SOME EXPERIMENTS ON FACTORS INFLUENCING THE EARLY REPRODUCTIVE CYCLE OF THE BENGALESE FINCH

by

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CONTENTS

INTRODUCTION

Reproduction in birds is usually limited to a few months in the year when conditions are at their most favourable. The onset of a period of reproductive activity is marked by an increase in the size of the gonads and their commencement of the secretion of sex hormones. That the spring recrudescence of the gonads in many temperate zone species occurs in response to increasing day-length has long been realised (Rowan, 1938; Marshall, 1942), and a strong body of evidence has accumulated indicating that light, acting through the hypothalamus, stimulates the release of gonadotropins from the adenohypophysis, and that this in turn leads to the growth of the gonads (Farner, 1961, 1964; Wolfson, 1964, 1966).

Approaching the equator, fluctuations in day-length, weather and food supply become less great, and in many species breeding may be found at any time of year (Moreau, 1950; Miller, 1963), while in others the cycle of reproductive activity is not annual (Ashmole, 1963; Chapin, 1954; Dorward, 1962). In certain tropical species which do show seasonal periodicity, it has been found possible to correlate breeding seasons with specific factors in the environment. Rainfall is the most common, some species breeding only during the rainy season and others only outside it (Chapin, 1959; Marchant, 1959; Miller, 1963). *Quelea*, a species breeding in the rainy season, comes into condition only in response to green grass which it uses for nest building, rain without green grass being ineffective (Marshall & Disney, 1957). Natural selection has presumably favoured individuals which do not attempt to breed at those times of year when their reproductive effort would usually be wasted and their own survival put in jeopardy. The abundance of food is clearly an important factor in producing such selective forces (Lack, 1954).

There is thus considerable diversity in the timing of breeding in birds, and likewise different factors doubtless operate in different species to bring them into reproductive condition at the time most appropriate to successful breeding. But while the selective advantage of breeding at one time of year and not another is often apparent, rather little is known of the proximate factors acting on tropical species to bring them into condition (Farner, 1967), the case of *Quelea* being an exception.

External stimuli which lead to the recrudescence of the gonads at the start of the breeding season, have been termed *primary timing mechanisms* by Farner (1964). They may bring about full spermatogenesis in males (Riley & Witschi, 1938; Burger, 1949), but have not been found to result in full ovarian development or ovulation in females (Lehrman, 1959). Further environmental stimuli, such as those provided by mate, nest-site and nest material, are usually required for ovulation and for the orderly progression of behavioural and physiological changes occurring during the breeding cycle. Farner (1964) refers to these as essential supplemental mechanisms, and it is with these that we shall be primarily concerned in this thesis.

Within a given breeding season there may be several cycles. Lack (1954, 1966) argues convincingly that the reproductive rate of birds is geared to producing the maximum possible number of young. Parental behaviour, linked with the rather low reproductive rate that it seems to necessitate, doubtless evolved as leading to a greater number of surviving young than the huge number of eggs laid and left uncared for by most invertebrates and many lower vertebrates. In birds, parental behaviour has reached a high level of sophistication with incubation of the eggs and care of the young lasting some weeks or even months. The succession of events during the single breeding cycle, from courtship and nest building, to egg-laying and incubation, to hatching and the feeding and brooding of the young, relies for its efficiency on a high degree of integration between the members of a pair and their surroundings. The achievement of this has been shown by a great weight of research to depend on a subtle interplay between external stimuli and endocrine secretions (Eisner, 1960a; Lehrman, 1961, for reviews).

Changes in hormone levels in the blood stream are considerably slower than changes in the nervous system following stimulation, but they are adapted to provide sustained signals of a type ill-suited to the nervous system. Because of the time taken for hormone levels to build up, the stimulus leading to hormone secretion is likely to be found hours or days before a behavioural response which is dependent on the presence of that hormone. It cannot therefore be assumed that this stimulus is present in the immediate context in which the response occurs. It is in fact often necessary to name two stimuli: that bringing about the appropriate physiological state and that eliciting the immediate response. Only after the first has acted on the endocrine system, will the action of the second elicit the response by means of the nervous system. In a highly organised and synchronised pattern of changing behaviour, such as the breeding cycle of a bird, there is no reason why the stimuli leading to these two responses should be the same, indeed the very structure of the cycle often suggests that they are not. For example, it has been shown for several species (e.g. Poulsen, 1953; Tinbergen, 1953), that the reaction of a bird to eggs placed in its nest is indifference up to a certain time before laying, but incubation after that time. The latter response being immediate and not found where no egg is present, this indicates that the immediate stimulus for incubation is the egg, but that it is unimportant in bringing about the correct endocrine background. The main requirement for a stimulus to become linked to hormone secretion is that it should occur a predictable time before the hormone is needed. Selection has no doubt favoured those animals reacting to environmental cues that are reliable indicators of conditions which are to follow.

What then are the external stimuli responsible for the further development of the gonads and other associated changes occurring after growth has started under the influence of primary timing mechanisms? The most important are undoubtedly those provided by the mate. Even in males, where full spermatogenesis can be achieved in a number of species under the influence of light alone (Lehrman, 1959), the presence of females may accelerate the effect (Burger, 1953). Other important influences are those connected with territory, nest-site and nest building (Lack, 1933; Lehrman, 1959).

The majority of experimental work on this subject has been carried out on pigeons and doves. As early as 1904, Harper noted that egg-laying followed about eight days after pairing, and that two females placed together sometimes laid. Whitman (1919) concluded that "the stimulus to ovulation is to be found in the preceding sexual activities". He noted that isolated pigeons may occasionally lay and that in pairs of females both copulation and egg-laying sometimes occur, the bird assuming the masculine role in copulation tending to lay less often than its partner. Craig (1911) had previously found that copulation is not essential to normal egg-laying in heterosexual pairs. Matthews (1939) deduced that the stimulus to ovulation is visual: the lower level of stimulation provided by a companion of the same sex, or by isolation in the presence of a mirror, led to longer delays before laying, despite the continued presence of the calls of other pigeons. This conclusion is certainly not valid for the ring dove, the sound of a breeding colony and the presence of a mirror together having been found to lead to normal egg-laying, while either of these factors on its own was inadequate (Lott & Brody, 1966). Erickson and Lehrman (1964) found that stimulation from intact males led to greater increase in oviduct weight and incidence of ovulation than that from castrates.

In an elegant experiment, and one of the very few so far performed on the external stimuli leading to incubation, Lehrman, Brody and Wortis (1961) found oviduct growth, ovulation and incubation behaviour to occur only in the presence of a mate and to be brought forward slightly by the additional presence of nest material. Their results suggest that the external stimuli leading to incubation are similar to those inducing ovulation and oviduct development.

In other species, Burger (1942) on the starling and Polikarpova (1940) on the house sparrow reported that ovarian growth would only reach a certain level under the influence of light alone and that the presence of males led to further development. Polikarpova (1940) found that a nest was required for ovulation. Vaugien (1948) discovered that isolated female serins did not lay, but would do so if placed with another bird of either sex. Removal of nests stopped all laying, as did deprivation of the down normally used to line the nest.

In the domesticated canary, egg-laying is delayed in pairs given no nest material and further so in those without a nest pan (Hinde & Warren, 1959). Pairs with nest pan and nest material but not allowed to build a nest, laid at about the same time as normal pairs, suggesting that the completed nest is unimportant (Hinde & Warren, 1959). The presence of a male accelerates the occurrence of nest building - in this species largely carried out by the female (Hinde, 1958) - as well as completion of defeatherisation of the brood patch and egg-laying. The influence of the male on egg-laying appears to be largely through his effect in causing the female to build, building in turn leading to laying (Warren & Hinde, 1961b). In the budgerigar, auditory stimuli are clearly important for ovarian development (Vaugien, 1951; Ficken et al., 1960), and Brockway (1965) found this to be largely attributable to one vocalisation from the male repertoire, the 'soft-warble', normally associated with pre-copulatory behaviour.

From these studies it is apparent that both mate and nesting facilities are important as external stimuli leading to the physiological and behavioural changes occurring early in the breeding cycle. Complete lack of either has been shown to stop laying altogether in some species, and increased latencies have been found with varying degrees of partial deprivation. In the case of incubation a further factor must be taken into account: that of the presence of eggs in the nest.

Eggs are clearly important as an immediate stimulus to incubation. Odd cases arise in the literature of birds incubating on empty nests (Whitman, 1919; Owen, 1940), but it is difficult to decide from the descriptions whether these birds were actually incubating or just sitting on the nest (Simmons, 1955). Steel and Hinde (1966a), however, found that in some cases canaries brought into reproductive condition in winter sat without laying. They behaved as if glued to the nest, could be lifted off by hand and even removal of the nest for up to four days did not break the habit (Steel, <u>in litt</u>.). Incubation without eggs can therefore happen under certain circumstances, although such behaviour is presumably disadvantageous, especially in view of the fact that incubation has been found to inhibit further egg-laying: eggs presented in advance of laying may be adopted and the clutch subsequently laid has been found to be diminished in size or suppressed altogether (Paludan, 1951; Poulsen, 1953; Weidmann, 1956; Nelson, 1965). From these observations it seems that readiness to incubate develops in advance of egg-laying but is not usually expressed until the eggs are present. Lehrman (1958a) found that ring doves, which had previously associated with mate and nest material for seven days, were prepared to incubate within a few minutes of being given eggs. As most of the birds had not yet laid themselves, the presence of eggs cannot have been responsible for the development of readiness to incubate. Tendency to incubate therefore develops as a result of stimuli from mate and nest material in the ring dove. Several studies have shown that it does not appear suddenly, as the accounts of naturalists might suggest, but develops gradually over some days (Gurr, 1954; Tinbergen, 1953; Snow, 1960; etc.), and increases in intensity during the incubation period as well (Breitenbach, Nagra & Meyer, 1965). For these reasons, the lack of an exact criterion for incubation in many of the older studies makes them hard to interpret.

Discussion has so far been limited to the changes in physiology and behaviour occurring early in the reproductive cycle and the external stimuli to which they are attributable: we shall now consider the hormones which mediate these changes. Activity mimicing that of mammalian Follicle-stimulating hormone (FSH) and Luteinising hormone (LH) has been found in birds (van Tienhoven, 1961), although it is possible that in birds these hormones exist only as a complex (Nalbandov, 1959; Farner, 1967). Injected FSH leads to ovarian growth and LH induces ovulation in hypophysectomised hens (Opel & Nalbandov, 1961a, b). Winter injection of pregnant mare's serum has been found to lead to ovarian development and in some cases egg-laying in serin (Vaugien, 1957) and canary (Steel & Hinde, 1966b). Lehrman (1959) postulated that courtship leads to FSH secretion and thence oestrogen, while nest building causes secretion of LH and thence progesterone. A number of links in this chain, particularly that suggesting that LH induces progesterone secretion, remain to be verified.

A period of courtship is often required before nest-building begins, suggesting that oestrogen may be necessary for nest-building. Lehrman (1958b) did in fact find that ring doves began nest-building at once when paired after a course of oestrogen injections. Warren and Hinde (1961a) found the presence of a male to augment nest-building in canaries during the natural breeding season but to have no effect on nest-building induced by oestrogen injection in winter. The hormonal basis of nest-building in this species is not simple, however, as oestrogen is effective only in near lethal doses and its effect is not enhanced by progesterone or prolactin (Warren & Hinde, 1959; Steel & Hinde, 1963). Both these last mentioned hormones are undoubtedly present at around this time and they seem to play a part in other changes which occur concurrently with nest-building.

Progesterone has been detected in the blood of laying hens (Fraps _et al_., 1948; Lytle & Lorenz, 1958), and in extracts of their ovaries (Layne _et al_., 1957). Fraps _et al_. (1949) found this hormone in cocks and Lofts and Marshall (1959) discovered it in the seminiferous tubules of hypophysectomised male pigeons, thus also pointing to it being gonadal in origin, though the exact site of its secretion is still unknown (van Tienhoven, 1961). The pituitary content of prolactin has been found to be low during laying and high during incubation in hens (Saeki & Tanabe, 1955; Burrows & Byerly, 1936), turkeys (Cherms _et al_., 1962) and pheasants (Breitenbach & Meyer, 1959). These results could as well mean a high level in the blood stream as a low one, depending on whether a high pituitary content is due to a high rate of secretion or a block to release (Follett _et al_., 1967).

Bailey (1952) found that a full brood-patch could only be obtained in hypophysectomised passerines if both oestradiol and prolactin were injected. Vascularity of the canary brood-patch seems to be under the control of oestrogen alone (Steel & Hinde, 1963); defeathering appears due to oestrogen and prolactin (Steel & Hinde, 1963, 1964), and increased sensitivity to progesterone and oestrogen (Hinde & Steel, 1964, 1966).

These results, originally obtained with intact individuals, have since been confirmed using birds ovariectomised before injection (Hutchison et al., 1967). Both progesterone and prolactin augment the effect of oestrogen-induced oviduct growth at low doses (Steel & Hinde, 1963). Work on fowl (Mason, 1952; Brant & Nalbandov, 1956) and dove (Lehrman & Brody, 1957) has suggested that oestrogen and progesterone is the important synergism here. Rothschild and Fraps (1949) found injected progesterone to lead to ovulation, and this effect has also been obtained when the hormone is introduced into the hypothalamus (Ralph & Fraps, 1960). Feedback of progesterone may in fact lead to LH secretion and thus ovulation (Fraps, 1955). Although fragmentary and not always clear cut, the evidence therefore points to growth of the gonads and oestrogen secretion resulting from courtship, and oestrogen, either alone or in combination with progesterone or prolactin, leading to oviduct growth, brood-patch formation and nest-building.

The first suggestion that a hormone was involved in incubation behaviour came when Lienhart (1927) showed that the blood of incubating hens could induce incubation in non-incubating birds. Riddle et al. (1935) obtained the "full expression of broodiness" in actively laying hens of broody genotypes when they were injected with prolactin at doses sufficient to suppress ovarian activity. A partial effect was obtained with other hens and with cocks. Eigemann (1937) and Riddle (1937) found progesterone to be ineffective in bringing about broodiness and van Tienhoven (1958) actually found this hormone at high doses to disrupt established incubation in turkeys.

Although the work on the domestic fowl is now somewhat old, it points to prolactin being the important hormone in this species. One disturbing factor, apparent in retrospect, is the failure of many workers to distinguish between incubation and brooding behaviour (Eisner, 1960a). Nalbandov and Card (1945) induced cocks of broody genotypes to adopt young but not to incubate eggs. Yet care of young can often be induced without hormone treatment whereas incubation seems more dependent upon it (Collias, 1946; Saeki & Tanabe, 1955). That the success in inducing cocks to adopt chicks by prolactin injection may be due to the anti-gonad effect of this hormone is suggested by the fact that the behaviour is annulled by FSH or androgen (Nalbandov, 1945), and can equally well be induced by castration (Goodale, 1916).

Extending their work to the ring dove, Riddle and Lahr (1944) implanted pellets of progesterone, testosterone or desoxycorticosterone in females, and found 43 out of 62 birds to be ready to incubate within three weeks, mostly within nine days. As they observed crop growth to occur as well as incubation in every case, they concluded that these hormones led to the release of prolactin and that this in turn led to incubation as they had found it to do in the fowl.

Subsequent work has shown this conclusion to be invalid. Lehrman (1958b) treated doves with oestrogen or progesterone for seven days and then placed them in pairs to test for incubation. A control series, treated with neither hormone, sat with the normal latency of 4-10 days. The results of those given oestrogen showed a bimodal distribution, all birds sitting in either under four days or over ten days, although none was observed to do so on the day of pairing. By contrast, all 14 pairs given progesterone sat on the day of pairing and, significantly, none had an increased crop weight at the time of doing so. Lehrman and Brody (1961), in a long series of systematic experiments, found an increase in crop weight to result from 10 i.u. of prolactin, a dose level which proved insufficient to bring about an increase in incubation behaviour. While a dose of 400 i.u. was adequate to bring about full crop development, only 40% of such birds incubated compared with 70% in previous experiments using progesterone. An important difference between these experiments and those of Riddle and Lahr (1944) is that Lehrman tested all his birds in heterosexual pairs after the period of treatment whereas Riddle and Lahr placed their birds in monosexual pairs from the outset, under which conditions incubation may occur without hormone treatment.

While it is safe to conclude that the onset of incubation occurs under the influence of progesterone and not prolactin in the ring dove, prolactin is probably important in its maintenance once started. Patel (1936) concluded that incubation itself brings about secretion of this hormone and thus crop growth. Lehrman and Brody (1964) found that birds isolated for 12 days during incubation lost the tendency to incubate unless they were treated with prolactin, in which case their readiness to do so was maintained.

Results on other species are scarce. In two out of four canaries tested, desoxycorticosterone (DCA) made them "become broody and begin to sit on the nest for the greater part of the day" (Kobayashi, 1952). Progesterone, though structurally very closely similar to DCA was found ineffective. The criterion employed and the small number of birds used leave much to be desired, however. Robinson and Warner (1964) found a thirtyfold increase in incubation behaviour in young brown-headed cowbirds treated with prolactin, but no increase in adults. The relevant hormones for parental behaviour are present in this species, a brood parasite, but the target organs appear to lose sensitivity in the adult (Hohn, 1962; Selander, 1960; Selander & Kuich, 1963).

Hohn and Cheng (1965) support prolactin as the important hormone in the incubation of most species, finding male phalaropes to produce more prolactin than females and the pituitary weights of three diverse species to be greater in the incubating sex. This circumstantial evidence is of little use, however, as the prolactin production may as well be connected with brood-patch formation as with incubation behaviour. The extent to which normal incubation depends on sensory feedback from the brood-patch is doubtful, though the work of Medway (1961) points to it being rather unimportant. The brood-patches of male phalaropes are apparently induced by testosterone and prolactin and this treatment is also effective on females which normally have no brood-patch and do not incubate (Johns & Pfeiffer, 1963).

From this survey it will be seen that the ring dove is the only species in which the hormonal basis of incubation is at all clear. The dove may well be exceptional, however, for such results as do exist on other species do not confirm the role of progesterone in inducing incubation. As doves belong to the only avian order producing crop milk, there are particular dangers in generalising from them to other birds. Prolactin undoubtedly causes crop milk production (Riddle, 1937), and it is thus not surprising that the onset of incubation in doves occurs under the influence of another hormone. Such considerations might lend support to the view that prolactin is involved in the onset of incubation in other species, and some results do point to this. Nevertheless, the testes of incubating males remain large in a number of passerine species, suggesting that prolactin, the anti-gonad effect of which is well established, may not be involved (Eisner, 1960a).

One factor which complicates the issue is the close association between prolactin and progesterone which has been shown to exist in mammals (Everett, 1961), and may well do so in birds. In mammals prolactin supports the corpus luteum from which progesterone is secreted. The situation in birds undoubtedly differs, the amount of progesterone in the ruptured follicle being small (van Tienhoven, 1961), but there is nonetheless circumstantial evidence that an interplay exists between the two hormones. A number of effects have been found to occur to a greater or lesser degree after the injection of either (Lehrman & Brody, 1961; Steel & Hinde, 1963; Hinde & Steel, 1964). These effects may well be due to one stimulating release of the other but evidence is lacking on this point. Lehrman (1963) speculated that the incapacity of prolactin to produce incubation in hens which are not laying may be because its effect is through the release of progesterone which can only occur from active ovaries.

In this brief review it will have become apparent that work on the reproductive behaviour of birds has been carried out on very few species. The difficulty of breeding all but a few in captivity, a necessary prerequisite for adequate control of the situation, has undoubtedly been of influence here, and many of the results obtained have been on very small numbers. A more systematic approach has been largely limited to the fowl and the dove (see Eisner, 1960a; Lehrman, 1961), with the recent work of Hinde and his co-workers on the canary (reviewed by Hinde & Steel, 1966; Hinde, 1967) extending it to the Passeriformes. The need for further species to be studied is apparent from the equivocal nature of some of the results obtained so far, and the wide diversity in breeding behaviour and physiology found amongst birds (Lehrman, 1961). The differences between altricial and precocial species (Lehrman, 1958a), and between those with and without brood-patches (Bailey, 1952; Eisner, 1960a), is particularly relevant and interesting here. There is also a wide variety in patterns of parental behaviour, from those species in which the male plays no part to those in which he alone incubates and tends the young (Kendeigh, 1952; Skutch, 1957), with the interesting intermediate stage in which the male does not incubate but returns to feed the nestlings (Skutch, 1953).

The experiments to be described in the present thesis were stimulated by the above considerations. The Bengalese finch is a particularly suitable animal for such an investigation. Its behaviour differs in a number of respects from that of the canary and, while it is similar to the ring dove in the share of the sexes in nest-building and incubation and in its failure to form a full brood-patch, the two species are certainly phylogenetically distant. A thorough examination of the effects of various external stimuli during the early part of the reproductive cycle has therefore been undertaken. The results obtained will be described here, as will those of a few preliminary attempts to stimulate behaviour changes by the injection of hormones.

Chapter 2

MATERIALS AND METHODS

The Bengalese Finch

The ancestry of the Bengalese finch has been a subject of some doubt, but Eisner (1957) argued convincingly in favour of the present stock being derived exclusively from some sharp-tailed finches (Lonchura striata) taken from China into captivity in Japan some 250 years ago. The uncertainty which previously existed appears to have been due to the fact that piebald, white and fawn individuals are common in the Bengalese, while not being found in the wild. The evidence points to these forms having arisen in captivity from the 'self-chocolate' type which closely resembles the sharp-tailed finch.

The Bengalese has a number of features which make it a useful animal for laboratory study. Not the least of these is the fact that it breeds throughout the year, a characteristic apparently shared with wild Lonchura striata (Eisner, 1960b). Other useful points are its short generation time, young birds being ready to breed at three to four months of age, and the ease with which it may be kept and bred in captivity. The wide differences in plumage between individuals make it possible to separate them from a distance.

While variation in plumage is useful, there are other points of difference between individuals which are less welcome from an experimental point of view. The time taken from pairing until the first egg is laid was found by Eisner (1960b) to vary between 1 and 21 days and the clutch size from 2 to 9 eggs in pairs with breeding experience (I have since on two occasions noted incubation proceeding normally with a single egg). In most clutches the eggs are laid on consecutive days, but gaps in laying of up to ten days are not uncommon. This leads to difficulty in defining a clutch, for a succession of eggs with a gap in laying in the middle could be regarded as a single clutch or as two clutches, the first of which was not incubated. The majority of clutches do not hatch, apparently due to infertility, and eggs or chicks are sometimes deserted in mid-cycle. It is quite common for chicks to die through failure of the parents to start feeding them after they hatch. These features, as with variations in song and plumage, are doubtless due to alteration or reduction in selective forces compared with those in the wild. The fact that the Bengalese differs greatly from any wild species does not of course diminish its usefulness as an experimental subject, but the individual differences mean that care must be taken in selecting the parameters for study so that significant information can be gained from the comparatively small number of replicates possible in studies of this type.

Housing and Maintenance

Towards the end of the study a sound-deadened room became available and this was used for experiments involving auditory isolation. In all other experiments the birds used were visually isolated but in the same room as the stock cages.

Natural daylight entered this room through a window and further lighting was provided by a fluorescent strip controlled by a time-switch. During the summer (1st May to 15th August) this was set to give 15½ hours light per day, and in winter (15th November to 1st February) light was maintained at 10 hours. Between these periods it was increased and decreased with the natural day-length. This is the same regime as that used by Eisner (1960b) but with a rather longer day-length in mid-winter. The room was ventilated by an extractor fan and heated by radiators attached to the ceiling which maintained the temperature in the range 19-24°C. Noise from outside the room, which was on occasion considerable, was dulled to some extent by the sound of the extractor fan.

All cages were provided with water for drinking and bathing, sand, cuttlefish bone and seed. This last was a mixture of white millet and canary seed to which cod-liver oil and wheat-germ oil were added as well as Kal-min mineral mixture. Thomas's conditioning food and Sluis Universal food were given occasionally in a moist mixture. Birds feeding chicks did so almost entirely with this and others ate it in small quantities. All birds were given lettuce about once a fortnight.

The size of the colony was around 100 birds throughout the study and it was maintained as far as possible by breeding, although some birds were bought from dealers. Two stock cages were used, enabling males and females to be kept separately. Each measured 180 x 45 x 30 cm and, although there were sometimes up to 50 individuals in this space, there was no evidence that they were overcrowded. All the birds in each stock cage habitually went to roost in the three nest boxes provided. Lonchura striata is noted for its sociability and this character is thought by Immelmann (1962) to be accentuated in captivity.

Young birds were kept separately from adults until two to three months old, by which time the males had started to sing and were moved to the male stock cage. Birds which were not seen to sing at this stage were assumed to be female and subsequent egg-laying showed this to be so in every case. All birds used in experiments were put through at least one breeding cycle beforehand. First cycles tend to be more variable than later ones with the delay before the first egg usually much longer. Birds which failed to construct nests, lay eggs or incubate during this initial cycle were given further pairings until they did so or else not used in experiments. Shortage of experienced stock necessitated the use of the same birds in several experiments, though not as far as possible in similar situations. Appendix I shows the birds used in each experiment. Pairs were selected which had neither parent in common, had never been together before and in which each bird had spent at least a month in its stock cage since last paired. Where possible all females used were under three years old and in no case was any over five years old.

Birds to be used in experiments were generally taken direct from the stock cages and placed in breeding cages 60 cm long x 45 cm high x 30 cm wide. A nest box of 10 cm^3 dimensions was placed in one of the upper corners at the rear of each cage. This was open at the front but for a lip 4 cm in height with a perch mounted on it. Each box was backed with a glass plate and, the area behind the cages being blacked out, observations could be made through this at close quarters without disturbing the birds. Unless otherwise mentioned, periods of observation to detect incubation behaviour were carried out from behind. All other observations were made from in front of the cages at a distance of about 6 feet so that behaviour both inside and outside the nest box could be noted. The twelve experimental cages were arranged in three rows of four: the top row was too high for observation within the nest box from in front or behind so that these were not used in experiments on incubation. The birds did not appear to be disturbed by the presence of the observer either in front of or behind their cages, but five minutes were always left between the observer taking his place and the start of a watch. Following usual practice, the day of the commencement of each experiment is termed day 0. The dates on which experiments were started are shown in Appendix I.

