Body mass and immune function, but not bill coloration, predict dominance in female mallards

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ABSTRACT

Competition over indivisible resources is common and often costly. Therefore, selection should favor strategies, including efficient communication, that minimize unnecessary costs associated with such competition. For example, signaling enables competitors to avoid engaging in costly asymmetrical contests. Recently, bill coloration has been identified as an information-rich signal used by some birds to mediate aggressive interactions and we evaluated this possibility in female mallards *Anas platyrhynchos*. Specifically, we conducted two rounds of competitive interactions among groups of unfamiliar adult female ducks. By recording all aggressive behaviors exhibited by each individual, as well as the identity of attack recipients, we were able to assign dominance scores and evaluate links between numerous physiological, morphological, and experimental variables that we predicted would influence contest outcome and dominance. Contrary to our predictions, dominance was not linked to any aspect of bill coloration, access to dietary carotenoids during development, two of three measures of immune function, or ovarian follicle maturation. Instead, heavier birds were more dominant, as were those with reduced immune system responses to an experimentally administered external immunostimulant, phytohemagglutinin. These results suggest that visual signals are less useful during the establishment of dominance hierarchies within multi-individual scramble competitions, and that immune function is correlated with contest strategies in competitions for access to limited resources.

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1. Introduction

Intraspecific competition for food, space, or mates drives the evolution and elaboration of traits that increase the likelihood of winning aggressive interactions and securing resources (Hardy and Briffa, 2013; Huntingford and Turner, 1987). For example, armaments may allow individuals to physically dominate competitors, increasing the likelihood of gaining access to contested resources (e.g., Emlen, 1997; Plard et al., 2011). However, because engaging in fights is energetically expensive and bears risk of injury, evolution frequently favors the production of signals that mediate aggressive or competitive interactions via information transfer, thus potentially avoiding the risks associated with physical combat (Searcy and Nowicki, 2005). Such status signals aid conflict participants by providing them with additional information about their oppo-

nents, allowing individuals that are likely to lose an encounter to avoid injuries inflicted by markedly superior animals, and enabling individuals that are likely to win encounters to avoid expending energy in unnecessary escalation (Rohwer, 1975). Though status signals have typically been examined in males (Briefer et al., 2008; Ligon and McGraw, 2013; Ord and Evans, 2003; Rick and Bakker, 2008), females of many species can be territorial (Murphy et al., 2009a,b), compete for mates (Chancellor et al., 2009; Pryke, 2007), and produce signals related to dominance status (Murphy et al., 2009b). Examining the role of female ornaments in modulating intraspecific, female–female competition may facilitate new insights regarding the evolution of these characters, specifically whether such traits evolved as socially selected signals within females or as by-products of sexual selection on males (Amundsen, 2000; Clutton-Brock, 2009; LeBas, 2006).

Status signals frequently manifest as visual signals, often in the form of colorful patches of integument. Animal coloration is produced by several mechanisms, including pigment deposition (Hill and McGraw, 2006) or physical arrangement of nanostructures (i.e., structural colors; Prum, 2006). Among pigmentary colors,

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two frequently studied modes of animal coloration are melanin-based coloration (McGraw, 2006a) and carotenoid-based coloration (McGraw, 2006b). Melanins, large molecules that can be synthesized *de novo* in many vertebrate species, produce many of the black, grey, and rufous colors in vertebrate skin, hair, scales, and feathers (McGraw, 2006a). In contrast, carotenoids must be acquired from the diet and are responsible for many of the vibrant yellow, orange, and red colors in vertebrate integuments (McGraw, 2006b). Both melanin- and carotenoid-based coloration have been linked to social status in males (Ardia et al., 2010a; Liker and Barta, 2001), and several mechanisms linking coloration and behavior have been investigated, including putative links between circulating levels of testosterone and melanin-based coloration in both males (Lindsay et al., 2011) and females (Muck and Goymann, 2011). Because testosterone can increase aggressive behaviors (Ardia et al., 2010a), the same hormone that drives aggression can also increase melanin-based coloration, allowing for such colorful patches to honestly signal status. Similarly, testosterone levels in males have been linked to increased bioavailability of carotenoids (Blas et al., 2006) and expression of carotenoid-based ornaments (Martínez-Padilla et al., 2010), suggesting a similar potential for honest signaling.

To date, most studies linking coloration and behavior have focused on relationships between adult body condition and the concurrent expression of colorful ornaments (e.g., Hill and Montgomerie, 1994; Weiss, 2006). However, conditions experienced during development can also shape adult phenotype, a phenomenon termed developmental plasticity (Monaghan, 2008). While a variety of dietary manipulations (e.g., access to protein; Ohlsson et al., 2002) can affect adult phenotype, developmental access to dietary carotenoids in particular can shape carotenoid physiology (e.g., the ability to assimilate, circulate, or mobilize carotenoids; Blount et al., 2003), immune function (Butler and McGraw, 2012a,b), and even the color of eggshells laid at adulthood (Butler and McGraw, 2013a). Carotenoid supplementation during development can also affect behavior, with carotenoid-supplemented individuals exhibiting increased pecking behaviors (Fenoglio et al., 2002) and improved song performance (Van Hout et al., 2011). Thus, it seems plausible that carotenoid access during development may shape adult coloration or dominance status because individuals that consume high quantities of carotenoids during development may be able to produce more colorful bills and may also be in better overall health or condition.

