Variation in pre-laying behaviour of hens: implications for control and motivation

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Declaration

I confirm that all the work presented in this thesis is of my own composition. All advice and help given has been acknowledged.

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Abstract

Considerable variation in pre-laying behaviour of domestic hens (*Gallus gallus domesticus*) is observed within individuals, between individuals and between different husbandry systems. The contributions of internal and external factors to this variation were considered. Part of this variation is in relative amounts of searching and nesting, so criteria were defined to separate pre-laying behaviour into a searching and sitting phase.

The main internal factors considered related to lag and position of eggs in sequences. The duration of the sitting phase and the total time spent in the nest box was related to lag. The duration of pre-laying behaviour was longer for the first egg of a sequence than for the other eggs. This was mainly due to a longer searching phase in which hens performed more nest examinations. The duration of the sitting phase was longer for the last egg of a sequence than for other eggs; this may have been related to a longer lag found for the last egg.

Effects of competing behavioural tendencies on pre-laying behaviour involve both internal and external factors. A series of experiments was conducted to investigate the performance of pre-laying behaviour while altering the availability of food and motivation to feed (length of food deprivation). Hens always interrupted their pre-laying behaviour in order to feed when food was presented. Length of deprivation did not influence the duration of feeding or pre-laying behaviour, that is, even "satiated" hens stopped their pre-laying behaviour and fed. However, the delay in oviposition was found to be greater when food was presented in the later, rather than the earlier, stages of pre-laying behaviour. The duration of pre-laying behaviour and of the searching phase was longer if hens were food deprived than if food was available. These findings suggests that the expression of pre-laying behaviour is determined by the tendency to perform pre-laying behaviour competing with other behavioural tendencies.

External factors examined included the effects of conditions that facilitate searching and nesting behaviour on pre-laying behaviour. Hens provided with an unlittered nest box showed an extended pre-laying behaviour and searching phase, more searching behaviour and nest examinations and more nest entries of a shorter duration than when provided with a littered nest box. These results suggested that in the absence of a suitable nest site, hens delayed, and showed an incomplete transition from searching to nesting behaviour. Providing an exploratory walkway to facilitate searching behaviour resulted in the searching phase starting earlier than expected, and in the occurence of more searching behaviour and nest examinations during this time. Environments that facilitate searching behaviour may provide external cues that allow the motivation to perform pre-laying behaviour to be expressed earlier than in barren environments.

The strength of hens' motivation to reach a nest box was assessed with the aid of aversive stimuli. Hens were required to pass through an empty corridor, or past a dominant, subordinate or unfamiliar hen to reach a nest box. Hens delayed their approach to the nest box and made more attempts to find alternative routes to the nest box when required to pass a dominant or unfamiliar hen. Thus social factors were found to influence access to the nest site and pre-laying behaviour. Hens appeared to be only weakly motivated to reach the nest site during the searching phase; motivation to reach the nest site increased near the start of the sitting phase.

The effects of social interactions on access to a nest site and on the pre-laying behaviour of hens in small groups was investigated. Evidence of competition for the nest site was found when more than one hen was showing pre-laying behaviour. Subordinate hens walked more in the last hour before oviposition and sat less in the last 25 minutes when other hens were also showing pre-laying behaviour than when no other hens were in the pre-laying phase. Dominant hens in the pre-laying phase remained nearer the nest when other hens were showing pre-laying behaviour than when none were doing so. Thus social interactions during the pre-laying phase result in variation in pre-laying behaviour in both directions; subordinate hens do not settle into the expected nesting phase whereas dominant hens stay nearer the nest.

A motivational theory of pre-laying behaviour is proposed in which pre-laying behaviour is controlled by an interaction between the tendencies to perform searching and nesting behaviour. The tendency to start searching behaviour is influenced by internal factors, competition between motivational systems and external cues for exploration. The tendency to start nesting behaviour is influenced by the availability of a suitable nest site and social factors. It is suggested that a certain amount of behavioural priming is normally required before oviposition can occur. The implications of this model for the welfare of laying hens is discussed.

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CHAPTER 1: **General introduction**

1.1 Abstract

1) Motivational theory is introduced as a "black box" approach used to study the mechanisms that control behaviour. Two models of motivation are described briefly to show how models help in our understanding of these mechanisms. Motivational theory also provides an important framework for the study of animal welfare.

2) Domestic hens (*Gallus gallus domesticus*) perform an elaborate sequence of activities before oviposition termed pre-laying behaviour. In extensive environments pre-laying behaviour has two distinct phases: a searching and sitting (nesting) phase. However, pre-laying behaviour varies considerably between environments.

3) In the absence of a nest site, hens show activities indicating frustration and are prepared to work to gain access to a nest site. This suggests that welfare is compromised if hens are denied access to a nest site.

4) A brief description of genotypic variation and physiological control of pre-laying behaviour is given. However, this offers a poor explanation of the variation in pre-laying behaviour in individual hens between different environments. The application of motivational theory might help in understanding the mechanisms that control pre-laying behaviour.

5) It is suggested that changes within pre-laying behaviour are best accounted for by considering that hens have a demand for searching and nesting behaviour. Evidence that hens have a demand to perform these two activities is discussed.

6) An outline of the thesis is given. The effects of internal factors, competing behavioural tendencies, environmental complexity and social factors on pre-laying behaviour will be examined. Findings will be discussed with respect to a motivational model of pre-laying behaviour.

1.2 Control of behaviour

The approach employed in the study of animal behaviour depends on the area of interest. Tinbergen (1963) suggested that there are four different approaches to the study of behaviour; causation, survival value, ontogeny and evolution. This thesis is primarily concerned with interpreting the control of behaviour (i.e. how behaviour arises) in other words with the area of causation. Two very different yet complementary approaches have been used to try to understand how behaviour arises and how it is organised. One approach, that of neuropsychologists, involves examining how the nervous system organises and gives rise to behaviour (reductionist "circuit-breaking" approach). This technique has proved useful for identifying the control of simple actions, such as jumping in locusts (Hoyle, 1978). The other approach is to treat the animal as a "black box" and try to deduce the mechanism by which behaviour arises is from the way that they behave. This can give rise to detailed predictions about behaviour, which can ultimately be investigated physiologically. This proved to be the case in determining the organisation of behaviour in the marine gastropod (*Pleurobranchia californica*, Davis, 1976). This gastropod has a small behavioural repertoire and observations revealed that certain activities overide others. Further studies demonstrated the physiological basis for the relationship between activities.

For animals with complex nervous systems, the use of a circuit-breaking technique to reveal the control of all behaviour is unlikely to be achieved. Nonetheless this technique can be used to examine restricted aspects of higher function. One example is the work by Hubel and Wiesel (1974) which located orientation columns in the visual cortex of monkeys and cats. This discovery proved essential for the understanding of how a two dimensional image on the retina can be interpreted so as to allow the orientation of behaviour. It is not only the complexity of the mechanisms involved that have led scientists to employ a black-box approach, but also because a full understanding of behaviour can only arise when we examine the animal as a whole. For example, although a circuit-breaking approach may lead to an understanding of the internal and external cues responsible for a bird to feign an injury to lure a potential predator from the nest site, only by employing a whole animal approach can we discover what the wider implications of this behaviour are. A field experiment conducted on plovers (*Charadrius wilsonia*) and piping (*Charadeius melodus*) using human intruders found that the feigning response shown by the birds was highly dependent on the behaviour and positioning of the intruder (Ristau, 1991). Ristau (1991) found that the bird's display was made in a direction that would make the intruder move away from the nest, the birds also monitored the intruders behaviour and changed their behaviour accordingly (such as displaying nearer to the intruder if the intruder was not distracted). These subtleties in behaviour could only have been identified by employing a whole animal approach.

1.3 Motivational theory

One problem of using a black box approach to study the mechanisms that control behaviour is defining the terminology used. In the study of motivation, intervening variables such as hunger or thirst are used to speculate on the mechanisms of the black box. For example, one answer as to why an animal may not eat when food is presented is that it is not hungry. This term is nothing more than a hypothetical construct which serves to identify some phenomenon that is as yet unidentified. The way in which such phenomena are invoked to explain the mechanisms that control behaviour is at the heart of motivational theory. Motivation can be defined as the "causal state that is generated by all the stimuli [internal and external] which impinge upon an animal" (Toates, 1986). Motivation therefore attempts to explain how behaviour patterns arise, how they are maintained and eventually replaced by other patterns. From the animal's point of view, doing the right thing at the right time is essential for survival (Manning and Dawkins, 1992).

So how does motivational theory help in explaining the occurrence of behaviour? Let us assume that at a particular point in time an animal is performing activity A. The motivation to perform this particular activity is determined by a variety of factors. Internal factors, such as daily cycles or physiological imbalance, and external factors, such as the presence of the consummatory substrates may influence the tendency (motivation) to perform this behaviour. Also, different motivational systems will interact and have either a positive (priming) or negative (competitive) effect on the tendency to perform activity A. These interactions can occur at a variety of levels (McCleery, 1983). At the periphery of the nervous system the performance of one behaviour may affect the causal state for the other, such as feeding creating a water deficit. Interactions may also occur within the nervous system, serving to anticipate a future state such that it is avoided (for example a thirsty animal may avoid eating as this would make the water deficit worse). It has been suggested that tendencies to perform various activities come together at a common effector mechanism that decides which activity is to be expressed: the behavioural final common path (McFarland and Sibly, 1975).

The relationship between internal and external factors and their effect on behavioural output have usually been represented in the form of models. These models serve to predict the operating characteristics of the system being investigated. They can therefore help in understanding how behaviour is controlled, helping to bridge the gap between behaviour and neurophysiology (Toates, 1986). There have been two main types of models of how motivational systems work (Toates, 1986).

Firstly, there are the models in which the build up of some sort of energy acts as a force which drives behaviour. For example, in Lorenz's psychohydraulic model (Lorenz, 1950), fluid (representing action specific energy) accumulates over time. As the level of the fluid builds up, pressure opens a valve (which represents reaching the threshold for that activity) and the fluid is discharged (behaviour). Weights (representing releasing stimuli) act on the valve such that it alters how much force is needed to open the valve. At first sight, this model satisfactorily explains the occurrence of activities which show cyclical fluctuations. For example, feeding behaviour is reasonably well explained by this model. However, the model fails when examining feeding behaviour in more detail. For example, this model predicts that once behaviour is initiated it will continue until all the action specific energy has been released. However, behaviour can cease prematurely; such as dogs not eating as much as expected (thereby expressing less feeding behaviour) after food has been placed directly in their stomach (Janowitz and Grossman, 1949).

Another major criticism of this model is that no provision is made to permit behaviour itself to regulate motivation (i.e. in this model changes in the environment arising from earlier actions do not feedback and regulate behaviour). This concern is of primary importance in the second type of model, the homeostatic model (McFarland, 1971; Fitzimmons, 1972; McFarland and Sibly, 1975). Simple homeostatic models assume that animals can monitor the difference between ideal and actual values for some condition (e.g. water deficit). Behaviour is triggered when this difference is large and serves to restore the actual value to the ideal, predetermined value (i.e. to achieve homeostasis). This type of model is a good predictor of the behaviour of a dog that has had food directly placed in its stomach described in the previous paragraph. In practice, the mechanism by which a homeostatic model could control behaviour is much more complicated than is at first apparent. There is the problem of the time-lag that exists between the performance of behaviour and the restoration of homeostasis. For example, drinking behaviour stops long before the water deficit in the body is corrected. Also, there is considerable evidence that animals perform behaviour despite the presence of consummatory stimuli (e.g. Hughes *et al* (1989) showed that domestic hens will perform nest building behaviour despite the presence of a preformed nest).

1.4 Animal welfare and motivational theory

Duncan (1987) highlights the problem in defining animal welfare by stating that it is probably impossible to give it a precise scientific definition and favours a broad working definition. However, a broad working definition should be precise enough to ensure that measures mean the same thing to different researchers (Mason and Mendl, 1993). One problem has been that some researchers view the physical condition of the animal as important in describing its welfare. For example, Fraser and Broom (1990) defined the welfare of an individual as "its state as regards to its attempts to cope with its environment". This implies that welfare is a continuum and physical states (e.g. injury, low energy resource) may represent poor welfare even if the animal is unaware of its condition (e.g. anaesthetised). For others, an animal's welfare is only impaired if it is experiencing an unpleasant mental state (Dawkins, 1990; Duncan and Petherick, 1991). Part of the reason why researchers have rejected this strategy may be that, as stated by Duncan and Petherick (1991), the existence or otherwise of awareness is crucial in this debate. The study of awareness in non-human animals is difficult (Dennett, 1983) and has often been described as unscientific. This problem in definition illustrates the fact that animal welfare lies at the intersection of science and ethics. That is, although some researchers view the mental state of the animal as all important in defining welfare, ethical considerations may restrict researchers from treating poor welfare by making the animal unaware of its condition (e.g. by the use of drugs).

Lately, much of the impetus for the construction and testing of motivational models has come from a concern for the welfare of animals (e.g. Hughes and Duncan, 1988). The Brambell Report (HMSO, 1965) suggested that animals have the need to perform behaviour and prevention of these needs would result in reduced welfare. Dawkins (1983) has defined behavioural need as "a high causal factor to perform a particular behaviour", though this may be an over-simplified definition. Hughes and Duncan (1988) have argued that the question of behavioural need only arises when there is a strong internal causal factor to perform that behaviour pattern. Behaviour patterns that are governed purely by external causal factors (e.g. escape from predators) would not be elicited in a constraining environment hence the question of behavioural needs (and welfare concerns) would not arise. Thus, Hughes and Duncan (1988) concluded that "the concept of ethological need can be clarified only if it can be subsumed within the context of a satisfactory model of motivation".

There is a consensus that welfare is reduced if animals are strongly motivated to perform behaviour but are unable to do so (Dawkins, 1990; Duncan and Petherick, 1991). However, different models provide different predictions for when animals become strongly motivated to perform a particular activity, thus they have different implications for welfare. A psychohydraulic model implies that action specific energy would build up until, in a constraining environment, it is released in the absence of external stimuli (as vacuum activities). Pre-laying behaviour of hens in cages fits this model well; the frustration observed (Duncan, 1970) may represent the build up of energy and sometimes vacuum nest building behaviour is observed in the later stages of pre-laying behaviour (Wood-Gush, 1975a; Meijsser and Hughes, 1989). The homeostatic model, however, has very different implications for welfare. Baxter (1983) has extended the central theme of this model to its logical conclusion. That is, if the functional consequences of some behaviour patterns are to alter the environment, then providing an "ideal" environment would make these behaviour patterns redundant, such that behavioural needs, and hence welfare problems would not arise. However, evidence is mounting that animals do indeed have a need to perform certain behaviour patterns (Hughes *et al*, 1989; Young, 1993).

Thus models of motivation explain how and why animals become strongly motivated to perform particular behaviour patterns. An understanding of these issues is essential for predicting when behavioural needs and hence welfare problems arise. Models can also be used to predict what happens when behavioural needs are not met. For example, the

Hughes and Duncan (1988) model predicts that if an activity does not lead to the appropriate functional consequences, the animal will be in a closed loop and perform that behaviour pattern in a repetitive and stereotyped fashion. In this thesis motivational theory will be developed in relation to a specific behaviour pattern in a commercially important species, namely pre-laying behaviour in the domestic hen (*Gallus gallus domesticus*).

1.5 Pre-laying behaviour

Domestic hens perform an elaborate sequence of activities before oviposition collectively termed pre-laying behaviour. In extensive or semi-extensive husbandry systems with nest sites, this period lasts for about 1.5 hours (Wood-Gush, 1954; Kite *et al* 1980; Meijsser and Hughes, 1989). At the start of pre-laying behaviour the hen becomes restless and gives a particular "pre-laying call" (Wood-Gush and Gilbert, 1969). She examines potential nest sites with the neck held in a horizontal position and side to side movements of the head. These nest inspections become more frequent with time and lead to nest entries. Eventually, the hen will sit on a nest site and remain there without much movement. Occasionally she will perform nest building activities; rotating while pressing the keel down on the ground and making outward scraping movements with the feet to form a hollow scrape (Wood-Gush, 1975b). She will also gather litter and feathers and place it on the sides of the nest or on her back so that it falls on the nest. The hen will usually change her posture (to a laying squat) to lay the egg about 45 minutes after the start of nest entries. Modern laying hybrids will usually leave the nest straight after oviposition, sometimes while cackling (Wood-Gush, 1971).

Pre-laying behaviour of hens in conventional battery cages differs considerably from that described above. Hens in cages may show more locomotor activity including repetitive pacing (Wood-Gush, 1975a) and pre-laying behaviour can be extended (Wood-Gush and Gilbert, 1969). Hens also spend less time sitting and may perform nest building activities in the apparent absence of a suitable substrate (vacuum activities, Wood-Gush, 1975a; Meijsser and Hughes, 1989). Light hybrids also show a peak in aggression just prior to oviposition which falls immediately after laying (Hughes, 1979). This pre-oviposition surge in aggression has been interpreted as arising from frustration (frustration-aggression hypothesis, Duncan, 1970).

Differences in pre-laying behaviour have been described between hens in wire cages and alternative systems, such as perchery, modified cages and free range (Brantas, 1980; Hughes, 1980; Meijsser and Hughes, 1989; Reed, 1991), and between hens in similar environments (Meijsser and Hughes, 1989; Appleby, 1990; Sherwin and Nicol, 1993b). Consideration of these differences in pre-laying behaviour raises three issues (relating to welfare, production and control) that highlight the importance of a clear understanding of the control of pre-laying behaviour. Firstly, to what extent do these differences in pre-laying behaviour indicate reduced welfare? Secondly, what are the implications of variation between systems on production? Lastly, do differences in pre-laying behaviour represent adaptive responses to the cues experienced in different environments (Baxter, 1983; Dawkins, 1988)? These questions can only be answered when we have a clear understanding of the factors governing onset and changes within pre-laying behaviour.

1.5.1 Welfare

The Farm Animal Welfare Council (1986) concluded that the inability to perform normal pre-laying behaviour in cages was one of the most important problems for the welfare of laying hens. Two lines of research have been employed to assess welfare during the pre-laying period. One method is to compare the behaviour of birds experimentally subjected to stressful situations with the behaviour of hens in commercial conditions (Duncan, 1980). Hens thwarted in their attempts to feed show repetitive pacing which has been interpreted as indicating frustration (Duncan and

Wood-Gush, 1971). Pacing is sometimes also observed during the pre-laying period of hens in wire cages or in hens that have been denied access to a previously open nest box (Duncan, 1970; Duncan and Wood-Gush, 1972).

Another method of assessing welfare involves quantifying the demand for the nest site. This can be done by allowing the hens to work to gain access to the nest site; by walking long distances (Duncan and Kite, 1987) or opening doors (Smith *et al*, 1990) or squeezing through narrow gaps (Cooper and Appleby, 1994a). Overcoming such obstacles indicates a high demand to reach a nest site, thus welfare may be compromised if hens are denied the opportunity to nest there (Dawkins, 1983). One problem with this approach has been that although hens may work to gain access to a nest site, it is unclear if they regard the attainment of a nest site as necessary for the expression of behaviour. Dawkins (1983, 1990) has proposed the use of consumer demand theory to resolve this issue. If hens regard the nest box as necessary for the performance of pre-laying behaviour then they will persevere with gaining access to it in the face of an increasing cost (inelastic demand). If hens, however, have a low priority in gaining access to the nest box then they will cease gaining access to it in the face of an increasing cost (elastic demand). Cooper and Appleby (1994a) applied this technique to assess whether hens regarded the nest box as necessary for the expression of pre-laying behaviour. They found that hens persisted in reaching the nest box despite an increasing cost, thereby showing that hens have an inelastic demand for that resource.

1.5.2 Production

The development of the battery cage for laying hens affected much of the early research into pre-laying behaviour. Of primary concern was the excessive pre-laying pacing observed in some hens. This increased energy expenditure aswell as increasing the risk of injury to the hens (Mills *et al*, 1985). Researchers attempted to determine whether the observed differences in pre-laying behaviour from that in other systems were indicative of reduced welfare (e.g. Duncan, 1970). More recently, the lower egg production obtained in most alternative systems (Appleby *et al* 1988, 1993; Tauson and Abrahamsson, 1993) has redirected much of the research into pre-laying behaviour. Floor laying in non-cage systems can have major effects on production, and as many as 30% of eggs may be laid on the floor (Perry *et al*, 1971a,b). The collection of floor eggs is labour intensive and the eggs are often broken or dirty which reduces hatchability (Hodgetts, 1981) and economic value. This has led to an increase in research into the factors determining the choice of nest site by the laying hen.

Eggs may be laid on the floor due to social pressures and hens being displaced from preferred nest sites (Perry *et al*, 1971b; Kite *et al*, 1980; Rietveld-Piepers *et al*, 1985). Failure to lay in the nest boxes may also arise from an inability of some hens to perch and gain access to raised nest boxes (Kite *et al*, 1980; Appleby *et al*, 1983). This problem can be alleviated by allowing hens the opportunity to perch during rearing (Appleby *et al*, 1986). Alternatively, some individuals may lay on the floor because they may not recognize the nest box as a suitable nest site (Wood-Gush, 1954, 1975a; Appleby *et al*, 1986). The use of the nest site is affected by nest box design (e.g. roll-away or littered) and size (individual or communal, see Appleby, 1984 for a review) or the quantity of litter (Petherick *et al*, 1993). These factors therefore appear to be important in determining a hen's perception of a "suitable" nest site. Recent research has suggested that floor layers are strongly motivated to find a suitable nest site but fail to recognize normal nest boxes as suitable (Cooper and Appleby, 1994b). A thorough understanding of the factors that influence nest site choice by the laying hen would thus be necessary in developing nest sites to suit all hens.

1.5.3 Control

Surprisingly, as first reported by Wood-Gush (1963) the presence of an egg ready to be laid is not required for pre-laying behaviour to occur. It was later revealed that pre-laying behaviour is controlled by the release of oestrogen and progesterone from the post-ovulatory follicle (Wood-Gush and Gilbert, 1975). These hormones act on the central nervous system (perhaps the hyperstriatum, Wood-Gush and Gentle, 1978) which initiates pre-laying behaviour about 24 hours after ovulation. Meanwhile egg formation occurs independently of this process though also largely controlled by the presence of oestrogen and progesterone (see Bahr and Johnson (1991) for a review). Prostaglandin released from the ovarian follicles initiate oviposition which is usually appropriately synchronised with pre-laying behaviour (Shimada and Saito, 1989). However the exact mechanism determining the timing of oviposition is not known.

Pre-laying behaviour is often described as being relatively "preprogrammed" in its genetic control (Appleby *et al*, 1993). This may partly stem from the appearance of pre-laying behaviour for the oviposition of the first egg without any discernible developmental stage (but see Rietveld-Piepers, 1993; Sherwin and Nicol, 1993a). Most of what is known about the inheritance of pre-laying behaviour has been driven by interest in welfare and production. Wood-Gush (1972) identified strain differences in pacing prior to oviposition. Pacing may be indicative of frustration and poor welfare (Duncan, 1970; Duncan and Wood-Gush, 1972) and may have deleterious effects on production; such as increased energy expenditure, food consumption, feather loss and an increased risk of death due to trapping (outlined in Mills *et al*, 1985). Mills (1983) performed selection experiments on two strains; S line, which showed stereotypic pacing and T line which showed little or no pacing before oviposition. He significantly increased these traits in both lines indicating genetic variability. Furthermore, crosses and backcrosses between the two lines indicated that these traits were inherited separately. Whether selection against this pacing reduced frustration during pre-laying behaviour or merely reduced the outward display of frustration remains to be resolved.

1.6 A motivational theory of pre-laying behaviour

Wood-Gush (1963) showed that the tendency to perform pre-laying behaviour is dependent on physiological factors. However, the expression of pre-laying behaviour is strongly influenced by external factors. This is illustrated by the large variation in pre-laying behaviour between environments described above. However, our understanding of the onset and changes within pre-laying behaviour are minimal. The application of motivation theory might help in understanding the mechanisms that control pre-laying behaviour. Pre-laying behaviour is sometimes described in terms of two phases, a searching (involving exploratory behaviour) and sitting (involving nesting behaviour) phase. I will propose that laying hens have a demand to perform both these phases. This distinction serves to help in the explanation of the mechanisms that control pre-laying behaviour.

A number of studies have shown that hens have a strong demand for a nest site (Duncan and Kite, 1987; Smith *et al*, 1990; Cooper and Appleby, 1994a,b); however, the exact role of the nest site is undetermined. To expand on this, a nest site may serve both as the subject of exploratory behaviour and for the release of behaviour associated with the sitting phase. Thus it is unclear if a hen's motivation to reach the nest site represents a demand to explore possible nest sites or a demand to be in a nest. So what evidence is there that hens have a demand to perform both these phases?.

There is substantial evidence that hens have some demand to exhibit nesting behaviour. Vacuum activities are activities performed in the apparent absence of releasing stimuli and may represent a strong motivation to perform these activities (Manning and Dawkins, 1992). In conventional cages vacuum nest building activities are often observed (Brantas, 1980; Meijsser and Hughes, 1989; Appleby, 1990) which may reveal a demand for this behaviour. Hughes *et al* (1989) compared pre-laying behaviour of hens presented with a previous day's preformed nest and a flat littered nest. They found that hens performed more litter tossing and gathering and equal amounts of rotations and scrapping movements when presented with a preformed nest. The authors concluded that the performance of nest building activities is reinforcing in itself and is therefore important to the hen. Cooper and Appleby (1994a) found that when the cost of reaching the nest site was increased, hens approached the nest later in their pre-laying behaviour but the duration of the sitting phase was unaffected (supposedly due to the delay of oviposition). These results show that hens may have a requirement to exhibit the full duration of the sitting phase. Thus hens appear to have a demand to perform nesting behaviour, such that they will exhibit this behaviour irrespective of the presence or absence of the appropriate stimuli.

The performance of behaviour can be a rough measure of the demand for that activity (Manning and Dawkins, 1992) and can correlate with other measures of motivation (as for feeding behaviour, Savory, 1979). Hens perform exploratory behaviour in environments with nest sites (Meijsser and Hughes, 1989), even if hens use the same nest repeatedly (Wood-Gush, 1954). Although the searching phase is reduced in environments with nest sites compared with environments lacking in nest sites it is not completely absent in these environments (Appleby, 1990; Sherwin and Nicol, 1993b). The existence of exploratory behaviour despite a supposedly obvious and repeatedly used nest site is difficult to explain unless a demand for exploratory behaviour is inferred. Preventing access to the nest site results in an extension of the searching phase (Duncan, 1970). This extension of the searching phase is similar to that of floor layers with access to a nest box (Kite *et al*, 1980). Both floor layers and nest layers without a nest box show an equal willingness to overcome a narrow gap to explore their surroundings (Cooper and Appleby, 1994b). The latter study concluded that both floor and nest layers are equally motivated to find a nest site. These findings suggest that exploratory behaviour is terminated when the hen encounters the stimuli that allow the sitting phase. Thus the available data would suggest that hens have some demand to express exploratory behaviour, but after a short duration this may be terminated by environmental cues that allow the expression nesting behaviour.

1.7 Thesis plan

In this project, the effects of a number of stimuli on the expression of searching and nesting behaviour will be examined in order to understand better the control of pre-laying behaviour. The above representation of the mechanisms that control pre-laying behaviour can be used to formulate testable hypotheses. The effects of internal factors, preventing the expression of competing behavioural tendencies, environmental complexity and social factors on pre-laying behaviour will be examined. These findings can then be applied to problems of welfare and production as outlined above.

Chapter 2 provides a description of the general methods employed in this thesis.

In chapter 3, the relationship between lag (interval between ovipositions) and pre-laying behaviour will be investigated, as well as pre-laying behaviour for eggs of different position in the sequence. These findings will be used to assess to what extent variations in pre-laying behaviour due to environmental factors may be attributable to internal variation.

Chapter 4 investigates the interaction between feeding and pre-laying behaviour. A high demand to perform other activities around the time when the searching phase is due to start may influence the onset of pre-laying behaviour. Various models have been proposed to explain how animals switch from one activity to another (Dawkins, 1976; McFarland, 1974b; Colgan, 1989). A competition model proposed by McFarland (1974b) suggests that switches occur when the causal factors for an activity become stronger than the causal factors for the ongoing activity. The basis of this model is that performance of an activity decreases the tendency for that activity whereas the tendency increases while an activity is not being performed (though there may be positive feedback initially (Wiepkema, 1971)). The ability of this model to describe the timing of the onset of pre-laying behaviour will be examined by altering the causal factors for feeding behaviour around the time when pre-laying behaviour is due to start.

Also, if the demand to perform some activity unrelated to pre-laying behaviour increases above the demand to sit on the nest, then, according to a competition hypothesis, it is predicted that the hen would leave the nest and perform that activity. However, in the later stages of pre-laying behaviour the hens may be highly motivated to sit on the nest, such that the demand for a second activity would have to be greater than during the earlier stages to produce a switch in behaviour. This was tested by presenting food after various durations of food deprivation (thereby altering the motivation to feed) to hens showing pre-laying behaviour.

Chapter 5 has two objectives; to examine the effects of nest quality and other cues for exploration on pre-laying behaviour. As pre-laying behaviour starts with searching behaviour, factors influencing searching behaviour will affect the onset of pre-laying behaviour. Hence an environment with many possible nest sites may provide the external cues which increase the motivation to perform searching behaviour, such that less of an internal influence may be needed to start the searching phase. It is therefore hypothesised that pre-laying behaviour will start earlier in environments that provide the external cues that elicit exploratory behaviour.

Similarly, the sitting phase may be influenced by factors that allow (or inhibit) its expression. It is predicted, therefore, that the absence of cues that elicit nesting behaviour (i.e. a suitable nest site) would result in the searching phase being extended, whereas the sitting phase would be reduced. Perhaps, under such circumstances oviposition may be delayed to allow the full duration of the sitting phase (as suggested by Cooper and Appleby (1994a)).

Chapter 6 investigates the effect of social inhibition on the start of pre-laying behaviour and approach to the nest site. It is suggested that the presence of dominant hens prevents searching behaviour being expressed until the motivation for it is high enough to overcome this inhibition. Thus, it is hypothesised that hens will delay moving past a dominant or unfamiliar bird to gain access to a nest site until later in their pre-laying behaviour than compared with a subordinate bird.

In chapter 7, the effect of social factors on nest occupancy will be examined. Social factors may inhibit the performance of nesting behaviour, either by preventing hens from sitting on the nest until the demand to perform nesting behaviour overcomes these social factors (Rietveld-Piepers *et al*, 1985) or by the displacement of hens already settled on the nest (Perry *et al*, 1971b).

Chapter 8 offers a brief summary of the findings reported in this thesis. These findings are discussed with respect to a descriptive model for the control of pre-laying behaviour. The implications of the results presented in this thesis for welfare and production in laying hens is also discussed.

CHAPTER 2: **General methods**

2.1 Abstract

1) This chapter describes the subjects and basic methods used to record the various elements of pre-laying behaviour. Variations from this methodology are described in the materials and methods section of each chapter.

2) An instantaneous sampling technique was used to record the location of the hen and searching behaviour at 1 minute intervals. This allowed a good recording of events with approximate durations of pre-laying behaviour and searching and sitting phases.

