The Underpinnings of Breeding Behaviors

Brandi Van Roo

On an early morning in the Appalachian Mountains in southwest Virginia, I was walking trails and listening for the familiar *here I am, where are you?* of vireos. Turning a bend in the path and entering a rhododendron stand, I heard those phrases sung with a patient pause between them. It was a Blue-headed Vireo, a species I had been studying there for five years. This male (vireo females do not sing) was easy to locate a short distance from the trail, about twelve feet up in a rhododendron (which can reach up to twenty feet tall). He did not stay there for long, and soon I was bushwhacking across the mountain, following the steady song as he delineated his territory. On we went, I stomping, climbing, and running, all the while with my binoculars poised in one hand. He would fly to a tree and move from branch to branch singing and foraging. We stopped occasionally when he gleaned a moth from a leaf or branch and paused to pull off the wings, which fluttered to the ground at my feet like the maple tree seeds. Then he beat the moth's body against his perch, back and forth with dramatic slaps, before swallowing the meal. On to the next tree he moved, alternating branches, momentarily disappearing behind a clump of leaves, then popping out from the other side (Figure 1).

Figure 1. Blue-headed Vireo (*Vireo solitarius*) male in Appalachian Mountains, Virginia.

Abruptly, he began making soft mewing noises as he continued his movements through the trees. My excitement grew: this was a signal that we were near his nest. Our chatty fellow disappeared behind a clump of leaves and emerged from the other side rather quiet. In earlier years I would have continued to follow my target, unaware that "he" was now "she"; the parents had switched places, and the female would lead me away from the nest. Having since learned the significance of those contact calls, I ignored the female as she left. Instead, I worked my way through the underbrush until I gained a full view of the pendant cup nest, suspended by a fork near the tip of the branch. I settled down to watch the father dutifully arrange his brood patch (large but less swollen than the female's) over the eggs. In twenty minutes he stood up to turn the eggs, distributing his heat more evenly and preventing the embryos inside from sticking to the shells. Little else happened during the forty-five minutes until the female arrived emitting the same soft contact calls, which the male returned in kind. Their mutual contact calls coordinated the deceptive changing of the guard: one parent arriving from one direction, and the other parent departing to the opposite direction, creating the illusion of a single bird continuing on its way through the forest.

During my two- or three-hour observation, I witnessed several of these "switches." Incubation bouts in this species may be as short as twenty minutes or as long as an hour and a half. The key is, whatever the length of time, both members of the pair incubate for the same duration, ensuring that each provides equal investment at this stage of caring for their offspring.

Birds, as a group, demonstrate impressive cooperation between parents, with over ninety-five percent of bird species performing some form of biparental care. Despite this, Blue-headed Vireo males are still champions among fathers because they not only perform *some* care, as do males of most species; they contribute approximately fifty percent to *all* forms of care including building the nest, incubating the eggs, and feeding the young. This contrasts with my next day's observation.

I did not get far along the trail before hearing *here I am, where are you?* this time sung in rapid succession. In early summer in Virginia, this could only be the Red-eyed Vireo, a species I had been studying there for four years. Although the song was easy to identify, obtaining a view of the male was quite another thing. I craned my neck backwards until my chin pointed skyward and watched the forty-five foot tops of maples and oaks, following the nonstop song for fifteen minutes before I saw his profile flit across a gap in the canopy. Red-eyed Vireo males will not lead you directly to the nest at this time of year because they do not contribute to parental care until the eggs hatch, at which time both parents begin feeding the young. Despite this, I followed his song, trying to get a glimpse of him through my binoculars. With their small territories and high breeding densities, I needed to be sure this was not a male I had already studied. So I not only needed to see *him*, I needed to see his *legs* to determine whether or not he had been banded.

Determining whether a 3mm-wide piece of plastic on a moving target, forty-five feet overhead, backlit by a clear, bright sky, is light blue or light green is not a task I would wish upon the most enthusiastic of birders. However, while doing so, I reaped a different reward. The male's incessant singing was suddenly interrupted by another bird's harsh cat-like whine, after which the male fell silent. I had only seconds to determine the location of this sound and fix my binoculars upon it; otherwise I would miss the female leaving the nest to forage. I did so and narrowed the nest site down to a particular clump of branches thirty-five feet up in a maple. I chose a good vantage point and settled in, knowing that she would make the same brief announcement upon her return in ten to fifteen minutes. If I missed her return, it would be another hour before the female would forage again. I saw her return to the same spot, but the canopy foliage was too dense to follow her to the nest. Settling down, again expecting an hour's wait, an unusual stroke of luck occurred: a Blue Jay flew into the surrounding area and prompted alarm calls from the female. By the time the Blue Jay moved out of the area, I had found the nest; in another twenty minutes I had found a place to clearly view the parent over the rim of the nest. I observed the nest for two more hours as the female alternated between long incubation bouts and short foraging bouts. The nest was left unattended while she foraged because, upon hearing her call, the male accompanied her. How different from their cousins, the Blue-headed Vireos!

