Intensity of Nest Defense in Indigo Buntings Increases with Stage and Not Number of Visits

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Many parent birds respond to the approach of potential predators by giving alarm calls or distraction displays. Detailed research has usually revealed that the intensity of the birds' reactions increases as the offspring get older (e.g. Barash 1975; Curio 1975; Gottfried 1979; Weatherhead 1979, 1982; Andersson et al. 1980; Grieg-Smith 1980). Two hypotheses have been proposed to explain this increase. First, levels of nest defense could reflect the increasing value of the offspring to the parents, either measured by past investment (Trivers 1972) or future expected benefits (Dawkins and Carlisle 1976, Boucher 1977, Maynard Smith 1977). Alternatively, the parents' increased defense might be a response to an increase in danger from predators (Harvey and Greenwood 1978) as the young get older.

Recently, Knight and Temple (1986) proposed that increases in nest defense might be an artifact of the methods employed by investigators to measure nest defense. In most studies, researchers either approached the nest themselves or presented a potential predator. Then they measured the birds' responses and removed the stimulus without harming the contents of the nest. Throughout the nesting cycle, this process was repeated many times. Thus, the observed increases in intensity of defense might have resulted from positive reinforcement and loss of fear. In other words, the parents learn that their behavior deters the "predator" and that the "predator" is unlikely to harm them.

Knight and Temple (1986) performed an experiment on Red-winged Blackbirds (*Agelaius phoeniceus*) and American Robins (*Turdus migratorius*). They found that, regardless of the stage in nesting cycle, the responses of birds whose nests were visited frequently were stronger than the responses of birds whose nests were visited only once. These data were taken as evidence that repeated visits affected the defense intensity of parent Red-wings and robins. If repeated visits commonly affect nest defense in this way, then a number of empirical studies of nest defense will have to be reexamined. I present data from an experiment on the nest defense of male Indigo Buntings (*Passerina cyanea*) that indicate repeated visits do not affect the male's behavior as strongly as does the stage in nesting cycle. I will discuss the disparity between these results and those of Knight and Temple (1986).

I studied nest defense of male Indigo Buntings in a population at the Mason Farm Biological Reserve, 3 km southeast of Chapel Hill in Orange County, North Carolina. The study area comprised a series of cultivated fields along the floodplain of Morgan Creek. Large hedgerows of roses (*Rosa multiflora*) separated the fields throughout the area. Bottomland hardwoods bordered the fields, and the edges along these borders often contained large patches of blackberries (*Rubus* spp.), goldenrod (*Solidago* spp.), and hardwood saplings. The study area contained two loops of dirt road, only one of which was traveled frequently. Most of the study area was not disturbed by human use.

I began observations of male and female buntings in early May 1987. I attempted to find nests by following females that carried nesting material. Nests found in this way were checked periodically until the first egg was laid. Some additional nests were found after incubation had begun. Because bunting eggs have partially translucent shells, I could judge, to within a day or two, the stage of development of the eggs in these nests. Eggs with no visible embryo had been incubated for only one or two days. By day 8 of incubation, the embryo and associated blood vessels filled the eggshell. I treated nests with recently laid eggs (within 3–4 days of the onset of incubation; $n = 6$) the same as nests found before egg laying ($n = 5$). Nests found at a later stage ($n = 4$) were omitted from tests of the effects of visits on nest defense.

At the end of each day, nests in which the first egg had been laid that day or that had been found that day were assigned randomly to one of three groups without replacement (i.e. matched for time of season). The groups were categorized as experimentals, control-incubation, and control-nestlings. Experimental nests were visited frequently throughout the nesting cycle (usually every other day). Control nests were divided into two groups. The first group, *control-incubation*, included nests visited for the second time (first visit occurred when nest was found) during the 3-day period before hatching; the second, *control-nestlings*, included nests visited for the second time when the young were 4–5 days old. I initially assigned nests in groups of 5 (2 nests each to experimental and control-nestling groups, 1 to control-incubation) because nest predation was expected to eliminate more of the later stages before measurement. This assignment of nests was changed midway through the season when I was not obtaining enough nests in the control-incubation category because of lower predation on the later stages than expected. From that point on, each new nest had an equal chance of being assigned to each group.

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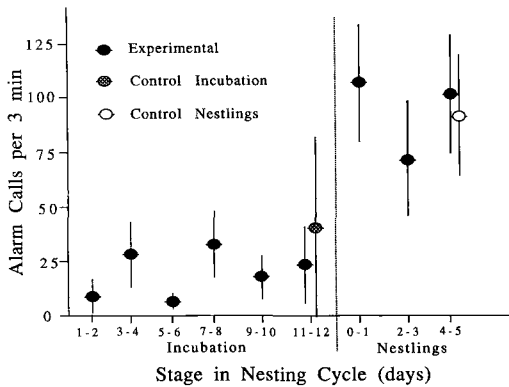
Fig. 1. Number of alarm calls ($\bar{x} \pm$ SE) given by male Indigo Buntings by the days since completion of egg laying (incubation) and days since hatching (nestlings). Experimental nests were visited every 2 days, whereas control nests were visited just once after discovery (see text).

During each visit, I approached the nest quickly, while I scanned the territory for the male. When I was 1 m away, I started to measure the male's responses. After 3 min, I checked the contents of the nest by hand and left the territory by the same path as I arrived. I always faced the male during the measurement phase of the visit. Male response was measured by counting the number of alarm calls given by the male in each of the 1-min periods. Other defense behaviors measured by Knight and Temple (1986), such as dives or strikes, were not recorded because buntings rarely, if ever, dive at or strike potential predators (either a human or a model bluejay) near their nests (Westneat unpubl. data). I did not measure the female's responses because her proximity to the nest was expected to be different between incubation (female is usually on the nest) and the nestling stage (female is usually away from the nest).

