Introduction

Partly concealed in the hole she has excavated, a female (foreground) lays an egg while the male stands at the edge of the hole. In the background, a second female has come onto the mound, taking advantage of the egg-laying female's inability to remove her while in the act of laying.

Males in some species typically behave aggressively toward females in a mating context, and some of this aggression may be characterized as sexual coercion. The term *sexual coercion* usually refers to coercion directed specifically at obtaining copulations and is generally understood to take three forms: forced copulation, harassment and intimidation (Clutton-Brock and Parker, 1995). Sexual coercion of females by males can be viewed as an additional form of competition giving rise to sexual selection, alongside male-male conflict and males competing to be chosen by females. Sexual coercion is typically viewed as an alternative or sub-optimal tactic employed by males who are in a weak position relative to male competitors (e.g. Pelletier et al., 2006), but in Australian brush-turkeys it appears to be employed by dominant males, namely those who own incubation mounds.

Unlike sexual coercion, which focuses on the interests of the coercive male, sexual conflict focuses on the competing interests of both male and female. Sexual conflict arises where male-female interactions have an optimum outcome for males which is different from the optimum outcome for females, and both optima cannot be achieved simultaneously (Chapman, 2006; Parker, 2006). Sexual conflict is typically about mating/fertilization or parental investment, but other points of conflict are also possible, for example

reproductive resources, infanticide or mate cannibalism (Chapman, 2006). In brush-turkeys, sexual conflict appears to be about the male's coercive pursuit of copulations versus the female's interest in the mound as a reproductive resource.

Mate choice is typically defined as any pattern of behavior by members of one sex which results in a higher likelihood of mating with certain members of the opposite sex than with others (Halliday, 1983). Patterns of behaviour amounting to female choice may vary in their objects (principally males themselves as opposed to the resources controlled by males) and their benefits for females (principally genetic as opposed to immediate benefits). Brush-turkeys offer an interesting case-study in the relative importance of males and resources as objects of choice because males construct and maintain incubation mounds which they use as a means of attracting females.

Male-female sex roles concern the degree to which a sex is competitive or choosy, and are traditionally thought to depend on relative parental investment by each sex (Kokko et al., 2006; Trivers, 1972). Brush-turkeys once again offer an interesting case-study because females appear to be both competitive and choosy, consistent with a mating system in which male-female differences in parental investment are minor, while males appear to be exclusively competitive, consistent with a mating system in which female parental investment is substantially larger than that of males.

These themes, namely sexual coercion, sexual conflict, female mate choice and male-female sex roles are here addressed in an unusual study system, the mating behaviour of the Australian brush-turkey.

Australian brush-turkeys are member of the megapode clade (Family Megapodidae). Megapodes have attracted considerable scientific interest because, uniquely among birds, they incubate their eggs using environmental sources of heat, an adaptation which has had profound evolutionary consequences within the group (Jones et al., 1995). In some species, the environmental source is soil heated by natural volcanic action or beach sand heated by the sun, but the source in most species is microbial decomposition in a purpose-built mound of soil and organic matter (Jones et al., 1995). Environmental incubation requires a large, super-precocial chick able to dig its own way out of the mound and fend for itself on emergence, a large yolk-rich egg in order to support the large chick, and an unusually thin-shelled egg to support gas flow across the eggshell despite low levels of oxygen and high levels of carbon dioxide and moisture in the mound substrate (Jones and Göth, 2008). By removing the need for post-hatching care, environmental incubation has also influenced adult reproductive strategies, with females free to concentrate on producing many eggs, and males (or both sexes) free to construct

and maintain incubation mounds, where these are the preferred heat source (Jones et al., 1995).

Environmental incubation also has a strong influence on mating and sexual selection in the megapode group, with two distinct patterns identifiable (Jones and Birks, 1992). Most megapodes are monogamous, with males following and monopolizing individual females at the expense of defending heat sources. In some species, however, namely the Australian brush-turkey, *Alectura lathami*, and most probably species in the *Aepipodius* genus, males defend incubation mounds at the expense of monopolizing females (Jones et al., 1995). Malleefowl (*Leipoa ocellata*) males defend mounds but form monogamous pair bonds with individual females, an arrangement which has been interpreted as the brush-turkey system in an arid environment, where monogamy appears to be imposed by low male-female encounter rates (Jones, 1992). Chapter 5 contains a phylogeny of the megapode group, annotated with mating system information.

The mating system of the Australian brush-turkey combines male resource-defence with polygyny and polyandry, an unusual combination in most groups, but especially in birds. Where standard theories are advanced to explain frequently-encountered patterns, it is difficult to be sure that the correct causal factors have been identified without also testing the theories against unusual patterns. The brush-turkey mating system therefore provides a valuable research opportunity. Both sexes are emancipated entirely from post-hatching care and males provide pre-hatching care without monopolizing female reproductive potential, thereby potentially incubating eggs which are not their own (Birks, 1997). Females appear to have an unusual degree of freedom to choose both males and mounds, while dominant males appear to employ sexual coercion despite having the advantage of mound-ownership. While both males and females appear to be competitive when seeking mating opportunities, apparently only females are choosy. In addition to these unusual patterns, all copulations appear to occur on incubation mounds, where they are easily observed.

 Previous research into the Australian brush-turkey mating system has focused on mound-ownership patterns among males (Jones, 1990b), the degree to which males have sired the eggs in their own mounds (Birks, 1997), the female visiting pattern (Birks, 1996) and female-female aggression during mound visits (Birks, 1996). Female choice has been investigated, but with a strong emphasis on choice of mounds as opposed to males. Mounds are considered more important because females appear to invest considerable effort in mound assessment, tend to copulate only with the male in whose mound they are laying and generally do not mate with the same male over successive years (Birks, 1996; Göth, 2007). Consistent with these assumptions, females have been found to prefer

relatively warm, moist mounds (Birks, 1996), lay larger eggs in mounds with more favorable incubation conditions (Göth, 2007), and show no preference for larger males or males with larger home-ranges (Jones, 1994). Contrary to this, however, it has been argued (e.g. Jones, 1994) that the long-term availability of a mound for incubation is the most important criterion for female choice, as incubation quality varies little between individual mounds. On this view, a male's ability to defend his mound against rivals is critical for female choice (Jones, 1990b).

The female pattern of copulating predominantly with the male in whose mound she is depositing her eggs indicates that she is treating the male and his mound as a package. This in turn suggests that the influence of male and mound traits should be considered together, to explicitly include the relationship between the male and his mound in the analysis. For example, the degree to which a male maintains his mound, presumably a key influence on mound quality, is an attribute of the male-mound relationship, and not an attribute of either the male or the mound. Similarly, a male's ability to defend his mound against rivals is principally evidenced by male-mound information. One key question is therefore the relative influence of male, mound and male-mound traits on female choice (chapter 4). It is unusual to include male-resource traits in this way when analyzing female choice, examples from other taxa suggesting that this approach has broader applicability, and has been unduly overlooked.

Females may treat the male and his mound as a package because they are constrained to do so by male aggression toward them. It has been suggested, for example, that copulation is the price extracted by males when females attempt to investigate their mounds (Birks, 1996). Various other functions have been suggested for male aggression, including that it favours certain types of female visitors over others and extends the duration of female egg-laying visits, thus allowing time for the male's sperm to reach female sperm storage organs before passage of the egg flushes it from the oviduct (Jones, 1990c). These ideas, however, have not been subjected to careful testing. Before investigating female choice, therefore, it is important to ask to what degree male aggression toward females constitutes sexual coercion (chapter 1), what is the function of the male aggression which is characteristic of egg-laying visits (chapter 2), whether female responses succeed in ameliorating the aggression and to what degree sexual conflict in this species is a contest over perseverance, as appears to be the case (chapter 3).

A better understanding of female choice (chapter 4) then provides a basis for investigating why females are so much choosier than males about their mating partners, despite the male's large investment in pre-hatching care (chapter 5). As choosiness is generally considered the mirror image of competition, the bases of both male and female

competitiveness are also examined. These investigations lead to some surprising and interesting conclusions which appear to have broad implications, namely that while competition is driven by the limited availability of the opposite sex, often arising from the total or the largest reproductive cost of the opposite sex, choosiness is driven by a high marginal cost of reproductive events in the choosy sex. One implication of this is that choosiness is not the mirror image of competition.

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Chapter 1 - Paying the price? Attempted sexual coercion in Australian brush-turkeys

A male charges a female, who has started to take evasive action by leaping out of the hole in which she has been digging. Immediately prior to the charge, the female had her back turned toward the male.

