non-breeding sociality	0.30	0.10, 0.49	2.97	*0.0030*
	Breeding dispersion	0.24	-0.03, 0.50	1.72	*0.085*
	Sexual dichromatism	0.35	0.12, 0.57	3.04	*0.0024*
	Sexual size dimorphism	0.05	-0.16, 0.27	0.50	0.61
	Body size	-0.46	-0.80, -0.11	-2.57	*0.010*
Female					
	Non-breeding sociality	0.21	0.08, 0.35	3.13	*0.0018*
	Breeding dispersion	0.23	0.05, 0.41	2.48	*0.013*
	Sexual dichromatism	0.23	0.08, 0.39	2.99	*0.029*
	Sexual size dimorphism	0.08	-0.06, 0.23	1.10	0.28
	Body size	-0.31	-0.55, -0.07	-2.54	*0.011*
Male					
	Non-breeding sociality	0.15	0.03, 0.28	2.43	*0.015*
	Breeding dispersion	0.01	-0.16, 0.17	0.03	0.98
	Sexual dichromatism	-0.02	-0.15, 0.12	-0.22	0.82
	Sexual size dimorphism	-0.03	-0.16, 0.10	-0.45	0.65
	Body size	-0.21	-0.42, 0.01	-1.93	0.054