In all trials, the observer acted as the potential predator. Although this technique might have created problems because of unconscious biases of the observer that affected responses of the bird, I chose it for three reasons. First, measurement of the number of alarm calls is not as likely to be affected by observer bias as other measures of defense (e.g. the distance approached or the direction of distraction displays). Second, alternative methods, such as displaying a stuffed predator or using a tethered live predator, create additional disturbance around the nest while setting up. Finally, I felt the experiment would be adversely influenced if a second person acted as observer. Because buntings nest in fairly large territories and in brushy habitats, a second person would have to be on the focal bird's territory to observe its behavior. I reasoned that the presence of another person would provide a second and confusing stimulus to the focal bird.

No males were measured at more than one nest. Approximately 40% of the males on the study area had been color-banded the previous year (Westneat unpubl.). Most of the remainder could be identified by song or plumage. In any event, I focused my nest searches on territories where I had not found and visited nests previously.

Data were analyzed using nonparametric statistical tests (Siegel 1956), primarily because sample sizes were small and the number of alarm calls did not fit a normal distribution (many birds did not give any calls). All tests were two-tailed and were considered significant when $P < 0.05$.

I measured the defense behavior of males at 10 nests assigned to the experimental category. Three nests were visited a single time during late incubation, and 11 when the young were 4–5 days old. The average number of alarm calls given at each stage increased over the course of the nesting cycle (Fig. 1). I tested whether or not this increase was due to repeated visits by comparing the average number of alarm calls by experimental males (whose nests were visited at least 8 times) with the average number by control males (visited once after being found when the young were 4–5 days old). Experimentals ($n = 10$) averaged 102.6 alarm calls per 3 min, not significantly different from control males ($n = 11$) who averaged 91.4 calls (Mann-Whitney U-test, $U = 50$, $P > 0.36$). Comparison of experimentals (at least 4 visits, $\bar{x} = 26.5$ calls, $n = 10$) against controls ($\bar{x} = 40.0$ calls, $n = 3$) during late incubation also revealed no difference in the number of calls (Mann-Whitney U-test, $U = 14$, $P > 0.43$), but the number of controls was too small for a reasonable statistical test.

The comparison between control-nestlings and experimentals suggests that repeated visits do not cause the apparent increase in alarm-call rates through the nesting cycle. However, inability to reject the null hypothesis (no effect of repeat visits) is not convincing evidence that repeated visits have no effect and that stage does. When sample sizes are small, the probability of making a type II error, failing to reject the null when it is actually false, is high. Comparison of the responses of control-nestling birds to the responses of experimental birds on the first visit (in the first 6 days of incubation) provides a direct test of the effect of stage controlling for the number of visits (only one visit after nest was found). Control birds responded with significantly more alarm calls than experimental males during the first visit to the nest (Control: $\bar{x} = 91.4$ calls, $n = 11$; Experimental: $\bar{x} = 16.4$ calls, $n = 11$; Mann-Whitney U-test, $U = 23.5$, $P < 0.02$). This result indicates that stage had a strong effect on the birds' responses.

I surmise that increases in nest-defense intensity over the nesting cycle are influenced by the stage in the nesting cycle. Whether or not nest defense is also affected by repeated visits is not as clear. Experimental males did average 11 more alarm calls per 3 min than

controls. Given the small samples and the large variance in alarm-call rates, repeated visits could have had some effect and yet go undetected by the statistical test. However, it is clear that, in Indigo Buntings, nest-defense intensity is affected more by stage than by repeated visits.

A number of theoretical and methodological issues are raised by the difference between these results and those of Knight and Temple (1986). First, one key difference between my study and Knight and Temple's (1986) is in the statistical analysis of the data. Knight and Temple (1986) used two regression analyses to test the effects of stage. One regression was on the responses of birds whose nests were visited repeatedly and so tested the effects of both stage and repeated visits. The other regression was on responses at nests visited only once but at different stages, thus testing only the effect of stage. The regression testing the effects both of stage and of repeated visits was significant, whereas the test of stage alone was not. Knight and Temple (1986) concluded that stage had little effect and that repeated visits explained the increase in nest-defense intensity over the nesting cycle. However, this conclusion depends on an acceptance of the null hypothesis of no effect of stage in the test of stage alone. Care must be taken when interpreting this result because the probability of rejecting the null when it is false (i.e. Type II error) is not known for the test used. It is thus possible that stage has a strong effect and repeated visits have a small effect on the behavior of Red-winged Blackbirds and American Robins, but that the variance in responses is sufficient to mask the effect of stage when considered alone.

Nevertheless, the main issue Knight and Temple (1986) raise is still valid. Carefully controlled experiments (e.g. Curio 1975) that test for influences of methodology should be a prerequisite to detailed studies of the adaptive function of nest defense. In any study, similar care should be used when interpreting statistical tests that suggest no difference between groups.

I found that stage has an effect in one species. However, it is possible that different species may respond differently to repeated presentations of the same predator. In this context, the impact of repeated visits on parent birds may be of interest beyond being simply a potential problem in methodology. Lack of a response by male Indigo Buntings to repeated visits may indicate that buntings rarely interact with the same individual predators, whereas the opposite may be true in Red-winged Blackbirds and American Robins. Future researchers should heed Knight and Temple's (1986) suggestions about methodology, but should not ignore the possibility that successful nest defense may require increases in the intensity of defense as the young become older in some species and require increases with repeated stimuli in others.

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