ABSTRACT

Sexual coercion by males is directed specifically at obtaining copulations from females, and is generally understood to have three forms: forced copulation, harassment and intimidation. I studied Australian brush-turkeys, *Alectura lathami*, to determine whether male behaviour toward females at incubation mounds could sometimes be classified as aggressive, and whether aggressive behaviours enhanced male copulation success. I found that male behaviours toward females were either significantly associated with the cessation of female mound activity, and hence could be classified as aggressive, or were significantly associated with the commencement of female mound activity, and hence could be classified as enticing. Copulation was significantly more likely to be preceded by some forms of male aggression, typified by the male running at the female or making repeated copulation attempts. A different form of male aggression, however, namely maintaining a position close to the female and sometimes pecking her, was significantly more likely to follow copulation. Video records of successful and unsuccessful copulation attempts

suggested that forced copulation sometimes occurred, but was infrequent. No evidence could be found for intimidation. Sexual coercion in this species seemed to take the form of harassment, with repeated copulation attempts supplemented by running at the female or otherwise moving toward her. Although sexual coercion is generally understood to be a sub-optimal tactic employed by males who are in a weak position relative to male competitors, brush-turkey sexual coercion is employed by males who own incubation mounds, and therefore have dominant status. The explanation may lie in some combination of: limited male control over females, a favorable risk-reward balance for coercion given that the male has exclusive control over the mound, the role of aggression as a default solution to social conflict, the influence of phylogenetic inertia, and the opportunities for coercion afforded by lengthy female visits.

INTRODUCTION

Sexual selection arises where access to mates is competitive in the sense that access by one individual makes access by others more difficult (Andersson, 1994). In species where males are more competitive than females, most competition can be classified either as male-male conflict (often referred to as intra-sexual selection), or as males enhancing their prospects of being chosen as mates by females (inter-sexual selection). In some species, however, it may be necessary to consider a third form of competition, sexual coercion of females by males. The term *sexual coercion* usually refers to coercion directed specifically at obtaining copulations and does not include other male behaviours toward females which may also involve mating-related coercion, such as sequestering females to form a harem or mate-guarding of individual females (Clutton-Brock and Parker, 1995). Although the term includes behaviours such as infanticide in some usages (Smuts and Smuts, 1993), just three forms of sexual coercion are generally recognized by behavioural ecologists: forced copulation, harassment (males seeking to enhance their immediate mating success by repeatedly attempting to copulate) and intimidation (males attempting to enhance their future mating success by punishing females who refuse to mate with them) (Clutton-Brock and Parker, 1995). These three forms overlap with each other, and should not be regarded as exclusive categories. A focus on sexual coercion disrupts the simple two-way contrast between intra- and inter-sexual selection by introducing a distinction within the latter between males coercing females and males enhancing their prospects of being chosen by females.

Sexual coercion is typically viewed as an alternative or sub-optimal tactic employed by males who are in a weak position relative to male competitors. Among dominant male bighorn sheep (*Ovis canadensis*), for example, the optimal mating tactic is to defend a single estrus ewe. An alternative tactic used by subordinate rams is to disrupt this defense

and to seek forced copulations during an extended chase of the ewe. Although this tactic is much less successful for individual rams, those using it can collectively obtain up to 40% of paternities during a breeding season (Pelletier et al., 2006). Similarly, satellite male fallow deer (*Dama dama*) rely on harassment of individual females who have become detached from harems under the control of dominants (Clutton-Brock et al., 1988). Adult male Sumatran orangutans (*Pongo pygmaeus abelii*) are more likely to mate non-coercively with females within their home range than are sub-adult males, who usually rely on forced copulation (Fox, 2002). Sexual coercion is also more likely to be employed by subordinates than by dominants in chimpanzees, *Pan troglodytes verus* (Stumpf and Bösch, 2010). Sexual coercion is sub-optimal apparently because the male's prospects of success are generally low in relation to potential costs. Coercion involves a male-female contest which may be costly for the male as well as the female, and may either attract the attention of rival males, thus triggering a male-male contest (Clutton-Brock and Parker, 1995), or render both parties more vulnerable to predation (Griffiths et al., 2004). Copulation, moreover, is only a means to fertilization, and without long-term influence over the female, a coercive male's fertilization prospects are more vulnerable to female counter-tactics such as copulation with alternative males. Coercion may, nevertheless, be the only option for subordinate males who are attempting to make the most of a bad situation.

In birds, as in the mammalian examples thus far cited, sexual coercion is a tactic adopted by males in a competitively weak position, even though coercion is less common than in mammals (Caizergues and Lambrechts, 1999; Pradhan and Van Schaik, 2009). Among waterfowl (Anatidae), already-paired males are sometimes able to evade the mate-guarding efforts of rival males and force copulations with females who are not their social partner (McKinney and Evarts, 1997). Uniquely among birds, the New Zealand hihi or stitchbird (*Notiomystis cincta*) employs a face-to-face style of mounting exclusively for forced extra-pair copulations which often appear behaviourally complete, although lack of an intromittent organ renders insemination success especially uncertain (Low, 2005). Female white-fronted bee-eaters (*Merops bullockoides*) are sometimes forcibly mated by extra-pair males, as in a number of other colonially-breeding species, but cloacal contact is rarely achieved, and fertilization may be even more rare because paired individuals induce sperm competition by copulating more frequently in response (Emlen and Wrege, 1986). Lack of an intromittent organ among most birds (Briskie and Montgomerie, 1997) suggests that harassment and intimidation are more likely forms of sexual coercion than forced copulation. In free-ranging feral fowl (*Gallus gallus domesticus*), mating is promiscuous with subordinate males often obtaining copulations by harassment (Lovlie and Pizzari, 2007). Harassment by non-territorial males is also common in ring-necked

pheasants, *Phasianus colchicus* (Mateos, 1998) . In many monogamous species, extra-pair males incur no parental care costs and hence have a strong incentive to obtain copulations by any means. The degree to which extra-pair copulations rely on coercion and the degree to which they result in extra-pair paternity, however, are both controversial questions (Westneat and Stewart, 2003).

Australian brush-turkeys (*Alectura lathami*) provide an interesting case study in this context because males appear to use sexual coercion as a primary rather than an alternative mating tactic. Investigation of this behaviour requires placing it in the context of reproduction in the megapodes (Family Megapodidae), the clade to which the brush-turkeys belong (Birks and Edwards, 2002; Jones and Göth, 2008). Uniquely among birds, megapodes incubate their eggs using environmental sources of heat, usually microbial decomposition in a mound of soil and rotting vegetation (Seymour, 1985). On the basis of this ancestral pattern, variations have evolved. In the brush-turkey system, males construct mounds, control their temperature via ongoing maintenance, defend them against rival males and are polygynous (Jones et al., 1995). Females make frequent visits to mounds, often copulate with the male and periodically dig large holes in which they bury their eggs. Females are not subject to mate-guarding, do not pair-bond with the male and are polyandrous (Jones et al., 1995). Males evidently use their mound as a means of attracting females interested in the incubation properties of the mound (Jones, 1990a). Having attracted a female, a male typically behaves towards her in a way which suggests coercion, for example by charging at her or pecking her on the back or head (Jones 1991).

This male-female aggression is less extreme than that evident when male-male encounters occur near incubation mounds (Jones, 1987), but it is nevertheless substantial, as prolonged interactions can result in the female's death in captive situations where she is unable to escape (Jones et al., 1995). Aggression toward females is interspersed with non-aggressive displays apparently attempting to influence female choice. Both forms of behaviour are directed at each female, suggesting some co-ordination between the two. Where individual females can be confidently identified, it is clear that males are attempting to both entice and coerce each female, rather than selectively enticing some females and driving others away. This allows us to exclude male choosiness as an explanation for the coercive behaviour (for more details, see chapter 5). The male's aggression probably discourages visits from females unwilling or unable to tolerate it, hence biasing the copulations which occur, but it is not usually a form of rejection. Expulsion of a female from the mound is almost always followed by an attempt to entice her back (chapter 2).

Female mound visits may include both copulation and egg-laying, two activities critical for male reproductive success. The relevance of coercion for egg-laying is considered elsewhere (chapter 2). Here I focus on the possible role of coercion in enhancing male copulation success.

My objective has two elements. First, I seek to confirm that some male behaviours toward females are accurately characterized as aggressive, and second I test the hypothesis that this aggression functions to enhance male copulation success. With respect to the first element, I assessed male aggression by considering the impact of male behaviour on female behaviour. Male behaviour was classified as aggressive if it discouraged female mound activity, the digging, probing and scratching by means of which females appear to prepare a mound for egg-laying or assess it for future egg-laying. Conversely, male behaviour was classified as enticing if it encouraged female mound activity. This female-centric approach treated aggression as the proactive use or threat of coercion, and enticement as an attempt to attract by arousing interest. More formally, I ask the following questions:

1. Which male behaviours discourage female mound activity, and can hence be classified as aggressive, and which encourage female mound activity, and can hence be classified as enticing?
2. If male behaviours can be distinguished as above, do males attempt to obtain copulations coercively, and are they successful?