The Red-eyed Vireo males, however, reflect the type and amount of male care that is typical in most birds.

Hormones

In addition to documenting these behavioral differences using banded birds, my goal is to elucidate some aspects of *how*, physiologically, the different behaviors occur. What prompts males of two species of vireos to behave so differently from one another? The nervous system is ultimately responsible for stimulating behavior, but hormones modify the architecture of the nervous system and thereby influence the likelihood of particular behaviors occurring. Behavioral endocrinology is the study of how hormones alter behaviors.

An overarching trade-off exists regarding how wild animals use their time: parental care (to ensure the survival of current offspring) or mating (to create future offspring [Trivers 1972]). Typically, time spent doing one is time taken away from doing the other. Red-eyed and Blue-headed vireo males appear to navigate this trade-off differently. Blue-headed Vireo males invest heavily in parental care, while Red-eyed Vireo males invest in extrapair copulations with females other than their mates (Morton, Stutchbury, Howlett, and Piper 1998). I investigated two hormones in vireos, testosterone and prolactin, because each is associated with behaviors on opposite sides of this trade-off. Testosterone (T) stimulates male song, which is used for attracting a mate and for maintaining a territory used by females to choose a mate. Prolactin (PRL) has many physiological roles, among which is the formation of the brood patch and the stimulation of behaviors associated with parental care. Thus, it is believed that these two hormones are the underpinnings of the trade-off between mating and parental care. This idea is supported by the typical pattern of secretion of these hormones across the breeding season in north temperate species (Figure 2A, modified from Ball 1991). T is elevated early, while males are singing the most, defending territories, and courting females. The T declines, and PRL is elevated with the onset of parental breeding stages.

Natural Levels of Hormones in Vireos

In order to determine why Blue-headed and Red-eyed vireo males behave differently, I began by documenting the levels of these two hormones in their blood across the breeding season. Would the patterns of T and PRL differ between two species? Would they differ from the pattern typical of most temperate species?

At each breeding stage, I captured males by luring them to mist nets with playbacks of the song of that species. Upon capture, I immediately took a small blood sample by pricking a vein in the arm, much as a doctor might take a small sample from our finger (Figure 3). The birds were color-banded for individual identification; however, leg bands are not visible when males incubate, so they also received a stripe of correction fluid (White-out ®) on either side of the crown (Figure 4).

Levels of T and PRL were determined back in the lab using radio-immunoassays. As predicted, the patterns of both hormones in Red-eyed Vireo males were similar to those seen in most temperate species (Figure 2B), just as parental care in Red-eyed

