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IMPORTANCE OF TACTILE AND VISUAL STIMULI OF EGGS AND NEST FOR TERMINATION OF EGG LAYING OF RED JUNGLEFOWL

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ABSTRACT.—Experiments were conducted to separate the influence of tactile and visual stimuli emanating from the nest or eggs on the development of incubation behavior, the termination of egg laying, and the determination of clutch size. Red Junglefowl (*Gallus gallus spadiceus*) hens, whose eggs were left inside the nest (experiment 1), received both tactile and visual information, remained in the nest box longer, and stopped laying after eight days (or six eggs). Sixteen or 17 females incubated. Leaving only the first egg in the nest (experiment 2) gave similar results. When the eggs laid were placed under a wire-mesh basket (experiment 3) such that the hen could see but not touch the accumulating eggs, laying stopped two days (or one egg) later than in experiment 1, and most hens "incubated" on the empty nest until the nest box was removed. Surprisingly, when eggs were continually removed (experiment 4), hens also incubated progressively more, stopped laying after two weeks (or nine eggs), and then sat on the empty nest for one or more days. Stimulation of the brood patch by the nest alone led to more incubation and to termination of egg laying. Visual stimuli alone provided by eggs accelerated both processes and were sufficient for one-half of the hens to maintain full incubation behavior. Red Junglefowl do not appear to judge clutch size visually.
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DURING THE BREEDING SEASON, a female has to make two important "decisions": when to start laying, and when to stop laying. The period between the onset and the end of egg laying (as well as the interval between eggs) determines the number of eggs in a clutch (clutch size).

During the laying period, eggs accumulate in the nest, and females of both altricial (Haftorn 1981, Zebra and Morton 1983, Beukeboom et al. 1988, Meijer 1990) and precocial species (Parsons 1972, Caldwell and Cornwell 1975, Drent 1975, Afton 1980, Kennamer et al. 1990, Hall 1991, Meijer and Siemers 1994) sit on the nest for a longer time each day. At the end of the laying period, females incubate for almost the whole day and, in the ovary, follicle development becomes suppressed. The gradual increase of incubation is a good predictor of clutch size (Haftorn 1981, 1985, Meijer 1990, 1993, Meijer et al. 1990)

Visual and tactile stimuli can inform the laying female about the number of eggs in the nest (Klomp 1970, Murton and Westwood 1977). It is apparent from egg-removal and egg-addition experiments (for reviews, see Kennedy 1991, Haywood 1994) that females of a variety of bird species take notice of the number of eggs in the nest. The few experiments by which tactile information coming from the brood patch was manipulated by local anesthesia (Hall and Goldsmith 1983) or denervation (Hall 1987) failed to show a direct behavioral effect in incubating female domestic ducks (*Anas plathyrhynchos*). Denervation of the brood patch in laying turkeys (*Meleagris gallopavo*; eggs removed continually) shortened the time spent in the nest and inhibited full development of incubation behavior (Book et al. 1991). Experiments to test the influence of tactile and visual stimuli separately during the laying period are even rarer. Steen and Parker (1981), conducting experiments in which eggs accumulated under a wire-mesh basket beside the real nest, claimed that bantam (*Gallus domesticus*) hens can judge their clutch size by visual stimuli alone.

I present data on the egg-laying behavior of Red Junglefowl (*Gallus gallus spadiceus*; Kruijt 1964, Meijer and Siemers 1994). I tried to separate the influence of stimuli emanating from the nest from those provided by eggs. Also, I set up an experiment to distinguish the influence of visual and tactile information on the development of incubation behavior, the termination of egg laying, and the determination of clutch size.

TABLE 1. Summary of the main results for four experiments: (1) eggs accumulated inside nest; (2) only first egg laid left in nest; (3) eggs accumulated next to nest under wire mesh; and (4) eggs removed every day.