The role of enticement will also be examined. If it can be established that brush-turkey males are indeed engaged in sexual coercion, despite monopolizing an essential resource for female reproduction (namely the incubation mound) an intriguing ancillary question is raised: Why do brush-turkey males use sexual coercion as a primary as opposed to a secondary tactic, contrary to the pattern suggested above? This question will be a major focus in the discussion.

METHODS

Behavioural observation, recording and data compilation
Field studies were conducted on a free-living, individually colour-banded brush-turkey population at the town of Pearl Beach in New South Wales, Australia (33.54°S, 151.30°E). Observations were recorded remotely by five Sony HDR-SR7 video cameras mounted at active mounds and programmed to run daily for 3.5 hours from first light. Data are here drawn from the second half of the 2008-9 breeding season (18 mounds maintained by 15

males; there being three instances of a single male maintaining two mounds). To achieve sufficient statistical power when answering Question 2, data from the first half of the 2009-10 season were also included (16 mounds, each male owning a single mound). With some exceptions due to access difficulties or extreme weather, each mound was observed for seven consecutive days.

When reviewing video of female visits to mounds, behaviour was recorded on an all-occurrences basis to the nearest second. Where necessary the video was reviewed several times in order to record the behaviour of all individuals simultaneously on the mound. While within camera view, females could be uniquely identified but female visits were often interrupted by periods of absence. Observation from hides indicated that during these periods of absence females typically remained in the vicinity of the mound, often just out of camera view. If a period of absence was 22 minutes or less on the video record, the female's return was treated as the resumption of an existing visit so long as male enticement during her absence indicated that she was in the vicinity and likely to return (for details of male enticement, see below). Where female absences from the mound were associated with the presence of rival females, male enticement could not be used as an indicator, but observation from hides suggested that in these instances females also tended to remain in the vicinity and the 22-minute limit was therefore still applicable. Absences from the mound of greater than 22 minutes were treated as gaps between separate visits by the same female, as were absences during which there was no indication that the female remained in the vicinity. A frequency histogram of female absences is shown in Fig. 1, indicating that the majority of absences were 22 minutes or less in duration, and hence treated as temporary absences during a visit.
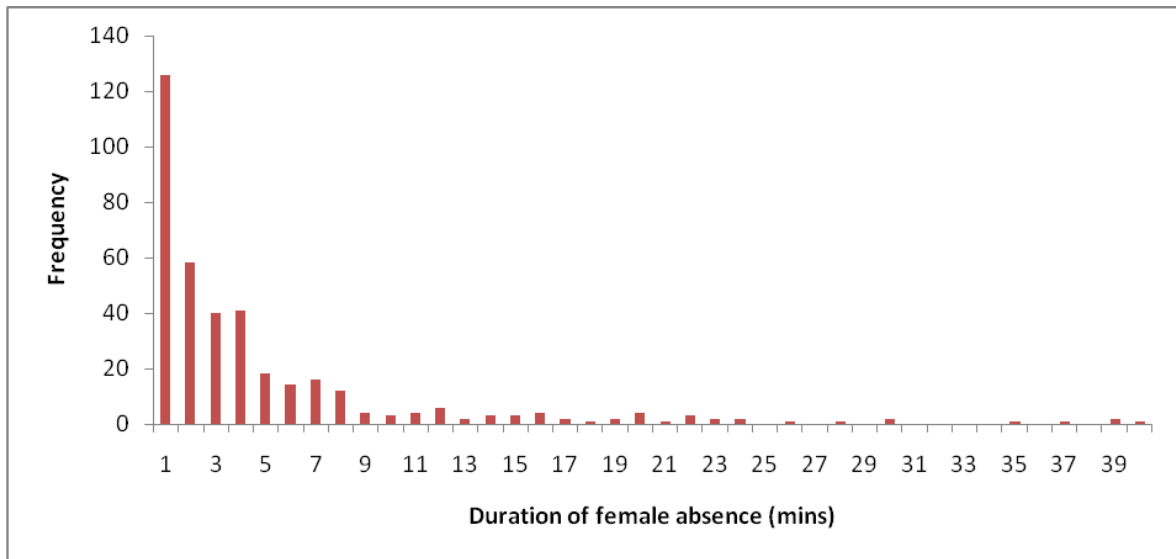
Figure 1 Frequency histogram of the duration of intervening periods of female absence from a mound, where females were present at the mound on multiple occasions on a single day. Absences in excess of 40 minutes are not displayed (there were 26 absences 41-181 minutes in duration). Absences of 22 minutes or less were treated as temporary absences during a visit, while absences greater than 22 minutes were treated as gaps between separate visits.

Where a female was unbanded (52.42% of visits), or her bands were unclear (9.95%), the following identification techniques were used in descending order and, where possible, in combination:

1. infer likely bands by using a combination of video observation of band colours and independently-known female home ranges
2. use identifying morphological characteristics; this was sometimes valid on an ongoing basis, for example when using distinctive bald patches on the head or neck, sometimes only on a daily basis, for example when using displaced feathers
3. use time and direction of arrival and departure, for example a female arriving from the direction of a specific landmark was likely to be the same female who departed in the direction of the landmark several minutes earlier, given the visiting pattern observed when females could be confidently identified

After these techniques had been employed, 23.66% of visits were by females with no distinguishing features.

The identity of male mound-owners could typically be confirmed when cameras were being set-up or removed, or footage was being downloaded, and could also be checked when reviewing video if there was reason to suspect that the male present at the mound was not the mound-owner. Interloping males were generally easy to spot on video because their behaviour differed from that of the owner, for example they might maintain the mound more or less vigorously than the owner.

Only pre-defined events or states were recorded (see Table 1). The standard distinction between events and states was employed (behaviours deemed to have frequencies vs. behaviours deemed to have duration) (Martin and Bateson, 2007), but a rule of thumb was used when applying the distinction. Behaviour was treated as a state if it tended to occur in bouts, and as an event if it did not. Male pecking of the female was treated as a state when distinguishing aggression from enticement (Question 1 below), where the emphasis was on identifying temporal sequences, making it necessary to focus on when the behaviour commenced and concluded. When comparing behaviour pre- and post-copulation, however (Question 2), it was treated as an event, enabling a count of the number of pecks.

Recording of behavioural states was subject to specific rules. If two states cannot occur simultaneously (e.g. a female cannot dig and scratch at the same time), it is arguably necessary to record a separate digging state each time she briefly interrupts her digging with scratching, even though it is both accurate and much simpler to record that she is predominantly digging. The following approach was therefore adopted. A single state was recorded only if the pre-defined behaviour was performed for more than 75% of the recorded duration and was not interrupted for longer than 15 seconds. This allowed other pre-defined behaviours and miscellaneous behaviours to occur during the recording period, but not be recorded, e.g. if female digging was interspersed with female scratching, the scratching was ignored if it occupied less than 25% of the recorded duration, and each interruption was for less than 15 seconds. Thus only a single state could be recorded at any given time for each individual. It was possible, however, for no state to be recorded for an individual for a given period, in which case the period was assigned to a 'non-focal' state. The data was checked to ensure that the sum of state durations, including 'non-focal', equaled total visit time.

There were two exceptions to the rule that only a single state could be recorded at any given time. The male state *hover*, defined as maintaining a position within one body-length of the female, could occur simultaneously with other male states, but was subject to all other rules. The male state *ground-peck*, defined as repeated pecking at the mound substrate, occurred in long bouts as a background accompaniment to other behaviours, and was treated as occurring simultaneously with other male states.

Table 1 Pre-defined behaviours which are the focus of this chapter, distinguished according to whether they are performed by males or females and whether they are events or states. The definitions are similar to, but in some cases amplify or simplify those in Jones (1987). The collective term *bird-peck* refers to male behaviours (peck tail, body and head, plus bite) which are here classified as states, but when comparing behaviour pre- and post-copulation were treated as events, enabling the number of pecks to be counted. The collective term *female mound activity* refers to female behaviours which either prepare a mound for egg-laying or assess it for future egg-laying.