The Normal Breeding Cycle

The courtship of the Bengalese finch has been described by Morris (1958), and the breeding biology is given in general terms by Eisner (1960b, 1961), who has also studied its parental behaviour in rather more detail (Eisner, 1963). Although she found no evidence of seasonal fluctuations in breeding condition, certain parameters associated with breeding did show annual cycles, though not in phase with one another. From the point of view of the present study, the most important of these was the delay between pairing and the first egg which was longer in winter than in summer. The medians are similar (7 days for 27 pairs set up between April and September; 9 days for 39 pairs set up between October and March), but the two groups differ significantly and this must be borne in mind when comparing data from different times of year (data from Eisner, 1960b).

In most pairs, therefore, a period of several days must elapse before egg-laying and incubation commence. Some females allow copulation on the day of pairing but it is more usually observed only after a gap of some days. Jefferies (1967) reports that within 10 minutes of pairing 90% of males were seen to court, 37% attempted copulation and 22% were apparently successful. Manipulation of nest material and carrying may also be seen from the first day, but most nest building activity is concentrated into the week before egg-laying, so that in pairs showing a long lag to the first egg, relatively little nest building occurs in the few days after pairing. The male does by far the majority of the carrying of material to the nest box (Table 2.1), and the sexes share in the shaping of the nest within the box. Up to 9 eggs are laid at intervals of one day or more. Incubation then proceeds for about 16 days before the eggs hatch, but if they fail to do so it lasts for around 30 days. Both sexes share in incubation, the part played by each being roughly equivalent. The eggs are never left uncovered during the incubation period, the two partners alternating in their duties with individual stints usually less than an hour in length. The ventral apterium in both sexes is devoid of feathers at all times, but no increase in vascularity or oedema is noticeable in incubating birds, although it is of course possible that the apterium becomes more sensitive. At about the time of the start of incubation singing by males declines considerably and is rare when incubation is well underway. Carrying ceases almost entirely at the same time.

TABLE 2.1: Comparison between sexes of number of pieces of nest material carried to the nest box during observation period of ½ hour, 10 minutes after addition of new supply of material.

Pair	Date	Day after pairing	Carrying	
			♂	♀
♂234 ♀275	13/2/68	5	72	0
♂181 ♀229	13/2/68	5	39	3
♂255 ♀245	13/2/68	5	48	1
♂233 ♀206	14/2/68	6	28	2
♂254 ♀256	14/2/68	6	45	0

Chapter 3

EGG-LAYING

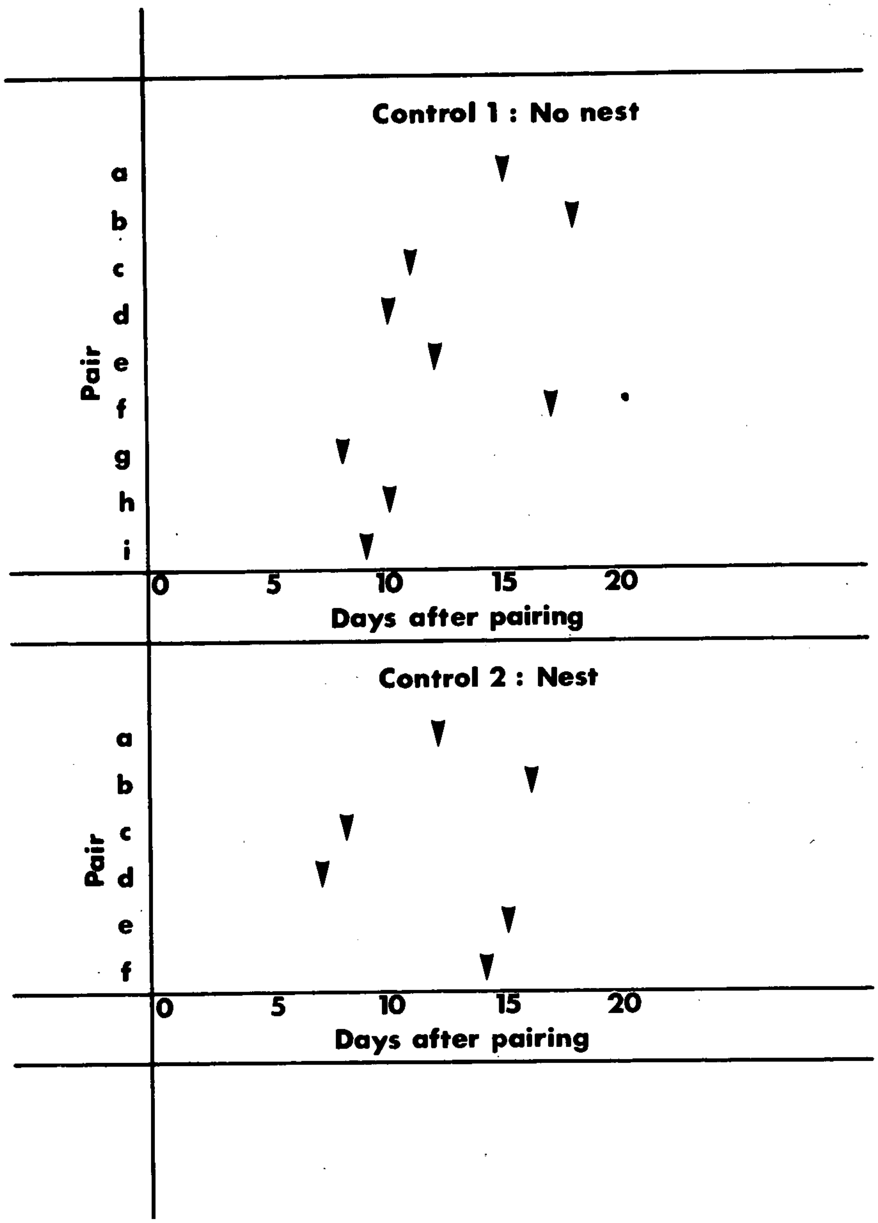
Introduction

Eisner (1960b) found a delay of up to 21 days between pairing and the laying of the first egg in experienced pairs provided with a nest box and nest material. Although the median lag was eight days, in only 7% of cases was the first egg laid less than six days after pairing. Eggs, or the remains of them, are rarely found in the female stock cage (see also Jefferies, 1967). From this it is clear that external factors prevalent in the first few days after pairing are influential in bringing about egg-laying.

Basically the factors which could be important here may be split up into those emanating from the mate and those from nest material. Both of these may stimulate the female in a number of different ways, and the experiments to be described in this section involved total or partial deprivation of one or a number of these stimuli to determine their relative effects on egg-laying. The cages in which the females were kept were inspected daily for the appearance of eggs.

As a control, nine normal pairs were set up with nest material and empty nest box. The results obtained are shown in Figure 3. 1, compared with six pairs which were used as

FIGURE 3.1: Egg-laying by pairs given empty nest box and nest material (Control 1), and by those given a rough nest as well (Control 2). Arrows indicate day on which first egg was laid by each pair.


Control 1 : No nest
Pair
a
b
c
d
e
f
g
h
i
0
5
10
15
20
Days after pairing
Control 2 : Nest
Pair
a
b
c
d
e
f
0
5
10
15
20
Days after pairing

controls in the incubation experiments and given a nest as well. The two groups do not differ significantly from each other. The median lag of 12 days is rather longer than that found by Eisner (1960b). Her use of the same pairs more than once, and use of birds only a brief period after they were last paired, might have been factors leading to this shorter gap. Thus differences found in the experiments I shall describe here are best compared with my own data.

The Effect of the Mate

Any influence which the male may have on the day of laying could be exerted in a number of different ways. Singing, courtship and copulation are the most obvious ones, but his mere presence may itself be important, and his manipulation of nest material and carrying of it to the nest box are also stimuli that the female receives at this time. As the male does by far the majority of the carrying, his presence may also be necessary if experience of building within the box is an important stimulus to the female, as she can only do this after material has been carried to it.

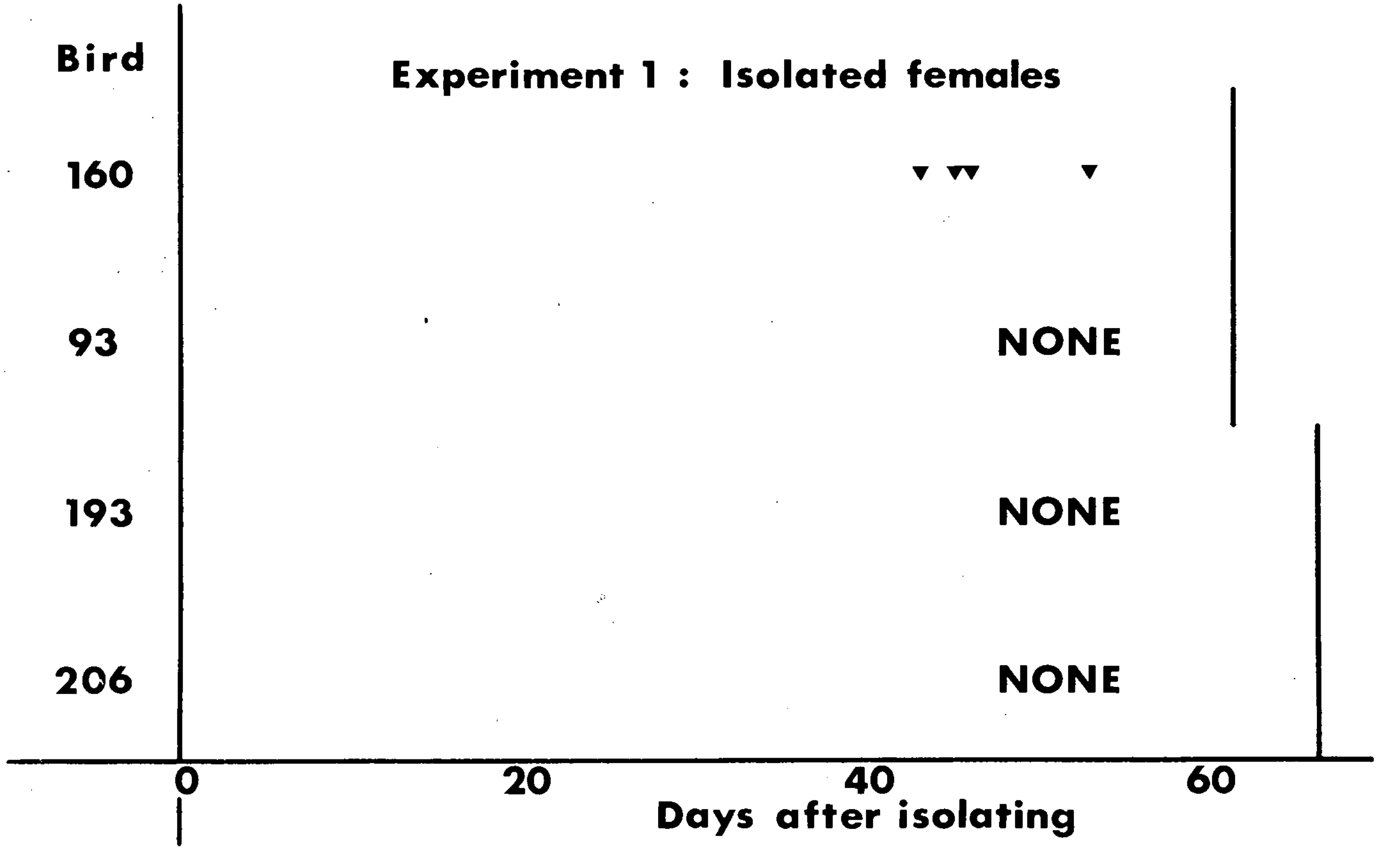
In an attempt to decide which of these factors are of importance three different techniques were employed. It is not of course usually possible in a complex situation such as this to remove one factor without altering others, so that the experiments performed depended on varying degrees of deprivation to assess the importance of factors removed relative to those that remained. In the first experiment, females were isolated in cages by themselves but supplied with a nest and nest material to ascertain whether the presence of a male was of any influence at all. In the second, monosexual pairs of females were set up to determine the extent to which stimuli inducing egg-laying are singular to the male. The third situation involved pairs separated by bars and these experiments will be dealt with later as this also allowed some control of nest building experience.

a) Isolated birds (Experiment 1)

Four females were placed singly in cages provided with a nest box containing a rough hand built nest and with nest material scattered on the floor of the cage. These birds were visually isolated from all other birds but in the same room as the stock cages and therefore subject to auditory stimulation from courting and singing males. All these birds were kept in isolation for over 60 days, the nest being replaced on occasions when it was found to have been dismantled. This was in fact necessary at least once in every case and none of the birds was noted to have added material to the nest.

As shown in Figure 3. 2 only one bird (♀160) laid, and

FIGURE 3.2: Egg-laying by isolated females given nest material and nest box containing rough nest (Experiment 1). Arrows indicate days on which eggs were laid; vertical lines show the finish of each experiment.


Experiment 1 : Isolated females
Bird
160
93
NONE
193
NONE
206
NONE
0
20
40
60
Days after isolating

even then only after the abnormally long delay of 43 days. ♀93 not only failed to lay on this occasion, but also during the two subsequent pairings before she died, indicating that she might have become incapable of doing so. After 67 days without laying, ♀193 and ♀206 were placed together and laying took place on day 9 for one of them and day 11 for the other (the appearance of two eggs in the nest on day 11 indicated that both birds participated).

These results stress the importance of the male in stimulating egg-laying by the female. The fact that one of these birds did lay and that eggs are sometimes found in the female stock cage points to visual stimuli from males not being an absolute prerequisite for laying, but they are clearly necessary if it is to occur at the normal time.

b) Monosexual pairs (Experiment 2)

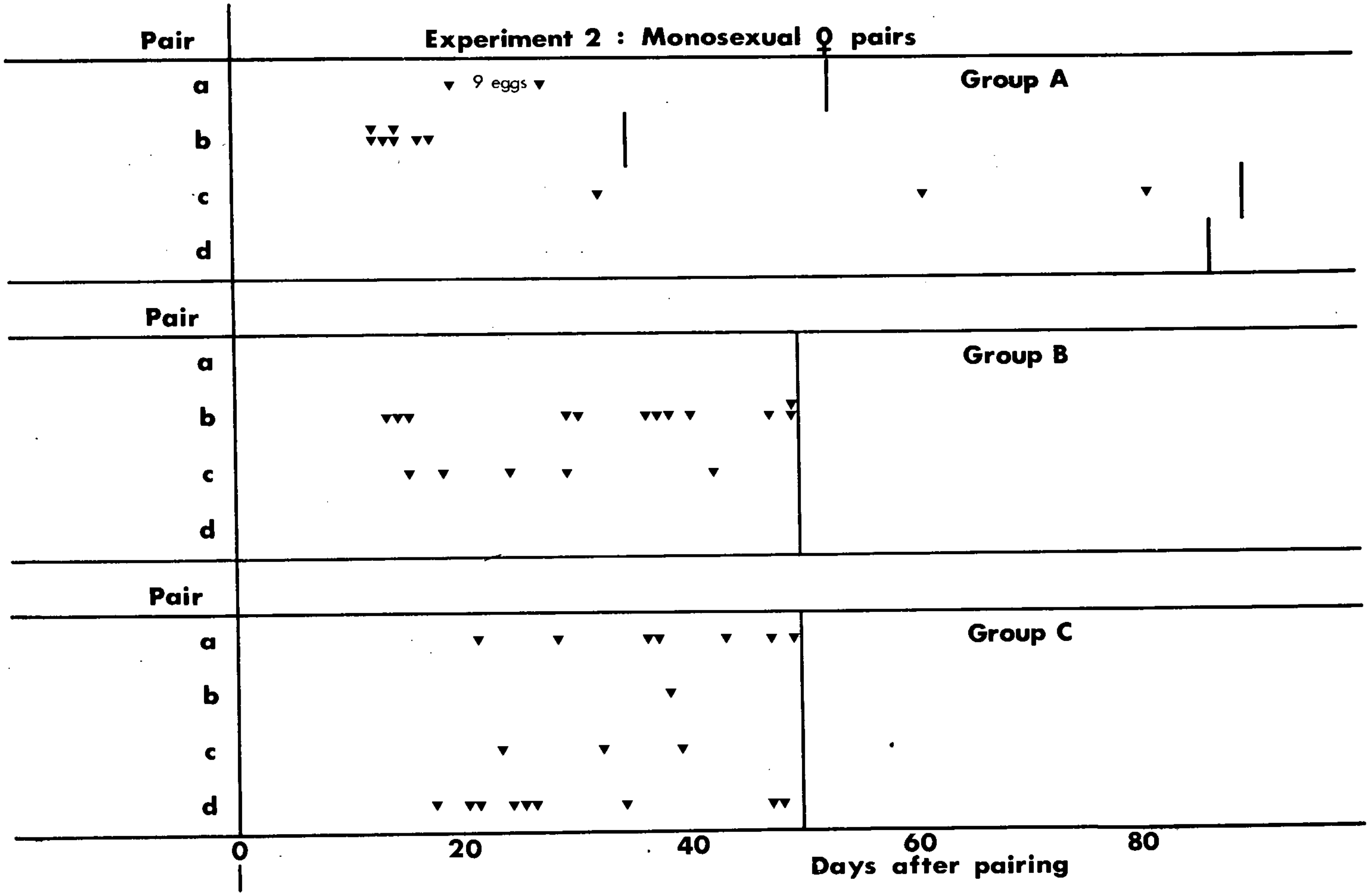
The discovery that two females which did not lay in isolation did so when put together, after a delay which would have been normal in a heterosexual pair, pointed to courtship being unimportant in stimulating egg-laying. Several further pairs of females were set up, with nest and nest material, to discover whether this phenomenon was widespread. Group A was tested in the same room as the stock cages, Group B was in a sound-deadened room with 12 males also present but visually isolated from them, and Group C was in the sound-deadened room with 12 females present but visually isolated from them. The aim in using these different conditions was to determine whether the stimuli leading to egg-laying were partly attributable to male vocalisations in the same room or totally to the other female.

Figure 3. 3 shows the results for these birds. In Group A eggs which were laid were left in the nest but in the other two groups they were removed as laid. This stopped incubation from interrupting laying and also avoided any ambiguity which might arise from an egg being lost from the nest on the same day as another was laid.

The first two pairs of Group A laid clutches in which both birds were involved, and incubated them as would a heterosexual pair. The other two pairs were left together for almost three months: in one case three eggs were laid, in the other none at all. On day 42 three of these four birds became ill, as did others in the colony at the same time. Two which died were sent for autopsy and one was found to have had a chill, the other prolapse of the oviduct. The latter being a characteristic complaint of laying birds and all those affected being paired females and showing the same symptoms (panting deeply and crouching in a fluffed up posture on the floor of the cage), it seems likely that the three birds which became ill in these

FIGURE 3.3: Egg-laying by monosexual pairs of females given nest material and nest box containing rough nest (Experiment 2). GROUP A: With males audible, eggs laid left in nest. GROUP B: With males audible, eggs removed on days laid. GROUP C: With no males audible, eggs removed on days laid. Arrows indicate days on which eggs were laid; vertical lines show the finish of each experiment.

(Note: Although the exact pattern of laying is not known for pair _a_ of Group A, 11 eggs were laid during the course of nine days, and both birds must therefore have been participating.)


Experiment 2 : Monosexual ♀ pairs

experiments were also suffering in this way. ♀218 (pair d) died on day 86 and was found to have a large shell-less egg at the distal end of the oviduct.

Groups B and C were an extension of the above tests under more carefully controlled conditions to note the effect of male vocalisations in the same room. In Group B laying was recorded in two pairs, involving at least three birds. In Group C laying occurred in all four pairs, though two eggs were never laid in the same nest on the same day. The presence of another female seems therefore to raise the incidence of laying above that in isolated birds even where no auditory stimulation from males is possible. The form which stimulation from another female takes cannot be stated with certainty, but the lack of laying in the female stock cage points to the necessity of some form of bond arising between the two birds involved or the over-riding importance of nest material. Laying was not found amongst the 12 females kept communally in the same room as Group C even though nest material was provided from day 34 to day 49. Attempts to place nests in their nest boxes were fruitless, however, as they were rapidly dismantled. Nests given to the monosexual pairs also tended to be taken to pieces: in pair a of Group B, where no laying took place, the nest had to be replaced 12 times in seven weeks. Addition of material to the nest was noted in only one case (Group B: pair c).

Whether stimulation of egg-laying involves copulation in monosexual pairs or not must remain in doubt, for Masatomi (1957) records homosexual behaviour in a female of this species. In none of the pairs tested here was this observed, although mutual soliciting (adopting a horizontal posture with tail fluttered in a vertical plane) was noted on one occasion. This is a normal prelude to copulation which usually appears as a result of male courtship but occasionally occurs without it and when it does so often stimulates the male to start courting.

The Effect of Nesting Facilities

All the birds discussed so far were provided with a constant supply of nest material on the floors of their cages. They were also given a nest box and, as females carry little, monosexual pairs and isolated birds were given a nest placed in this. In early experiments the floor of the cage was also covered with newspaper and strips were often torn off this for building. Thus, while the stimuli received from the mate were altered, those from nest material were kept as far as possible the same as during normal breeding.

To note the effect of nesting facilities all birds used were in heterosexual pairs but deprived of some factor important to nest construction. The various factors relevant here which could influence egg-laying are: the nest site, the completed nest, manipulation of nest material, and building experience within the nest box. The small amount done by females makes it reasonable to exclude carrying. The main experiments to separate the other factors involved setting up pairs with material but without a nest box, with nest box but without material and with both but the material removed from the box three times daily. In several of these pairs observations were carried out to detect the influence of the treatment on the behaviour of the birds. The results on this will be discussed separately after the data on egg-laying.

a) Pairs without nest material (Experiment 3)

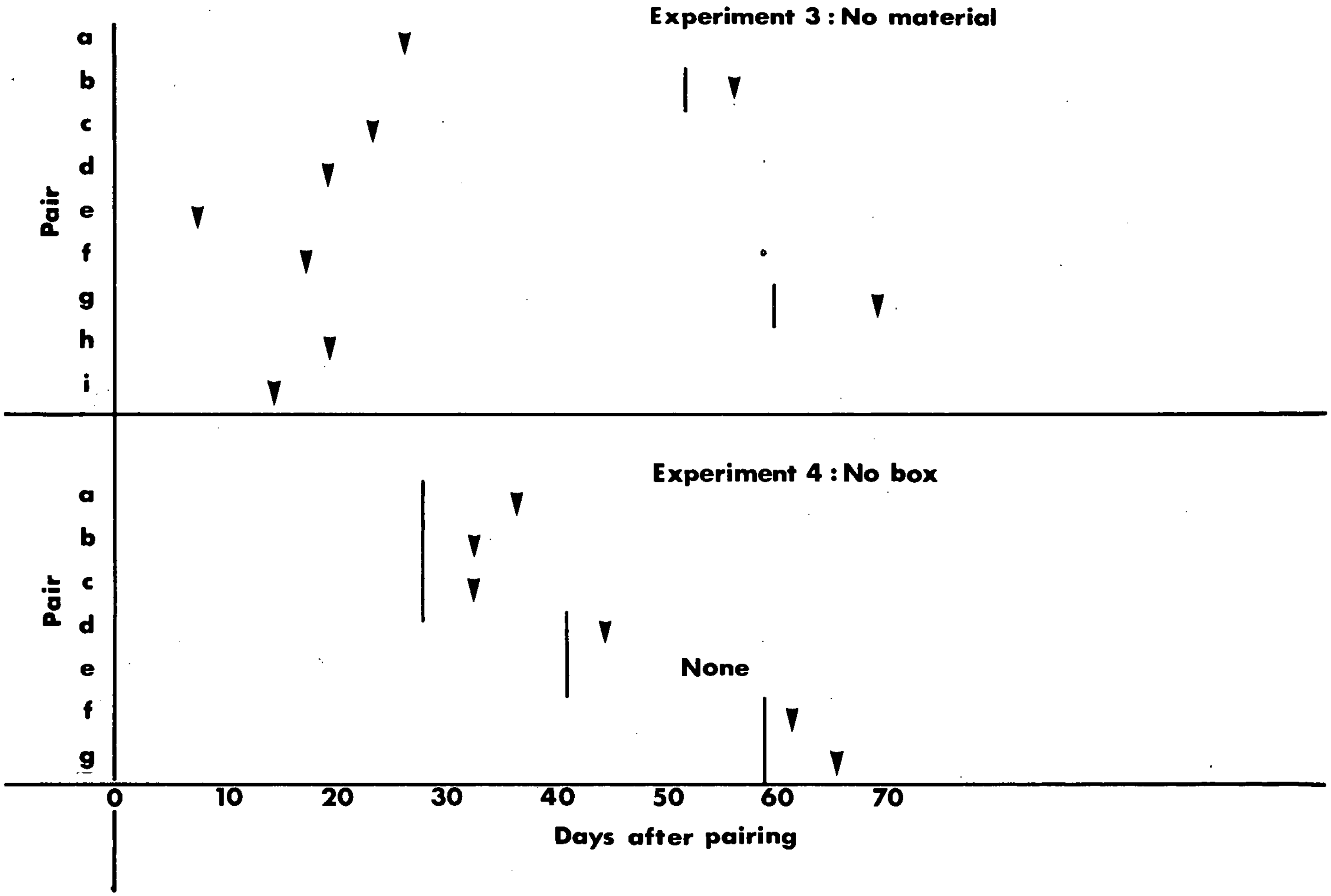
Nine pairs were tested in this way, but deprivation of nest building experience proved more difficult than was at first supposed. Although no hay was provided for building and the floor of the cage was not covered with newspaper, the birds were found to carry seed, lettuce and faeces to the nest box. In the box they were often seen to go through the movements of building using this material, their own or their mates' feathers or no material at all. In the last case the most characteristic movement observed was an upward thrusting of the head with the bill touching the wall of the box. This normally serves to push material up and so create the covered over appearance of the nest. In this context it is presumably released by the failure of the wall of the box to conform to the stimulus pattern of a finished nest. In some cases birds were seen to push their mates bodily around the nest box and manipulation of the mate's wing and tail feathers was common, leading to a very abraided appearance in many cases. Despite these factors, and the accumulation of seed and faeces within the nest box (lettuce being removed where this was carried), no nest can be achieved in this way, and depriving the birds of hay certainly cuts down nest building experience even if it does not eliminate it.