While coloration may be linked to dominance status via shared physiological processes (e.g., high testosterone levels), color could also signal other aspects of phenotype that contribute to variation in dominance hierarchies. For example, carotenoid- and melanin-pigmented ornaments can signal immune function (Butler and McGraw, 2011), parasite load (Mougeot et al., 2010), or nutritional history (Ohlsson et al., 2002), and behavioral tests have shown that such factors can affect dominance hierarchies (Dolnik and Hoi, 2010; Royle et al., 2005). Thus, dominance may be associated not only with carotenoid- or melanin-based coloration, but may be more strongly influenced by, and correlated with, other aspects of phenotype. Here, we tested whether carotenoid- and melanin-pigmented bill coloration is related to dominance status in female mallards *Anas platyrhynchos* and whether dietary carotenoid manipulation during development influenced the expression of aggressive behavior. However, despite our initial predication that developmental access to carotenoids would influence adult beak coloration, no such relationship was detected (these results are described in detail in Butler and McGraw, 2013b). This absence of a developmental influence on adult beak coloration thus allowed us to separately assess how developmental diet and adult beak coloration relate to adult aggressive behavior and dominance.

The carotenoid-pigmented bill of male mallards is used as a signal during mate choice (Omland, 1996a, 1996b), but the potential signaling function of the female bill is currently unknown. Because female bills contain dark, putatively melanin-pigmented patches in the center, surrounded by orange, carotenoid-pigmented coloration (Butler and McGraw, 2013b), it is possible that female bill coloration may have a signaling function similar to the social signaling function of male bill coloration. To test this possibility, we conducted a series of behavioral tests using adult female mallards to assess the relative influence of bill coloration, developmental nutrition, immune function-based indicators of quality, body mass, and reproductive status on female aggressive behavior and dominance.

2. Methods

2.1. Husbandry and experimental protocol

This study was conducted in accordance with the Institutional Animal Care and Use Committee at Arizona State University under protocol 10–1094R. We acquired 48 one-day-old female ducklings from Metzer Farms (Gonzales, CA) in December 2009 and housed them as described in Butler and McGraw (2009). Ducklings were reared indoors in randomly selected groups of five ducklings per cage ($60 \times 60 \times 60$ cm) until they were two weeks old, three per cage until they were four weeks old, and two per cage until they were seven weeks old, at which point all birds were moved outside and individually housed in these same cages to allow for normal sexual maturation (Butler and McGraw, 2009). Light:dark regime was 13L:11D when ducklings were housed indoors, and natural photoperiod thereafter (10.5L:13.5D at 7 weeks old to 13.5L:10.5D at 20 weeks old). Individuals received *ad libitum* access to a base diet that contained low levels of carotenoids (described below) unless otherwise specified.

Individuals were randomly assigned to one of four developmental treatment groups that varied in dietary carotenoid content (none of which had any detectable influence on adult bill coloration; Butler and McGraw, 2013b). Per Butler and McGraw (2013b), individuals received carotenoid-supplemented diets during either the period of maximal growth (EARLY; 3–6 weeks old; N = 10), minimal growth and nuptial plumage acquisition (MIDDLE; 8–11 weeks old; N = 12), or nuptial plumage acquisition (LATE; 13–16 weeks old; N = 12). CONTROL (N = 12) birds did not receive carotenoid-supplemented diets at any point during the study. The sample size for the EARLY treatment was 10 instead of 12 because two ducklings had their diets mistakenly switched and were removed from the study. We prepared diets by mixing a base diet of dry food (Mazuri Waterfowl Starter: Richmond, IN, USA, weeks 0–7; Mazuri Waterfowl Maintenance thereafter) with ORO-GLO dry pigmenter (2% carotenoids by mass, predominately lutein; Kemin AgriFoods North America, Inc., Des Moines, Iowa, USA) suspended in sunflower oil to achieve concentrations of 25 µg/g of carotenoids (upper quartile of carotenoids in mallard duckling diets in the wild; Butler and McGraw, 2010). Circulating levels of carotenoids in mallards receiving non-supplemented diets were in the lower range of those found in wild ducklings, while supplemented levels were in the higher range (Butler and McGraw, 2010; Butler and McGraw, 2013b). Whenever any particular treatment group was receiving carotenoid-supplemented diets, all other individuals received food mixed with sunflower oil as a sham control.