3) A behavioural sampling technique was used to record the number of nest entries and inspections in each 1 minute period.

4) A behavioural sampling technique was also used to record rarer aspects of pre-laying behaviour. These data were used to help in the identification of the various phases of pre-laying behaviour.

5) The time of oviposition was also recorded wherever possible. However, in some experiments this could only be estimated from related events.

2.2 Subjects

All subjects used in the experiments were commercial laying strains (either light or medium hybrids). Hens were reared by the breeders in littered pens until 14 to 18 weeks of age. Experiments, however, were carried out on hens no younger than 26 weeks of age (in order to avoid the early period of laying when pre-laying behaviour can be very unsettled). Most experiments were on isolated birds except for the two experiments on social interactions (Chapters 5 and 6). Hens also had access to an individual nest box (except for Chapter 4, experiments 1 and 2).

2.3 Methods

Data extraction was performed from video recordings for all the experiments reported. The specific behaviour elements recorded depend on the nature of the experiment and environment in which the hens were kept and are described in the materials and methods section for each chapter. However, general procedures were employed for all the experiments reported in this thesis and these are described in this section. Two recording methods were used simultaneously.

A time sampling procedure (instantaneous sampling) was used to record some aspects of pre-laying behaviour. This method was used as it allows the recording of various behaviour patterns while giving a good approximation to the proportion of time spent performing a behaviour pattern (Martin and Bateson, 1986). The sample interval used (unless stated otherwise) was 1 minute. This was found to be an adequate interval after examining the duration of the several behaviour patterns recorded. At each sample point, the following was recorded:

(1) Location of the hen (cage/pen or nest box). A nest entry was recorded when both feet were in the nest box.

(2) Searching (exploratory) behaviour. This is characteristic of the earlier stages of pre-laying behaviour and is accompanied by increased locomotion and the nesting call. It includes side to side movement of the head: walls are examined keeping the keel up high and possible nest sites are examined with the neck held straight in a horizontal position (Wood-Gush, 1975b).

A behavioural sampling technique was used to record various activities. The number of bouts of these activities were recorded per minute (during sample intervals), in order to obtain an estimation of time in relation to other aspects of pre-laying behaviour. The activities recorded by this method were:

(1) Nest examinations (inspections). One nest examination involved placing the head over an imaginary line demarcating the front of the nest box and withdrawing the head fully past this line.

(2) Nest entries that were not registered by the instantaneous sampling method were recorded. One nest entry involved placing both feet into the nest box and then stepping (both feet) out of the nest box.

Several activities were also recorded by a similar behavioural sampling method as that described above. The purpose of recording these activities was to help in the identification of the various phases of pre-laying behaviour and they were not analysed statistically (unless stated otherwise). Thus a precise definition of a bout was not necessary. The activities recorded were:

(1) Escape behaviour, including back and forth pacing and pushing against the side of the enclosure (Kite *et al*, 1980).

(2) Crouching under other hens, also called "nuzzling under" by Wood-Gush (1954).

(3) Nest building behaviour. This involves producing a shallow scrape by rotating whilst sitting to create a depression with the keel bone, usually accompanied by outward scraping movements with the feet.

(4) Litter tossing (onto back) or gathering and placing around the nest. This activity was also observed in caged hens without access to a suitable substrate (as a vacuum activity).

Data obtained by the time sampling procedure was used to calculate the durations of the searching (exploratory) and sitting (nesting) phases of pre-laying behaviour. Three samples of searching behaviour (or recordings of nest occupancy) within a 5 minute period, followed by some aspect of pre-laying behaviour in the next 10 minutes and every 10 minutes thereafter until oviposition was designated as the start of pre-laying behaviour. Thus the duration of pre-laying behaviour was from this point until oviposition. The time from the start of pre-laying behaviour until the first nest entry was designated as the duration of the searching phase. The time from the first nest entry until oviposition was designated as the duration of the sitting phase.

The time of oviposition was also recorded for all records of pre-laying behaviour. In some experiments this was recorded directly by observing the hen straining and oviposition. Sometimes this could not be observed and the time of oviposition had to be estimated, such as by recording when the egg rolls out of the cage.

CHAPTER 3: **Effects of lag and egg number in the sequence on pre-laying behaviour**

3.1 Abstract

1) Domestic hens lay their eggs in a sequence, separated by one or more non-laying days. The difference in time of day of ovipositions of two successive eggs in a sequence is called the lag.
2) This study examined the relationship between lag and egg number in the sequence on various elements of pre-laying behaviour.
3) The pre-laying behaviour of all eggs of a sequence for twenty one Hisex White laying hens individually kept in modified cages was recorded.
4) Lag was found to be greater at the beginning and ends of a sequence (97.1(±9.0) and 117.1(±12.7) minutes respectively than in the middle (37.2(±10.0) minutes). The mean difference in time of day between first and last eggs of a sequence was 7.31 hours (±29.9 minutes), agreeing well with a similar time period in which ovulation can occur.
5) The duration of the sitting phase and the total time spent in the nest box were found to be positive correlated to lag (gradient 0.21(±0.08) and 0.18(±0.05) respectively, $P<0.05$).
6) The duration of pre-laying behaviour was found to be longer for the first egg of the sequence than for the middle or last eggs (75.1(±7.1), 54.9(±6.1) and 62.6(±7.4) minutes respectively, ANOVA dfs=2,19, $P<0.001$). This was largely due to a longer searching phase for the first egg (28.7(±5.3) minutes) than for the middle or last eggs (7.5(±2.2) and 5.6(±1.2) minutes respectively, ANOVA dfs=2,19, $P<0.001$). More nest examinations were also observed during the searching phase for the first egg of a sequence than for the middle or last eggs (8.7(±2.0), 4.4(±0.8) and 3.9(±0.8) minutes respectively, ANOVA dfs=2,19, $P<0.01$).
7) The duration of the sitting phase was longer for the last egg of a sequence than for the first or middle eggs (56.9(±7.7), 46.6(±3.8) and 47.4(±5.6) minutes respectively, ANOVA dfs=2,19, $P<0.05$). However, this may have been attributed to the longer lag at the end of the sequence described earlier.
8) It is suggested that lag is related to the later stages of pre-laying behaviour. Also, searching behaviour may be under the control of a priming mechanism which reduces the extent of searching behaviour for all but the first egg of a sequence.

3.2 Introduction

The egg production cycle of the domestic hen is best considered by starting with the ovulatory cycle. Progesterone acts via the hypothalamus to stimulate lutenizing hormone release from the anterior pituitary, which indirectly triggers ovulation 6-8 hours later (Etches, 1990). Ovulation is initiated by a surge of progesterone from the largest pre-ovulatory follicle (Fraps, 1955; Wilson and Sharp, 1976). Ovulation independently determines the regulation of pre-laying behaviour and oviposition. The post-ovulatory follicle releases oestrogen and progesterone, which cause pre-laying behaviour about 24 hours after ovulation (Wood-Gush and Gilbert, 1964 and 1973). The ovum is ready to be laid about 20 hours after ovulation (Bahr and Johnson, 1991). Oviposition is initiated by the release of prostaglandins which bind with uterine membrane receptors and cause contractions of the uterus (Shimada and Saito, 1989). Pre-laying behaviour and oviposition are usually synchronised despite being regulated by different mechanisms.

Successive ovipositions form a sequence. Sequences may range in length from one or two eggs to over 100, separated by non-laying days (lapse days, Bahr and Johnson, 1991), though such long sequences are rare and sequence length decreases after about one year of age (Yoo *et al*, 1988). The first egg of a sequence is laid early in the morning, with successive eggs usually being laid slightly later than the previous egg. The difference in time of day of oviposition of two successive eggs is called the lag. The ovulatory cycle is usually entrained (but not exclusively, Bhatti, 1987) by the light/dark cycle, with dusk being the most important cue (Naito *et al*, 1984). Pre-laying behaviour and oviposition (and hence the interval between oviposition of the first and last eggs of a sequence) are restricted to about 8 hours of the day (Lillpers, 1991). This corresponds to a similar interval (open period) over which ovulation can occur (Fraps, 1955; Etches, 1990). The open period may arise out of a circadian rhythm in sensitivity preventing lutenizing hormone release during the closed period (Fraps, 1955).

On any laying day (except for the last egg of a sequence), ovulation occurs about 30 minutes after oviposition. The exact interval between oviposition and the associated ovulation is correlated to the lag (Warren and Scott, 1935). Differences in lag have also been related to some elements of pre-laying behaviour (Wood-Gush, 1963). This suggests that the mechanism that determines lag is related to the expression of pre-laying behaviour. The post-ovulatory follicle may be responsible for the relationship between lag and pre-laying behaviour, as it is important in the timing of ovulation, oviposition and pre-laying behaviour. However a more central mechanism, which controls the action of the post-ovulatory follicle may be involved.

Wood-Gush (1963) found that the duration on the nest and the time taken over nest examinations was positively correlated to lag. There are two factors of the environment used in his study which might have masked any other relationships between pre-laying behaviour and lag. Firstly, social interactions may have modified pre-laying behaviour as the hens were in groups. Indeed, there were a high number of observations inconsistent with the majority of the results in the pen with the largest group size. The author notes that in some cases these were certainly due to competition for the nest sites. Secondly, the use of trap-nests may not allow the full expression of pre-laying behaviour. Although hens learn to examine the nest boxes without entering them, it denies them the opportunity to enter more than one, a behaviour which is commonly observed in many husbandry systems (e.g. Meijsser and Hughes, 1989).

The results of Wood-Gush (1963) suggest that physiological factors modify the expression of pre-laying behaviour on a daily basis. These variations in pre-laying behaviour are related to the temporal relationship between ovulation and the preceding oviposition. Thus other relationships between the ovulatory and oviposition cycles might also be expected to have an effect on pre-laying behaviour. The relationship between ovulatory and oviposition cycles differs for the first and last eggs of a sequence as compared with other eggs. Oviposition is absent around the time of ovulation of the first egg of the sequence, whereas for other eggs (except the last egg of a sequence) it preceeds ovulation by about 30 minutes. The timing of the first ovulation seems to be determined by the start of the open period (Fraps, 1955). Ovulation of the first egg of the sequence is therefore not preceded by oviposition. For the last egg of a sequence, oviposition is not followed by ovulation until the next day. The largest pre-ovulatory follicle has an important role in the timing of oviposition (Fraps, 1955). As the largest pre-ovulatory follicle is not about to rupture just after oviposition of the last egg of a sequence, it may exert a different influence on pre-laying behaviour and oviposition than for other eggs of the sequence.

The first objective of the study reported here was to examine the relationship between lag and number and position of eggs in a sequence. The second objective was to extend the findings of Wood-Gush (1963) in clarifying the relationships of the elements of pre-laying behaviour with the daily lag. Many aspects of pre-laying behaviour in the absence of social influences were correlated with lag. The third objective was to identify variations in pre-laying behaviour corresponding to the first, middle and last eggs of a sequence.

3.3 Materials and methods

Twenty-one HiSex White light hybrid laying hens reared and kept until 45 weeks of age in deep litter pens with littered nest boxes were used in this study. At 45 weeks of age the hens were housed individually in Edinburgh Modified Cages (Appleby, 1993). The nest box in this system consists of an enclosed box with a sloping floor lined with astroturf. Food and water (from nipple drinkers) were provided *ad lib*. The artificial lighting regime was 14 hours light: 10 hours dark with lights coming on at 08.30 hours.

A daily record of eggs was kept from when the hens were 51 weeks of age. Observations started two weeks later at 53 weeks of age and lasted for 12 weeks.

A video camera and VCR (Panasonic NV100) were set up to record the pre-laying behaviour of a particular hen. Filming was from lights-on until oviposition. If neighbouring hens were about to commence a sequence, the field of view was enlarged to include them. A maximum of 4 hens could be filmed on any one day using this technique. However, only one or two hens were usually filmed on any one day. There were two aims to the observations. Firstly, to obtain the laying times of all eggs of one clutch for each hen. Secondly, to obtain a record of pre-laying behaviour for all eggs of a clutch.

From the video recordings, activities were recorded from lights-on until oviposition. The position of the hen (cage, nest box) was recorded at one minute intervals. Searching behaviour was also recorded if observed at these intervals. All nest examinations (insertion of head into the nest box) were also recorded. Time of oviposition (evident by egg rolling out of nest box) was also recorded. However, this might not have always been accurate as the hen's body or feet may have prevented immediate rollaway.

3.4 Analysis of results

Laying times for all eggs of a sequence were obtained for all 21 hens. These data were used to examine various characteristics of the relationship between lag and number of eggs in the sequence. Full records of pre-laying behaviour for all the eggs of a sequence were obtained for 7 hens and these records were used to examine the effect of lag on pre-laying behaviour. Various aspects of pre-laying behaviour with lag were plotted and a least squares regression line calculated for each individual (Minitab, Ryan *et al*, 1976). The gradients of the regression lines for each individual were analysed by a one-sample t-test (Null hypothesis, mean=0) for various aspects of pre-laying behaviour.

Records from 12 hens were used to analyse the effect of egg position in the sequence on pre-laying behaviour. Three records were counted as missing values for the first egg of the sequence, due to pre-laying behaviour starting before lights-on. There were also three missing values for the last egg of the sequence, due to the video finishing before the end of pre-laying behaviour. The start of pre-laying behaviour was designated as described in chapter 2. The data for first, middle and last eggs were analysed using an ANOVA with individual hens forming the block structure. Pairwise comparisons for significant ANOVA results were performed using a related-samples t-test.

3.5 Results

Hens with complete records of pre-laying behaviour for all eggs of a sequence were used to examine the effect of lag (N=7). In a separate analysis records from twelve hens, including the above seven, were used to examine the relationship between position in the sequence and pre-laying behaviour.

3.5.1 Relationship between lag and number of eggs in a sequence

Firstly, the difference in lag between different positions in the sequence was examined. This was performed by comparing the lag for the second, middle (or (n/2)+1 if an even number of eggs in the sequence) and last eggs of the sequence. Mean lags were 97.1(±9.0), 37.2(±10.0) and 117.1(±12.7) minutes for the second, middle and last eggs respectively. Thus lag was greater at the beginning and end of the sequence. Related-samples ANOVA on the lag for the second, middle and last eggs was highly significant (F=15.2, degrees of freedom 2, 19, P<0.0001). This is likely to have been due to the shorter lag for the middle egg, as the differences between the lag for the second and last eggs were not significant (related samples t-test T=1.56, N=21, P=0.13).

Eggs were laid progressively later in the day and negative lags were only observed for one hen. The mean difference in time of day between the first and last ovipositions was 7.31 hours (±29.9 minutes). This agrees well with the earlier suggestion that oviposition is restricted to about 8 hours of the day. There was considerable variation between hens in mean lag times. Mean lags for all hens ranged from 15.2 to 137.4 minutes, with the number of eggs in a sequence varying from 4 to 31. Figure 3.1 shows that for sequences up to about 10 eggs, the mean lag decreased with increasing egg number. For sequences of more than 10 eggs, the mean lag appears to be relatively constant at about 18 minutes.

3.5.2 The relationship between lag, position in sequence and pre-laying behaviour

Figure 3.2 shows the time of oviposition of all eggs of a sequence for 7 hens with full records of pre-laying behaviour. From the sequences of the 7 hens, the range in lag was -5 minutes to 163 minutes (mean 94.0 (±16.2) minutes). Of all the various aspects of pre-laying behaviour examined, only the durations of pre-laying behaviour, sitting phase and duration in the nest box appeared to be affected by lag (Table 3.1). These durations appeared to increase with increasing lag. However only increases in the duration of the sitting phase and duration in the nest box proved significant when tested with a one-sample t-test (Table 3.1).

There were significant differences in the duration of pre-laying behaviour between first, middle and last eggs of a sequence (Table 3.2). Pairwise comparisons revealed the duration of pre-laying behaviour to be significantly longer for the first egg (Table 3.3). Examination of means showed that this difference was largely due to a significantly longer searching phase for the first egg. Hens also examined the nest significantly more for the first egg than for the others during the searching phase (Table 3.3). Thus the searching phase for the first egg was longer and involved more nest examinations than for other eggs in the sequence. One possible explanation for this observation would have been seen if the searching phase was longer early in the morning than later in the day. If so, then a plot of duration of searching phase for the first egg against time after lights-on should show a negative correlation. In fact, such a plot showed a slightly positive correlation (Pearson product moment correlation coefficient, $r = +0.19$, $N=9$). Thus there was no evidence that time of day influenced the duration of the searching phase for the first eggs in sequences.

There was also a significant difference in the duration of the sitting phase for the first, middle and last eggs (Table 3.2). Pairwise comparisons revealed that the sitting phase was significantly longer for the last egg than for the first or middle egg (Table 3.3). This increase in duration of about 10 minutes was partly due to an increase (not significant) of about 3 minutes in the duration of nest occupancy. However, the increased duration of the sitting phase may be related to lag as suggested in the introduction. Lag was 53.0(±13.6) and 109.2(±19.3) minutes for the middle and last eggs respectively.

3.6 Discussion

Lag was found to be, on average, greater for the second and last eggs than for the middle eggs of a sequence. The pattern in oviposition times that this produces is the most common pattern observed (85% of hens, Lillpers and Wilhelmson, 1993a). In the study reported here, the interval (in time of day) between the first and last eggs in a given sequence was found to be about 7.3 hours. This agrees well with the findings of Lillpers (1991), which found that oviposition under normal commercial light regimes was restricted to about 8 or 9 hours of the day. Oviposition times, however, are strongly influenced by age of hens (Lillpers and Wilhelmson, 1993b) and genetics

(Lillpers, 1991; Yoo *et al*, 1988). This restriction in laying times is thought to be due to a similar restriction in ovulation (the open period, Fraps, 1955). Not surprisingly, the mean lag was found to be negatively correlated with the number of eggs in the sequence for short sequences (less than about 10 eggs). However, for longer sequences, mean lag appeared to remain constant at about 18 minutes (Figure 3.2). This result was unexpected and suggests that hens laying later every day with pause days (some lay every day at roughly the same time, Lillpers and Wilhelmson, 1993a) may have a limit on the rate at which they can produce an egg. Thus selection for short lag may increase production in this type of hens.

The second objective of the study reported here was to examine the relationship between various components of pre-laying behaviour and lag in time of lay. A significant relationship between the total duration in the nest box and lag was found which confirmed the findings of Wood-Gush (1963). The present study however found that the duration of the sitting phase increased with increasing lag. This increase in the duration of the sitting phase accounts for the large (but not significant) relationship between the duration of pre-laying behaviour and lag. These findings suggest that the later stages of pre-laying behaviour are strongly related to the lag. However the actual number of nest entries was not found to be related to lag. Thus the time taken from the first nest entry until oviposition (which includes the time taken to enter the nest various times), but not the actual number of entries, was related to lag. This conclusion could not be reached in the study by Wood-Gush (1963) as the use of trap-nests prevented more than one nest entry.

Thus, variations in physiological factors which determine patterns of lag also determine aspects of pre-laying behaviour. However, the exact mechanism by which the relationship between pre-laying behaviour and lag arises remains unclear. One possible mechanism involves progesterone release from the post-ovulatory follicle. Lag is related to the interval between oviposition and the associated ovulation (Warren and

Scott, 1935). Progesterone from the post-ovulatory follicle influences the timing of oviposition (Shimada and Saito, 1989) and may thus determine lag. Progesterone from the post-ovulatory follicle also initiates pre-laying behaviour (Wood-Gush and Gilbert, 1964). Thus progesterone release is linked to lag and pre-laying behaviour. It may be that variations in this, such as the timing of progesterone release brings about variations in lag and pre-laying behaviour.

Wood-Gush (1963) found a relationship between nest examination time and lag. This component of pre-laying behaviour is equivalent to the duration of the searching phase in this study, which was not found to be related to lag. This discrepancy is likely to be due to the different environments used in the two studies. In the study reported here, the environment was less complex as it was smaller, more barren and contained only one nest. This may inhibit exploration during pre-laying behaviour (Chapter 5) resulting in reduced variation which may have masked any relationship between the duration of the searching phase and lag.

The third objective of this study was to investigate if variations in pre-laying behaviour corresponded to egg number in the sequence. It was found that pre-laying behaviour was longer for the first egg of the sequence than for all other eggs, due to a significantly longer searching phase involving more nest examination. Assuming that a sequence is analogous to a clutch, this seems an adaptive strategy by the ancestors of the domestic fowl. That is, a longer searching phase for the first egg may serve to ensure that the hen finds a nest site that proves safe during incubation. Subsequent eggs in the clutch are usually laid in the same site, thus searching behaviour would not be as important and it may be advantageous to the hen to reduce the amount of searching behaviour for later eggs (in terms of an unnecessary predation risk and energy loss). However, the mechanism by which a longer searching phase may arise for the first egg of a sequence remains puzzling.

As suggested in section 3.2, variations in pre-laying behaviour between the first and other eggs of a sequence may be related to variations in hormones around the time of ovulation of these eggs. It may also be that the duration of the searching phase is linked to the time of day. As the first egg is usually laid earlier in the day than the remainder, a mechanism whereby searching phase is negatively correlated to time of day may ensure that the searching phase is longer for the first egg of the sequence. This hypothesis was examined by correlating the duration of the searching phase for the first egg with time from lights-on (Figure 3.2), though no evidence for it was found.

Alternatively, it may be that some mechanism (either neural or hormonal) that is influenced by the performance of behaviour governs the duration of the searching phase. In clarification, searching behaviour for the first egg could have an inhibiting effect on searching behaviour for subsequent eggs in the sequence. Thus pre-laying behaviour for subsequent eggs in the sequence show less searching behaviour, resulting in a shorter searching phase. On pause days, this inhibiting effect could be abolished such that searching behaviour is expressed with full vigour for the first egg of the next sequence. This hypothesis could be examined by investigating what factors change as predicted.

The duration of the sitting phase was found to be significantly longer for the last egg of the sequence compared to other eggs. This increase in duration of about 10 minutes was partly due to an increase (not significant) of about 3 minutes in the duration of nest occupancy. One possible explanation of this finding is that the largest pre-ovulatory follicle has a different influence on pre-laying behaviour for the last egg than for other eggs of a sequence. This may arise because the largest pre-ovulatory follicle is not about to ovulate around the time of the last oviposition of a sequence. However, as observed in this study and elsewhere (e.g. Lillpers, 1991), lag at the end of the sequence is greater than in the middle. As lag was shown to influence the duration of the sitting phase and duration on the nest, this explanation for the observed results cannot be rejected. Comparisons between the last egg and a middle egg of approximately equal lag was attempted but long lags in the middle were rare and a considerable disparity in mean lag remained.

In conclusion, there may be a limit on the mean lag for hens that show sequences with ovipositions later in the day. Possible mechanism by which the later stages of pre-laying behaviour, but not the number of nest entries, are related to lag are discussed. Searching behaviour was suggested to be under the control of a priming mechanism, which serves to produce a longer searching phase for the first egg than for other eggs of a given sequence.

Table 3.1: Mean gradients of regression lines of various aspects of pre-laying behaviour plotted against lag. Results of one-sample t-test on individual gradients is shown.

Variable	Mean gradient	T-test Probability
Duration of pre-laying behaviour	0.24(±0.10)	0.06
Duration of searching phase	0.10(±0.10)	0.34
Duration of sitting phase	0.21(±0.08)	0.04*
Number of nest examinations during the searching phase	-0.0006(±0.02)	0.9
Number of nest examinations during the sitting phase	0.02(±0.02)	0.3
Number of nest entries	0.005(±0.007)	0.5
Total duration in nest box	0.18(±0.05)	0.02*

* $P<0.05$.

Table 3.2: Means of various aspects of pre-laying behaviour for the first, middle and last eggs of the sequence (durations in minutes). Results of ANOVA are presented.

	Egg number in sequence			ANOVA
Variable	First	Middle	Last	Sig. level
Duration of pre-laying behaviour	75.1(±7.1)	54.9(±6.1)	62.6(±7.4)	0.001
Duration of searching phase	28.7(±5.3)	7.5(±2.2)	5.6(±1.2)	0.001
Duration of sitting phase	46.4(±3.8)	47.4(±5.6)	56.9(±7.7)	0.05
Number of nest examinations during the searching phase	8.7(±2.0)	4.4(±0.8)	3.9(±0.8)	0.01
Number of nest examinations during the sitting phase	7.3(±1.6)	5.9(±1.6)	4.7(±1.0)	N.S.
Number of nest entries	3.6(±0.8)	3.3(±0.6)	3.5(±0.9)	N.S.
Total duration in nest box	33.9(±3.2)	33.5(±3.2)	36.2(±3.8)	N.S.

Table 3.3: Pairwise comparisons between first, middle and last eggs of a sequence. Results of related-samples t-test and probabilities are shown.

Variable	Pairwise comparison	t-test	Significance level
Duration of pre-laying behaviour	First vs. Middle	4.5	0.001
	First vs. Last	2.8	0.01
	Middle vs. Last	1.7	N.S.
Duration of the searching phase	First vs. Middle	4.8	0.001
	First vs. Last	5.3	0.001
	Middle vs. Last	0.4	N.S.
Duration of the sitting phase	First vs. Middle	0.2	N.S.
	First vs. Last	2.4	0.05
	Middle vs. last	2.1	0.05
Number of nest examinations during the searching phase	First vs. Middle	3.6	0.01
	First vs. Last	4.0	0.01
	Middle vs. Last	0.4	N.S.

Figure 3.1: Mean lag plotted against number of eggs in the sequence for all hens.

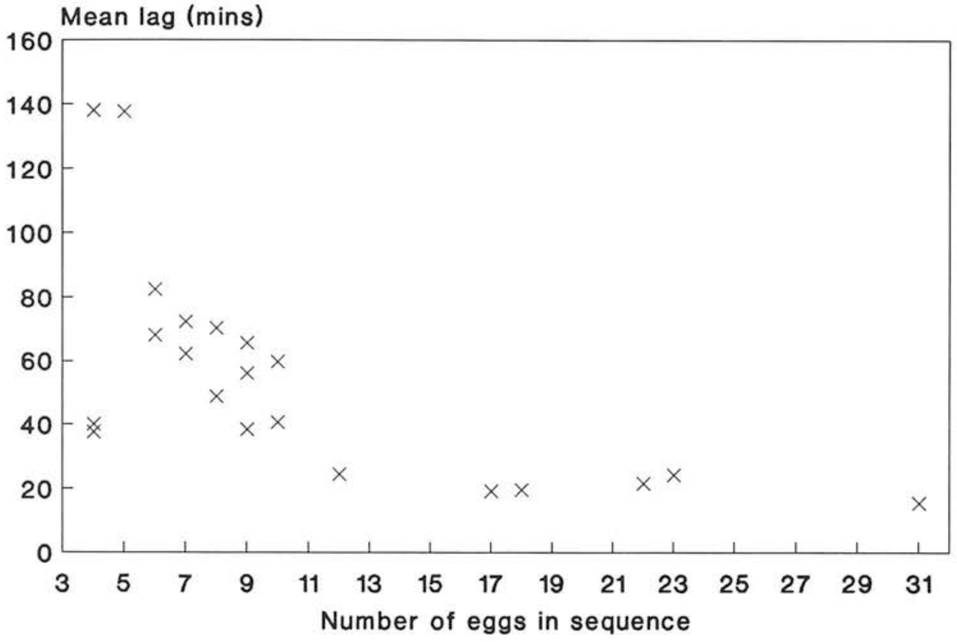
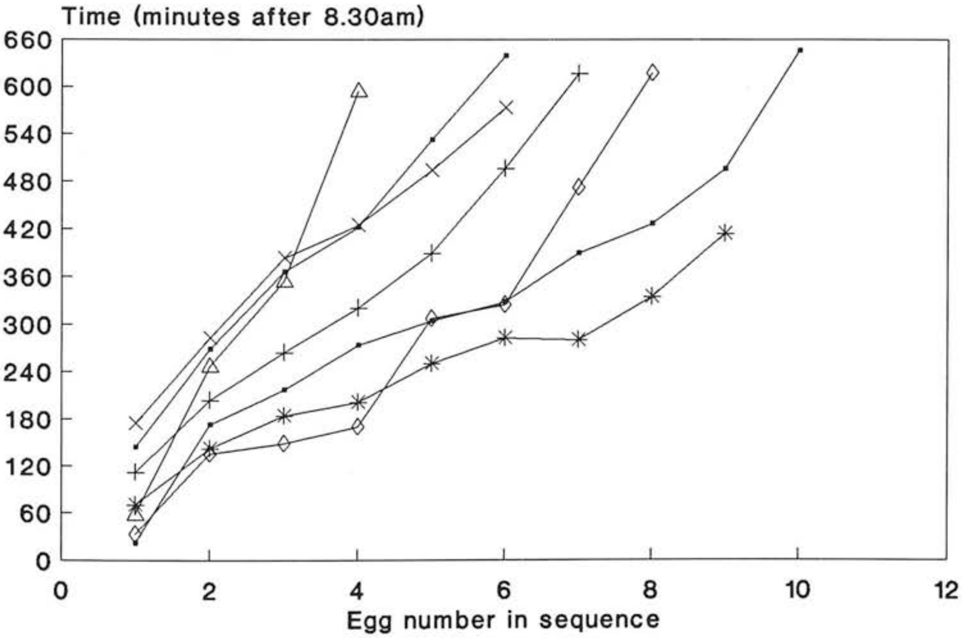


Figure 3.2: Oviposition time of all eggs of a sequence for 7 hens with complete records.



CHAPTER 4: **Interaction between feeding and pre-laying behaviour**

4.1 Abstract

1) A series of experiment were conducted to investigate pre-laying behaviour after food presentation and under various levels of food deprivation.

2) In the first experiment, food was presented to 8 laying hens showing pre-laying behaviour after various durations of food deprivation.

3) Hens were always observed to interrupt pre-laying behaviour and feed after the presentation of food. After 0 (food topped up) and 1.5 hours of deprivation, oviposition was delayed compared to control records of pre-laying behaviour (by 24.0(6.8-31.2) and 32.0(28.5-37.3) minutes respectively, Wilcoxon N=6, P<0.05). The relationship between the delay in oviposition and the stage during the pre-laying behaviour sequence when food was presented was studied in the second experiment.

4) The first two records for 17 hens individually housed in battery cages after interruption of pre-laying behaviour by the presentation of food were analysed. It was found that when food was presented near to oviposition, the delay in oviposition was greater than if food was presented further away from the expected oviposition (oviposition delayed by 24.3(±2.8) and 11.9(±4.2) minutes respectively, t-test N=10, P<0.01).

5) In the third experiment, the pre-laying behaviour of 20 hens individualy housed in modified cages with or without access to food was studied. The duration of pre-laying behaviour was longer in hens that had been food deprived for short (food removed 15 minutes after lights-on) or long (food removed previous night) periods of time than for hens with access to food (98.3(±9.0), 110.7(±9.3) and 72.6(±7.0) minutes respectively, ANOVA dfs=2,22, P<0.001). This was largely due to a longer searching phase during short or long food deprivation periods than when food was available (32.2(±7.7), 28.8(±5.0) and 8.6(±2.3) minutes respectively, ANOVA dfs=2,22, P<0.001). Hens also performed more nest examinations during short or long deprivations than when food was available (11.6(±2.7), 12.1(±3.0) and 6.9(±2.2) respectively, ANOVA dfs=2,22, P<0.05).

