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Female-Female Interactions at Nest Sites of Breeding
Wood Ducks (*Aix sponsa*)

by

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THESIS

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Abstract

Reproduction is perhaps one of the most energetically expensive activities that an individual performs in a lifetime. Many species have evolved behaviors and tactics to offset the energetic and physiological costs of reproduction while optimizing long-term reproductive success and survival. Some individuals do so by parasitizing the reproductive efforts of other individuals, thereby reducing the costs of reproduction while still maintaining some reproductive output. Conspecific brood parasitism (CBP) is one such alternative reproductive tactic whereby some females lay eggs in the nests of females of the same species to avoid the cost of rearing and caring for those offspring. The evolution of this behavior has received considerable theoretical attention, but surprisingly there have been few quantitative studies examining the interactions between parasitic females and the host of the nest. We used motion sensor cameras and radio frequency identification devices to record the behavior interactions at the nest among female wood ducks (*Aix sponsa*) in a population in California. Our observations revealed a remarkable range of responses in female-female interactions. We classified females as “owners” if they incubated the nest and as “intruders” if they entered the nest, potentially laid eggs but did not incubate the nest. There were significant differences in behavior and variation of response for owners and intruders; intruders typically exhibited passive, nonaggressive behaviors and avoided interactions with the owner, while owners often behaved aggressively, although they also showed a wider variation in response. Repeatability (measured as the interclass correlation coefficient) of female behavior was significant and high for owners, but low among intruders. We tested several hypotheses on the possible factors that might account for variation in responsiveness, including the effects of status, age/experience, body size/phenotype/resource holding potential, history with the nest/previous ownership, and nest site timing. Status had a

strong influence on all behavioral dynamics. Absolute age and body weight/condition did not influence the level of response, but relative differences in age and body weight/condition between owners and intruders had a strong effect. Owners were increasingly more aggressive when they were relatively older or larger than a given intruder. However, these same relative differences in age and size did not affect the behavioral response of intruders. Owners were also more aggressive to intruders during incubation than during the egg-laying period. There was no effect of previous history of the female or use of the box. Intruders laid an egg during 37 of the 57 observed encounters with owners, indicating that the intruders were, in at least most cases, acting as brood parasites. Owner aggressiveness did not prevent intruders from laying an egg. The passive avoidance of intruders in all interactions suggests strongly that these were parasitic interactions and not simply instances of two females fighting for possession of a nest site. The variation in the observed level of aggressiveness among pairs of females is intriguing and suggest that owners may act differently to some females; future research will explore the pattern of kinship and social relationships among these females.

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Introduction

Contests in the animal kingdom often arise when resources that are critical for survival or reproduction are limited. Such resources may include food, territory, shelter, or even mates. Given the extensive literature on territoriality and mate defense (Briffa & Sneddon 2010), contests among males are typically studied (Taborsky & Brockmann 2010, Engqvist & Taborsky 2016). However, access to limited resources is no less important for females and contests among females also occur, although female-female competition has been far less studied. Perhaps one of the most essential resources required for reproduction is a nest site and these are often limited. This is particularly true for secondary cavity-nesting birds that rely on other species (cavity excavators), human intervention (nest boxes), or natural processes (tree decay) to create nest sites. Nest site availability is further constrained for large cavity nesting birds for which suitably sized cavities are often scarce. In many species, females choose a suitable site, and are the sex that lays eggs and incubates the clutch; accordingly, female reproductive investment and success is closely tied to nest site access and quality. Hence competition for quality nest sites may be as intense as they are vital. As females search for suitable nest sites, or select among those available, they undoubtedly encounter other females, and interactions occur frequently among females at nest sites. These interactions have been found to vary from contest to cooperative behavior among many species (Andersson 2001, Zink & Lyon 2015).

One demonstration of potential competition among females for nest sites is a behavior known as conspecific brood parasitism (CBP), in which some females lay eggs in the nest of other females of the same species, seemingly bypassing the need to acquire a nest and avoid the cost of caring for offspring. CBP has been observed in over 250 bird species (Yom-Tov 1980,

Yom-Tov 2001, Lyon & Eadie 2008) with a large proportion of species in the family Anatidae (Eadie et al. 1988, Lyon & Eadie 2017). Numerous hypotheses have been proposed to describe the occurrence, ecology, and evolution of CBP (Andersson 1984, Eadie et al. 1988, Zink 2000, Arnold & Owens 2002, Lopez-Sepulcre & Kokko 2002, Lyon & Eadie 2008, Andersson & Ahlund 2012). However, there are still relatively few detailed quantitative studies of the behavior in wild populations, much less with a focus on behavioral interactions among females.

There is ongoing debate whether interacting females are simply competing for a nest site or whether brood parasites are pursuing a fundamentally different reproductive tactic, potentially involving stealthy behavior. In common goldeneyes (*Bucephala clangula*), for example, host females and parasitic females were found to behave differently (Ahlund 2005). Parasitic females never placed down, laid eggs at different times of the day compared to hosts, and only host females increased their time spent on the nest up to incubation (Ahlund 2005). These behavioral differences indicate that some goldeneyes are laying eggs parasitically as an alternative reproductive tactic (ARTs), a type of fixed or flexible trait variation found within populations (Engqvist & Taborsky, 2016). Poysa and Eadie likewise suggested that CBP was an alternative reproductive tactic in both common and Barrows goldeneyes (*B. islandica*) (Eadie 1989, Eadie & Fryxell 1992, Eadie et al. 1998, Poysa et al 2014). In contrast, Semel and Sherman (2001) argued that CBP in wood ducks (*Aix sponsa*) was largely an epiphenomenon of contests for nest sites and that ‘parasitic’ egg-laying was an inadvertent outcome of two (or more) females attempting to claim the same nest site, but only a single female ‘winning’ the nest. Semel and Sherman (2001) concluded that parasitism was a side effect of competition for nest sites. Indeed, there have been reports of injuries and even fatalities among incubating wood duck females and intruding hens (Bellrose & Holm 1994), although these escalated contests might also be a result

of host females vigorously defending their nest (and reproductive investment) from an intruding parasitic female.

Understanding the behavioral interactions among females at the nest site may be critical to resolving this debate – are females engaging in contests to secure the nest for their own use, or are females following different reproductive tactics with associated differences in their behavioral responses? In the past, direct observation of the interactions between females at a nest proved difficult “...since it requires individual identification of the females involved as well as constant observation of the nest.” (Yom-Tov 1980). Technological advancements such as smaller cameras, radio frequency identification devices (RFID), and passive integrated transponders (PIT tags) have allowed us to obtain more direct observations of interactions and identify individuals. In this study, we employ these technologies to provide detailed behavioral records of female-female interactions at nests. In doing so, we then test several hypotheses to account for variation in the pattern and intensity of these interactions within and among females.

Wood ducks as a model system

Wood ducks are among the most thoroughly studied waterfowl in North America that exhibit CBP (Andersson 1984). Wood duck nest in tree cavities in flooded hardwood forest bottomlands in the east and in riparian oak woodland habitat in the west. Females reach reproductive age one year after hatch and return to their natal area in search for suitable nest sites in early spring. Females typically lay only a single clutch of eggs in a breeding season with an average clutch size of 9-12 eggs. However, nest boxes have been found with a range of 7-31 eggs and an upward count of 40-50 eggs, revealing the existence of extensive parasitic nesting (Bellrose & Holm 1994, Eadie et al. 1998). Access to a nest site is a vital component for wood

ducks, yet suitable nest sites may be scarce. Nest box programs are widely used for populations of wood ducks and other cavity nesting birds where natural cavities are scarce or nonexistent. Many populations now depend on nest boxes, making them a critical resource for reproduction. Wood ducks exhibit several life history characteristics that may contribute to the prevalence of brood parasitic behavior (CBP) including having precocial young that require relatively little post-hatch parental care, large clutches, easily discoverable nests, lack of territorial defense, and natal philopatry (Andersson 1984, Andersson et al 2019).

Hypotheses - What Might Determine Level of Interaction?

Numerous hypotheses have been proposed to explain the prevalence and variation of CBP. Factors such as age, population density, ecological conditions, and personality have all been speculated to account for variation (Arnold & Owens 2002, Wolf & Weissing 2012, Lyon & Eadie 2017). In addition to individual variation, certain life history traits may be responsible for the prevalence of this behavior among avian species. Nesting in cavities, precocial young, natal philopatry, and strong social networks may all be important life history traits that contribute to the evolution of this behavior. Additionally, CBP may involve interactions and collaboration among kin (Andersson 2001). Waterfowl are unusual among birds in that it is the females, not the males, that are the philopatric sex and return to their natal area to breed. Accordingly, females in a population may be closely related. Andersson (2001) developed a theoretical model suggesting that parasitic interaction among kin might facilitate CBP – hosts might accept eggs of related females if by doing so their inclusive fitness is increased. In contrast, hosts might reject parasitic egg-laying by unrelated females and may defend their nest against intrusions. Though this idea has been popular, support has been mixed and often based on comparisons of patterns

of kinship among hosts and parasites (Andersson 2019). One study (Andersson & Ahlund 2015) examined interactions among females at nests using video recordings of common eiders (*Somateria mollissima*) and found that host females did respond differently to related versus unrelated “parasites”. However, there is still little quantitative data as evidence of variation in host response to intruding hens.

One approach to gain a deeper understanding of the context of these contests is to quantify the types of interactions that occur at a nest, identify the participants, and examine potential factors that may influence the intensity and outcomes of these interactions. We do so here, noting that we do not address the role of kinship in this study. Rather, we begin by documenting and exploring variation in contest behavior among wood duck females, and we test several hypotheses about the factors that might account for some of this variation. We view this as a necessary and informative first step to better understand the behavioral dynamics involved, beyond simple assays of relatedness.

Below, we list the hypotheses considered, the rationale underlying each hypothesis, and we propose alternative predictions where appropriate. We emphasize that these hypotheses are not mutually exclusive, and it is likely that several factors (i.e., age, status, body conditions) act in concert to shape a female’s response. At the risk of some redundancy, we consider each factor separately to explain the potential influence of each variable more clearly.

In the following analyses, we identify females as “owners” or “intruders”. Typically, the literature on CBP makes a distinction between “host” and “parasite”, or “primary” and “secondary” females. However, in doing so, there is an implicit assumption that the secondary females are acting as brood parasites, when that might not be the case (i.e., females may be

prospecting for nest sites). Accordingly, in this paper, we designate ownership using the term “owner” to the female that was recorded on the nest at time of hatch, and any other females are designated as “intruders”. For one hypothesis regarding reproductive strategy, we evaluate whether intruders are parasites as determined by evidence of laying an egg in the nest.

Hypothesis 1: Females vary in responsiveness and do so consistently

Bellrose and Holm (1994) reported several observations of females fighting and even killing each other, whereas other females have been reported to be tolerant and even co-incubate in the same nest. Despite these natural history reports, there is remarkably little direct observational or quantitative data. As a first step in our analyses, we tested the hypothesis that females do, indeed, vary in their responsiveness to the presence of another female in a nest box. Further, we explored whether females are consistent in their behavior. There is a large and growing literature showing that individuals respond in very different ways to the same stimuli and do so repeatedly. If wood duck females are in fact pursuing alternative reproductive trajectories (i.e., host, parasite, etc.), consistent differences in individual behavior at the nest might be expected.

Hypothesis 2: Variation in responsiveness is related to status

Prediction 2A: Owners will be more aggressive than intruders

Variation in behavior may be determined by the status of the female (owner vs. intruder). Intruders differ consistently in behavior from incubating females in common Goldeneye (Ahlund 2005) and may do so for other species. Owners may be more aggressive than intruding females as the nest site may be more valuable to the owner. The incubating female may also have access

to more information on the quality of the nest site and therefore a greater ability to defend the nest site (Semel & Sherman 2001, Briffa & Sneddon 2010). A greater investment by an owner in the nest would also reduce their ability to invest in future nests and so owners would obtain greater relative return on continued investment than leaving and searching for a new nest. Further, continued current investment in the nest means less additional investment needed to complete the nest (vs. starting over) and so owners again obtain greater relative return on continued investment than leaving. Owners may be protecting known assets versus the uncertainty over the availability and quality of alternative nest sites.

Prediction 2B: Intruders will be more aggressive than owners

In contrast, it is possible that intruders would be more aggressive than owners if the nest site is more valuable to the intruder. This may occur if the intruding hen does not have a nest site and has few other options. In a contest for a limited resource, an intruder may have to expend more effort to win an occupied nest and therefore aggressive behavior may be the only way to gain access to a nest site (Poysa et al. 2014). Alternatively, if intruders are acting specifically as a brood parasite, aggressive intrusions may be the only way to gain access to the nest and lay an egg in the presence of host defense.

Hypothesis 3: Variation in responsiveness is related to age/experience

Prediction 3A: Older females will be more aggressive than younger females

Age and experience may grant benefits to females in being able to compete successfully for a nest site. Older females perhaps have more experience and skill in nest defense as well as being better able to assess the value or history of a nest site. Further, older females have reduced

residual reproductive value (RRV) and so nests are more valuable as females age. Fewer future opportunities to reproduce may pressure older females to defend the resources they do have more aggressively.

Prediction 3B: Younger females will be more aggressive than older females

If younger females are attempting to take over a nest, they may have to fight harder to succeed. Wood ducks in our study population live, on average, to two or three years of age. Therefore, an average female may only have one or two chances to breed. The current opportunity will be more valuable than any uncertain future opportunity so young females may be more willing to invest heavily in fighting for a nest at an early age, especially if early success/ownership confers any future advantages.

Early life conditions might influence a female's phenotype which can in turn affect how that female allocates investments in nesting (Brockmann 2001, Lyon & Eadie 2017).

Individuals operate in a "risk-reward trade-off". Higher risks yield more rewards and vice versa. This accounts for certain individuals being bolder, typically reproducing at a younger age. Other individuals may remain cautious and may hold off on reproducing. Bold individuals are more risk prone while cautious individuals are risk averse (Sih et al. 2020). This could relate to the dynamics of behaviors of younger and older individuals interacting with one another within a population as well.

Hypothesis 4: Variation in responsiveness is related to body size, phenotype, and resource holding potential (RHP)

Prediction 4A: Larger females in better condition will be more aggressive than younger females

Body size is known to typically play a role in the outcome of interactions in the context of competition (Briffa & Sneddon 2010). Larger females are expected to be in better condition. Being in better condition could lead the hen to have greater ability, resources, and RHP than smaller counterparts and so these hens may be more capable of being aggressive when it comes to defending the nest.

Prediction 4B: Smaller females in lower condition will be more aggressive

Smaller females or those in poor condition may have limited options and may be making the best of a bad situation by going “all-in” if the chances of success are greater than the likelihood of surviving to reproduce in a future year (Lyon & Eadie 2008). Accordingly, smaller females in poor condition may be more aggressive as a last chance at reproducing.

The intensity of interaction between an intruding hen and the owner may depend on the RHP of the females and the perceived resource value (RV). Resource value can vary between old and young individuals, between owner and intruder, or by body size (Briffa & Sneddon 2010). Age, body size, body condition, breeding experience, or other biological characteristics may be factors of variation. Size variation has been shown to strongly select for the emergence of alternative reproductive tactics of male competitors among major animal taxa (Engqvist & Taborsky 2016).