Behaviour	*Description*
Male events:	
toward	move toward female without running; male's intention remains unclear, either because the female avoids him or he suspends action
failed copulation with no contact	move toward female to copulate, but without making physical contact; movement is usually rapid, with neck outstretched
failed copulation with contact	as immediately above but achieving at least physical contact, and in some cases nearly succeeding in copulating
barge	run toward female, resulting in significant physical contact because she fails to move sufficiently quickly
full charge	run toward female, requiring her to move in order to avoid contact
half-charge	run toward female, but not requiring her to move in order to avoid contact
rush-and-dig	run into hole, flap wings rapidly or hold in a spread position, and commence rapid digging (see below for definition of *dig*)
rush-and-scratch	run, flap wings rapidly or hold in a spread position, and commence rapid scratching (see below for definition of *scratch*)
imminent arrival (female present)	female is at the mound while the male owner is absent, and female observes his approach
Male states:	
hover	maintain a position within one body-length of female
peck tail	peck with downward stroke to female's tail
peck body	peck with downward stroke to female's body
peck head	peck with downward stroke to female's head
bite	use bill to grasp female's neck or head, sometimes pulling her across the mound
bird-peck	a collective term which covers *peck tail*, *peck body* and *peck head*, as well as *bite*; bird-peck is usually delivered while hovering
maintain	dig, probe or scratch, as defined for *female mound activity* below
flattened	lower body to mound substrate, and lie motionless with wings out and tail spread
motionless	a simplified version of *flattened*, with emphasis on lack of movement, usually in a crouched position in a hole
ground-peck	peck repeatedly at the mound substrate but without foraging
Female mound activity (all treated as states):	
dig	move substrate with a backward motion of the legs, resulting in commencement or expansion of a hole
probe	insert head into hole, apparently to test temperature
scratch	move substrate with a backward motion of the legs without commencing or expanding a hole

The effect of male behaviour on female mound activity

By recording both male and female behaviour against the same timescale, it was possible to consider the effect of male behaviour on female behaviour, and hence to test the hypothesis that male behaviour partitions into aggressive and inviting components. The key observation is that whereas males seem focused on exploiting their control over the mound as a way of maximizing the number of copulations (Jones, 1990b), females seem focused on the qualities of the mound as an incubator, as evidenced by their digging, probing and scratching in the mound, either to prepare a hole for egg-laying, or to explore the incubation properties of a mound apparently with a view to future egg-laying (Birks, 1996). This group of female behaviours will henceforth be referred to collectively as *female mound activity* (Table 1). Only female behaviour directed toward the mound is included under this category, which therefore excludes behaviours directed at the male and miscellaneous behaviours such as standing or walking. Prevention of female mound activity by males must impose a cost on females because they can no longer assess the quality of the mound or prepare it for egg-laying. Any male behaviour which causes mound activity to cease, therefore, must potentially impose a greater cost and can be classified as aggressive. Conversely, any male behaviour which causes mound activity to commence can be classified as enticing. The focus here is not on correlations between male and female behaviour, but on correlations between male behaviour and ***changes in*** female behaviour. Because the female change occurs after the onset of the male behaviour, it is also possible to be more confident about causality.

For the purpose of allocating female responses, male behaviours were considered in the following sequence:

1. Male events were considered first, as female changes in response were usually obvious.
2. Male states:
    a. In general, any female event or state change was a potential response to a male state if it occurred during this state and had not been recorded as a response to some other male behaviour. Male states might elicit a female response, but might then continue beyond that response.
    b. Male pecking of the female (*bird-peck*) was subject to a specific rule, i.e. her behaviour was considered a response to bird-peck if it caused him to cease bird-peck. The same rule applied to the male behaviour of maintaining a position close to the female (*hover*), except where it occurred simultaneously with bird-peck, in which case the response for hover was considered not identifiable; in effect, it was assumed that, if

both of these behaviours were ultimately found to be aggressive, bird-peck was the more extreme form of aggression

c. Again more specifically, where hover or ground-peck occurred simultaneously with other male states, they were assumed to be background accompaniments to those states, and hence conceded explanatory priority to them.

d. Where multiple female changes qualified as responses to a male state, the first change was recorded, being the closest in time to the commencement of the male behaviour

3. If the female's behaviour was unaffected by the male event or state, the female was recorded as having continued her behaviour.

Where females responded, each male event or state could have only one female response, being an event, the cessation of an existing state, the commencement of a new state, or the continuation of an existing state. Conversely, each female behaviour could be a response to only one male event or state, unless the female's response was to continue her current behaviour (because females were often resistant to change, a female state might persist through many male behaviours).

The hypothesis that certain male behaviours encouraged female mound activity, while others discouraged it, was assessed using a binomial test of frequencies. It was necessary to consider each pre-defined male behaviour separately, although some aggregation was required to attain sufficient statistical power. It was also necessary to focus on female commencement or cessation of mound activity, ignoring all other female responses. For this purpose, commencement was defined narrowly to exclude arguably ambiguous events such as entering a hole without starting to dig. Conversely, cessation was defined narrowly to exclude leaving a hole where there was no prior digging activity, and hence no cessation of digging. On the other hand, some male behaviours only occurred early during female visits, when mound activity was unlikely to have commenced. Female arrivals and departures from the mound were therefore also included as responses, these being respectively precursors to mound activity or confirmation that it had ceased. The dataset for Question 1 was the second half of the 2008-9 breeding season.

An alternative method of investigating the effect of male behavior on female behavior would be to compare female mound activity in the presence vs. the absence of males. Later evidence (chapter 3) indicates, however, that females who visit while the male is absent are generally not the same females who visit while the male is present, so that we would not be comparing behaviour exhibited by the same females

Do males attempt to obtain copulations coercively, and are they successful?

To ensure a sufficient number of copulations and failed copulation attempts, the dataset for Question 2 was expanded to include behaviour at all 16 mounds during the first half of the 2009-10 breeding season. Five approaches were used to assess the broad effect of male behaviour on copulation. The first approach was to analyze male behaviour before and after copulation. As assessed by a paired t-test, greater incidence of a behaviour pre- as opposed to post-copulation was taken as evidence that males employed the behaviour as a way of achieving copulation. Conversely, greater incidence of a behaviour post-copulation might indicate that males avoided the behaviour as a way of achieving copulation. For each category of male behaviour, frequencies or durations were calculated for 1-, 5-, 10- and 20-minute intervals both prior to and subsequent to each copulation, and then compared with each other.

Preliminary analysis revealed that the five-minute interval was the most likely to provide a sample of male behaviour which was sufficiently large to minimize random effects arising from a key behaviour falling just inside or just outside the period boundary, while longer intervals tended to include confounding events. To further control for confounding effects, the five-minute interval was reduced where certain events occurred during it (visit commencement, visit termination, egg-laying and any other copulation). Because of this variability in interval length, the incidence of behaviours was expressed as a rate per minute. Copulations were excluded from the analysis altogether if they occurred within two minutes of the confounding events listed above. An interval of two minutes or less was considered insufficient to minimize random effects arising from a key behaviour falling just inside or just outside the period boundary. The second method used to investigate the impact of male behaviour on copulation was to graph male and female behaviour against visit time in an attempt to identify patterns in the timing of copulations, particularly in relation to male aggression and enticement. To enable both events and states to be shown on the same scale, behaviour was expressed in cumulative terms. Once a pattern was identified, visits were split at multiple points to determine which yielded the strongest contrast, as determined by a paired t-test.

Increased or reduced incidence of a behaviour post-copulation may indicate a post-coital effect rather than a male attempt to influence the likelihood of copulation. This possibility was tested by comparing pre- and post-copulation incidences, not only with each other, but also with mean incidences for the visit, using a paired t-test. If a behaviour was significantly elevated post- as opposed to pre-copulation, but this difference arose principally from a significantly reduced incidence pre-copulation, relative to the visit mean, it was unlikely to represent a post-coital effect. As with all paired t-tests carried out for Question 2, test assumptions were verified prior to analysis.

Fourthly, the association between male behaviours and male copulation success was assessed for all female visits during which both male and female were jointly present during the 2008-9 and 2009-10 seasons. The technique employed was simultaneous multiple regression.