Figure 3. 4 shows the day of the first egg for these pairs. The median of 19 days is significantly longer than that of the normal pairs in Control 1 (Figure 3. 1) ($p < .01$ on a Mann-Whitney U test), though there is a considerable spread and one female actually laid on day 7. Particularly striking is the fact that in two of these pairs no egg had been laid by day 52 and day 60; they were then given nest material and eggs were laid on day 56 and day 69 respectively. It seems therefore that nest material, while not essential, has an accelerating effect on the egg-laying of normal pairs.

b) Pairs without nest box (Experiment 4)

These pairs were kept well supplied with nest material but given no nest box until a considerable period after pairing. The floor of the cage was inspected daily for eggs or their

FIGURE 3.4: Egg-laying by pairs deprived of nesting facilities.
Experiment 3: Pairs given nest box but no nest material. Vertical lines indicate days on which nest material was given to two pairs which had not laid.
Experiment 4: Pairs given nest material but no nest box. Vertical lines indicate days on which each pair was given a nest box.
Arrows show the day on which the first egg was laid for each pair.


Experiment 3 : No material
Pair
a
b
c
d
e
f
g
h
i
Experiment 4 : No box
Pair
a
b
c
d
e
f
g
None
0
10
20
30
40
50
60
70
Days after pairing

remains and, to make this easier, the metal tray covering the floor was washed every three days (normally once a week is adequate). As shown in Figure 3. 4 no egg was laid by any female before a nest box was provided. Subsequent laying showed six out of the seven females to be capable of doing so: in the pair separated before laying (Pair e), the female did not lay on a subsequent pairing either.

To make certain that it would be possible to detect egg-laying in the absence of a nest box, the four pairs which had not yet been given a box were tested with an egg placed on the floor of each cage on day 35. In two cases this egg was intact and in the other two it was broken by dropping from the height of the top perch. All pairs immediately approached the egg, began to peck at it where it was whole and to eat it where it was broken. Twenty-four hours later three of the eggs had been eaten and one was still intact. Yolk was found on the floor of the two cages given broken eggs and a little albumen was in the water dish of the fourth pair. This result indicates that it would have been apparent had the birds without boxes been laying, despite their interesting reaction to eggs not in the nest.

Although lack of a nest box seems to stop laying altogether, the gap between the box being given and the first egg was shorter than in newly paired birds. This points to some ovarian growth having taken place in its absence.

c) Nest removed three times daily (Experiment 5)

Three pairs were given nest boxes and nest material and allowed to build but for the removal of all material from the nest box at 9.30 a.m., 12.30 p.m. and 4 p.m. on each day. These experiments were carried out during February, when the longest day experienced was from 6.45 a.m. to 5.30 p.m. Thus the longest period of building without removal of the nest was three and a half hours. In this way it was hoped to give the birds some experience of building at the same time as depriving them of the complete nest.

This proved not to be possible as, towards the time of egg-laying, the rate of building increased so much that a complete nest was present in the box each time material was to be removed. In the three pairs egg-laying occurred on day 9, day 12 and day 15 respectively, suggesting that the treatment had been without effect.

Three possibilities emerge to explain this. Either the completed nest is of no importance to egg-laying, or the lack of stimulation from the completed nest was compensated for by the increased building experience or the nest was complete for a sufficient time to provide adequate stimulation.

The Effects of Deprivation of Nest Material on Behaviour

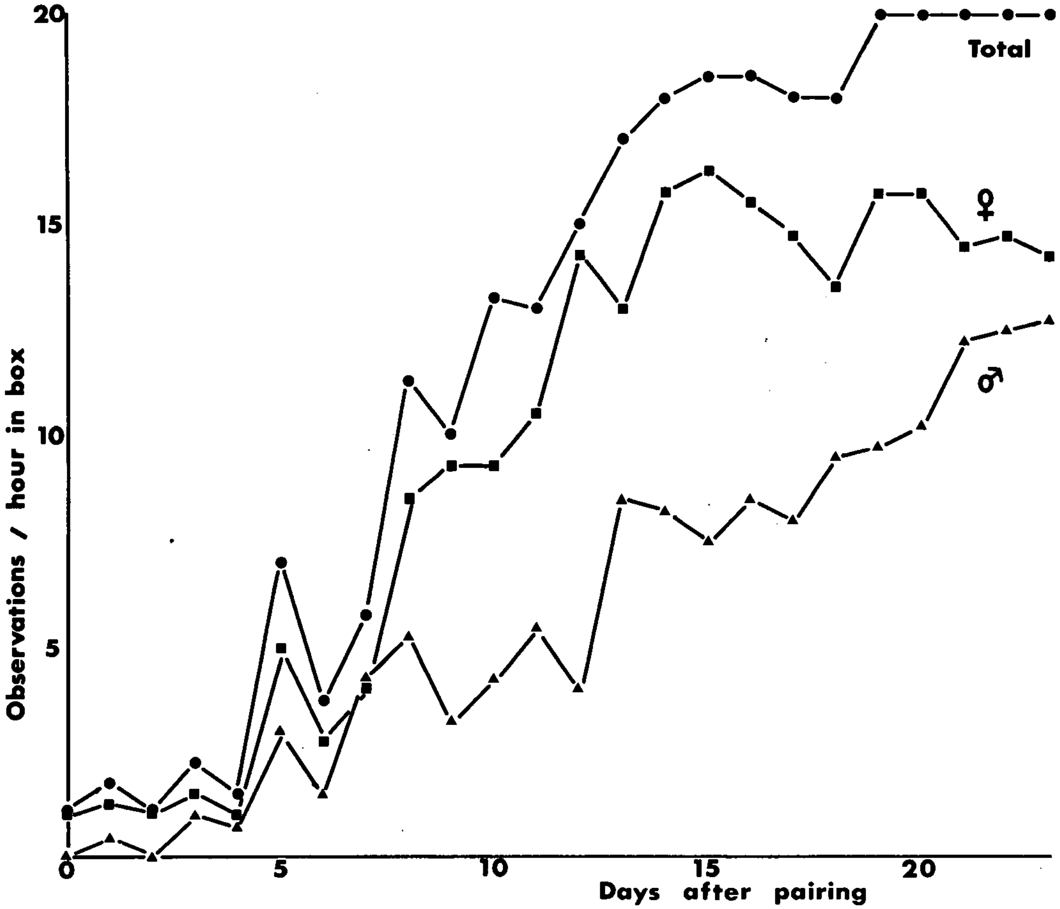
To determine how behaviour was affected by varying degrees of deprivation of nest material daily observations were carried out on several pairs. At the outset twelve pairs were set up, but one bird died and another pair failed to settle down, the birds showing no interest in each other, nest material or nest box during three weeks of pairing. The ten remaining pairs were: three without nest material (Experiment 3: pairs a, b and c), three with nest removed three times daily (Experiment 5: pairs a, b and c) and four under normal conditions (Control 1: pairs a, b, c and d).

All these pairs were watched at the same time, a scanning technique being used to obtain equal data on each. Every three minutes for an hour each pair was observed and the position within the cage of each bird, and whether or not it was manipulating nest material, were noted. Thus twenty observations on each of 24 birds were made during the course of an hour. This method is particularly useful to indicate changes in the amount of time spent within the nest box and with nest material as the time passes after pairing.

Figures 3. 5 - 3. 7 show the mean number of observations when the box was occupied by either bird, by the male and by the female for every day in each experiment. The differences between individuals are in most cases too large to allow all

FIGURE 3.5: Pairs given nest material and empty nest box and allowed to build. Data plotted are the means of four pairs from Control 1.

Number of observations (out of a possible of 20 made at three minute intervals during a daily watch of one hour) of female in the nest box (♀), male in the nest box (♂) and of the nest box being occupied by one or both birds (Total).

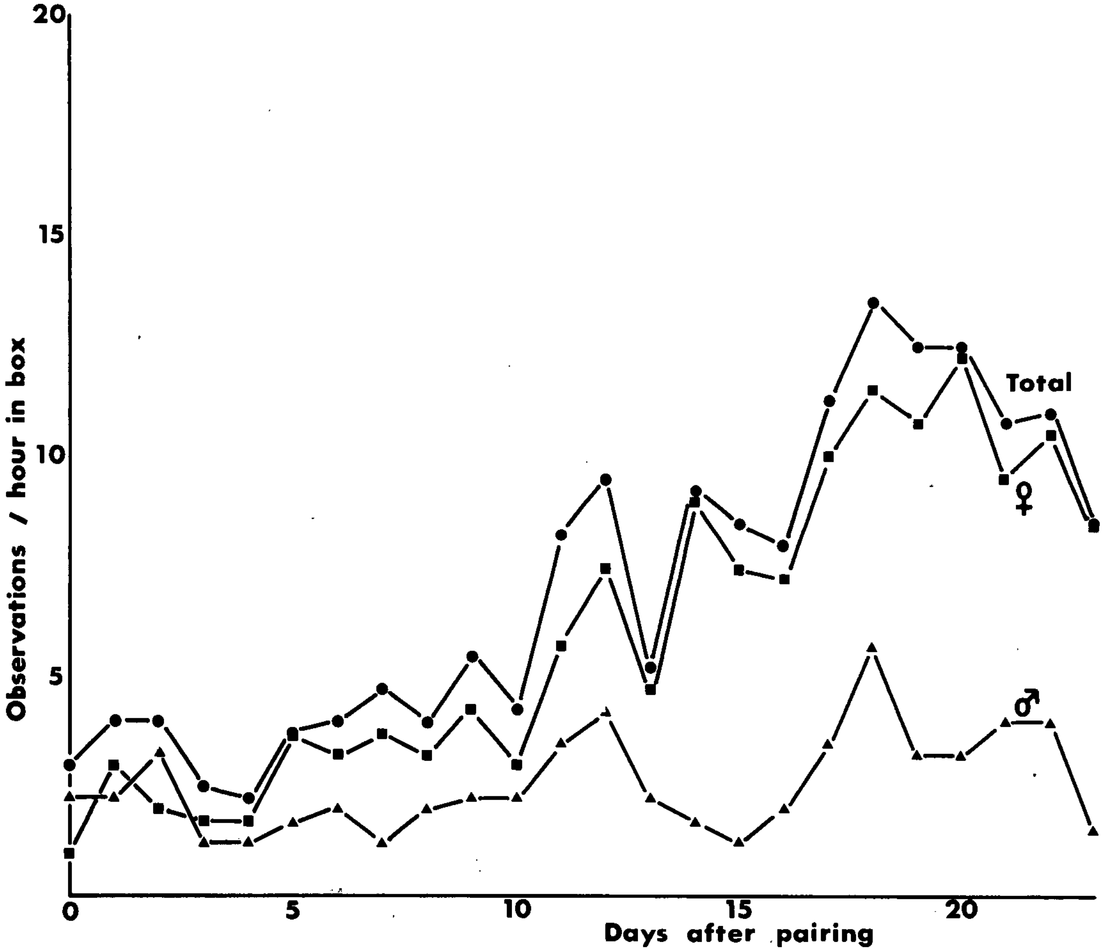

20
15
10
5
0
5
10
15
20
Observations / hour in box
Days after pairing
Total
♀
♂

those under the same experimental conditions to be massed to make comparisons between the sexes and between experiments, but these figures indicate certain differences which may exist between the groups. In the normal pairs (Figure 3. 5), neither bird visited the nest box much during the first five days, but thereafter there was a gradual rise. Comparison between day 0-11 and day 12-23 on a Mann-Whitney U-test shows there to have been a significant rise between the two periods for every individual. The mean number of observations of females within the box is greater than that of males every day after day 8. Three pairs show a difference between the sexes on this measure on a Wilcoxon matched-pairs test for the full 24 days. In the fourth pair, however, there are periods both at the beginning and end of the experiment when the male was seen in the box more often than the female. But from day 8 to day 16 the female was in the box more on every day than the male, giving a significant difference ($p < .01$). Data to be mentioned in Chapter 4 point to females averaging only a few minutes per hour longer than males in incubating. These results seem to point therefore to females spending more time in the nest box than males during the nest building phase. The total time for which the box is occupied also rises and reaches 100% for the period of incubation.

All three graphs for pairs without nest material show a markedly more gradual rise (Figure 3. 6). Only one pair had

FIGURE 3.6: Pairs given nest box but no nest material. Data plotted are means of three pairs from Experiment 3.

Number of observations (out of a possible of 20 made at three minute intervals during a daily watch of one hour) of female in the nest box (♀), male in the nest box (♂) and of the nest box being occupied by one or both birds (Total).



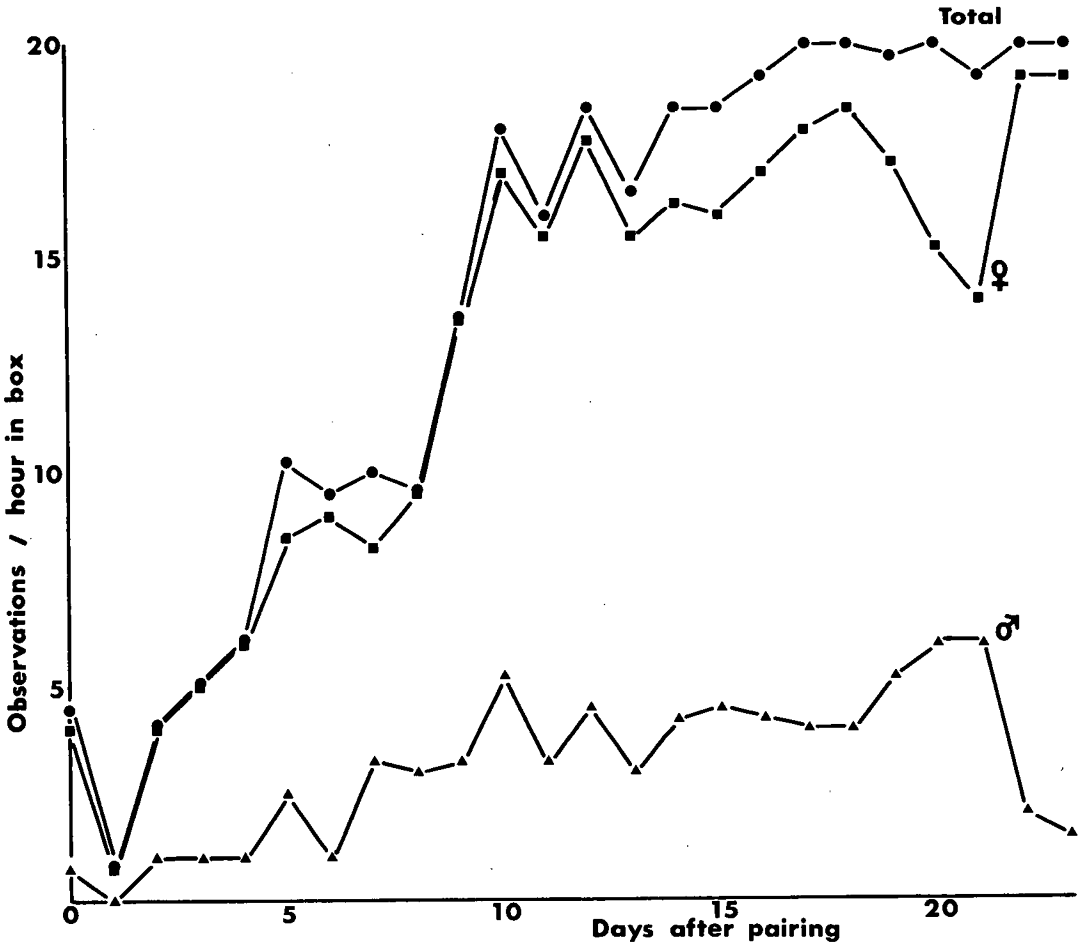
20
15
10
5
Observations / hour in box
Total
♀
♂
0
5
10
15
20
Days after pairing

laid by day 23 after which observations were ceased. All three females showed a significant rise in the number of observations within the nest box between days 0-11 and days 12-23 and each was noted in the box on more than half the twenty observations during several watches. One of the males showed a significant fall in this measure between the first and second halves of the experiment but the other two showed rises. These were, however, slight and no bird was seen in the box more than eight times in one hour. Thus time in the nest box is slower in rising in pairs deprived of nest material, although once again the female tends to spend more time in it than the male.

All six birds with nest removed three times daily (Figure 3. 7) showed an increase in the number of sightings within the nest box between the first and second halves of the experiment. In males this was very slight with only two of the 72 bird hours (three birds each watched for one hour on 24 consecutive days) including ten or more sightings compared with 24 out of 96 in normal pairs. Females were strikingly different from males, each being seen in the box more than her mate on every day after day 9, but for a single exception on one day. If anything the females here spent more time in the box than those in normal pairs, the male making a rather small contribution to the approximately normal rise in the total time for which the box was occupied. One reason for this was doubtless

FIGURE 3.7: Pairs given nest material and nest box, but with nest removed three times daily. Data plotted are means of the three pairs in Experiment 5.

Number of observations (out of a possible of 20 made at three minute intervals during a daily watch of one hour) of female in the nest box (♀), male in the nest box (♂) and of the nest box being occupied by one or both birds (Total).

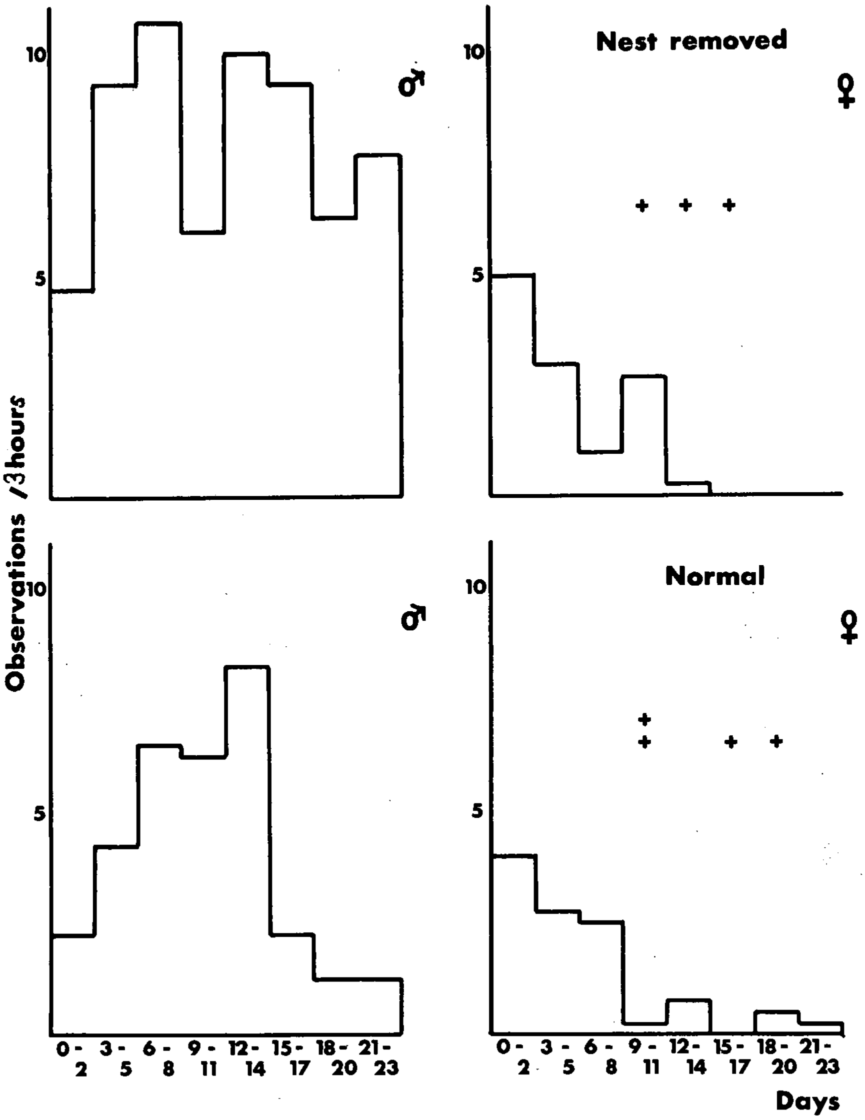

Total
♀
♂
Observations / hour in box
20
15
10
5
0
5
10
15
20
Days after pairing

increased nest building after nest removal. Also, the box was occupied for the full number of observations within the hour by each pair on several days after egg laying, as is normal, suggesting that the female was compensating for lack of attentiveness on the part of the male which was still preoccupied with nest building.

For the two experimental situations in which nest material was provided, it was also possible to compare the number of observations made of birds manipulating nest material outside the nest box (it was not possible to see birds within the nest box clearly enough to determine whether or not they were doing so). The data on this are shown in Figure 3. 8: the columns are means for each group of birds summed over three day periods. Where the nest was removed, males continued to manipulate nest material at a high level without any obvious peak, while males in normal pairs showed a peak at around the time of egg laying. The fairly smooth decline shown for females from the day of pairing is not so apparent if the results are expressed as a percentage of the observations outside the box rather than the full number. Thus the fall may be, at least in part, due to the increasing amount of time spent by females within the box. Treatment in this way does not markedly alter the data on males.

FIGURE 3.8: Observations of birds manipulating nest material outside their nest boxes. Data are means of three pairs with nest removed three times daily (from Experiment 5), compared with means of four pairs under normal conditions (from Control 1).

Number of observations (out of a possible total of 60, made at three minute intervals during a daily watch of one hour, and summed over three day periods) of male with material (♂), and of female with material (♀). + : period during which first egg was laid by one bird in the group indicated.


Observations /3 hours
10
5
♂
Nest removed
♀
+ + +
Normal
+
+ + +
0- 2
3- 5
6- 8
9- 11
12- 14
15- 17
18- 20
21- 23
Days

Pairs Separated by Bars (Experiment 6)

These experiments were instigated to determine whether copulation is a necessary prerequisite for egg-laying, as it has been shown to be in some mammals (Everett, 1961). The technique employed was to place a male and a female in adjoining cages with the partition between them replaced by bars of the same type as the cage front, the space between the bars being ½" wide. This arrangement also allowed some control of nest building experience so that one bird could be given nest material and the other deprived and the nest built by the male could be either passed to the female, by exchanging the two nest boxes, or withheld from her.

The pairs were tested in three groups. In Group A both birds were given nest material and the male's nest was given to the female after it was completed. Group B was in the same situation but with the male's nest withheld from the female and in Group C only the male was given nest material, his nest being given to the female after 39 days.

Table 3. 1 shows the results. The first noteworthy point is that, in contrast to isolated males (see p. 70), all the males here built nests, in most cases within five days of pairing. Where these nests were passed to the females at this stage (three pairs in Group A), laying followed on day 10, day 20 and day 21 respectively. In one case (pair <u>c</u>) the nest

Table 3.1: Pairs separated by bars

GROUP A. Both birds with material, ♂ nest given to ♀.

Pair	Day ♂ nest complete	Day ♂ nest given to ♀	Day ♀ laid
a	5	5	10
b	5	5	20
c	1	35	49+
d	3	5	21

GROUP B. Both birds with nest material, ♂ nest not given to ♀.

Pair	Day ♂ nest complete	Size of ♀ nest	Day ♀ laid
a	5	½ full	12
b	4	full	9
c	4	¼ full	18

GROUP C. Only ♂ given material.

Pair	Day ♂ nest complete	Day ♂ nest given to ♀	Day ♀ laid
a	10	39	40
b	16	39	42
c	24	39	42
d	3	-	21

was withheld until day 35 and the female had not laid by day 49 when the pair was separated, but she may have been incapable of doing so as she laid no eggs in the only other pairing before she died.

When females were allowed nest material but not given the male's nest (Group B), they all laid within 20 days. One had built a complete nest by the time of doing so and in the other two the box contained about a quarter of the usual amount of material in a complete nest. It is probable that these females carried more than they would have done had a male been present in the same cage.

As this lasttechnique failed to deprive the females of nests, in Group C they were given no material. In fact a little always fell through the bars from the male's cage, but this was always removed if it was taken to the box, so that no building or stimulation from a completed nest was allowed. Only one of these birds laid before day 39. The others were then given the male's nest and all three laid within three days thereafter. This stresses the importance of personal experience with nest material rather than the mere sight of the male manipulating and carrying it. Once again the brief gap between the giving of the nest and the first egg indicates that some ovarian growth had occurred in the absence of nest material under the influence of stimuli from the male.

The most interesting and important conclusion here is that copulation is unnecessary for egg-laying, this taking place in Groups A and B after a normal interval. This confirms the tentative deduction made from the data on monosexual female pairs.

Discussion

In this chapter I have given an account of all the experiments carried out to assess the effects of external stimuli on egg-laying. Several of these involved few pairs because it became apparent that they were providing little information. Only by such pilot tests was it possible to discover how the birds would react to the experimental situation and to what extent they were able to compensate for deprivation of normal stimuli by changes in behaviour. The variability in the latency of egg-laying, which occurs even under normal circumstances, also means that an effect must be striking if it is to be detected with a small number of birds. Figure 3. 9 summarises those factors which may be said to have been removed by the various experimental situations and those in which deprivation was partial or doubtful.

Striking results were obtained in two experiments. No laying occurred in normal pairs without a nest box (Experiment 4) and only one of the four isolated females laid (Experiment 1).

FIGURE 3. 9: Summary of factors removed in the various experiments on egg-laying and results obtained.
(X : stimulus indicated was effectively removed.
? : stimulus indicated was cut down upon and possibly removed.)