Hypothesis 5: Variation in responsiveness is related to history/previous ownership

Prediction 5A: Females with prior ownership will defend the nest more aggressively than new females

Females that have history at a nest site might be able to defend the nest more aggressively than newer females. Previous ownership confers advantages in prior knowledge of the site, familiarity, and quality of the resource that may enhance the ability to defend the nest site successfully (Briffa & Sneddon 2010). Therefore, the nest site might be more valuable to owners.

Prediction 5B: Prior ownership conveys no advantage

On the other hand, ownership may hold no advantage and may not correlate with behavior as contests may not be determined based on the previously stated factors and may only be dependent on the current characteristics of the interacting females.

Hypothesis 6: Variation in responsiveness is related to nest state and timing

Prediction 6A: Owners will be more aggressive during egg-laying

Timing of an interaction in relation to the nest state may influence the intensity of a contest. Owners may be more aggressive during egg-laying if there is a greater cost of caring for eggs for a longer period. If more eggs are added during the egg-laying period, this could also reduce hatching success due to less efficient incubation. Additionally, more offspring may reduce the effectiveness of parental care (Arnold & Owens 2002).

Prediction 6B: Owners will be more aggressive during incubation

Conversely, owners may be more aggressive during incubation, as additional eggs that are asynchronous to the rest of the nest may impact the development of the owner's eggs and incubation efficiency. Once committed to incubation, the option to abandon and lay eggs

elsewhere is limited. Increased investment, and therefore defense, of the nest would have a higher payoff relative to accepting a larger egg load by not responding defensively.

Hypothesis 7: Variation in responsiveness is related to female reproductive strategy

Prediction 7A: Hosts will be more aggressive than parasites

Though akin to our hypothesis for status (Hypothesis 2), here we are explicitly assuming that intruders are acting as brood parasites by laying eggs in the nest of owners who become hosts. In this case, parasitic intrusions represent a potential cost to the host and so the intensity of host-parasite contests may be magnified. If there are high costs of parasitism (extra eggs and additional parental care costs), then hosts might be expected to defend their nests more aggressively to avoid being parasitized (Sorenson 1997).

Prediction 7B: Parasites will be more aggressive than hosts

If intruders are seeking access to a nest in which to lay an egg, levels of aggressive intrusion might also be higher if this is the only pathway to gain access to reproduce. If intruders are not investing in parental care elsewhere, they may be re-allocating the energy they would have used to gain access to other nests.

Prediction 7C: Interactions between host and parasites will vary among individual pairs

The level of interaction between hosts and parasites may be highly dependent on the individual pairs. This could be due to individuals differing in personality or in the risks they are willing to take in a contest (Sih 2020). It might also be due to familiarity of females with each other, relating again to the possibility of kinship among some pairs of females. We will evaluate

the kinship hypothesis in a future study (data not yet available), but a correlated prediction of the kinship hypothesis is that hosts would be aggressive to some females (presumably unrelated) but not to others (presumably kin). Finding such a pattern is a necessary precursor to testing the kinship hypothesis once relatedness estimates are available. If there is no variation among pairs of females, this would weaken the kinship hypothesis.

As noted, these hypotheses are not mutually exclusive. These factors might be correlated, and more than one factor may influence the pattern and intensity of female interactions at a nest. Our goal is to test each of these hypotheses to evaluate the overarching hypothesis that aggressiveness and contest behavior among female wood ducks at a nest site varies among females and can be predicted by one or more of the factors described. A summary of these hypotheses can be found in Table 1.

Table 1: Summary of hypotheses on factors that could account for variation in contest behavior among female wood ducks

Hypothesis 1: Females vary in responsiveness and do so consistently

Hypothesis 2: Variation in responsiveness is related to status

Hypothesis 3: Variation in responsiveness is related to age/experience

Hypothesis 4: Variation in responsiveness is related to body size, phenotype, and resource holding potential (RHP)

Hypothesis 5: Variation in responsiveness is related to history with the nest/previous ownership

Hypothesis 6: Variation in responsiveness is related to nest state and timing

Hypothesis 7: Variation in responsiveness is related to female reproductive strategy

Technological Advancements

Nest cameras are efficient and effective tools that can be used to collect accurate and detailed observational data and allow us to gain more insight on the behavior, life-history, and ecology of a species. Video surveillance has been used in many studies and provides an invaluable opportunity for data collection (Semel & Sherman 2001, Ahlund 2005, Andersson 2015). Video files can be referred to repeatedly, human error can be reduced and collected data can be re-analyzed after the fact. Nest cameras have been used to document predation and nesting behavior of various bird species, however, only relatively few of these studies have focused on waterfowl and even less in cavity nesting species (Ellis-Felege & Carroll, 2012). In addition to the benefits of using cameras to monitor behavior, RFID has become more applicable and commercial for use in the biological sciences (Bridge et al. 2019). These technologies have allowed for identification of individuals and near constant observation desired for evidence to study this behavior.

By placing motion sensor cameras within nest boxes for a population of wood duck hens fitted with PIT tags we focus on observing the level of intensity of interactions between nesting females. RFID will be used to identify individuals by their PIT ID and keep track of the timing of hens entering and leaving nest boxes. Interactions of females will be scored overall along with individual response scores given to hens involved in the interaction. Characteristics and demographics of hens that are interacting will then be analyzed for correlations that may explain the variation in behavior among these interactions.

Methods

Location & Study Area

Roosevelt Ranch (Figure 1) is a 2,700-acre privately owned wetland located in Zamora, California that provides accommodations for recreational activities such as birdwatching and hunting. 100 nest boxes for breeding wood duck are placed annually at designated locations marked by poles. 50 boxes are placed in ‘high density’ arrangements, and 50 boxes in ‘low density’ arrangements.



Figure 1. Aerial photograph of Roosevelt Ranch, Zamora, CA provided by Google Earth. The property borders are highlighted in yellow. Figure on right shows locations of nest boxes in both low- and high-density configurations.

Females are weighed (net weight in grams) and measured (culmen, tarsus, wing, and eye patch length and width) after two weeks of incubation for each nesting season. Ducklings are implanted with PIT tags subcutaneously in the region of the back between the scapula (Bridge et al. 2019). Blood samples are also collected from hens and ducklings for genetic analysis.

Cameras

During the field season (March 2020-July 2020) up to 17 Apeman H68 Trail Cameras were deployed across the study area. Cameras were fixed on the inside lid of nest boxes once eggs were found within the nest box. If feather down was placed by a female before a camera had been set up, that nest was bypassed since important interactions might have been missed at the beginning of laying. Cameras were kept on nests until the end of the first week of incubation. Videos started recording once motion was detected at 30 second intervals with 15 seconds between each recording at 1920x1080p resolution and saved on SD cards (64GB). Cameras were removed if there was no activity recorded after a week. If no new nests were found, cameras were left on active nests for the duration. Batteries and SD cards were changed every 3-4 days. A divider was slipped between the box and the removable lid if the incubating female was present when removing the camera or changing the batteries or SD card.

RFID and PIT tags

Radio-frequency identification devices (RFID) have been attached to nest boxes at Roosevelt Ranch since 2014. All boxes are outfitted with RFID readers attached to the side of the nest box within water-tight plastic boxes. Hand-wrapped, plasti-dipped, tuned antennas were fixed around the nest entrance with zip ties. When a PIT-tagged hen moves through the entrance, the PIT ID code, date, and time are stored on an SD card. RFID readers are powered by 12V batteries housed in a plastic box on the ground and recharged with an attached solar panel (Bridge et al. 2019). The functionality and upkeep of the RFID readers are checked routinely throughout the nesting season to maintain accuracy. Timestamps for cameras and RFID were synchronized with a 30 second margin of error on average.

Classification of Females

Data from previous and current years are archived on FileMaker Pro 18. Age and incubation status was determined by searching individual PIT IDs. Hens that were tagged as ducklings in 2019 were classified as hatch year (HY) hens, those that were PIT tagged as ducklings or captured as adults in previous years were classified as after hatch year (AHY) birds. Hens that were captured in 2020 without a USGS band or PIT tag were classified as unknown (UNK) age. Females were classified as owners by being present at the nest box at time of hatch. All other females were labeled as intruders for that nest. Intruding hens that were recorded on camera but showed no PIT reading (untagged) were also classified as UNK for age and incubation status. Females that are strictly parasites were not captured for measurements and therefore no demographic information was collected for them for this year.

Behavioral Observations

Raw video footage was sorted through to find instances of activity between two or more females. The video clips (30 seconds each) were compiled into an individual video file for observation. Interactions that had a clear start and end time and clear corresponding time stamps for the camera and RFID readings were chosen for analysis. FileMaker was consulted to discover which female had incubated the nest at hatch to determine the owner. Owners and intruders were given a score for the first 30 seconds of the interaction and response overall. The intensity of the interaction was also categorized. Response and intensity scores were given based on the following criteria (Table 2). For this thesis, one observer reviewed all videos and assigned response scores. To validate the scoring, a second observer independently scored 14 videos (See Study Limitations).

Table 2. Scoring criteria for each interaction. Descriptions of scores corresponding to owners and intruders on a scale of 0 to 5, and scores of the overall intensity of the interaction on a scale of 1-5 are shown.

Response Scores

Owner

0	The owner does not engage with or contact the intruder regardless of engagement from intruder (shoving, adjusting substrate, or adjusting eggs)
1	The owner does not engage with the intruder for most of the duration with little to no engagement from intruder (shoving, adjusting substrate, or adjusting eggs)
2	The owner initially engages with the intruder before disengaging and not re-engaging. Engagement typically occurs on intruder entering or exiting.
3	The owner occasionally engages with the intruder with obvious breaks in between interaction to adjust substrate or preen. May loudly call but not only when engaging.
4	The owner repeatedly and diligently engages with the intruder while calling but engagement is not enough to move intruder.
5	The owner constantly engages with the intruder with sustained movement of both individuals. Owner may be loudly calling while obviously biting and/or tugging at the other hen.

Intruder

0	The intruder actively avoids or tries to move away from owner
1	The intruder does not engage with or contact the owner regardless of engagement from owner (shoving, adjusting substrate, or adjusting eggs)
2	The intruder initially engages with the owner before disengaging and not re-engaging. Engagement typically occurs on intruder entering or exiting.
3	The intruder occasionally engages with the owner with obvious breaks in between interaction to adjust substrate or preen.
4	The intruder repeatedly and actively engages with the owner while calling.
5	The intruder constantly engages with the owner with sustained movement of both individuals. Owner may be loudly calling while obviously biting and/or tugging at the other hen.

Intensity

1 - Very Low	No interaction at all; completely ignore each other
2- Low	Minimal interaction, some passive response, moving, nibble, not aggressive
3- Medium	Continual interaction but not very aggressive; calling and bill probing; clearly responding to each other but not a fight
4- High	Constant aggression by host (biting, calling); parasite may not interact or may actively move to avoid
5- Very High	Full out fight and scramble, pulling aggressively on feathers, loud calling

Time budgets of the actions and vocalizations of each hen in an interaction (Table 3, Figure 2) were observed and recorded using an instantaneous sampling rule every 10 seconds for the full period of the observation. The frequency and proportion of each behavior was then calculated for each hen (Table 3).

Table 3. Descriptions of behaviors recorded for time budget observations

Fight	Full fight, squealing, pulling feathers, biting hard, wrestling, pulling at tail or head feathers
Bite	Biting hard at the other female, pulling feathers but not a full-on hyperactive fight
Bill Jab	Probing or jabbing at another female; may not be hard or aggressive
Nibble	Light almost preening nibble at head (around eye, bill, or body) of another female; not aggressive
Preen self	Preening own feathers; may be a displacement activity or just nonresponsive to another female
Sit	Sitting still (often quiet, although calling may occur); not actively moving or turning away
Move	Moving and turning in nest, may be moving away, towards or even under another female
Adjust nest	Either female adjust nest material or eggs with bill; may be under another female
Avoid	Facing away, hiding head, turning body, may actively move away or just “hunker”
Loud call	Calls may co-occur with any of the above. Loud calls are ‘Oooeek’ squeals; very active calls
Soft call	Softer calls, but could also be “whimper” like calls; not loud

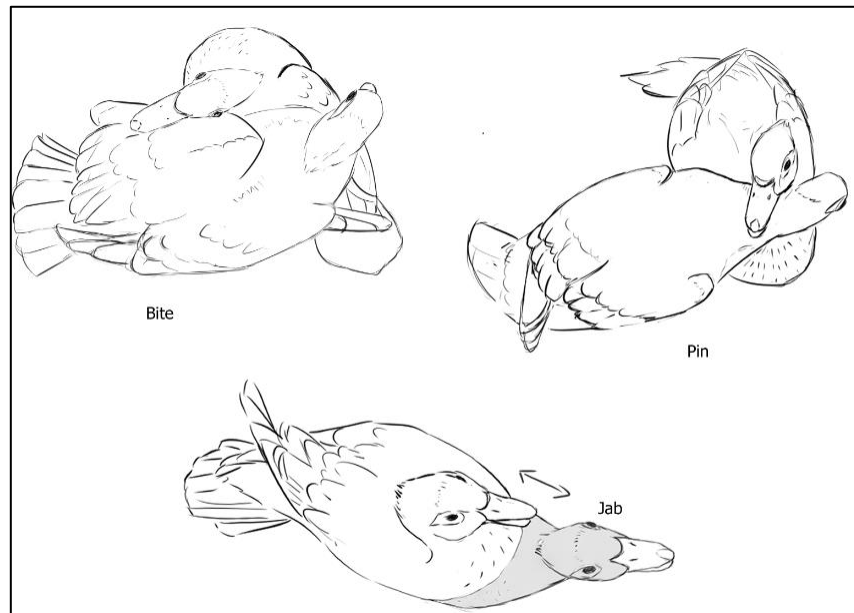


Figure 2. Illustration of the position and posture of each hen showing *Bite* (upper left), *Pin* (upper right) and *Jab* (bottom) behaviors.

In addition to ranking the hen's behaviors and the intensity of each interaction, videos were also observed to determine if an egg was laid by the intruder (indicating that the female was a parasite). An egg was confirmed to have been laid by noting a female's pelvic depressions or the appearance of an egg from the female after 5 minutes.

Analyses

We considered three response variables in our analyses: *1st 30s* – the response score (0-5, Table 2) for the first 30 seconds of interaction determined for the owners and intruders separately; *Overall Response* – the overall response score (0-5, Table 2) for the entire period of observation determined for the owners and intruders separately; and *Intensity* – the intensity score (1-5, Table 2) for the interacting pair over the entire period of observation. Intensity is a

property based on the pair and so is not determined separately for the owners and intruders. Intensity classifications were converted to numerical values to allow for analysis of continuous variables (1- very low to 5- very high).

Every female was identified individually by her PIT tag code. Interactions for each unique pairing of two individuals were given an individual 'pair number' (1-49) for analysis.

We examined how response variables varied among females and among pairs using two sets of analyses. For most analyses we considered each recorded interaction as an independent observation, but we controlled for repeated observations of the same female and the same pair of females by considering female ID and pair ID as random effects in statistical models. In seven observations, one of the females was untagged and we could not assign identity to those females; accordingly, those observations were excluded in any models with female ID as a random effect. In a smaller number of other analyses, we were interested in the specific pairs of females. In most cases, we had only a single observation for a specific pair (39 of 49 observations of pairs), while for six pairs we had two observations and for four pairs we had three observations. In analyses of pairs, we either included the pair ID as a random effect or we calculated the average of each response variable for pairs with multiple observations such that each unique pair comprised a single data point.