In addition to these open-ended approaches, specific evidence was sought for each of the three recognized types of sexual coercion. For this purpose, it is useful to describe copulation in this species. Typically, the female braced to take the male's weight by standing tall with a straight back at an angle of 60-70 degrees to the surface of the mound, wings brought forward but only partially extended; the male walked up her back; as the female tilted her head toward the ground, keeping her back straight, the male grasped the loose skin at the back of her neck in his bill; it was at this point, while the posterior end of the female's body was tilted upward, that females exposed the cloaca by raising their tail at an angle to their back and moving the tail to one side; the female then bent her legs and lowered herself to the mound surface while the male also lowered himself to sit on her back and, with his bill firmly attached to her neck, obtained cloacal contact. For forced copulation, the female's ability to avoid copulation was assessed by examining video of failed copulations, while the degree of her acquiescence was assessed by examining successful copulations. For harassment, the frequencies of attempted and actual copulations were compared. For intimidation, the visit record for each female was examined, to determine whether visits where copulation occurred early (within the first quarter of visit elapsed time) were more likely to be preceded by a visit during which the rate of male aggression was higher than the mean rate for the male.
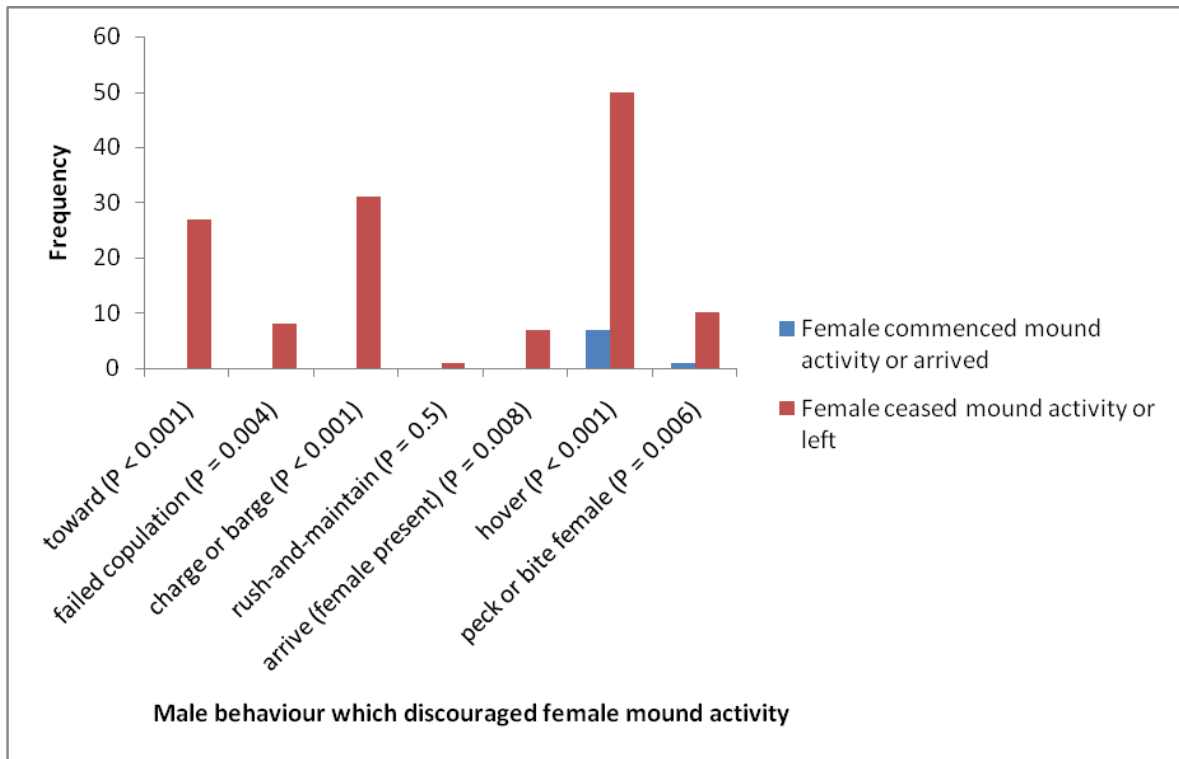
RESULTS

Hours of video recorded were 355.3 for the 2008-9 season (mean of 20.9 per mound) and 361.3 for the 2009-10 season (mean of 22.58 per mound). Total number of female visits over the two seasons was 372. Females individually identifiable in the context of a mound were 26 for 2008-9 and 47 for 2009-10.

The effect of male behaviour on female mound activity
With only one exception, all male behaviours were either significantly associated with the cessation of female mound activity, and hence could be classified as aggressive, or were significantly associated with the commencement of mound activity, and hence could be classified as enticing (Figure 2). To ensure that this result was not dominated by a small number of aberrant individuals, I confirmed that the pattern reported here was evident in each of the 15 males separately. The exception to this pattern was male *rush-and-maintain* (a combination of *rush-and-dig* and *rush-and-rake*), which tended to occur when females were in the process of leaving the mound, making it difficult to observe female responses. These two behaviours were excluded from the analysis of copulations (Question 2 below).

Although male behaviours clearly partitioned into aggression and enticement, this result was obtained by focusing on changes in female behaviour in response to male behaviour. When all female responses were considered, including those involving no change, females were more likely than expected by chance to continue whatever they were doing prior to the onset of the male behaviour, whether or not their prior behaviour constituted mound activity. Continuation of prior behaviour accounted for 57.6% of all female responses to male behaviour, leading to rejection of the null hypothesis of an equal response frequency (Table 2). As above, this result was not attributable to a small number of aberrant males. When reviewing the separate results for each of the 15 males, it was found that continuation of previous behaviour was the most common female response for all but two males, and both differences and frequencies were low for these males.
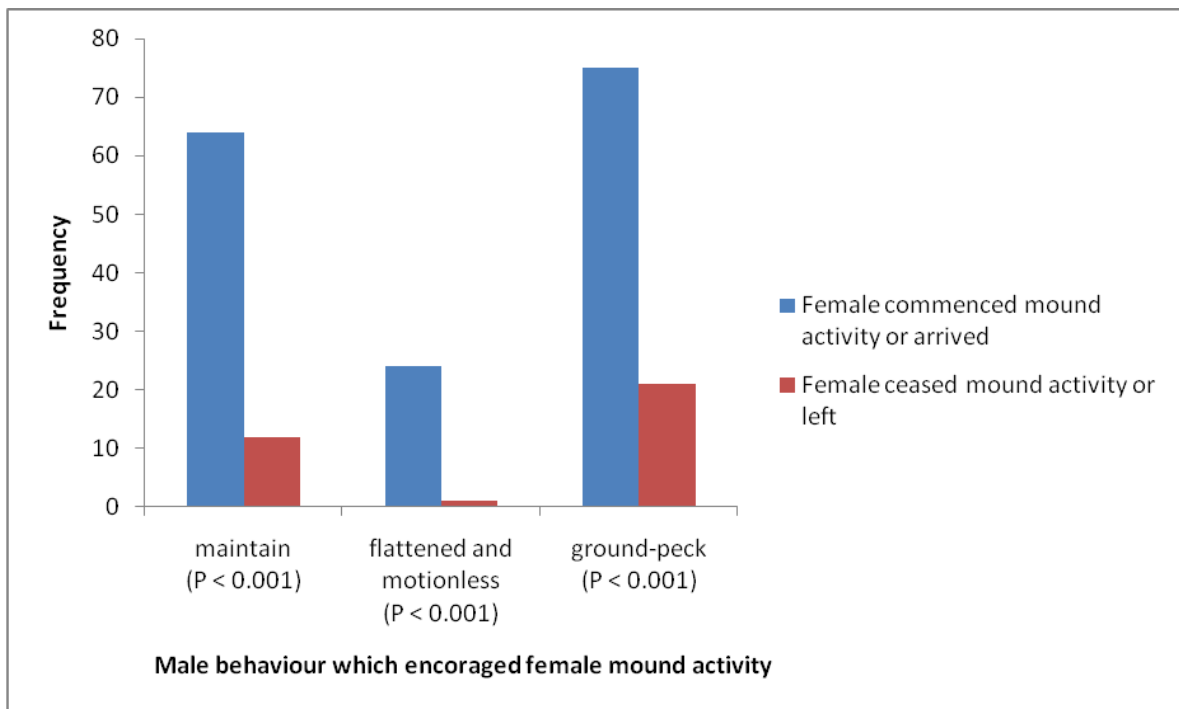
Fig 2 Frequencies of female responses to pre-defined male behaviours. Male behaviours are distinguished according to whether they discouraged (A) or encouraged (B) female mound activity. *P* values report binomial test results.

Table 2 Frequencies of female responses to all pre-defined male behaviours, in descending frequency order. Each female response is also expressed as a percentage of all female responses. For frequencies, $\chi^2 = 1238$, d. f. = 4, $P < 0.001$

	Female behaviour in response					
	Continue current behaviour	Cease mound activity	Commence mound activity	Response unclear	Switch mound activity	Total
Frequencies:	767	208	191	130	38	1334
Percentages:	57.6	15.6	14	9.7	2.8	100

Do males attempt to obtain copulations coercively, and are they successful?