6) The presentation of food was found to suppress pre-laying behaviour: the resulting delay in oviposition was found to be greater if food was presented nearer, rather than further, to the expected time of oviposition.

7) Pre-laying behaviour was found to start earlier in the absence of food. It is suggested that the onset of pre-laying behaviour is determined by the tendency to perform pre-laying behaviour competing with other behavioural tendencies.

4.2 Introduction

At any particular point in time, an animal may perform a number of alternative behaviour patterns. Deciding what behaviour an animal should do at any particular time is essential for that animal's survival and reproductive success. The mechanism by which animals make these decisions has been widely discussed (see Colgan, 1989 for a review). A general assumption is that animals perform the behaviour for which they are most motivated. However this is an oversimplification of the mechanisms involved as there is a cost to changing (such as in terms of time) which an animal must overcome in switching from one behaviour to another (McFarland, 1989). This study attempted to learn about the motivation for pre-laying behaviour by experimentally altering the tendency to perform another behaviour.

Restricting the food intake of laying hens is an accepted management practice for controlling weight and reducing food costs. Considerable research has been conducted which has found that egg production and oviposition is affected by food restriction and feeding time. Feeding in the afternoon has been found to cause a delay in oviposition time in broiler breeders (Wilson and Keeling, 1991). This suggests that the oviposition time can be affected by feeding time. This is also the case in continous light, when a restricted feeding schedule can act as a zeitgeber absent if hens are fed *ad lib* (see Bhatti, 1987 for a review). Egg production can also be affected by feeding time. Egg production is greater if hens are fed for 4 hours in the evening than if fed 4 hours in the morning (Daniel and Balnave, 1981). These results suggest a link between egg production, oviposition time and availability of food. This link may arise because feeding activity is affected by the egg formation cycle even under continuous light (Duncan and Hughes, 1975).

As feeding activity and egg production are closely related, interactions between feeding activity and pre-laying behaviour might also be expected. Hens with continuous access to food show decreased food intake during pre-laying behaviour which may signify a self-imposed suppression of feeding activity (Wood-Gush, 1954). After oviposition food intake is high which may signify compensation for this suppression (Wood-Gush and Horne, 1970). If pre-laying behaviour can suppress feeding behaviour, the reverse might also be expected if the tendency to feed were increased. Evidence that feeding activity can suppress pre-laying behaviour comes from the unpublished observations of A. Rasmussen. She found that pre-laying behaviour was shorter for eggs laid in the hour after feeding than for other eggs. Food presentation resulted in increased feeding activity, which may signify a high tendency to feed (Savory *et al*, 1993). Rasmussen also found that on 5 occasions there was no pre-laying behaviour at all for eggs laid after feeding. This suggests that feeding activity may be able to suppress pre-laying behaviour fully such that the egg is expelled while the hen is engaged in activities unrelated to pre-laying behaviour.

Preventing access to food for 8 hours during the day results in large changes in behaviour during the whole day (Preston, 1987). One interpretation of this is that in the absence of food hens are unable to express feeding behaviour and so exhibit behaviour controlled by the next highest tendency. As already mentioned, feeding is rare immediately prior to oviposition so it is unlikely that the absence of food will have any effect on pre-laying behaviour. However, initiation of bouts of pre-laying behaviour may depend on the motivation to perform pre-laying behaviour surpassing the motivation to perform the behaviour being exhibited at the time. This conflict in interests (whether to nest or not) may account for the displacement activities observed at this time (Wood-Gush, 1954). Hens would be unable to express feeding motivation in the absence of food and this may result in pre-laying behaviour starting earlier than in the presence of food. However if hens have been food deprived for a considerable length of time, the motivation to search for food may be high enough to prevent this advanced onset of pre-laying behaviour.

A series of experiments was conducted to investigate the interaction between the tendencies to feed and to perform pre-laying behaviour. The first experiment was a pilot study to examine the effects of food presentation on pre-laying behaviour and oviposition time. The second experiment examined the relationship between variations in pre-laying behaviour and the time before expected oviposition at which feeding took place. Finally, pre-laying behaviour was compared between hens that had food available or had been food deprived for short and long durations.

4.3 Experiment 1: Effects of food presentation on pre-laying behaviour and oviposition time.

The results of Rasmussen (Unpublished) suggest that the presentation of food results in increased feeding activity and a suppression of pre-laying behaviour. The objectives of the experiment reported here were to extend these findings. It was predicted that the presentation of food during pre-laying behaviour would result in hens feeding until the tendency to feed fell below that for pre-laying behaviour. Birds deprived of food for longer durations should thus have had longer feeding sequences, resulting in greater delays of oviposition.

4.3.1 Materials and methods

Eight 30-week-old ISABrown medium hybrid laying hens had been housed individually in battery cages (0.3m x 0.45m) without a nest box. Water was available *ad lib* and food was provided in individual feeders which could be removed. Food was topped up at 15.00h daily. Sufficient food was provided at each feeding to ensure that food was still present by the next feeding. The lighting regime was 16 hours light: 8 hours dark with lights coming on at 08.30h. A daily record of eggs was kept from when the hens were 36 weeks of age. Behavioural observations started when the hens were 38 weeks of age.

Observations on behaviour were carried out on alternate days when one of four treatments was presented. The control treatment involved observing the hens from 09.00h (hens did not start pre-laying behaviour before this time) till 13.30h for one day. The second treatment (0 hours of food deprivation) involved feeding (i.e. topping up food) at one of the following times: 10.15h, 11.00h, 11.45h or 12.30h. The third treatment involved feeding at the same times but after 1.5 hours of food deprivation. The fourth treatment involved depriving the hens of food for 3 hours and feeding at 11.45h or 12.30h. The order of presentation of the treatments was random, with only one treatment being presented on any one day. Each feeding treatment was repeated once for each specified feeding time.

The hens were scanned every minute in order to record behaviour. During each scan one of the following activities was recorded: feeding, searching behaviour (see chapter 2 for a description), standing (other than during searching or feeding behaviour) and sitting (vacuum nesting activities, such as rotating and pecking were noted and used to assess whether this sitting was part of pre-laying behaviour). The time of oviposition was also recorded. Recording of behaviour started 1 hour before feeding and continued until all hens that were fed during pre-laying behaviour had laid. The start of pre-laying behaviour was defined as occurring when 3 scans of pre-laying behaviour (searching or nesting behaviour) within a five minute period were observed, followed by some aspect of pre-laying behaviour in the next 10 minutes and every 10 minutes thereafter.

Records of pre-laying behaviour for the control treatment were obtained for all hens. However only six hens had pre-laying behaviour interrupted by just the presentation of food (0 hours of deprivation). Six hens (five of which were the same as for 0 hours of deprivation) had pre-laying behaviour interrupted by the presentation of food after 1.5 hours of deprivation. Only 2 hens had pre-laying behaviour interrupted by the presentation of food after 3 hours of deprivation, which was an insufficient sample size for statistical analysis. The data were not normally distributed and so were analysed by non-parametric statistical methods. Wilcoxon matched-pairs test was used to compare differences between feeding treatments and controls. Comparisons between 0 hours and 1.5 hours of food deprivation could not have produced a statistically significant result with the Wilcoxon matched-pairs test due to the small sample size (N=5).

4.3.2 Analysis of results

The durations of pre-laying behaviour, standing, searching, feeding and sitting was compared between feeding treatments and the control. For each record of pre-laying behaviour that was interrupted by feeding an observed and expected duration of the above activities was recorded. The observed durations were the actual durations of the above activities from the time of food presentation until oviposition. The expected durations were obtained from the control treatment. Expected durations were recorded from the point at which food was presented for the interrupted record (measured from the start of pre-laying behaviour) until oviposition. Subtracting the expected durations from the observed durations produced a difference in the duration of various activities for each hen. A difference of zero would represent no difference in duration, whereas a positive difference would represent a longer observed duration and a negative difference a shorter observed duration.

4.3.3 Results

The presentation of food alone resulted in a significant increase in the duration of pre-laying behaviour, compared to the expected duration (Table 4.1). Hens spent more time standing, searching, feeding and sitting but only the differences in searching and feeding were significant. Feeding activity during pre-laying behaviour was uncommon during the control treatment (median feeding duration 0 minutes). However after the presentation of food all hens fed for at least 2 minutes. This increase in feeding does not fully explain the large delay in oviposition. Feeding after 1.5 hours of deprivation also significantly increased time spent feeding and searching, but also time spent standing. Feeding after 3 hours of food deprivation resulted in comparable changes in the two birds observed in the duration of pre-laying behaviour and activities until oviposition (median increase above expected in duration of pre-laying behaviour 20.0 minutes, standing 2.0 minutes, searching 5.5 minutes, feeding 7.5 minutes and sitting 6.0 minutes).

Statistical analysis of the differences between the three feeding treatments was not possible due to the small sample sizes. Examination of the medians does not suggest that there are any large differences between feeding treatments. This may be because the durations of food deprivation used were not sufficient to induce large differences in feeding motivation. However, hens not showing pre-laying behaviour spent increasingly more time feeding after the presentation of food for 0 hours, 1.5 hours and 3 hours of deprivation (Figure 4.1) and this difference was significant (Friedman non-parametric test, dfs = 3, $P<0.01$). Higher feeding durations after the presentation of food may represent high feeding motivation. Furthermore, food deprivation treatments (1.5 or 3 hours) do not appear to cause any large differences in pre-laying behaviour when compared to just the presentation of food. (Table 4.1 suggests that there may be differences between the 0 and 1.5 hours deprivation treatments, but such effects are not supported by the results for the 3 hours of deprivation treatment).

Lastly, a plot of the difference in the duration of pre-laying behaviour against time before expected oviposition (estimated by comparing the duration from the start of pre-laying behaviour until oviposition in interrupted and control records) suggested that the delay in oviposition was related to the timing of feeding (Figure 4.2). A Spearman rank-order correlation on the first interrupted record of pre-laying behaviour for each hen irrespective of deprivation treatment (7 hens had food presentated during pre-laying behaviour) found a strong correlation between the delay in oviposition and the expected time to oviposition (r = -0.86, N=7, P<0.05). However this correlation is confounded by the different deprivation treatments as the first interrupted records were used.

4.3.4 Discussion

Hens were shown to interrupt pre-laying behaviour and feed after the presentation of food despite having continuous access to food. These results suggest that the presentation of food was a strong stimulus for feeding which increased the tendency to feed such that pre-laying behaviour was inhibited. The duration of feeding during pre-laying behaviour appeared to be unrelated to the level of food deprivation, with no apparent differences in the amount of feeding after 0, 1.5 or 3 hours of food deprivation. However hens not showing pre-laying behaviour showed an increasing duration of feeding with increasing deprivation. Duration of behaviour performed when given the opportunity to do so has been interpreted as being a measure of motivation (Manning and Dawkins, 1992), which has been found to correlate well with other measures of feeding motivation (Wood-Gush and Gower, 1968).

Thus the deprivation durations used appeared to produce fluctuations in feeding motivation. It was suggested in the introduction that pre-laying behaviour would resume when the tendency to feed fell below that to perform pre-laying behaviour. This would suggest that hungry hens should take longer before resuming pre-laying behaviour than less hungry hens. There were two possible reasons why this was not found to be the case. Firstly, after long deprivations hens may have fed at a faster rate than hens deprived for shorter periods of time. Fast feeding rates may have resulted in feeding motivation falling more quickly and this could account for the lack of a longer delay before pre-laying behaviour was resumed. Indeed, Savory *et al* (1993) found that the rate of eating in the 10 minutes after food presentation was correlated consistently with the preceding period of deprivation. Alternatively, presentation of food may have elicited feeding, inhibiting pre-laying behaviour. Feeding activity may thus have continued for some time until it terminated itself and so disinhibited the second highest motivational priority (McFarland, 1974b). However, such time-sharing explanations for switches from one behaviour to another have not received much support (Colgan, 1986).

Presentation of food during pre-laying behaviour resulted in a delay in the time of oviposition. This delay was unrelated to the level of hunger, yet could not be attributable to just an increase in the time spent feeding. Large increases in the duration of standing and searching were also observed. Hens were often seen to stand for a short duration between bouts of feeding and pre-laying behaviour and this may have accounted for the increased time spent standing. The finding that delay of oviposition was related to the point during the pre-laying behaviour sequence when food was presented suggested a possible link between the two. For food presentation during the early stages of pre-laying behaviour (searching phase), hens may have been able to resume pre-laying behaviour at the stage where the interruption occurred. However, for food presentation during the sitting phase, hens may have to perform some searching behaviour before proceeding with sitting on the nest. In the ancestor of the domestic hen, such an adaptation would ensure that the hen sits in a desirable nest site (Wood-Gush, 1983) even if it is interrupted during pre-laying behaviour. The second experiment examined in more detail the relationship between delay in oviposition and time of feeding.

4.4 Experiment 2: The relationship between the delay in oviposition and stage during the pre-laying behaviour sequence when food was presented.

The results of experiment 1 suggested that the delay in oviposition was related to the stage during the pre-laying sequence when food was presented. A second experiment was performed with a larger sample size and not confounded by various treatments to examine this effect. Interrupted pre-laying records were obtained when food presentation (topping up of food troughs) occurred during pre-laying behaviour. These records were compared with un-interrupted records of pre-laying behaviour.

4.4.1 Materials and methods

Seventeen HiSex Brown laying hens, reared in deep litter pens and kept in modified cages (Edinburgh Modified Cage, Appleby, 1993) from 18 weeks old were used in this study. At 40 weeks old hens were individually housed in battery cages (without nest boxes). Food and water were provided *ad lib* with the hens being fed at 15.00h every day. Lights were on from 8.00h till 00.00h. A daily record of eggs for each hen was kept from 46 weeks old with observations starting two weeks later.

Observations on the hens were performed on five days per week to record pre-laying behaviour under two treatments. On two of these days hens were fed at 15.00h (control), on the other three days (interrupted) the food was topped up at 11.10h. Sufficient food was given to the hens so that they never ran out. The order of presentation of the treatments was random over the five days. Hens were fed at 15.00h on the two days per week that no observations were made. This was repeated for 6 weeks.

Observations were performed from 10.00h (pre-laying behaviour rarely started before this time) until the oviposition of all hens that had started pre-laying behaviour before 12.30h. Hens were scanned every minute and the following activities recorded: posture (standing, sitting or laying squat), feeding and searching behaviour.

The data were handled as in experiment 1 to calculate the difference (observed minus expected) in the duration of pre-laying behaviour, standing, searching, feeding and sitting. The duration of the sitting phase was the duration from the first observation of sitting on the nest site until oviposition. Control and interrupted pre-laying behaviour records were obtained for 11 hens, with two or more interrupted records obtained for 10 of these hens. The data fitted the requirements for parametric statistics and were analysed using the related samples t-test.

4.4.2 Results

The first interrupted nesting record was used to confirm the findings of experiment 1. Again, presenting food during pre-laying behaviour resulted in a significantly longer duration of pre-laying behaviour (Table 4.2, Figure 4.3). This increased duration was partly due to significant increases in the duration of standing, searching and feeding. The duration of sitting after food presentation was not significantly greater than expected.

A regression analysis was performed using all the data obtained (25 interrupted records from 11 hens). The observations were therefore not independent, but were used to give an indication of the relation between delay in oviposition and stage during the pre-laying behaviour sequence when food was presented. The delay in oviposition increased as food presentation became closer to the expected time of oviposition (linear regression, F=14.22, P<0.001, Figure 4.3). Figure 4.3± suggested that a curve might account for the variability better than a straight line. Delays when fed 15 minutes or more before the expected oviposition appeared to be about 0 minutes, whereas if fed nearer to oviposition delays are considerably greater. A logistic curve was found to offer the best fit (Gompertz curve, Genstat 5 committee, 1987). The logistic curve had upper and lower asymptotes of delay in oviposition of 22.8 and -0.1 minutes respectively. The point of inflexion was 15.8 minutes before expected oviposition. This coincided reasonably well with the mean duration of the sitting phase for control records of pre-laying behaviour (mean= 13.0±3.8 minutes). However the amount of variability in delay which was attributable to variability in timing of food presentation was the same as for a least-squares regression line (r-sq.= 36.5%). Thus a logistic curve was no better than a straight line at predicting the delay in oviposition from the stage during the pre-laying behaviour sequence when food was presented.

The first 2 interrupted nesting records for each hen were used to examine the above mentioned interaction in a balanced analysis. Firstly, a related-samples t-test was performed on the differences between the first two interrupted records. Analysis showed that the probability of a significant result was not less than 30%. Secondly, the two records for each hen were divided according to when food was presented. The nearer category represented food presentation nearer to the expected time of oviposition than the further category. Food presentation for the nearer category was on average 2.2(±2.3) minutes before expected oviposition compared to 11.9(±3.5) minutes for the further category. T-tests showed that the delay in oviposition was significantly longer for the nearer category. This category also showed a considerably longer duration of searching behaviour (Table 4.3).

Two eggs (from two different hens) were laid without any obvious pre-laying behaviour after food was presented. For one of them food was presented 1 minute before oviposition was expected. The hen then fed for 11 minutes continuously before the egg was dropped also while feeding. For the other record food was presented 5 minutes before oviposition was due. The egg was dropped 25 minutes later while the hen was standing in the front of the cage. The hen spent 13 minutes of this time feeding and the remainder was spent standing. Both eggs appeared normal (not banded or dusted) after examination.

4.4.3 Discussion

The first interrupted records of 11 hens were analysed and the results confirmed the findings of experiment 1. A delay in oviposition after feeding was again observed with significant increases in the duration of standing, searching and feeding. The delay in oviposition was found to be related to the stage during the pre-laying behaviour sequence when food was presented.

Feeding near to the expected time of oviposition resulted in a longer delay in oviposition than if birds were fed further from the expected time of oviposition. Hens sat for similar amounts of time after resuming pre-laying behaviour for both categories and this was not significantly greater than expected. Thus sitting appeared to be unaffected by food presentation and continued for the expected length of time when pre-laying behaviour was resumed. This is different to what appeared to occur to searching behaviour. Feeding near to the expected time of oviposition produced a greater difference in the duration of searching before oviposition than feeding further from the expected time of oviposition. It therefore seemed that hens could, to some extent, resume pre-laying behaviour from where the interruption took place if fed early during pre-laying behaviour. However if fed near oviposition hens showed a large amount of searching behaviour when pre-laying behaviour was resumed. One interpretation of this is that a certain amount of searching behaviour was necessary before hens continued sitting on the nest site. The performance of searching behaviour so late in pre-laying behaviour may have served to ensure that the hen lays in a "good" nest site even after interruption ("good" refers to qualities of the nest which convey good reproductive success, Wood-Gush, 1983).

The finding that a logistic curve describes the relationship between the delay in oviposition and the stage during the pre-laying behaviour when food is presented offers some support for the above suggestion. The point of inflexion of the logistic curve roughly coincided with the start of the sitting phase. Thus perhaps the delay in oviposition is related to the phase of pre-laying behaviour at which food is presented. If food is presented during the searching phase, hens may resume pre-laying behaviour at the stage where they would be if there had been no interruption, thus oviposition is not delayed. However, if the interruption occurred during the sitting phase, hens may have to perform some exploratory behaviour before resuming with the sitting phase. Oviposition would then be delayed, assuming that oviposition time can be regulated voluntarily such that it occured when the hen was on the nest. This conclusion is tentative as the logistic curve was no better at predicting the relationship between delay in oviposition and timing of feeding than a straight line. A more conclusive result might be obtained with a larger sample size which overcomes the large amount of unaccounted variation observed.

On two occasions pre-laying behaviour was overridden as suggested by Rasmussen (unpublished). No pre-laying behaviour was observed on either of these occasions after feeding. Interpretation of these results requires an understanding of the mechanisms that delay oviposition and control pre-laying behaviour. Delay of oviposition may occur due to a variety of stressors or disturbances, such as social interference or being handled, experienced before or during pre-laying behaviour (Perry *et al*, 1971b; M. Reynard, personal communication). Feeding behaviour has been observed to delay oviposition in restricted-fed birds (Wilson and Keeling, 1991), though it does not appear to influence the number of floor eggs (Hearn, 1981). It may be that for the two records mentioned above suppression of pre-laying behaviour was greater than the delay in oviposition, resulting in the egg being laid during feeding behaviour. The reasons why oviposition was not delayed enough, or pre-laying behaviour did not resume as quickly as in other hens remains puzzling.

Alternatively, oviposition during feeding behaviour may have been due to the termination of pre-laying behaviour. Pre-laying behaviour without oviposition has been observed on a number of other occasions (personal observation). Pre-laying behaviour under these circumstances has ended abruptly after an unusually long duration and the egg (which has usually been banded) has been laid during the course of other activity about 3-5 hours later. Ovulation can only occur during a certain period of the day

(Fraps, 1955; Etches, 1990), so it is likely that pre-laying behaviour, which depends on ovulation can also only occur in a certain period of the day. If oviposition is delayed by mechanisms unrelated to ovulation (Shimada and Saito, 1989) beyond this period it will not be preceded by pre-laying behaviour. This may provide an explanation for the two occasions when eggs were laid without pre-laying behaviour. That is, feeding behaviour may have suppressed oviposition and pre-laying behaviour past its termination point, such that oviposition occurred without pre-laying behaviour. Although this provides a possible explanation for the observed phenomenon, there are several discrepancies between the records reported here and other records of pre-laying behaviour presumably ending before oviposition. In this study pre-laying behaviour was not greatly extended, the egg was laid less than 30 minutes after the termination of pre-laying behaviour and the egg had a normal (non-banded) appearance.

4.5 Experiment 3: Pre-laying behaviour in the absence or presence of food.

The previous two experiments have shown that pre-laying behaviour can be suppressed by feeding activity. This suppression is interpreted as hens feeding until the tendency to feed falls below that for pre-laying behaviour. It is unlikely that the inability to feed would influence pre-laying behaviour once the latter has started, as feeding appears to be suppressed during pre-laying behaviour (Wood-Gush and Horne, 1970). However the onset of pre-laying behaviour may depend on the competition for expression of pre-laying behaviour and other activities. The hypothesis being tested in the following experiment is that pre-laying behaviour would start earlier in the absence of food than in its presence. Furthermore, if hens are highly motivated to search for food (i.e. after a long deprivation), the possibility that the start of pre-laying behaviour may be delayed will be examined.

4.5.1 Materials and methods

Twenty ISABrown medium hybrid laying hens reared in deep litter pens were individually housed in cages with enclosed littered nests from 17 weeks of age (point of lay at about 18 weeks). Water and food were provided *ad lib* in individual feeders which could be removed. Lights were on from 08.30h till 00.30h. A daily record of eggs laid in these cages and their location (nest or cage) was kept from 22 weeks of age. Hens were 26 weeks old at the start of the experiment which lasted for 5 weeks.

Each hen was presented with each of three treatments. For T1 (control), hens had continuous access to food which was topped up at 15.00h. For T2 (long deprivation), food was removed at midnight, then being replaced and topped up at 15.00h. For T3 (short deprivation), food was removed at 08.45h, again being replaced and topped up at 15.00h. Presentation of each treatment involved three days of familiarisation followed by 4 days of filming to record behaviour. Treatments were presented consecutively in the following order, T1-T2-T1-T3-T1. The control treatment was repeated to identify any variations in pre-laying behaviour with time or as a consequence of testing.

Behaviour was recorded with the aid of two video cameras and video cassette recorders. Ten hens were filmed from 08.30h until 15.30h on filming days. Thus a maximum of 2 nesting records were obtained for each hen per presentation of each treatment. The video recordings were used to record behaviour during three different periods:

1) Pre-laying behaviour (from 8.45h until oviposition). The location (cage or nest box) of the hen and occurrence of searching behaviour (Chapter 2) were recorded at 1 minute intervals. The number of nest examinations and entries per minute were also recorded. The time of oviposition (revealed by the hen squatting and straining) was also recorded.

2) Post-laying behaviour (recorded for 30 minutes from the last nest box exit). This was only recorded for the egg laid nearest to 11.00h for each treatment. The number of steps and bouts of cage inspections were recorded for each 5 minute period. Cage inspections involved inspecting the cage corners, under the feeder and front of the cage. They were performed with an outstretched neck accompanied with sideways movement of the head. A bout consisted of moving towards a suitable inspection site (e.g. corner of the cage), inspecting it and then moving away from it.

3) Feeding behaviour. This was recorded once for each hen for each treatment. The number of pecks at the food in a 1 minute period was recorded 5, 10 and 15 minutes after food was replaced or topped up.

Complete nesting records for all three treatments were obtained for 12 hens. Of the 8 hens that failed to produce a complete set of data, one hen laid intermittently whereas the remainder laid on most days but started pre-laying behaviour before lights-on. The start of pre-laying behaviour and the different phases were determined as described in chapter 2. The data fulfilled the requirements for parametric analysis and were analysed using an ANOVA test with block structure provided by individual hens.

4.5.2 Results

4.5.2.1 Pre-laying behaviour

The duration of pre-laying behaviour was significantly longer in the food deprived situations (Table 4.4). Much of this variation was due to significantly longer searching phases in the food deprived situations, with sitting phase duration being similar between treatments. The later stages of pre-laying behaviour were largely unaffected with the number of nest examinations during the sitting phase, nest entries and total time in the nest box not varying significantly between treatments.

During the searching phase hens spent significantly more time searching (Figure 4.4) and performed significantly more nest examinations (Figure 4.5) in the food deprived situations. This suggests that the searching phase in the food deprived treatments is being extended. This extension is likely to be due to pre-laying behaviour starting earlier for the food deprived treatments when compared to the control. The alternative possibility, that nest entry was delayed was unlikely as the nest occupancy was unaffected by the treatments (Figure 4.6).

4.5.2.2 Post-laying behaviour

Increased exploratory behaviour in the earlier stages of pre-laying behaviour could be due to a general increase in exploratory behaviour during food deprivation. To test this, exploratory behaviour was examined in the 30 minutes after oviposition (during this time food was absent for T2 and T3). No significant differences were found in the number of steps or cage inspections during this time (Table 4.5). Figures 4.7 and 4.8 suggest that hens in the control treatment were walking less and performing fewer cage inspections immediately after oviposition. This reduced activity for the control treatment was likely to be due to hens feeding repeatedly immediately after oviposition, as has been reported elsewhere (Wood-Gush, 1975a; Meijsser and Hughes, 1989); in the experimental treatments food was not available.

4.5.2.3 Feeding behaviour

The number of pecks at the food after feeding was measured to provide an estimation of feeding motivation. The results suggested that hens were more hungry in both food deprivation treatments than in controls (Table 4.6). There were no significant differences in the rate of pecking between the two deprivation treatments 5 and 10 minutes after food presentation (related-sample t-test, N=20, t = 1.4 (P=0.2) and t =0.4 (P=0.7) respectively). However the difference between these two treatments was significant after 15 minutes (related-sample t-test, N=20, t =3.6, P<0.01).

4.5.3 Discussion

In the absence of food, the durations of pre-laying behaviour and the searching phase were longer than in the presence of food. Hens also performed more searching behaviour and made more nest examinations under these circumstances. These results indicate that in the absence of food the searching phase either starts earlier or nest entry is delayed. Significant differences in nest occupancy and the duration of the sitting phase were not found between treatments. Differences in nest occupancy and the duration of the sitting phase would be expected if the first nest entry was being delayed. Therefore the results suggest that pre-laying behaviour is starting earlier in the absence of food rather than nest entry being delayed. This is consistent with the hypothesis presented in the introduction that pre-laying behaviour starts earlier than expected if hens are unable to express their tendency to feed. A second interpretation of these results is that hens are strongly motivated to feed in the absence of food. This tendency to feed may be redirected (through an increase in arousal) for the expression of pre-laying behaviour (Lorenz, 1950).

There is another possible explanation for the finding that pre-laying behaviour starts earlier in the absence of food than in its presence. It may be that hens generally perform more exploratory behaviour in the absence of food. This was assessed by examining the number of steps and nest inspections in the 30 minutes after oviposition, when food was still absent for the food deprived treatments. No significant differences in the number of steps or bouts of cage inspections were found between treatments. Thus there was no indication that hens are indeed more active in the absence of food. Other studies of the behaviour of caged laying hens confirm this finding. Preston (1987) found no differences in locomotor activity between food deprived and *ad lib* fed hens.

Two levels of food deprivation were used in this experiment. It was hypothesized that if hens were very hungry they would be strongly motivated to search for food, resulting in pre-laying behaviour starting later than if hens were food deprived but not as hungry. However, although the results suggested that the deprivation treatments used increased feeding motivation, there appeared to be little differences between them. This is likely to account for the lack of any significant variations in pre-laying behaviour between the two deprivation treatments. There are more accurate methods of assessing feeding motivation than the one used in this study (e.g. by applying consumer demand theory, Dawkins, 1990). These methods of assessing feeding motivation did not seem appropriate as the method used in this experiment has been found to correlate well with other methods for hens (Wood-Gush and Gower, 1968; Savory *et al* 1993) and hamsters (DiBattista and Bedard, 1987).

4.6 Summary

The presentation of food during pre-laying behaviour confirmed the findings of other studies that showed that feeding behaviour can suppress pre-laying behaviour (A. Rasmussen, Unpublished). However, for hens showing pre-laying behaviour the duration of feeding was found to be unrelated to the length of deprivation, though the rate of feeding may have varied. Oviposition was delayed considerably as a result of feeding activity. This delay was found to be related to the phase during the pre-laying behaviour sequence when food was presented. Results suggested that hens interrupted during the early stages (searching phase) of pre-laying behaviour resumed pre-laying behaviour at the point at which interruption took place. Hens interrupted during the later stages (sitting phase) of pre-laying behaviour performed more searching behaviour than expected upon resuming pre-laying behaviour. This may be an adaptation which ensures that hens lay in a suitable nest site after interruption. In the absence of food, pre-laying behaviour was found to start earlier than in its presence.

This suggests that the tendency to perform pre-laying behaviour interacts with other behavioural tendencies to determine the start of pre-laying behaviour.

Table 4.1: Effect of feeding on pre-laying behaviour in experiment 1: difference (experimental minus control) in the duration (mins) of various activities produced by feeding birds after 0h or 1.5h deprivation. Medians (inter-quartile range) are presented.

	Duration of food deprivation			
Activity	0 hours	P.	1.5 hours	P.
Pre-laying behaviour	24.0 (6.8-31.2)	*	32.0 (28.5-37.3)	*
Standing	3.0 (0.8-6.4)	+	5.0 (2.5-15.5)	*
Searching behaviour	6.5 (1.8-13.1)	*	3.0 (2.0-6.8)	*
Feeding	5.5 (4.3-11.0)	*	8.0 (4.8-17.5)	*
Sitting	0.5 (-1.0-8.5)	N.S.	9.5 (5.3-16.5)	+

+ P<0.1, * P<0.05, by Wilcoxon test (N=6).

Table 4.2: Effect of feeding on pre-laying behaviour in experiment 2: Difference (experimental minus control) in the estimated duration (minutes) of various activities produced by feeding birds. Means (S.E.M.) are presented.

Activity	Mean (S.E.M)	P.
Pre-laying behaviour	22.8(±4.0)	***
Standing	5.1(±1.5)	**
Searching behaviour	5.5(±1.4)	**
Feeding	6.2(±1.5)	**
Sitting	3.7(±2.8)	N.S.