We measured body mass to the nearest gram immediately prior to each of two rounds of dominance trials (21–22 weeks). We calculated the average of these two body mass values to determine adult mass for each bird. For immune assessment, conducted two weeks before dominance trials, we quantified primary and secondary antibody response to the benign antigen keyhole limpet hemo-

cyanin (KLH), as well as inflammatory response (i.e., amount of swelling) due to administration of the T-cell mitogen phyothemag-glutinin (PHA). See Butler and McGraw (2013b) for complete details on methodology. In addition, we collected 600 μl of whole blood from each individual each time we recorded mass, and blood was stored on ice until centrifugation for 3 min at 10,000 rpm, and then aliquots were stored at −80 °C until analysis.

To assess their reproductive status, we euthanized all animals using isoflurane when they were 22.5 weeks old, collected their ovaries, placed these samples on ice, and stored them at −80 °C. Several days later, we used digital calipers to measure the five largest follicles in each ovary to the nearest 0.01 mm. We then used a principal components analysis (PCA) to generate a single variable that reflected degree of ovarian development (Butler and McGraw, 2013a,b). Specifically, we used the size of the five largest follicles in the ovary to produce a single principal component score (PC1) that had an eigenvalue = 4.70, had an eigenvector that loaded positively and relatively uniformly for all five follicle measurements (all loadings between 0.43 and 0.45), and accounted for 94% of the total variation in follicle size.

2.2. Dominance trials and behavioral analyses

We performed two rounds of dominance trials in an outdoor arena (2.4 m × 3.7 m × 1.2 m) when the ducks were 21 and 22 weeks old. The arena was constructed of wooden supports and shade cloth, and placed under an aluminum-roofed shelter. Running through the central minor axis of the rectangular arena was a plywood board upon which a round food bowl (approx. 20 cm diameter) and a rectangular water bath (approx. 23 cm × 33 cm × 8 cm) were affixed and filled with Mazuri Waterfowl Maintenance food and tap water, respectively (Fig. 1). The food and water were placed within the arena in an attempt to make the central portion of the arena valuable to contest participants and all individuals were food-restricted beginning at 1800 h the night before the trial in an attempt to increase the potential value of this central area. However, conflicts occurred throughout the trial arena with no apparent concentration near the food dish. All trials were completed the following morning between 0700 and 1000 h.

Prior to each trial, we glued a randomly assigned identifying patch (circle of white poster board, 10 cm in diameter, with a design drawn with permanent black marker) to the back feathers of each bird. To begin each trial, four birds were placed in inverted cardboard boxes located in each corner of the arena and left to acclimate in the dark for 2 min, after which the boxes were raised by a remote pulley system. Individuals were then allowed to interact for

approximately 44 min without visual contact from humans or any other ducks not actively involved in the trial.

In the first round of trials, ducks were placed into groups of four birds from each of the four developmental treatment groups so that we could directly test for an effect of dietary access to carotenoids during the developmental period. In addition to having one duck from each developmental treatment group, we attempted to match individuals for mass within these groups (mean intra-trial range, the difference between the most and least massive ducks in a given trial, was 93 g). Because there were only 10 individuals from the EARLY treatment group, we conducted 10 trials in this round. In round 2, individuals were again matched for mass (mean intra-trial range, 104 g), but all individuals within a trial were from the same treatment group (N = 11 trials). Those individuals who were able to participate in round 2, but not round 1 (owing to logistical constraints of conducting 10 trials containing 4 birds each) were exposed to a mock dominance test prior to participating in round 2 so that all individuals would have similar experience with the arena during round 1 (no experience) and round 2 (one previous trial).

We recorded behaviors in the arena using two digital camcorders (Canon ZR830, Canon U.S.A., Inc., Lake Success, NY, USA; JVC Everio, JVC U.S.A. Inc., Wayne, NJ, USA) placed approximately 1 m off the ground at both ends of the major axis of the arena. These two visual inputs were routed through a video splitter (Color Quad Splitter, #QC900, Clover Electronics, CA) to synchronize both view-points into a single screen, and the resulting video was burned to a DVD (DVD Recorder, #DR570KU, Toshiba, Tokyo) for subsequent analysis.

At the completion of all trials, we (RAL and MWB) independently scored all videos for aggressive interactions blindly with respect to treatment group and duck identity. Specifically, for every interaction between any two ducks, we recorded which individual initiated and which was the recipient of the following behaviors: Full body lunges (striking movement with the head that is accompanied by forward propulsion by the feet), neck lunges (striking motion with the head only), bill fighting (two individuals biting at each other's heads at the same time), biting (biting another individual without any reciprocation), chasing (moving quickly and directly at another individual, but without contact), bill threatening (opening the bill in the direction of a nearby individual), and avoiding (quickly moving away from a slowly approaching individual; chasing and avoiding could not happen simultaneously). All behaviors were weighted equally, and the sum of all wins and losses per dyad were recorded for each trial (wins being instances where an individual was aggressive and losses being instances where an individual received aggression). Scores were repeatable between observers for all physical (total of full body lunges, neck lunges, bill fighting, and biting) and non-physical (chasing, bill threatening, and avoiding) interactions, as well as the sum of physical and non-physical interactions (all R > 0.6, all P < 0.0001; Lessells and Boag, 1987). Therefore, we used the average between the two observers for all subsequent analyses.