All statistical analyses were conducted using JMP Pro 16.2 (2021 SAS Institute Inc). For analyses where we examined the influence of female status, age, weight, prior experience, or timing, we treated these as main effects in linear mixed models fitted using restricted maximum likelihood (REML) and included female ID and pair ID as random effects. We tested distribution of response variables using goodness-of-fit tests and accessed best fit using the smallest AIC_c value. For all three response variables, the data were better fit with a normal 2 mixture model

than a simple normal distribution. However, we show below that was largely influenced by difference in female status, a variable we were particularly interested in, and so we continued parametric analyses of these responses cautioning that statistical inferences and p-values should be viewed cautiously. Because of the non-normality of the response scores, we repeated many of these analyses using simple non-parametric Wilcoxon Rank tests. In almost all cases, the assessment of the main effects in these analyses mirrored those using the linear mixed models and so we are confident in the overall outcomes of our analyses.

There were 12 owners and 15 intruders that were recorded more than once. We also determined the intraclass correlation coefficient (ICC) using the Measurement System Analysis function in JMP 16 Pro.

Results

A total of 86 of the 100 nest boxes were occupied in the 2020 nesting season. Cameras were placed on 65 of these boxes, 21 of which (12 low density 9 high density) recorded interactions between two or more hens, resulting in 60 videos and a total of 42 individually PIT tagged hens. This yielded a total of 126 observations – one for each female in an interaction – for 49 unique pairs of females.

The results are presented in two sections. We first explore the variation among females in their behavior when encountering a second female on a nest and we focus on the influence of female role or status (owner or intruder). In the second set of analyses, we examine contextual and phenotypic factors that might influence these interactions.

I. Variation among females

The intensity of interactions among females was highly variable

We observed a large range in the intensity of interactions among females when a second hen entered the nest. Some interactions occurred with very high intensity while others were low intensity (Figure 3). Of the recorded interactions, most were low intensity (31%) with 22% of interactions classified as high intensity, 19% medium intensity, 17% very high intensity, and 11% very low intensity (Figure 3). The pattern was bimodal, and a normal 2-mixture distribution fit the data better than a simple normal distribution ($\Delta AIC_c=13.7$).

Females differed individually and consistently in their level of response/aggression

A similar pattern emerged when we examined the responses of individual females, with some females being highly aggressive (scores > 4) and others being quite passive and not aggressive (scores 0 or 1). This was true both for the first 30 seconds of an interaction (1st 30s) as well as for the overall response (Figure 4). Again, there was a strong bimodal pattern, and a normal 2-mixture fit was much better than a simple normal distribution (1st 30s: $\Delta AIC_c=75.8$; Overall Response: $\Delta AIC_c=36.1$). In the first 30 seconds, most females scored a 1 or 0 while others scored a 3 or more (Figure 4, top). A similar trend is observed for overall response with many females scoring a 1 and others a response of 3 or more (Figure 4, bottom).

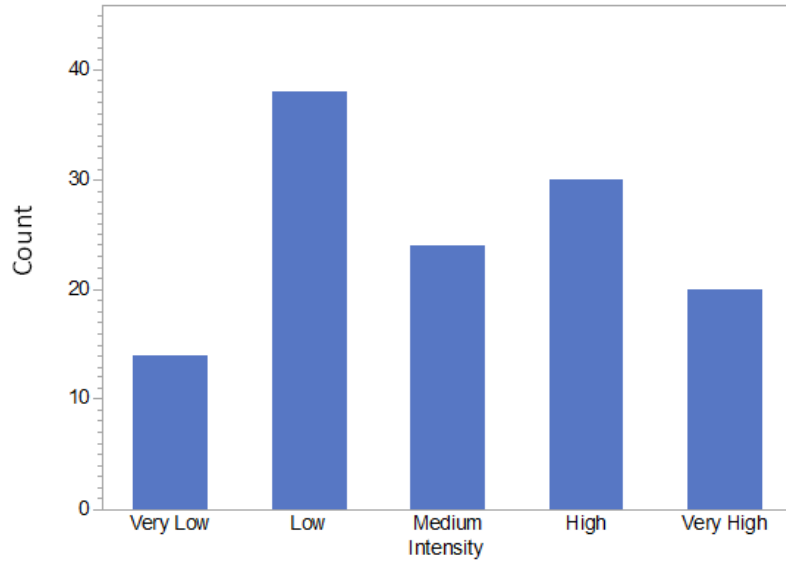


Figure 3. Frequency of the intensity of interactions among females when an intruder entered a nest.

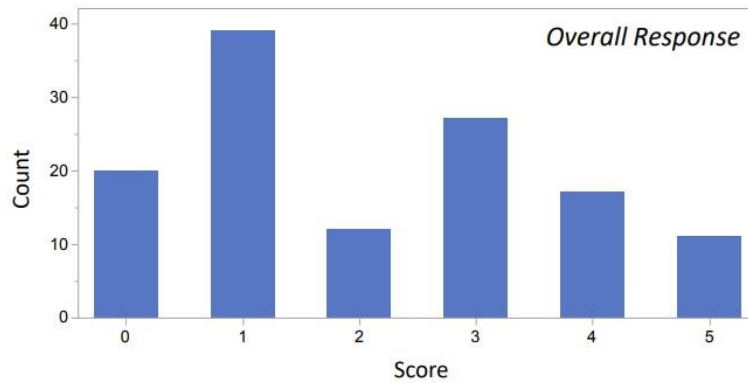
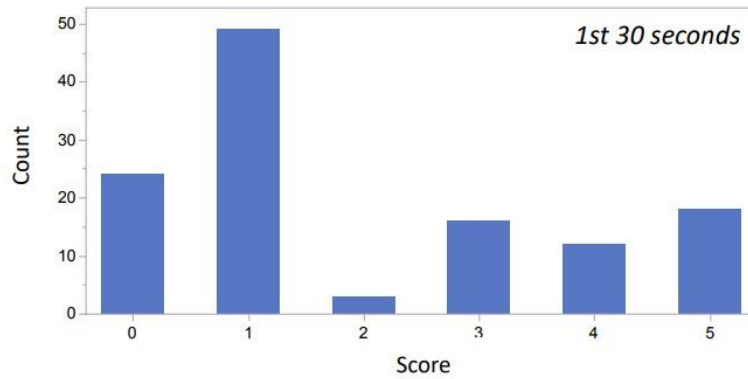


Figure 4. Variation among females in level of responsiveness/aggression (response score) in the first 30 seconds (top) and overall response (bottom).

Individual females differed and were consistent in their responses. Scores for the first 30 seconds differed significantly among females ($F=5.90$, $n=25$, $P<0.0001$, $R^2_{adj}=0.55$) and were highly repeatable (intraclass correlation coefficient $ICC=0.53$). Likewise, overall response scores were significantly different among females ($F=4.91$, $n=26$, $P<0.0001$, $R^2_{adj}=0.49$) and highly repeatable ($ICC=0.47$). Among all females, scores during the first 30s were correlated with overall response scores ($R^2_{adj}=0.49$, $N=115$, $F=303.2$, $P<0.0001$; Figure 5) indicating that both scores measure similar levels of response, although the scores were not identical. Most females had the same level of response in the first thirty seconds and overall. There were about the same number of cases of females starting with a higher score in the first 30 seconds and ending with a lower score overall and some females scored low in the first 30 seconds but by the end of the observation period, their level of response increased. Most response scores only changed by one point with a few exceptions (for example, see the cluster of 13 observations with a score of 1 for *1st 30s* and a score of 3 for *overall response* in Figure 5), suggesting that occasionally an increase in activity and/or response occurred as the interaction continued.

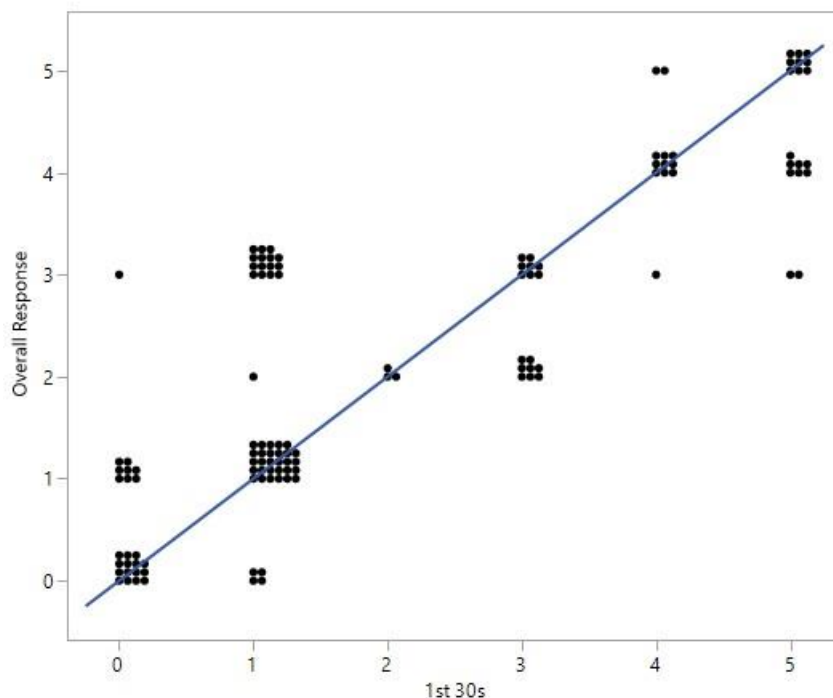


Figure 5. The relationship between scores for the first 30 seconds of an interaction and overall response for the full observation period for individual females. The blue line is the 1:1 slope for 1st30s and overall response.

Variation in responsiveness is strongly related to status (owner/intruder)

The status of a female (owner or intruder) had a very strong influence on how females respond to each other when interacting on the nest and this was consistent throughout several analyses. Overall, we found females classified as owners were significantly more aggressive than intruders (Figure 6, Table 4). Owners scored a 3 on average while intruders scored a 1. The range of owner response spread from scores 0-5 while intruder response score was restricted to 0, 1, or 3. There was no instance of an intruder with a score of 2 or higher than a score of 3 (Figure 6).

Table 4. REML linear mixed models to examine the effect of status on female response scores. Random effects included female identity and pair number.

a) 1st 30 Seconds

Predictor Parameter Estimates

Term	Estimate	SE	df	t Ratio	95% Lower	95% Upper	P
Intercept	1.903	0.145	30.17	13.15	1.608	2.198	0.61
Status	1.307	0.127	84.53	10.23	1.055	1.562	<0.0001

Random Effects Variance Component Estimates

Variance Component	Variance Component	SE	95% Lower	95% Upper	Wald p-Value	Percent of Total
Female	0.476	0.191	0.102	0.851	0.013	34.89
Pair Number	-0.047	0.085	-0.214	0.120	0.589	0.000
Residual	0.889	0.173	0.628	1.135		65.11
Total	1.365	0.215	1.025	1.907		100.0

b) Overall Rank

Predictor Parameter Estimates

Term	Estimate	SE	df	t Ratio	95% Lower	95% Upper	P
Intercept	2.077	0.145	25.61	14.37	1.780	2.374	<0.0001
Status	0.966	0.139	86.18	6.95	0.689	1.242	<0.0001

Random Effects Variance Component Estimate

Variance Component	Variance Component	SE	95% Lower	95% Upper	Wald p-Value	Percent of Total
Female	0.495	0.212	0.079	0.911	0.0198	29.37
Pair Number	-0.178	0.061	-0.299	-0.058	0.0037	0.000
Residual	1.190	0.203	0.875	1.714		70.63
Total	1.685	0.248	1.287	2.303		100.0

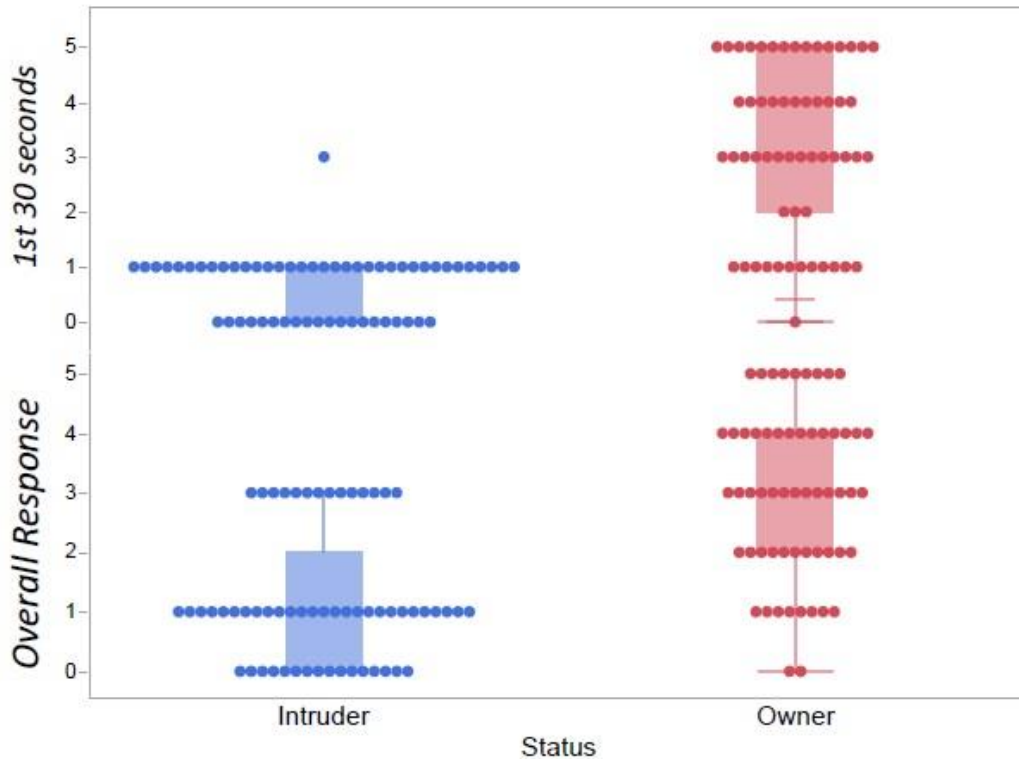


Figure 6. Response scores of intruders and owners in the first 30 seconds of an interaction (top) and the overall response for the full observation period (bottom). Scores range from 0 (low) to 5 (high). Box plots show the interquartile range (IQR) from the 25th to 75th quartile, vertical whiskers are 1.5*IQR, and individual points are shown for each female in each status class (intruder and owner).

Intruders rarely (once) scored higher than 1 within the first thirty seconds (Least Square Mean (LSM)= 0.59 ± 0.19 SE) and none scored above 3 (LSM= 1.12 ± 0.20). Owners varied more but still consistently scored higher in the first thirty seconds (LSM= 3.21 ± 0.20) and overall (LSM= 3.02 ± 0.21).

Analyses of the effect of status were conducted using REML linear mixed models with status as the main effect and females and pair ID as random effects (see methods). Untagged females were excluded. Status (owner or intruder) had a strong and significant effect on female

response for both the 1st 30s ($F_{1, 84.53}=104.6$, $P < 0.0001$, $R^2_{adj}=0.757$) and the overall response ($F_{1, 86.18}=48.28$, $P < 0.0001$, $R^2_{adj}=0.526$; Table 4). Female identity accounted for 34.9% of total variation in response in the 1st 30s and 29.4% of variation in overall response, whereas pair identity, although significant, account for a tiny fraction of the variation.