Experiment	No. hens	No. hens (%) on nest at night	Clutch size ($\bar{x}$ ± SD)	Day when stopped laying ($\bar{x}$ ± SD)
1	17	16 (94)	6.1 ± 0.9	8.2 ± 1.3
2	10	8 (80)	5.6 ± 1.3	8.4 ± 3.1
3	13	11 (85)	7.2 ± 1.9	9.8 ± 2.8
4	14	7 (50)	9.0 ± 2.8	13.9 ± 5.9

METHODS

Experiments were conducted using 21 pairs of Red Junglefowl hatched from eight clutches in the early summer of 1992 (see Meijer and Siemers 1994). The birds lived in large outdoor aviaries in three mixed groups over the winter and started to lay during late December and January (see Sharp 1993) at an age of six months. Eggs were removed daily. From 25 February, they were held pairwise (one male and one female) in adjacent outdoor aviaries (1.5 m wide × 4.5 m long × 3.0 m high) under natural daylight and temperature conditions. Each pen contained a shelter (1.0 × 1.0 × 1.5 m) with roosting perches and a nest box (27 × 50 × 23 cm). Commercial chicken food and water were provided *ad libitum* daily.

As a measure of incubation behavior (see fig. 1 in Meijer and Siemers 1994), nest temperature was recorded every 5 min with NiCrNi thermistors situated on the bottom of the nest cup and connected to a 16-channel datalogger (Squirrel 1205, Grant England). To separate tactile from visual stimuli nest boxes were divided into two halves, one of which was covered with wire mesh (see Steen and Parker 1981). The bottom of the wooden nest box was covered with a rubber pad having fingers (3.5 mm diameter, 15 mm height) topped with a nipple (0.9 mm diameter, 1.0 mm height; Vencomatic, The Netherlands), over which was place a layer of hay. In the late afternoon (1600–1700 MET), nest boxes were checked, eggs were marked, and eggs weighed to the nearest 0.01 g (Sartorius Pt 120). Between late March and early June, four experiments were conducted in which: (1) eggs were put back, so that eggs accumulated inside the nest (n = 17 different females); (2) only the first egg laid was put back and others were removed (n = 10); (3) eggs were put back into other half of nest box, under wire mesh, so that accumulating eggs were within sight of the hen, but she could not touch them (n = 13); (4) eggs were continuously removed (n = 14).

When females stopped laying, they were allowed to incubate for a maximum of four to five days, after which the nest box (with eggs) was removed for a period of five days. When hens started to lay again (after two to three weeks), one of the other three experiments was carried out (the order of which was randomized).

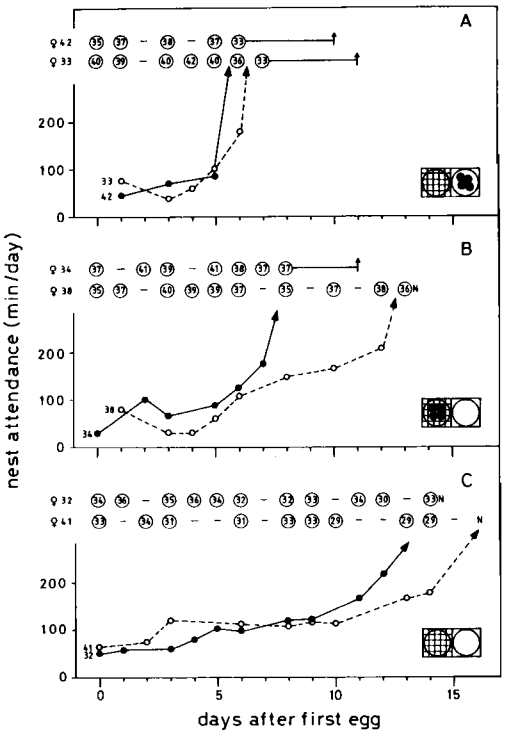

Fig. 1. Representative examples of egg-laying patterns (top of each diagram) and development of incubation behavior when: (A) eggs accumulated inside nest (experiment 1); (B) eggs accumulated next to nest under wire mesh (experiment 3); and (C) eggs removed every afternoon (experiment 4). Egg mass (g) shown within egg symbol; pause days indicated with dash. Full incubation, which lasted until nest box removed (indicated by arrow) is represented by solid line. At end of laying, females 38, 32, and 41 attended empty nest only for one night (indicated with N).