	Copulation	Courtship	Presence of partner	Sound of males	Partner building	Nest site	Manipulation	Building	Complete nest	Delay to first egg
Controls 1 & 2										NORMAL
Experiment 1	X	X	X		X					GREATLY INCREASED
Experiments 2A & 2B	?	?								POSSIBLY INCREASED
Experiment 2C	?	?		X						POSSIBLY INCREASED
Experiment 6A	X									NORMAL
Experiment 6B	X								?	NORMAL
Experiment 6C	X						?	X	X	INCREASED
Experiment 3					?		?	?	X	INCREASED
Experiment 4					X	X		X	X	NO LAYING
Experiment 5									?	NORMAL

Thus both mate and nesting facilities are important to the occurrence of egg-laying at the normal time. Both may in fact be essential if laying is to occur at all: the isolated female which did lay had been subject to male vocalisations and would have been able to see her own reflection in the glass backing the nest box, the water dish and the dull metal of the floor.

The nest box is not the only facet of nesting facilities which stimulates egg-laying, for when it is present but no material is provided there is still a delay (Experiment 3), though not so great a one as in its absence. A delay is also apparent when females without material are separated by bars from a male which they can see manipulating and carrying (Experiment 6C). This points to personal experience with material within the nest box being the important feature here. The apparently longer delay in these pairs compared with those normal ones deprived of nest material (Experiment 3) was probably due to the latter gaining building experience within the box with feathers, etc. It is unfortunately not possible to determine the extent to which nest material exerts its influence through building as opposed to the completed nest. An attempt to deprive the birds of a nest but let them build was not successful (Experiment 5). The nest being an enclosed structure, it is reasonable to point out that the nest box bears a resemblance to it in form, even before any building has been carried out. The short delay without nest material compared with that in the absence of a nest box suggests that the completed nest is the important factor, the nest box accelerating laying through its resemblance to one. Also, in pairs separated by bars where the male's nest was given to the female (Experiment 6A), this was done when the nest was complete. In no case was it noted to be dismantled or greatly reorganised and these females had not carried and built within the box before it was given to them, yet the lag to laying was not markedly increased. These arguments are largely speculative, however, as conclusive evidence on these points is lacking.

In pairs separated by bars with the nest of the male withheld until day 39 (Experiment 6C) and in pairs without nest boxes (Experiment 4), laying followed very shortly after the deficiency was made up. This showed that experience prior to the addition had been of some influence. Egg-laying after a normal interval in pairs separated by bars, the female given the male's nest (Experiment 6A), points to copulation being unimportant and certainly not essential. Masatomi's (1957) result makes it doubtful exactly which factors had been removed in the monosexual pairs of females (Experiment 2). Homosexual behaviour, if it did occur, was not observed and must have been infrequent: Masatomi observed it once, towards the end of two months of observations, in a pair of females in close proximity to a normal breeding pair. On the other hand, he saw the tail-quivering female display frequently and it would seem more likely that this and the male courtship pattern share the ability to induce sexual arousal in the partner and that this in turn stimulates egg-laying. The greater incidence of laying in monosexual pairs than in the stock cage may be due to a requirement for some particular attachment to a partner unlikely to arise with other birds present. At least as many eggs were laid by monosexual pairs without males in the same room (Experiment 2C) as by those with (Experiment 2B), pointing to singing, which is auditorily very similar to courtship, being unimportant at least in this context.

The main conclusions from this series of experiments are therefore that the presence of a mate, though not necessarily one which is able to copulate, and that of both nest site and nest material, are important if egg-laying is to occur at the normal time.

Chapter 4

INCUBATION

Introduction

The move from the courtship and nest building phase to the incubation phase of the reproductive cycle involves several striking changes in behaviour. Nest building, song and courtship decline and are rare when incubation is well established, the birds spend increasing amounts of time in the nest box and the various motor patterns associated with sitting on the eggs appear and rise in frequency. All these changes occur at around the time of laying. The onset of incubation being gradual it is not possible to define a specific time at which it may be said to take place. It is therefore necessary to devise criteria which may be used as markers in this process of development to determine when birds under different experimental conditions have reached equivalent stages.

Eisner (unpublished) used an automatic device which gave a five hour record of visits to the nest box and also indicated which member of the pair was in the box at any given time. Towards the end of egg-laying a pattern of attentiveness is set up whereby the eggs are never left uncovered, each bird remaining in the nest box until the return of its mate. Such a record may be used to detect the appearance of this rhythm and Table 4. 1a shows the day on which she first found it in relation to the day of the last egg for clutches in which all eggs were laid on consecutive days. It occurred most commonly on the day of the last egg but tended to be earlier in large clutches and later in small ones. In larger clutches it was later in relation to the first egg (Table 4. 1b).

This technique has several disadvantages: the records are laborious to interpret, the birds have to be trained to the use of special nest boxes and a constant eye must be kept on the apparatus for failures of recording pens, photo-cells and transistors. The criterion which it uses (that the box is never left unoccupied during five hours) is useful where the members of a pair are synchronised with one another but difficult to apply to individual birds. It also appears late in the development of incubation so that its use as sole criterion would take no account of earlier events.

For these reasons, this criterion was considered to be inadequate as the only one for the commencement of incubation. It is useful, however, as an indicator of fully developed incubation and was therefore employed in this role. Instead of using the automatic nest record, an hour of observation was carried out daily and if the nest box was never left unoccupied during that period, this criterion, referred to as <u>full establishment</u>, was said to have been reached. This might be

TABLE 4.1

a) Day of full establishment in relation to that of the last egg (data from 21 clutches with eggs laid on consecutive days. From Eisner, unpublished)

Clutch size	-2	-1	Day of last egg	+1	+2
3					1
4			3	1	
5		1	3		
6		3	4		
7	1	3			
8		1			

Number of pairs reaching criterion on day indicated.

b) Day of full establishment in relation to that of the first egg

Clutch size	+3	+4	+5	+6
3		1		
4	3	1		
5	1	3		
6		3	4	
7		1	3	
8				1

Number of pairs reaching criterion on day indicated.

expected to happen earlier on a one hour watch than on a five hour record, but observations on six pairs under normal conditions showed no tendency in this direction. It is therefore probable that full establishment develops rather rapidly towards the end of the egg-laying period.

At the outset of this project an attempt was made to design a device which would give a guide to the development of incubation in terms of the temperature in the nest. Several circuits were built on the potentiometer principle with one of the resistances being a thermistor embedded in an artificial egg which could be placed in the nest. When the temperature of the thermistor reached a threshold level, which could be altered by using a variable resistance in one of the other positions in the circuit, a relay was closed. In this way a pen record could be made indicating periods when the incubating bird had warmed the eggs to above the chosen temperature. This method was rejected as an experimental technique because of the difficulty of correlating the behaviour of the bird with the temperature of the egg. Before incubation begins the birds may spend periods standing or crouching in the nest cup, a form of behaviour distinct on a number of grounds from incubation. It was therefore necessary to look at the behaviour of the bird to decide what it was doing and set the thermistor circuit to fire only when true incubation was observed. In view of this, and the fact that, in at least some species of birds, the temperature transmitted to the eggs rises during the first few days of incubation (Lehrman, 1961), it was decided that it was best to use direct observation of behaviour to assess the development of incubation before full establishment.

The posture of incubation is characteristic, the bird sitting low in the nest with head and body hunched up and tail protruding over the edge of the nest cup. But some of these features may be seen in birds crouching on the floor of the nest cup before the eggs are laid and an objective distinction is not easy. Early observations showed the most useful and definitive behaviour pattern associated with incubation to be the movements seen as a bird settles on the eggs. The feathers are raised from the ventral apterium, the body and tail are shifted from side to side and the bird lowers itself on to the eggs. These movements always precede a bout of incubation and occur at intervals during it as the bird rises and rearranges the eggs under it. Thus, on the basis of the same daily one hour watches as used to test for full establishment, the first observation of settling movements was taken as indicating the <u>start</u> of incubation.

In six pairs watched under normal breeding conditions, the first observation of settling movements was found to be at various stages during the laying of the clutch. For three birds it took place the day the first egg was laid, but I have never recorded it in these or any other pairs before eggs are in the nest, and their presence is probably essential to the occurrence of these movements. This does not necessarily indicate that egg-laying is a prerequisite for incubation behaviour. A distinction must be drawn between readiness to incubate and the start of incubation itself. The former may develop some time in advance of laying but the presence of eggs may be essential to reveal it. For this reason, in testing for readiness to incubate, the experimental pairs were given eggs in their nest boxes before they laid themselves.

The criterion for the start of incubation has the advantage over that for full establishment that it may be applied to each bird separately. The attainment of full establishment, as previously defined, relies on the co-operation of both birds. In theory, the full establishment of one bird is the stage at which it ceases to leave the box before its mate has returned, but in practice this can only give a rough indication as the behaviour of one member of a pair can, of course, be affected by that of the other in this respect. In several experimental pairs the female followed the male into the nest box when he was building and remained there until after he had left. Such behaviour gave the impression that he was fully established, while the pattern set up was almost entirely due to the behaviour of the female. A further possibility is that this criterion might be reached by chance alone as the number of times an incubating bird leaves the box during an hour is usually very small. This criterion can thus be used only as a general guide when applied to individual birds. The decision on whether it has been reached or not must take into account the behaviour of the bird and of its mate when in the box, as well as when entering and leaving it.

The external stimuli which might lead to readiness to incubate are the same as those considered as possibly affecting the onset of egg-laying, with the addition of the eggs themselves. Before testing the effects of mate and nest material, an experiment was set up to make certain that incubation does develop during the reproductive cycle and is not an ever-present response to eggs in the nest (Experiment 7). It was also necessary to investigate the possibility that the eggs, as well as being the immediate stimulus to incubation, might also be the stimulus inducing its appropriate physiological state (Experiment 8).

To facilitate accurate observation of whether or not the two criteria were fulfilled, observations in most experiments were carried out from the darkened area behind the cages. This precluded the taking of detailed notes and observation of the birds outside the nest box. However, twelve pairs (six from Experiment 7 and all those from Experiment 8) were watched from in front. The results of these allow some conclusions on the general changes in behaviour associated with the onset of incubation. They will be presented after the data on the induction of incubation in these two experiments.

The Effect of Eggs

a) Pairs given eggs on day 0 (Experiment 7)

To see whether readiness to incubate can be demonstrated before laying, eight pairs were set up with nest material and a nest box containing a rough nest in which six artificial eggs were placed on the day of pairing. Building tends to obscure eggs placed in the nest at such an early stage and so the box was inspected half an hour before each daily watch and the eggs were uncovered if necessary.

In most cases both settling movements and full establishment were recorded every day following that on which they were first seen, but in three cases both full establishment and egg-laying failed to follow the normal pattern. When pair c was separated on day 37 the female had laid 12 eggs in two separate groups 11 days apart, yet she was not fully established while the male had been so for 30 days. Full establishment of both birds was recorded for a period of four days around the end of the second clutch and on five single days earlier. These can be put down to particular assiduity on the part of the male and the female was certainly not fully established between clutches.

The period of four days full establishment started on day 33: whether this or 37+ is taken as the timing is immaterial to the group median (see Table 4.2). In pair h five eggs were laid daily from day 9 and full establishment proceeded for seven days from day 10, the female then broke it for seven days before laying four further eggs starting on day 24. As both incubated equally for at least the latter part of the first period of full establishment this appeared normal and it is taken as dating from day 10, though the female deserted later and laid again. In pair g two eggs were laid: on day 16 and day 27. Full establishment proceeded for at least 23 days from day 30 onwards, but was noted also on isolated earlier days between which attentiveness fluctuated considerably. It thus seems most reasonable to discount these earlier days (on one of which the male incubated for the full 60 minutes), and consider full establishment as dating from day 30, except for the male which did not leave the box empty on day 29 either. These considerations, and the fact that the decisions reached leave room for some doubt, lay stress on the difficulty of this criterion, cases like these failing to conform to the general pattern and having to be assessed on their merits.

The data for all eight pairs are shown in Table 4. 2. It is apparent that egg-laying is not essential to incubation: six out of eight females and seven out of eight males started to incubate in advance of their own first egg. In two of

TABLE 4.2: Delay before egg-laying and incubation in pairs given nest box, nest material and six eggs from day 0 (Experiment 7)

Pair	Day of first egg	Day of start of incubation		Day of full establishment		Day of last egg
		♂	♀	♂	♀	
a	7	10	10	12	12	13
b	11	2	9	7	9	13
c	15	4	13	7	37+	37+
d	11	3	9	10	11	14
e	None*	4	6	5	6	None*
f	None*	10	15	17	17	None*
g	16	9	17	29	30	27
h	9	3	8	7	10	13
Median	11	4	9.5	8.5	11.5	13.5

* These results are excluded in calculating the medians at the foot of the columns.

these cases incubation proceeded normally without the female herself laying and in the others there was some indication that a reduction in clutch size had taken place (see Table 4.3). Although there is not a marked difference in the timing of full establishment between the sexes, the male started to incubate earlier than the female in all but one instance ($p < .01$ on a Wilcoxon matched-pairs test). All of the five birds which started before day 5 were males, which suggests that the physiological state of males is more labile than that of females at this early stage.

b) Pairs given eggs on day 4 (Experiment 8)

There are two possible reasons for the start of incubation being advanced by the presence of eggs in the nest: either the birds are in the physiological state appropriate to incubation before the eggs are laid, or this state is brought about by stimuli from the eggs themselves. The next experiment was designed to decide which of these was the case. The birds were placed under identical conditions to those in the last experiment except that eggs were not placed in their nests until day 4 - the stage by which half the males in Experiment 7 had started to incubate. In this case they were also deprived of nest material after day 4, in an effort to avoid the eggs being covered over by later building and so maximise the chances that birds ready to incubate at this stage would be seen to settle.

Two males and two females, representing three out of the six pairs tested, were seen settling on the day the eggs were placed in the nest. A further male started on day 5 and two females on days 6 and 7 respectively. These last two birds began their own clutches on the same day as they started to incubate, but none of the birds which started to incubate before day 6 had eggs of their own in the nest when they did so. The deprivation of nest material after day 4 was probably responsible for the fact that two females did not start their clutches until days 28 and 32 respectively. Perhaps also for this reason, full establishment of both birds either did not appear or became disrupted in four out of the six pairs.

Pair d incubated without laying. Combining pairs from this experiment with those from the last gives seven pairs in which the female started incubating before the day of her first egg but did in fact lay. In Table 4. 3 the clutch size is compared with the mean previous clutch size for each of these females and in every case the number of eggs laid here is found to be smaller ($p < .01$ on a Wilcoxon matched-pairs test). Thus the presence of eggs in the nest led to reduction or suppression of the clutch in cases where the female began to incubate before laying.

As mentioned previously (p. 54), some of the control birds

TABLE 4.3: Pairs given eggs before laying. Comparison of clutch size with mean previous clutch size for females which started incubating early but laid also.

Pair	♀	Size of clutch laid	Previous clutches	Mean previous clutch size
7b	102	3	6, 4, 4	4.3
7c	47	4	7,7,8,8,7,7,7,6	7.1
7d	129	4	5, 7, 5	5.7
7h	150	5	6,9,7,6,10,3,9,6,4	6.7
8a	87	4	3, 6, 6, 6, 5	5.2
8c	137	5	6	6.0
8e	131	3	7	7.0

not given artificial eggs were seen to settle on the day of their first egg, and here four birds responded within twenty-four hours to eggs placed in their nests before laying. Thus it appears that readiness to incubate normally develops before laying and that the stimuli evoking this condition come from the mate and nesting facilities rather than the eggs themselves.

Behaviour Changes Early in the Cycle

Six of the pairs given eggs on day 0 and all those with eggs from day 4 were the first experimental pairs studied in this project. They were observed from in front of the cages with a rather lower nest box lip than in later pairs, making it possible to note the occurrence of settling movements. Watching from in front also allowed a more detailed record of the behaviour of the birds to be taken than was possible in the darkened area behind the cages. Thus songs, copulations, carrying excursions and visits to the nest box were also recorded during the daily one hour watches on each of these pairs.

Results of all twelve pairs watched in this way are combined in Table 4. 4 to indicate the time per hour which each bird spent in the box before it was first seen to incubate, and the relation of this to the delay before the first copulation was seen and that before incubation started. The time per

TABLE 4.4: The relation between the day incubation was first seen, the day copulation was first seen and the time per watch which each bird spent in the nest box on the days before it started incubating. Data from those pairs in Experiments 7 and 8 watched from in front of the cages.

Pair	Day copulation first seen	Day male first seen incubating	Time/hour male in box before start of incubation (minutes)	Day female first seen incubating	Time/hour female in box before start of incubation (minutes)
7a	0	10	8.75	10	9.25
7b	1	2	19.75	9	20.0
7c	5	4	13.75	13	12.0
7d	0	3	12.5	9	19.0
7e	0	4	18.5	6	23.5
7f	6	10	2.25	15	9.75
8a	6	21	3.25	25	4.5
8b	1	5	22.25	6	25.5
8c	4	11	4.5	11	2.75
8d	0	4	23.75	4	18.0
8e	16	-	-	4	6.5
8f	3	4	13.5	7	15.75

hour in the box up to the start of incubation is inversely correlated with the delay before incubation in both sexes (Product moment correlation: ♂ $r = -.75$ $.01 > p > .001$; ♀ $r = -.72$ $.02 > p > .01$). Thus birds which are slow to start incubating also average less time in the nest box over the period before they start to do so. The measure of the timing of the first copulation is, of course, a very rough one and the delays may be much shorter than indicated. In both sexes the delay before incubation correlates positively with this however (♂ $r = +.61$ $.05 > p > .02$; ♀ $r = +.70$ $.02 > p > .01$). Pair 8e has been excluded in the calculation of all these correlations as the male did not start to incubate and was probably ill for at least part of the experiment. The data in Table 4. 4 thus suggests that the longer delay in starting to incubate in some pairs is a feature of general slowness to settle down as reflected by other measures.

The normal timing of egg-laying, incubation and nest-building was upset in Experiment 8 by removal of nest material after day 4. But in Experiment 7 this was not done and various other parameters could be looked at, bearing in mind that a nest and eggs were provided from day 0. The total amount of material carried to the nest box during watches is shown for each pair in Table 4. 5. This points to the same sex difference as seen in Table 2. 1 but the amount of material carried is very much less here, probably partly due to the

TABLE 4.5: Total number of pieces of nest material seen carried to the nest box by six pairs in Experiment 7.

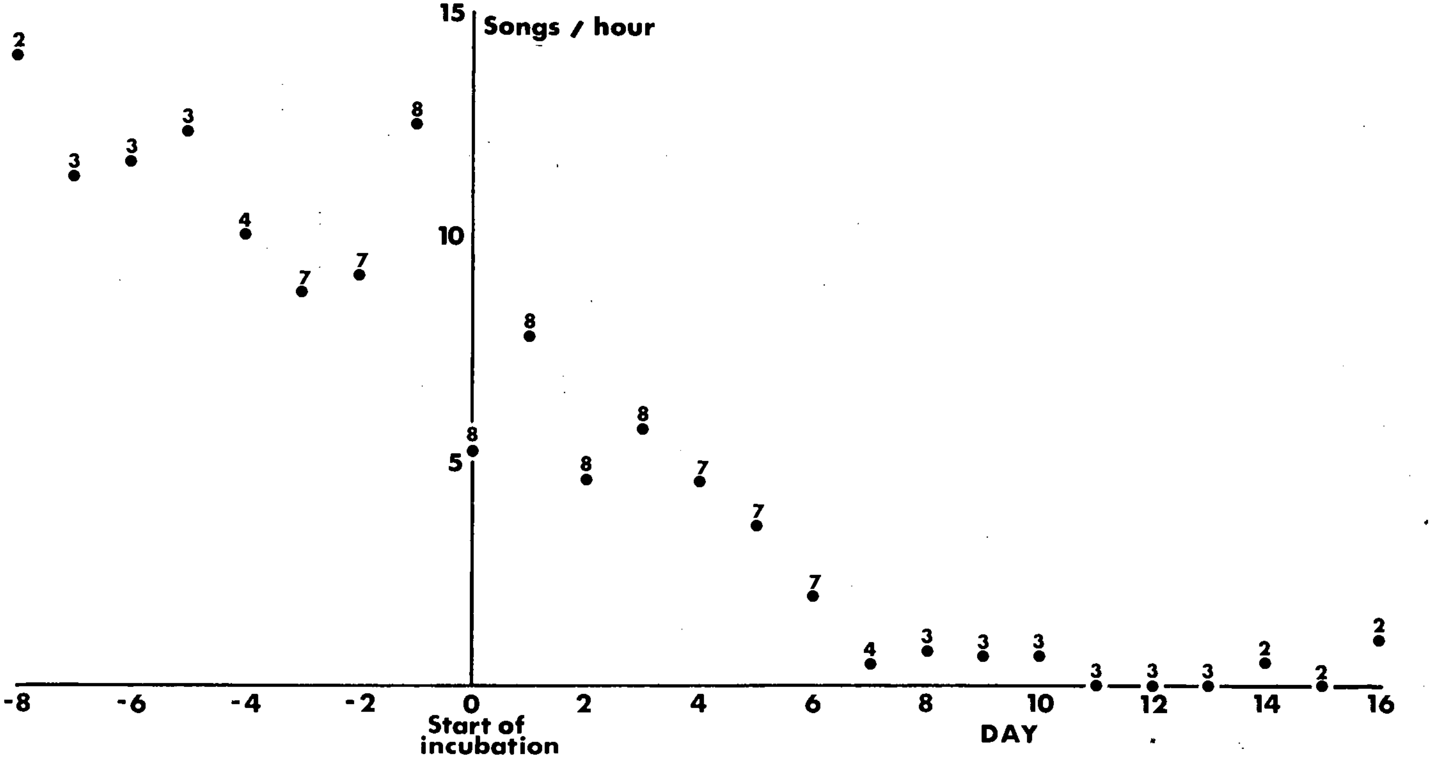
Pair	Number of one hour watches carried out	Total number of pieces carried by male	Total number of pieces carried by female
7a	16	17	0
7b	16	2	0
7c	38	180	12
7d	22	203	0
7e	11	53	1
7f	26	185	0

provision of a nest from the outset but also because the pairs here were not given fresh material before each watch.

The fact that songs decline markedly at around the start of incubation is illustrated in Figure 4. 1. The data here is based on the mean of six pairs from Experiment 7 and two normal pairs. It is arranged around the day on which each male started to incubate so that the number of birds on which the means are based declines towards the edges. In these same eight pairs a record was taken of the days on which the females were seen feeding at the cuttlefish bone provided in each cage: none of the males was seen to do so. Excluding the two pairs in which the female neither laid nor ate cuttlefish bone (7e and 7f), the others showed a strong relation between these two activities. Of a total of 116 days of observations, cuttlefish bone was taken on 36, of which 32 were on the day an egg was laid, the day before or the day after. Of the total of 116, 54 days came into this category giving a significant relation between egg-laying and the eating of cuttlefish bone ($X^2 = 44.44$, $p < .001$).

A consideration of visits to the nest box for the pairs in Experiment 7 gives rather fuller data than could be obtained with the scanning technique used in Chapter 3. The main inaccuracy in assessing the amount of time in a one hour observation period spent by each bird within the box arises

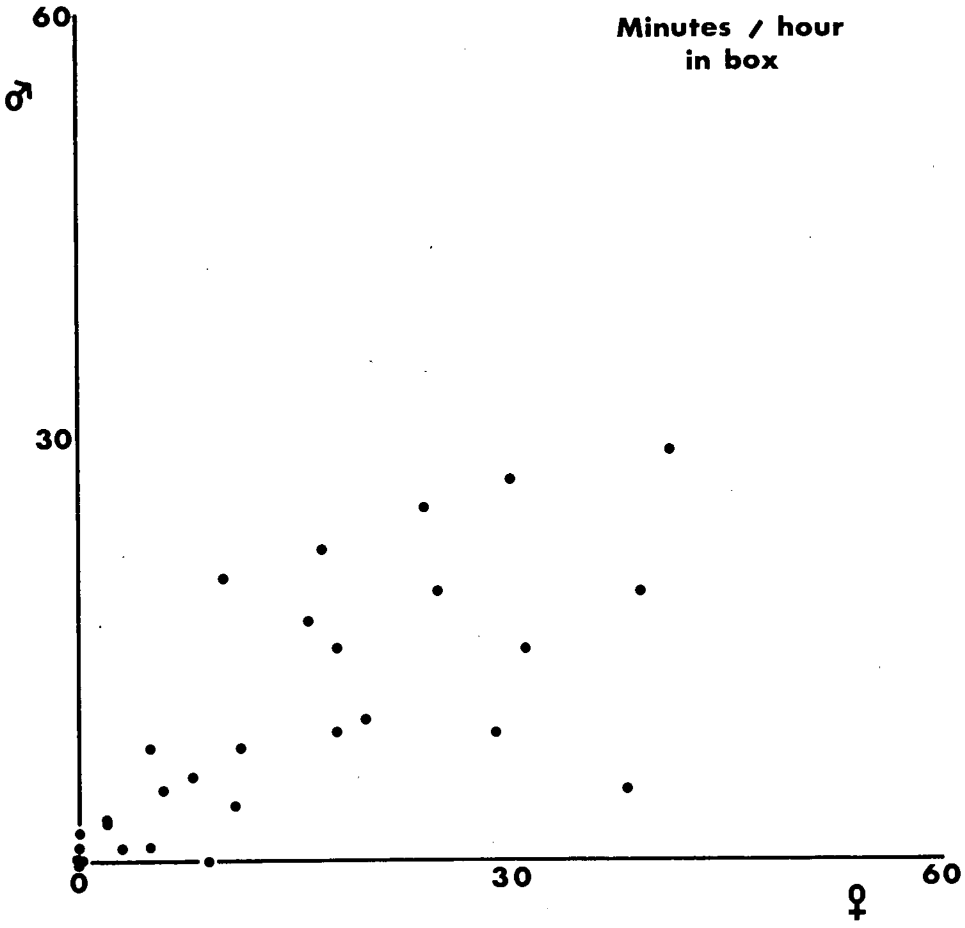
FIGURE 4.1: Mean number of songs produced by eight males during one hour of observation daily. Means are calculated for each day relative to the day on which each male started incubating. The figure above each point indicates the number of birds involved in the calculation of that mean.