Because of the non-normal distribution of the response scores, we repeated these analyses using non-parametric Wilcoxon rank tests and the results were unchanged. Response scores of owners were significantly higher than intruders in both the 1st 30s (Wilcoxon rank test S statistic 872.5, $P < 0.0001$) and overall response (Wilcoxon rank test S statistic=763.0, $P < 0.0001$). To control for possible differences among pairs, we also analyzed the direct contrast between owner and intruder within each pair using paired t-tests. In the first 30 seconds, owners averaged a score of 3.31 while intruders averaged a score of 1.16 (paired t-value 10.51, $N=61$, $P < 0.0001$). For the overall response, owners averaged a score of 3.08, while intruders averaged a score of 0.66 (paired t-value 6.92, $N=63$, $p=0.0001$). Untagged females were included in this analysis since we used pairwise comparisons instead of ID/PIT tag. All the above analyses clearly demonstrate strong and significant differences in the response of owners and intruders, with owners being much more aggressive while intruders were passive and avoided aggression by the owners.

Time budget analysis revealed further variation among owners and intruder

More detailed analysis of the different behaviors expressed by owners and intruders (Table 3) also revealed striking differences. Mean behaviors in the time budgets of owners were Bite (31%), Sit (29%), and Nibble (16%). Adjust Nest (8%), Preen Self (8%), and Bill Jab (4%). Fight and Move accounted for only 2% of time budget behavior on average. Pin was below 1% of the time budget behavior and Avoid was a behavior that was not present in owner time budgets (Figure 7). In contrast, the mean proportion time budget of intruders was dominated by Sit (56%). Avoid (17%) and Move (13%) accounted for more than a quarter of intruder time budgets on average. Adjust Nest (7%) and Pin (4%) were also relatively represented in intruder time budget behavior. Nibble accounted for 2% of time budget behavior for intruders while Fight accounted for 1%. Bill Jab and Preen Self were below 1% of time budget behavior for intruders while Bite was a behavior that was not present in intruder time budgets (Figure 7).

The difference in the behavioral time budgets of owners and intruders is best contrasted by calculating the differences in the proportion of time for each behavior among intruders and owners (intruder proportions were subtracted from owner proportions; Figure 8). Bite, Nibble, Preen Self, Bill Jab, and Fight, were heavily weighted as owner behavior on average. Sit, Avoid, Move, and Pin were weighted overall as intruder behavior. Adjust Nest was represented for both owner and intruder (Figure 8).

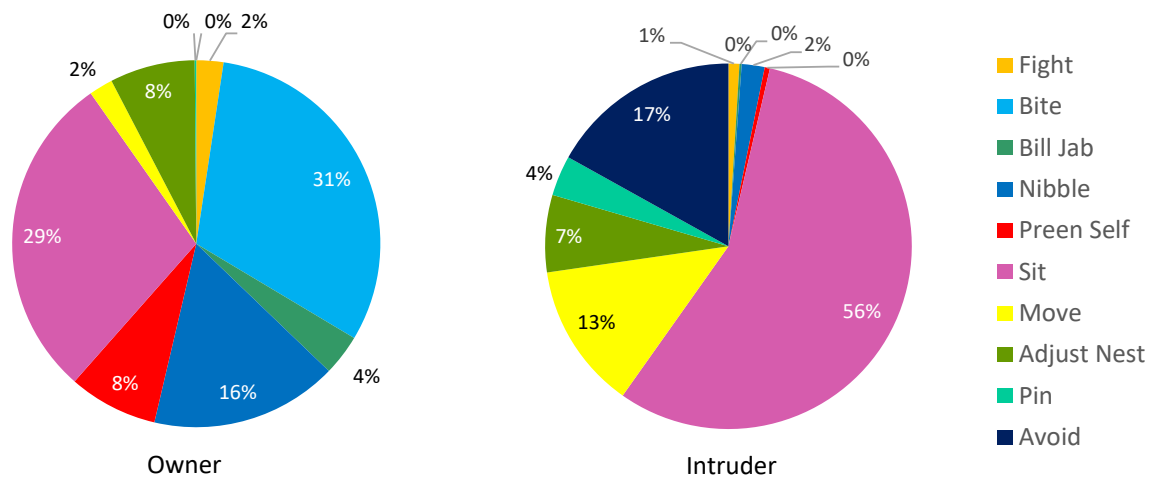


Figure 7. Mean proportions for time budgets of behaviors expressed by owners (left) and intruders (right). Activities are described in Table 3.

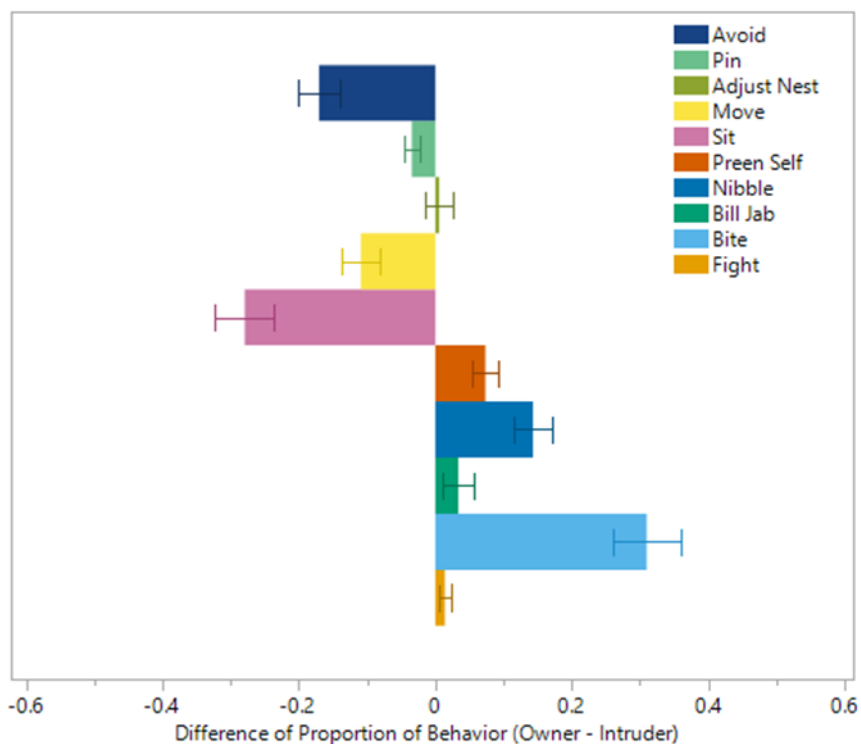


Figure 8. Differences in the mean proportion of time spent in each behavior by owners and intruders. Positive values indicate that owners spent more time in that activity, negative values indicate that intruders spent a greater proportion of time in that activity.

Status also influenced consistency of female responses

Our analyses (Table 4) revealed that females differed significantly and repeatedly from each other when we did not consider the effects of status and all females were included (excluding untagged). However, a different picture emerges when we analyzed the variation and consistency of female responses for owners and intruders separately. Owners varied more and did so repeatedly while intruders varied much less (Table 5). For both response measures (1st 30s, overall response), there were significant differences among females for owners, but not for intruders. Likewise, repeatability was high for both response variables for owners (0.50 – 0.63), but close to zero for both measures for intruders. Adjusted R² values indicate that individual variation accounts for 45% to 58% of the variation in owner scores, but effectively none of the variation in intruder scores (Table 5).

Table 5. Summary of analyses to examine among-female differences and repeatability of responses when owners and intruders are considered separately. Untagged females were excluded from this analysis.

	F ratio	df	p value	adjusted	ICC
Owner Only					
1st 30 seconds	3.48	20, 60	0.0004	0.452	0.531
Overall Response	5.19	20, 62	<0.0001	0.575	0.649
Intruder Only					
1st 30 seconds	0.90	26, 53	>0.60	-0.052	0
Overall Response	0.76	26, 55	>0.75	-0.130	0

Visual examination of response scores for each female helps to explain these differences (Figure 9). As noted, scores of intruders were low while scores of owners were high. Moreover, there was little variation among intruder females with most averaging a score of 1 over the entire observation (this is even more apparent when only the first 30 seconds are considered). In contrast, scores of owners ranged from 1 to 5, although individual females appeared quite consistent (indicated by small standard errors for females with repeated observations in Figure 9). Thus, it appears that the largest source of individual difference is from the differences between owners and intruders. It is the response of owners, not intruders, that underlies much of the variation observed among female interactions in our population of wood ducks.

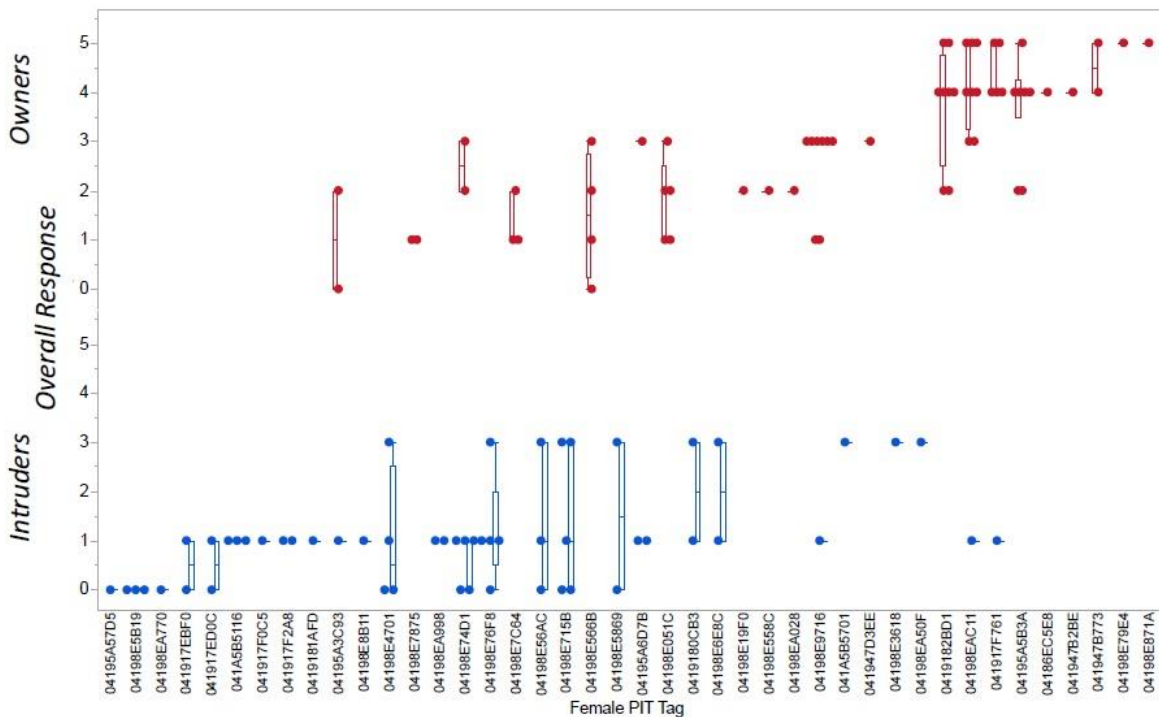


Figure 9. Variation in overall response among individual females identified by their unique PIT tag code. Owners (top) varied more and were more consistent than intruders (bottom). Points show the response score for individual females in each interaction with box plots where there was more than one interaction recorded for a given female.

Although owners appear to be more consistent in their response, we found that this is highly context specific. Six owners (04195A3C93, 04198E74D1, 04195A6D7B, 04198E9716, 041917F761, 04198EAC11) were also recorded acting as intruders at another nest and when they did so, their behavioral response changed dramatically (Figure 10). When acting as an owner, these hens typically scored a rank of 2 or higher, but when they were acting as an intruder at other nests, they all scored 0 or 1. In the first thirty seconds, their score when in owner status was 3.70 ± 0.24 SE but when in intruder status, their score dropped to 0.60 ± 0.37 SE (t-test, $t=7.08$, $df=31$, $P<0.0001$; Wilcoxon rank test, $Z=3.35$, $P<0.0001$). Considering their overall rank score, when acting as owners their score was 3.36 ± 0.22 SE while when acting as an intruder their score was 0.82 ± 0.32 SE ($t=6.35$, $df=34$, $P<0.0001$; Wilcoxon rank test $Z=3.35$, $P<0.0001$). These results show clearly that females adopt different behavioral responses when in different roles and that even if aggressive as owners, they become passive and non-aggressive when acting as an intruder (Figure 10). These results are consistent with the hypothesis that intruding behavior is not simply a case of females in equal roles competing for a nest site and that both inter-female variability and status/role strongly influence female behavior.

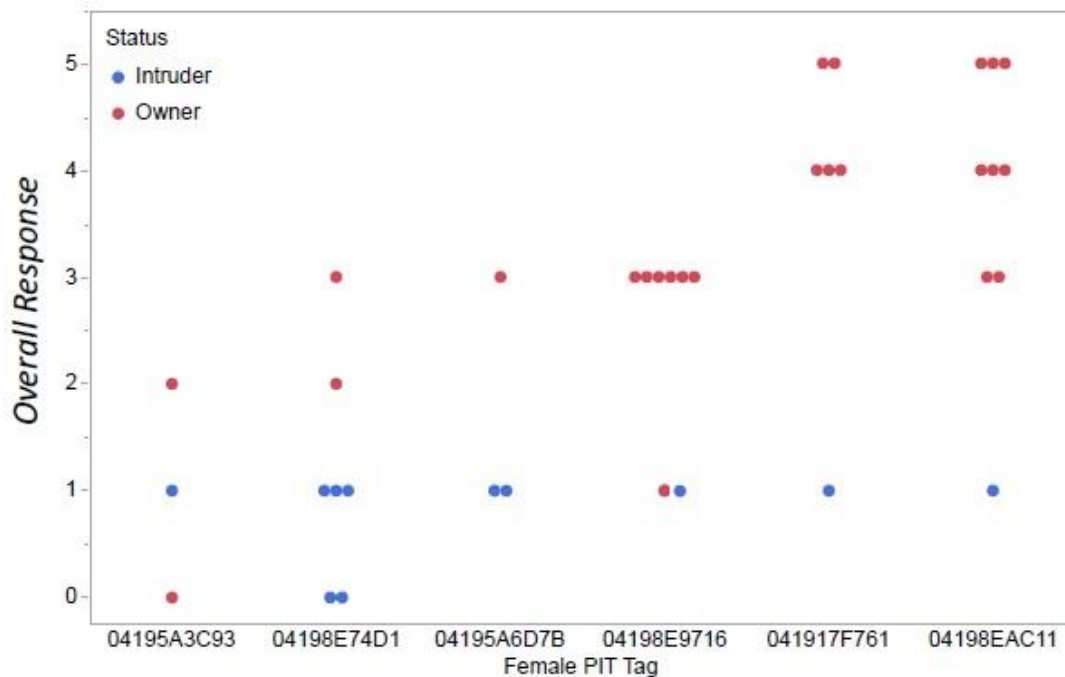


Figure 10. Overall response scores for the six females that acted both as owners and as intruders. Each point represents a different observation for each female when acting as an owner (red) or intruder (blue).