** P<0.01, *** P<0.001, by Wilcoxon test (N=11).

Table 4.3: Means (S.E.M.) of the difference (experimental minus control) in the estimated duration (minutes) of various activities divided into two categories depending on feeding time (nearer and further from the expected laying time). Table also shows probability using a related samples t-test (N=10).

Activity	Nearer	Further	P.
Pre-laying behaviour	24.3(±2.8)	11.9(±4.2)	**
Standing	3.8(±1.5)	1.7(±1.7)	N.S.
Searching behaviour	6.8(±1.6)	2.7(±1.6)	*
Feeding	6.9(±1.2)	5.9(±1.9)	N.S.
Sitting	2.6(±1.5)	1.2(±1.2)	N.S.

* P<0.05, ** P<0.01.

Table 4.4: Means (S.E.M.) for various aspects of pre-laying behaviour (estimated durations in minutes) for 3 treatments and results of ANOVA (degrees of freedom, 2, 22).

Activity	Food deprivation (treatment) None	Short	Long	ANOVA P.
Duration of pre-laying behaviour	72.6(±7.0)	98.3(±9.0)	100.7(±9.3)	***
Duration of searching phase	8.6(±2.3)	32.2(±7.7)	28.8(±5.0)	***
Duration of sitting phase	62.6(±6.5)	63.3(±7.3)	73.5((±7.7)	N.S.
Number of nest box entries	4.0(±1.0)	3.1(±0.4)	3.4(±0.4)	N.S.
Total duration in nest box	45.9(±4.1)	45.4(±4.5)	53.7(±5.6)	+
Number of nest examinations during searching phase	6.9(±2.2)	11.6(±2.7)	12.1(±3.0)	*
Number of nest examinations during sitting phase	5.5(±1.4)	9.1(±3.4)	7.9(±2.0)	N.S.

+ $P<0.1$, * $P<0.05$, *** $P<0.001$.

Table 4.5: Mean (S.E.M.) number of steps and bouts of cage inspections in the 30 minutes after oviposition for 3 treatments. Results of ANOVA are presented.

Activity	Food deprivation (treatment) None	Short	Long	ANOVA P.
Number of steps	10.9(±1.2)	16.0(±1.9)	16.0(±3.7)	N.S.
Number of cage inspections	17.6(±1.2)	18.0(±1.8)	16.7(±1.9)	N.S.

Table 4.6: Mean (S.E.M.) number of pecks at food per 1 minute at three time periods after food presentation for 3 treatments. Results of ANOVA are presented.

		Food deprivation		ANOVA
Time after feeding	None	Short	Long	P.
5 minutes	56.6(±3.4)	72.4(±3.6)	79.1(±4.0)	***
10 minutes	45.7(±3.8)	56.9(±3.7)	59.1(±5.8)	*
15 minutes	17.3(±4.5)	60.0(±3.1)	43.2(±4.9)	***

* $P<0.05$, *** $P<0.001$.

Figure 4.1: Estimate of the mean duration of feeding behaviour in the 30 minutes after the presentation of food.

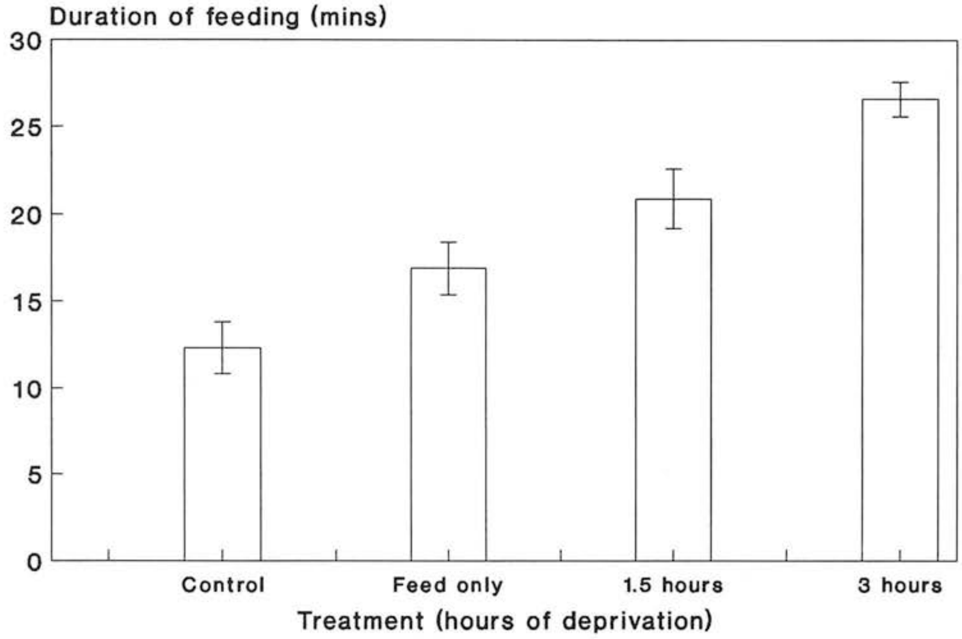


Figure 4.2: Effect of the timing of feeding on the delay in oviposition (showing first values for each hen).

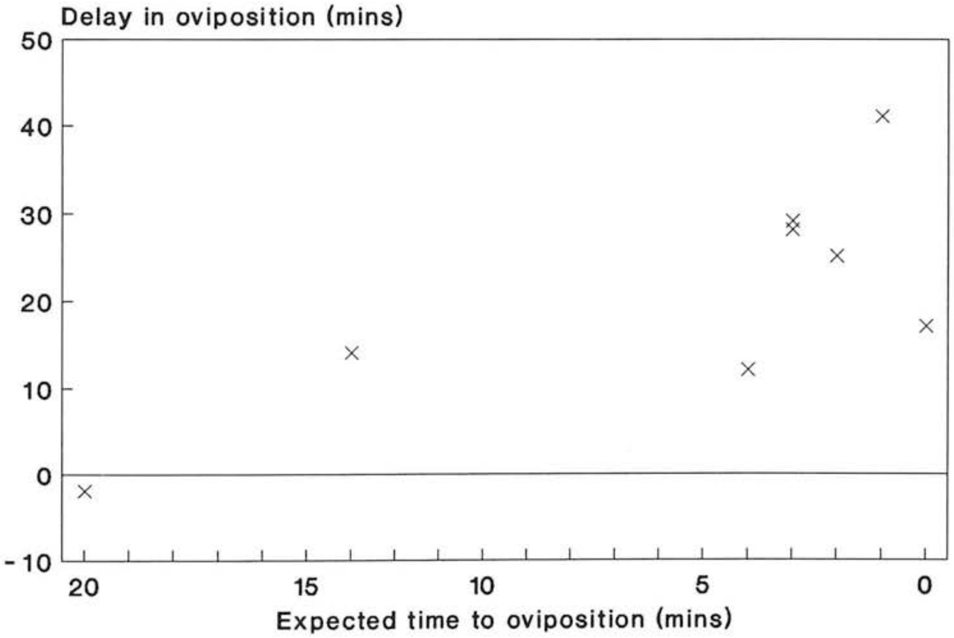


Figure 4.3: Effect of the timing of feeding on the delay in oviposition (experiment 2).

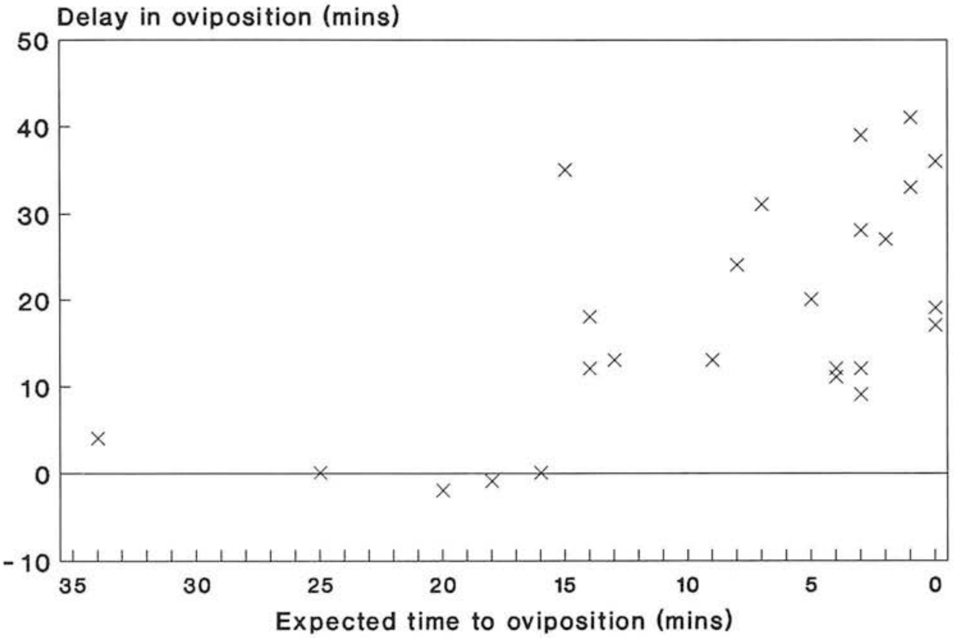


Figure 4.4: Estimation of the percentage of time spent searching in the 2 hours before oviposition.

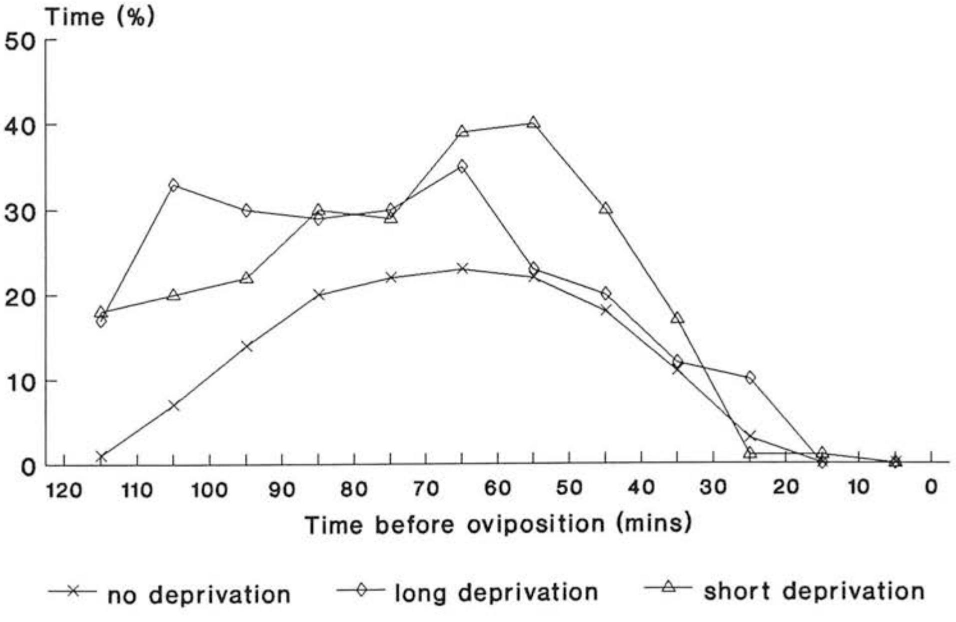


Figure 4.5: Mean number of nest examinations per bird in the 2 hours before oviposition.

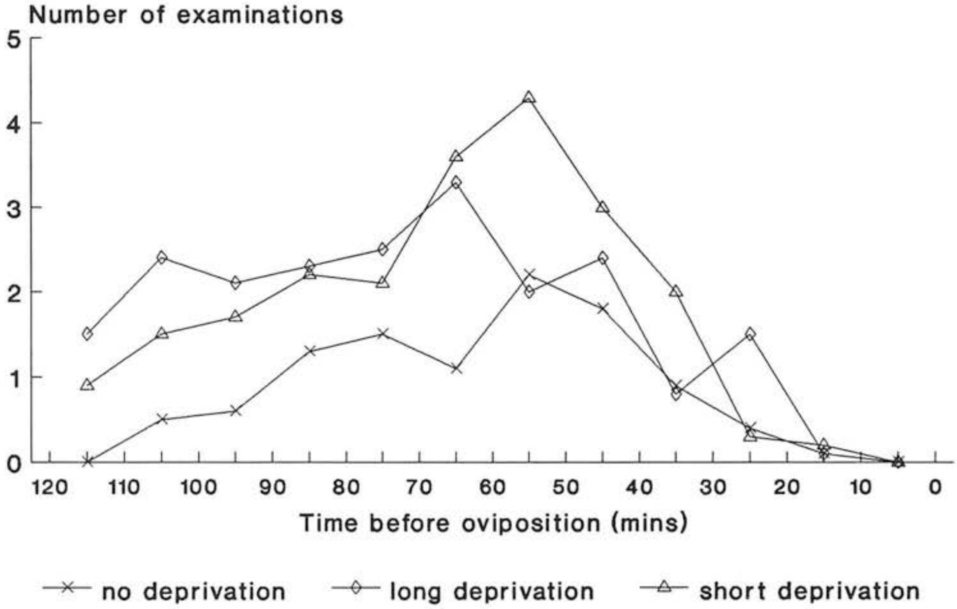


Figure 4.6: Estimation of the percentage of time spent in the nest box in the 2 hours before oviposition.

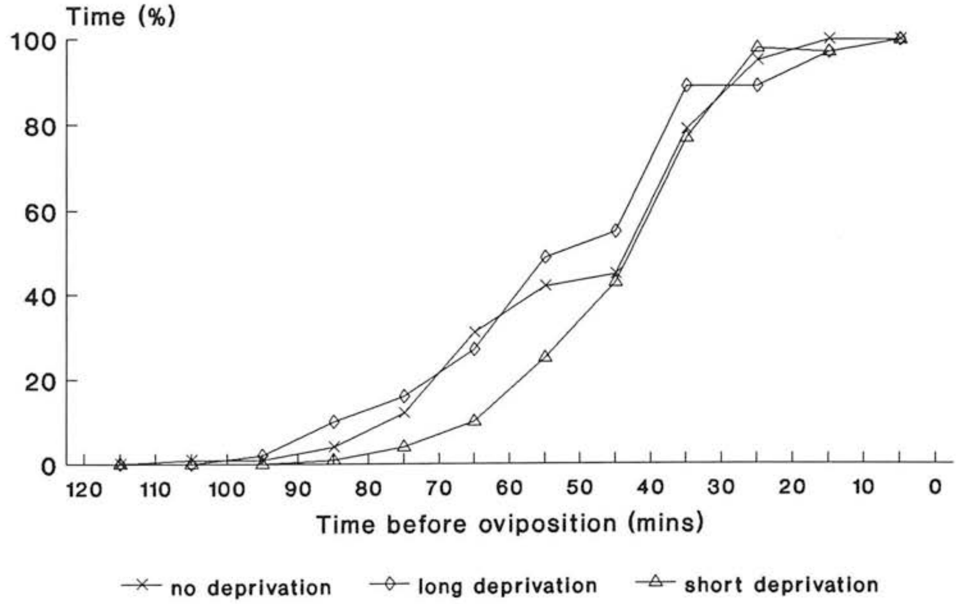


Figure 4.7: Mean number of steps in each 5 minute period over the 30 minutes after oviposition.

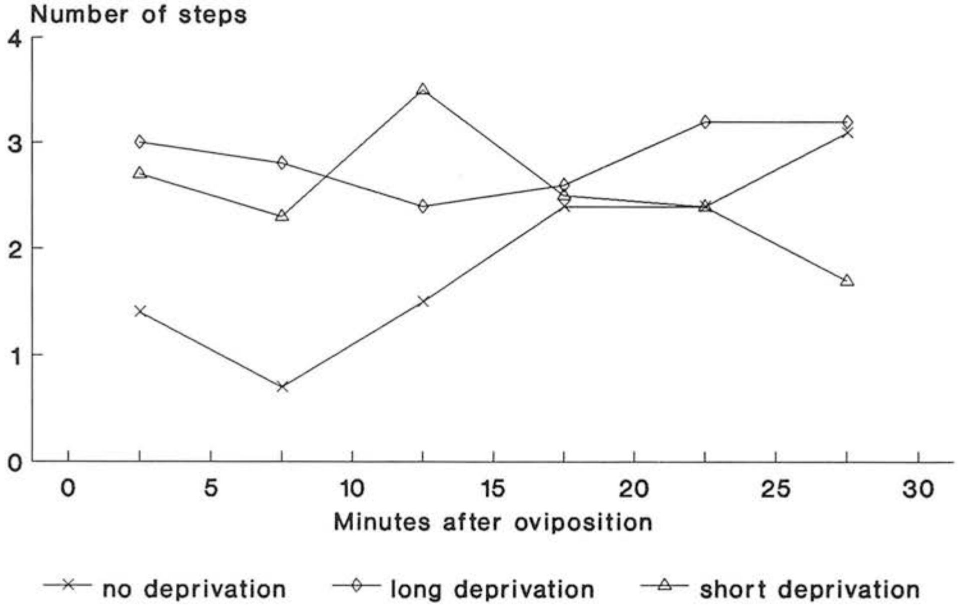
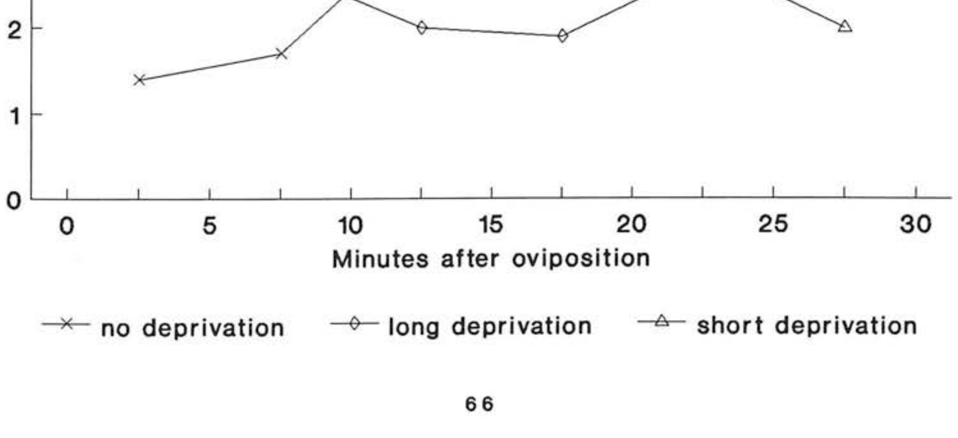


Figure 4.8: Mean number of bouts of cage inspections in each 5 minute period over the 30 minutes after oviposition.



CHAPTER 5: **Effects of nest quality and other cues for exploration on pre-laying behaviour**

5.1 Abstract

1) The persistence of searching behaviour during the pre-laying period in the presence of a suitable nest site may indicate that hens are strongly motivated to perform this behaviour. This was investigated by observing behaviour in environments with or without a suitable nest site and other cues for exploration.

2) Eleven hens were tested individually in four environments determined by a 2 by 2 experimental design: presence or absence of litter and presence or absence of an exploratory walkway. The order of testing in each of the four environments was balanced as far as possible.

3) Comparing pre-laying behaviour in unlittered with that observed in littered nest boxes, longer durations of pre-laying behaviour (151.2(±20.2) compared to 110.7(±13.9) minutes, ANOVA dfs=1,27, $P<0.05$) and searching phase (31.8(±5.8) compared to 19.0(±3.3) minutes, ANOVA dfs=1,27, $P<0.05$) were observed. Hens also spent more time searching (26.6(±4.6) compared to 14.3(±4.1) minutes, ANOVA dfs=1,27, $P<0.01$) and performed more nest examinations (26.7(±7.9) compared to 9.6(±3.1), ANOVA dfs=1,27, $P<0.01$) in environments with a littered nest box. More entries were recorded into unlittered nest boxes (11.0(±2.0) compared to 7.2(±1.4), ANOVA dfs=1,27, $P<0.05$) though of a shorter mean duration (8.4 (±1.9) compared to 16.2(±5.9) minutes, ANOVA dfs=1,27, $P<0.01$).

4) The duration of the searching phase was longer in environments with the walkway than without (34.7(±6.0) and 16.1(±4.1) minutes respectively, ANOVA dfs=1,27, $P<0.01$). In environments with the walkway, more searching behaviour (28.2(±5.1) compared to 12.7(±3.0) minutes, ANOVA dfs=1,27, $P<0.001$) and more nest examinations (7.9(±1.5) compared to 4.1(±1.0), ANOVA dfs=1,27, $P<0.01$) were recorded.

5) It is suggested that in the absence of an appropriate nest site the motivation to perform searching behaviour remains high. This high motivation for searching may disrupt the later stages of pre-laying behaviour. However, when compared to barren environments, environments that provide cues for exploration result in the motivation to perform pre-laying behaviour being expressed earlier.

5.2 Introduction

In the early stages of pre-laying behaviour, domestic hens show increased locomotion and searching behaviour, exploration with characteristic movement of the head, usually accompanied by the nesting call and nest examinations (Wood-Gush, 1975b). In environments with nest boxes this behaviour starts about two weeks before the first oviposition and may serve to gather information about potential nest sites, homologous to nest-searching behaviour in non-gallinaceous birds (Rietveld-Piepers, 1993). However unlike many other birds, domestic hens and other gallinaceous birds will show searching behaviour before each oviposition. Indeed, this behaviour is observed in penned hens that lay successive eggs in the same nest (Wood-Gush, 1954) and in caged hens laying in a nest box provided (Appleby, 1990).

Searching behaviour can be called an appetitive behaviour, its function being to obtain information about a nesting site (reinforcer). It has been argued that the performance of behaviour is a strong reinforcing stimulus itself (Hughes and Duncan, 1988). For example laying hens will show similar amounts of nest building behaviour if provided with a preformed nest or a flat litter surface (Hughes *et al*, 1989). Thus the persistence of searching behaviour in the presence of a suitable nest site may signify that the hen is strongly motivated to perform this behaviour. If so, does a small barren environment provide the external factors that allow the full expression of this behaviour?

In the absence of external cues that act as releasers for the performance of appetitive behaviour, animals may redirect their appetitive behaviour towards other stimuli. For example, Breland and Breland (1961) reported that pigs taught to pick up wooden tokens and deposit them in a bank to obtain a food reward started to root at the tokens and showed slower responses after a few weeks. This and other findings (see Wood-Gush and Vestergaard, 1989 for review) indicate that animals are strongly motivated to perform appetitive behaviour. When there are more appropriate stimuli, appetitive behaviour may increase. This may account, for example, for the large amount of time that hens fed from troughs spend pecking in litter containing very little food (Wennrich, 1980). Thus a decrease in the amount of behaviour performed in a barren environment, when compared with an environment containing more appropriate stimuli may indicate that this behaviour is not being fully expressed. Expression of behaviour may be an indicator of motivation (Dawkins, 1990) and it provides a valuable starting point for further measures of motivation.

In battery cages some hens show excessive pacing during pre-laying behaviour which may be indicative of frustration (Duncan, 1970). This frustration appears to arise because hens don't find the relevant stimuli that takes them from the searching phase to the next stage. Allowing these hens access to a nest box greatly reduces the incidence of this pacing and increases the duration of sitting on the nest (Sherwin and Nicol, 1993b). Thus there is no doubt that the provision of a nest site helps in the satisfactory expression of pre-laying behaviour (Appleby, 1990). However, the number of times that hens sit down on the nest during pre-laying behaviour varies considerably between environments with nest sites (Meijsser and Hughes, 1989; Sherwin and Nicol, 1993b). Meijsser and Hughes (1989) reported that hens in a perchery system made more nest entries than hens in deep litter or strawyard systems. The perchery system had considerably smaller floor area and was more crowded, thus is likely to represent an environment less suited to exploratory behaviour. All these environments contained littered nests but there were also nest sites with other substrates in the perchery and deep litter systems which may have been a complicating factor. Nonetheless the less settled sitting on the nest observed in the perchery may have resulted from a failure to extinguish the motivation to perform searching behaviour.

The differences in pre-laying behaviour of hens with or without access to nest sites has received considerable attention (Brantas, 1980; Meijsser and Hughes 1989; Appleby 1990). These differences in behaviour have led to suggestions that in the absence of nest boxes pre-laying behaviour is poorly expressed, which may be detrimental to welfare (Meijsser and Hughes, 1989), or that the behaviour expressed is indicative of frustration (Brantas, 1980). Hens show a strong preference to lay in nest boxes with loose litter (Appleby *et al*, 1988b; Breden *et al*, 1993; Petherick *et al*, 1993). This preference may signify a strong motivation to lay in a littered nest. If so, this may present a problem for the design of welfare improving modified cages (e.g. Appleby, 1993) as these designs usually have rollaway nests which cannot incorporate litter. Pre-laying behaviour in environments with or without littered nest has not been investigated in detail. Differences in pre-laying behaviour between these environments may indicate whether hens are strongly motivated to lay in a littered nest.

The aims of the experiment reported here were to assess firstly whether hens perform more searching behaviour in environments that provide more stimuli for exploratory behaviour and secondly, whether more searching behaviour results in a more settled sitting phase (fewer nest entries for longer duration). Lastly, are there differences in pre-laying behaviour in environments with a littered or an unlittered nest box? A 2 by 2 factorial experimental design was used. The presence or absence of litter provided the two levels of one factor and the presence or absence of an exploratory walkway provided the two levels of the other factor.

5.3 Materials and methods

Sixteen ISABrown medium hybrid laying hens were wing tagged to allow identification of individuals. Hens were placed in a deep litter pen (home pen) measuring 2.7m x 3.0m with 8 individual nest boxes in two tiers. Food and water were supplied *ad libitum*. Lights were on for 16 hours from 09.00h until 01.00h. Hens were kept under these conditions for 4 weeks before the start of training at 30 weeks of age.

Training was performed to familiarise the hens with laying in a new environment after being removed from the home pen. The training environment consisted of a small pen measuring 0.6m x 0.7m and made from wire mesh 0.6m high with an individual littered nest box attached. Food and water were provided *ad libitum* in troughs outside the pen (Figure 5.1). Training involved two stages. Firstly, hens were palpated and a hen without a hard shelled egg was removed from the home pen and placed in the training pen for 4-6 hours. This was repeated once for each hen. Secondly, a hen with a hard shelled egg (detected by palpation) but which was not showing pre-laying behaviour (deduced after 5 minutes of observations) was removed from the home pen and placed in the training pen until oviposition. This was also repeated once for each hen. All hens laid in the nest box during the second stage of training.

After training, hens (now 34 weeks of age) were tested in four different experimental pens. The procedure for testing involved watching hens in the home pen for 5 minutes from lights-on to assess whether they were showing pre-laying behaviour. A hen with a hard shelled egg but not showing pre-laying behaviour was removed and placed in a previously assigned experimental pen until oviposition. A video recorder was used to record activity throughout this time.

All experimental pens consisted of a pen as used in the training situations. One factor being examined was the influence of access to a walkway 0.6m high and constructed from wire mesh with a plastic lining 0.3m high (Figure 5.1). Hens could gain access to this walkway from the experimental pen via two openings. Another factor being examined was the influence of litter in the nest box. Litter was present in some nest boxes (about 2cm deep) and absent in others (substrate being flat wooden floor). The two levels (presence or absence) of each factor (nest quality and pen layout) were tested in a 2 x 2 factorial design.

Eleven hens were tested on all four treatments (one hen stopped laying just after training) with at least two days between presentation of each treatment. The design was balanced as far as possible by testing roughly equal numbers of each treatment for each position (i.e. first trial, second etc.). The order of presentation of treatments was randomised within this structure to ensure that any carry-over effects influence the treatments equally.

From the video recordings, the position of the test bird was noted at one minute intervals (nest box or pen). It was also noted if the hen was showing searching behaviour at each of these observations. Nest examinations (inserting head into nest box) were recorded whenever they occurred. Nest entries that were not registered by the interval recordings were noted as entries with a duration of less than one minute. The time of oviposition was also recorded.

The start of pre-laying behaviour was defined as in chapter 2. Statistical analysis was performed by an ANOVA for a 2 x 2 factorial experiment (Genstat 5 committe, 1987). This was decided after checking for normality and homogeneity of variance of the data.

5.4 Results

Treatments affected pre-laying behaviour in a number of ways (Table 5.1).

5.4.1 Effects of littered nest

The duration of the searching phase was significantly longer in environments with an unlittered nest box (ANOVA, $F=6.9$, $dfs=1,27$, $P<0.05$); this was largely responsible for a longer total duration of pre-laying behaviour in these environments (ANOVA, $F=6.2$, $dfs=1,27$, $P<0.05$). Although the duration of the sitting phase did not vary significantly (ANOVA, F=3.9, dfs=1,27, N.S.), differences in behaviour were observed throughout the whole of the pre-laying period with respect to nest quality.

Component behaviour patterns shown during the searching phase were examined in more detail. An unlittered nest significantly increased the duration of searching behaviour (ANOVA, F=10.4, dfs=1,27, P<0.01) and number of nest examinations during this phase (ANOVA, F=21.6, dfs=1,27, P<0.001, Figures 5.2 and 5.3). The percentage of time spent performing searching behaviour did not differ significantly with respect to nest quality (ANOVA, F=0.2, dfs=1,27). This suggests that the searching phase is indeed longer in environments with an unlittered nest, rather than such a result arising from an inaccurate estimation of the start of pre-laying behaviour. The number of nest examinations during the sitting phase was also significantly greater in environments with unlittered nest boxes (ANOVA, F=12.7, dfs=1,27, P<0.001). Thus behaviour associated with the searching phase was more frequently observed throughout the whole of pre-laying behaviour in environments with the unlittered nest.

Marked differences in behaviour were also observed throughout the sitting phase. There were significantly more nest entries into the unlittered nest box than into the littered nest box (ANOVA, F=6.2, dfs=1,27, P<0.05). The mean duration per entry was significantly longer in environments with the littered nests (ANOVA, F=8.4, dfs=1,27, P<0.001). Also, the percentage of time (excluding duration in the nest box) spent performing searching behaviour was significantly greater in the environment with an unlittered nest box (ANOVA, F=15.9, dfs=1,27, P<0.001). These observations suggested that hens in environments with unlittered nests showed an unsettled sitting phase.

Plots of individual hen's data for various aspects of pre-laying behaviour for treatments with littered nests against treatments with unlittered nests were made. Spearman's rank correlation coefficients were calculated (Ryan *et al*, 1976) to identify within hen consistency. Table 5.3 shows that there were no significant correlations.

5.4.2 Effects of walkway

The presence of a walkway seemed to influence only the earlier stages of pre-laying behaviour. The searching phase was significantly longer in environments with the walkway (ANOVA, F=13.0, dfs=1,27, $P<0.001$) though the sitting phase and total duration of pre-laying behaviour did not differ significantly. Again, the percentage of time spent performing searching behaviour during the searching phase did not differ significantly. This finding does not support the possibility that a longer searching phase was recorded due to an inaccurate estimation of the start of pre-laying behaviour.

During the searching phase, the duration of searching behaviour and number of nest examinations (ANOVA F=16.5 and 13.0, dfs=1,27, $P<0.0001$ and $P<0.001$ respectively) were greater in environments with the walkway. This suggested that hens were indeed performing more exploratory behaviour during the searching phase in environments with the walkway. There was a greater number of nest examinations during the sitting phase with a walkway but this was not significant (ANOVA, F=4.1, dfs=1,27, $P<0.07$). However, the percentage of total pre-laying behaviour duration (outside the nest box) spent performing searching behaviour was significantly greater in environments with the walkway (ANOVA, F=5.5, dfs=1,27, $P<0.05$, Figures 5.2 and 5.3). Thus, hens spent more time exploring other possible nest sites and the nest box (though not significant) in the environments with the walkway.