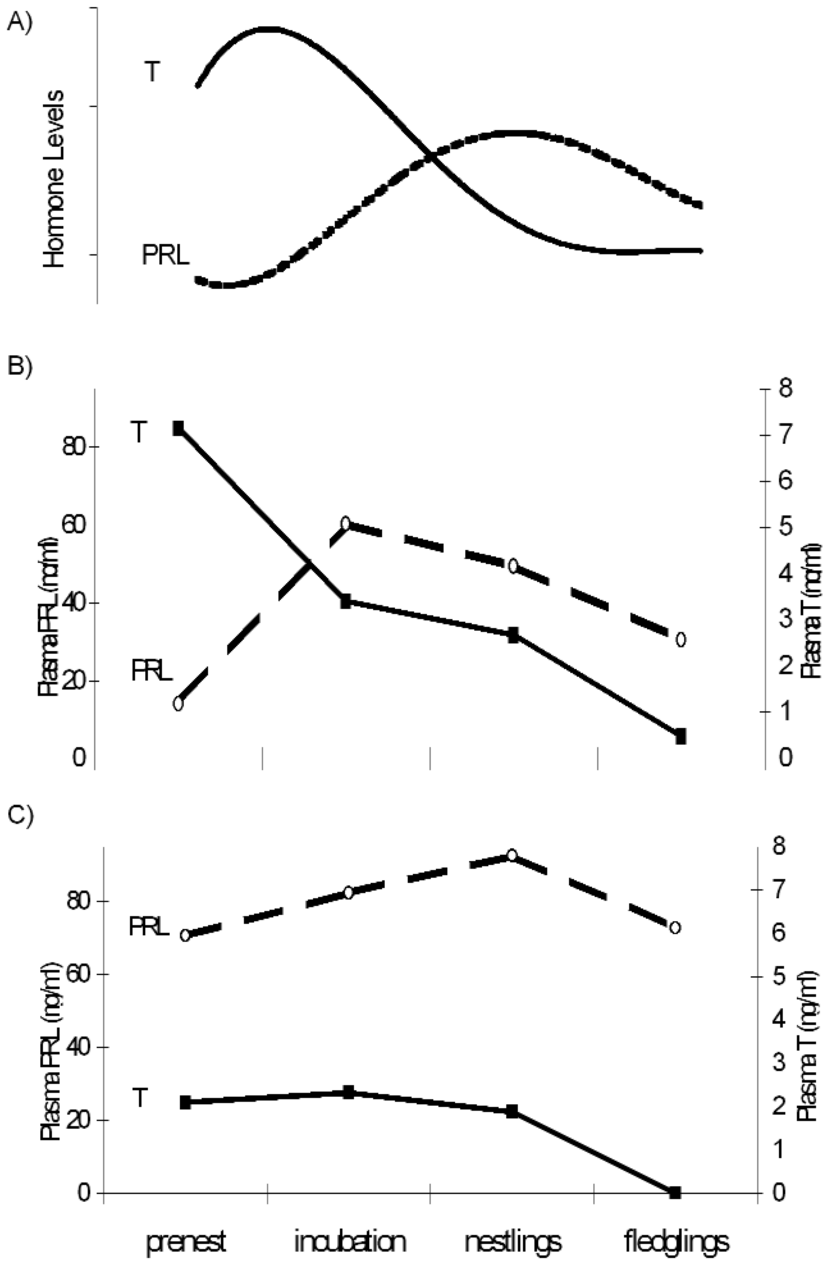

Figure 2. Breeding season profiles of testosterone (T) and prolactin (PRL): A) generalized for males of north temperate species, B) measured in Blue-headed Vireo males, and C) measured in Red-eyed Vireo males.

Vireo males is typical of most bird species. Patterns of T & PRL in Blue-headed Vireo males (Figure 2C), however, differed starkly from those of Red-eyed Vireos and most species. The early season peak in T was absent in Blue-headed males and remained low across all breeding stages. Further, PRL was not delayed until later breeding stages but was relatively high at the start of the breeding season. The unusually extensive parental investment demonstrated by these males, including early season behaviors such as nest building and incubation of eggs, was reflected in the underlying hormones: suppressed T and elevated PRL throughout the season (Van Roo, Ketterson, and Sharp 2003).

Figure 3. Collection of a blood sample from the brachial vein of a male Blue-headed Vireo.

Figure 4. A male Blue-headed Vireo marked with correction fluid and color leg bands.

As satisfying as these results were in many respects, they also prompted countless new questions. For example, why does PRL rise during the incubation stage in most species even though the males do not provide care until the nestling stage? Perhaps PRL needs time to alter the nervous system in preparation for hatching; surely, not responding until several days after hatch would be detrimental to the nestlings. Also, why can Blue-headed Vireos get by with low T during territory establishment and courtship? Is this because of their larger territories and lower breeding densities?

Testosterone's Effects on Parental Care

Perhaps most intriguing to me was the question of why T isn't lower in Blue-headed Vireo males than in Red-eyed Vireo males during the parental stages. I ask this because we know, from studies in a wide variety of temperate species, that artificially elevating T in males during parental stages can inhibit their normal levels of parental care (they run around singing and ignore the young). During the nestling stage, males of both species provide the same *type* of care, but Red-eyed Vireo males feed nestlings *less* than Blue-headed Vireo males do. Are Blue-headed males simply not as sensitive to T? I could find this out by giving them artificial T and observing whether parental behaviors declined. On the other hand, perhaps there's no benefit to lower T