Both aggressive events ($t_{paired} = 4.432$, $P <0.001$) and enticement ($t_{paired} = 5.201$, $P <0.001$) occurred at a significantly higher rate pre-copulation as opposed to post-copulation (Fig. 3). In contrast, both hover ($t_{paired} = -5.987$, $P <0.001$) and associated bird-peck ($t_{paired} = -3.065$, $P = 0.003$) occurred at a significantly higher rate post-copulation as opposed to pre-copulation (Fig. 3). With one exception, these findings were consistent with what was found when male behaviour and female behaviour were graphed against visit time. Both copulations and the male aggressive events which tended to precede copulation were more likely to occur earlier during visits, while the male aggression which tended to follow copulation was more likely to occur later during visits. The strongest contrasts were found after 45% of visit time had elapsed, with the rate of copulations ($t_{paired} = 6.224$, $P < 0.001$) and aggressive events ($t_{paired} = 4.053$, $P = 0.001$) significantly elevated prior to that point and the rate of both hover ($t_{paired} = -6.558$, $P <0.001$) and bird-peck ($t_{paired} = -2.038$, $P = 0.045$) elevated subsequent to that point. There was no significant difference in the rate of enticement ($t_{paired} = -1.706$, $P = 0.092$).
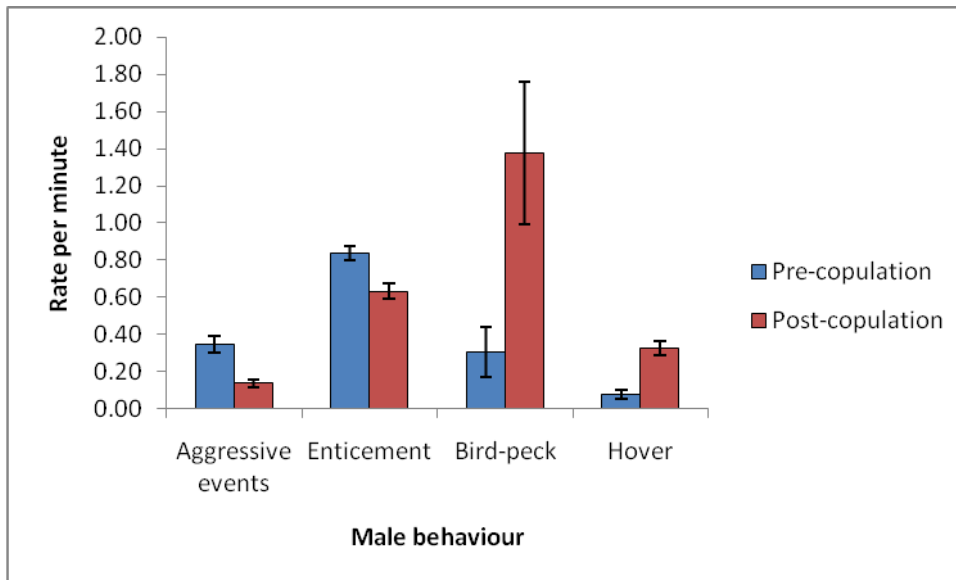
Figure 3 Mean rates of specified male behaviours compared pre- and post-copulation for 76 copulations

On 24 of 122 occasions, copulation occurred very early in the female's visit, within two minutes of her arrival. On a further 16 occasions, copulation occurred within two minutes of the female resuming a visit which she had temporarily interrupted. In five of these cases, a male aggressive event triggered the female's departure, or conversely, the female's departure triggered a male aggressive event.

Aggressive events were significantly elevated pre-copulation when compared with the visit mean, but not significantly reduced post-copulation (t_{paired} = 4.261, P <0.001 pre-copulation and t_{paired} = -1.840, P = 0.07 post-copulation). Meanwhile, both hover and bird-peck were significantly reduced pre-copulation when compared with the visit mean, but not significantly elevated post-copulation (t_{paired} = -9.237, P <0.001 and t_{paired} = -5.088, P <0.001 respectively pre-copulation and t_{paired} = -0.781, P = 0.437 and t_{paired} = 0.809, P = 0.421 respectively post-copulation). When compared with the visit mean, male enticement was both significantly elevated pre-copulation (t_{paired} = 4.326, P < 0.001) and significantly reduced post-copulation (t_{paired} = -2.352, P = 0.021).

For female visits during the 2008-9 and 2009-10 seasons, simultaneous multiple regression was used to assess the association between male behaviours and male copulation success, treating copulation rate as the dependent variable. Three independent variables were included in the model, all measured as rates: male aggressive events, male enticement and a combined average of male hover and bird-peck. For female egg-laying visits during which both male and female were jointly present (n = 24),

there was no significant association between any of the male behaviours and copulation success while controlling for the effect of other independent variables. For aggressive events, for example, the standardized correlation coefficient (β) was 1.56, $P = 0.134$. A reliable model could not be generated for non-laying visits, but could be generated for non-laying visits during which at least one copulation occurred (n = 51). Here too there was no significant association between any of the male behaviours and copulation success while controlling for the effect of other independent variables, β for aggressive events being 1.345, $P = 0.185$.

Prompted by the finding above that both copulations and male aggressive events were elevated during the first 45% of visit duration, multiple regression was repeated for just this portion of visits. There was again no significant association between any of the male behaviours and copulation success while controlling for the effect of other independent variables, β for aggressive events being 0.04, $P = 0.74$.

Video records of both successful and unsuccessful copulation attempts were examined for evidence of forced copulation. Where the male attempted copulation but failed to make physical contact with the female (n = 220), responsibility for this failure could be attributed to the female's movement to avoid him, although this interpretation was not entirely clear in some cases because the male abandoned his attempt so quickly. Where the male made contact with the female, but ultimately failed in his copulation attempt (n = 54), responsibility for this failure could clearly be attributed to avoidance by the female (binomial test, $P < 0.001$), the single exception being a male who failed to obtain a secure bill-hold on the loose skin at the back of the female's neck. Where copulation was behaviourally complete, there was almost always movement from the female to expose the cloaca, indicating that she accepted the male (95 of 100 cases, after excluding those in which a clear view was obstructed, binomial test, $P < 0.001$). Only five cases involved sufficient coercion to be classified as forced copulations.

A check for evidence of harassment revealed that males made 38 unsuccessful copulation attempts compared to 21 successful copulations in the 2008-9 (late season) dataset and 239 unsuccessful copulation attempts compared to 101 successful copulations in the 2009-10 (early season) dataset. The overall ratio of unsuccessful attempts to successful copulations was therefore 2.27:1. Where copulation occurred early in a visit (within the first quarter of visit elapsed time), the immediately preceding visit by the same female at the same mound was found in the study datasets in 41 cases, enabling a test for the role of intimidation. Whether the preceding visit was an egg-laying visit made a clear difference to the result. Where the preceding visit was egg-laying, there was a non-significant trend for the rate of male hover aggression to be higher during these visits

than the mean rate for the male (t_{paired} = -2.007, *P* = .092). A slightly stronger result was found for male bird-peck aggression (t_{paired} = -2.354, *P* = .057), but the rate of aggressive events during these preceding visits was significantly lower than the mean rate for the male (t_{paired} = 4.581, *P* = .004). These results were obtained despite there being only seven cases where the preceding visit was an egg-laying visit. Where the preceding visit was not egg-laying (n = 34), rates of male hover and bird-peck aggression were significantly reduced during the preceding visit (t_{paired} = 7.116, *P* < .0001 and t_{paired} = 6.848, *P* < .0001 respectively), and there was no difference for rates of aggressive events (t_{paired} = -1.166, *P* = .252).

DISCUSSION

Aggression and sexual coercion

My observations suggest that the behaviour of male brush-turkeys toward females at mounds has elements which are coercive as well as those which are not merely non-coercive, but appear to entice females onto the mound. Analysis revealed that individual male behaviours could be partitioned into aggressive and enticing categories (Fig 2), even though the most frequent female response to male behaviour, whether aggressive or enticing, was to resist male influence and continue prior behaviour (Table 2). Examples of aggression included running at the female or pecking her, while examples of enticement included pecking at the mound substrate or digging, probing and scratching in the substrate. The classification of a behaviour as aggressive could be confirmed by observing the evasive tactics adopted by females when resisting male influence. Females often sidestepped the male when he ran at her, or raised a protective wing when he pecked her, in order to continue their current activity in spite of the aggression.

Male sexual coercion is defined as the use or threat of coercion directed specifically at obtaining copulations (Clutton-Brock and Parker, 1995). It was found that copulation was preceded both by higher rates of male enticing behaviour and by higher rates of certain male aggressive events. These events were running at the female (regardless of whether she was required to move in order to avoid contact), running at her with the result that significant contact occurred, unsuccessfully attempting copulation (regardless of whether contact was achieved) and moving toward the female with unclear intentions and without running. One aggressive event was not associated with copulation, namely the arrival of the male mound-owner while the female was present at the mound.

Male behaviours which were elevated pre-copulation arguably represented a male attempt to achieve copulation, especially as they were reduced post-copulation, once success had been achieved. An alternative explanation of this pattern, however, is in terms of post-coital behaviours. It is possible that male hover and bird-peck, both of which were elevated post-copulation, represented post-coital behaviours which displaced aggressive events and enticement after copulation, and the observed pattern therefore had no connection with male attempts to obtain copulations. If this were so, one would expect post-copulation hover and bird-peck to be significantly elevated, not only in relation to pre-copulation levels, but also in relation to mean values for the visit, and this was not the case. The relative rise in hover and bird-peck post-copulation did not seem to represent a post-coital effect, as it reflected a significant reduction pre-copulation rather than a significant rise post-copulation, relative to mean values for the visit. Assuming that there are no other post-coital behaviours which have gone unrecorded, this suggests that

the observed pattern of enhanced male aggressive events and enticement pre-copulation did reflect a male attempt to obtain copulations.