2.3. Carotenoid titer and color assessment

We quantified plasma carotenoid concentration from blood collected at adulthood (prior to both immune assessment and behavioral trials). To do so, we extracted carotenoids from 50 μl of plasma using 1:1 hexane:methyl tert-butyl ether and measured concentrations using high-performance liquid chromatography (McGraw et al., 2008). Detectable amounts of lutein, zeaxanthin, a lutein isomer, and ß-cryptoxanthin were positively correlated with total carotenoid titer (all R > 0.624, all P < 0.0001). We thus used the total circulating carotenoid concentration for subsequent analyses.
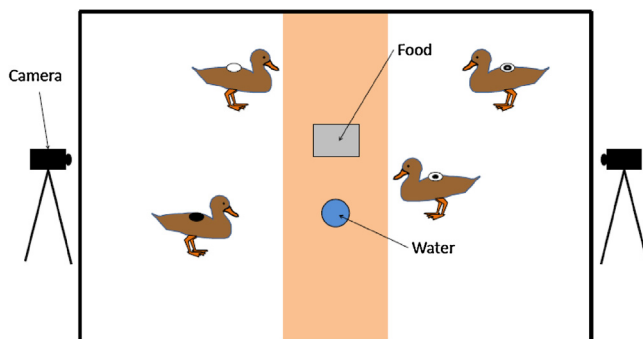

Fig. 1. Schematic of the experimental trial arena where behavioral interactions were recorded. Each duck had a small paper identifier affixed to her back enabling individual identification and directionality of all aggressive behaviors when videos were later scored (blindly). Food and water were affixed to the floor in the central portion of the arena.

Ornamental bill coloration in mallards begins to develop by 10 weeks of age (Drilling et al., 2002) and is completed in all birds by 16 weeks (MWB, pers. obs.; J. Metzer, pers. comm.). When ducks were 20 weeks old, we measured carotenoid-based bill coloration of adults using an Ocean Optics (Dunedin, FL, USA) USB2000 spectrometer with a PX-2 pulsed xenon light source to measure reflectance from $\lambda = 300$–700 nm. We measured a 1 cm band of the right dorso-lateral surface of the bill between the nares and the bill tip, and binned all measurements into 1 nm increments using CLR-files (Montgomerie, 2008). We then used CLRvars (Montgomerie, 2008) to calculate the brightness (B1), saturation (S1B), and hue (H4b) scores that are most closely correlated with carotenoid content in the male mallard bill (Butler et al., 2011). While male (yellow) and female (orange) bill coloration differ in appearance, both use predominately lutein and zeaxanthin as pigments (MWB and KJM, unpublished data), and thus we elected to use these same coloration metrics for female mallards in this study.

Shortly before behavioral trials, we took two digital photographs (Nikon Coolpix P3, Nikon Inc., Melville, NY) of the top of each duck's bill (5 days apart within the 21st week). Pictures were taken against a uniformly gray background under standardized illumination. Using Adobe Photoshop v. 8 software (Adobe Systems Inc., San Jose, CA), we quantified the proportion of the bill that was heavily melanized (the 'saddle') by selecting the central area on the bill that was black or uniformly dark and dividing the number of pixels associated with the black patch by the total number of pixels in the entire bill. The selection of the black saddle of the bill was performed using the 'magic wand' option (tolerance = 10) in Adobe Photoshop, which selects all adjacent pixels that have a similar coloration, and the lasso marquee to select the outline of the bill.

2.4. Analyses

To identify the biological factors most closely associated with dominance, we conducted a two-step analysis. In the first stage, we used Bradley-Terry models to obtain overall dominance scores for each duck in our study (see below). In the second stage, we used an information-theoretic model averaging approach to identify the phenotypic or experimental factors associated with dominance (see below).

2.4.1. Bradley-Terry dominance scores

We first tested whether the dominance interactions between the ducks met the critical assumption of linearity (i.e., dominance is transitive) using the *transitivity* function in the R package *Perc* (Fujii et al., 2016). Following confirmation of linearity (number of intransitive dominance triangles = 0), we obtained individual dominance scores using Bradley-Terry analysis (Bradley and Terry, 1952; Firth, 2005) to identify the underlying 'ability' parameters (representing the likelihood of one individual beating another) from a matrix of aggression outcomes (*sensu* Ligon and McGraw, 2013). Specifically, we obtained Bradley-Terry scores using the BradleyTerry2 package (Turner and Firth, 2011) in R (R Core Team, 2015).