As already mentioned the duration of the sitting phase was unaffected by the presence of a walkway. Examining this phase in more detail revealed that there were no significant differences in behaviour during this phase. The number of entries, duration spent in the nest box and mean duration per entry did not differ significantly between environments with or without a walkway. There was however consistency within hens in behaviour shown in treatments with and without the walkway. The duration of searching, number of nest examinations, duration of the sitting phase and mean duration per entry into the nest box were all significantly correlated between treatments (Table 5.3).

5.4.3 Interaction

F ratios for interaction were significant for the total number of nest examinations (ANOVA, F=4.4, dfs=1,27, $P<0.05$) and for the percentage of searching phase duration spent performing searching behaviour (ANOVA, F=19.0, dfs=1,27, $P<0.001$). The significant interaction for the total number of nest examinations suggest that in environments with an unlittered nest box, the walkway results in more exploratory behaviour than expected (Table 5.3). As already mentioned there was a significantly greater total number of nest examinations in environments with unlittered nests. Examination of the means showed that the total number of nest examinations was much greater than expected in the environment with the walkway, accounting for the significant interaction. Examining the means of the percentage of searching phase duration spent performing searching behaviour suggests that the significant interaction resulted as an artefact of the data being examined. Searching phase durations varied considerably between treatments and the extremely short duration in the environment with a littered nest and no walkway is likely to have produced a very high percentage of time spent searching.

5.5 Discussion

There was evidence that exploratory behaviour was more fully expressed in environments with the walkway. There were two possible explanations for the longer duration of the searching phase in environments with the walkway. Firstly, hens may have entered the nest box later in their pre-laying behaviour. Secondly, the walkway may have provided stronger cues that released searching behaviour causing the behaviour to be expressed earlier. Although it was impossible to distinguish between these two possibilities in this study, the first option seems unlikely. If hens were indeed entering the nest box later in their pre-laying behaviour, other differences related to the nest box such as a delay in other aspects of the sitting phase or oviposition may be expected. However, the sitting phase duration, oviposition time and all variables related with entering or sitting on the nest examined failed to show any significant variation between treatments.

During the searching phase hens performed more searching behaviour and made more nest examinations in treatments with the walkway. Hens also showed a higher rate of searching behaviour during the sitting phase for these treatments. Thus it appears that an environment more suited for exploration resulted in hens expressing appetitive behaviour more fully. This could be interpreted as the walkway providing the environmental cues that stimulate searching behaviour. Alternatively it may be that the walkway facilitates the expression of searching behaviour, such that hens express searching behaviour even if the internal stimulus for it is low. This study does not, nor intended to distinguish between these two possible explanations. However such a distinction is important in understanding the motivational basis for the performance of exploratory behaviour.

Further evidence that the walkway allows a fuller expression of appetitive behaviour comes from the significant interaction in the total number of nest examinations (which can also be termed appetitive behaviour). This showed that there were considerably more nest examinations in the environment with an unlittered nest box and walkway than expected. In the absence of a suitable consummatory stimulus (littered nest box) appetitive behaviour is likely to be extended, as the results suggested. However, the significant interaction suggested that this appetitive behaviour is more fully expressed in the environment with the walkway. Nest examinations have also been interpreted as intention movements to enter the nest box (Wood-Gush, 1975b). If so, they cannot be termed as an appetitive behaviour but rather as the onset of consummatory activity. Thus the high number of nest examinations in the treatment with an unlittered nest box and walkway may indicate more hesitation in commencing the sitting phase, perhaps because the motivation to perform appetitive behaviour remained high.

It was suggested in the introduction that a failure to express appetitive behaviour fully may interfere with the performance of the consummatory behaviour. Thus the sitting phase was expected to be less settled in the environment without the walkway. This was not found to be the case; the number of nest entries, total time in the nest box and mean duration of each entry were not significantly affected by the presence or absence of a walkway. Pigs in the operant conditioning situation reported in Breland and Breland (1961) showed appetitive behaviour out of context after about two weeks. A failure to observe changes in the sitting phase in the experiment reported here might therefore only have arisen after a long time period of not being able to express full searching behaviour. It was likely that hens were able to express searching behaviour fully in the home pen as it was large and contained many nests. Thus one day without being able to express appetitive behaviour fully may not be enough for this behaviour to interfere with the sitting phase.

The absence of a littered nest box had a large effect on pre-laying behaviour. Studying behaviour shown during the sitting phase provided evidence of restlessness and agitation in the absence of a littered nest. There were a considerable number of nest entries into the unlittered nest (mean 34.6) which has also been observed in environments with "unsuitable" nest sites (Appleby, 1990). The mean duration per entry and total duration of sitting were significantly shorter in unlittered nests. Although hens sat on the nest for less time, the duration of the searching phase was significantly longer in environments without a littered nest. Furthermore, there was more behaviour associated with the searching phase shown during the sitting phase in treatments with an unlittered nest. This suggested that hens were not settling down into the normal sitting phase pattern in treatments with unlittered nests.

A reduction in sitting and increase in searching is observed in battery cages (Brantas, 1980; Meijsser and Hughes, 1989) and has been attributed to hens not finding the cues that allow them to progress from the searching phase to the next stage (Wood-Gush, 1975b). Behaviour shown under such circumstances has led to the suggestion that a strong pre-laying motivation is being thwarted (Duncan, 1970). An unlittered nest box also results in the incomplete expression of pre-laying behaviour, though not to the same extent as in a bare cage. Thus the differences in pre-laying behaviour observed may result from hens being thwarted in their motivation to lay in a littered nest site. However, it seems that an unlittered nest allowed a fuller expression of pre-laying behaviour than the complete absence of a nest site. These findings suggest that hens were strongly motivated to lay in a littered nest and this could be examined by studies specifically aimed at measuring motivation.

There was considerable evidence to suggest that hens were consistent in pre-laying behaviour between environments with and without the walkway. This may reflect the similarity between these two treatments. However hens did not show consistency in behaviour between treatments with littered or unlittered nests. This lack of consistency may indicate the large effect that environmental factors have on the expression of pre-laying behaviour.

In conclusion, the earlier start of pre-laying behaviour in the presence of many cues for exploration suggests that searching behaviour is under the control of a motivational system that is influenced by external cues. There is no smooth transition between searching and nesting behaviour in the absence of a suitable nest site. Evidence presented here suggests that the motivation to sit on the nest site does not increase as much in the absence of a nest site as when a suitable nest site is present. The effect of other external cues on pre-laying behaviour is discussed in the next chapter.

Table 5.1: Means (S.E.M.) for nest quality (L= litter, N-L= no litter) and pen layout (W= walkway, N-W= no walkway) and significant effects, from ANOVA (degrees of freedom 1,27). Durations are in minutes.

Variable	Nest quality		Pen	
	L	N-L(P)	W	N-W(P)
Duration of pre-laying behaviour	110.7 (±13.9)	151.2* (±20.2)	143.9 (±21.2)	118.0 (±14.4)
Duration of searching phase	19.0 (±3.3)	31.8* (±5.8)	34.7 (±6.0)	16.1** (±4.1)
Duration of sitting phase	91.7 (±12.4)	120.1 (17.3)	109.8 (±18.4)	102.0 (±12.6)
Number of nest examinations during the searching phase	3.6 (±1.2)	8.5*** (±1.3)	7.9 (±1.5)	4.1** (±1.0)
Number of nest examinations during the sitting phase	9.6 (±3.1)	26.7** (±7.9)	22.2 (±8.0)	14.1 (±4.3)
Number of nest entries	7.2 (±1.4)	11.0* (±2.0)	10.3 (±1.6)	11.7 (±2.0)
Total duration in nest box	61.8 (±8.4)	58.2 (±10.8)	54.0 (±9.3)	65.9 (±9.6)
Mean duration per entry into the nest box	16.2 (±5.9)	8.4** (±1.9)	12.8 (±4.4)	11.7 (±5.1)
Duration of searching behaviour during the searching phase	14.3 (±4.1)	26.6** (±4.6)	28.2 (±5.1)	12.7*** (±3.0)
Percentage of searching phase time spent searching	82.6 (±5.1)	84.1 (±3.5)	80.1 (±4.3)	86.7 (±4.2)
Percentage of total time spent searching	65.2 (±4.7)	83.2** (±4.5)	79.5 (±4.1)	68.9* (±5.8)

* P<0.05, **P<0.01, ***P<0.001.

Table 5.2:Means (S.E.M.) of all treatments for (a) number of nest examinations during the sitting phase and (b) percentage of searching phase spent searching. The 2 by 2 factorial analysis performed showed a significant interaction for these two variables.

(a) Number of nest examinations during the sitting phase.

		Nest quality	
		Litter	No-litter
Pen Layout	Walkway	7.5 (±3.0)	36.8 (±9.9)
	No-walkway	11.7 (±3.3)	16.5 (±5.1)

(b) Percentage of searching phase spent searching.

		Nest quality	
		Litter	No litter
Pen Layout	Walkway	71.7 (±4.2)	88.4 (±2.8)
	No walkway	93.5 (±3.8)	79.8 (±3.8)

Table 5.3: Spearman's rank correlation coefficients on variables for individual hens. Correlations between littered or unlittered nests (nest quality) and walkway or no-walkway (pen layout) are presented. Durations are in minutes.

Variable	Nest quality r (P)	Pen r (P)
Duration of pre-laying behaviour	+0.23	+0.58
Duration of searching phase	+0.31	+0.32
Duration of sitting Phase	+0.29	+0.56
Number of nest examinations during the searching phase	+0.30	+0.69*
Number of nest examinations during the sitting phase	+0.34	+0.64*
Number of nest entries	+0.11	0.60
Total duration in nest box	+0.44	+0.58
Mean duration per entry into the nest box	+0.45	+0.65*
Duration of searching behaviour during the searching phase	+0.56	+0.83**

* P<0.05, **P<0.01.

Figure 5.1: (a) Plan of the pen used in the training and test trials and (b) plan of the walkway which was joined to the pen as shown by the dotted lines.

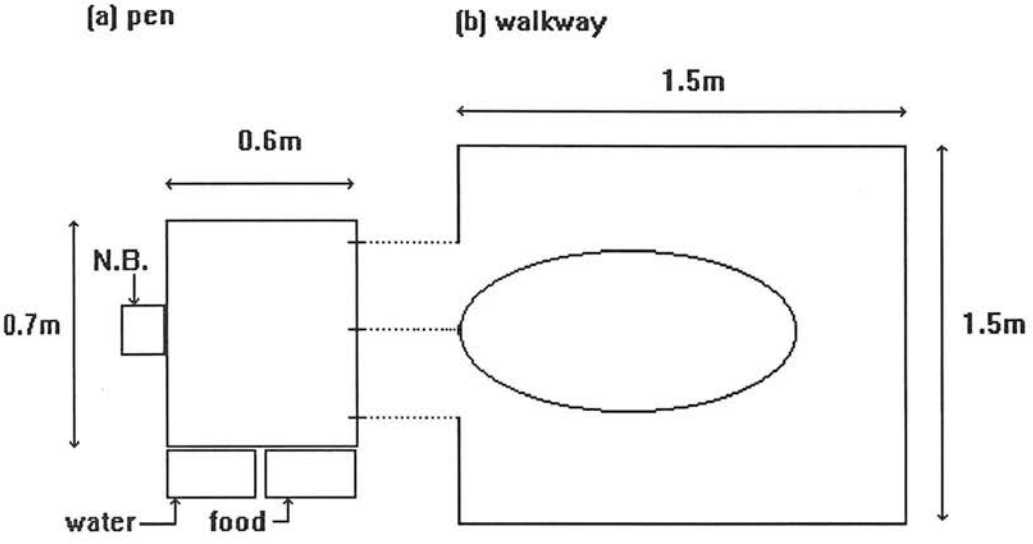


Figure 5.2: Percentage of time spent searching in each 20 minute period over the 2 hours before the last nest entry.

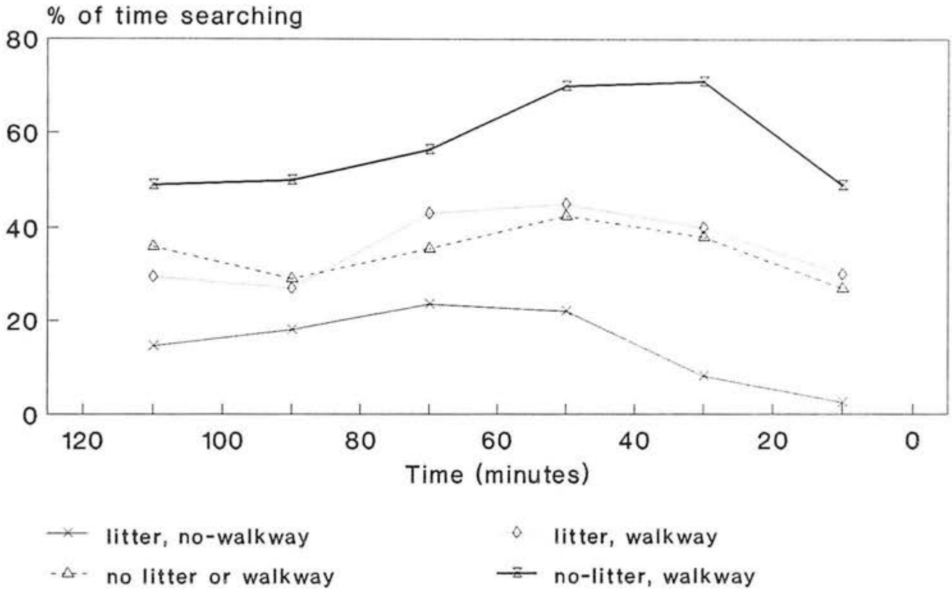
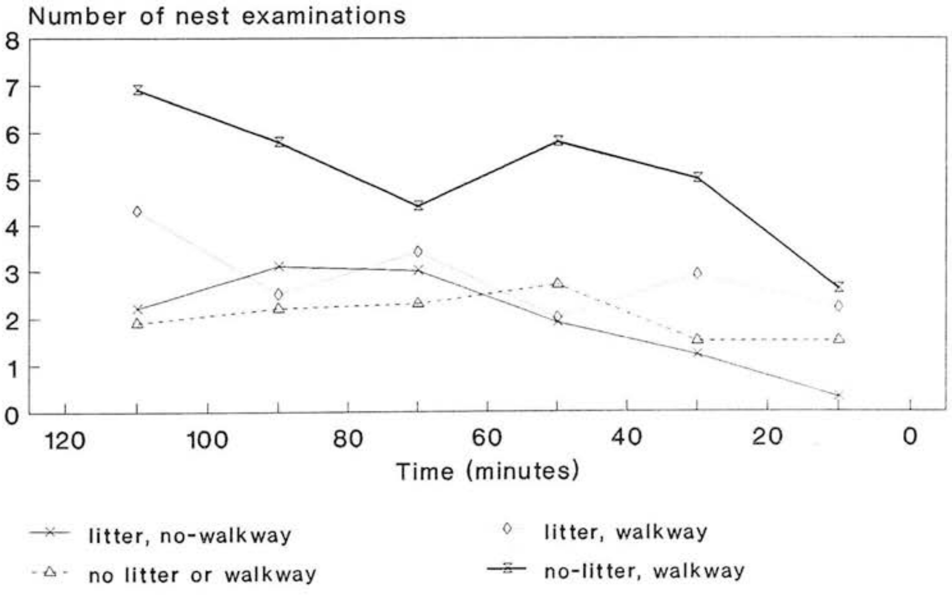


Figure 5.3: Number of nest examinations each 20 minute period over the 2 hours before the final nest entry.



CHAPTER 6: **Effects of social interaction on the approach to the nest site**

6.1 Abstract

1) Overcoming an aversive obstacle to reach a nest box may indicate a high motivation to reach that resource. The effect on pre-laying behaviour of performing such a task was examined.

2) Twelve hens were tested in four treatments presented as a Latin squares design. The test arena was arranged such that hens could only approach the nest box along one corridor, and return to the home pen by another. The four treatments consisted of leaving the corridor leading to the nest box empty, or placing a dominant, subordinate or unfamiliar hen to the test bird in it.

3) The duration from the start of pre-laying behaviour until the first entry into the corridor leading to the nest box was longer when there was a dominant or unfamiliar stimulus bird than with a subordinate or an empty corridor (42.3(±8.9), 76.5(±20.3), 26.9(±11.6) and 20.5(±4.8) minutes respectively, ANOVA dfs=3,15, $P<0.01$).

4) Hens also made more attempts to find alternative routes to the nest box during the searching phase when there was a dominant or unfamiliar stimulus bird, than with a subordinate stimulus bird or empty pen (11.3(±4.4), 16.7(±5.1), 0.5(±0.3) and 0.0(±0.0) respectively, ANOVA dfs=3,15, $P<0.05$).

5) Total number of entries into the corridor leading to the nest box showed that hens were reluctant to encounter an unfamiliar stimulus bird, but not a dominant or subordinate stimulus bird nor an empty pen (5.0(±1.2), 10.6(±4.0), 11.8(±3.6), 13.3(±4.0) respectively, ANOVA dfs=3,15, $P<0.05$).

6) It is suggested that hens are slightly motivated to reach the nest site during the searching phase. However, the motivation to gain access to a nest site increases near the start of the sitting phase.

6.2 Introduction

"Fowls live primarily and foremost in a social world" as quoted in McBride (1970) shows the importance of the social environment to domestic hens. A central feature of the social environment is the peck-order, which has been suggested to form a social control system (McBride *et al*, 1969) that restricts behaviour within a certain distance of other birds. Variations in many aspects of behaviour have been reported to be related to position in the peck-order (Collias, 1944; Guhl, 1953; Eskeland, 1977).

The effect that the social environment has on pre-laying behaviour has received less attention. It has been reported that dominant hens have priority at the nest site (Guhl, 1953; Perry, 1971b). This leads to certain changes in pre-laying behaviour which are more pronounced in the later stages of pre-laying behaviour and appear to arise out of competition for the nest site (Chapter 7). Competition for the nest site seems to depend on various environmental factors, such as enclosure and light intensity of the nest site and nest to bird ratio. In most husbandry systems competition for the nest site is rare. However group-housed hens showing pre-laying behaviour would (almost) always encounter other birds as they search for a nest site. The study reported here was performed to investigate what effect these encounters may have on pre-laying behaviour. In the experiment reported here a social stimulus was used as an obstacle for hens attempting to reach the nest site.

Hens showing pre-laying behaviour were required to interact with an unfamiliar, dominant or subordinate bird to reach a nest site. Several studies have shown that hens avoid encountering unfamiliar birds (Hughes, 1977; Dawkins, 1982; Bradshaw 1992) and show signs of fear during such encounters (Siegel and Siegel, 1961; Jones 1986), suggesting that this is aversive (Grigor, 1993). Evidence that hens find encountering dominant hens aversive is less conclusive, though the distribution in a floor pen is influenced by high ranking hens (Mankovich and Banks, 1982; Keeling and Duncan,

1989) and hens are unwilling to approach them (King, 1965). It is unlikely that hens find encountering subordinates aversive, though they may take longer in passing a cage containing a subordinate hen as opposed to an empty cage to reach a preferred pen (Grigor, 1993), perhaps because there is a delay involved in recognising other hens.

The use of social stimuli as aversive obstacles may prove to be a more useful measure of motivation to add to other aversive stimuli presently used to measure motivation. Allowing animals to make a "natural" response to obtain a reinforcement has been argued to improve the accuracy with which motivation is measured (Young, 1993). The main line of support for this comes from observations on the constraints on learning. The reinforcer used in an operant-learning experiment can become a conditioned stimulus that will elicit an innate set of responses (Breland and Breland, 1961). Even complex behaviour such as feeding or courting can become conditioned to the reinforcer (Moore, 1973). Motivation to perform behaviour associated with the reward may compete with motivation to perform other conditioned responses, as in the case of autoshaping. Thus operant responses which incorporate "natural" behaviour may eliminate this conflict ("natural" refers to appetitive behaviour normally associated with the reinforcer used). A similar argument can be presented for the use of aversive obstacles which require responses normally associated with the reward. Overcoming an aversive obstacle to reach a resource is one way of measuring motivation to gain access to that resource (Dawkins, 1990). Using this technique to measure the motivation to reach the nest site has involved hens having to overcome arbitrary obstacles such as water baths, blasts of air and small gaps (Duncan and Kite, 1987; Cooper and Appleby, 1994a). Although in these experiments hens are not required to respond in a specific way to obtain reinforcement (as in operant experiments), they are still required to respond (i.e. walk across water gap). These responses are not "natural" and may interfere with what the animal is motivated to perform and result in an inaccurate measure of motivation. In the experiment reported here the response (e.g. approaching bird) would be common for group housed hens in pre-laying behaviour.

This eliminates the conflict created when a hen in pre-laying behaviour is required to perform a response normally unassociated with pre-laying behaviour.

The aim of the experiment reported here was to investigate the effect on pre-laying behaviour of encountering hens of different social status while approaching the nest site. Hens had to pass through an empty pen or a pen containing another hen of either higher or lower social status or unfamiliar in order to reach the nest site. The literature suggests that passing an unfamiliar hen or dominant hen is aversive. Success at this task in order to reach the nest site would thus show that hens are (strongly) motivated to reach this resource.

6.3 Materials and methods

Twenty four 50-week-old HiSex Brown medium hybrid laying hens were wing-tagged to allow identification of individuals. Hens were randomly assigned to 3 equal sized groups. Each group was housed in a rectangular littered pen measuring 1.1m x 2.0m with 3 littered nest boxes and water and food available *ad lib*. Two weeks later observations on aggressive pecks (pecks to the head), together with the identity of the individuals involved were noted. At least six hours of observations spread over 3 days were performed on each group. Dominance-subordinance hierarchies were identified and each bird was assigned a social rank index (Lee *et al*, 1982). This was based on the number of birds whom an individual was dominant and subordinate to (i.e. the bird that was dominant to all others was ranked 1, the bird that was dominant to all except one was ranked 2, etc.). Although some triadic relationships were found, they involved hens whose exact rank was not important for this experiment. Throughout the experiment observations on aggressive interactions were carried out once a week (for two hours) to ensure that the dominance-subordinance hierarchies had not changed.

The layout of the arena used in the training and test situations consisted of a littered pen (pen 1) containing food and water. A hen could move into two other pens (pens 2 and 3) and into a littered nest box connected to pen 3 (Figure 6.1). Movement was restricted with three one-way doors (door 1 for access to pen 2, door 2 for access to pen 3, and door 3 for access to pen 1) such that a hen could only move from pen 1 into pen 2 then pen 3 and back into pen 1. This ensured that a bird would always have to pass through pen 2 in order to reach the nest box from pen 1. The swing-doors were hinged about 20cm high and consisted of 4 vertical bars about 5cm apart which extended down to a 4cm step on the floor which prevented the door from opening in one direction.

Training began three weeks after the groups were formed and lasted for about 10 days. Hens were palpated at lights-on to identify those which had a hard shelled egg in the shell gland. Pairs of hens with eggs were placed in pen 1 and left in the arena until both had laid, after which they were moved back to their original pens. This was repeated three times for each bird. All birds laid normal (non-banded) eggs in the nest box on all three training days. The stimulus-birds used as part of the treatments (described later) were tethered in pen 2 with a plastic cable tie fastened around one leg which was tied to the pen wall with a nylon string, restricting the movement of these birds to pen 2. These birds were tethered in pen 2 for over 4 hours on the day before testing to allow them to become familiarised with this procedure. Although no formal records of the behaviour of these birds was noted, they tended to be flighty when first tethered but this subsided after about 10 minutes.

Once all birds were trained, ranks 2-6 of each group were tested on four treatments presented in a Latin square design. The treatments were as follows:

T1: pen 2 empty

T2: hen ranked 1 from the same group as the test-bird was tethered in pen 2;

T3: hen ranked 8 from the same group as the test-bird was tethered in pen 2;

T4: A hen unfamiliar to the test-bird was tethered in pen 2.
At lights-on, a stimulus-bird was placed in pen 2 for T2-T4 and tethered so that it could not pass into pen 3. The hens were observed for 5 minutes in their home pen, then a test-bird that was not showing pre-laying behaviour was palpated to ensure that it had an egg and placed in pen 1 of the apparatus. A video camera and VCR (Panasonic NV100) were used to record the bird's movement and behaviour until oviposition. A total of 12 hens (4 middle ranking hens of each of the 3 groups of 8 hens) was tested on all four treatments.

At 1 minute intervals, the position of the test-bird (pen1, 2 or 3, nest box) was recorded from the video recordings. Searching behaviour (Chapter 2) was recorded at 1 minute intervals for hens in pen 1. The number of steps taken in the first 20 seconds of every minute for hens in pen 1 was also recorded. The number of pushes on door 3 from pen 1 (i.e. attempting to reach pen 3 directly, which was not possible because the one-way door prevented such movement) was frequently observed in some treatments and recorded. Occupation of the nest box which were not registered by the interval recordings taken every minute were also noted (as entries with a duration of less than 1 minute). Aggressive interactions (pecks to the head) between the birds were recorded as unidirectional (with identity of aggressor) or as fighting (both hens emit pecks to the head).

6.4: Results

Ten records of pre-laying behaviour had to be counted as missing values so an ANOVA test was performed which took into account this large number of missing values (Genstat 5 committee, 1987). Treatment or trial number (position at which treatment was presented) did not appear to have any influence on the pattern of these missing values. Seven of these were due to hens laying banded eggs, in which "pre-laying behaviour" was greatly extended (4-6 hours) and terminated abruptly without oviposition. The egg was dropped 3-7 hours later without any pre-laying behaviour and had a dusted band. For the other three records the start of pre-laying behaviour was undefined as the hens moved into pen 2 very early and stayed there for a considerable length of time before emerging and entering the nest box.

Three hens did not interact aggressively with the unfamiliar stimulus-bird but the remaining nine test-birds fought on the first entry into pen 2. On six occasions this fighting gave way to unidirectional aggressive pecks, with an equal number of wins and losses for the test-birds. A clear winner could not be identified from the other three interactions with the unfamiliar stimulus-bird. Aggressive pecks involving the other stimulus-birds was always unidirectional and emitted by the (previously identified) higher-ranked individual. Two hens received aggressive pecks from the dominant stimulus-bird and 5 hens pecked the subordinate stimulus-bird. The means and standard deviations of the results are shown in Table 6.1. Table 6.2 shows the results of the ANOVA (degrees of freedom= 3,15) and the pairwise t-tests (N = 11).

There were no significant differences between treatments in the total duration of pre-laying behaviour. Also the durations from the start of pre-laying behaviour to the first nest entry and from the first nest entry to oviposition did not differ significantly between treatments (Table 6.2). This showed that there were no differences in the durations of the searching or sitting phases. Indeed, the sitting phase appeared to be unaffected by the treatments with the number of nest entries and amount of time spent in the nest box not differing significantly between the four treatments (Table 6.2). The largest treatment variations occurred at the earlier stages of pre-laying behaviour.

The duration from the start of pre-laying behaviour until the first entrance into pen 2 was significantly different between the four treatments. T-tests were performed to identify which treatment or treatments were having an effect. With an unfamiliar stimulus-bird, hens took significantly longer before moving into pen 2 than for T1 and

T3. There was also a significant difference between T1 and T2, showing that hens took longer to move into pen 2 when there was a dominant hen there when compared to it being empty. There is no evidence that the onset of pre-laying behaviour is being falsely estimated for some treatments as the percentage of time spent performing searching behaviour before going through to pen 2 did not differ significantly between treatments (Table 6.2). Hens that delayed entry into pen 2 did not show any behaviour associated with attempting to nest in pen 1 (sitting, litter gathering or making a scrape). Indeed, such behaviour was not seen at all in pen 1 (except for hens that laid banded eggs). Most of the time from the start of pre-laying behaviour until the first entrance into pen 2 was spent performing searching behaviour (about 75%). For T3 and T4, the extra time in pen 1 not spent searching was spent in activities unrelated to pre-laying behaviour (e.g. standing, feeding, drinking). Hens also showed significantly more pushes on door 3 from pen 1 for T4 when compared to T1 and T3, and for T2 when compared to T1 before the first entry into pen 2 (Table 6.2).

Once they entered pen 2 for the first time, hens in T2 and T4 were significantly quicker in entering the nest box than for T1 and T3. Hens in T4 showed significantly fewer passes through door 1 during pre-laying behaviour than all the other treatments. This may be a result of the aversive nature of having to approach an unfamiliar bird. Comparing the number of passages into pen 2 between hens that won encounters with the unfamiliar hen (less aversive) with those that continued fighting or lost (more aversive) supports this point. The number of passes into pen 2 (as a percentage of the number of passes into pen 2 in T3) for hens that won encounters with the unfamiliar hen was greater (100%, 100%, 266%) than those that lost or continued fighting (10%, 38%, 50%, 15%, 50%, 54%).

The number of steps counted before going into pen 2 did not differ significantly between treatments despite T4 showing a considerably higher number of steps. Examining the raw data shows that all but two hens showed more steps in T4 than in both T1 and T3. It appears that the large individual differences observed were probably responsible for the lack of a significant result. Such a large discrepancy between treatments was not found for the number of steps counted in pen 1 during the total duration of pre-laying behaviour. This is as expected if hens are approaching the nest box near the sitting phase for T4, whereas they are approaching it as part of the searching phase for the other treatments.

6.5 Discussion

No treatment effects were found on the durations of pre-laying behaviour, searching and sitting phases. Indeed results failed to show any variations in the sitting phase, with no significant differences in the number of nest box entries or their duration. Evidence reported in the introduction suggested that encountering an unfamiliar hen (and to a lesser extent a dominant hen) is aversive. Thus the hens persistence in laying in the nest box shows that hens are willing to overcome an aversive stimulus in order to reach the nest site, as reported in other studies (Duncan and Kite, 1987; Cooper and Appleby, 1994a). This persistence of nesting in the nest box when the animal is required to pay a cost to reach it indicates a high motivation to nest there, though it is impossible to speculate on the strength of this motivation with so little variation in the "aversiveness" of the stimulus. It is quantifying the strength of this motivation that is important in understanding the importance of a nest site to the welfare of hens. That is, persistence of a behaviour in the face of a rising cost (such as in terms of aversiveness) indicates a high motivation to perform that behaviour, and denial of the stimuli that elicits this behaviour may result in poor welfare (Dawkins, 1990). Secondly, these findings failed to show that the variations in searching behaviour observed later had any effect on the sitting phase (as suggested in chapter 5).

Results showed that hens made more attempts at trying to find an alternative route to the nest site for T4 than for the other treatments. This behaviour may indicate the aversiveness of the task and is supported by the finding that hens that won encounters with the unfamiliar stimulus-bird made at least as many entries into pen 2 as in T2. Hen's delay in approaching the nest box with an aversive obstacle (T2 and T4) could be interpreted in terms of motivation theory. That is, motivation to inspect the nest may not have initially been high enough to overcome the aversive stimulus of T2 and T4. However, motivation to nest on a suitable site may eventually have been sufficient to overcome the aversive task.