15
Songs / hour
10
5
2
3
3
3
4
7
7
8
8
8
8
8
7
7
7
4
3
3
3
3
3
3
2
2
2
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
Start of incubation
DAY

from the very brief nest building visits of males. When summed these might amount to a considerable period within the box but accurate estimation of the duration of each is not possible when a notebook and stop clock are used for recording. Thus, in practice, only visits of more than 15 seconds in length were used and the time within the box for each bird was taken as the sum of these. Females seldom visit the box for less than 15 seconds and it is rare for males to do so after incubation has commenced. Thus the only data liable to be altered by ignoring shorter visits is that on males during the nest building phase.

In fact the total time spent by the male within the box between pairing and the day on which he started to incubate was less in all six pairs than that of the female. The difference is comparatively small (means from massed data of all six pairs for total of 29 bird days: 9.8 minutes for males, 14.3 minutes for females/hour). It may be in part due to failure to include the shorter visits which are more characteristic of the male, but the major factor is doubtless the previously mentioned conclusion that females spend more time building in the box than males. Massing of data from the six pairs also gives a strong positive correlation between the time spent in the nest box by the male and that by the female on each day ($r = +.80$ on a Spearman rank correlation $p < .01$). These data, shown in Figure 4. 2, point to interest in the nest box by the

FIGURE 4.2: Number of minutes in a one hour watch which the male spent in the nest box compared with the number spent by the female during the period before either bird had started incubating. Massed data from six pairs in Experiment 7.

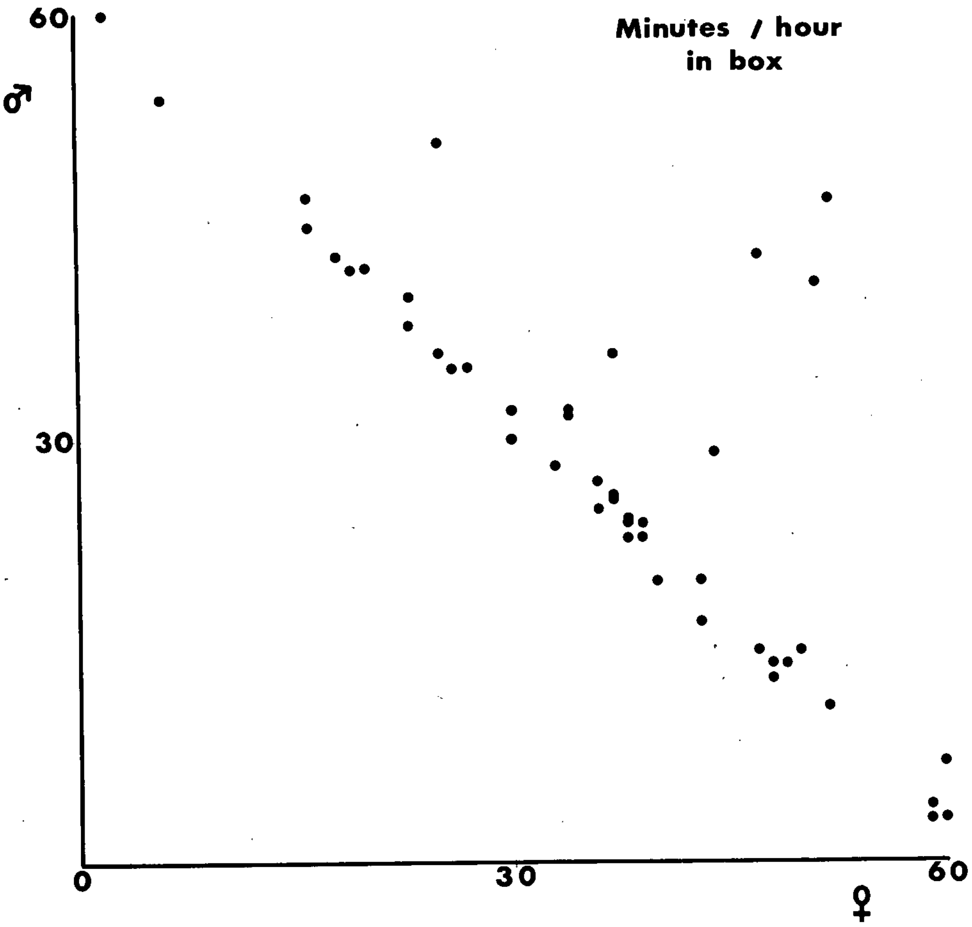

Minutes / hour
in box
60
♂
30
0
30
60
♀

two members of a pair mounting in concert in the few days after pairing.

In both sexes the time spent in the nest box is greater between start and full establishment than before that stage and rises further after full establishment. In the particular situation of Experiment 7, males tended to become fully established earlier than females. The mean time per hour in the box for the five which did so over the period between their own full establishment and that of their mates was 37.2 minutes for 26 bird days. As the mean for males after full establishment of both birds was only 28.8 minutes this indicates that the male was compensating for failure on the part of the female during the interim period. This was particularly noticeable in some pairs where departure of the female from the box before the male's return elicited alarm calls from him and his immediate return to the nest.

In all the six pairs from Experiment 7 observed in detail, the female averaged more time than the male in the box after the full establishment of both birds. Over a total of 45 bird days on which the box was occupied for the full sixty minutes, males averaged 28.8 minutes per hour and females averaged 36.3. The sum of these two figures being only marginally over 60 minutes, it is not surprising that the time spent by one bird in the box correlates negatively with that by the other for

FIGURE 4.3: Number of minutes in a one hour watch which the male spent in the nest box compared with the number spent by the female in the period after both birds had become fully established. Massed data from six pairs in Experiment 7.


Minutes / hour
in box
60
♂
30
0
30
60
♀

different days ($r = -.72$ on a Spearman rank correlation $p < .01$). The data on which this correlation was carried out are shown in Figure 4. 3.

The Effect of the Mate

The influence of the mate in bringing about readiness to incubate is hard to assess because isolated birds very seldom visit their nest boxes and to watch for the start of incubation in these circumstances is laborious. This is particularly noticeable with isolated females, which usually show no interest in nest box or nest material. The isolated female which laid (Figure 3.2) was an exception, being seen in the nest box frequently at around that time, though never observed to incubate.

Isolated males provide an interesting case as they show a wide range of interest in the nest box and nest building, some birds ignoring the material and box altogether while others complete nests. Of twelve males isolated till day 14 (Experiment 9A: details in Appendix I), three built nests and the rest showed no tendency to do so. The three which had built were then given six eggs and three of those which had not were given a nest pan (a shallow wooden cup serving to keep the eggs in the centre of the floor) and six eggs. Each nest box was watched for quarter of an hour daily to look for incubation.

Only one of those which had not built was ever seen in the box before they were replaced in the stock cage on day 29; it was only seen in once and certainly did not incubate. Two of those which had built covered the eggs over by further building every day and, despite their being uncovered, still failed to incubate. The last bird started to incubate on the same day as it was given the eggs and was recorded as doing so daily thereafter. Although each of these birds had been in the male stock cage for at least six weeks prior to the experiment, this result suggests that they had been in different physiological states at the outset. As two males which built nests failed to incubate, it cannot be decided whether the bird which did so had reached the correct physiological state through its building experience or had been in it from the start.

The six other birds which had not built by day 14 were placed in monosexual pairs. Two pairs built a nest within a week, they were given eggs on day 28 and all four birds were seen incubating on day 29. The third pair had not built by day 28 and neither bird was seen in the nest box before separation on day 29. Both the building of complete nests and incubation behaviour were recorded in seven other pairs of monosexual males which were set up (Experiment 9B: see Appendix I). Two males which had not built at all during 67 days of isolation completed a nest within three days of being paired with one another. Thus it seems that it is the presence of another bird which encourages carrying by the male in normal circumstances, only 25% of isolated males having been found to complete nests. The only isolated bird recorded as incubating was a male which had built and his experience in doing so may have been responsible for this. Monosexual male pairs build nests and incubate eggs placed in them and the two pairs of females which laid and whose eggs were left in the nest (Figure 3.3) incubated them like a heterosexual pair.

Some of the pairs separated by bars were tested with eggs, and several birds of both sexes were seen settling under these conditions. In some cases egg-loss occurred from the female's nest, perhaps due to its diminishing size without the presence of a male to replace lost material. This may have been partly responsible for the fact that only three females were seen settling and only in one case (♀206, see below) did the female maintain incubation for more than two days. Incubation was noted in six of the seven males given eggs during the period when their females were laying but again it ranged from a single observation of settling movements to sitting closely for over a month. The chances of desertion thus seem to be increased by these conditions. Isolated birds, birds separated by bars and birds whose mates are removed or die, incubate particularly assiduously if they do not desert. Single birds compensate for the lack of a mate by sitting for very protracted periods and it becomes unusual to see them off the nest. Thus, while the full establishment criterion is not applicable in these birds, it is certainly clear to even casual observation that they are incubating when they have reached an equivalent stage.

To see how necessary the sight of the mate is to the maintenance of incubation, the bars separating two pairs were replaced by partitions. In one case (♂172, ♀206) the male was given six eggs on day 27 and started to incubate; the female had laid three eggs by day 35 and was incubating them. The partition was introduced on day 49 and both continued to incubate, the female last being seen to do so on day 60, the male on day 67. In the other pair (♂222, ♀184) the male was given eggs on day 32, started to incubate on day 35 and continued to do so until day 66 despite the introduction of a partition from day 41 onwards. Thus in pairs separated by bars it is not essential that the mate be seen for incubation to be maintained.

The data from these experiments suggest that the internal state for incubation is attributable at least in part to the presence of a mate, incubation in isolated birds being rare. There is a possibility that the action of the mate in males is through causing them to carry which they do not normally do otherwise.

The Effect of Nest Material

Because of the different roles which the sexes have in nest building and the finding that males are ready to start incubating sooner than females after pairing, the part played by nest material is particularly interesting. This was examined in some detail.

a) Pairs without nest material (Experiment 10)

Seven pairs were given a shallow nest pan with six eggs, placed in their nest boxes on the day of pairing. They were given no nest material so that carrying and building experience was minimised but, as in the case of previous pairs without nest material (Experiment 3), certainly not eliminated.

These pairs showed great variation both in egg-laying and in the degree to which they achieved and maintained full establishment. In pair d both birds were noted as being fully established daily after it was first recorded and a clutch of five eggs was laid on consecutive days. Pair e also reached this criterion smoothly and continued to fulfil it, in this case without laying. The other pairs were more variable. Pair a was not fully established until day 27 although laying had begun on day 12: the clutch of four eggs begun then was laid on consecutive days and followed by single eggs on days 23 and 26. Although first seen settling on day 9, the female was only observed to do so on six days before day 27. In pair b full establishment of both birds proceeded for eight days from day 14 without laying having taken place, but the female broke it during the watch on day 22, laid an egg the next day and then resumed. This being the only disruption, full establishment is taken here as dating from day 14. The female of pair c laid seven eggs in two groups and both birds were fully established for five days around the end of the second clutch. Both shared equally in incubation during this period and it is thus considered as normal full establishment although they ceased to incubate after it. The laying of pair f was spread out, seven eggs appearing between day 11 and day 22. Full establishment was continuous from day 21, but as the male incubated for the full 60 minutes on this and the next day, its exact timing in the female is doubtful. Pair g was exceptional in that the female, though recorded as settling every day after day 5, did not start laying until day 27 and neither bird was noted as fully established before day 32. Thus both the period of development of incubation and the gap between the start of incubation and egg-laying was unusually protracted in this case.

Table 4. 6 shows the data for these pairs after all these points are taken into consideration. One pair incubated without laying. The latency of the first egg in the other six pairs is significantly greater than that in control pairs without eggs in their nests but supplied with nest material (Controls 1 and 2:

TABLE 4.6: Delay before egg-laying and incubation in pairs given nest box and six eggs from day 0, but deprived of nest material (Experiment 10).

Pair	Day of first egg	Day of start of incubation		Day of full establishment		Day of last egg
		♂	♀	♂	♀	
a	12	16	9	24	27	26
b	23	7	5	9	23	23
c	34	35	31	37	43	45
d	31	22	16	28	32	35
e	None*	10	11	13	15	None*
f	11	13	9	14	21	22
g	27	7	5	32	32	31
Median	25	13	9	24	27	28.5

* These results are excluded in calculating the medians at the foot of the columns.

Figure 3.1) ($p < .001$ on a Mann-Whitney U-test). This effect having also been found in the egg-laying experiments and not being observed in pairs given nest material and eggs in their nests, it appears to be independent of the artificial eggs and to derive from the lack of nest material.

Despite this delay in egg-laying, no difference could be found in the timing of the start of incubation between the females here and those in Experiment 7, where they were given nest material as well ($p > .35$ on a Mann-Whitney U-test). But the mean period of development of incubation (gap between start and full establishment) for females appears to be protracted. It was 5.5+ days with nest material present and 14.0 days without it. This difference is on the verge of significance ($p < .03$ on a Mann-Whitney U-test) even on the assumption that the female in pair <u>c</u> of Experiment 7, which did not become fully established before the end of the experiment, showed a greater gap than all those here. In both experiments it is noticeable that the full establishment of females tends to occur at around the time of the last egg: the only case in which the results shown in Tables 4. 2 and 4. 6 point to it being more than four days earlier is in pair <u>b</u> of this experiment. As previously mentioned, this female broke her full establishment, reached eight days earlier, on the day before her only egg. That a delay in egg-laying should correlate with a delay in full establishment links with the earlier finding that clutch reduction or suppression follows an earlier start to incubation to suggest that egg-laying and fully developed incubation behaviour cannot occur concurrently.

Turning now to males it is immediately apparent that the start of incubation has been delayed by the removal of nest material compared with that in those which were given it (Experiment 7) ($p < .005$ on a Mann-Whitney U-test). Six out of seven males here started later than their mates, whereas seven out of eight started earlier than theirs when material was provided. But the period of development of incubation (mean of 6.1 days with nest material, 6.7 days without) is not significantly greater ($p = .14$ on a Mann-Whitney U-test). Where there was a sex difference, the males reached this criterion before the females as they did in Experiment 7.

Deprivation of nest material thus results in delays in egg-laying, in the start of incubation of males and in the full establishment of females. Full establishment of males occurred later with respect to the day of pairing but not in relation to the start of incubation. In females the presence or absence of material appears to be irrelevant to the timing of the start of incubation.

b) Pairs given string (Experiment 11)

That lack of nest material should have no effect on the start of incubation in females but cause a delay in males pointed strongly to the manipulation of nest material outside the nest box and carrying of it to the nest box being the important influences in the male. To test this two experiments were set up in which the only nest material provided was string attached to the bars of the cage. In Group A seven pairs were given three pieces of string, each about 25 cm in length: long enough to be carried to the perch outside the nest box but not into the box. The pairs in Group B were provided with two pieces of string, each about 35 cm in length. Here the string was long enough for the end of it to be carried into the nest box. These experiments were performed during the summer months under a rather longer day than the previous ones, so that the seasonal changes found by Eisner (1960b) must be borne in mind when comparing them.

Egg-laying was delayed in both groups, as was full establishment of females; but the data on full establishment cannot be compared as the experiments were terminated in a number of cases before it was achieved. Where the string was short, the full establishment of males was also retarded to a mean of at least 13 days later than the start of incubation (in three cases the pairs were separated before the males were fully established: pair a, day 34; pair c, day 34; pair d, day 41). This increased lag is probably accounted for by the unnatural situation, as the males continued trying to carry the string into the nest box right up to the end of the experiment. Under normal conditions incubation would have been fully established at this stage and nest building would have ceased almost entirely.

The time of the start of incubation in females does not differ significantly between the two groups (Table 4.7). Nor do the results in this experiment, where nest material was provided (Experiment 7) and where there was no material at all (Experiment 10) show a difference from each other (on an analysis of variance following logarithmic transformation), even without making allowances for the differences in photoperiod. This confirms the conclusion that nest material is not important to the timing of the start of incubation in females.

A comparison between these two string experiments with regard to the start of incubation in males shows a striking difference. Where the string was long all the males started on day 7 or before while all those with short string started on day 8 or after, giving a strongly significant difference ($p < .001$, on a Mann-Whitney U-test). Thus actual experience in carrying material into the nest box is probably the important factor in accelerating readiness to incubate in males. Increased experience within the nest box could also be of importance but this is unlikely. The birds were not often

TABLE 4.7: Timing of egg-laying and the start of incubation in pairs in which the only material provided was string attached to the bars of the cage (Experiment 11).

Group A: String too short to reach nest box.

Pair	Day of first egg	Day of start of incubation	
		♂	♀
a	34+	9	9
b	11	8	6
c	11	9	7
d	36	11	6
e	20	26	6
f	14	17	13
g	33	12	9
Median	20	11	7

Group B: String long enough to reach box.

Pair	Day of first egg	Day of start of incubation	
		♂	♀
a	18	4	4
b	10	4	12
c	16	7	5
d	23	2	7
e	17	3	11
f	35+	7	14
g	13	6	7
Median	17	4	7

seen manipulating the string in the box and it was in fact seldom in the box for long, as the males continually pulled it out from outside, as they normally do with material straggling over the lip of the box.

Discussion

From the data presented so far it is possible to construct a network of interactions indicating which external stimuli are relevant to the changes in behaviour occurring early in the reproductive cycle. In doing this of course one is disregarding the physiological states mediating these changes and simply indicating which stimuli are important in leading to these states. One must also bear in mind the two ways in which stimuli can act: to induce a behaviour pattern or leading to the physiological state appropriate to the appearance of such a pattern.

Nest-building, egg-laying and incubation are not usually found in isolated birds provided with nest material though they may all be found in some cases (the commonest being building which was found in 25% of isolated males). It is exceptional not to find nest building in males when paired, regardless of the sex of the mate or whether or not copulation is allowed. Thus stimuli from the mate induce nest-building in males: the fact that the response is not usually immediate points to a physiological change being required first (Experiment 9). In most cases copulation does not take place immediately either: in pairs given nest material and eggs in their nests before laying a positive correlation was found between the delay to the first observation of settling movements and that of copulation for both sexes (Table 4.4).

While copulation is not essential, egg-laying does not usually occur in isolated birds or pairs without nest boxes and is delayed in the absence of nest material. Nest material may affect it either through stimulation from building or from the completed nest. (It was delayed in pairs given only string and in birds separated from their mates by bars, whose only experience with material was manipulation of the few pieces which fell through the bars from the male's cage. This points to manipulation outside the nest box not being the important factor.) In either case these stimuli depend on the male, as it is largely through his carrying that nest material reaches the box. Thus the female stimulates the male to carry and carrying by the male accelerates laying by the female through her experience with the nest material in the box.

By the time the first egg is laid both birds have become ready to start incubating. In the female this situation is particularly interesting, for while egg-laying and the start of incubation normally occur at the same time, the external factors affecting them are different. Lack of nest material delays egg-laying but has no influence on readiness to start incubation (Experiment 10). Incubation can thus start well in advance of the first egg if a clutch is provided but, as in pairs given nest material (Experiment 7), it does not reach full establishment until shortly before the last egg is laid. Thus fully developed incubation is to some extent incompatible with egg-laying: while it may be reached and maintained in the absence of laying, if laying does occur after it has been proceeding for more than a few days it is associated with a disruption in the full establishment of the female.

Readiness to incubate in males is delayed considerably in the absence of nest material (Experiment 10), the important feature here being the carrying of it into the nest box as shown by the string experiments (Experiment 11). It is possible that carrying is the only important influence, for an isolated male which built a nest also incubated eggs placed in it, without any stimulation from a mate at all (Experiment 9, p. 71). Nevertheless the mate would still be of importance under normal conditions to elicit the carrying in the first place. Not surprisingly, the full establishment of males is not affected by egg-laying as is that of females: whatever the timing of the start of incubation it tended to follow this after a short interval.

Chapter 5

GENERAL DISCUSSION ON EXTERNAL STIMULI

The main aim of the experiments described so far was to discover how various factors in the external environment interact with the behaviour of the members of a pair early in the reproductive cycle. Chapter 3 covered those stimuli inducing females to lay eggs and in Chapter 4 an account was given of experiments on the factors affecting development of readiness to incubate. The results obtained were discussed briefly at the end of the relevant chapters: here they will be compared with the results obtained by workers on other species.

Female Bengalese finches do not normally lay eggs either in their stock cages or when kept singly even when nest material is present. In heterosexual pairs laying occurs within three weeks and it takes place at approximately the normal time when the two birds are separated by bars so that copulation is prevented. Laying is also more common in monosexual female pairs than in the stock cages or isolated birds and its occurrence appears to be independent of whether males can be heard or not.

Many other results stress the importance of the partner in stimulating laying. Lehrman <u>et al</u>. (1961) found 100% of ring doves with mate and nest material to have ovulated by day 8, whereas none of the isolated control females had ovulated and they showed no increase in oviduct weight or tendency to incubate. When copulation was disallowed by placing a glass plate between the birds 65% had ovulated by day 7 but only 10% of females with castrated males had done so (Erickson & Lehrman, 1964). Matthews (1939) also showed copulation to be unnecessary for egg-laying in pigeons. Polikarpova (1940) reported oviduct growth in the house sparrow to be enhanced by the presence of males and Burger (1942) found the ova of paired starlings to increase in size more than those of isolated females, groups of caged females or females in heterosexual groups. In this last case six or eight birds in cages of 4800 cubic feet did not form pairs. This suggests, as did the results reported here, that some form of pairing relationship is necessary for egg-laying. The results on monosexual pairs do not point to auditory stimulation being an important influence in this context. This seems to be at variance with results on the budgerigar in which male vocalisations accelerate ovarian development in isolated females (Vaugien, 1951), groups of females (Brockway, 1965) and normal pairs (Ficken et al., 1960). Colony noise has been found to increase ovarian development in ring doves (Lott et al., 1967). The effect of colony noise on normal breeding pairs was not examined in the Bengalese, all experiments, but for two on monosexual pairs, being carried out in the same room as the stock cages.

In the canary stimuli from the male accelerate reproductive development, but females caged alone do eventually lay (Warren & Hinde, 1961b; White & Hinde, 1968). This seems not to be the case in the dove (Lehrman *et al*., 1961), and the difference here may be that the female canary does most of the nest building and this stimulates ovarian development. In the dove, on the other hand, Lehrman (1959) suggests that the influence of nest material is not directly on the female but through its effect on the male's behaviour. In the Bengalese finch, as in the dove and canary, egg-laying is delayed in the absence of nest material. As it was also delayed in pairs separated by bars when only the male was given nest material, it seems that direct experience by the female is the important stimulus here. It was not possible to decide whether such experience was through building or stimulation from the completed nest. Whichever is the case, however, the presence of the male is of indirect importance as the female cannot build within the nest box until the male has carried material to it. This he is in turn stimulated to do by the presence of the female.

Nesting facilities seem of relatively little importance to laying in the ring dove, as 80% of females with a mate but without a nest pan or nest material ovulate by day 8, thus showing only a small difference from those birds given nesting facilities (Lehrman *et al*., 1961). Hinde and Warren (1959) found canaries to lay later when deprived of nest material and even more so in the absence of a nest pan. This situation is similar to that found in the Bengalese finch, except that here no females without a nest box were recorded as laying. Lack (1933) noted delayed nesting in two colonies of arctic terns whose usual nesting sites remained waterlogged late into the spring and cites other examples of laying being postponed in the absence of nest sites. Geese of several different species which had not laid during years of captivity were found by Berry (1943, 1944) to do so when provided with artificial nests. In these cases, however, it is possible that the birds had laid but that the eggs were undiscovered.

When pairs of Bengalese finches were given eggs in their nests before laying they started incubating early and their subsequent clutches were diminished in size or suppressed altogether. A number of workers have obtained similar results. Poulsen (1953) reports that pigeons which have adopted artificial eggs will either lay a full clutch of two eggs or no eggs at all. Nine out of 21 gannets given an egg before laying accepted it and four of them did not lay (Nelson, 1965). If eggs given to black-headed gulls are adopted more than one day before laying a reduction in clutch size occurs and in some cases laying does not take place (Weidman, 1956). Because of clutch suppression it was not possible to determine the earliest time in relation to the first egg that the birds could be induced to sit. In the herring and lesser black-backed gulls, however, Paludan (1951) found that clutches given more than ten days before laying were either eaten or built into the nest, but after this they were normally incubated. Even when incubation proceeded for ten days before laying the maximum reduction recorded in clutch size was only one egg. Results from the tri-coloured redwing indicate that addition of one or two eggs to the nest before or during egg-laying may reduce clutch size, but this effect might stem from increased egg loss due to overcrowding in the nest (Emlen, 1941). These results, as well as those of the present study, point to there being an incompatability between egg-laying and incubation. Patel (1936) and Saeki and Tanabe (1955) give evidence that incubation leads to prolactin secretion. Eisner (1958) suggested that prolactin, produced as a result of incubation, leads to clutch termination. Unfortunately the variation in clutch size of the Bengalese finch makes it impossible to assess the exact effect of early incubation on subsequent clutch size.

In the present thesis males were found to settle earlier than females where nest material was provided and the results of Weidmann (1956) suggest that, where a sex difference existed in the black-headed gull, it was the male which sat first. Noble _et al_. (1938) reported that the male black-crowned night heron settles to incubate immediately when an egg is placed in the nest before the female has laid. On the same theme, Lehrman _et al_. (1961) found that a higher proportion of males than females were prepared to incubate on days 3 to 5 when pairs of ring doves were given nest pan and nest material from the day of pairing. When no nest pan or nest material were provided until the birds were tested, a higher proportion of females than males sat on days 3 to 6. This result is similar to that obtained here, where the timing of readiness to incubate in females was apparently unaltered by deprivation of nest material, while that in males was greatly delayed, thus reversing the sex difference.