2.4.2. Information-theoretic model averaging approach

We used Akaike's information criterion corrected for small sample size (AICc) (Burnham and Anderson, 2002) to evaluate models exploring links between dominance scores (see above) and experimental and biological correlates. Specifically, we modeled relationships examining the influence of dietary treatment, immune function, body mass, reproductive status, and bill color using linear models. We evaluated our complete dataset (n = 46 ducks) using models with four or fewer predictor variables (following rule of thumb described in Bolker et al., 2009; Harrell, 2001).

Multiple models may be well-supported by the data within an information-theoretic framework, so we evaluated the relative importance of multiple variables with a model averaging approach (Burnham and Anderson, 2002; Burnham et al., 2010). Model averaging allowed us to incorporate parameter estimates from multiple models, each weighted by the support for that model (Burnham and Anderson, 2002). In this framework, parameter estimates from highly supported models will contribute relatively more to multi-model parameter estimates. Information-theoretic approaches generally provide accurate parameter estimates even when dealing with collinear predictor variables, but we omitted additive models that included highly correlated variables (r > 0.5; Supplementary Table 1) because of the increased variance among parameter estimates when models include highly correlated predictors (Freckleton, 2010).

Evaluating multiple models also allowed us to calculate relative importance (RI) values for each predictor variable within our model set. Specifically, we calculated RI values by summing the Akaike weights (w_i) for all of the models in which that variable appeared. Akaike weights for a given set of models add up to 1, so RI values range from 0 to 1 (where RI values that are close to 0 indicate variables that occur infrequently or in poorly-supported models and RI values near 1 indicate variables commonly represented in well-supported models). RI values were calculated from our 95% confidence set, that is, for the set of models for which the cumulative Akaike weight was ~0.95. A cumulative Akaike weight of 0.95 indicates a 95% probability that the best individual model explaining dominance among females was contained within this set. All statistical analyses were conducted within the R computing environment (R Core Team, 2015) and model selection was performed using the MuMIn package in R (Barton, 2013).

3. Results

Female mallards were highly aggressive toward one another during our staged interactions, exhibiting an average of 125.19 ($\pm$ SEM 15.15, median = 112.50) aggressive behaviors (collectively) in each trial. To evaluate how individual characteristics influenced the dynamics of these aggressive behaviors, we employed a model averaging approach (see Section 2.4.2) that yielded the set of models which, collectively, had a cumulative AIC weight of 0.95. This 95% confidence set included 32 different models linking dominance scores and body mass, bill coloration, developmental diet treatment, follicle development, and immune function. Mass was included in all 32 of these models, leading to a relative importance (RI) value of 1.00. The average parameter estimate linking mass and dominance was 2.72, indicating that dominance was positively linked to body mass (Fig. 2). A single linear model designed to uncover the strength of the relationship between body mass and dominance revealed that mass explained 36% of the variation in dominance scores ($F_{1,43} = 24.38$, $R^2 = 0.36$, $p < 0.0001$). Interestingly, one metric of immune system responsiveness was also linked to dominance. Specifically, the size of the wing-web swelling that occurred in response to an administration of phytohemagglutinin (PHA) was negatively linked to dominance (RI = 0.98, average parameter estimate = -1.52). Swelling explained a relatively small, but significant portion of the variation in dominance scores ($F_{1,43} = 6.27$, $R^2 = 0.13$, $p = 0.016$; Fig. 3). Bill coloration, circulating carotenoid levels, immune responsiveness to KLH injections, and developmental diet treatment were unrelated to dominance scores (Table 1).

Table 1
95% confidence set of best-ranked models (the 32 models whose cumulative Akaike weight, cum w_i, ≈ 0.95) used to determine the variables that best explain female mallard dominance scores. The null model is also included for reference.