Higher rates of both enticement and aggressive events prior to copulation suggested that males adopted a dual entice-then-coerce tactic. Without enticing the female onto the mound, and then ensuring her continued presence, the male was unable to coerce her. Such a strategy is highly unusual, but not unique. Male climbing camel-spiders (*Oltacola chacoensis*), for example, combine both sexual coercion and luring behaviour in a single behavioural sequence (Peretti and Willemart, 2007). Females appeared obliged to tolerate the male, the alternative being to avoid the coercion by departing, but at the cost of not accessing the incubation mound. The foundation for the male's tactics, therefore, appeared to be his control of a limiting resource which was essential for reproductive females (Jones, 1990b).

Despite the evidence for aggressive events and enticement as a male tactic for achieving copulation, there was no evidence that the tactic was successful. When copulation was regressed on aggressive events, enticement and a combined measure of hover and bird-peck, none of the independent variables was significantly associated with copulation success. This was true, even during the first 45% of female visit duration, despite both copulations and aggressive events being elevated during this period. This suggests that females were able to resist male attempts at coercion, a theme which will be taken up in Chapter 3 on sexual conflict. In summary, we have evidence of male attempts at sexual coercion but no evidence of success, possibly because of female resistance.

When applying the term *sexual coercion*, authorities either assume that a link has been established with copulation success (Clutton-Brock and Parker, 1995) or explicitly require such a link to be established (Smuts and Smuts, 1993). In the brush-turkey case, the term *attempted sexual coercion* is therefore appropriate. Whether a sexual coercion attempt is successful or unsuccessful, the question nevertheless arises as to which type of coercion is being attempted.

Three forms of sexual coercion are generally recognized in the literature: forced copulation, sexual harassment and intimidation (Clutton-Brock and Parker, 1995). Unusually among birds, male brush-turkeys have a phallus, but it is non-intromittent (Brennan et al., 2008), suggesting that forced copulation is unlikely to be a frequent tactic. During successful copulations, females almost always exposed the cloaca in the manner described in Methods above. Even in five cases which were classified as successful forced copulations, the female appeared to expose the cloaca to some degree. What distinguished these cases was the speed of the male's movements, generally giving the female no time to brace herself while attempting to escape from a hole, and therefore

vulnerable to being pinned against the side of the hole. In one case, the female's highly unusual response of leaving the mound immediately after copulation seemed to confirm that the copulation was forced.

Where the male made physical contact with the female, but did not ultimately succeed in copulating, the key to female success in avoiding copulation appeared to be a refusal to tilt her back head-down in the required manner, even when taking the full weight of the male for periods of up to five seconds. This might be supplemented by walking forward and wing-flapping. In some cases, the female simply sat at the bottom of a hole and refused to move while the male stood or sat on her. In one case, the full sequence was completed, except that the female managed to dislodge the male by tilting to one side while prone on the mound surface. Such an extreme ability to avoid copulation suggests that the small number of forced copulations might have been due to female inexperience, inattention, or a decision that further resistance risked injury. Much of this evidence required slow-motion video replay. Previous studies have assumed either that successful copulations not solicited by the female were forced (Jones, 1987) or appeared to assume that unsuccessful attempts involving male-female physical contact were forced attempts (Birks, 1996). The above findings, however, were independent of both of these circumstances. In feral fowl, *Gallu gallus domesticus*, females accept or reject sperm by cloacal contractions within five seconds of ejaculation. A female usually does so while the male is still on her back or in the process of dismounting (Pizzari and Birkhead, 2000). Unfortunately, it was not possible to observe whether a similar behaviour occurred here, given the distance between the camera and the birds observed.

Where copulation occurred early in a visit, it was possible to test for the role of intimidation by measuring the rate of male aggression in the immediately preceding visit by the same female and comparing it with the mean rate for the male (for a definition of *visit*, see Methods above). The results, however, revealed more about the distinction between egg-laying and non-laying visits than they did about the role of intimidation. As will be seen in chapter 2, egg-laying visits are characterized by a higher rate of male hover and bird-peck aggression when compared with non-laying visits. Because they are also relatively long, and are dominated by the egg-laying process, they are also characterized by a lower rate of the aggressive events which tend to precede copulation. This is just the pattern which was observed for the preceding visits in this case. Even where the preceding visit was non-laying, there was no significant elevation in the rate of aggressive events during the preceding visit. There was therefore no evidence that males were even attempting intimidation. One further comment which can be made is that for intimidation to work, aggression during the preceding visit would need to be remembered by the

female, and hence presumably to stand out as in some way exceptional. Male aggression, however, is frequent and seemingly routine, and females apparently habituate to it.

Having considered forced copulation and intimidation as ways of classifying brush-turkey attempted sexual coercion, we are left with sexual harassment. Although its apparently routine nature suggests harassment, the male's behaviour did not unreservedly qualify as harassment because the frequency of copulation attempts was not markedly high (2.27 unsuccessful attempts for each successful copulation). This is likely to underestimate copulation attempts, however, as it did not include occasions when the male moved toward the female with unclear intentions, either because she avoided him or he suspended his movement. More importantly, if the objective of harassment is to wear down female resistance, and this might be accomplished by a wider range of aggressive behaviours than attempted copulation alone, then that wider range of behaviours would seem to qualify as harassment. On this line of reasoning, the relevant measure is the frequency of all of the aggressive events which tend to precede copulation, including failed copulation attempts, which results in a much higher ratio of 5.96 events for each successful copulation.

In summary, certain types of male aggression seemed to be directed at obtaining copulations, even if there was little evidence that such tactics were successful. *Attempted Sexual coercion* therefore seems an appropriate category for this behaviour, even though the coercion is unusual in that it is combined with male attempts to entice the female to visit and remain at his mound, a resource which is essential for her breeding success. *Harassment* seems the most appropriate sub-category.

Males also employed two additional forms of aggression, namely maintaining a position close to the female and pecking the female. The former behaviour often occurred without the latter. Although the latter behaviour sometimes occurred without the former, in these cases taking on a character similar to the aggressive events listed above, it generally accompanied the former. The role of these behaviours was unclear. Their rate was significantly lower pre-copulation as opposed to post-copulation, which might be evidence that their relative absence enhanced the likelihood of copulation. It seems more likely, however, that causality operated in reverse. Copulation allows the female greater access to the mound and hence tends to be followed by an increase in female mound activity. This in turn provokes an increase in these forms of male aggression, hence producing the pattern observed. These themes are discussed further in chapter 2.

Sexual coercion as a primary tactic
 If brush-turkey males are indeed attempting sexual coercion, as argued here, it follows that sexual coercion is a primary mating tactic, as the males in question are mound-

owners, and therefore have a high dominance status. This is surprising, as one would expect that any sexual coercion will be employed by males in a weak competitive position, namely non-owners. What is even more puzzling is that non-owners do not appear to employ sexual coercion at all. Indeed, it is difficult to find evidence for any copulation away from mounds. Despite substantial opportunities for observation by various researchers when carrying out population counts or surveying for new mounds, and despite the fact that copulation in this species is not furtive, there being no pair bond, only one researcher has reported copulation away from mounds, and in the reported cases, males probably owned mounds in the vicinity (Dow, 1988). Moreover, non-owning males are rejected by females when they masquerade as mound-owners while the actual owner is absent (Jones 1990b, confirmed on 30 occasions across all datasets for the present study), suggesting that females are also likely to reject them when encounters occur away from mounds. It therefore seems that mound ownership is so essential for male mating success that non-owning males either do not attempt coercion, or the success rate is negligible. This is probably attributable to the female's ability to exercise choice, as seen in her ability to evade copulation on the mound. If coercion is employed as a copulation tactic only when combined with enticement, which presupposes ownership of an essential reproductive resource, it is unlikely to be employed without that resource.