Nest inspections are common during pre-laying behaviour and even occur in the days before hens come into lay (Rietveld-Piepers *et al*, 1985). This seems to be important in determining later use of the nest for laying (Sherwin and Nicol, 1993a). This suggests that nest inspections may serve to identify suitable nest sites. Furthermore, nest inspections are more frequent during pre-laying behaviour in environments with many nest sites (Meijsser and Hughes, 1989; Sherwin and Nicol, 1993b) and appear to increase with an increasing number of choices (Unpublished data, R. Freire and M. Appleby). Thus nest inspections may only be observed in environments in which the choice of one preferred nest site is difficult, perhaps because such environment provide the cues that increase the motivation to inspect nests. Futhermore, nest inspections are not obvious in feral domestic fowl living in an environment with distinct nest sites (Duncan *et al*, 1978). In the experiment presented here nest inspections may arise because the hens had limited experience of laying in this environment. Alternatively, the lack of a nest site clearly visible from pen 1 may have increased the motivation to perform nest inspections, such that hens moved towards the nest. For T2 and T4, the motivation to inspect the nest did not appear to be sufficient to overcome the aversive task in the early stages of pre-laying behaviour.

No significant differences in any of the variables reported were found between T1 and T3 (pen 2 empty or containing a subordinate hen). However when compared with T1, hens in T2 (dominant stimulus-bird) showed a significantly longer duration before going through to pen 2 and more attempts on door 3 which was not found when compared to T3, suggesting non-significant differences between T1 and T3. There are a number of reasons why hens should react differently to an empty cage and a subordinate stimulus-bird. Firstly, the subordinate hen may have been near door 1 and obstructing the one-way door thus causing a delay in entering pen 2. Secondly, entering another hen's "personal space" (Keeling and Duncan, 1989) requires identification and (perhaps) modification of the hen's posture (McBride *et al*, 1963), which may cause hesitation in approaching the subordinate bird. To enter into pen 2, hens appeared to crouch through the one-way door thus exhibiting a posture similar to that which displays subordinance. The large number of aggressive interactions with the subordinate stimulus-bird may have served to reinforce social hierarchies after this unusual approach.

The effect on pre-laying of having to pass a dominant hen is not immediately clear. As mentioned before, significant results in the duration until the first entry into pen 2 and attempts on door 3 were only found when compared with T1. This small effect was strengthened by the finding that hens entered the nest box significantly more quickly after going through for the first time than in T1 and T3. Thus when we take the first nest entry as our reference time rather than the start of pre-laying behaviour, hens in T2 were entering the nest later in their pre-laying behaviour than T1 and T3. So, were hens moving through to the nest in order to nest in it rather than as part of searching behaviour? This did not appear to be the case with the number of visits to pen 1 being statistically identical to T1 and T3. Thus for T2, hens delayed their first passage towards the nest and showed more attempts on door 3 (when compared to T1) suggesting that this may be aversive, but then showed a similar number of return visits to pen 1. It therefore seems that encountering a dominant bird is aversive (as studies cited in the introduction would suggest). However as little aggression was seen, hens appeared to lose this aversion and showed similar movement around the pens as with T1 and T3, albeit in less time.

In conclusion, hens were willing to overcome an aversive social stimulus to reach the nest site, showing a high motivation to lay in the nest site. The results indicate that hens are reluctant to overcome an aversive obstacle in the earlier stages of pre-laying behaviour. This suggests that the motivation to perform nest inspections was insufficient to overcome the aversive obstacle. However, the motivation to inspect nests near the beginning of the sitting phase was higher and sufficient to overcome the aversive obstacle.

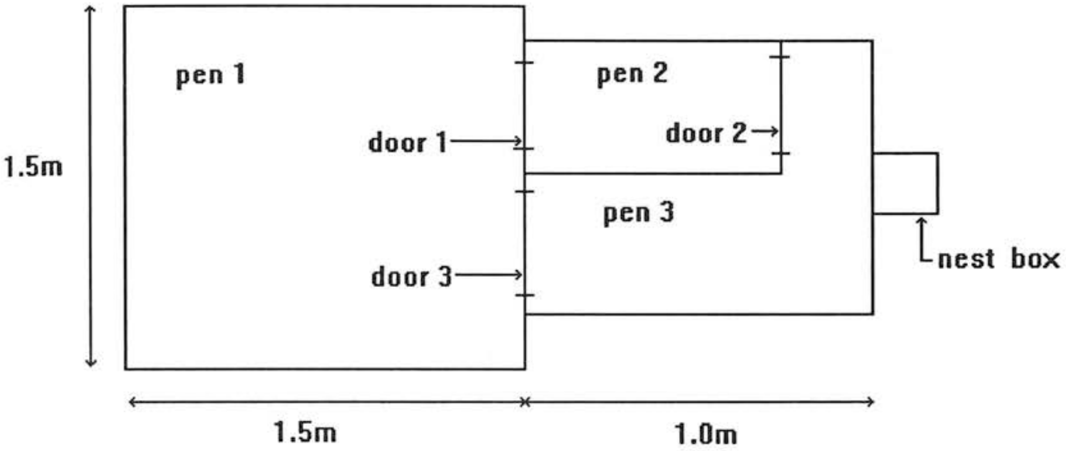
Table 6.1: Mean (S.E.M.) for the variables reported for each treatment. Durations in minutes.

Variable	None	Treatment (stimulus-bird) Dominant	Subordinate	Unfamiliar
Duration of pre-laying behaviour	128.8 (±10.1)	124.3 (±27.2)	140.0 (±16.4)	155.6 (±16.4)
Duration of the searching phase	65.6 (±5.8)	63.6 (±73.4)	66.6 (±13.2)	103.7 (±16.1)
Duration of the sitting phase	68.6 (±10.1)	60.7 (±12.1)	73.4 (±20.0)	50.9 (±9.5)
Duration to first entry into pen 2	20.5 (±4.8)	42.3 (±8.9)	26.9 (±11.6)	76.5 (±20.3)
Number of steps counted to first entry into pen 2	62.5 (±18.1)	101.8 (±22.6)	56.1 (±25.1)	234.5 (±103.5)
Number of attempts on door 3 to first entry into pen 2	0.0 (±0.0)	11.3 (±4.4)	0.5 (±0.3)	16.7 (±5.1)
Duration from first entry into pen 2 to first nest box entry	46.8 (±8.4)	22.0 (±6.9)	40.1 (±7.6)	18.5 (±6.0)
Percentage of time spent searching before first entry into pen 2	73.2 (±6.0)	74.1 (±2.7)	78.1 (±6.4)	69.5 (±7.3)
Number of nest box entries	6.0 (±1.7)	3.9 (±1.1)	5.1 (±1.6)	2.5 (±0.9)
Total time spent in nest box	39.1 (±3.7)	32.9 (±5.0)	36.6 (±4.2)	29.6 (±3.6)
Total number of steps counted	185.8 (±43.7)	232.0 (±53.0)	161.8 (±50.6)	334.3 (±96.4)
Total number of passes through door 1	13.3 (±4.0)	10.6 (±4.0)	11.8 (±3.6)	5.0 (±1.2)

Table 6.2: Outcome of ANOVA (F ratios are shown) for the data summarized in Table 1. Pairwise comparisons using the t-test were performed to identify the sources of variation. Durations in minutes.

Variable	F	P	Treatment	t	P
Duration of pre-laying behaviour	0.8	N.S.			
Duration of the searching phase	2.2	N.S.			
Duration of the sitting phase	2.0	N.S.			
Duration to first entry into pen 2	5.9	P<0.01			
			T1 vs. T2	2.04	P<0.05
			T1 vs. T3	0.69	N.S.
			T1 vs. T4	4.06	P<0.001
			T2 vs. T3	1.20	N.S.
			T2 vs. T4	1.72	N.S.
			T3 vs. T4	2.69	P<0.05
Number of steps counted to first entry into pen 2	2.7	N.S.			
Number of attempts on door 3 to first entry into pen 2	3.6	P<0.05			
			T1 vs. T2	2.08	P<0.05
			T1 vs. T3	0.21	N.S.
			T1 vs. T4	2.87	P<0.001
			T2 vs. T3	1.74	N.S.
			T2 vs. T4	0.58	N.S.
			T3 vs. T4	2.17	P<0.05
Duration from first entry into pen 2 to first nest box entry	5.8	P<0.01			
			T1 vs. T2	2.90	P<0.01
			T1 vs. T3	0.61	N.S.
			T1 vs. T4	3.62	P<0.01
			T2 vs. T3	2.12	P<0.05
			T2 vs. T4	0.46	N.S.
			T3 vs. T4	2.40	P<0.05
Percentage of time spent searching before first entry into pen 2	0.1	N.S.			
Number of nest box entries	2.4	N.S.			
Total time spent in nest box	2.3	N.S.			
Total number of steps counted	2.4	N.S.			
Total number of passes through door1	4.2	P<0.05			
			T1 vs. T2	0.52	N.S.
			T1 vs. T3	0.08	N.S.
			T1 vs. T4	3.27	P<0.01
			T2 vs.T3	0.40	N.S.
			T2 vs.T3	2.46	P<0.05
			T3 vs.T4	2.61	P<0.05

Figure 6.1: Plan of the arena used in the training and test situations. Doors 1-3 refer to one-way doors (direction described in text).



CHAPTER 7: **Pre-laying behaviour of group housed hens**

7.1 Abstract

1) The aims of this study were to investigate the effects of social interactions on access to the nest site and pre-laying behaviour of hens in small groups.

2) Nine groups of 4 hens were placed in a littered round pen. Aggressive pecks, together with the identity of the individuals involved, were recorded for 8 hours of the day. Behaviour in the hour prior to oviposition was also recorded at 15 second intervals using a video camera and VCR.

3) Records of pre-laying behaviour were divided into 3 categories: (1) Dominant category, for hens whose pre-laying behaviour overlapped with that of subordinate pen-mates, (2) Subordinate category, for hens whose pre-laying behaviour overlapped with that of dominant pen-mates and (3) Undisturbed category, for hens that showed pre-laying behaviour when no other hens were showing it.

4) Dominant hens showed a pre-oviposition increase in the number of pecks emmitted in the hour before oviposition when compared to the hour after (13.0(3.0-30.0) and 1.0(0.0-10.0) respectively, Mann-Whitney W=70, N=9, $P<0.05$). Subordinate hens however, received more aggressive pecks in the hour before oviposition than in the hour after (21.5(10.0-37.0) and 0.0(0.0-5.0) respectively, Mann-Whitney W=55, N=9, $P<0.05$). Undisturbed hens showed no changes in aggressive pecks neither received or given between pre-and post oviposition time periods.

5) Subordinate hens were also displaced more times from the nest in the 30 minutes prior to oviposition than Undisturbed hens (7.0(4.3-12.5) and 1.5(0.8-2.3) respectively, Mann-Whitney W=55, N=9,6, $P<0.05$).

6) In the period 60-25 minutes before oviposition, Subordinate hens walked more than Dominant and Undisturbed hens (163.5(112.5-174.7), 85.0(43.0-221.5) and 59.0(18.5-74.5) steps respectively, Kruskal-Wallis H=7.7, dfs=2, $P<0.05$).

7) Subordinate hens also walked more than Dominant and Undisturbed hens in the last 25 minutes before oviposition (113.5(50.3-281.2), 14.0(9.0-15.0) and 43.0(20.0-59.5) steps respectively, Kruskal-Wallis H=6.4, dfs=2, $P<0.05$). For this time period, differences in the time spent sitting were also observed (Subordinate 10.0(4.1-14.6), Dominant 19.3(12.6-20.9) and Undisturbed 13.8(10.5-18.9) minutes, Kruskal-Wallis H=6.2, dfs=2, $P<0.05$).

8) Results suggested that hens compete for access to the nest site. Variations in pre-laying behaviour due to social interactions were observed in both directions: Subordinate hens showed an increased searching phase, whereas Dominant hens remained nearer the nest site.

7.2 Introduction

There may be considerable fighting when a number of unfamiliar domestic hens are grouped together. With time, aggression levels fall and aggressive pecks and threats between any two individuals are unidirectional. This indicates definite dominance-subordinance patterns between such individuals which form the basis for the peck-order (social hierarchy), as first reported by Schjelderup-Ebbe (1922). The peck-order shows flexibility in its structure depending on the environment. For example, in small flocks kept in deep litter pens there is little aggression and hens show stable and well defined linear social hierarchies. As group size is increased, triangular relationships are more common and birds may be unable to form relationships with other known birds. In cages, there may be one dominant hen (despot) that shows aggression towards its cage-mates, with little aggression seen between the other birds (O'Keefe *et al*, 1988).

For hens in a stable peck-order, dominance or subordinance is most commonly expressed when one hen enters within some distance of another. This is largely conveyed through postural signals (Foreman and Allee, 1959). For example, McBride *et al* (1969) reported that in feral fowl, males moving to within 6 metres of the alpha male (most dominant male) would stop, lower their tail and compress their feathers to show subordinance. In single sex groups of hens, dominant hens have also been observed to display such control over another hen's behaviour within a certain distance. Ylander and Craig (1980) found that subordinate pairs of hens showed reduced agonistic acts when within one metre of a dominant hen. Position in the peck-order can also influence other aspects of behaviour. For example low ranks spend less time feeding, scratching and dustbathing (Eskeland, 1977), and tend to feed after dominant hens have gone to roost or before they rise (Guhl, 1953). The role that the peck order has on the distribution of hens has also been well documented (Mankovich and Banks, 1982; Grigor, 1993). Thus the peck-order forms the basis for a complex system of spacing, one of its effects being to restrict and control the behaviour of hens in close proximity to each other.

It is widely assumed that, to quote Wood-Gush (1971) "high ranking birds which deliver most threats and win most fights also have priority for food, nest sites, roosting places and greater freedom of the pen". Considerable research has shown that dominant hens do indeed have priority for food, roosting places and greater freedom of the pen (Collias, 1944; Guhl, 1953; Banks *et al*, 1979) though these priority rights may not be identical for the different resources (van Kreveld, 1970). Displacement of hens from the nest site has been reported (Appleby and Smith, 1991), and such displacement has been observed to indicate that dominant hens have priority at the nest site. For example Perry *et al* (1971b) noted that in a flock of broiler breeders "one particular dominant bird evicted all other [nesting] birds from her territory on the floor and then proceeded to remove birds from nest boxes 1 to 7". However, systematic research investigating such priority at the nest site has not supported such observations. Banks *et al* (1979) tested whether dominant hens had priority of access to various resources experimentally held in short supply. They found no competition for the nest site, though two aspects of their methodology would make it unlikely for a positive result as, (1) hens were placed in the experimental pen 5 hours after lights-on, by which time most hens had already laid, and (2) hens were watched for 10 minutes only, not enough time for hens to settle into pre-laying behaviour after the disruption caused by being moved into the observation pen. Thus although there is considerable evidence suggesting that hens are displaced from the nest site, it is unknown how much of this displacement is due to competition and how much is due to nesting hens being disturbed by hens not showing pre-laying behaviour.

Feral domestic (and Red Junglefowl) hens showing pre-laying behaviour are usually escorted to the nest site and back to the flock by the male (McBride *et al*, 1969). During the earlier stages of pre-laying behaviour there is an increase in locomotion, which may result in a rise in the number of social interactions. The male's presence may serve to prevent aggressive interaction between the hen showing pre-laying behaviour and other hens. Furthermore, the pre-laying behaviour of domestic hens kept in single sex groups can be very different from that shown by feral hens. Some strains show high levels of aggression prior to oviposition in certain environments (Hughes, 1979). The cause of this may be frustration or competition for the nest site but it is uncertain what effect this has on pre-laying behaviour. In similar environments, the pre-laying behaviour of group housed and individually housed hens may be very different. Group housed hens show less standing, sitting and preening and more pacing and restlessness in pre-laying behaviour than single housed birds (Ramos and Craig, 1988). One marked difference between these environments that may be responsible for the variations in pre-laying behaviour is the social environment. In the absence of a male, group housed hens may experience social control of pre-laying behaviour, perhaps through increased aggression prior to oviposition. Social control might be expected to have different effects depending on the rank of other individuals also showing pre-laying behaviour. If so, this may present a welfare problem as hens may not have adapted to cope with this social control of pre-laying behaviour.

The aim of the experiment reported here was to examine the effect of social interaction on the pre-laying behaviour of hens in small groups. Records of pre-laying behaviour were assigned to three categories, depending on the number of hens and an individual's relationship (in terms of social rank) to hens also showing pre-laying behaviour. Differences in pre-laying behaviour between the three categories were then examined. As all eggs were laid in one nest, it was decided to investigate whether dominant hens have priority of access to this site when more than one hen is showing pre-laying behaviour.

7.3 Materials and methods

A flock of 80 HiSex White light hybrid laying hens was housed in a deep litter pen measuring 4.2m x 3.7m with 16 nest boxes from 20 weeks old. All birds were wing-tagged to allow identification of individuals. At 26 weeks old, 9 groups of 4 were randomly chosen for observations, which lasted 7 weeks.

Groups were placed in a round pen made from wire mesh, 0.6m high and lined with a 0.4m high plastic sheet to reduce litter loss, for three days. The stocking density was the same as in the home pen (7 birds/m sq.). Several measures were taken to make the environment bare and allow maximum interaction, for example, scattering pelleted food on the ground at lights-on and mounting drinkers externally with access via three equally spaced holes in the pen wall. The light regime was 14 hours light: 10 hours dark. On the third day hens were filmed for eight hours from lights-on using a Panasonic video camera and VCR (NV100). The hens were viewed from above using a mirror placed above the pen at 45°.

All aggressive pecks (pecks to the head) were noted, together with the identity of the individuals involved for the eight hours of video recordings. The data were collected for ten minute periods in order to analyse temporal changes in aggressive interactions. The location of these aggressive pecks was noted for four fixed quadrants marked by placing an acetate sheet on the monitor screen. The location of the nest (where the hen laid) was also noted with respect to these quadrants. Dominance-subordinance relationships were identified between any two individuals on the outcome of these interactions. As all aggressive pecks between any two hens were unidirectional, the emitter and recipient of the pecks were respectively referred to as the dominant and subordinate hens. Furthermore, the number of pecks received while sitting on the nest that resulted in the hens standing up and moving away from the nest site was noted.

From the video recordings, the time of oviposition was identified for each hen. Posture (standing or sitting) was recorded at 15-second intervals in the hour prior to oviposition together with any of the following activities:

(a) escape behaviour. Pushing against plastic lining or putting head in drinking holes and pushing outwards.

(b) searching head movements. Characteristic movement of head during pre-laying behaviour usually accompanied by the nesting call. Involves examining the walls keeping the keel up high or examining possible nest sites with the neck held straight in a horizontal position (Wood-Gush, 1975b).

(c) rapid changes in direction of approximately 180°.

(d) crouching underneath another hen.

(e) nest building behaviour. Producing a shallow scrape by rotating whilst sitting to create a depression with the keel bone, usually accompanied by outward scraping movements with the feet.

(f) litter tossing. Involves collecting litter in the beak and placing it on the back. Other litter gathering behaviour was rarely observed during these observations and were not recorded.

The number of steps for every 15 second period between samples was also noted.

7.4 Analysis of results

Of the 36 hens observed, 28 complete records of the last hour before oviposition were obtained. Twenty four of these records were divided into three categories using the dominance-subordinance relationships for pairs of hens as follows (the remaining four records were undefined): (1) Dominant category, for hens whose pre-laying behaviour overlapped with that of subordinate pen-mates, (2) Subordinate category, for hens whose pre-laying behaviour overlapped with that of dominant pen-mates, (3) Undisturbed category, for hens that showed pre-laying behaviour when no other hens were showing it. Records were included in the first two categories if at least 50 minutes of the last hour overlapped with the pre-laying behaviour of another hen as described. The groups from which pre-laying behaviour records for these three categories and the four Undefined records were obtained are shown in Table 7.1.

To examine the effect of social interaction on both the searching and sitting phases the data were analysed for two time periods. This was decided after calculating the mean sitting time (mean 25.2±2.1 minutes, N=28) and studying Figures 7.1-7.4. These were 60-25 and 25-0 minutes before oviposition. The experimental design yielded data that were unbalanced with respect to groups making statistical analysis difficult. Kruskal-Wallis tests were performed on the data to test for any significant group effects, though none were found. This offers some support for the assumption that there are no group effects. Results displayed non-normal distribution, so non-parametric tests (Kruskal-Wallis one-way analysis of variance) were used to test for statistical differences in various parameters for the three categories. Pairwise comparisons between the three categories were not performed as the small sample size and the unbalanced design may have produced misleading results.

7.5 Results

7.5.1 Aggressive behaviour

Although the pen was bare (and without a nest box) there was a distinct nest site for each group in which all the eggs were laid. The nest was a shallow scrape made by hens that had nested there previously. It also contained some eggs laid either earlier that day or on previous days. The position of the nest site varied between groups perhaps because all the eggs were removed and fresh litter added before a new group was introduced into the pen. No eggs were laid outside of the nest. The presence of just one nest site made it possible to examine competition for this site.

Figures 7.5 and 7.6 shows the number of aggressive pecks received and given for a four hour period including the time of oviposition. Aggressive pecks outside of this were very rare and were not plotted on the figures. Mann-Whitney non-parametric tests were performed to compare aggressive interactions (both the number of aggressive pecks given and received) in the hour before oviposition with the hour after. The differences for the Dominant and Subordinate categories were significant (dominant category medians (inter-quartile range) before and after oviposition 13.0 (3.0 to 30.0) and 1.0 (0.0 to 10.0) respectively; Mann-Whitney W=70; N=9, $P<0.05$; and Subordinate category medians (I.Q.R.) before and after oviposition 21.5 (10.0 to 37.0) and 0.0 (0.0 to 5.0) respectively; Mann-Whitney W=55.5: N=6, $P<0.01$). The Undisturbed category however did not show a significant difference in the number of aggressive interactions before and after oviposition (medians (I.Q.R) 3.5 (0.3 to 8.5) and 1.5 (0.3 to 3.75) respectively; Mann-Whitney W=76.5; N=9, N.S.). Thus a pre-oviposition increase in aggression was only observed when more than one hen were simultaneously showing pre-laying behaviour; suggesting that there may be competition for a limited resource.

Table 7.5 shows that the number of pecks received by Subordinate hens in the quadrant in which they eventually laid compared with the mean for the other three quadrants was significantly greater in the 30 minutes prior to oviposition, but not for the other time periods. During this period it was common for hens to attempt to sit at the nest site and the increase in aggression received around the nest may be because they were being displaced or prevented from settling on the nest. There were no significant differences in the number of aggressive pecks given in the quadrant in which Dominant hens eventually laid as compared with other quadrants (Table 7.5), though this was almost significant for the first period (P=0.06). During this period hens were still showing searching behaviour and the increased aggression observed may be to displace or prevent a subordinate hen from settling there.

The data on aggressive pecks received while on the nest were analysed to examine whether Subordinate hens were being displaced from the nest site. Aggressive pecks received that caused a hen to get up and leave the nest were compared between Subordinate and non top-ranked Undisturbed hens (i.e. Undisturbed hens that had no dominant pen mates were omitted, as these did not receive any aggressive pecks at all). It was found that Subordinate hens were displaced through aggressive pecks significantly more than the Undisturbed category (median (I.Q.R.) pecks received 7.0 (4.3 to 12.5) and 1.5 (0.8 to 2.3) respectively; Mann-Whitney W=55; N=9,5, $P<0.05$). Thus hens that show pre-laying behaviour at the same time as a dominant pen mate are being displaced more from the nest site than hens that show non-overlapping pre-laying behaviour.

7.5.2 Searching phase, 60-25 minutes before oviposition.

Table 7.2 shows that there is a significant difference in the number of steps taken between the three categories. The data suggest that Subordinate hens take more steps than the other two categories. Furthermore, Figure 7.1 shows that Dominant hens showed a decrease whereas Subordinate hens showed an increase in the amount of walking performed as oviposition approached. The difference between the slopes of individual regression lines for the 50 minutes before oviposition was significant (Dominant and Subordinate slope medians (I.Q.R.) -0.37(-0.7 to -0.13) and 0.08(-0.3 to 1.3) respectively; Mann-Whitney W=54; N=9,9, $P<0.05$).

Table 7.3 shows the outcome of Kruskal-Wallis tests on the incidence of other activities during this time period. Only the time spent crouching under other hens showed a significant difference between the three categories, with Subordinate hens appearing to have spent more time under other hens than the other two categories.

7.5.3 Sitting phase, 25-0 minutes before oviposition.

Again there was a significant difference in the number of steps taken in the 25 minutes before oviposition between the three categories (Table 7.2), with Subordinate hens appearing to take more steps. As expected this coincided with a significant difference in the amount of time spent sitting during this period (Table 7.4). This period of pre-laying behaviour usually consists of the hen sitting on the nest and nest building (as the Dominant and Undisturbed hens appear to be doing here). However, as Figures 7.2 and 7.3 show, Subordinate hens appeared to be walking more and sitting for less time than the other two categories of hens. A less pronounced difference can be seen between the Dominant and Undisturbed categories in the number of steps taken, with Dominant hens appearing to be walking less than the Undisturbed hens. There was no apparent difference between these two categories in the amount of time spent sitting on the nest.

There is a significant difference in the amount of time spent crouching under other hens (Table 7.4, Figure 7.4) as oviposition approaches. The data again suggest that Subordinate hens are spending more time crouching under other hens than the other two categories. Possible functions of this behaviour are discussed below. There were no other significant differences between the three categories for this time period.

7.6 Discussion

The finding that all eggs were laid in one site for each group is unexpected considering that the pen was round and uniform. Such clumping in egg distribution has usually been attributed to an attraction to unknown features of a particular nest site, though this is unlikely as the sites were different between groups. Hens may have been attracted to a particular nest site because they had laid there before (conservatism). This behaviour is common in feral domestic fowl which show conservatism within a clutch and also to a lesser extent between clutches (McBride *et al*, 1969; Duncan *et al*, 1978). However, conservatism is observed in semi-intensive environments (e.g. Wood-Gush, 1954) though not as often as expected. Alternatively, the clumping observed in this study may be the result of hens being attracted to the presence of eggs, which is known to make nests more attractive and increase their use (Cunningham, unpublished; Reed, 1991). Domestic hens can also show gregarious nesting behaviour (Appleby *et al*, 1984). However even Undisturbed hens, that laid when no other hens were showing pre-laying behaviour laid at the communal nest site. This attraction to nesting hens may arise through hens recognising that nesting hens may have eggs underneath them and so may be exhibiting an attraction to eggs. Perhaps the simplest explanation is that the hens are attracted to the nest itself (consisting of a depression in the litter) and hens have indeed been found to show a preference for pre-formed nests over flat litter (Hughes *et al*, 1989).

The factors governing nest-site choice are complex and often difficult to disentangle. There is the added difficulty that hens show large individual variation in their preference for particular nest sites (Rietveld-Piepers *et al*, 1985; Cooper and Appleby, 1993). Communal nest boxes are common in semi-intensive systems and displacement from them is rare. However in the experiment reported here there were a high number of displacements from the nest site and hens were rarely seen nesting together. This lack of tolerance is unexpected as light hybrids seldom show aggressive interactions in nest boxes (e.g. Rietveld-Piepers *et al*, 1985), though the low light intensity in nest boxes may result in hens being unable to recognise individuals or establish the eye contact necessary for an aggressive response (McBride *et al*, 1963). The lack of aggression in communal nest boxes may also be because hens are constantly close together, and don't approach into the space of another which may be the trigger for an aggressive response (Hughes and Wood-Gush, 1977). This persistence of hens to nest at a particular site despite repeated displacements may indicate that hens have a high demand to nest there. Alternatively, gregarious nesting behaviour may arise because of the absence of a large, complex environment and a male to help in nest site choice. Thus hen's inability to find a new nest site may show that they are unadapted to cope with being displaced from the nest site as this would not have occurred in their ancestors.

Subordinate hens were displaced significantly more from the nest site than Undisturbed hens. One possible explanation for this is that hens showing pre-laying behaviour were competing for the nest site. Stronger evidence of this would have been obtained if the identity of the hen causing the displacement had been noted. However the finding that aggressive interactions were more prominent in the nest site quadrant suggests that competition was indeed taking place. Subordinate hens received significantly more aggressive pecks in this quadrant during the later stages of pre-laying behaviour, which may be due to the hens being displaced from it by a dominant hen also showing pre-laying behaviour. Indeed, Dominant hens showed more aggressive pecks (though not significant) earlier in their pre-laying behaviour. These hens were still performing searching behaviour and the increased aggression observed may be to displace subordinate hens from the nest site. However, in the last 30 minutes before oviposition these hens spent most of their time sitting on the nest and are unlikely to be approached by hens subordinate to them (hens were rarely seen sitting at the nest together), which may account for the observed drop in the number of aggressive pecks given.

The finding that the Dominant and Subordinate categories showed a pre-oviposition increase in the number of aggressive interactions is consistent with the results of Hughes (1979). However, the findings reported here expand on this phenomenon in three ways. Firstly, Hughes (1979) found a pre-oviposition increase in aggression after introducing a strange bird (which enhances the level of aggression), and suggested that the frustration (which lead to aggression) was latent, becoming observable only when a stranger was added. In the experiment reported here no stranger was added, though hens may be frustrated due to not having settled into their environment after two days of acclimatisation. Secondly, Hughes (1979) found a pre-oviposition increase in aggression in a group of battery housed hens and not in deep litter housed birds. In the experiment reported here a pre-oviposition increase was found in a littered pen with adequate space. Lastly, Undisturbed hens did not show this pre-oviposition increase in the number of aggressive interactions, suggesting that it is not due to environmentally induced frustration. Instead, it appears to be related to competition for a limited resource, as suggested earlier.

There were marked differences in behaviour between the three categories. Subordinate hens took more steps than the other two categories in the hour before oviposition and this increased (as opposed to the decrease shown by Dominant hens) as oviposition approached. Not surprisingly, there was a significant difference between the three categories in the amount of time spent sitting with Subordinates appearing to sit for less time than the other two categories during the sitting phase of pre-laying behaviour. This prolonging of walking and reduction in sitting as oviposition approaches suggests that hens are not settling down during the sitting phase as expected. A similar phenomenon has been observed in group housed hens when compared with individually housed hens (Ramos and Craig, 1988). The data presented in this study suggest that it is not the mere presence of other hens that is responsible for the behaviour observed but that it is a consequence of competition for the nest site.

The behaviour observed in Subordinate hens is similar to that observed in hens without a nest (i.e. battery cages, Meijsser and Hughes, 1989) and hens prevented from reaching a nest (Duncan, 1970). Duncan (1970) interpreted this behaviour as showing frustration and found that hens would show longer pre-laying behaviour when prevented from reaching the nest site. Unfortunately, the duration of pre-laying behaviour was not recorded in this experiment so it is impossible to say if this was the case. Frustration may serve to increase locomotion (and hence increase the chances of finding a new nest) while extending the time till oviposition. Thus it may be an adaptive strategy to increase the chances of finding a suitable nest during the later stages of pre-laying behaviour. Alternatively, hens may be able to withhold the egg voluntarily while they search for a nest site and frustration may only arise when a suitable nest site is still not found. Unfortunately, our knowledge of the physiological mechanisms that regulate oviposition do not help in the differentiation of these two possible explanations.