Model[a]	k	Log L	ΔAIC$_c$	w_i
Mass + Swelling	4	−117.142	0.00	0.119
Mass + Swelling + Carotenoid concentration	5	−116.117	0.49	0.093
Mass + Swelling + Black area	5	−116.455	1.17	0.066
Mass + Swelling + KLH2	5	−116.499	1.25	0.064
Mass + Swelling + Black area + Carotenoid concentration	6	−115.239	1.41	0.059
Mass + Swelling + Brightness	5	−116.783	1.82	0.048
Mass + Swelling + Black area + KLH2	6	−115.511	1.95	0.045
Mass + Swelling + Hue	5	−116.861	1.98	0.044
Mass + Swelling + Saturation	5	−116.926	2.11	0.041
Mass + Swelling + Brightness + Carotenoid concentration	6	−115.734	2.4	0.036
Mass + Swelling + KLH1	5	−117.094	2.44	0.035
Mass + Swelling + Carotenoid concentration + KLH1	6	−115.775	2.48	0.034
Mass + Swelling + Carotenoid concentration + KLH2	6	−115.775	2.48	0.034
Mass + Swelling + Carotenoid concentration + Hue	6	−115.859	2.65	0.032
Mass + Swelling + Carotenoid concentration + Saturation	6	−115.867	2.66	0.031
Mass + Swelling + KLH2 + Hue	6	−116.106	3.14	0.025
Mass + Swelling + KLH2 + Saturation	6	−116.115	3.16	0.025
Mass + Swelling + KLH2 + Brightness	6	−116.281	3.49	0.021
Mass + Swelling + Brightness + Black area	6	−116.385	3.7	0.019
Mass + Swelling + Hue + Black area	6	−116.397	3.72	0.018
Mass + Swelling + Saturation + Black area	6	−116.414	3.76	0.018
Mass + Swelling + KLH1 + Black area	6	−116.437	3.8	0.018
Mass + Swelling + KLH1 + Brightness	6	−116.656	4.24	0.014
Mass + Swelling + Saturation + Brightness	6	−116.711	4.35	0.014
Mass + Swelling + KLH1 + Hue	6	−116.846	4.62	0.012
Mass + Swelling + KLH1 + Saturation	6	−116.916	4.76	0.011
Mass	3	−121.32	5.94	0.006
Mass + Black area	4	−120.235	6.19	0.005
Mass + Brightness	4	−120.666	7.05	0.004
Mass + Saturation	4	−120.728	7.17	0.003
Mass + +Swelling + Treatment	7	−116.822	7.39	0.003
Mass + Hue	4	−120.974	7.67	0.003
Null model	2	−131.425	23.85	0.000

[a] Mass = body mass, Swelling = wing-web swelling size in response to phytohemagglutinin (PHA) injection, Carotenoid concentration = circulating carotenoid titre, KLH1 =&9617.primary antibody response to injection of keyhole limpet hemocyanin, KLH2 = secondary antibody response to injection of keyhole limpet hemocyanin, Saturation = saturation of yellow bill color, Hue = hue of yellow bill color, Brightness = brightness of yellow bill color, Black area = proportion of bill that is black, Treatment = developmental carotenoid supplementation treatment group.
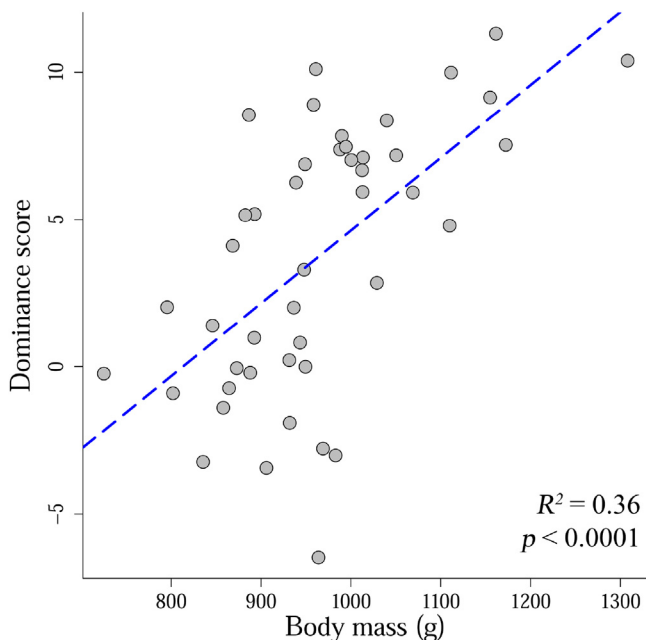

Fig. 2. Body mass of female mallards was positively linked to dominance. Dominance scores were obtained from Bradley-Terry models designed to identify underlying 'ability' parameters from a matrix of aggression outcomes.
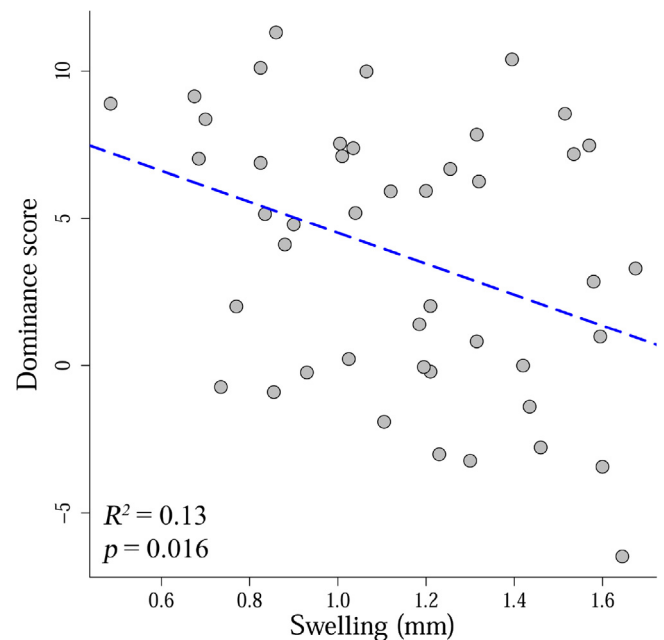
Fig. 3. The size of the swelling in response to an injection of phytohemagglutinin, a measure of immune function, was negatively linked to dominance in female mallards. Dominance scores were obtained from Bradley-Terry models designed to identify underlying 'ability' parameters from a matrix of aggression outcomes.