From these considerations one can conclude that the effects of various stimuli on egg-laying and readiness to incubate in the Bengalese finch are similar to those found in other species. Such differences as do occur are mainly of degree. An example of this is the differing effect of nesting facilities on egg-laying in the Bengalese finch, the ring dove and the canary. Other dissimilarities are connected with the different roles of the sexes, for instance in nest building. The female canary is stimulated directly by nest building; in the dove the female is apparently stimulated largely by the effect material has on the behaviour of the male. The Bengalese shows an intermediate stage, the female being stimulated directly by the material but only after it has been carried by the male.

The complex effects of mate, eggs and nest material on egg-laying and incubation are the product of natural selection.

It is not surprising to find a system in which changes in behaviour and physiology are affected by many different external factors to varying degrees. Where a number of factors are required for successful breeding, selection will favour that individual which is stimulated by each according to its relative importance. A bird must have both a mate and a nest if its eggs are not to be wasted. Thus growth of the ova is enhanced by those stimuli which predict the occurrence of mating and the completion of a nest. A great many stimuli are included in this category: selection cannot pick on one but attends to the whole.

Chapter 6

HORMONE EXPERIMENTS

Introduction

Previous work, reviewed in Chapter 1, pointed to oestrogen, progesterone and prolactin being both present in female birds, and important in the induction of physiological and behavioural changes early in the breeding cycle. To gain an insight into the part played by various hormones in the Bengalese finch attempts were made to inject these, and also testosterone in males, in the absence of external stimuli previously found to be important, and so determine whether behaviour changes could be induced in this way.

Probably because males tend to be less involved in parental behaviour, and because of the endocrine and economic interest of egg-laying, most information on hormones and reproduction in birds refers to females. This suggested that it might be fruitful to attempt some analysis of the situation in males. In females egg-laying doubtless adds to the complexity of the endocrine background, and the development of incubation beyond the appearance of settling movements was found to show some interaction with egg-laying (Chapter 4). The simpler situation in males, particularly in regard to the striking increase in carrying resulting from the presence of a mate and the necessity for a mate to be present before incubation will occur, prompted endocrine investigation. Most isolated males do not build nests and ignore eggs given to them, the change occurring after pairing is slow and suggests a hormonal basis. By injecting hormones into isolated birds, it was hoped to induce these changes in the absence of the mate, whose presence is normally required for them to take place. The discovery that the incidence of a behaviour pattern is altered by hormone administration is several stages removed from the deduction that the hormone injected is normally involved in that behaviour change. The interactions between hormones are complex and the injection of one almost certainly alters the levels of several others within the bloodstream. But this approach is a first step in the analysis: techniques at present available enable it to be extended by noting the effects of different hormones, alone or in combination, of organ removal and of hormone implants within the brain.

Materials and Methods

The exact conditions, schedule of injections and programme of observations varied between experiments and these will therefore be summarised with the results for each. Six different experiments were conducted: one with testosterone, one with oestrogen, two with progesterone and two with prolactin. The small number of birds used in each case was partly due to a paucity of cages open to observation and partly to the difficulty of carrying out concurrent observations on more than four birds when detailed notes were being taken. It was planned to extend the experiments to further birds if the results suggested that this might be profitable.

Observation periods to detect incubation were carried out from behind the nest boxes: the birds were provided with eggs and settling movements were looked for. Several different measures were used to assess nest building and these were recorded by observation from in front of the cages. The number of pieces of nest material carried into the nest box and the total amount of time spent in the box were recorded for each bird, as well as the number of songs produced by males. The time which each bird spent manipulating nest material was also recorded, but this is difficult to assess accurately as birds will pick up and drop material repeatedly. In all experiments where nest material was provided the box was inspected daily for signs of building and, in the experiment using oestrogen, an attempt was made to assess the amount of material within the box using a method to be described later.

Hormones were injected in solution into the pectoralis muscles on alternate sides. Because of the small size of the bird (in the region of 12 grams), no more than .05 ml was ever injected at one time. The volume of liquid required was accurately delivered by an Agla micrometer syringe on which one revolution of the micrometer head was equivalent to .01 ml. The syringe was mounted firmly on a retort stand and liquid from it was delivered through a 27 gauge needle, the ventral apterium of the bird having been exposed by dampening and displacing the feathers surrounding it. The needle was inserted close to the keel and at as acute an angle as possible to the skin. It was also left in the muscle for a few seconds after the solution had been delivered. Despite these precautions no position for the needle was found which entirely ruled out the possibility of bleeding from the wound and, in birds injected with oil, a small proportion of the solution frequently welled up out of the wound as the needle was withdrawn. Most birds showed no adverse after-effects, but in some song numbers fell and they seemed less active. One or two adopted an unusual, very straight-legged posture. None of the birds died during the course of treatment.

The following hormone preparations were used:

Testosterone Propionate B.D.H.: Solution of 10 mg/ml in 20% Ethyl Alcohol/80% Arachis Oil.

Stilboestrol B.D.H.: Oily solution of 5 mg/ml.

Progestin B.D.H.: 1st Experiment - Solution of 5 mg/ml in Ethyl Oleate. 2nd Experiment - Solution of 50 mg/ml in 3% Benzyl Alcohol/7% Absolute Alcohol/90% Ethyl Oleate.

Prolactin (N.I.H., Bethesda, Maryland): Mean potency =

24.3 i.u./mg. 50 mg dissolved in 2.025 ml of chick ringer giving 600 i.u./ml.

Testosterone

Lehrman (1961) has drawn attention to the correlation between the periods of copulation, maximal follicle growth and nest building in birds, and suggested that these occurrences may have a common endocrine background. It seems reasonable to postulate that nest building, where it occurs in male birds, may be under the influence of testosterone, the hormone which normally induces male sexual behaviour. Little evidence is available on this point, though Noble and Wurm (1940) found testosterone to enhance nest building in both sexes of the black-crowned night heron. In the male Bengalese finch, the rise in nest building after pairing does not appear to coincide with other behaviour changes. There is certainly often a delay before copulation; but this is probably due to lack of receptivity on the part of the female, as most males court, and will copulate if the female is receptive, within a few minutes of pairing. Nevertheless, the possibility exists that testosterone levels rise after pairing and that nest building appears only after a certain threshold has been reached.

Four males were isolated, in cages supplied with nest material and a nest box, and watched daily thereafter for one hour from in front of the cages. Each bird was given an injection of 0.02 ml on day 2 and again on day 4. In two birds (♂158 & ♂159) this contained 0.2 mg of Testosterone propionate: in the other two it was of solvent only (♂147 & ♂156).

The birds showed marked differences between each other:

Experimentals:

♂158: On none of the twelve days of observation was this bird seen to carry or visit the nest box. The time spent manipulating material was greater on day 1 than thereafter on any one day, and there was no suggestion of an increase following the injection.

♂159: The three parameters associated with nest building all seemed to change in this bird following the injections. No carrying was seen before day 2 or after day 9, but it was observed daily on the seven days in between. A total of 46 pieces of material were taken to the nest box during this period, although most of these were subsequently removed and no nest was built. The time spent in the nest box and manipulating material outside it were also higher during this period than either before or after on a comparison of means, but some high figures at other times preclude a statistically based conclusion.

Controls:

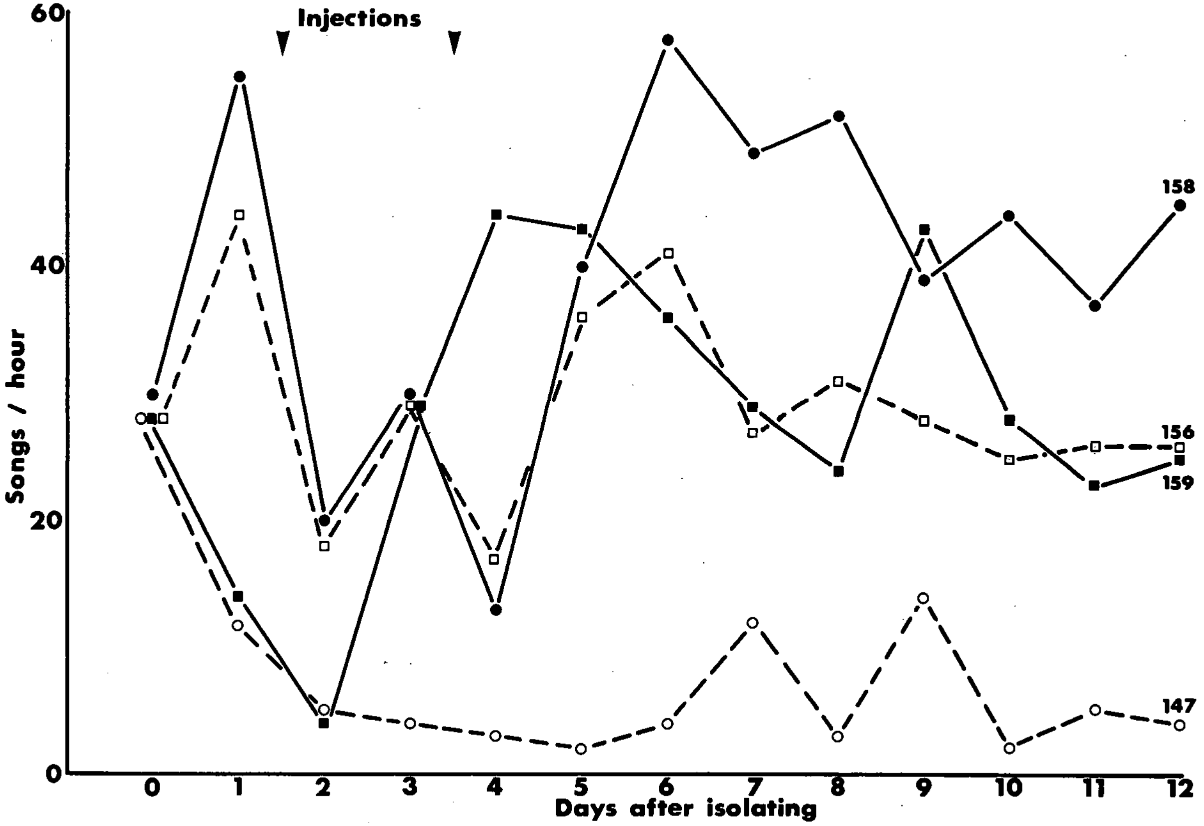
♂156: This bird fluctuated greatly from day to day on the three nest building measures and no overall trend was discernible. On ten days no carrying was performed but on days 6, 8 and 12, 6, 21 and 45 pieces were carried to the box respectively.

♂147: This bird appeared to be adversely affected by the injections, its song frequency being depressed well below that of the other birds and a normal rate, to give a mean of 5.3 per hour for the 11 days following the first injection (see Figure 6.1). It was never seen to carry or visit the nest box, and only manipulated nest material briefly on three days.

None of the birds had built a nest before day 12, when they were each given one containing six eggs. During observations from behind the nest boxes for one hour on each of the subsequent three days no incubation was observed. At the end of these three days ♂♂156 and 159 had removed all material from their boxes, and the other two birds, which had shown no interest in the nest box during earlier observations, had dismantled their nests by day 25.

Thus, while one of the birds injected with testosterone (♂159) appeared to show greater interest in nest material as a result, this was not found in the other bird, nor did the effect lead to the construction of a nest. Marked day to day fluctuations in nest building behaviour suggested that bouts of

FIGURE 6.1: Number of songs produced by testosterone-injected birds (solid points and lines), compared with number by control birds (open points and dotted lines) during daily one hour watches. Arrows indicate timing of injections.



60
40
20
0
Songs / hour
Injections
0
1
2
3
4
5
6
7
8
9
10
11
12
Days after isolating
158
156
159
147

it are too infrequent to be adequately examined by daily one hour observation periods. Figure 6. 1 shows the daily number of songs for the four birds. If any increase occurred as a result of testosterone treatment it was clearly very slight. It is possible nevertheless that singing by males is elicited by testosterone, but that normal secretion of the hormone is sufficient to maintain song at a maximal rate, beyond which further hormone will not raise it. Figure 6. 1 also shows that injections depress the number of songs, and some tendency (particularly comparing ♂156 with ♂158) for the day to day fluctuations of different birds to be in concert with one another. Song frequency is very susceptible to external influences, birds tending to sing more immediately following a disturbance and also when there is environmental noise, both from within the colony and from outside it.

Stilboestrol

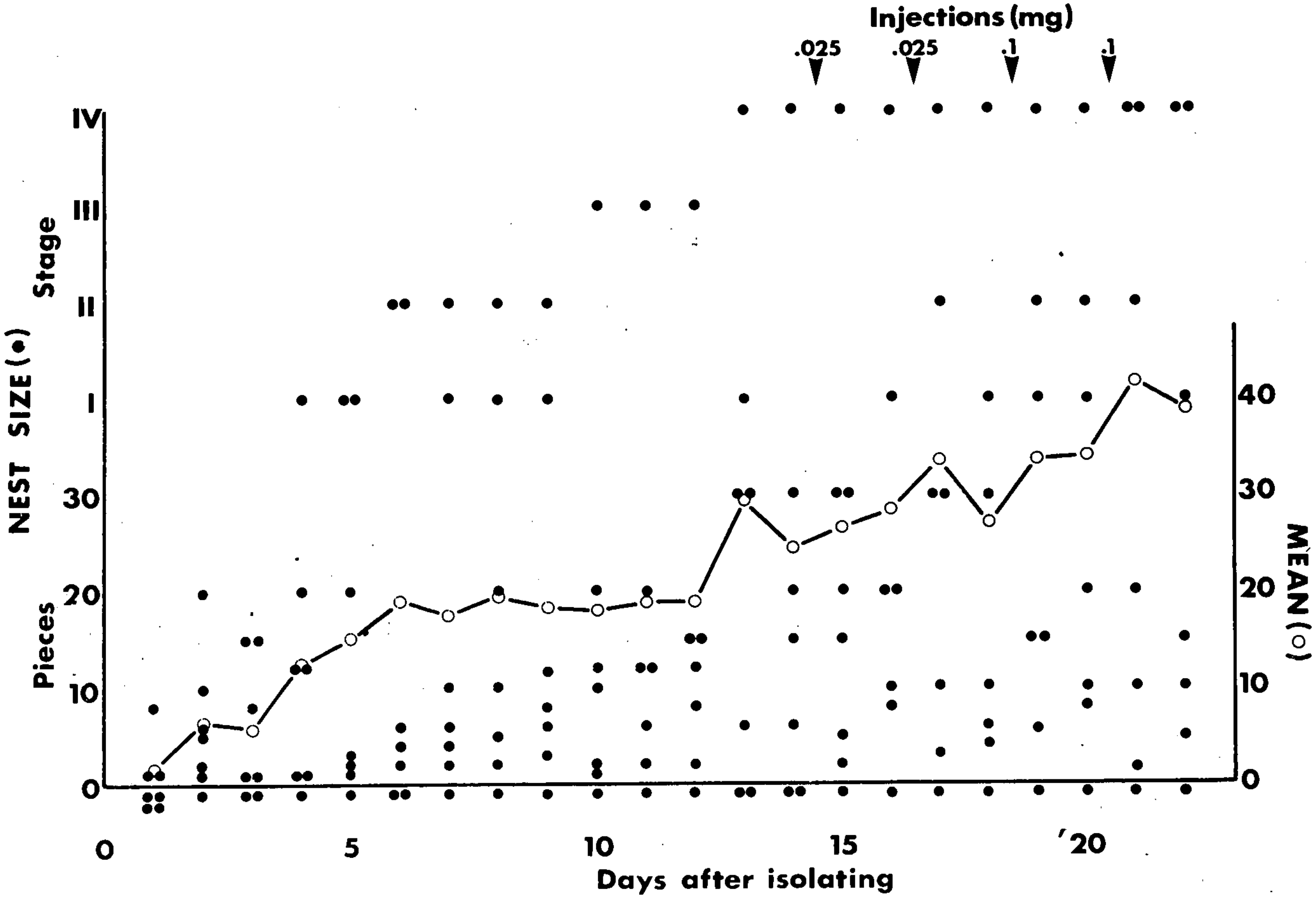
Warren and Hinde (1959) found that 0.2 - 0.5 mg of oestradiol benzoate injected three times weekly stimulated nest building in female canaries, but the high dose required indicates that the effect was not entirely natural. In the ring dove, Lehrman (1958b) was able to induce building with 0.4 mg of diethylstilboestrol daily. This result was obtained with both sexes and suggested that oestrogenic hormone might be involved in the carrying of the male Bengalese, the roles of the sexes in building being similar to those in the ring dove.

Eight males were isolated in cages provided with nest box and nest material. One died before injections were commenced and the others were injected with 0.025 mg of stilboestrol on days 15 and 17, and 0.1 mg on days 19 and 21. Stilboestrol, though not in fact a steroid, is a synthetic molecule showing oestrogenic activity. The period of isolation before injections began stemmed from the discovery, made after the testosterone experiment, that some isolated males build nests. Two of the birds here showed a tendency to do so, one (♂212) having a complete nest by day 13 and the nest box of the other (♂191) containing an approximately half full nest by day 6, but only about 30 pieces of material on day 14. All but one of the others showed some nest building activity as gauged by this measure, but none had more than 30 pieces of material in the box on the day injections were started. If there were up to 30 pieces of material in the box it was possible to count them roughly: beyond this the nest size was estimated as being a proportion of the finished size. A platform of material covering the floor of the box was termed Stage I; Stage II was a nest cup with material at the sides rising not higher than the lip of the box; a Stage III nest had some material forming sides and a roof; a Stage IV nest had sufficient material in the box to make it hard to see into the nest through the glass plate behind it. This last is roughly the situation in the complete nest of a normal pair.

Figure 6. 2 shows the estimated nest size of these birds for each day of the experiment. To indicate the trend, means have been included for each day computed, including those nests too large for their material to be counted, by assigning these a minimum number of pieces thought to comprise them (Stage I = 40; II = 60; III = 80; IV = 100). While it is clear from this that nest size rose during the three weeks, no rise in the rate is detectable after injections began.

Each bird was also watched for half an hour on alternate days from day 9, giving three half hour observation periods prior to the injections and four on the days of the injections. To maximise the chances that nest building would occur during this period, a new supply of material was placed in the cage ten minutes before each watch, and the remains of that which had been there for two days was removed. But for the lack of a mate, the situation was thus comparable with those males tested for nest building earlier (Table 2.1).

Three birds were never seen manipulating or carrying nest material at all. Of these only two visited the nest box, in each case once, one before and one after injections had begun. Of the four birds which did carry, two did so less after injections had begun and two did so more. There was also no discernible increase in the amount of time spent manipulating


Injections (mg)
.025
.025
.1
.1
NEST SIZE (●)
Stage
IV
III
II
I
Pieces
30
20
10
0
MEAN (○)
40
30
20
10
0
0
5
10
15
20
Days after isolating

nest material. Songs showed a slight decline on average, though in two birds there was actually an increase. In none of the measures noted was there a change which was consistent for all individuals. There is thus no evidence to suggest that oestrogen can induce nest building behaviour in the male Bengalese finch.

Progesterone

This hormone has not been found to induce nest building behaviour, Warren and Hinde (1959) having found it ineffective both alone and in combination with oestrogen. That it is present at around the time of nest building is indicated by its involvement as a secondary hormone in the induction of various aspects of reproductive development in the canary (Hinde & Steel, 1966). Its injection has also been found to lead to incubation in the ring dove. That it might lead to incubation, and perhaps nest building, in the Bengalese therefore seemed a possibility.

1st Experiment

Four males were placed singly in cages supplied with nest box and nest material. None had any material in the box by day 6 and they were then each given a nest and six eggs. Two (♂179 & ♂104) were injected with 0.1 mg of progestin on days 6, 8, 13 and 15, the others (♂175 & ♂142) receiving carrier injections at the same time. Watches of one hour were carried out four hours after the injections on day 6 and day 8, to look for nest building particularly, and thereafter daily observations were made from behind to detect incubation behaviour.

This was in fact the first hormone experiment carried out and it was designed with a view to detecting any behaviour changes which might result rather than with a specific point in mind. One of the hormone-injected birds showed no interest in nest material on either of the one hour watches, sat still on the same perch throughout the second, and was not seen to sing. The other appeared rather ruffled for a period immediately following each injection but seemed to have recovered before the observation periods. In the watch after the first injection, it carried 18 pieces of material to the box and spent eight minutes within it, but without being seen to incubate. In the light of the behaviour of one of the controls to the testosterone experiment (♂156, see p. 97), it seems that carrying of this intensity is normal occasionally in untreated birds. One of the controls here took no interest in nest material; the other manipulated it a little and carried material away from the box, dismantling the nest by day 8.

Observations of the nest between days 8 and 15 for a total of half an hour daily failed to reveal any incubation behaviour.

2nd Experiment

A possible explanation for failure to obtain any effect in the above experiment despite a comparatively large dose was that much of the hormone had failed to diffuse out of the oil. Steroids are relatively insoluble in water compared with oil, and doubtless the amount in the bloodstream depends not only on the absolute quantity injected but also the concentration within the oil compared with that in the blood. In this second experiment therefore, a ten times more concentrated solution was used and the injections were of 0.01 ml containing 0.5 mg. Four birds were again used, two (♂208 & ♂135) injected with this solution and two (♂170 & ♂230) with solvent alone.

The birds were isolated on day 0 and injected on days 7, 9 and 11. An additional control here was five daily one hour watches on days 0-4 enabling comparison to be made between behaviour of the same bird before and after injection. They were also watched daily from day 7 to day 11. Up to day 11 they had no nest or eggs but were supplied with material: after the watch on day 11 the two experimentals were paired with each other, as were the two controls. They were then supplied with nest pan and eggs and watches were carried out to detect incubation. The reason for pairing them was that it was felt that the presence of another bird might be important as an immediate stimulus in the elicitation of incubation.

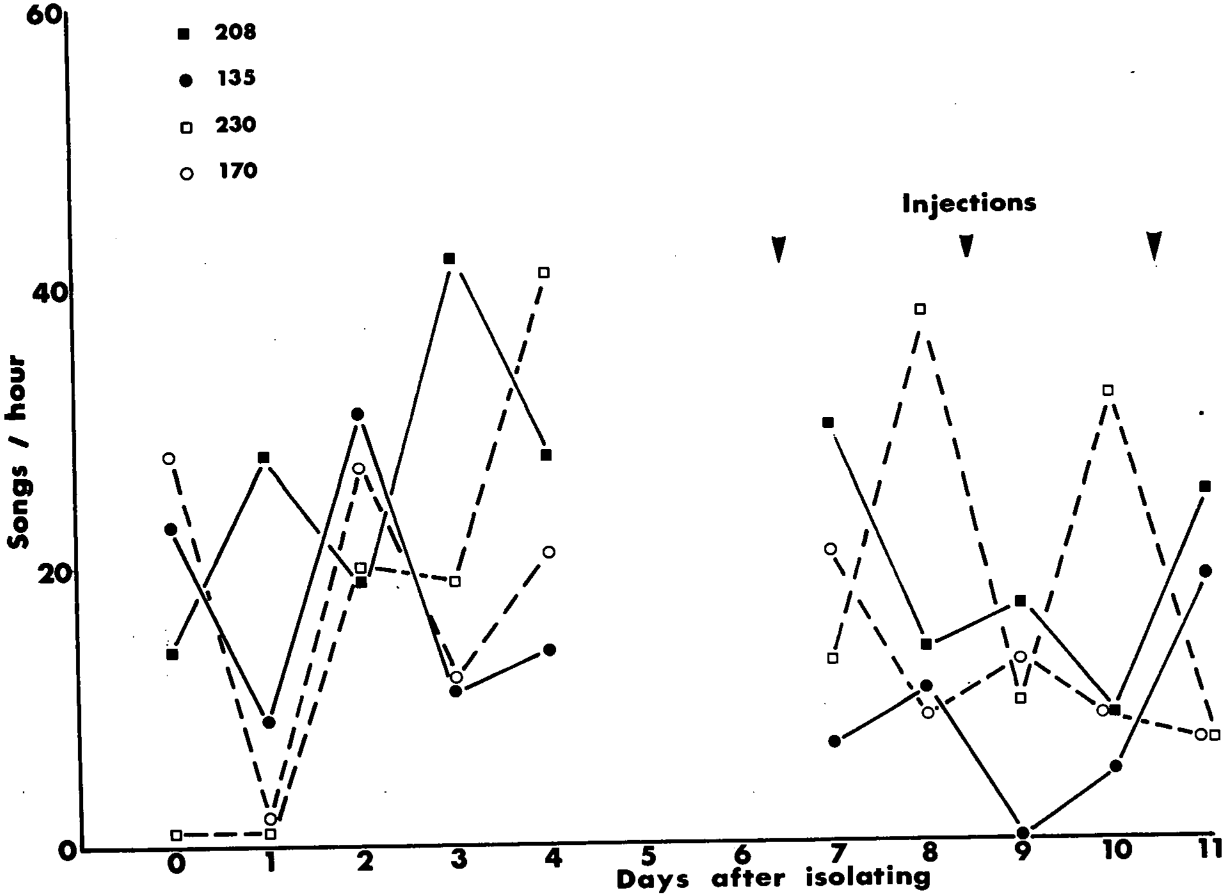
As in previous experiments, the time spent manipulating nest material and that within the nest box varied greatly between birds and from day to day. None of the birds was seen to carry at all, either before or after hormone treatment, and no change could be detected in song frequency (Figure 6.3).

Nest building did not occur immediately after pairing on day 10: on day 13 the experimentals had 20 pieces of nest material in their box and the controls had none. The experimentals had completed a nest by day 18 and the controls had done so by day 25. Incubation was first recorded in both experimental birds on day 24 and in the controls on day 25, both delays being usual for monosexual male pairs, so that progesterone cannot have been said to have advanced it. From the results of Lehrman (1958b) one might have expected a positive result to be manifest within 48 hours of pairing: certainly no incubation occurred during two hours of observations within that period.

Prolactin

Failure to detect incubation following progesterone injection has also been reported for the domestic fowl (Riddle, 1937; Eigemann, 1937) and the canary (Kobayashi, 1952). In the former prolactin appears to be the incubation inducing

FIGURE 6.3: Number of songs produced by progesterone-injected birds (solid points and lines), compared with number by control birds (open points and dotted lines) during one hour watches on days indicated. Arrows indicate timing of injections.