There were also differences between the Dominant and Undisturbed category in the number of steps taken in the last 25 minutes before oviposition, with Dominant hens walking less. This however does not coincide with an increase in the amount of time spent sitting during this time. This suggests that Dominant hens are staying near the nest more than Undisturbed hens. Hens did not always succeed in displacing subordinate pen-mates from the nest site through aggressive interactions, thus this staying near the nest may be to prevent other hens from sitting there. Furthermore, hens rarely approached dominant hens sitting on the nest, and two hens were never observed sitting at the nest site together. Thus although Dominant hens appear to have priority at the nest site, observations suggest that they prefer to stay near it and prevent occupation of it. This strategy seemed more successful than displacing subordinate hens from the nest site once they had already settled there. Hens may also try to avoid displacing hens from the nest site as the disruption created may trigger an attack by a dominant third party (Ylander and Craig, 1980).

Thus social interactions are having strong influences on the pre-laying behaviour of Subordinate hens and, to a lesser extent on Dominant hens when compared with the Undisturbed category. The data suggest that differences between the three categories arose because of varying degrees of access and competition for the nest site. Another aspect of social interaction that may be having an effect on the three categories is social facilitation of pre-laying behaviour. In social facilitation, the stimulus of one hen performing pre-laying behaviour would make a nearby hen more likely to perform a similar behaviour. Little is known about social facilitation of pre-laying behaviour, though as physiological control is important in the expression of this behaviour it may only occur in hens due to lay at similar times. The data presented in this study suggest that social facilitation is not occurring as the hens most likely to show this, the Dominant and Subordinate hens are showing the most marked differences in behaviour. It may be that social facilitation of pre-laying behaviour does indeed occur, but in this study any such effect may have been masked by the larger effect resulting from competition for the nest site.

The amount of time spent under other hens in the hour before oviposition was significantly different for the three categories. Subordinate hens appeared to be spending more time under other hens during both time periods. This behaviour has often been interpreted as hens looking for enclosure (Wood-Gush, 1954; Meijsser and Hughes, 1989), which has been shown to be an important stimulus in nest site selection (Appleby and McRae, 1986). However, in Subordinate hens this behaviour was often preceded by the hen receiving an aggressive peck suggesting that it may serve to deter further attack, perhaps through a change in posture (Syme and Syme, 1979). Although a large peak in the amount of time spent crouching under other hens coincides with the peak in aggressive pecks received (30-35 minutes before oviposition), this is also a time of intense searching behaviour. Further research is required to identify the function of this behaviour.

In conclusion, the pre-oviposition increase in aggression did not appear to be due to environmentally induced frustration as previously suggested but due to competition for the nest site. There were a large number of displacements from the nest in this study and this appears to be related to the open nest sites and overlap in pre-laying behaviour. The hens persistence in nesting on the nest site may show a high demand to sit there. The findings reported here identify one source of variation in pre-laying behaviour and demonstrate that social pressures can result in variations in both directions when compared with Undisturbed hens. That is, when more than one hen is showing pre-laying behaviour at the same time, the least dominant hen showed increased locomotion and unsettled pre-laying behaviour whereas the most dominant hen stayed nearer the nest.

Table 7.1: Groups from which the pre-laying behaviour records of 28 hens were obtained and categorised as shown.

	Category			
Group	Dominant	Subordinate	Undisturbed	Undefined
1	1	*	1	1
2	1	1	1	1
3	1	*	1	1
4	1	1	1	*
5	1	1	1	1
6	*	*	2	*
7	*	*	2	*
8	3	1	*	*
9	1	2	*	*

* no observations

Table 7.2: Medians (I.Q.R.) for the number of steps and 180° changes in direction in the hour before oviposition and outcome of the Kruskal-Wallis tests (dfs=2). Results are presented for two time periods: 60-25 and 25-0 minutes before oviposition.

Variable	Time Period	Category medians (I.Q.R.) Dominant	 Subordinate	 Undisturbed	Kruskal-Wallis H
Steps	60-25	87.0 (43.0-221.5)	163.5 (112.5-174.7)	59.0 (18.5-74.5)	7.7*
	25-0	14.0 (9.0-15.0)	113.5 (50.3-281.2)	43.0 (20.0-59.5)	6.4*
Changes in direction	60-25	9.0 (0.5-28.5)	10.5 (5.5-15.8)	2.0 (0.5-11.0)	1.9
	25-0	1.0 (0.0-12.5)	12.5 (1.8-31.3)	3.0 (1.5-4.0)	4.5

* $P<0.05$.

Table 7.3: Medians (I.Q.R.) for the estimated duration in minutes of various activities in 60-25 minute period before oviposition and outcome of the Kruskal-Wallis tests (dfs=2).

	Category median (I.Q.R.)			Kruskal-Wallis
Variable	Dominant	Subordinate	Undisturbed	H
Sitting	8.0 (2.9-17.7)	9.9 (0.8-17.4)	12.5 (6.4-19.5)	0.5
H.M.	14.8 (11.4-23.4)	10.5 (2.0-18.0)	9.0 (3.4-13.1)	5.1
U.O.	0.0 (0.0-1.6)	1.1 (0.4-5.5)	0.0 (0.0-0.4)	7.8*
E.B.	0.5 (0.0-1.2)	0.1 (0.0-1.3)	0.0 (0-0)	3.3
N.B.	0.6 (0.2-2.9)	0.4 (0.2-2.0)	2.5 (0.7-4.1)	4.3
L.T.	0.8 (0.0-4.0)	0.0 (0.0-0.6)	2.8 (0.4-9.3)	5.5

$P<0.05$.

Key: H.M.= head movements. U.O.= crouching under other hens, E.B.= escape behaviour, N.B.= nest building behaviour, L.T.= litter tossing.

Table 7.4: Medians (I.Q.R.) for the estimated duration in minutes of various activities in 25-0 minute period before oviposition and outcome of the Kruskal-Wallis tests (dfs=2).

	Category median (I.Q.R.)			Kruskal-Wallis
Variable	Dominant	Subordinate	Undisturbed	H
Sitting	19.3 (12.6-20.9)	10.0 (4.1-14.6)	13.8 (10.5-18.9)	6.2*
H.M.	9.8 (3.5-13.1)	7.8 (4.9-11.3)	7.5 (3.5-8.6)	1.2
U.O.	0.0 (0.0-1.3)	2.0 (0.8-2.7)	0.3 (0.1-0.9)	8.7*
E.B.	0.3 (0.0-1.0)	0.1 (0.0-0.9)	0.0 (0-0)	3.7
N.B.	1.1 (0.1-3.1)	1.8 (0.3-2.4)	2.9 (0.6-4.9)	2.1
L.T.	4.3 (1.6-10.9)	0.5 (0.0-3.5)	6.3 (2.3-11.3)	5.6

* P<0.05.

Key as in Table 3.

Table 7.5: Medians (I.Q.R.) number of aggressive pecks given and received in the nest site quadrant and in other quadrants. Results are presented for three time periods: (1) 60-30, (2) 30-0 minutes before oviposition and (3) 0-30 minutes after oviposition.

(a) Aggressive pecks received by Subordinate hens.

Time period	Quadrant median (I.Q.R.) Nest-site	 Other	Mann-Whitney W
1	2.0(0.0-7.7)	1.8(0-2.8)	11
2	6.0(3.8-12.8)	1.3(0.2-2.2)	21*
3	0.0(0.0-0.8)	0.0(0.0-0.4)	2

(b) Aggressive pecks given by Dominant hens.

Time period	Quadrant median (I.Q.R.) Nest-site	 Other	Mann-Whitney W
1	3.(1.0-8.0)	1.0(0.3-1.6)	15
2	2.0(0.0-3.0)	0.3(0.0-2.6)	15
3	0.0(0.0-2.0)	0.0(0.0-1.6)	3

* $P<0.05$.

Figure 7.1: Number of steps taken per 5 minutes in the hour before oviposition.

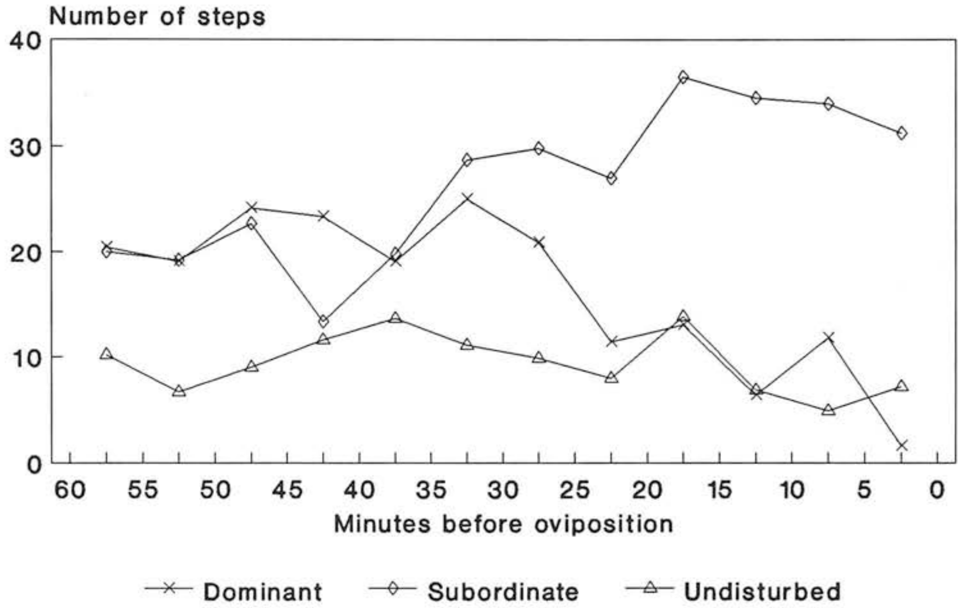


Figure 7.2: An estimation of the mean percentage time spent sitting per 5 minutes in the hour before oviposition.

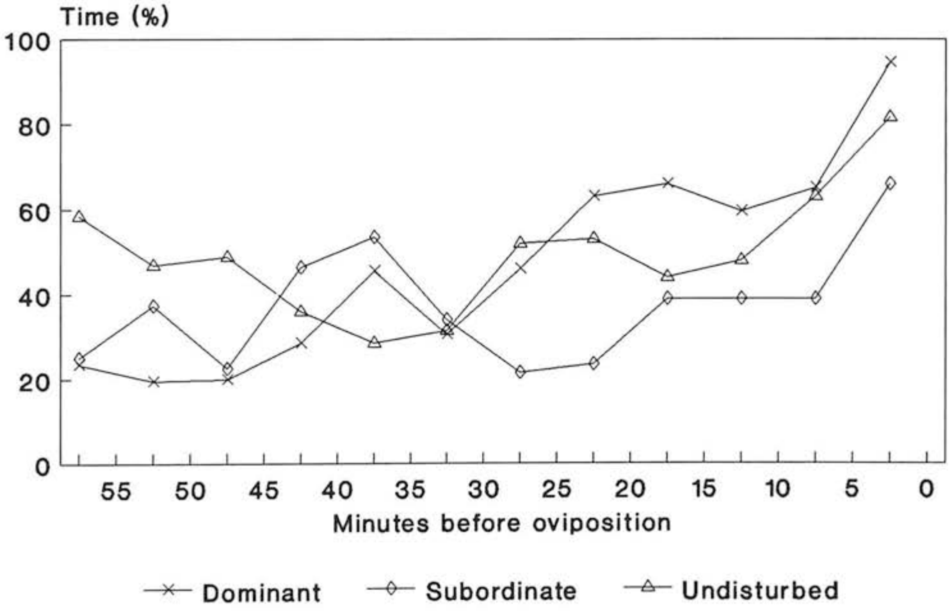


Figure 7.3: An estimation of the mean percentage time spent performing head movements per 5 minutes.

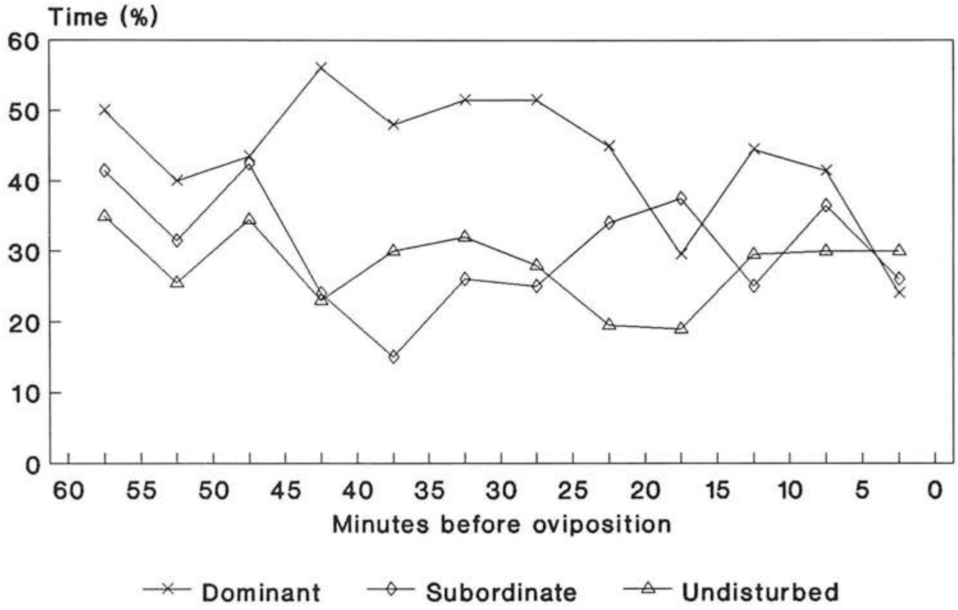


Figure 7.4: An estimation of the mean percentage time spent crouching under other hens per 5 minutes.

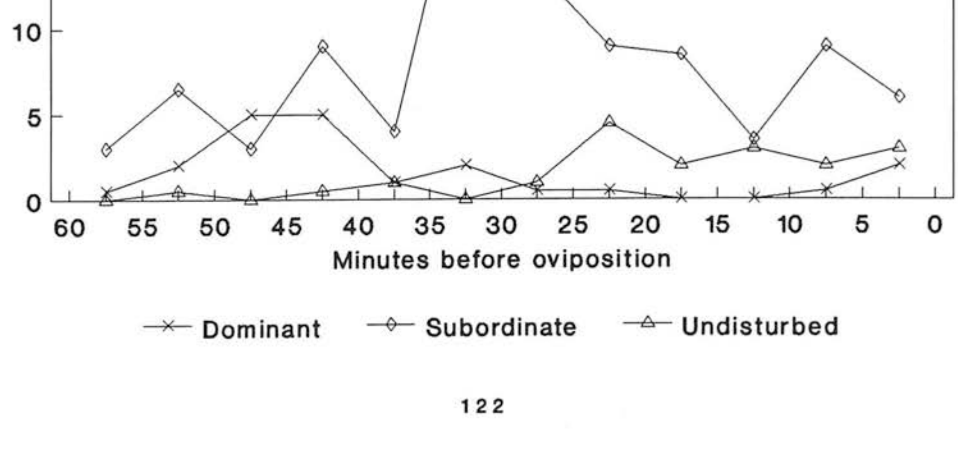


Figure 7.5: Mean number of aggressive pecks emitted in a three hour period starting two hours before oviposition.

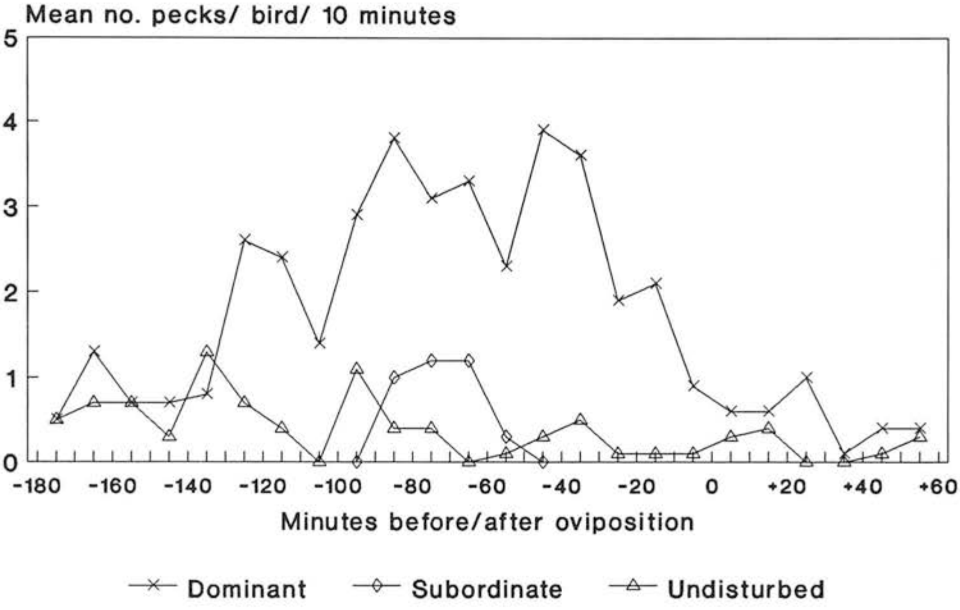
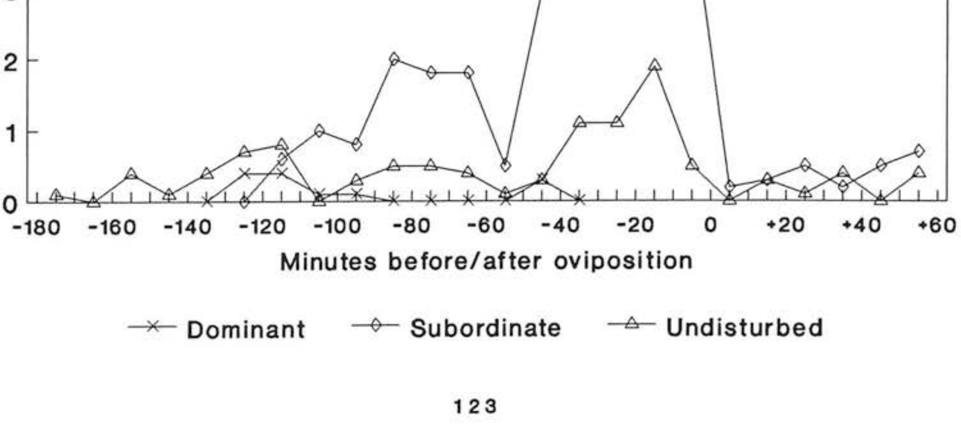


Figure 7.6: Mean number of aggressive pecks received in a three hour period starting two hours before oviposition.



CHAPTER 8: **General discussion**

8.1 Abstract

1) The first section provides a brief summary of how the factors studied in this thesis influence pre-laying behaviour in laying hens. Internal factors, interaction with other motivational systems, environmental complexity and social factors were all found to affect pre-laying behaviour.
2) These results are discussed in relation to a model proposing that pre-laying behaviour is controlled by 3 motivational systems.
3) Evidence is presented suggesting that the start of pre-laying behaviour is determined by the motivation for searching behaviour competing with other motivational systems. Increasing the tendency to search and preventing the expression of competing behavioural tendencies were found to advance the start of pre-laying behaviour.
4) It is suggested that the tendency for nesting behaviour increases as oviposition approaches, resulting in searching being replaced by nesting. The possibility that in the absence of a suitable nest site the tendency for nesting does not increase as much as in the presence of a suitable nest site is discussed.
5) Oviposition was delayed by presenting food during pre-laying behaviour or in the absence of a suitable nest site. It is suggested that a certain duration on the nest is required before oviposition can occur.
6) The inability to express all of the aspects of pre-laying behaviour in cages is one of the most important problems for the welfare of laying hens. The implications of the findings reported in this thesis for welfare and production in laying hens in commercial husbandry systems is discussed.

8.2 Summary of factors studied in this thesis and their effect on pre-laying behaviour

8.2.1 Relationship between lag and egg number in the sequence and the different elements of pre-laying behaviour

The physiological control of the egg production cycle is relatively well understood (Wood-Gush and Gilbert, 1973; Shimada and Saito, 1989; Etches, 1990). Ovulation is caused by a surge of luteinizing hormone from the anterior pituitary (Fraps, 1955). The ovum spends varying amounts of time in each segment of the oviduct and is ready to be laid about 20 hours after ovulation (Bahr and Johnson, 1991). Oviposition is initiated by the release of prostaglandins from the largest pre and post-ovulatory follicles (Shimada and Saito, 1989). Laying hens usually lay one egg per day in a sequence of several days, each sequence separated by one or more pause days. The difference in the time of oviposition of two successive eggs minus 24 hours is called the lag.

The duration of the sitting phase and total time spent in the nest box were found to be positively correlated to lag (Chapter 3). No other significant correlations between lag and the various aspects of pre-laying behaviour were found. These results therefore demonstrate that variations in physiological factors which determine patterns of lag also determine aspects of pre-laying behaviour. Physiological state of the hen varies between the first and other eggs of the sequence, as ovulation of the first egg is not preceded by oviposition and this may also affect pre-laying behaviour. Hens showed longer pre-laying behaviour for the first egg of a sequence than for other eggs, largely due to a longer searching phase with more nest examinations. Increased searching for the first egg of a clutch might be expected to convey an evolutionary advantage in wild species, as the extended duration of searching may lead to the identification of "good" nest site (Wood-Gush, 1983). Subsequent eggs are likely to be laid in the same site, so that a long searching phase may be disadvantageous as it could attract predators. The variation in pre-laying behaviour within a sequence therefore suggests that a sequence (commonly called a clutch in the poultry industry), although separated from previous and subsequent sequences by only one day, has some characteristics in common with a natural clutch. Thus the control of onset and offset of the elements of pre-laying behaviour are partly physiological. However, the large variations in pre-laying behaviour between husbandry systems (e.g. Meijsser and Hughes, 1989) suggest that the control of this behaviour is modulated by environmental events.

8.2.2 Competition between the tendency to feed and to perform pre-laying behaviour

The effects of internal variables (hunger) and external cues (presence or absence of food) on pre-laying behaviour were examined in chapter 4 within a framework of interacting motivational systems. The first experiment examined how varying the causal factors for feeding affected the pre-laying behaviour of hens. Hens showing pre-laying behaviour were found to feed after the presentation of food, although the duration of feeding was found to be unrelated to the length of deprivation. Even relatively satiated hens stopped pre-laying behaviour to feed for at least two minutes. It may be that the presentation of food is a strong stimulus for feeding, perhaps through conditioning (by repeated pairings). This seems unlikely, as hens never ran out of food and so could feed at any time. It may be that the disturbance created by topping up the food troughs is enough to initiate feeding activity and this may induce feeding activity in all the flock, perhaps through social facilitation. For example, Hughes (1971) found that changing observers stimulated feeding activity in one bird and this appeared to act as an initiator of group activity.

The above findings suggest that competition between the tendencies for feeding and pre-laying behaviour determine switches between these two activities. The delay in oviposition was related to the stage during the pre-laying behaviour sequence when food was presented, which was tested in the second experiment. The delay in oviposition was found to be greater when food was presented near, rather than much before the expected time of oviposition. It is suggested that hens require to search before resuming nesting behaviour and oviposition after an interruption (i.e. behavioural priming of nesting behaviour and oviposition is required). The hypothesis that the onset of pre-laying behaviour is governed by competition between the tendency for pre-laying behaviour and other behavioural tendencies was tested in the third experiment. It was found that the durations of pre-laying behaviour and searching phase were greater if food was absent than if it was present, supporting the original hypothesis.

8.2.3 Effects of nest quality and other cues for exploration on pre-laying behaviour

In chapter 5, the effects of several environmental factors on pre-laying behaviour were examined. The hypothesis that the motivation to search would remain high and nesting behaviour would be disrupted or absent in the absence of a suitable nest site was tested. It was found that with unlittered nest boxes, pre-laying behaviour and the searching phase, comprising more searching behaviour and nest examinations, were longer than with littered nest boxes. Hens also performed more nest entries of a shorter mean duration into unlittered nest boxes, though the total duration on the nest was not affected by the treatments. Thus in the absence of a suitable nest site the searching phase was extended and the sitting phase was fragmented and unsettled. This agrees well with the findings of the comparative study by Meijsser and Hughes (1989); they found that searching was extended in the absence of a nest site (cage) or if the preferred nest to bird ratio was high (perchery). There was also a greater number of bouts of sitting or nest entries in these two systems. Furthermore, as for chapter 5 of this thesis, the duration of sitting or total time on the nest were not affected by the absence or shortage of preferred nest sites. The absence of an effect of nest quality on the duration on the nest is discussed below (section 8.3.3).

Pre-laying behaviour was also compared between barren environments and environments that provide many cues for exploration. It was found that the searching phase was longer (and more searching behaviour and nest examinations were performed) in environments that provided many cues for exploration. It is suggested that the longer duration of searching behaviour is due to pre-laying behaviour starting earlier; as no changes in the later stages of pre-laying behaviour were observed which would be expected if hens were delaying entry into the nest box. It was concluded that environments that provide cues for exploration result in the motivation to perform pre-laying behaviour being expressed earlier than in barren environments.

8.2.4 Effects of social interactions on pre-laying behaviour

Overcoming an aversive stimulus to reach a nest box may indicate a strong motivation to reach that resource. This was examined in chapter 6, in which the aversive task involved passing a dominant or unfamiliar hen (Grigor, 1993). It was found that hens were willing to overcome these aversive stimuli in order to reach the nest site. Before approaching the nest box however, hens made more attempts at finding alternative routes to the nest box with a dominant or unfamiliar stimulus bird. These findings support the results of Grigor (1993) that passing a dominant or unfamiliar hen is aversive. The time from the start of pre-laying behaviour until the first approach to the nest box was longer when required to pass a dominant or unfamiliar hen than an empty cage or subordinate hen. However, hens were still approaching the nest box about 20 minutes before the first nest entry with an aversive stimulus, suggesting that they were approaching the nest box to examine it. Thus hens are weakly motivated to inspect the nest box in the earlier stages of pre-laying behaviour but this was insufficient for them to overcome the aversive stimuli presented. However, the motivation to reach the nest site increased with time such that hens overcome these aversive stimuli towards the start of the sitting phase.

The effect of social interactions on access to the nest site and pre-laying behaviour of hens in small groups was investigated in chapter 7. A pre-oviposition surge in aggression was observed when more than one hen was showing pre-laying behaviour, but there was no indication that aggression increased before oviposition when only one hen was showing this behaviour. Hens were displaced more from the nest site when there were dominant hens also in the pre-laying phase than when no other hens were showing this behaviour. The pre-oviposition surge in aggression observed here differs from that reported in Hughes (1979) in several ways. Hughes (1979) observed a pre-oviposition surge in aggression in caged layers (and not in hens in littered pens) after the introduction of a strange bird (which enhances aggression). In chapter 7, a pre-oviposition surge in aggression was observed in litter housed hens only when more than one hen was showing pre-laying behaviour. These findings extend the results of Hughes (1979) by suggesting that a pre-oviposition surge in aggression can also result from competition for the nest site.

When hens were in the pre-laying phase they walked more in the last hour before oviposition and sat less in the last 25 minutes when dominant hens were also showing pre-laying behaviour than when no other hens were in the pre-laying phase. Subordinate hens showed an increase in pacing (180° changes in direction) but a similar number of exploratory head movements in the last 25 minutes before oviposition compared with the other two categories in chapter 7. This suggests that the increase in locomotion observed in subordinate hens was due to thwarting of the motivation to nest rather than an extension of the searching phase. Increases in the number of steps taken and other activities indicating frustration are also observed when hens are thwarted in their attempts to reach a previously available nest site (Duncan, 1970). Dominant hens in the pre-laying phase remained nearer the nest when other hens were showing pre-laying behaviour than when none were doing so. This could perhaps be interpreted as "guarding" the nest site. As displacement attempts were not always successful, guarding of the nest site may ensure that dominant hens have constant access to the nest. Thus competition for the nest site influences pre-laying behaviour in both high and low-ranking birds, but in different ways.

8.3 Implications for a motivational theory of pre-laying behaviour.

It was argued in chapter 1 that hens have evolved to express both searching and nesting behaviour. Oviposition can also be viewed as an action, and it is proposed that it too is controlled by a motivational system in its normal form; if it is disrupted or prevented it can sometimes occur without the normal behaviour in the course of other activities. The expression of these three actions controls and accounts for variability in pre-laying behaviour. Thus pre-laying behaviour begins with searching behaviour. The switch from searching to sitting terminates searching behaviour and also determines the duration of sitting on the nest. Oviposition terminates pre-laying behaviour and in commercial laying strains is usually followed by activities unrelated to nesting. Within this framework I shall discuss the factors that determine the expression of pre-laying behaviour.

8.3.1 Onset of pre-laying behaviour

A descriptive model of the presumed variation in the tendencies to perform pre-laying, feeding and other activities around the time of the start of pre-laying behaviour is presented in Figure 8.1. This model proposes that the tendency to perform pre-laying behaviour arises and increases due to physiological events (initiated by ovulation 24 hours earlier). Tendencies to perform various activities are assumed to compete with each other and the strongest tendency will be expressed in behaviour. According to this model, pre-laying behaviour will start when the tendency for this behaviour exceeds the tendency for the current activity. Therefore, onset would be governed by causal factors for pre-laying behaviour as well as causal factors for competing behavioural tendencies.

It was found (chapter 3) that pre-laying behaviour started earlier for the first egg of a sequence than for other eggs. This suggests that the internal causal factors for searching behaviour begin to rise earlier for the first egg of a sequence than for other eggs. The role of internal factors in controlling pre-laying behaviour of hens was first demonstrated by Wood-Gush (1963). Internal factors are also important in controlling the nesting behaviour of canaries, such that the intensity of nesting behaviour is linked to the intensity of the hormonal cue (Hinde, 1958). As discussed in chapter 3, a longer searching phase for the first egg of the sequence would appear to be adaptive for the ancestor of the domestic hen or feral hens if sequences are comparable to natural clutches. However, pre-laying behaviour is difficult to study in the wild and the time taken to choose nest sites is unknown in feral hens (Duncan *et al*, 1978).

As Figure 1 shows, pre-laying behaviour would start when the tendency to perform pre-laying behaviour surpasses the tendency to perform the current activity. There is substantial evidence to suggest that feeding is the activity that is usually suppressed by pre-laying behaviour. Firstly, feeding occurs mainly at the start of the day (Savory, 1979), when pre-laying behaviour usually takes place. Intermittent bouts of pre-laying behaviour and other activities are observed in the early stages of pre-laying behaviour, suggesting that the competing motivations are evenly balanced (Wood-Gush, 1954). Lastly, the high feeding activity when the hen comes off the nest (Wood-Gush and Horne, 1970), and the strong motivation to reach food (Cooper *et al*, 1993) suggests that feeding has been suppressed during the pre-laying period and that feeding motivation has built up. Thus, in the absence of food the start of pre-laying behaviour would be governed by competition between the tendency for pre-laying behaviour and other activities (Figure 8.1). The tendency to perform these other activities is less than the tendency to feed. Thus pre-laying behaviour would start earlier in the absence of food than if it were present. This was tested in chapter 4, in which the searching phase was found to be longer when food was not available to the hens than if it was present. The lack of changes to the later stages of pre-laying behaviour suggested that pre-laying behaviour was indeed starting earlier when food was absent rather than the hen entering the nest box later in the sequence. Thus, the model offers a good explanation for the start of pre-laying behaviour in the absence of the usual competing behavioural tendency.