Owner response and intruder response were negatively correlated

Owner and intruder responses were further influenced by the reaction of the other hen. When we compared owners and intruders in pairwise interactions, there was a significant negative relationship between intruder and owner response both in the first 30 seconds ($R^2_{adj}=0.468$, $N=61$, $P<0.0001$) and the overall response ($R^2_{adj}=0.303$ $N=63$, $P<0.0001$; Figure 11). The most frequent owner-intruder response combinations were (O= owner I= intruder) O5-I0, O4-I0, O4-I1, O3-I1, and O2-I1. When owners respond aggressively (score of 4 or 5) overall, intruders tend to score low (0 or 1). However, intruders in some instances had a higher score than owners O0-I3, O1-I3, O2-I3 (Figure 11).

If the owner was overall non-aggressive in response (score of 1-3), the intruder scored a 1 or 3. In some of these interactions, the owner left the nest box while the intruder was still present. In many of these instances, the intruder was seen nudging the owner (causing the owner to move), moving her head under the owner to move the eggs with her bill, and/or nibbling the owner while the owner remained relatively inactive.

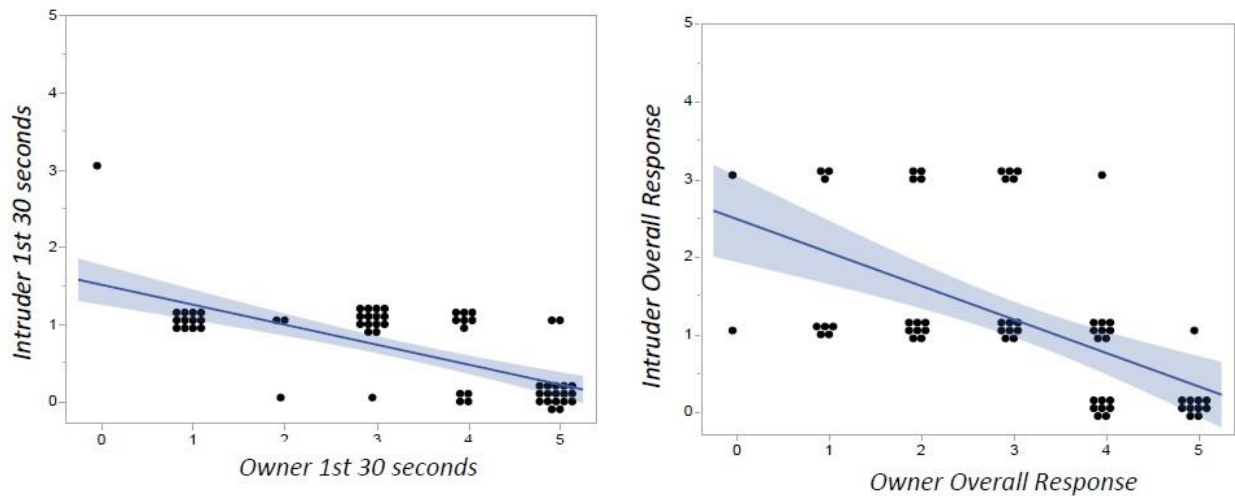


Figure 11. The response of owners and intruders were negatively related both in the first 30 seconds (left) and overall (right). Each point represents a separate interaction between an owner and intruder. A simple linear regression line with 95% CI (shaded) is shown.

The intensity of the interaction was influenced strongly by owner response, less so by intruder response

The overall intensity of each interaction (Figure 12) was strongly and positively correlated with owner response ($R^2_{adj}=0.84$, $n=63$, $P<0.0001$), but negatively correlated with intruder response ($R^2_{adj}=0.26$, $n=63$, $P<0.0001$). When both owner and intruder response were included in the same model, owner response remained strongly influential on the intensity of the interaction (estimate = 0.834 ± 0.06 SE, $F_{1,62}=208.8$, $P<0.0001$) whereas the influence of intruder

response was small and statistically insignificant (estimate = -0.02 ± 0.08 SE, $F_{1,62}=0.045$, $P>0.80$). Hence, when owners are more aggressive, the overall intensity of interactions increases, whereas when intruders become more aggressive, the intensity of interactions decreases. These results indicate that owners and intruders jointly adjust their behaviors in response to the actions of the other female.

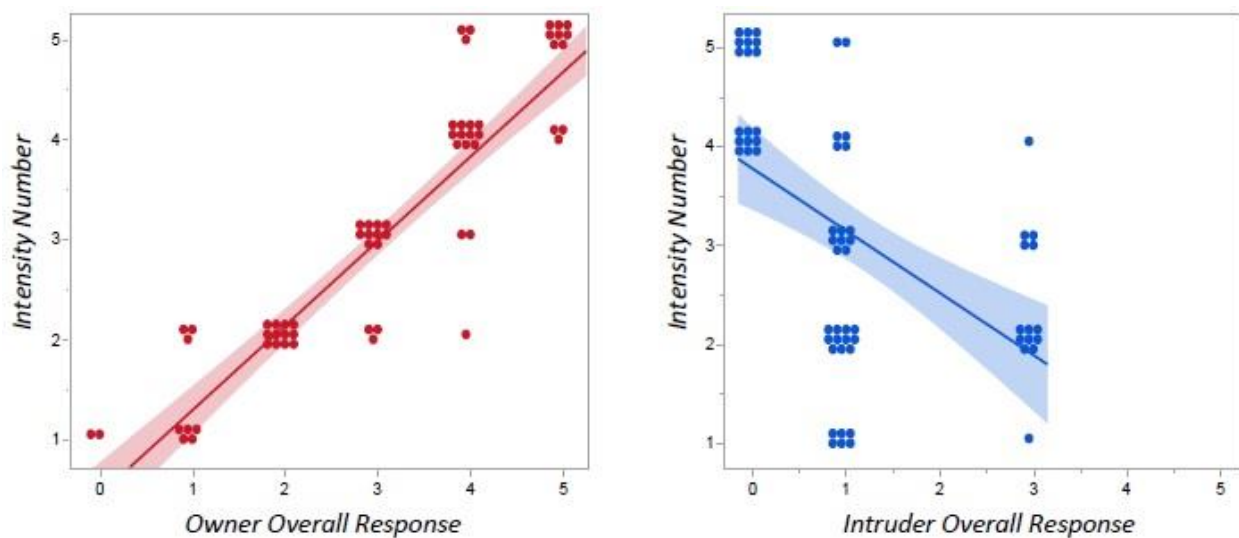


Figure 12. The intensity of female interactions was influenced differently by the response of owners (left) and intruders (right). Each point represents a separate interaction. A simple linear regression line with 95% CI (shaded) is shown in each panel.

II. Individual VS Contextual Factors Accounting for Variation

Age differences between females had a strong influence on owner but not intruder response

Of the females observed during this study, 20 were hatch year birds and 20 were after-hatch year birds with two hens of unknown hatch year (females captured as adults for the first time in 2020). Most interactions were between similarly aged birds with the majority of interactions involving hatch year birds. A large proportion of interactions occurred between 1

year old owners and 1 year old intruders, 1 year old owners and 2 year old intruders, and 2 year old owners and 2 year old intruders (Figure 13).

Analyses using REML linear mixed models with age as the main effect and female ID and pair ID as random effects indicated that age, on its own, did not significantly influence female response scores in the first 30s ($F_{4, 41.36}=1.47$ $P=0.23$, $R^2_{adj}=0.667$), overall ($F_{4, 41.35}=1.22$, $P=0.32$, $R^2_{adj}=0.605$) or the intensity of the interaction ($F_{4, 76.09}=0.086$, $P=0.982$, $R^2_{adj}=0.87$). Interactions with unknown aged hens were excluded from these analyses.

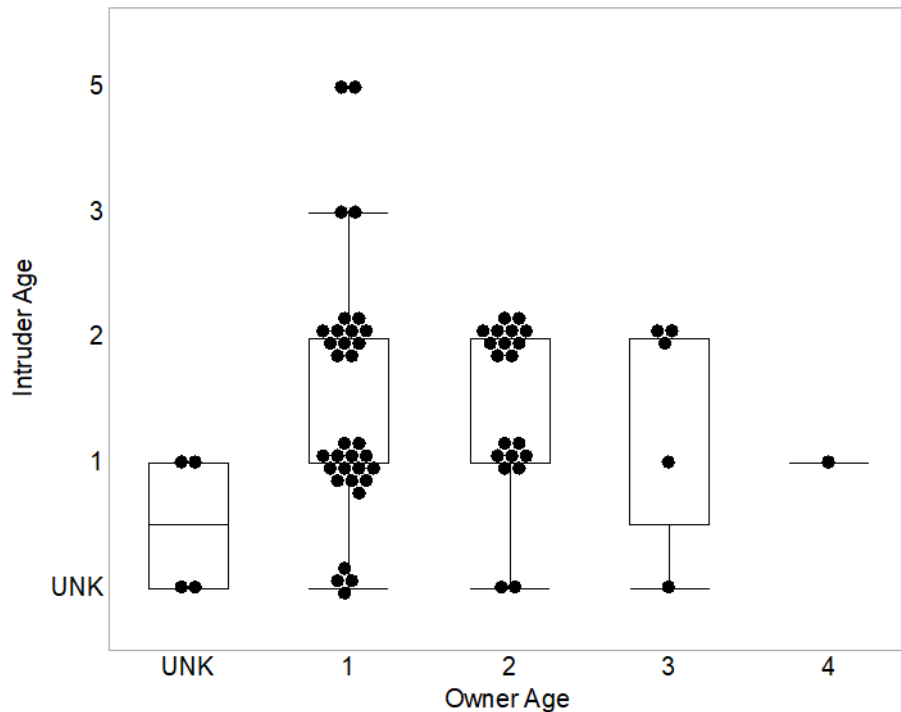


Figure 13. Ages of owners and intruders in each interaction (pairwise). Points represent a single interaction between a pair. Box plots show the interquartile range (IQR) from the 25th to 75th quartile, vertical whiskers are 1.5*IQR and horizontal lines are medians. UNK=unknown.

However, age differences between interacting pairs of females revealed an influence of age on owner's responses. Age differences were calculated by subtracting intruder age from owner age. Positive values indicate interactions in which the owner was older than the intruder

and vice versa for negative values (Figure 14). The majority of interactions were between females of equal age or one year apart. The largest age difference between owner and intruder was 4 years.

The age difference between females in an interaction influenced the response score for the owner when we analyzed pairwise interactions and controlled for repeated observations within each pair (REML linear mixed model with age difference as main effect and pair ID as random effect). As the difference in age between owners and intruders increased, the response score of owners in the 1st 30s also increased ($F_{1, 42}=11.93$, $P=0.0013$, $R^2_{adj}=0.4295$), as did the overall response ($F_{1, 38.06}=6.00$, $P=0.019$, $R^2_{adj}=0.761$), and overall intensity ($F_{1, 49.25}=7.89$, $P=0.0003$, $R^2_{adj}=0.150$). However, there was no effect of the age difference on the intruder's response score in the first 30s ($F_{1, 41.35}=1.17$, $P=0.29$, $R^2_{adj}=-0.001$) or overall response ($F_{1, 35.25}=0.024$, $P=0.88$, $R^2_{adj}=-0.871$). Pairs with untagged females were excluded from this analysis.

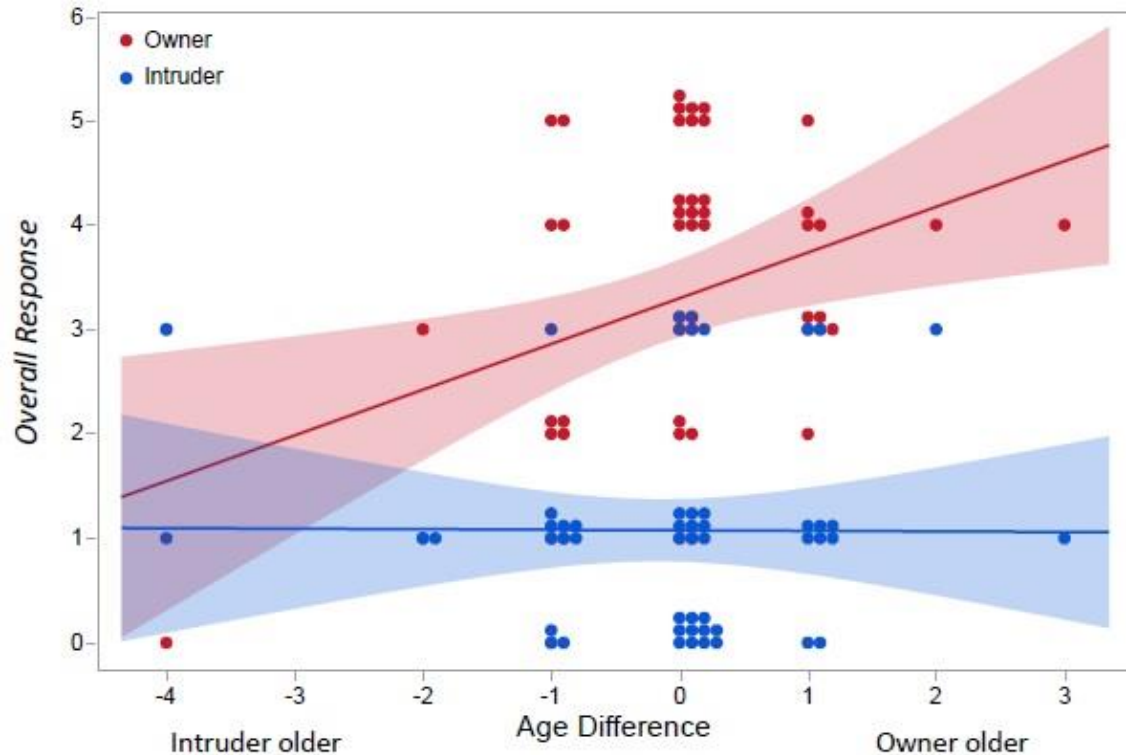


Figure 14. Age difference between owners and intruders influenced the response of owners but not intruders. Each point represents a pairwise interaction. Simple linear regression lines and 95% CI (shaded) are shown.

Intruders differed from owners in morphology and body condition

Several morphological traits differed significantly between intruders and owners (REML linear mixed models with Pair ID as a random effect; Table 6, Figure 15). Overall, intruders were heavier, had larger bills (culmen length and width) and longer wings, although tarsus length did not differ (Table 6). Interestingly, the area of the white eye ring in females was also larger (marginally significant) in intruders than owners.

We conducted a principal component analysis (PCA) to reduce and simplify the structural measurements. Two principal components (PCs) accounted for 67.6 % of the variation in the original data (Table 7). All variables loaded strongly and positively on PC1, indicating that it

represents a general index of structural size. The second PC loaded positively on tarsus measurements and negatively on wing and culmen measures, indicating that this PC further separated females with relatively larger tarsi and shorter wings and bills, and vice versa (Table 7). We then calculated an index of female body condition by regressing net weight on PC1 ($\text{NetWt} = 522.52 + 11.99 * \text{PC1}$; $R^2_{\text{adj}}=0.23$, $P<0.0001$) and used the residuals of this regression as an index of body condition, as has been employed in numerous other studies.

Table 6. Comparison of morphological traits of owners and intruders using REML linear mixed models with Pair ID as a random effect. Measurement data was only collected on females that incubated, therefore, there is no data on females that were only intruders.