RESULTS

Experiment 1: Full tactile and visual stimulation.—In the control situation, when females were allowed to keep their eggs inside the nest, they stopped laying after an average period of 8.2 ± SD of 1.3 days (n = 17; see Table 1). During this period, females remained in the nest box for a longer period each day (see Fig. 1A), and incubated also during the night on the day the penultimate or the last egg was laid. Sixteen of 17 females incubated continuously from the moment the last egg was laid. The other female (no. 30) attended the nest box 30 to 50 min while

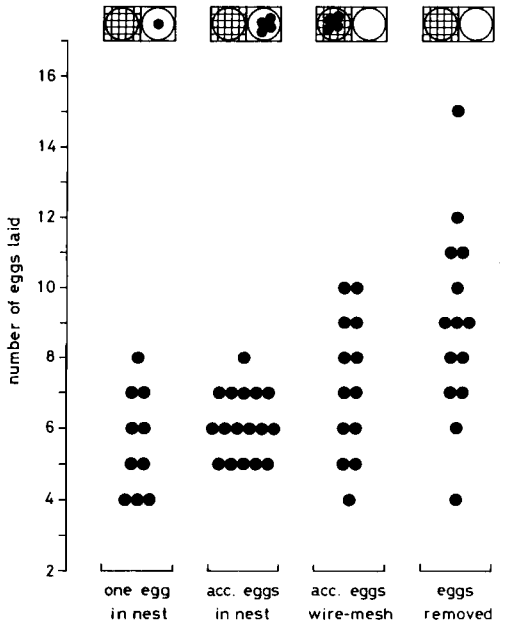

Fig. 2. Number of eggs laid by females when: only first egg laid left in nest (experiment 2); eggs accumulated (acc.) inside nest (experiment 1); eggs accumulated next to nest under wire mesh (experiment 3); and eggs removed every day (experiment 4). Each dot represents clutch size of single hen.

laying each of the first four eggs, and over 200 min for the last (eighth) egg, but did not incubate during the night.

Clutches contained 6.1 ± 0.9 eggs (Fig. 2). The masses of the last eggs in a clutch were markedly lower (Fig. 1A). After removal of the nest box, females were forced to stop incubation, and started to re-lay after a period of 13.4 ± 2.8 days.

Experiment 2: Tactile and visual stimulation of first egg only.—When only the first egg laid was left in the nest box (all subsequent ones were removed), the hens reacted similarly to females in experiment 1. Incubation behavior developed slowly during the laying period. The birds stopped laying after 8.4 ± 3.1 days, clutches contained four to eight eggs (n = 10; see Fig. 2), and last eggs were again much smaller than the first ones.

Eight of 10 females started to incubate during the night (from two days before until two days after the last egg was laid). They incubated continuously, on one egg only, until the nest box was removed. Of the other two females, one (no. 13) incubated for longer and longer periods (from 20 to over 250 min); however, after being driven off the nest twice at the end of laying (during the afternoon inspection), she did not start full incubation. The other female (no. 9) stopped laying after five eggs, without any increase in incubation behavior.

Experiment 3: Permanent visual stimulation of eggs.—When the eggs were put under the wire mesh in the afternoon so that females could only see but not touch them, the hens stopped laying after 9.8 ± 2.8 days (range 6–14 days, 7.2 ± 1.9 eggs, n = 13; Fig. 2). All 13 females showed an increase in incubation behavior during the laying period (see Fig. 1B), two did not start night incubation, five incubated during one (e.g. no. 38; see Fig. 1B) or two nights, and the other six incubated next to the clutch on an empty nest until the nest box was removed (e.g. no. 34; see Fig. 1B).

Experiment 4: No visual stimulation.—When I continually removed the eggs laid (in the late afternoon), hens stopped laying after 13.9 ± 5.9 days (range 6–29 days, 9.0 ± 2.8 eggs, n = 14; see Fig. 2). Nest attendance slowly increased, and egg mass decreased as laying progressed (Fig. 1C). Five of 14 females did not return to the empty nest after being gently driven off during the afternoon control (they had incubated the last egg at that moment for 198 ± 107 min). Another seven sat on the empty nest continuously at the end of the laying period for one to three days. All females stopped "incubation" on their own before the empty nest box was removed. Also, in this situation, females waited almost two weeks (11.2 ± 4.5 days) before laying new eggs.

Females reacted significantly differently in the four experiments with respect to the period in which laying stopped (ANOVA, $F_{3,50} = 7.80$, $P < 0.001$). Posthoc tests (LSD test for unequal sample sizes; Sokal and Rohlf 1981) showed significant differences only between the laying period of females in experiment 4 (eggs removed every day) compared to those of experiment 3 (eggs under wire mesh; $P = 0.02$), experiment 2 (first egg only left in nest; $P = 0.005$), and experiment 1 (control; $P < 0.001$). The same result was found for the number of eggs laid per "clutch" (ANOVA, $F_{3,50} = 8.45$, $P < 0.001$).