■ 208
● 135
□ 230
○ 170
Injections
Songs / hour
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
11
Days after isolating

hormone (Riddle et al., 1935), and it was therefore decided to examine this possibility in the Bengalese finch. In the first experiment its effect on nest building was also investigated, and song counts were carried out in both to look for evidence of the anti-gonad effect of this hormone, which has been found in several species of birds (Eisner, 1960a).

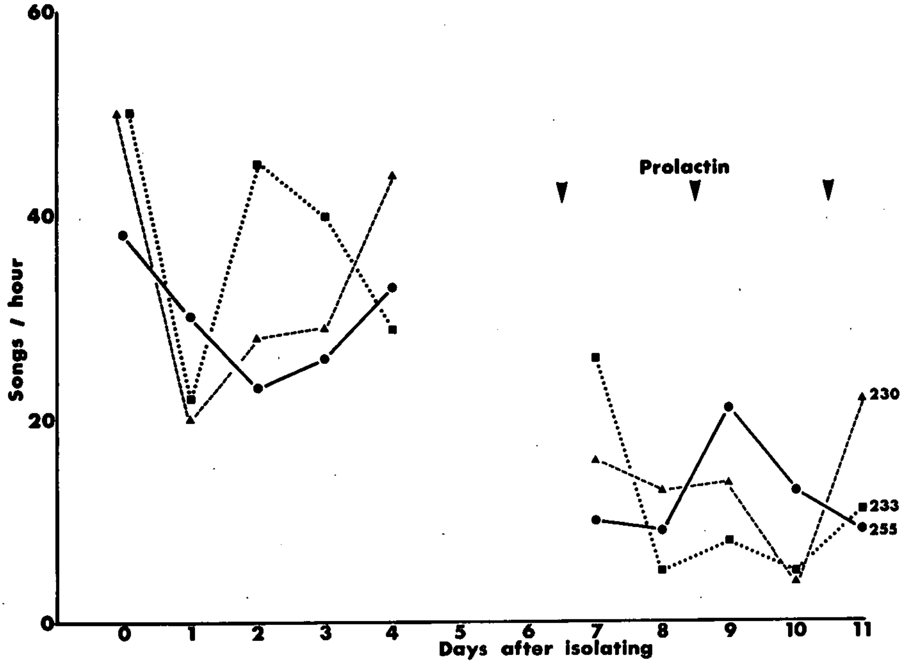
1st Experiment

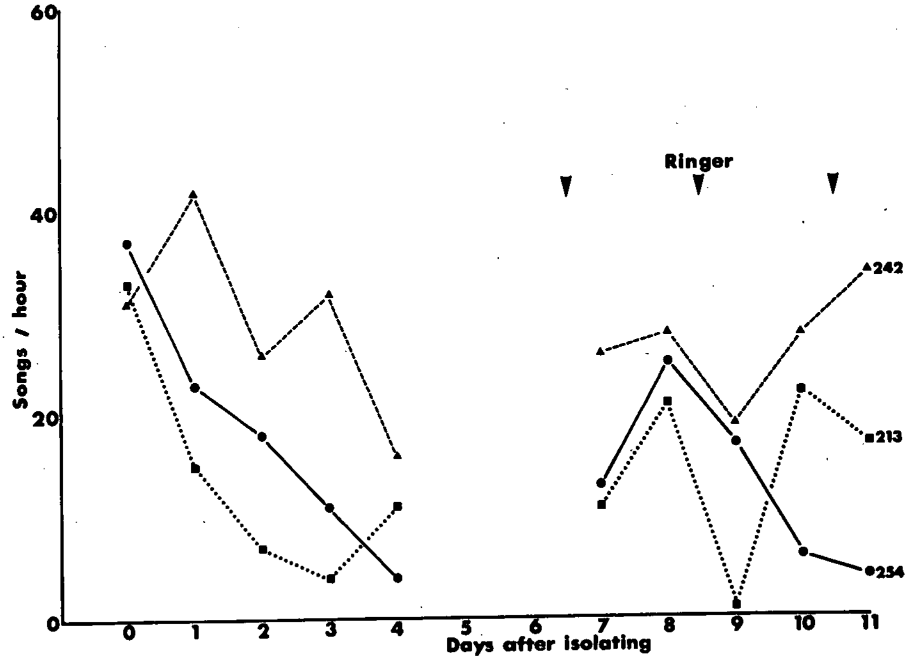
This experiment was designed, not to test for a specific effect, but in the hope that any behavioural changes resulting from the injections would become manifest. Three birds of each sex were isolated in cages supplied with nest box and nest material (a fourth pair was discarded as the male built a nest during the week before injections began). They were watched for one hour each afternoon from days 0-4 and again on days 7-11, each bird receiving 30 i.u. of prolactin four hours before the watches on days 7, 9 and 11. After the watch on day 11, the birds were placed in heterosexual pairs in cages supplied with a nest box containing six eggs in a nest pan, but without nest material. During the next three days observations were made from behind the nest box to test for incubation. A control series of three pairs was treated likewise but injected only with chick ringer: because of shortage of observation cages this was not carried out at the same time.

One of the prolactin-injected birds (♂255) had a Stage I nest at the time of pairing, but this was probably not due to the injections as there were about 15 pieces of material in its box before they commenced. One control bird (♂242) built a full nest before injections began, but was injected nonetheless. Nest material was continuously present prior to pairing but not replaced before each watch: the only carrying observed was one piece by ♂255 on the first day of isolation. There was no evidence of a rise in the time spent manipulating nest material or in the nest box after the injections began.

Figure 6. 4 shows the number of songs produced by each bird in each watch. In all the experimentals there was a marked decline in the number of songs per hour between the first and second weeks. Numbers by the controls fell off during the first week and tended to remain low during the second week in two of the three birds. Thus it appears that song is most frequent immediately after isolation and declines subsequently. Five of the total of six males watched here sang more on day 0 than on any subsequent day. It is possible that, due to an unfortunate choice of individuals, the three prolactin injected birds were ones which normally sang more frequently than the three controls. This hypothesis might suggest that, after an initial peak of singing, the birds settled down to their normal rates on day 1 or 2, this being maintained in the controls but depressed by the prolactin in

FIGURE 6.4: Number of songs produced by prolactin-injected birds (upper figure), compared with number by control birds (lower figure) during one hour watches on days indicated. Arrows indicate timing of injections. Data from 1st Prolactin experiment.


60
40
20
0
Songs / hour
Prolactin
230
233
255
0
1
2
3
4
5
6
7
8
9
10
11
Days after isolating


60
40
20
0
Songs / hour
Ringer
242
213
254
0
1
2
3
4
5
6
7
8
9
10
11
Days after isolating

the experimentals. Another possibility is that, the controls and experimentals having been performed at different times, the low rate of singing by the controls might have been due to extrinsic factors not prevalent at the time of the experimentals.

Whichever of these explanations is correct, the pronounced decline after prolactin treatment seems to indicate an effect of the hormone. The difference between the first and second weeks was significant for each of the experimental birds on a Mann-Whitney U test (♂255: $p = .004$; ♂233: $p = .008$; ♂230: $p = .008$), but not so for the three controls (♂242: $p = .345$; ♂254: $p = .274$; ♂213: $p > .60$). One interesting result, which can be seen in Figure 6. 4, is the tendency for prolactin-injected birds to sing more on the day of the injection than the day after (four hours after as opposed to 28), while the song levels of controls tended to be more depressed on the days they were injected. This last situation was also found in the testosterone-injected birds and their controls (Figure 6.1). The different result after prolactin treatment points to the diminution being an effect of a slow build up in hormone in the bloodstream after the injection.

Immediately after pairing on day 12, both experimentals and controls were watched for ten minutes from in front of the cages: all the males courted but in no case did copulation occur. Four half-hour watches were made from behind the nest boxes on day 12, three on day 13 and two on day 14. None of the experimental females was seen to incubate during these. One male (♂230) was first seen settling within an hour of pairing and did so again in all later watches. Neither bird in the two other pairs visited the nest box during the first two watches, and they were each then given five pieces of nest material to encourage them to do so. One (♂223) was first seen settling on day 13 (24 hours after pairing), and ♂255 incubated on the morning of day 14. None of the controls showed signs of incubating on day 12 but three birds, one from each pair (♂242, ♂213, ♀225), were seen to settle on day 13.

This result was unexpected. While the appearance of incubation in the three prolactin-injected males within 48 hours of pairing, seemed to suggest that the hormone had brought it forward, the controls behaved in a similar fashion, so invalidating this conclusion. The most likely reason for this was that nest material had been present throughout. It is possible that a fortnight in the presence of nest material, even without building taking place, followed by a very brief period with a mate, might be sufficient to induce incubation early.

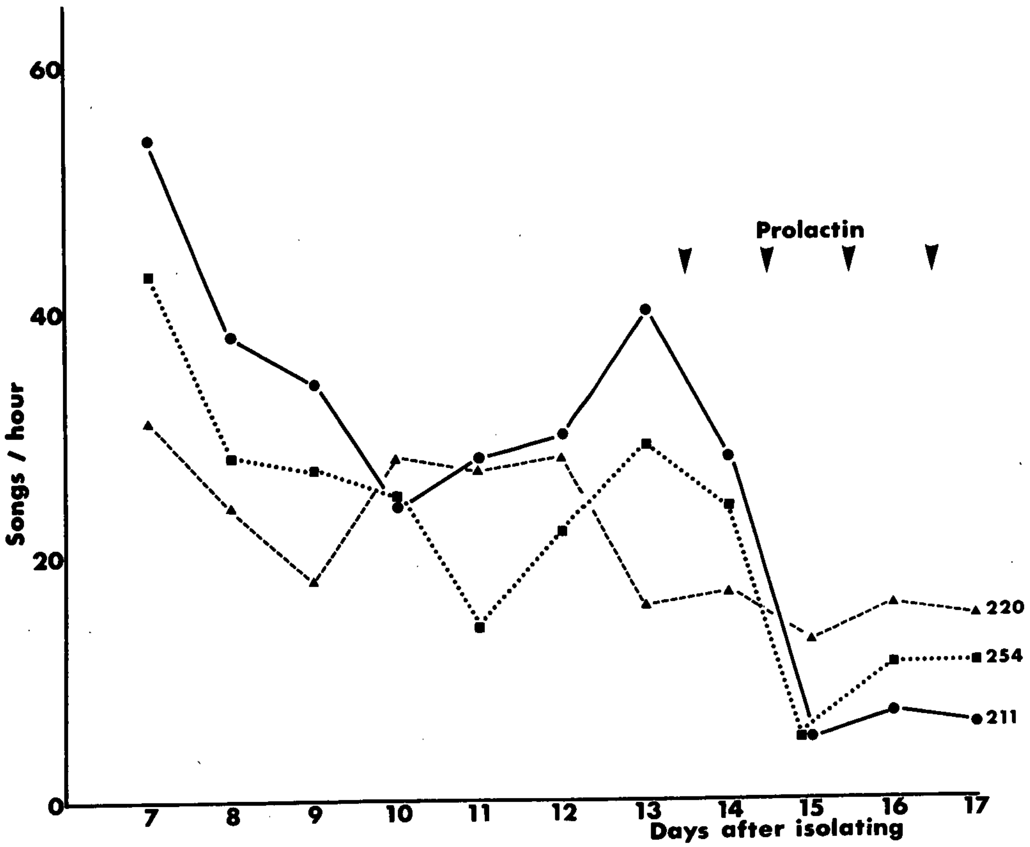
2nd Experiment

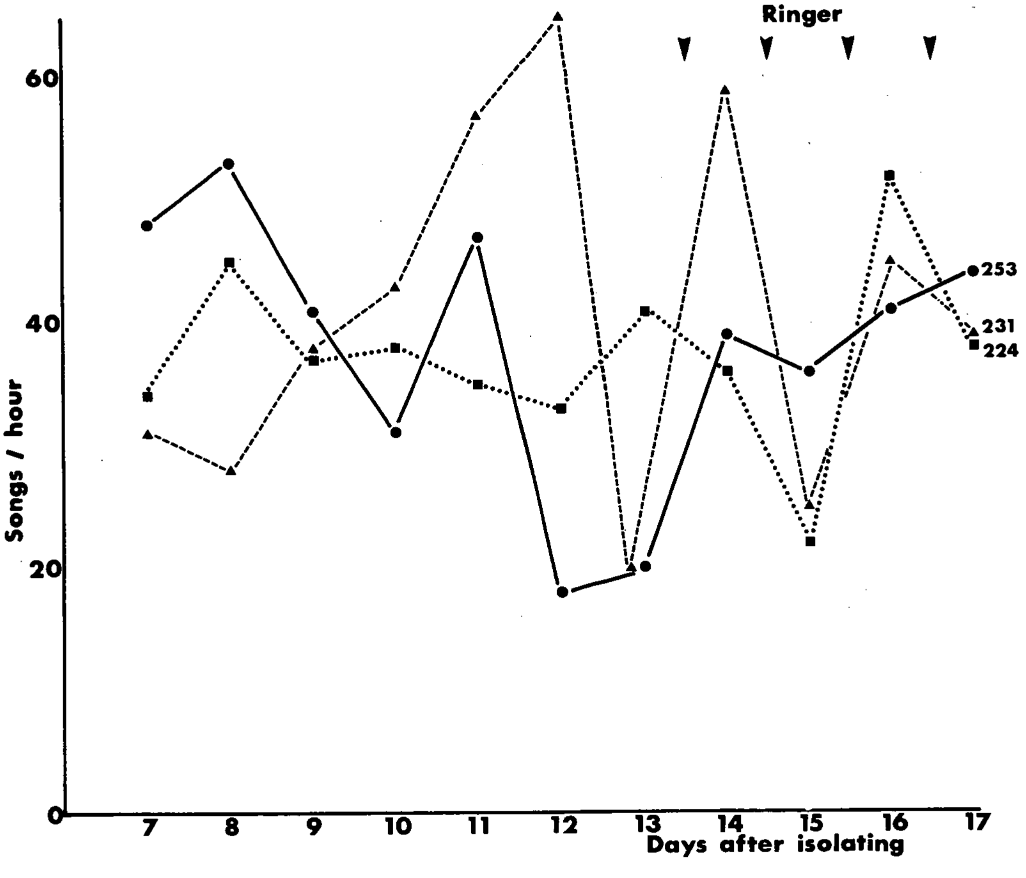
Some modifications were introduced here to make conditions more stringent, the major aim being to determine whether prolactin affected readiness to incubate. The birds were given no nest material to cut out the possibility that the presence of this alone might bring forward the start of incubation. They were isolated a week before observations began and the males were then observed from day 7-17 to count songs. It was hoped that in this way the initial decline in singing would be eliminated, and that song frequency would have stabilised before watches began.

Once again cage shortage precluded the carrying out of experimentals and controls at the same time. Three males and three females were used in the experimental series and four pairs in the controls. One of these last pairs was rejected, however, as the male became unwell during the first week of isolation. Four injections were given on days 14, 15, 16 and 17 and, as before, each of those given to the experimentals contained 30 i.u. of prolactin. Each bird was given a nest pan and six eggs at 11.30 a.m. on day 18, they were watched from behind the nest boxes from 2.00 - 2.30 p.m. and then paired. By using this method it was hoped that isolated birds might be seen to incubate. It was also possible that the presence of eggs before pairing might increase the attention paid to the box, one of the difficulties in these experiments being that some birds do not visit the box so that incubation cannot be detected.

The number of songs for each male during each watch is shown in Figure 6. 5. Despite the one week gap before observations began, the song level of each experimental bird was higher on day 0 than on any later day. This seems to imply that the presence of the observer is an important factor here. So many external stimuli affect song production that it seems quite possible that any novel environmental factor may cause an increase. Such an effect was not found with the controls, however. The two days on which they showed the greatest reduction were days 13 and 15. That on day 13 was probably due to failure of the heating in the experimental room, and that on day 15 may have been connected with the second injection, although the other three did not depress song numbers. The results of two prolactin-injected birds (♂254, ♂211) confirm that song is reduced by this hormone and reaffirm that the maximal effect is achieved with a latency of around 24 hours. The third bird did not show such a clear effect: although singing was infrequent after the injections, it was as common as on the day immediately prior to them. Nevertheless, comparison of data from the seven days before injections began with the four days after gave a significant decline on a Mann-Whitney U test for each of the three experimental birds (♂220: $p = .006$; ♂254: $p = .012$; ♂211: $p = .006$). No change could be detected in the controls (♂224: $p = .464$; ♂253: $p = .394$; ♂231: $p > .50$).

FIGURE 6.5: Number of songs produced by prolactin-injected birds (upper figure), compared with number by control birds (lower figure) during one hour watches on days indicated. Arrows indicate timing of injections. Data from 2nd Prolactin experiment.


Prolactin
Songs / hour
0
20
40
60
7
8
9
10
11
12
13
14
15
16
17
Days after isolating
220
254
211


Ringer
Songs / hour
0
20
40
60
7
8
9
10
11
12
13
14
15
16
17
Days after isolating
253
231
224

Observations on the nest box after pairing were for half an hour on day 18, one hour on day 19 and half an hour on days 20 and 21. All the prolactin-injected males courted immediately after pairing and in one case (♂220, ♀189) the female solicited and copulation occurred within 10 minutes of pairing. No incubation was observed on day 18 but on day 19 both ♂220 and ♀189 were seen settling, on day 20 ♂242 started and ♂211 did so on day 21.

Copulation was seen within half an hour in all three control pairs: in one case three times within the first 15 minutes. This is in itself unusual and suggested that the females had become receptive in isolation. No incubation was observed on day 18, but both members of one pair (♂224, ♀256) were seen settling on the morning of day 19, and during subsequent watches, and a further bird (♂253) was first seen incubating on day 21.

Yet again therefore it is not possible to draw a conclusion as to whether prolactin provides the hormonal basis of incubation in this species. From the experiments on external influences leading to readiness to incubate it appeared that a gap of 48 hours or more were required after pairing before this behaviour could be elicited in males and rather longer in females. The present experiments, however, indicate a shorter latency with several birds first settling within 24 hours of pairing, regardless of whether injected with prolactin or ringer. One bird in the 1st Experiment, having been injected with prolactin, sat within an hour of pairing. The explanation for these curious results seems to lie in the fact that all the birds were isolated for two or three weeks before they were paired. Certainly in the 2nd Experiment this seems to have led to heightened responsiveness on the part of females, as copulation was observed in four pairs within half an hour of pairing, and three of the females in these cases were seen soliciting vigorously. As this is unusual, it could be that readiness to incubate also appeared earlier purely as a result of the period of isolation prior to pairing.

The effect which the prolactin had on the song production of males was evidence that it was reaching the bloodstream and showing some potency. It is interesting, however, to note that, although song numbers were reduced, in neither experiment did the course of injections before pairing stop the males from courting immediately they were placed with females.

Discussion

Apart from a possible connection between testosterone and nest building, the main conclusion from these hormone experiments is that the singing of males was diminished by treatment

Failure here may to some extent have been due to experimental technique. Day to day fluctuations and inter-individual differences in the various measures of nest building used made it hard to detect changes resulting from the injections. Any which did occur must, however, have been small for no sudden increases in the amount of material within the box, such as occur after pairing, were recorded during hormone treatment. Likewise with incubation, while lack of a clear result may have been partly due to problems of experimental design, the tendency of injected birds to incubate cannot have reached such a high level as that found by Lehrman (1958b). He reported doves given progesterone and then paired to go immediately to the nest and usually to be incubating within about five minutes.

It is possible that the hormones injected in the present experiments were not reaching the bloodstream in sufficient concentrations to give behavioural changes. The doses used were based on those found to be effective with other species with appropriate modifications for differences in size. As hormone levels may differ markedly from species to species, as Rivarola <u>et al</u>. (1968) have found between different vertebrate groups, especially where size differences are involved, it would have been preferable to try a number of different doses of the same hormone. It was felt most likely to prove productive, however, if experiments were carried out with all the hormones liable to be involved in the early part of the breeding cycle. Prolactin, which was injected in saline solution, having been shown to have an effect, was certainly getting into the bloodstream. The steroids were injected in oil, however, and no conclusive evidence was collected to show that they were influencing behaviour. Solutions of steroids in oil being widely used both clinically and experimentally, it seems certain that at least some of the hormone was reaching the bloodstream. As the amount diffusing out of the droplet presumably depends on the relative concentrations in the oil and in the blood, it is difficult to conclude just what proportion of the hormone would actually leave the oil.

A further possibility is that, while an increase in the circulating hormone was achieved, factors in the situation were incorrect for behavioural changes to become manifest. In ring doves, Bruder and Lehrman (1967) report that more prolactin-injected birds incubate when tested in pairs than when tested singly. If a bird's partner incubated it was also more likely to do so. In the experiments on egg-laying described in Chapter 3 it was concluded that eggs are laid more by birds in pairs, whether normal, monosexual or separated by bars, than by either isolated females or those in the stock cage. This suggested the necessity for some form of attachment to form between the two individuals involved. The attachment to the mate, built up in the few days after pairing, may be an important factor in eliciting various aspects of reproductive behaviour, which may not appear without it even if the endocrine background is correct. In hormone experiments it is not possible to recreate the exact situation found in normal pairs. The experiential factors which might lead to the secretion of a hormone are removed and the hormone is then injected in the hope that a behavioural change will occur in the absence of the external stimuli normally necessary. The possibility that these external stimuli may be important at a purely neural level as well as leading to hormone secretion cannot be discounted.

Arguing against these considerations is the fact that some males build nests in isolation and that one of these was found to incubate having done so. This last bird (♂234) was in fact also scheduled to be used in the first prolactin experiment but rejected as it again built a nest and incubated some eggs placed in it. In this case it had been back in the stock cage for only three weeks, but in the other it had been there for five weeks. Thus, despite periods in the stock cages to equate the physiological states of individuals as far as possible, marked differences in behaviour appeared when they were subsequently used. It is not possible to state whether these differences were due to variation between individuals in the stimuli necessary to elicit a given behaviour pattern or in the physiological state at the time of pairing or isolation.

SUMMARY

1. This study was undertaken to try to elucidate the ways in which internal and external stimuli interact in the integration of the reproductive cycle of the Bengalese finch.

2. Egg-laying is unusual in isolated females and females kept in large groups. It is more common in monosexual pairs, and its incidence in them is not markedly altered by whether or not males can be heard.

3. Females in heterosexual pairs usually lay within three weeks of pairing. A similar lag was found in pairs separated by bars, pointing to copulation being unnecessary.

4. Egg-laying is delayed in the absence of hay for nest building. A delay was also found in pairs separated by bars, in which only the male was given nest material: this indicates that personal experience by the female is important.

5. Heterosexual pairs deprived of nest boxes, but given nest material, were not recorded as laying.

6. Incubation normally starts early during egg-laying and becomes fully established at around the end of the clutch.

7. Readiness to start incubating, detected by providing eggs before laying, develops in advance of egg-laying in both sexes,

but earlier in males than in females. Its development is independent of the presence of eggs.

8. Females which start incubating before laying subsequently lay smaller clutches or no eggs at all.

9. The timing of readiness to start incubating in females is unaltered in the absence of nest material, but its further development is slowed down, so that full establishment does not occur until around the time of the last egg, which is itself later.

10. Readiness to start incubating is delayed in males in the absence of nest material, but its further development is not protracted. Carrying into the nest box appears to be the most important way in which nest material stimulates readiness to incubate in males.

11. Carrying in this species is mainly done by males. It is unusual in isolated birds, but occurs in monosexual and heterosexual pairs, and in pairs separated by bars.

12. It is concluded that egg-laying and readiness to incubate are stimulated by mate and nest material in a complex fashion. The various stimuli these provide interact to a greater or lesser degree to give the physiological and behavioural changes observed.

13. Attempts to induce nest building and incubation behaviour in isolated birds by hormone injection were unsuccessful.

14. Song frequency in males was depressed by prolactin.

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Photography of the figures as they appear in this thesis was the expert work of Mr D.F. Cremer. Help in attending to the daily needs of the birds was given by Miss May Petrie.

Some of the results described in this thesis were obtained while I was Shaw Macfie Lang Fellow, and I am grateful to the University Court for granting me this award. Some of the experiments included in Chapter 4 form the basis of a paper already published (Slater, 1967: Appendix III).

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APPENDIX I

List of birds used and dates on which experiments were set up.

CONTROL 1.

Pairs given nest material and empty nest box.

Pair	Male	Female	Date started
a	179	163	2/2/65
b	142	133	2/2/65
c	64	143	2/2/65
d	175	93	2/2/65
e	254	193	19/4/67
f	234	275	8/2/68
g	255	245	8/2/68
h	233	206	8/2/68
i	254	256	8/2/68

CONTROL 2.

Pairs given nest material and nest in nest box.

Pair	Male	Female	Date started
a	120	155	11/2/66
b	164	166	11/2/66
c	135	160	11/2/66
d	121	163	11/2/66
e	142	145	11/2/66
f	81	153	11/2/66

EXPERIMENT 1.

Isolated females with nest material and nest in nest box.

Female	Date started	
160	20/5/65	
93	20/5/65	
193	22/9/66	paired 28/11/66
206	22/9/66	

EXPERIMENT 2.

GROUP A. Monosexual pairs of females given nest material and nest in box. In same room as stock cages; eggs left in nest.

Females	Date started
153 / 162	23/4/65
143 / 161	16/6/65
198 / 229	22/9/66
226 / 218	22/9/66

GROUP B. Monosexual pairs of females given nest material and nest in nest box. In sound-deadened room with songs of males audible; eggs removed as laid.

Females	Date started
226 / 229	16/10/67
206 / 249	16/10/67
248 / 251	16/10/67
182 / 195	16/10/67

GROUP C. Monosexual pairs of females given nest material and nest in nest box. In sound-deadened room with only females present; eggs removed as laid.

Females	Date started
216 / 247	4/1/68
189 / 225	4/1/68
204 / 217	4/1/68
184 / 269	4/1/68

EXPERIMENT 3.

Pairs given no nest material and empty nest box.