	Owner \pm SE	Intruder \pm SE	F	df	R^2_{adj}	P
Net Weight (g)	510.28 \pm 4.47	545.66 \pm 5.97	34.37	59.2	0.612	<0.0001
Culmen Length (mm)	31.72 \pm 0.18	32.83 \pm 0.25	16.77	64.27	0.431	<0.0001
Culmen Width (mm)	15.37 \pm 0.06	15.63 \pm 0.08	7.17	78.54	0.139	0.009
True Tarsus (mm)	34.37 \pm 0.18	34.62 \pm 0.24	1.01	62.45	0.468	0.32
Full Tarsus (mm)	41.65 \pm 0.16	41.73 \pm 0.22	0.09	75.05	-0.014	0.77
Wing Length (mm)	221.45 \pm 0.68	225.58 \pm 0.93	14.19	73.64	0.275	0.0003
Eye Ring Area (mm ²)	215.09 \pm 6.14	230.27 \pm 8.07	3.69	58.78	0.604	0.0595
PC 1	-0.35 \pm 0.18	0.68 \pm 0.25	12.15	78.54	0.185	0.0008
PC 2	0.19 \pm 0.15	-0.36 \pm 0.21	6.09	58.84	0.421	0.0165
Residual (WT PC1)	-7.65 \pm 3.96	13.50 \pm 5.46	12.03	76.12	0.359	0.0009

Table 7. *Principal component analysis (PCA) of morphological traits. Eigenvalues, percent variance accounted for by each principal component (PC) and component loadings for each morphological trait are shown.*

	Principal Component 1	Principal Component 2
Eigenvalue	2.073	1.307
Percent of Variance	41.54	26.14
Culmen Length (mm)	0.632	-0.413
Culmen Width (mm)	0.749	-0.130
True Tarsus (mm)	0.468	0.736
Full Tarsus (mm)	0.715	0.508
Wing Length (mm)	0.618	-0.566

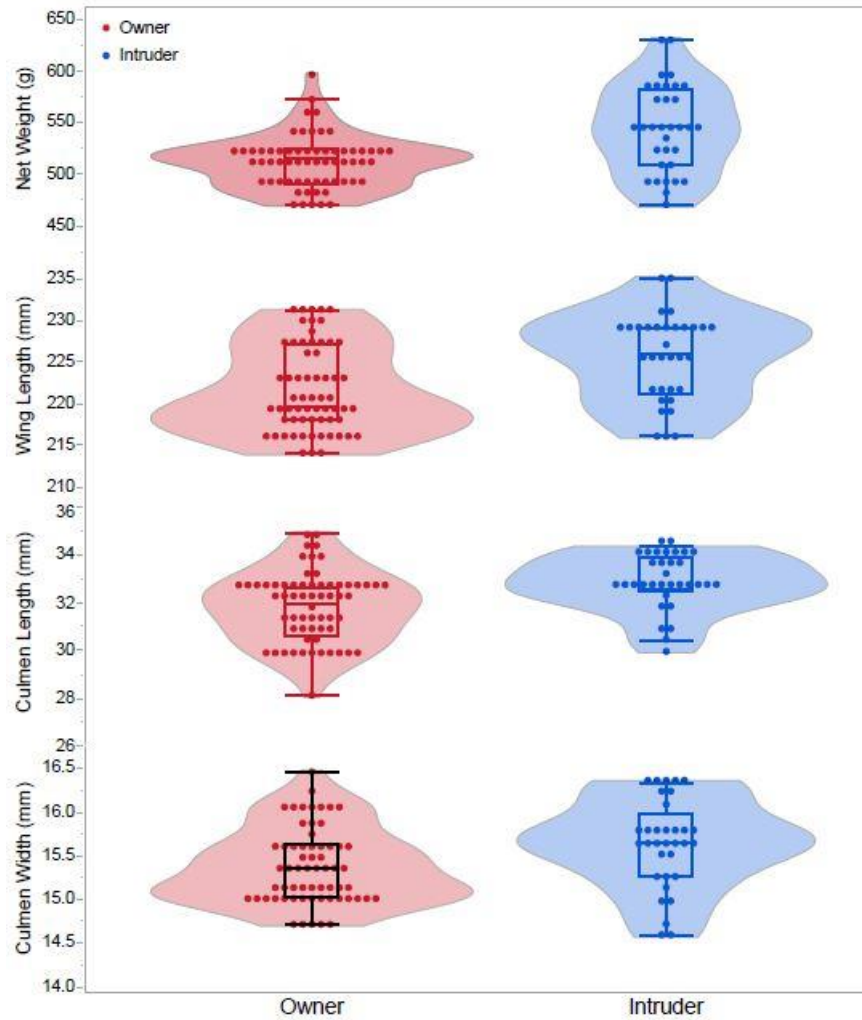


Figure 15. Comparison of morphological traits of owners and intruders. Each point represents an individual. Box plots show interquartile range. Violin plots (shaded) illustrate the density of data by plotting symmetric kernel estimates of the probability density function at each point (JMP Pro 16, 2021).

Body condition, as measured by the residuals of the regression regressing of net weight on PC1, was significantly higher for intruders than owners (Table 6, Figure 16). We caution that body measurements were only available for females that either incubated a nest (owners) or incubated and intruded other nests (net weights for two females, 04198E9716 and 04198E19F0, were incorrect in 2020 and we used weights from 2019). We did not have weight or structural measures for females that only acted as intruders as they were never caught on a nest in 2020. Intruders that also nested being larger and in higher condition holds important implications as for our understanding of conspecific brood parasitism and different tactics of brood parasitic hens (see discussion).

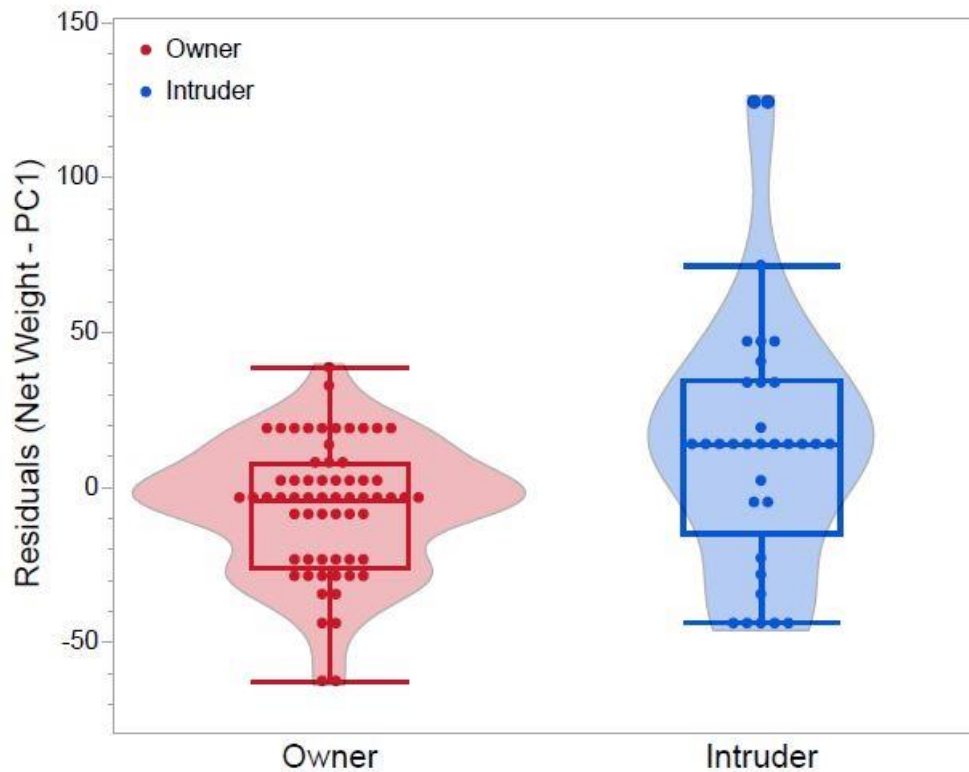


Figure 16. Comparison of body condition (residuals of weight – PC1 regression) for owners and intruders. Each point represents an individual. Box plots show interquartile range. Violin plots (shaded) illustrate the density of data by plotting symmetric kernel estimates of the probability density function at each point (JMP Pro 16, 2021).

We next examined how morphological differences might have impacted the responses of owners and intruders. In these analyses we included status as a main effect given that there are differences in morphological traits among intruders and owners (Figure 15) and we included female ID and Pair ID as random effects. In all cases, there was no absolute effect of any of the morphological traits alone on any of the three response variables when status was included in the model (Table 8). In all analyses of response in the first 30 seconds and overall response, the effect of status (owner or intruder) was highly significant ($P < 0.001$) whereas the effect of morphology was minimal and not significant (Table 8).

Table 8. Analysis of the effects of morphological traits on the response of females based on REML linear mixed models including status as a main effect and female ID and Pair ID as random effects. Only the F value, df and P value for the effect of each morphological value is shown.

	F	df	P
Response in 1st 30 seconds			
Net Weight	0.12	77.27	0.73
PC1	0.92	26.7	0.35
PC2	0.15	22.69	0.70
Body Condition (Residual)	1.18	32.16	0.29
Overall Response			
Net Weight	0.70	50.63	0.41
PC1	0.30	27.02	0.59
PC2	0.18	22.0	0.67
Body Condition (Residual)	0.78	28.34	0.38
Intensity of Interaction			
Net Weight	0.11	70.75	0.75
PC1	0.01	4.06	0.91
PC2	1.20	92.0	0.28
Body Condition (Residual)	0.001	92.0	0.98

However, when we considered the differences in morphology for each interacting pair and examined owner and intruder responses separately (as we did for age differences above), several trends emerged. Specifically, there was a marginally significant trend of increasing owner response in the 1st 30s as owners became more similar or greater in size ($F_{1, 19.52}=3.34$, $P=0.083$, $R^2_{adj}=-0.734$) and body condition ($F_{1, 19.05}=3.19$, $P=0.09$, $R^2_{adj}=0.737$) relative to intruders (in all analysis pair ID was treated as a random effect). Similarly, there was a

marginally significant trend of increasing owner response score overall as owners became more similar or greater in size ($F_{1, 19.05}=3.34$, $P=0.083$, $R^2_{adj}=0.772$) and body condition ($F_{1, 18.76}=3.67$, $P=0.071$, $R^2_{adj}=0.779$) relative to intruders (Figure 17). In contrast, in all comparisons, intruder response in the 1st 30s or overall response did not vary significantly with the differences in net weight or body condition (all $P > 0.15$; Figure 17). Thus, although owners are often smaller or in lower condition relative to intruders, their level of response and aggression increases as they become more similar or greater in size and body condition, like the pattern found for age differences (Figure 14).

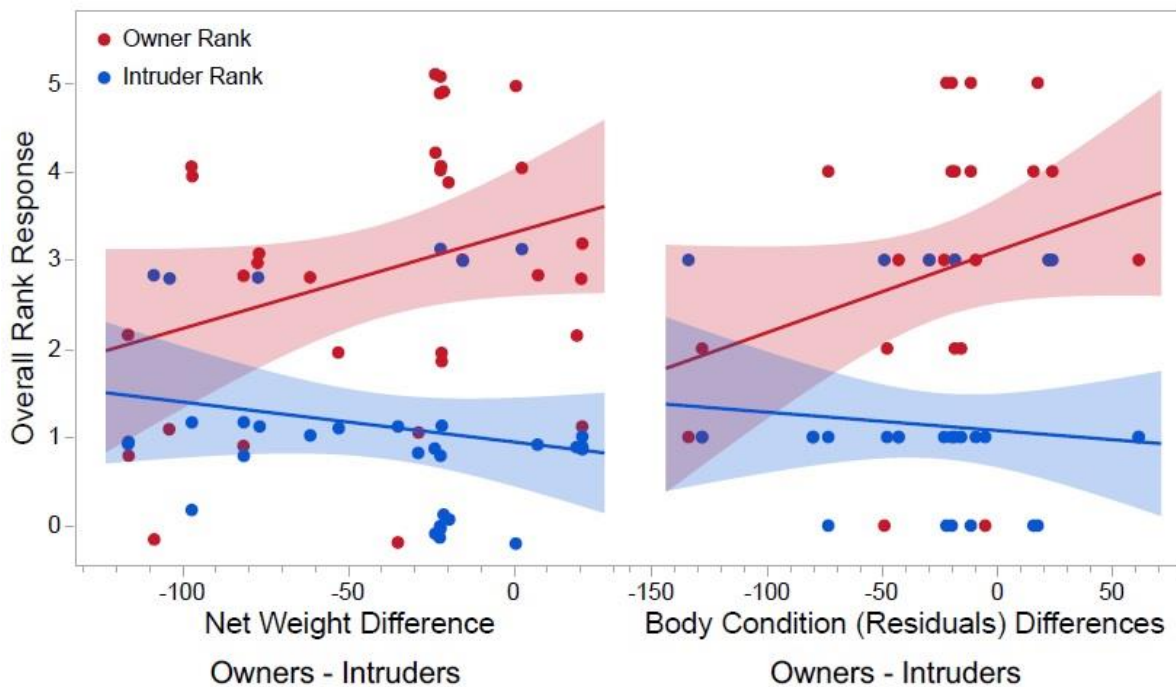


Figure 17. Response of owners and intruders (overall response) as owners became more similar in weight and body condition (x-axis shows the differences between owners and intruders; owners increase as values increase to the right). We used a pairwise function of analysis for each interaction. Simple linear regression lines and 95% CI are shown.

Previous ownership and breeding experience do not influence owner or intruder responses

We examined whether the previous ownership or previous breeding experience of an owner influenced their response to an intruder. There were too few observations of intruders with known previous ownership or experience to examine the influence of their breeding history on levels of responses. We analyzed responses of both owners and intruders using REML linear mixed models with pair identity included as a random effect.

We found no evidence of any significant influence of previous ownership on owner or intruder behavioral response (Table 9). Likewise, there was no evidence that previous experience of incubating a nest influenced the response of owner or intruder females (Table 9).

Table 9. Pairwise analysis of the effects of the owner’s previous ownership (main effect) and previous breeding experience on the response of owners and intruders based on REML linear mixed models with pair ID as a random effect.

	F	df	P
Owner – Response in 1st 30 seconds			
Owner - Previous ownership	0.79	40.01	0.38
Owner - Previous experience	0.72	39.87	0.40
Owner Overall Response			
Owner - Previous ownership	0.51	39.98	0.48
Owner - Previous experience	0.39	39.08	0.54
Intruder – Response in 1st 30 seconds			
Owner - Previous ownership	0.02	38.60	0.87
Owner - Previous experience	0.70	36.65	0.41
Intruder Overall Response			
Owner - Previous ownership	0.90	36.69	0.48
Owner - Previous experience	1.75	36.68	0.19
Intensity of Interaction			
Owner - Previous ownership	0.05	39.73	0.83
Owner - Previous experience	0.32	39.73	0.58

Owner responses were stronger during incubation

Nests were checked on average once a week and the timing of incubation was estimated once down and warm eggs were found. Not all nests or pairs of interacting hens had data recorded both before and after incubation, and only two interacting pairs of females were observed both before and during incubation. However, we found an influence on owner response depending on the time of interaction. Owner response was higher once incubation had started as was the overall intensity of interactions (Table 10). There was some indication that intruders

were slightly more active if an interaction occurred during egg-laying, but this was mostly evident in the first 30s (Table 10).

Table 10. Pairwise analysis of the effects of time of interaction (before or after incubation) on the response of owners and intruders based on REML linear mixed models with pair ID as a random effect. Least square means ± 1 SE are shown for each behavioral response.