4. Discussion

Female mallards placed in arenas containing multiple individuals engaged in numerous aggressive behaviors in an apparent attempt to establish dominance hierarchies. By evaluating these aggressive behaviors using Bradley-Terry models and a model-averaging information theoretic approach, we were able to evaluate the relative importance of body mass, bill color, reproductive state, developmental diet, and immune function on the dominance scores of individual ducks. Across two rounds of contests, we found that more massive females had significantly higher dominance scores. Additionally, one aspect of immune-responsiveness (swelling in response to a PHA injection) was negatively linked to dominance, indicating that dominant females had a reduced response to PHA relative to subordinate females. Interestingly, none of the putative visual signals we measured, including multiple indexes of bill coloration or melanization, were linked to dominance. Likewise, neither follicular development, immune responsiveness to KLH injections, nor variation in carotenoid access during development had any significant influence on female dominance scores.

Previous investigations of the factors associated with dominance among male mallard ducks indicate that more dominant males circulate higher levels of testosterone, but are not necessarily more massive (Poisbleau et al., 2005a). However, our findings suggest a very important role for mass in determining rank within dominance hierarchies for females. More massive females may have greater energetic reserves (Witter and Cuthill, 1993; though we did not collect the raw data on body condition required to test this possibility) which could allow them to undertake more aggressive actions towards conspecifics (Rosvall, 2011) or, alternatively, might have greater energetic requirements (Taylor et al., 1982) necessitating competitive exclusion of opponents from valuable food resources (e.g., the food within the trial arena). This latter possibility seems unlikely in the current experimental framework, however, because we rarely observed dedicated defense of food within the trial arena (RAL and MWB *personal observation*). Regardless of the underlying reasons for their dominance, massive female mallards hold a competitive advantage in intrasexual contests.

As well as being more massive, dominant females showed less PHA-induced swelling than subordinate females. From a proximate perspective, this link between behavior and immune response could arise in at least two ways. First, some underlying physiological parameter may provide a mechanism connecting lower PHA response and increased dominance. For example, testosterone could mediate aggression *and* influence immunity. Aggression has frequently been tied to testosterone levels (Ardia et al., 2010b; Johnsen and Zuk, 1995; Poisbleau et al., 2005a; Sandell, 2007) and testosterone can be correlated with PHA response (though the direction of such correlation varies depending on sex or body mass of the individuals; Li et al., 2014; Roberts and Peters, 2009). Alternatively, selection for particular behavioral traits may exert pleiotropic effects on physiology (Sild et al., 2011; van Oers et al., 2011), resulting in the observed correlation between dominance and immunoresponsiveness. Though we did not record the behaviors necessary to classify individuals with respect to 'personality', it would be informative to simultaneously compare the endocrine, immune, and behavioral types of individuals across contexts (i.e., not just competitive interactions). Likewise, future work manipulating these variables will shed light on the directionality and interdependence of behavioral strategies, endocrine function, and immune status in explaining different life-history variation within and among species. Both of the potential explanations we mention above depend upon an unidentified parameter linking immune response and dominance, rather than a direct causal link between PHA response and subsequent behavior. It is unlikely that a reduced investment (i.e., lower response) during the PHA test directly contributed to higher dominance levels for two reasons. First, birds were fed *ad libitum* and would likely be able to replace any caloric or nutritional deficits imposed by higher PHA responses during the two week interval between immune testing and the behavioral trials. Second, wing-web swelling in response to PHA injection peaks within one to two days post-injection and typically returns to baseline levels within about five days (Biard et al., 2009), suggesting a lack of any major physiological changes that would affect aggressive interactions. Thus, the PHA response itself likely did not drive subsequent aggressive behavior.

The negative correlation we uncovered between dominance and PHA-response also warrants investigation from an ultimate perspective. One such interpretation of our results is that higher-quality birds may simply be more aggressive and also able to minimize their response to an immune challenge. For years, eco-immunologists interpreted a larger immunological response as indicative of a superior immune system (Martin et al., 2011), but recently the costs associated with immune responses, including energetic investment (Martin et al., 2003) and increased oxidative damage (Costantini and Dell'Omo, 2006; but see Perez-Rodriguez et al., 2008), have been viewed as reasons for individuals to limit the degree of immune response (Adelman et al., 2013). Thus, high-quality individuals may be those that are naturally more dominant and these individuals may be best equipped to minimize the costs associated with injection of a pro-inflammatory molecule. This relationship may be exaggerated when individuals are exposed to multiple competitions wherein earlier outcomes can influence subsequent outcomes through winner- or loser-effects (Chase et al., 1994), which may manifest differently in high and low-quality individuals (Rutte et al., 2006).