Alternatively, Figure 8.1 shows that increasing the tendency for searching behaviour would result in the current activity being replaced by pre-laying behaviour earlier. Searching behaviour can be described as an appetitive behaviour, which serves to attain stimuli (nest site) necessary for consummatory behaviour (laying). There is increasing evidence that hens have a requirement to perform appetitive behaviour (as discussed in chapter 1). It has been proposed that appetitive behaviour is enhanced by appropriate stimuli (Wood-Gush and Vestergaard, 1989). For example, hens kept on litter spend much time pecking in the litter where there is little food (Wennrich, 1980), suggesting that the appetitive elements of feeding behaviour increase when there are more appropriate stimuli to trigger the activity. Likewise, sitting and some patterns of nest building are increased in a pre-formed nest with an egg compared with a flat litter surface (Hughes *et al*, 1989). Although animals may be motivated to perform appetitive behaviour, it may also be maintained and reinforced by expectation and performance of consummatory behaviour. For example, feeding behaviour can be reinforced by the expectation of food, such that feeding behaviour is more likely to occur at one place if the animal has found food there before. Feeding behaviour is also reinforced by the consumption of food at the beginning of feeding (Wiepkema, 1971). Thus, it is suggested that providing many external cues for an appetitive behaviour increases the tendency for that activity.

This was tested in chapter 5 in which pre-laying behaviour was observed in barren environments and environments with an exploratory walkway. Pre-laying behaviour was found to start earlier in environments with the walkway. This advanced the start of pre-laying behaviour as illustrated in Figure 8.1. Hens also performed more nest examinations during the searching phase in environments with the walkway. This suggests that hens were indeed performing more exploratory behaviour in environments with the walkway, rather than the alternative explanation that searching behaviour was more obvious in environments with the walkway. These findings agree well with the results of Hughes *et al* (1989), which reported that some patterns of nest-building and the duration of the sitting phase increased in pre-formed nests with eggs compared with flat litter nests. Similarly, nest building starts earlier in sows (*Sus scrofa*) with a pre-formed nest than without (Arey *et al*, 1991). Thus the presence of many appropriate stimuli release particular patterns of behaviour sooner than poor stimuli.

Evidence presented in this section suggests that the tendency to search at the start of pre-laying behaviour increases as suggested in Figure 8.1. The purpose of searching behaviour is, presumably, to bring the hen into contact with a suitable nest site on which the hen can perform nesting behaviour. The factors that determine the start of the sitting phase (nesting behaviour) are discussed in the next section. Is there any evidence that hens are motivated to examine possible nest sites during the searching phase, and that this tendency increases gradually? One method of assessing this would be to for hens to overcome an aversive obstacle in order to reach a nest site (Dawkins, 1983). It was found (Chapter 6) that hens approached the nest site about 20 minutes before the start of the sitting phase when required to pass a dominant or unfamiliar hen in order to reach the nest box. However, the large number of attempts at finding alternative routes to the nest box before successfully proceeding to the nest box suggests that hens were motivated to reach the nest site for some time before actually doing so. These results suggest that the tendency to examine possible nest sites increases with time, such that only later in the searching phase was this tendency sufficient for the hens to overcome an aversive obstacle.

The view presented in this section that there is a definite point at which pre-laying behaviour starts is a simplified one. Instead, the hen is often observed to show bouts of searching and other activities unrelated to pre-laying behaviour, with bouts of searching becoming longer and more frequent with time. Preening is also observed around this time, which has been suggested to be a displacement activity arising from the tendency to show pre-laying behaviour competing with other tendencies (Wood-Gush, 1954). Assuming that feeding is the primary conflicting tendency in the early stages of pre-laying behaviour, a model is proposed to account for these observations (Figure 8.2). This model proposes that hunger increases when the hen is not feeding and decreases when it is. In the early stages of pre-laying behaviour, the tendency to search may increase at a slow rate such that it is overtaken by the tendency to feed. This would result in feeding replacing pre-laying behaviour. As oviposition approaches, the tendency to perform pre-laying behaviour may increase more rapidly such that it is not replaced by feeding.

8.3.2 Onset of sitting phase

A descriptive model of factors involved in determining the switch from searching behaviour to nesting behaviour during the pre-laying period is proposed in Figure 3. The model proposes that the tendency to search increases as shown. The tendency to perform nesting behaviour increases later and at a faster rate than that for searching. This ensures that nesting behaviour follows searching. Factors that increase the tendency to search would thus delay the onset of nesting behaviour. Factors that increase the tendency for nesting behaviour however, may be expected to advance the onset of nesting behaviour.

In chapter 3, it was suggested that nesting behaviour is not identical for all eggs. The duration of the sitting phase and total time spent in the nest box were found to be positively correlated to lag. The mechanism by which the relation between nesting behaviour and lag arises remains unclear. Also, it is impossible to determine if the tendency for nesting behaviour varies between different eggs. For example, Figure 8.3 predicts that nesting behaviour would be longer if the tendency for it were increased (the line for nesting behaviour would be shifted up). Alternatively, the observed increases in nesting behaviour could also arise if lag was related to the time of oviposition. That is, if large lags delayed oviposition with respect to pre-laying behaviour, then nesting behaviour would be extended. Thus it remains unclear to what extent the tendency for nesting behaviour is influenced by internal causal factors.

There is good evidence that in the absence of consummatory stimuli, the motivation for appetitive behaviour remains high and appetitive behaviour continues. For example, Cooper and Appleby (in press) found that hens persisted with searching behaviour and were prepared to work to explore their environments in the absence of a nest site. Similarly, sows show more nest preparation and less nest building in environments with no substrate than when a substrate is provided (Jensen, 1993). The interaction between the searching and nesting elements of pre-laying behaviour was investigated in chapter 5 by observing pre-laying behaviour in the presence and absence of a suitable nest site. The searching phase was found to be longer in environments without suitable nest sites. The findings reported here and those of Cooper and Appleby (in press) suggest that searching behaviour is maintained in the absence of a nest site. In chapter 5, searching was expressed even during the sitting phase, when hens searched and examined the nest more in the absence of a suitable nest site than if a suitable nest site was provided. The sitting phase, however, consisted of more nest entries of shorter duration in the absence of a suitable nest site. Thus, in the absence of a suitable nest site, a high tendency to search appears to compete with the tendency for nesting behaviour.

Hens show incomplete nesting behaviour in the absence of a suitable nest site (chapter 5) or when prevented from reaching the nest site (chapter 7). Even in cages that provided few cues for nesting, hens perform some nesting behaviour (Meijsser and Hughes, 1989). There are two possible explanations for the occurence of nesting behaviour in the absence of appropriate cues. Firstly, hens may exhibit a low behavioural threshold for nesting, such that the behaviour is expressed even if only poor cues for it are available. However, hens are prepared to overcome aversive stimuli (Cooper and Appleby, 1994a; chapter 6) to gain access to a nest box suggesting that they are motivated to find better cues for nesting behaviour. Secondly, there may be internal factors that promote the change from searching to nesting (i.e. the motivation for nesting increases as in figure 3). Such an explanation is in agreement with the work of Hinde on the nesting behaviour of canaries. Hinde (1958) classified nesting behaviour into a number of categories. He found that the transition from one activity to the next was only partly dependent on external factors.

Some hens fail to show normal nesting behaviour even if a "suitable" nest site is provided. For example, Appleby (1990) reported that a few hens in modified cages performed an excessive number of nest entries during pre-laying behaviour. Also, Cooper and Appleby (1994a) found that floor layers with access to a nest box were as motivated to search for a nest site as nest layers with no nest box. These findings raise the question as to what hens regard as suitable nest sites, as well as pointing out that there is individual variation in this. Individual hens may have some internal representation of a suitable nest site, perhaps as a search image. This search image could be loosely defined in terms of qualities of the nest site, such as enclosure, loose material and mouldability. Only when hens have found a site with appropriate qualities is nesting behaviour elicited. This search image may be formed before the hen comes into lay, as early exposure to nesting cues affects nest site selection (Rietveld-Piepers *et al*, 1985; Sherwin and Nicol, 1993a).

Evidence that the start of the sitting phase is controlled by interaction between the tendency to search and to build a nest is provided in the study by Hughes *et al* (1989). They found that the sitting phase was longer in pre-formed nests than in flat littered nests. The results suggest that a longer sitting phase was due to it starting earlier in the pre-laying behaviour sequence. Similarly, nest building starts earlier in sows provided with a pre-formed nest, though they gather less material than when no nest is provided (Arey *et al*, 1991). Other external cues such as the degree of shelter and weather are also important in modifying the nest building behaviour of sows (Jensen, 1989). Thus, providing cues for nesting seems to allow the motivation to build nests to be expressed earlier. In sows, these cues can also modify nesting behaviour suggesting that it is under feedback control. Hughes *et al* (1989), however, failed to find a reduction in the nest building activities of hens provided with a pre-formed nest. Thus providing cues that trigger the sitting phase results in the motivation to express nesting behaviour being expressed earlier in hens, though it unclear to what extent this behaviour is under feedback control.

The results of chapter 7 emphasise that it is not just the external cues for nesting behaviour, but the actual performance of this behaviour that terminates searching behaviour. It was found that locomotion was increased and nesting behaviour reduced if access to the nest site was restricted by dominant hens also showing pre-laying behaviour. Under these circumstances, hens had visual contact with a suitable nest site but would have been prevented from reaching it. This restriction in access to the nest site might be expected to result in the hen moving to another site. However, this did not appear to be the case as hens were rarely seen to attempt to nest in another site. One possible explanation for this is that the cues to nest at one particular site, such as the presence of another hen and eggs and that the hen may have laid there before, were very strong. Alternatively, the increased locomotion may not have been part of searching behaviour but due to frustration at not being able to settle on a particular nest site. For example, closing the trap-nests of hens used to laying in them results in increased pacing and preening similar to those of hens in other frustrating situations (Duncan, 1970).

The presentation of food is a strong cue for feeding in hens. Indeed the presentation of food resulted in hens interrupting pre-laying behaviour to feed (Chapter 4). After such an interruption, it is predicted that pre-laying behaviour would resume when the tendency for it surpasses the tendency to feed. Thus, the duration of the interruption may be related to the length of deprivation. However this was not found to be the case (Chapter 4), even though the durations of deprivation used achieved variable levels of hunger in hens not showing pre-laying behaviour. One possible explanation for this may be that the rate of feeding is related to hunger (Savory *et al*, 1993), such that hens may have eaten more after long period of deprivation but in a similar time period as less hungry hens. Thus after long deprivations, the tendency to feed may fall at a faster rate than after short deprivation, resulting in no difference in the length of interruption.

This section summarized the factors involved in determining the switch from searching to nesting behaviour and a model which describes when this switch occurs is proposed. Evidence is provided which suggests that in the absence of a suitable nest site, hens continue showing searching behaviour until oviposition. The persistence of searching behaviour until oviposition could arise in two ways. Firstly, in the absence of a suitable nest site, the tendency for nesting behaviour may not increase enough to suppress searching behaviour (Figure 8.4a). Alternatively, the tendency to search may increase as oviposition approaches if the hen does not encounter the appropriate consummatory stimuli (Figure 8.4b). The results from chapter 5 do not support the latter alternative: although more searching was observed during the sitting phase in environments with unlittered nest boxes, no increase in the percentage of time (outside the nest box) spent searching was found. Large amounts of searching behaviour during the sitting phase may thus represent an extension of searching, rather than an increase in the tendency for it. As the performance of behaviour can be a rough measure of motivation more intense searching behaviour (i.e. a higher percentage of the time) would be expected in the later stages of pre-laying behaviour according to Figure 4b.

8.3.3 Oviposition

A greater duration of pre-laying behaviour is observed if hens do not have a suitable nest site (Chapter 5). The initial stages of pre-laying behaviour appeared as in environments with suitable nest sites, though the later stages were strongly influenced by nest quality. Thus I believe the longer duration of pre-laying behaviour observed in environments with unsuitable nest sites to be due to oviposition being delayed. Oviposition is also delayed in hens deprived of access to a nest site (Duncan, 1970).

Surprisingly, the total duration in the nest box did not differ between hens in littered or unlittered nest boxes, though hens performed more nest entries into unlittered nest boxes (chapter 5). Furthermore, the duration of sitting was never observed to be significantly more than expected after an interruption of pre-laying behaviour (either in experiment 1 or 2 of chapter 4). This is surprising as the duration of all the other activities recorded increased after an interruption. Similarly, Meijsser and Hughes (1989) did not find any significant differences in the duration of sitting between four different husbandry systems. Sherwin and Nicol (1993b) also noted the similarity in the duration of time spent sitting between hens kept in conventional cages, modified cages and alternative systems. This stability in the duration of sitting despite delays in oviposition and other changes in the sitting phase may indicate that duration on the nest is relatively fixed. A certain amount of behavioural priming may be required before normal oviposition can occur, such that hens may require to sit on the nest for a certain duration before oviposition. However, individual hens were not found to be consistent in duration in the nest box in different environments (Chapter 5).

The delay in oviposition observed after the presentation of food was related to the stage during the pre-laying behaviour sequence when food was presented (Chapter 4). However, as suggested in chapter 4, it was searching behaviour which differed significantly between the two interrupted records (feeding near or further from the expected oviposition time). Thus a certain amount of searching behaviour is necessary before the hen resumes sitting on the nest after an interruption. However, if the interruption occurs earlier in the pre-laying behaviour sequence, the hen resumes pre-laying behaviour from where she left off. The functional implications of this seem adaptive: if a hen is disrupted while on a nest it may well be advantageous (in terms of reproductive success) to search for a new nest site.

Further research is required to assess if a certain duration of sitting on the nest site is required before oviposition. This is represented in Figure 5, in which hens sit for a proportionate amount of time after the interruption of pre-laying behaviour as would be expected in undisturbed pre-laying behaviour. Figure 5 also shows that searching behaviour follows an interruption of nesting behaviour as suggested in chapter 4. Although this seems adaptive, the mechanism whereby this characteristic arises remains undetermined.

8.4 Implications for welfare and production

It was proposed (Chapter 1) that welfare is reduced if animals are strongly motivated to perform behaviour but are unable to do so. This may not affect production directly, but it may lead to behavioural changes indicative of frustration which increases energy requirements and may damage the animal (Duncan, 1970; Mills *et al*, 1985). This section summarizes the implications of the findings presented in this thesis on the welfare and production of laying hens in commercial husbandry systems.

Substantial evidence is accumulating to suggest that hens are strongly motivated to lay on a nest site (Duncan and Kite, 1987; Smith *et al*, 1990; Cooper and Appleby, 1994a). This was supported in chapter 6, in which hens were willing to perform an aversive task in order to reach the nest site. Performing aversive tasks in order to reach a resource has been interpreted as showing a high demand for that resource (Dawkins, 1983). A number of studies have been reported to identify the qualities of the nest site which the hen would perceive as "suitable" (Duncan and Kite, 1989; Petherick *et al*, 1993). In chapter 5, searching behaviour was extended and observed in the later stages of pre-laying behaviour in environments with unlittered nest boxes. This suggests that an unlittered nest box does not allow the expression of nesting behaviour. This coincides well with the findings of Petherick *et al* (1993) that hens preferred to lay in littered nest boxes. Thus hens appear to be strongly motivated to lay in a littered nest box (or at least a nest that can be moulded (Duncan and Kite, 1989)), denial of which may be detrimental to welfare. Also the large duration of searching behaviour in environments with unlittered nest boxes may affect production by increasing energy loss and the chance of damage to the hens.

It was suggested in the previous section that hens are motivated to express searching behaviour. In the absence of a suitable nest site, searching behaviour is extended and there is no smooth transition to nesting behaviour. Thus, in environments without suitable nest sites hens are unable to express nesting behaviour fully, which may be detrimental to welfare. Furthermore, this extension of searching has several implications. Firstly, the excessive locomotor activity may increase energy loss, and lead to more aversive encounters with dominant or unfamiliar hens (Chapter 6). The number of Gakel calls is greater in the later stages of pre-laying behaviour in cages (Meijsser and Hughes, 1989), and excessive nest entries may be observed in some hens in cages with nest sites (Appleby, 1990). These abnormal events may result from an extension of searching behaviour in the absence of a nest site that is perceived as suitable. Indeed, a great many nest entries are observed in floor layers, presumably because they fail to recognise the nest provided as suitable (Cooper and Appleby, 1994b). However, it remains to be confirmed if such changes in pre-laying behaviour indicate poor welfare.

There is probably an upper limit on the size of peck orders (Guhl, 1953) and in large flocks some hens may be regarded as unfamiliar. The results of chapter 6 suggest that hens avoid encountering dominant or unfamiliar hens during pre-laying behaviour and this may restrict their ability to carry out searching behaviour. Nesting behaviour may also be affected by social interactions. When more than one hen is showing pre-laying behaviour, the subordinate may be prevented from settling on the nest site (Chapter 7). In environments with a high bird:nest ratio, subordinate hens may not be able to find an available nest site. Thus social interactions during pre-laying behaviour can prevent the expression of searching and nesting behaviour, which may have deleterious effects on welfare. Furthermore, displacement of hens from nest sites may increase the number of floor eggs, which affects production (Appleby, 1984).

Lastly, the presentation of food was found to be a strong stimulus for feeding which interrupts pre-laying behaviour (Chapter 4). On two occasions, pre-laying behaviour was not resumed and the egg was dropped while performing other activities. A. Rasmussen (unpublished) also found that feeding behaviour suppressed pre-laying behaviour. This has important implications for production as feeding during the pre-laying period could lead to more broken eggs or eggs laid outside the nest sites.

8.5 Conclusions

Academic, welfare and production interests can all offer a basis for studying the mechanisms that control pre-laying behaviour. In this thesis, the effects of internal factors, competing behavioural tendencies, environmental complexity and social factors on pre-laying behaviour were examined. Results were interpreted within a framework of a motivational theory for the control of pre-laying behaviour. It is proposed that changes to pre-laying behaviour arise from 3 motivational systems that control the expression of searching, nesting and laying.

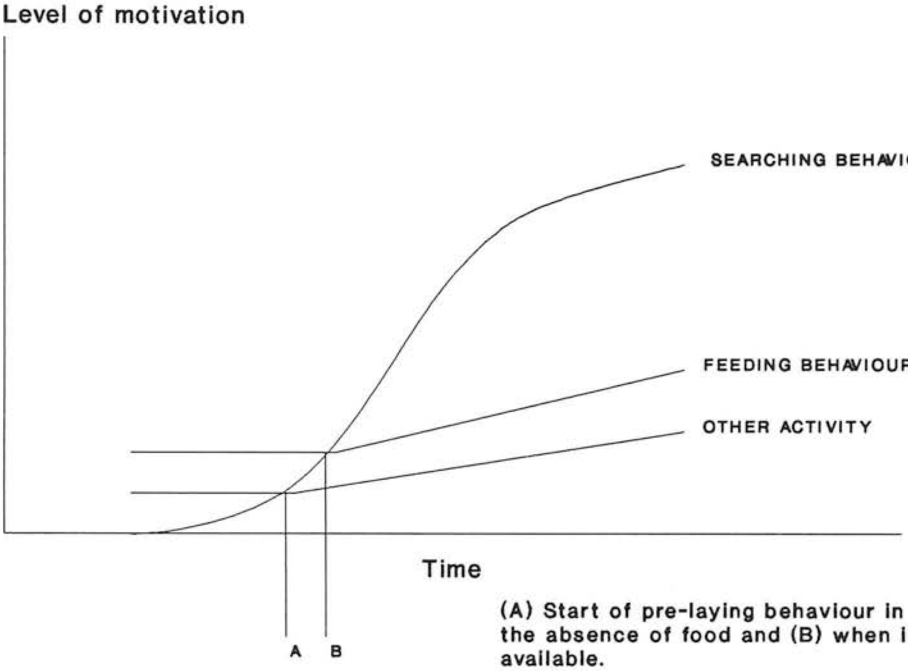
Internal factors influence the start of searching behaviour, such that more searching behaviour is expressed for the first egg of a sequence. This may be controlled by an inhibiting mechanism that reduces searching behaviour for later eggs of a sequence. The start of pre-laying behaviour appears to be determined by the tendency for searching ousting the current activity and this offers a good predictor for the start of pre-laying behaviour. Also, increasing the tendency for searching allows this activity to be expressed earlier in the pre-laying behaviour sequence, supporting this competition hypothesis. Evidence that hens are motivated to inspect nest sites during the searching phase was found. A model to account for bouts of searching behaviour often reported during the earlier stages of pre-laying behaviour is proposed.

The expression of nesting behaviour is related to lag, suggesting that the performance of this activity is influenced by internal events. The tendency to search competes with the tendency for nesting behaviour in the absence of a suitable nest site. If social factors prevent access to the nest site then nesting behaviour is reduced and locomotion is increased, either due to searching behaviour not being inhibited or frustration. It is suggested that the tendency for nesting behaviour may not increase enough in the absence of a suitable nest site to replace searching behaviour fully.

Oviposition can be delayed if pre-laying behaviour is interrupted or if a suitable nest site is not provided. These manipulations result in many changes to pre-laying behaviour, but not to the duration on the nest. It is suggested that oviposition can only occur after a certain duration on the nest. Furthermore, searching behaviour precedes resumption of sitting after an interruption. This may well be an adaptive strategy which ensures that nesting occurs on a suitable site.

The implications of the findings reported in this thesis for welfare and production in laying hens are discussed.

Figure 8.1: Competition model for the start of pre-laying behaviour in the presence and absence of food.



(A) Start of pre-laying behaviour in the absence of food and (B) when it is available.

Figure 8.2: Interaction of feeding and searching behaviour to account for bouts of pre-laying behaviour (see text).

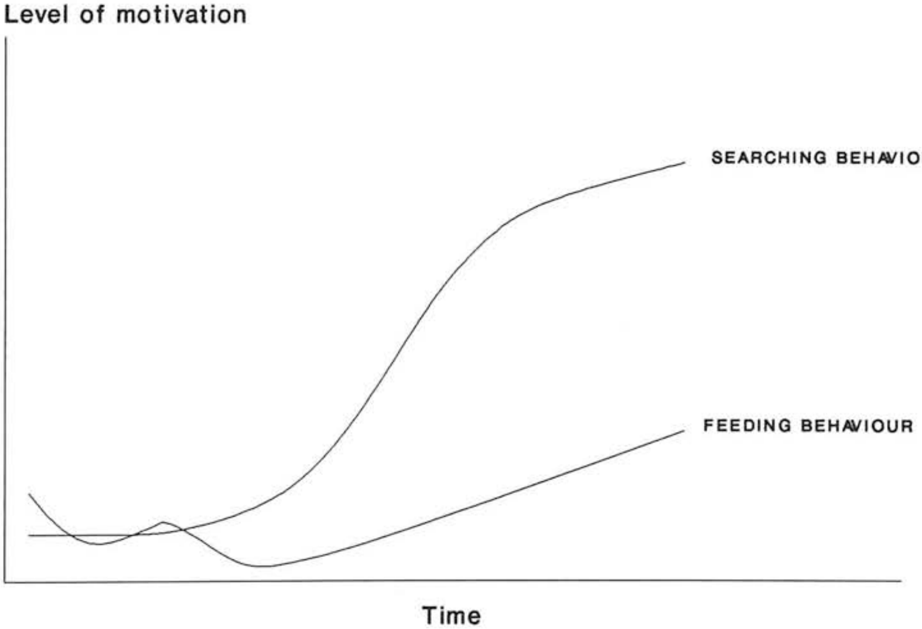


Figure 8.3: Model proposing the mechanisms that determine the switch from searching to nesting behaviour.

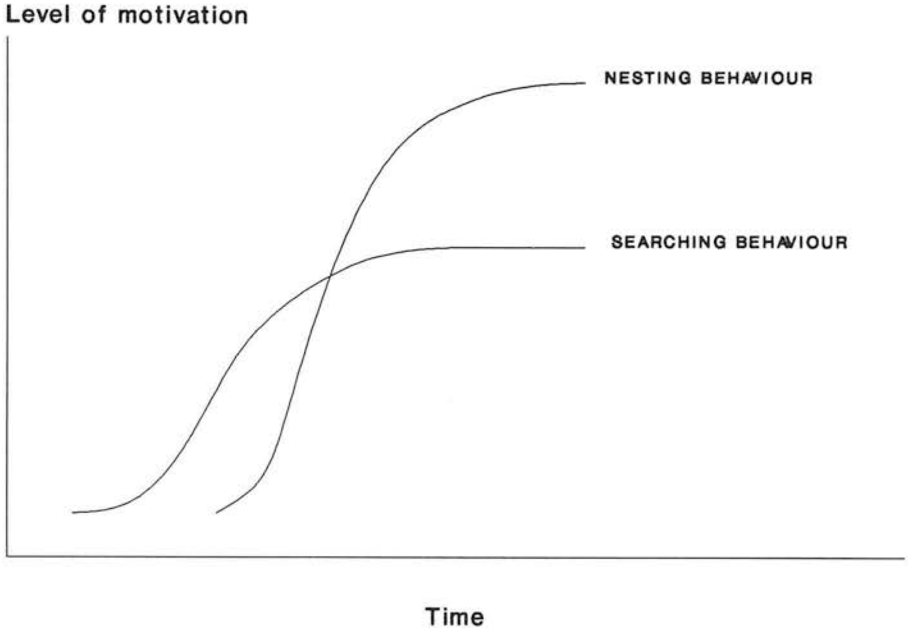


Figure 8.4(a): Model to account for searching behaviour during the sitting phase in the absence of nest (see text).

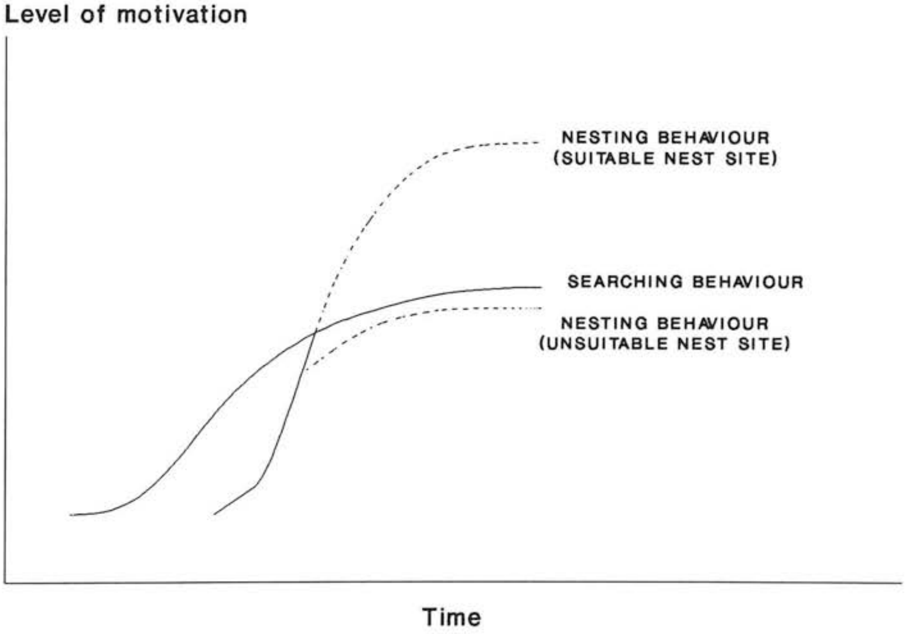


Figure 8.4(b): Model to account for searching behaviour during the sitting phase in the absence of nest (see text).

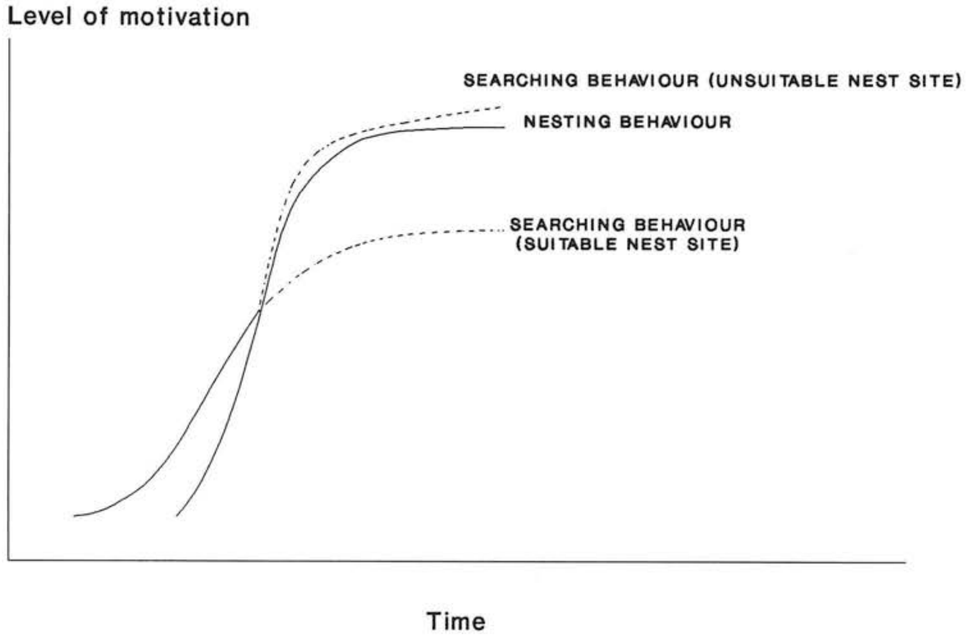
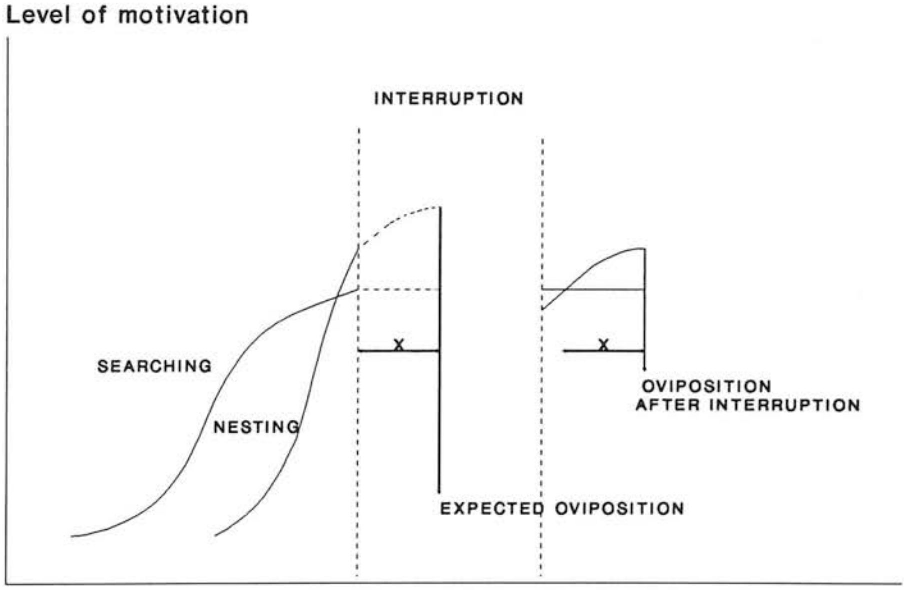


Figure 8.5: Representation of changes in pre-laying behaviour due to an interruption.



X represents equal amounts of time

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