	During Egg-laying	During Incubation	F	df	R ² _{adj}	P
Owner 1 st 30s	2.79 \pm 0.28	3.85 \pm 0.29	6.97	48.60	0.624	0.011
Owner Rank	2.48 \pm 0.10	3.65 \pm 0.10	9.58	52.84	0.835	0.003
Intruder 1 st 30s	0.76 \pm 0.10	0.48 \pm 0.10	4.14	43.40	0.252	0.048
Intruder Rank	1.26 \pm 0.23	1.11 \pm 0.23	0.22	52.31	0.468	0.64
Intensity	2.55 \pm 0.25	3.65 \pm 0.25	10.61	52.65	0.821	0.002

Are intruders acting as brood parasites?

We have, to this point, referred to females that did not incubate the nest as intruders because it is unclear whether these females were prospecting for a potential nest site of their own, or acting as a brood parasite seeking to gain access to a nest to lay an egg. However, using the video recordings and PIT tag information, we were able to document several instances in which we could confirm that the intruder laid an egg. In total, there were 37 instances where the intruding hen laid an egg in the presence of the owner, 20 instances that did not result in an egg being laid, and 5 instances where it was unclear. We found no differences in any response by either the owner or the intruder in cases where the intruder laid an egg or did not (Table 11). There are two implications from this analysis; first, the behavior of intruder was the same regardless of whether they were acting as a brood parasite (laid an egg) or investigating the nest without laying an egg. This suggests that many of these intrusions are parasitic (or potentially

parasitic) in nature. We have been cautious in our terminology, but these data suggest that owners are acting as hosts and intruders as parasites (see discussion).

Second, owner responses did not differ when an egg was laid or not, although there was an insignificant tendency for the response of owners in the 1st 30s and overall to be slightly greater in those instances when an egg was laid. Nonetheless, even though owners were very aggressive in some instances, this did not seem to deter parasitic intruders from laying an egg. Of the 11 interactions where the owner scored a 5, there were 7 instances of the intruder laying an egg, 2 interactions that did not result in a laid egg, and 2 that were unclear. In interactions where the owner scored a 0, both intruders successfully laid an egg. There were other instances where the owner scored low and there was no indication of the intruder laying an egg. In some interactions, the intruder left the nest almost immediately after entering and seemingly unprompted by the owner.

Table 11. Analysis of the response of owners and intruders in relation to whether the intruder laid an egg (REML linear mixed models with pair ID as a random effect). Least square means \pm 1 SE are shown for each behavioral response.

	Intruder Laid Egg	Intruder Did Not Lay Egg	F	df	R ² _{adj}	P
Owner 1 st 30s	3.40 \pm 0.28	2.79 \pm 0.36	1.86	53.92	0.782	0.178
Owner Response	3.15 \pm 0.24	2.61 \pm 0.32	1.86	53.47	0.718	0.179
Intruder 1 st 30s	0.59 \pm 0.11	0.81 \pm 0.13	1.52	52.35	0.567	0.223
Intruder Response	1.14 \pm 0.20	1.52 \pm 0.26	1.56	43.65	0.871	0.218
Intensity	3.11 \pm 0.22	2.79 \pm 0.29	0.84	46.98	0.864	0.364

Comparison of owner and intruder response in each pairwise interaction also suggests that the response of either female did not influence whether an egg was laid (Figure 18).

Although intruder response scores were lower on average than owners, there was no pattern

evident where lower response scores of owners or higher response scores of intruders resulted in an egg being laid or vice versa (Figure 18).

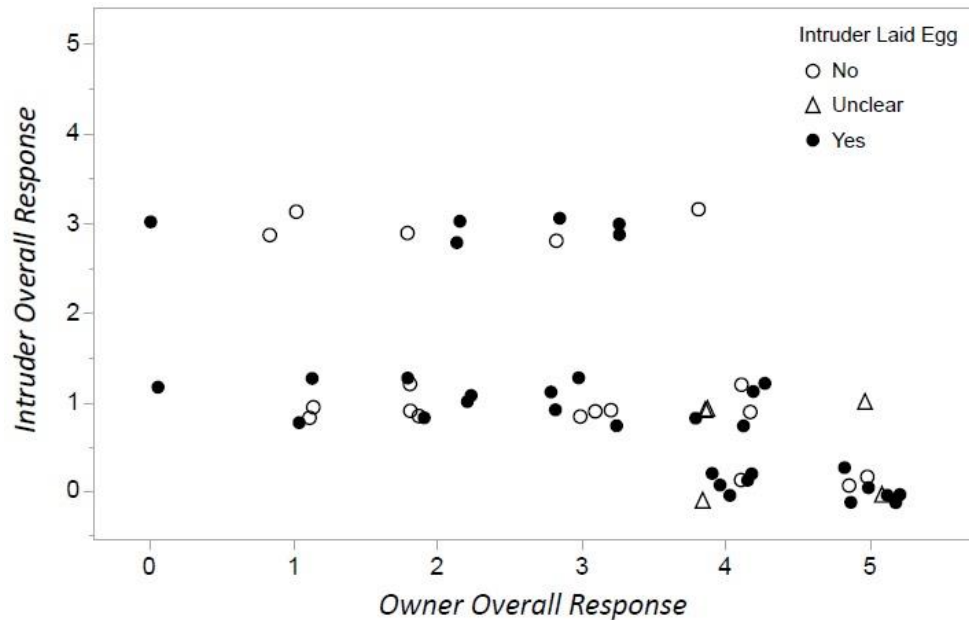


Figure 18. Pairwise comparison of owner response to intruder rank when an egg was laid by the intruder (solid circles), no egg was laid (open circles), or the status of a laid egg was unclear (triangles).

We also tested whether morphological traits (net weight, PC1, body condition and white eye ring area), age, or previous history experience of owners and intruders differed among females when an egg was or was not laid and there were no significant differences in any comparison (all $P > 0.15$ or higher, REML with pairs as a random effect to account for multiple observations of the same pairs). As reported above, intruders were heavier than owners but there was no influence of the difference in the weight of the owner and intruder on whether an egg was laid (Figure 19).

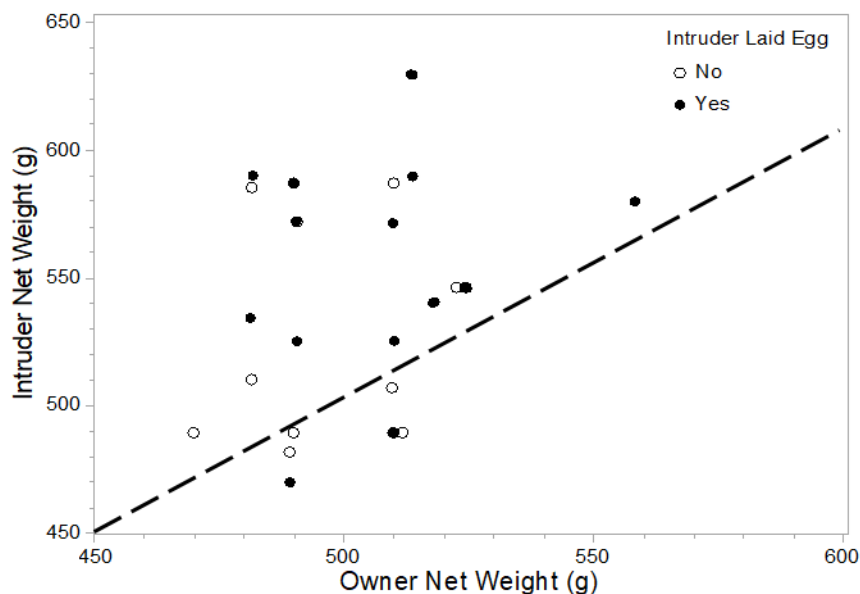


Figure 19. Comparison of owner weight to intruder weight when an egg was laid by the intruder (solid circles), or no egg was laid (open circles). The dashed line is the 1:1 slope for equal size.

Variation among pairs

Finally, we note that there was considerable variation among pairs in how owners responded. These patterns are implicit in many of the figures above where each data point represents an interaction among a single pair. We did not have enough pairs of multiple observations with the same two individuals to conduct a formal repeatability analysis. However, a visual evaluation of the intensity of interactions among pairs (Figure 20, top panel) and the average overall response score of each owner and intruder in those pairs (Figure 20, bottom panel) illustrates that interactions can vary between being extremely intense (scores 4 or 5), to very passive and low intensity (scores of 2 or less). Much of this variation is due to owners (bottom panel). In fact, when owners are most aggressive (right side of bottom panel) intruders tend to be least aggressive. Conversely, when interaction intensity is low overall (left side of figure), owners tend to be more passive and in several cases the intruders exhibit higher scores

(up to 3). The variation among pairs remains intriguing and does not seem to be explained by differences in morphology or history (see above). Further studies to explore the kinship and social connections among females of interacting pairs will be extremely informative.

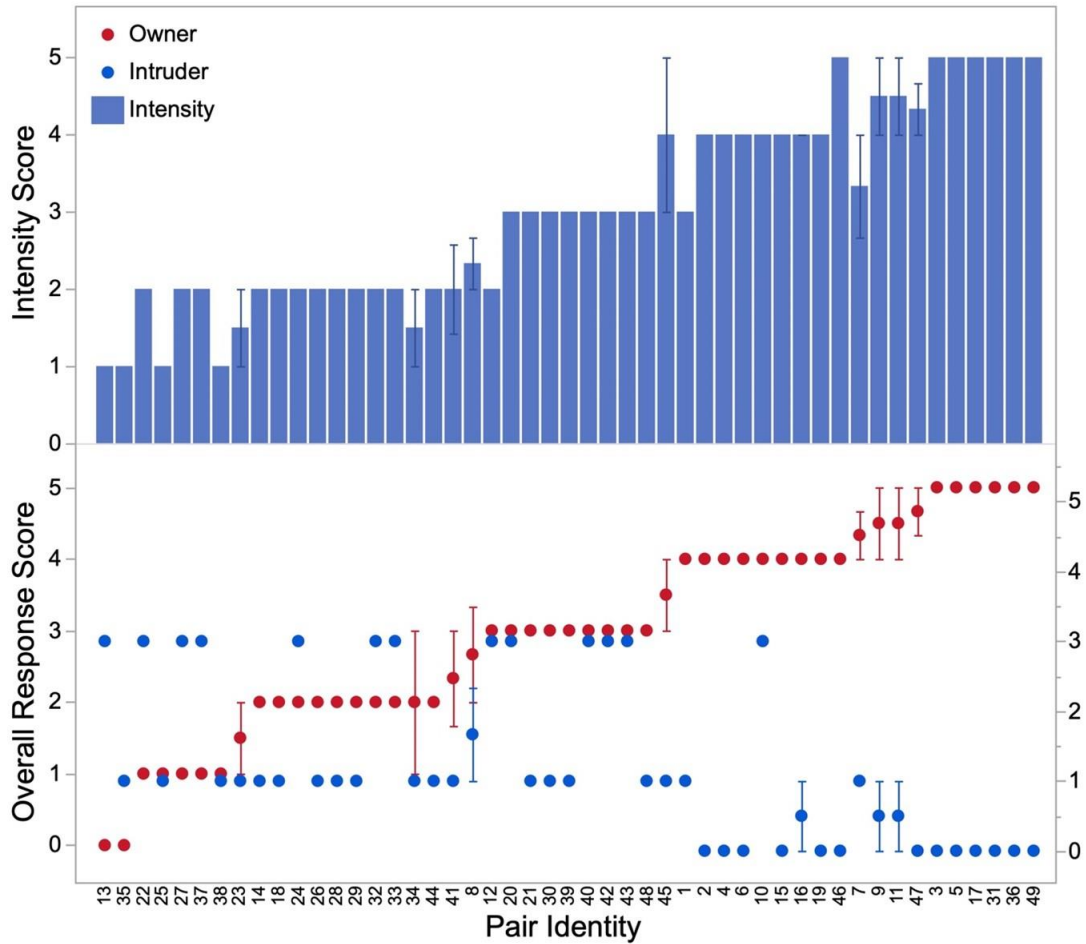


Figure 20. Variation among intensity of the interaction of each unique pair of individual wood duck females (top panel) and the mean overall response score of the owner (red) and intruder (blue) in those pairs. Pair ID was ordered by owner overall response score (ascending).

Discussion

Competition for critical resources such as a nest site, and the way that females interact to utilize these resources, may determine not only reproductive success, but also the reproductive strategy that females employ. However, prior to the advance of small in-nest camera systems and remote tagging of individuals by RFID and PIT tagging, we lacked quantitative information on female-female interactions. This is especially true for cavity nesting birds where most of the interactions occur hidden from view in a tree cavity or nest box. By using new technology, we are beginning to gain a better understanding of what might influence the scope and range of interactions that take place when female wood ducks encounter one another at the nest site. We found that females varied considerably and repeatedly in their response, behaved differently based on their status (owner or intruder), and responded with varying levels of intensity due to relative differences in age and morphology, and timing of the encounter (pre or post incubation). We consider each of these findings in the context of the hypotheses summarized in Table 1.

Hypothesis 1: Females vary in responsiveness and do so consistently - We found very strong evidence in support of this hypothesis. When all females, regardless of status, were included, differences in all response variables (1st30s, overall response) were significant and highly repeatable. Our results agree with anecdotal and observational reports indicating that intensities of interactions among female wood ducks can vary widely. Some interactions are described as full-on fights and occasionally even result in the death of a female, whereas other interactions are more docile and occur with apparently no aggression (Bellrose & Holm 1994, Semel & Sherman 2001). We observed similar variation among female-female interactions in our study; some fights (with scores of 5 on the intensity scale) involved biting and feather pulling

(targeting the face, wings, and legs), and were often accompanied by loud vocalization while other interactions (with scores of 1) involved very little or no engagement between females. There was no evidence of female-female conflict resulting in death for our study but there was one particularly intense interaction of a hen grabbing hold on the other hen's head and tossing it against the nest box wall during a fight.

The high repeatability of individual behavior also suggests that females vary in their “personalities” with some being aggressive, others quite docile. There has been a growing recognition in behavioral ecology that consistent individual variation may play important roles in many types of intraspecific interactions (Arnold & Owens 2002, Sih et al. 2012, Wolf & Weissing 2012). How an individual responds to ecological, environmental, and social factors largely affects that individual's fitness and the overall fitness of a population. Limited behavioral plasticity can pose a threat to populations in the face of changes such as human-induced rapid environmental change (HIREC) or invasive species (Sih et al. 2011, Sih et al. 2012). Consistency among and between individuals and variation in individual behavior allows for a population's overall success in a broader range of environmental and ecological conditions (Sih et al. 2020).

Hypothesis 2: Variation in responsiveness is related to status – As predicted, we found that status or role of the female had an overarching influence on every measure and almost every analysis we completed. When we examined the response of owners and intruders separately, we found the greatest source of variation and repeatability was due to role, especially among owners. Intruders were much less variable, and the differences in response due to status are likely the cause for the bimodality and repeatability of response of individuals (i.e., intruders primarily and consistently scored a response of 0 and 1 or occasionally 3).

Time budget analysis revealed further differences in behaviors of owners and intruders with greater variation in owner time budgets than intruder time budgets. Behavioral time budgets of owners reflected a prevalence of both antagonistic (Bite) as well as neutral behaviors (Sit). No owner exhibited avoidance behavior. In contrast, most intruders exhibited passive (Sit) or active (non-antagonistic, possibly defensive) behaviors (Avoid and/or Move) and no intruders expressing antagonistic behavior (Bite). The differences between the roles of intruders and owners are further reflected when analyzing the mean differences in time budget proportions. Bite, Nibble, and Preen Self were largely represented for owners while Sit, Avoid, and Move were far more common behaviors performed by intruders.