Discussion

Termination of egg laying.—When eggs were allowed to accumulate inside the nest (experi-

ment 1), the time spent on the nest increased rapidly; Red Junglefowl hens laid relatively small clutches of five to seven eggs (see also Meijer and Siemers 1994). This finding indicates that, directly or indirectly (Haftorn 1985), the increase in incubation behavior turns off follicular development in the ovary in 6 to 11 days. It has been argued that tactile stimulation of the brood patch from the accumulating eggs in the nest leads to an increase in incubation behavior (Klomp 1970, Murton and Westwood 1977). In bantams the onset of incubation is initiated by an increase in the secretion of prolactin, which also suppresses the secretion of LH, leading to regression of the reproductive system (Sharp 1980, Lea et al. 1981, 1982, but see Sharp et al. 1988).

Experiment 2, in which females kept only the first egg in the nest and all following ones were removed after being laid, demonstrates that without accumulating eggs in the nest the transition from laying to incubation occurred in a similar way. Even in a situation in which the female returned to the nest and saw but could not touch the (increasing number of) eggs beside it (experiment 3), incubation behavior still progressively developed and the ovary was turned off in a period of 6 to 14 days. Most surprisingly, when eggs were removed continuously (experiment 4), the laying female sat also on the nest longer and longer each day. Within 6 to 29 days ($\bar{x}$ = 14 days), all females stopped laying. Not only did female Red Junglefowl stop laying, but the increase in incubation behavior, the overnight sitting (in an empty nest), the smaller size of the last eggs, and the two-week pause before new eggs were laid strongly indicate that in this treatment females laid clutches of nine eggs.

The differences in the time needed to turn off the ovary recorded in the four treatments were small (on average 8 to 14 days). The biggest differences were in: (a) the period after which the hens remained in the nest box overnight; (b) the degree of disturbance by the afternoon inspection; and (c) the intensity of incubation at the end of the laying period. In the control situation (experiment 1), females started to incubate during the night on the day the last egg was laid and often one day earlier. In the other experiments, night incubation sometimes started one or even two days after the last egg was laid. When all the eggs were left in the nest (experiment 1), all incubating females returned to the nest after they had left it in the late afternoon during the nest check. When only the first egg remained in the nest (experiment 2), one hen stopped incubation after being driven off twice. This happened also once in experiment 3 (eggs under wire mesh). Of the 14 females whose eggs were continously removed (experiment 4), 5 stopped incubation after being driven off only once. When the incubating female could only see the eggs (experiment 3), 4 of 10 females stopped incubation on their own. When they could neither touch nor see the eggs (experiment 4), all 7 incubating females left the nest on their own. In experiments 1 and 2, all females were forced to stop incubation when I removed the nest box. These experimental findings indicate that to maintain incubation behavior visual stimuli of eggs are sufficient for one-half of the hens. Incubation continued in all females receiving tactile stimuli of the whole clutch. Tactile stimuli of only one egg in the nest had the same effect.

Does the hen "count" the number of eggs in a clutch?—To answer this question, Steen and Parker (1981) conducted experiments (my control experiment 1, and experiment 3 with the wire mesh) and suggested that bantam "hens observe the actual number of eggs by eye and used these observations to decide when the clutch had reached the desired size and possibly also to stimulate the onset of brooding." The outcome of experiment 2 (in which only the first egg laid stayed in nest box, and females laid normal number of eggs) does not support Steen and Parker's ideas.