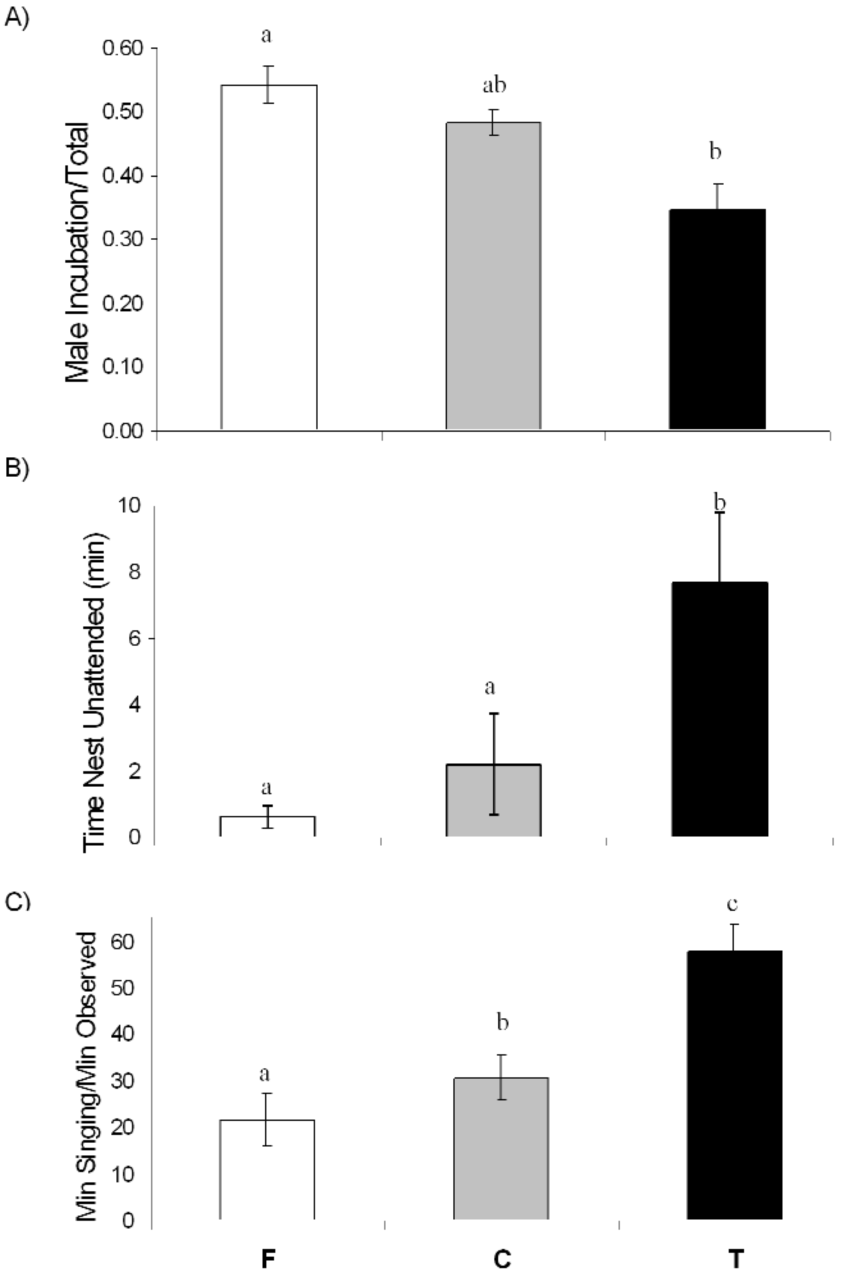

Figure 5. Behavior in male Blue-headed Vireos treated with flutamide (F), control (C), or testosterone (T) implants: A) relative duration of incubation bout (male/male + female), B) length of time that nests were left unattended, and C) percent time spent singing (minutes singing/minutes observed). Mean +/- SE.

in Blue-headed Vireos because they are already performing as much care as they are capable of. I could find this out by blocking their natural levels of T and seeing whether they perform greater levels of care or not.

I attempted to answer those particular questions in Blue-headed Vireos. I captured Blue-headed males in the same way as before, but this time, after taking the blood sample, I slipped a small hormone implant under their skin, much like how Norplant ® is administered to women. The implant looks like an uncooked macaroni noodle that I fill with T, which leaks out over time, providing a slow, even, long-term dose without having to recapture them. Males received one of three types of implant: empty (control), filled with T, or filled with flutamide (F), a chemical that blocks the androgen receptor. Flutamide does not actually lower the male's natural T, but it makes the natural T useless because it cannot bind to its receptor, effectively removing T from the playing field.