Pair	Male	Female	Date started
a	124	182	2/2/65
b	130	137	2/2/65
c	158	144	2/2/65
d	159	167	3/3/65
e	156	138	3/3/65
f	214	197	22/9/66
g	172	221	22/9/66
h	212	184	22/9/66
i	224	186	22/9/66

EXPERIMENT 5.

Pairs given nest material and empty nest box. Material carried to the box removed three times daily.

Pair	Male	Female	Date started
a	48	162	2/2/65
b	120	160	2/2/65
c	181	161	2/2/65

EXPERIMENT 6.

GROUP A. Pairs separated by bars: both birds given nest material and female given male's nest after it was completed.

Pair	Male	Female	Date started
a	121	176	13/4/65
b	48	163	20/5/65
c	191	198	19/4/67
d	242	263	8/2/68

GROUP B. Pairs separated by bars: both birds given nest material but female not given male's nest.

Pair	Male	Female	Date started
a	222	184	27/4/67
b	212	251	19/4/67
c	172	206	19/4/67

GROUP C. Pairs separated by bars: only the male given nest material.

Pair	Male	Female	Date started
a	81	133	23/4/65
b	191	207	16/10/67
c	230	245	16/10/67
d	241	271	8/2/68

EXPERIMENT 7.

Pairs given nest material and nest box containing nest and six eggs from day 0.

Pair	Male	Female	Day started
a	132	63	21/1/64
b	81	102	21/1/64
c	130	47	27/1/64
d	48	129	27/1/64
e	110	39	7/2/64
f	75	91	7/2/64
g	48	125	21/1/66
h	156	150	11/2/66

EXPERIMENT 8.

Pairs given nest material and nest box containing nest from day 0 and given six eggs on day 4.

Pair	Male	Female	Day started
a	120	87	18/2/64
b	134	138	18/2/64
c	94	137	4/3/64
d	55	122	4/3/64
e	83	131	5/3/64
f	147	144	17/3/64

EXPERIMENT 9.

Twelve males isolated with nest box and nest material but no nest. All were isolated on 15/9/67.

Male	
252 228	Neither had built by 29/9. Paired with each other. No nest by 13/10 when given nest pan and six eggs. Neither seen in box on 14/10.
181 212	Neither had built by 29/9. Paired with each other. Full nest by 3/10. Given six eggs on 13/10. Both seen incubating on 14/10.
178 173	Neither had built by 29/9. Paired with each other. Full nest by 3/10. Given six eggs on 13/10. Both seen incubating on 14/10.
222 158 241	None had built by 29/9: each then given nest pan and six eggs. 222 and 158 not seen in box subsequently; 241 seen in once on 13/10. No incubation recorded.
234 233 238	All had full nest by 29/9: each then given six eggs. 238 and 233 covered these over daily by building and were not seen to incubate. 234 adopted eggs on 30/9 and incubated daily thereafter.

All birds were replaced in the stock cage on 15/10.

EXPERIMENT 9B.

Monosexual male pairs given nest material.

Pair	Date of pairing	Day nest complete
181 / 64	16/6/65	14
228 / 178	22/9/66	14
222 / 211	22/9/66	16
124 / 164	10/11/65	14
120 / 142	10/11/65	21
94 / 147	12/5/65	1 (Both birds isolated for 15 days before pairing)
158 / 231	28/11/66	3 (Both birds isolated for 67 days before pairing)

EXPERIMENT 10.

Pairs given no nest material but nest box containing shallow nest pan and six eggs from day 0.

Pair	Male	Female	Date started
a	168	143	6/12/65
b	170	144	6/12/65
c	94	138	6/12/65
d	178	137	6/12/65
e	173	186	6/1/66
f	172	184	6/1/66
g	181	189	6/1/66

EXPERIMENT 11.

GROUP A. Pairs given no nest material but for pieces of string attached to the bars of the cage too short to be carried into the nest box.

Pair	Male	Female	Date started
a	170	137	13/4/66
b	178	161	13/4/66
c	181	206	13/4/66
d	214	182	13/4/66
e	168	198	13/4/66
f	142	205	13/4/66
g	81	207	13/4/66

GROUP B. Pairs given no nest material but for pieces of string attached to the bars of the cage and long enough to be carried into the nest box.

Pair	Male	Female	Date started
a	191	160	10/5/66
b	135	184	10/5/66
c	172	176	10/5/66
d	156	189	10/5/66
e	211	155	19/5/66
f	196	193	19/5/66
g	164	143	8/6/66

HORMONE EXPERIMENTS

TESTOSTERONE

Male	Treatment	Date started
158	Hormone	11/11/65
159	Hormone	11/11/65
147	Control	11/11/65
156	Control	11/11/65

STILBOESTROL

Males used were:

212; 230; 252; 228; 224; 191; 244

The experiment started on 12/2/68 and all birds were injected with hormone.

PROGESTERONE

Experiment 1.

Male	Treatment	Date started
179	Hormone	21/7/65
104	Hormone	21/7/65
175	Control	21/7/65
142	Control	21/7/65

Experiment 2.

Male	Treatment	Date started
208	Hormone	9/1/67
135	Hormone	9/1/67
170	Control	9/1/67
230	Control	9/1/67

PROLACTIN

Experiment 1.

Hormone injected birds:

Males: 255; 223; 230

Females: 245; 182; 248

The experiment was started on 3/7/67.

Control birds:

Males: 242; 254; 213

Females: 207; 225; 247

The controls were started on 28/7/67.

Experiment 2.

Hormone injected birds:

Males: 220; 254; 211

Females: 189; 204; 216

The experiment was started on 16/10/67.

Control birds:

Males: 224; 253; 231

Females: 256; 186; 194

The experiment was started on 13/11/67.

APPENDIX II

Dates of birth or purchase of birds used in experiments.

MALES

Code number	Date hatched or bought
48	Hatched 2/60
55	Hatched 6/60
64	Hatched 11/60
75	Hatched 3/61
81	Hatched 4/61
83	Hatched 6/61
94	Bought 1961
104	Hatched 11/61
110	Hatched 2/62
120	Hatched 4/62
121	Hatched 4/62
124	Bought 1/63
130	Bought 1/63
132	Bought 1/63
134	Hatched 3/63
135	Hatched 3/63
142	Hatched 3/63
147	Hatched 3/63
156	Hatched 7/63
158	Hatched 8/63
159	Hatched 8/63
164	Hatched 9/63
168	Hatched 10/63
170	Hatched 1/64
172	Hatched 1/64
173	Hatched 1/64
175	Hatched 1/64
178	Hatched 2/64
179	Hatched 3/64
181	Hatched 3/64
191	Hatched 9/64
196	Hatched 9/64
208	Hatched 2/65
211	Hatched 2/65
212	Hatched 2/65
213	Hatched 4/65
214	Hatched 4/65
220	Hatched 5/65
222	Hatched 7/65
223	Hatched 7/65
224	Hatched 7/65
228	Hatched 8/65
230	Hatched 8/65
231	Hatched 8/65
233	Bought 3/66
234	Bought 3/66
238	Bought 3/66
241	Bought 3/66
242	Bought 3/66
252	Hatched 3/66
253	Hatched 3/66
254	Hatched 5/66
255	Hatched 5/66

FEMALES

Code number	Date hatched or bought
39	Hatched 1/60
47	Hatched 2/60
63	Hatched 11/60
87	Hatched 6/61
91	Hatched 8/61
93	Hatched 8/61
102	Bought 1961
122	Bought 1/63
125	Bought 1/63
129	Bought 1/63
131	Bought 1/63
133	Hatched 3/63
137	Hatched 3/63
138	Hatched 3/63
143	Hatched 3/63
144	Hatched 3/63
145	Hatched 3/63
150	Hatched 5/63
153	Hatched 5/63
155	Hatched 7/63
160	Hatched 8/63
161	Hatched 8/63
162	Hatched 8/63
163	Hatched 8/63
166	Hatched 9/63
167	Hatched 9/63
176	Hatched 2/64
182	Hatched 3/64
184	Hatched 4/64
186	Hatched 6/64
189	Hatched 7/64
193	Hatched 9/64
194	Hatched 9/64
195	Hatched 9/64
197	Hatched 10/64
198	Hatched 10/64
204	Bought 2/65
205	Hatched 1/65
206	Hatched 2/65
207	Hatched 2/65
216	Hatched 5/65
217	Hatched 5/65
218	Hatched 5/65
221	Hatched 6/65
225	Hatched 7/65
226	Hatched 7/65
229	Hatched 8/65
245	Bought 3/66
247	Bought 3/66
248	Bought 3/66
249	Bought 3/66
251	Bought 3/66
256	Hatched 5/66
263	Hatched 10/66
269	Hatched 3/67
271	Hatched 4/67
275	Hatched 7/67

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EXTERNAL STIMULI AND READINESS TO INCUBATE IN THE BENGALESE FINCH

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It has long been known that the timing of bird breeding seasons is affected by outside factors such as light, but only recently has systematic work been started to determine the interactions of hormones and external stimuli giving rise to the finer changes in behaviour which occur during the course of a single reproductive cycle. Lehrman (1961) provides the most recent general review of the field, while Hinde & Steel (1966) have summarized the results which they and their co-workers have obtained with the canary. Few species have so far been studied in any detail; the diversity of reproductive behaviour amongst birds makes it important that a wide variety should be examined.

The experiments to be described here were carried out using a laboratory colony of Bengalese finches. In this species there is a delay of some days after pairing before egg-laying and incubation begin, and it is apparent that external stimuli prevalent at this time are instrumental in bringing these about. As eggs are not normally laid until the nest is complete and incubation does not begin until eggs are present, it is clear that both eggs and nest material are important influences here. The aim of the present study was to determine how the development of readiness to incubate is affected by these two factors and the extent to which it is independent of the egg-laying of the pair itself.

Method

Subjects

Present stocks of the Bengalese finch were considered by Eisner (1957) to be solely derived from some sharp-tailed finches (*Lonchura striata*) taken into captivity from south-east China about 250 years ago. The main advantages of this species for laboratory study are the ease with which it may be kept in captivity and the fact that it breeds throughout the year even when day length fluctuates.

The birds used in these experiments were mostly reared in this laboratory but some were obtained from dealers. All of them had had previous experience of at least one reproductive cycle.

Maintenance and Procedure

The experimental and stock cages were kept in a room the temperature of which varied between 19 and 24°C. Natural daylight entered through a window and further lighting was provided by a strip controlled by a time switch. During the summer (1 May to 15 August) this was set to give 15½ hr light a day, and in winter (15 November to 1 February) it was maintained at 10 hr. Between these periods it was increased and decreased with the natural daylength. This is the same regime as that used by Eisner (1960) but with a rather longer day in mid-winter.

Birds were taken direct from the stock cages, in which males and females are kept separately, and placed in pairs in breeding cages 60 cm wide × 45 cm high × 30 cm deep. These cages were visually isolated from each other and from the stock cages though all were in the same room. Each cage was constantly supplied with seed, water, sand and cuttlefish bone as well as more specialized items of diet as described by Eisner (1960). A nest box of dimensions 10 cm^3 was placed in one of the upper corners at the rear of each cage. This was open at the front but for a lip of 4 cm in height with a perch mounted on it. Each box was backed with a glass plate and, the area behind the cages being blacked out, observations could be made through this at close quarters without disturbing the birds. At first (experiments 1 and 2 below) the birds were watched from in front of the cages at a distance of about 6 ft with the nest box lip lowered to 3 cm, but in all later experiments the observations were made from behind.

Criteria for Incubation

The experiments to be described were designed to elucidate some of the factors affecting the onset of incubation. The technique employed was to make daily observations for 1 hr on each pair from the day of pairing and record changes in behaviour associated with the onset of incubation. First, several pairs were watched under normal breeding conditions to decide upon criteria which might be used to assess the timing f the development of incubation. The following wo features of incubation behaviour were picked s being the most characteristic and easily bserved.

(1) Settling movements. The side-to-side huffling movements seen as a bird settles down n eggs were used to indicate the *start* of inubation. A bird was said to have started ıcubating when these were observed one or ıore times during a given watch.

In pairs breeding normally these movements ave on several occasions been observed as arly as the first egg, but never before this, and is unlikely that they occur when there are o eggs in the nest. This does not necessarily ıdicate that the occurrence of egg-laying is ssential to incubation. A distinction must be rawn between readiness to incubate and the tart of incuation itself. The former may develop ometime in advance of egg-laying but the resence of eggs may be essential to reveal it. or this reason, in testing for readiness to inubate, the experimental pairs were given eggs ı their nest boxes before they laid themselves.

(2) The pattern of attentiveness. Towards the nd of the egg-laying period a rhythm is set up hereby the birds alternate on the nest so that ıe eggs are never left uncovered. This *full stablishment* was said to have been reached when he box was never left unoccupied during a atch. Data on the timing of full establishment ave been obtained by Eisner (unpublished) sing an automatic recording device to give a aily 5-hr record of visits to the nest box. She ound that it occurred most commonly on the ay of the last egg but tended to be later with maller clutches and earlier with larger ones. In he 1-hr watches used here, the criterion might e expected to be reached earlier but observaions on six pairs under normal conditions howed no tendency in this direction. It is herefore probable that full establishment develops rather rapidly in the last few days of eggıying.

It is useful to apply this criterion also to ıdividual birds in order to detect differences etween the sexes in the development of inubation. In theory, the full establishment of ne bird is the stage at which it ceases to leave he box before its mate has returned, but in ractice this can only give a rough indication s the behaviour of one member of a pair can, f course, be affected by that of the other.

Experimental Procedures

Five different experiments were performed:

Experiment 1. Pairs given a nest box containing a crude hand-built nest and six artificial eggs from the day of pairing (day 0), as well as a constant supply of hay for nest building scattered on the floor of the cage (eight pairs).

Experiment 2. Same as experiment 1 but with eggs not placed in the nest until day 4, and thereafter deprived of nest material other than that already in the box (six pairs).

Experiment 3. Pairs with nest box containing shallow nest pan and six eggs from day 0 but given neither nest nor nest material (seven pairs).

Experiment 4. Same as 3 but given three pieces of thin string, each about 25 cm in length, attached at one end to the bars of the cage. The length of the string was such that it could be carried to the perch outside the nest box but not into the box (seven pairs).

Experiment 5. Same as 3 but given two pieces of thin string, each about 35 cm in length, attached at one end to the bars of the cage. Here the string was long enough to be carried into the nest box (seven pairs).

In all cases a daily check was made to see whether any eggs had been laid, and, where nest material was provided, the nest box was inspected ½ hr before each observation period and the eggs uncovered if they had been obscured by building between watches.

Results

Experiment 1. The pairs in this group were tested with a view to deciding whether readiness to incubate can be demonstrated before egg laying. The results (Table I) show that six out of eight females and seven out of eight males did start to incubate in advance of their own first egg. In two cases incubation became established and proceeded normally without the female herself laying. In the other four pairs of which the females began incubating before laying, there was some evidence that the clutches subsequently laid were smaller than normal. In Table II these pairs are combined with those of experiment 2 which behaved likewise and comparison is made with previous clutches laid by the same females. This shows a reduction in size to have taken place ($P < 0{\cdot}005$ on a Wilcoxon matched-pairs test). In some cases, therefore,

Table I. Delay Before Egg-laying and Incubation in Pairs Given Nest Box, Nest Material and Six Eggs from Day 0 (Experiment 1)

Pair	Day of first egg	Day of start of incubation ♂	Day of start of incubation ♀	Day of full establishment ♂	Day of full establishment ♀	Day of last egg
a	7	10	10	12	12	13
b	11	2	9	7	9	13
c	15	4	13	7	36+	36+
d	11	3	9	10	11	14
e	none*	4	6	5	6	none*
f	none*	10	15	17	17	none*
g	16	9	17	29	30	27
h	9	3	8	7	10	13
Median	11	4	9·5	8·5	11·5	13·5

*These results are excluded in calculating the medians at the foot of the columns.

Table II. Comparison of Clutch Size with Mean Previous Clutch Size for Females which Started Incubating Early but Laid also (Experiments 1 and 2)

Experimental pair	Size of clutch laid	Previous clutches	Mean previous clutch size
1b	3	6, 4, 4	4·3
1c	4	7, 7, 8, 8, 7, 7, 7, 6	7·1
1d	4	5, 7, 5	5·7
1h	5	6, 9, 7, 6, 10, 3, 9, 6, 4	6·7
2a	4	3, 6, 6, 6, 5	5·2
2c	5	6	6·0
2e	3	7	7·0
2f	4	5	5·0

the presence of eggs in the nest before laying leads to reduction or suppression of the clutch.

Clearly, from these results, neither ovulation nor egg-laying is an essential prerequisite for incubation behaviour, though the start of incubation was found to take place earlier in males than in females ($P<0·01$).

Experiment 2. There are two possible reasons for the start of incubation being advanced by the presence of eggs in the nest: either the birds are in the physiological state appropriate to incubation before the eggs are laid, or this state is brought about by stimuli from the eggs themselves.

In experiment 2 the pairs were not given eggs until day 4—the stage by which over half the males in experiment 1 had begun incubating. Two males and two females, representing three out of the six pairs tested, were seen settling on the day the eggs were placed in the nest. A further male started on day 5 and two females on days 6 and 7 respectively. These last two birds began their own clutches on the same day as they started to incubate, but none of the birds which started to incubate before day 6 had eggs their own in the nest when they did so. The privation of nest material after day 4, in an ort to avoid the eggs being covered over by ter building was probably responsible for the ct that two females did not start their clutches til days 28 and 32 respectively.

The immediate response of four birds to the acing of eggs in their nests indicates that adiness to incubate develops prior to laying d that the stimuli evoking this condition must me from the mate and nest material rather an from the eggs themselves.

Experiment 3. These pairs were deprived of st material in order to determine how this fected the development of readiness to in- bate. Yet, although no hay was provided for ilding, complete deprivation was found to be possible in practice, as the birds carried seed, ttuce and faeces to the nest box. In the box ey were often seen to go through the movements of building using this material, using eir own or their mates' feathers or no material all. Nevertheless, no nest can be achieved this way, and depriving the birds of hay rtainly cuts down nest-building experience en if it does not eliminate it.

The results are summarized in Table III. hile one pair incubated without laying, there evidence of increased latency to the first egg the other six pairs, in it four of which came ter an interval greater than the 21-day maxi- um found by Eisner (1960). In experiments be reported in full elsewhere, I have also found an increased delay when artificial eggs are not present; the median latency of nine pairs tested was 19 days. In two of these pairs no eggs had been laid by day 52 and day 60. They were then given nest material and both females laid within 10 days.

Despite this delay in egg-laying, no difference could be found in the timing of the start of incubation between the females here and those in experiment 1. But the mean period of development of incubation (gap between start and full establishment) for females is 5·5+ days in experiment 1 and 15·3 days in experiment 3. This difference is significant ($P<0{\cdot}02$ on a Mann-Whitney U test), even on the assumption that the gap in pair Ic, in which the female did not become fully established before the end of the experiment, is greater than all those in experiment 3. Thus, although lack of nest material does not delay the start of incubation in females it leads to protraction of the gap before full establishment. This result is probably connected with the increased delay in egg-laying as no female in either experiment 1 or 3 became fully established more than 4 days in advance of her own last egg, although some birds in each experiment incubated without laying.

By contrast, the start of incubation in males was considerably later here than in experiment 1 ($P<0{\cdot}005$). But the mean period of development of incubation is not significantly greater (6·1 days in 1, 6·7 days in 3), so that in fact the males reached this criterion before the females here as in experiment 1.

Table III. Delay Before Egg-laying and Incubation in Pairs Given Nest Box and Six Eggs from Day 0 but Deprived of Nest Material (Experiment 3)

Pair	Day of first egg	Day of start of incubation ♂	Day of start of incubation ♀	Day of full establishment ♂	Day of full establishment ♀	Day of last egg
a	12	16	9	24	27	26
b	23	7	5	9	23	23
c	34	35	31	37	43	45
d	31	22	16	28	32	35
e	none*	10	11	13	15	none*
f	11	13	9	14	21	22
g	27	7	5	32	32	31
Median	25	13	9	24	27	28·5

*These results are excluded in calculating the medians at the foot of the columns.

Deprivation of nest material thus results in delays in ovulation, in the start of incubation of males and in the full establishment of females. Full establishment of males occurs later with respect to the day of pairing but not in relation to the start of incubation. In females the presence or absence of material appears to be irrelevant to the timing of the start of incubation.

Experiments 4 and 5. These experiments were carried out during the summer months under a rather longer day than the previous ones so that the seasonal changes found by Eisner (1960) must be borne in mind when comparing them. In experiment 4 the birds were only allowed to manipulate nest material outside the nest box, while in 5 they were allowed to take it into the box as well, although not to build a nest.

Egg-laying was delayed in both groups, as was full establishment of females; but the data on full establishment cannot be compared as the experiments were terminated in a number of cases before it was achieved. In experiment 4 the full establishment of males was also retarded to a mean of at least 13 days later than the day of the start of incubation (in three cases the pairs were separated before the males were fully established: 4a, day 34; 4c, day 34; 4d, day 41). This increased lag is probably accounted for by the unnatural situation, as the males continued trying to carry the string into the nest box right up to the end of the experiment. Under normal conditions incubation would have been fully established by this stage and nest building would have ceased almost entirely.

The results making the most interesting comparison are those for the start of incubation (Table IV). In neither experiment is the difference between the sexes significant but there is a marked difference when the males are compared between experiments. All the males in experiment 5 started on day 7 or before while all those in experiment 4 started on day 8 or after giving a highly significant difference ($P<0\cdot001$). Thus actual experience in carrying material into the nest box is probably the important factor in accelerating readiness to incubate in males. Increased experience within the nest box could also be of importance, but this is unlikely as the birds were not often seen manipulating the string in the box and it was seldom in the box for long as the males would pull it out from the outside, as they normally do with material straggling over the lip of the box.

As with experiments 1 and 3, the time of the start of incubation of females does not differ significantly between these two experiments. Nor do the results of all four experiments differ from each other (on an analysis of variance after logarithmic transformation), even without making allowances for the differences in photoperiod. This confirms the conclusion that nest material is irrelevant to the timing of the start of incubation in females.

Table IV. Delay Before Egg-laying and Incubation in Pairs Given Nest Box and Six Eggs from Day 0. Comparison Between Pairs Deprived of Nest Material Other than String too Short to Carry into the Nest Box (Experiment 4) and Those with String Long Enough to Carry into the Next Box (Experiment 5)

Pair	Experiment 4			Experiment 5		
	Day of first egg	Day of start of incubation ♂	♀	Day of first egg	Day of start of incubation ♂	♀
a	34+	9	9	18	4	4
b	11	8	6	10	4	12
c	11	9	7	16	7	5
d	36	11	6	23	2	7
e	20	26	6	17	3	11
f	14	17	13	35+	7	14
g	33	12	9	13	6	7
Median	20	11	7	17	4	7

Discussion

The discovery that incubation can be brought forward by placing eggs in the nest before laying, and that this tends to reduce or suppress the subsequent clutch, agrees with the findings of workers on other species. Paludan (1951) and Weidmann (1956) found this to occur with gulls, and Poulsen (1953) reported that domestic pigeons given eggs before laying incubated them and either laid a full clutch of two eggs or no eggs at all. Some female canaries brought into reproductive condition during the winter have been found to incubate on their empty nests without laying (Steel & Hinde, 1966). Such behaviour has not been observed in the Bengalese finch but whether one considers incubation to take place without eggs or not is probably largely a matter of the choice of criteria.

Males have also been found to be prepared to incubate earlier than females here, suggesting that the physiological condition of the male is more labile than that of the female early in the reproductive cycle. The start of incubation in males, however, was delayed considerably in the absence of nest material while that in females was unaltered. These results are basically similar to those of Lehrman *et al.* (1961) on the ring dove. With doves given a nest bowl and nest material continuously from the day of pairing, all birds of both sexes incubated when tested with eggs on day 7, though if tested for the first time on days 3, 4 or 5, a higher proportion of males than females sat. But in the absence of a nest bowl and nest material except during testing, 70 to 80 per cent of the birds of both sexes were still ready to sit on day 7, while a higher proportion of females than males sat in those pairs tested for the first time on days 3 to 6. The criterion employed by Lehrman *et al.* is more equivalent to that used here for the start of incubation than to full establishment.

In the Bengalese finch the male does nearly all the carrying of the material to the nest box. Males allowed to manipulate string but not to carry it into the box showed a delay in the start of incubation similar to that of those given no nest material. But in cases where the string was longer and could be carried into the box, the males started to incubate at the normal time. This suggests that the effect of nest material on males is primarily through experience in carrying it to the nest box. Manipulation of material outside the box, and building within it, appear relatively unimportant.

In normal breeding pairs not provided with artificial eggs, the start of incubation may be as early as the first egg and full establishment takes place on around the day of the last egg. Where artificial eggs were provided, the period of development of incubation of males varied greatly but did not appear to be affected by nest material. In females, on the other hand, lack of nest material protracted it considerably. This is probably connected with a similar delay in egg-laying also found under these conditions; although the start of incubation in females can take place well in advance of egg-laying, full establishment was not found more than 4 days before it.

Hinde & Warren (1959) found canaries to lay later when deprived of nest material and even more so in the absence of a nest pan. A slight delay in ovulation was found in the ring dove when nest bowl and nest material were not provided (Lehrman *et al.*, 1961). In the present experiments a nest box was always supplied, but if hay for nest building was not available as well egg-laying was retarded.

Summary

(1) Pairs of Bengalese finches were tested for incubation by placing artificial eggs in their nest boxes before laying. The effects of varying degrees of deprivation of nest material were noted.

(2) Readiness to incubate is present in both sexes before egg-laying, but earlier in males than in females. With females induced to incubate before laying the subsequent clutch is reduced in size or suppressed.

(3) Absence of nest material leads to a delay in egg-laying and in the start of incubation by males. In females the start of incubation is not affected by nest material but its further development is protracted where no material is present.

(4) Experiments in which pieces of string tied to the bars of the cage were the only material provided, suggested that the effect of nest material on males is through experience gained when carrying material into the nest box.

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