Our data strongly support prediction 2B that owners will be more aggressive than intruders. We hypothesized that there may be several reasons why this would be so. The nest site may be more valuable to the owner, the owner may have more access to information as to the quality of the nest site, and/or the owner may have a greater investment in the nest and protecting known assets (Briffa & Sneddon 2010). Owners of the nest typically (although not always) have more of their own eggs in the nest; therefore, the owner's fitness could potentially be negatively affected by parasitism (an increase in clutch size could lead to insufficient incubation and decrease hatching success) (Yom-Tov 1980, Bellrose & Holm 1994, Lyon & Eadie 2008), which in turn may elicit a strong, aggressive response from the owner. Despite the higher levels of aggressiveness of owners, many of the interactions were rather low intensity (scores of 0 and 1, Figure 4), which is consistent with the hypothesis that species with low parental care costs should also show low rates of retaliation towards intruders (Arnold & Owens 2002) and that aggressive behaviors (such as Bill Jab, Fight, and Bite) expend energy that some owners may be unable or unwilling to afford (Bellrose & Holm 1994).

Hypothesis 3: Variation in responsiveness is related to age/experience – This hypothesis predicted that female age would influence the response of owners and intruders. We found that it was not the absolute age of an individual female, but the relative differences in age between an intruder and owner of a given pair that was more relevant. Moreover, the age difference affected the response score for owners, but it had no effect on intruder response. In the context of competition, older owners would be expected to have more experience in nest defense and assessment of the nest site's value, as well as lower RRV leading to the nest site being more valuable to the owner. As the difference in age between owner and intruder increases, it may be that the benefit of greater experience of an owner or an increased ability to deter an intruder may have facilitated a more aggressive response by the owner. It was interesting that the age difference had little effect on the intruder's behavior. We might expect that intruders would behave more boldly (and perhaps more aggressively) when they are more similar in age, or even older, than owners. However, we found that age (absolute or relative) had no effect on response scores for intruders suggesting any asymmetry in age does not influence how intruders respond, even if owner aggression increases.

When owners were relatively older than intruders, and as age difference increased, the overall intensity of the interaction also increased. However, when the intruder was older, the owner's response and intensity of the interaction was relatively low. This may be expected as greater differences in age are expected to relate to greater differences in how the individual is valuing the nest as a resource (Briffa & Sneddon 2010) which likely affects response to intruders. Older owners may respond more aggressively to younger intruders as those younger intruders have higher RRV. An imbalance in the costs to an older owner and the benefits of a younger intruder may result in the aggressive responses we observed. Conversely, younger

owners when faced with an older intruder often had a non-aggressive response as they could have more to gain with an increase in clutch size. Though additional eggs can negatively impact incubation efficiency, parasitic young can increase the survival of the owner's offspring overall (Andersson 1984).

Hypothesis 4: Variation in responsiveness is related to body size, phenotype, and resource holding potential (RHP) – This hypothesis predicts that body size and/or condition will influence female response with two contrasting alternatives: either larger (better condition) or smaller (lower condition) individuals will be more aggressive. Larger females were predicted to be more aggressive if they were in better condition leading to the hen having more resources to allocate to nest defense. In contrast, smaller females were predicted to behave aggressively if poor body condition meant that the probability of surviving and reproducing in the next year was low. We would expect larger individuals to have higher RHP to compete (and in this context, perhaps, intrude) successfully than smaller individuals (Briffa & Sneddon 2010, Engqvist & Taborsky 2015). We found significant difference in morphology and condition between owners and intruders overall. However, absolute net weight did not affect response score and none of the morphological differences influenced the level of response. From the RFID data, we knew that several of the intruding females also had nests of their own – these were the only intruding females for which we could obtain body size measurements. These intruders were heavier than owners that were only nesting. Larger females above a certain body condition threshold may be able to lay additional eggs as brood parasites. Accordingly, these intruders may have been high quality females who had additional resources to also lay some eggs parasitically (Sorenson 1991, Lyon & Eadie 2008). Interestingly, the larger size and better body condition of these females did not lead them to be more aggressive – as intruders, their behavioral response

was muted and passive. We did not have body size or condition measurements for intruders that were never caught on the nest, and it would be interesting to determine if these intruders also differed in size or condition. Possibly, these intruders (without a nest of their own elsewhere) might be small or in poor condition if they were acting as parasite-only females, a prediction that would be in line with the model proposed by Sorenson (1991) and Lyon & Eadie (2008).

While there was no detectable influence of absolute size or condition on level of response, we found that *relative* differences in size/condition influenced how owners responded (a similar trend as we found for age difference). When owners were larger than intruders, they exhibited a stronger response. Conversely, intruder response was negatively correlated with net weight difference. When the owner was larger, intruders tended to respond more passively but when intruders were larger, the owner's response was lower, and intruder's response was higher. Body size/condition impacts an individual's RHP. As we saw with age and RRV, differences in RHP may affect how owners respond to larger or smaller intruders since the actions an individual can take tend to be limited by their state (Dall et al. 2004). Larger owners may respond to smaller intruders with more aggression because they have the energy stores to do so. Moreover, owners faced with larger intruders may already be at the threshold for their reproductive output and not have the energy for a strong defense response, resulting in a more docile interaction. Intruder response to larger or smaller owners may be similar, in that larger intruders were more active on the nest when the owner was smaller, and more passive when owners were larger and more aggressive.

Hypothesis 5: Variation in responsiveness is related to history/previous ownership – This hypothesis predicted that owners with previous breeding experience or history of use of a nest site might defend the nest more aggressively based on their greater knowledge of the value of the

site or their experience. Previous studies of wood ducks followed over multiple years have suggested have made claims that returning females prefer to reuse the same nest boxes (Semel & Sherman 2001) or that females simply prefer any box that has been used previously by any female (Roy et al 2009). However, none of the intruders in our study were recorded visiting nests they had previously incubated. Some of the owners were on nests they had used before, yet there was still no effect on owner or intruder behavior. These results suggest that the level of response during interactions at the nest is not influenced by owner experience or knowledge of the nest site. Perhaps past experience or knowledge of nest site use is not informative to females in assessing the current value of a nest site; if success is moderate or high for most sites, past use/success may be less informative than the current degree of competition or level of parasitism during the current year. Conversely, if nest success is highly variable among years (due to varying degrees of competition or parasitism), history or experience would not be a reliable predictor of current success for females to act upon. Social drivers, instead of environmental, might hold more precedence in female-female interactions.

Hypothesis 6: Variation in responsiveness is related to nest state and timing –

Timing/nest state was a factor attributing to the variation in owner response, supporting prediction 6B. Owners were relatively more aggressive during incubation and exhibited lower response scores before incubation and higher scores after incubation. In precocial birds such as wood ducks, where all young leave the nest together 24-48 hours after hatching, there must be nearly perfect synchrony for parasitically laid eggs to hatch along with the host clutch. Bellrose & Holm (1994) reported that the rate of injury and fatal interactions was correlated with the laying of parasitic eggs when the nest was already undergoing incubation. Even with precocial young, parasitism may be costly to the owner. Eggs laid after incubation have limited value to

owners or intruders that might gain some benefits inclusive fitness (if parasites and hosts are related) or a reduced risk of predation in a larger clutch size. Eggs laid after incubation might impact the efficiency, incubation, or developmental synchrony of eggs (Andersson & Ahlund 2012, Lyon & Eadie 2017). Further, the value of increased nest defense may be greater for females after incubation begins. Once committed to incubation, the option to abandon and lay eggs elsewhere is limited. Increased investment, and therefore defense, of the nest could have a higher payoff than the alternative of abandoning the nest or accepting a greater number of eggs that might reduce an owner's own hatch success.

Hypothesis 7: Variation in responsiveness is related to female reproductive strategy – As discussed earlier, owners and intruders behaved very differently. This is a pattern that Ahlund (2005) also discovered in a population of common goldeneyes. In the instances where individuals were recorded as both owners and intruders, there is clear evidence that behavior changes with role and that females are generally more passive when they are acting as intruders (even for hens that are aggressive as owners). Our observations strongly suggest that females in these different roles are not simply competing for the nest site but rather that owners and intruders are following very different behavioral pathways. Owners are acting as hosts defending their nest from intrusions, whereas intruders are behaving passively and avoiding host aggression while staying on the nest and often laying an egg. Parasitic females largely acted the same in both low and high intensity interactions. Owners were more aggressive than intruders overall, as might be expected, to deter parasitic laying and limit additional costs to their own reproductive success.

Interestingly, despite the higher levels of aggression of owners, there was no direct correlation with aggressive behavior and whether an intruder successfully laid an egg.

Aggressive behavior by owners did not deter intruders effectively. Of 63 interactions, there were 37 instances where intruders laid an egg, 20 where no eggs were laid, and 5 were unclear. Out of the 11 interactions where the owner's response scores a 5, there were 7 instances of an egg being laid. High levels of aggression therefore often did not prevent the intruder from laying an egg. General nest defense may still have deterred those females who did not lay an egg, although this variation could also depend on the motivation and the skill of the intruder more than the response of the owner.

Why isn't aggressive behavior by owners more effective in preventing parasites from laying an egg in the nest? And why do intruders often sit passively and avoid owner aggression, even when owners are vigorously biting and pulling at their feathers? Parasites may be hesitant to fight back or exit a nest while in the process of laying due to risk of in utero egg damage that can cause peritonitis, which may increase in risk due to increased activity and frantic movement (Fulton 2017). This may explain instances of drawn-out aggressive interactions where the intruder persisted until laying an egg, regardless of overtly antagonistic behavior from the host. The fact that most intruders were larger than the owners (in our sample of net weight measurements) could also explain how parasites are able to withstand attacks such as biting and feather pulling. Conversely, fights on the nest increases the risk of breaking eggs (Arnold & Owens 2002), so there is risk for both hosts and parasites. Even when eggs were not laid, those intruding hens may have still been parasitic hens evaluating a prospective nest to find a suitable host. Perhaps parasites are themselves evaluating the quality of the potential host by how vigorously the host defends the nest (as an indication of the quality of the host and the care she might provide for parasitic eggs and offspring).

Consistent with the third prediction for our final hypothesis (7C), we found that female-female interactions varied among individual pairs. Some of this variation may be due to the ability of an owner to defend the nest, depending on how she compares to the intruder. Owners were less aggressive when they were relatively similar or younger in age and smaller than the intruder. Furthermore, variation among pairs may be due to social or kin relationships among females, which may explain instances when the intruder was more active and the owner was more passive, or when the owner left the nest with the intruder still present. Kinship has been shown to influence behavior in other species of nesting waterfowl expressing CBP (Andersson 2015). Interactions where hosts were aggressive to parasites were found to have low relatedness between hens and more tolerant interactions occurred between highly related females. Females may gain inclusive fitness from offspring of related hens, a benefit which might outweigh other costs of additional young (i.e., lower hatch success). Life history patterns such as natal philopatry also might increase the rate of related females returning to the same nesting site (Andersson et al. 2019) which in turn might add to the prevalence of this behavior. Genetic analysis of females in the population is required to further answer this query, and to address this possibility (and these analyses are currently underway).

Study Limitations

In many of our analyses we used ordinal scores (e.g., 0-5) to measure female responses and levels of intensity. Ideally, we would have combined a larger number of measures, including time budget data, in a multivariate (PCA) analysis to construct continuous PC scores that would better capture the continuous range of behavioral responses of females. We have in fact, conducted preliminary analyses using such an analysis and the results reported here are not only corroborated but, in some cases, even stronger. Time limitations precluded including these

analyses in this thesis, but they will be incorporated prior to preparation of a manuscript for publication. Inter-observer reliability was calculated using Spearman's rank correlation (n=14, Owner 1st30s: 0.86 overall: 0.83; Intruder 1st30s: 0.34 overall: 0.73). Reliability was high for all scores except for intruders in the first 30 seconds. However, intruder scores were low, and limited to 0, 1, and 3, and therefore correlation low as well. More inter-observer scoring is required as is performing a second assignment of response scores from the initial observer for intra-observer reliability. Our study was originally designed to explore the relationship between owner and intruder response and kinship, but we were unable to analyze the genetic data at the time of the study due to laboratory closures in response to the COVID-19 pandemic. These analyses are forthcoming. Morphological data for females that only intrude (parasitize) are needed to understand the relationship between morphology and intruder response. Also, more detailed data on timing of the interaction would be helpful to evaluate the significance of timing on owner response (multiple interactions with the same pairs of females both before and during incubation). Finally, the limited number of cameras that could be deployed at a time, as well as issues encountered with SD cards, batteries, timestamps, etc., meant that some interactions were missed or unable to be used in our study due to discrepancies.

Summary

Our results do not support earlier claims that interactions at the nest among female wood ducks, and specifically instances of conspecific brood parasitism, are simply an inadvertent consequence of competition between females fighting for possession of the nest site (as Semel & Sherman 2001 suggested). Intruders and owners vary in their roles and behave differently when interacting with females in the opposite role. For hosts, time budgets largely consisted of

antagonistic behaviors such as bite and fight as well as more passive actions such as moving, adjusting the nest, or sitting. Intruders instead tend to behave passively or show avoidance behaviors when the owner is aggressive. Likewise, owners do not always deter intruders and there are some cases where the owners do not react to the intruder at all, allowing the parasite to lay an egg. Female-female interactions seem to be contextual with females responding to others in a consistent manner while also having the capacity to be flexible in their behavior.

There are several factors that may be contributing to the amount of variation and level of interaction that we observed in this population of wood ducks. The degree of interaction is modulated by differences in age and morphology, but only relatively. Older females were no more or less aggressive than younger females or vice versa and net weight had no singular effect on female response score. Rather, behavioral interactions depended more on the differences of age and size between owner and intruder and owner response increased as those differences increased. Of the females that were measured, those that acted as intruders and nesters were larger and in better condition than those that only acted as nesters. This suggests that RHP may play a role to some degree in the outcomes of these interactions.

Previous history or experience did not influence behavioral responses, suggesting that the information of nest quality was less important than the context of who was interacting with who. This is consistent with previous studies (Roy et al. 2009) that suggest that social attractiveness, as indicated by past use, was the single factor that predicted current use of a nest box. The quality of the nest site, at least in our population, did not seem to affect the response of owners to intruders and it is the social dynamic at each box that may ultimately determine whether it is a success or failure.

Our results strongly support the interpretation that intruders are acting as parasites or potential parasites and behaving in a passive manner for access to the nest. We also observed considerable variation in the intensity of the interaction with many interactions occurring with low intensity, but all levels of interaction are represented. All hens that enter a nest may not be intending to lay an egg and may be evaluating the state of the nest or status of the owner (host), which could account for short interactions. The fact that intruders persist long enough to lay an egg for most interactions (37/57) indicates intruder behavior as a parasitic tactic.

Cameras and radio frequency identification (RFID) recording of individuals provided a new and much richer picture of the interactions that occur at the nest. Without small cameras that could be inserted inside the nest box and record continuously (including at night), we would not have been able to quantify the scale and intensity of interactions among females, and without RFID tagging, we would not have been able to identify the interacting females. By combining these methods, we were able to track individuals and unique pairs of females throughout an entire breeding season and in doing so, evaluate multiple hypotheses that provide some insight into the factors that influence these female-female interactions.

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