Earlier, Moss and Watson (1982), in a reply to Steen and Parker (1981), showed that wild Red Grouse (*Lagopus lagopus scoticus*) lay clutches of similar size whether or not some eggs are removed from their nests. I think that this is the case for all determinate-laying species. However, Moss and Watson (1982) mentioned further that captive Red Grouse, all of whose eggs were continually removed from the cage, laid many more eggs than the number in the usual clutch (i.e. in captivity they behave as indeterminate layers). The evaluation of determinate versus indeterminate laying needs to be addressed using standard methods of egg removal and addition (see Kennedy 1991). The stage in the breeding season and in the laying period of one clutch when experiments are conducted can be critical. European Kestrels (*Falco tinnunculus*), for example, are indeterminate lay-

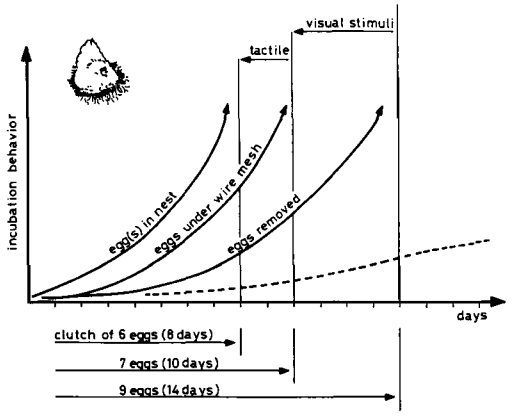

Fig. 3. Summary of development of incubation behavior, termination of laying, and number of eggs laid by Red Junglefowl held in pairs (one male and one female) under four experimental conditions. Broken line represents estimation of incubation behavior of hens living in mixed groups (data not given).

ers early in the season, but determinate later on (Beukeboom et al. 1988). European Starlings (*Stumus vulgaris*) switch from being indeterminate to determinate layers between the first and second egg of a clutch of four to five eggs (Meijer 1993). Another complication is that, as in the Red Junglefowl (experiment 4 versus 2), a number of bird species react differently to experiments in which all eggs or all but the first are removed (e.g. Blue Tits [*Parus caeruleus*]; Fox *in* Haywood 1993, see also Kennedy 1991).

Visual and tactile stimuli.—Figure 3 illustrates the importance of the tactile and visual information for the development of incubation behavior, termination of laying, and clutch size in the Red Junglefowl hen. When all eggs were continually removed (experiment 4), nest occupancy slowly increased to 3 to 4 h per day and, after occupying the nest overnight, females stopped laying on average within 14 days. In this situation, the hen returns to an empty nest, sits down, lays an egg, and leaves the nest and egg after a while. When the tactile information is exactly the same (i.e. only for brief period between laying of egg and leaving nest), but the hen sees the increasing number of eggs next to the nest (experiment 3), there is an acceleration in the development of incubation behavior and a significant advancement in the suppression of follicular development in the ovary (by about four days), resulting in smaller clutches (ca. two eggs fewer). When eggs accumulate inside the nest (experiment 1) and the laying female gets additional tactile stimuli from the eggs, there is a further (insignificant statistically) advancement of two days, and clutches become again another egg smaller. The tactile stimuli of one egg (and briefly of two eggs, for the time between laying an egg and leaving the nest subsequently; experiment 2) already are sufficient for a suppression of follicular development within eight days, a period for laying clutches of six eggs.

Surprisingly, Red Junglefowl held in small groups of three to six females and one male continued laying over more than two months (laying 45–50 eggs, without longer pauses; unpubl. data for 1992). Depending on the social situation under which the chickens were held, incubation behavior develops almost spontaneously and laying stops within two weeks (one male and one female), or incubation development is suppressed and laying continues for months (harem situation; see dashed line in Fig. 3). Also, in individually housed turkeys whose eggs were removed daily, the time spent on the nest progressively increased and laying stopped. The turkeys incubated in an empty nest. In contrast, hen turkeys with denervated brood patches visited nests the same number of times, but occupied the nest for a shorter time, showed no increase in nest occupancy, and none showed full incubation (Book et al. 1991). Stimulation of the brood patch by the nest alone probably increases incubation behavior, and ultimately leads to termination of egg laying.

The results of experiments 1 to 4 indicate that Red Junglefowl, in a situation in which eggs are removed continually, stop laying within two weeks after laying a "clutch" of nine eggs. The tactile stimuli of the nest alone can induce full expression of incubation behavior and termination of laying. When the hen can see, or see and touch, the increasing number of eggs, laying stops four to six days earlier, respectively. When only the first egg stays in the nest, laying is also terminated six days earlier. Thus, it is clear that visual stimuli alone accelerate the development of incubation behavior and the termination of laying; therefore, they are important in determination of clutch size. However, Red Junglefowl do not judge clutch size based on the visual observation of egg number, and the same is probably true for bantams.

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