In some contexts, bill color can serve as an important signal mediating intraspecific interactions among mallards. For example, female mallards prefer males that have brighter bills with fewer dark spots (Omland, 1996a, 1996b). However, we found no evidence that female mallards signal intrasexual dominance or aggression via bill coloration. Likewise, another putative integumentary signal was found to be unrelated to any aspect of individual quality or condition in female eiders *Somateria mollissima* (Lehikoinen et al., 2010), suggesting that not all visually-distinctive patches in female ducks necessarily provide information about individual condition. In the case of the eiders, there seems to be a strong, individual-specific component of wing bars (Lehikoinen et al., 2010), indicating that these patches may provide information about identity rather than quality (Dale, 2006). In our study, prior experience during development (when individuals were kept in small groups) may have influenced the importance of adult signals of quality during dominance interactions – but only if female mallards have the ability to recognize individuals based on characters that remain consistent throughout development and into adulthood. The existence of such characters has not been documented in any duck species, but previous experience (and the ability to remember individual-specific attributes) would decrease the importance of quality signals and reduce the likelihood of detecting any meaningful relationship between bill color and dominance. Nonetheless, the finding that female mallard bill color is not linked to dominance is somewhat surprising, given recent work documenting the signal value (Murphy et al., 2009b) and potential information content (Kelly et al., 2012; Lumpkin et al., 2014; Pham et al., 2014; Rosenthal et al., 2012) of bill coloration among female American goldfinches.

There are several potential explanations for the lack of a relationship between bill coloration and dominance in our study. First, bill color may serve as a signal *only* in males (Omland, 1996a, 1996b). This situation is exactly the opposite of that described by Murphy et al. (2014), where bill color appears to serve as a signal only for females—male bill coloration is used neither to mediate

competition nor in mate choice by females (Murphy et al., 2014). Second, recent work has linked chromatic attributes of female (but not male) duck plumage to indexes of condition (Legagneux et al., 2010), suggesting that the most informative signals of quality among female ducks may be plumage-, rather than bill-based (but see Lehikoinen et al., 2010). Third, the multiple competitor environment that we experimentally created may have limited the efficacy and utility of all signals, thereby increasing reliance on physical aggression and body size. Fourth, it is possible that mallard bill coloration is more dynamic than we realize (e.g. Rosenthal et al., 2012). If bill coloration in female mallards is a dynamic color signal that changes over short time periods (*sensu* Hutton et al., 2015), as it is for males (Butler and McGraw, 2012a), we may not have accurately captured the most relevant chromatic information from each duck by measuring bill coloration days ahead of the actual aggressive interactions.

Despite the potential for developmental conditions to influence adult phenotypes (Monaghan, 2008), including behavior (Marks et al., 2012), we found no effect of dietary access to carotenoids during the developmental period on dominance scores for female mallards during adulthood. This lack of carryover effect from developmental carotenoid supplementation is consistent with the previously documented lack of relationship between developmental access to carotenoids and adult immune function, ovarian development, circulating carotenoid levels, biliverdin concentration in the bile, and bill coloration (Butler and McGraw, 2013b). Perhaps these characters, as well as the behaviors that influence dominance, are simply so plastic that their expression is much more tightly linked to current conditions (e.g. stress hormone responsiveness; Poisbleau et al., 2005b). Similarly, it is possible that because all individuals experienced the same adult conditions (i.e., had access to the same food and had received the same immune challenges), expression of these characters was grouped tightly enough to minimize the chance of detecting relationships between these characters and behavior. For example, if we had run an experiment wherein only a subset of individuals had received adult immune challenges, it is possible that the subsequent increased variation in markers of adult immune function would have been linked to dominance.

Intrasexual contests between females, as well as their potential influence on the evolution of diverse ornaments and armaments among females, have recently begun to receive more attention by behavioral ecologists (e.g. Amundsen, 2000; LeBas, 2006; Clutton-Brock, 2009; Midamegbe et al., 2011). This attention is well-deserved, because a better understanding of dynamics of female contests, the resources over which females compete, and the adaptations favored by such competitive interactions will improve our understanding of the factors driving diversity in species with different mating systems. Developing a more complete framework for female competition is especially important because straightforward assumptions about the role of particular signal types (Muma and Weatherhead, 1989), physiological responses (Drea et al., 2002), or morphological adaptations (Packer, 1983) drawn from research on males may not hold for females. Consequently, a better understanding of the evolution and use of such characters among females will be possible only through detailed studies of female–female contests in carefully controlled experimental settings, coupled with findings from field research undertaken where such contests naturally occur.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.beproc.2016.08.010.

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