When I observed these males' behaviors, I found that T increased singing behaviors while decreasing male incubation behavior (Figure 5A), resulting in nests left unattended (Figure 5B), something that only usually occurred in Red-eyed Vireos. Testosterone also reduced rates of feeding young. Clearly, Blue-headed Vireo males are sensitive to T, so why aren't natural levels lower? Would it be possible for males to provide even more care? The F implants would determine this. Flutamide reduced singing (Figure 5C), indicating that F effectively inhibited T from doing its job. Flutamide influenced some parental behaviors in the predicted directions (e.g., longer incubation bouts, Figure 5A) but not statistically more so than controls. Thus, it appears that Blue-headed Vireo males are already providing as much parental care as possible, and inhibiting natural T can do little more in this regard (Van Roo 2004).

I am continuing my research on vireos here in Massachusetts. I hope to determine whether Red-eyed Vireo males would be better parents if their T was inhibited or their PRL was elevated. Also, I am bringing in a new player: the Warbling Vireo. There is reason to believe that while Warbling Vireo males incubate, they do not contribute a full fifty percent to incubation. Including this comparison allows me to increase the resolution of the questions. Are the effects of PRL apparent along a continuum: more PRL equals more care? Or is it threshold effect where PRL stimulates incubation and ecological pressures then determine how much. I will begin, as always, by documenting the natural levels of T and PRL in Warbling Vireo males. Then I would like to know whether artificially elevating PRL will result in Warbling Vireo males that perform more care, perhaps comparable to that of Blue-headed Vireo males? Or even more?

References

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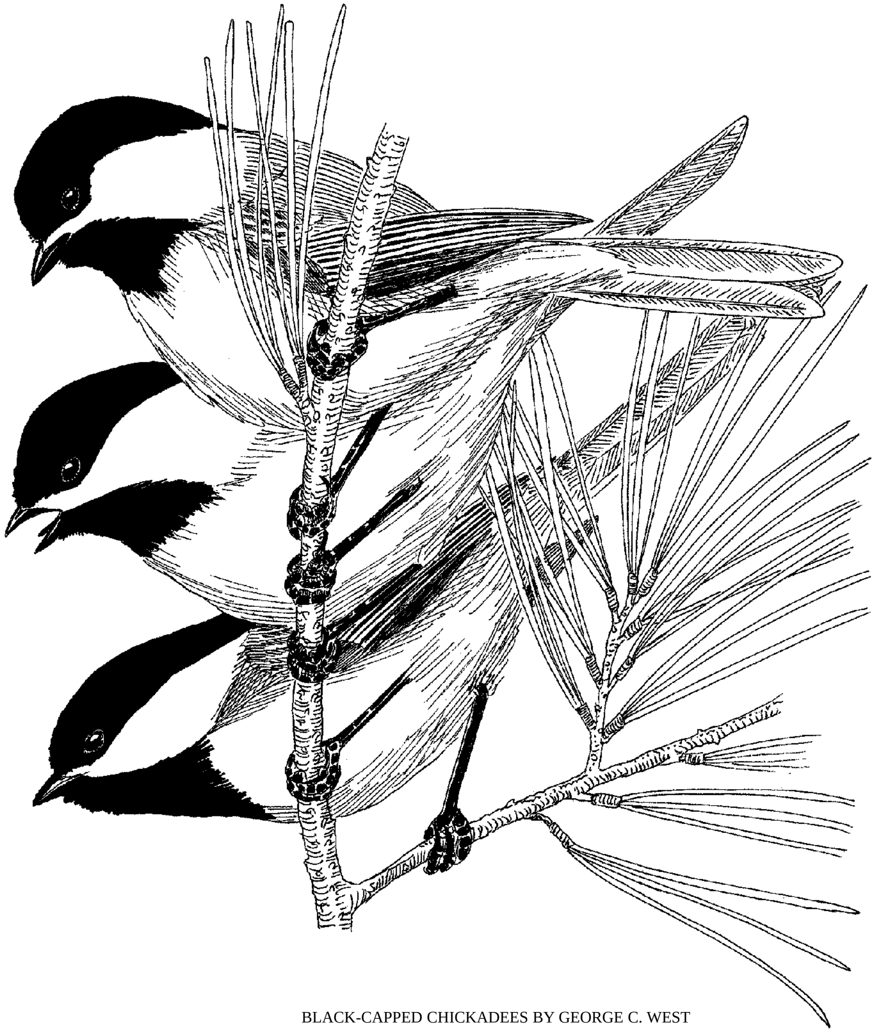

BLACK-CAPPED CHICKADEES BY GEORGE C. WEST