A relatively small number of taxa appear to employ sexual coercion as a primary tactic, and explanations for it are difficult to find. A review of the literature mostly reveals proximate mechanisms rather than an understanding of the circumstances under which sexual coercion as a primary tactic is most likely to evolve. Most copulations in mosquitofish (*Gambusia holbrooki*) are obtained by smaller, more agile males who are able to approach the female undetected from the rear (Pilastro et al., 1997); male water striders (*Gerris odontogaster*) (Arnqvist, 1989) and alpine bush crickets (*Anonconotus alpines*) (Vahed, 2002) have adaptations for grasping females for forced copulation; by restricting the female's ability to breathe, male garter snakes (*Thamnophis sirtalis parietalis*) induce cloacal gaping and can thereby force copulation (Shine and Mason, 2005). An evolutionary explanation for sexual harassment has, however, been proposed in the case of monarch butterflies (*Danaus plexippus*), namely that there is stronger selection on males to mate before a costly spring migration than on reproductively immature females to avoid mating (Solensky, 2004). It has been proposed that male Trinidadian guppies *Poecili reticulata* are more likely to use forced (sneaky) copulation where predation risk is elevated, thereby avoiding the cost of conspicuous courtship displays (Magurran and Nowak, 1991), but a study of eight wild populations found little evidence for this. It did, however, find that 44.5% of females had sperm in their gonoduct which could only have come from sneaky copulation, confirming that the behaviour is very common (Evans et al., 2003).

In an attempt to offer possible explanations in the brush-turkey case, we start with a reconsideration of the power relations around mound-ownership. There are several respects in which male ownership of a mound might change the risk-reward balance, sufficient to make coercion worthwhile as a primary mating tactic. Firstly, the prospects of successful coercion are arguably quite high, since the female loses access to the mound if she departs. In the case of egg-laying visits, loss of access potentially carries an extreme cost, namely the loss of an egg, although it is important to note that only 14% of all visits while the male was present were egg-laying visits. Secondly, the costs of coercion are reduced: if the male's control of the mound is secure, there is no danger of interference from rival males, and harassment is less likely than forced copulation to result in physical injury. Despite the coercion taking place at a predictable location, namely the mound, predation currently seems to pose minimal risk to adults in this species. Finally, although control of the mound gives the male considerable leverage over the female, it does not guarantee success. Among many bird species, control over a breeding territory readily converts to control over a female once she has started to nest in the territory, so long as mate-guarding and territorial defense are effective, as for example in pied flycatchers (*Ficedula hypoleuca*) (Askenmo, 1984), lark buntings (*Calamospiza melanocorys*) (Pleszczynska and Hansell, 1980) and prothonotary warblers (*Protonotaria citrea*). Control over a brush-turkey mound, however, does not convert to control over a female. Brush-turkey males remain at or in the vicinity of their mounds during the hours of peak daily activity in the breeding season, and have little knowledge of, or influence over, the behaviour of females away from their mounds (Jones, 1987). The male's window of opportunity for obtaining copulations is therefore restricted to periods when the female is not merely present in the vicinity, but present specifically on, or right next to, the mound. Females, moreover, can leave at any time, confident in the knowledge that, although the male may follow them, he will return to his mound shortly thereafter. All mound-owning males are in a similar position: there are no males who can obtain a competitive advantage by long-term control over females, e.g. by mate-guarding or harem formation. In short, although mound-owning males are in a strong competitive position relative to non-owning males, their competitive position relative to females is weaker than first appearances might suggest.

These considerations lead us to predict widespread sexual coercion in mating systems in which males defend resources which are essential for female reproduction and do not defend females. There is little evidence, however, of sexual coercion as a primary tactic in such systems. For example, male Australian toadlets (*Pseudophryne bibronii*) construct and defend nests, but do not appear to employ sexual coercion when females visit, despite all of the above considerations being applicable, namely lack of control over females away from the nest, lack of interference at the nest from rival males and the loss

which females suffer if they leave the nest (Byrne and Keogh, 2009). With the exception of male-male interference at the nest (Candolin, 1999), the three-spine stickleback (*Gasterosteus aculeatus*) system is broadly similar, although males behave aggressively toward gravid females (Bakker, 1986). Male Grevy's zebras (*Equus grevyi*) defend food patches and employ sexual harassment, but lactating females endure lower harassment levels by associating with one male, suggesting that this system grades into those in which coercion correlates with subordinate status and is therefore a secondary tactic (Sundaresan et al., 2007). Dominant male northern elephant-seals (*Mirounga angustirostris*) defend beaches at which females give birth to pups and forced copulation is employed by both dominant and subordinate males, i.e. as both a primary and a secondary tactic (Le Boeuf and Mesnick, 1991; Mesnick and Le Boeuf, 1991). Topi antelopes (*Damaliscus lunatus*) who defend resources also employ sexual harassment, but resource defense in this species is a secondary strategy, subordinate to holding a central or even a peripheral position on a lek (Bro-Jorgensen, 2003). It has been suggested that courtship herding in fiddler crabs (*Uca elegans*) may be a form of sexual coercion, but the alternative interpretation is that males are merely guiding acquiescent females to the correct home burrow (How and Hemmi, 2008).

This variability suggests that use of sexual coercion as a primary tactic depends on detailed features of a species' biology. For example, the key influence for elephant-seals (and for pinnipeds generally) appears to be the female's inability to evade the male while on land, combined with her need to give birth terrestrially. Are there similar specific factors which might explain the brush-turkey case? One possible explanation for the behaviour of male mound-owners is the prevalence of agonistic relations in brush-turkey social life generally (Jones, 1987), which may provide a bias toward aggression as the default solution to social conflict, including conflict over mating. As for all megapodes, brush-turkey chicks lead an independent existence from the time of hatching and generally do not aggregate on an ongoing basis until they have become juveniles (Göth and Jones, 2003). In contrast to most other megapodes, which are monogamous (Jones et al., 1995), no subsequent experience provides a countervailing influence for brush-turkeys, as no pair-bond ever develops. To an unusual degree, therefore, brush-turkey social life is marked by the absence of cooperation.

Another possible influence is phylogenetic inertia, with sexual harassment not infrequently observed among the closely-related galliforms, albeit usually as a secondary tactic (Lovlie and Pizzari, 2007; Mateos, 1998). Female golden Sebright bantams, a breed of *Gallus gallus domesticus*, are especially vulnerable to forced copulation when they descend from roost trees at first light, landing on the ground in a stooped position. In this case sexual coercion is a primary tactic, as it is the dominant harem-controlling males

which force copulation and they do so despite the female's ability to selectively eject sperm (K-L Smith, personal communication). It is also possible that female choice biases mating in favour of aggressive males, a possibility which will be considered in more detail in chapter 4.

Finally, females provide males with opportunities for repeated coercive interactions by spending substantial periods of time at mounds. In the present study, females collectively spent 3.1 hours at mounds for every egg laid (4.7 hours if one includes visits while the male was absent). Australian toadlets once again provide a counter-example. Pre-amplectant courtship in this species requires several hours, as does amplexus itself. Females then spend several hours laying the 70-100 eggs found in the typical nest (Woodruff, 1976). Although we are comparing single eggs with egg batches, the differences do not appear disproportionate, given that females toadlets may lay in the nests of two to eight males (Byrne and Keogh, 2009).

In summary, male brush-turkeys appear to use sexual coercion, specifically sexual harassment, as a mating tactic. While it is reasonably clear why subordinate males do not employ it, it is less clear why it is employed by dominant males. The most likely explanation is the role of aggression as a default solution to social conflict.This approach has the potential to explain not only sexual coercion as a primary tactic but also a second type of aggression (to be analysed in Chapter 2) and the female response to male aggression (to be analysed in Chapter 3). Further research is required to understand the circumstances in which sexual coercion evolves as a primary tactic in some taxa but not in others.

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Chapter 2 - Male mating aggression characteristic of egg-laying visits in Australian brush-turkeys: a non-adaptive by-product?

A male brush-turkey pecks at a female's head. Note the large hole excavated by the female for egg-laying.

ABSTRACT

Previous work on Australian brush-turkeys *Alectura lathami* has established that some male mating aggression is directed specifically at obtaining copulations, and can therefore be classified as attempted sexual coercion. However a second form of mating aggression by males, namely maintaining a position close to the female and sometimes pecking her, could not be explained in this way. Individual mate-guarding or harem formation similarly could not provide an explanation, as both are entirely absent from mating behaviour in this species. It was found that the unexplained form of aggression, referred to as *up-close aggression*, dominated egg-laying visits, was generally stimulated by female mound activity, and was most likely to be stimulated by female mound activity specifically during egg-laying visits. There was a clear switch from attempted sexual coercion to up-close aggression during egg-laying visits, this switch apparently signifying male recognition that the female intended to lay, and acceptance that further copulations were unlikely. The egg-laying female's increasing focus on the egg-hole seemed to enable the switch to up-close aggression by allowing the male to maintain a position close to her.

Many hypotheses were tested, but failed to provide a functional explanation for these findings. Male up-close aggression did not seem to protect the mound from excessive disruption by females, represent an assertion of male dominance, protect eggs already laid in the mound, increase the duration of egg-laying visits to allow time for sperm to reach sperm-storage organs before passage of the egg, reduce the duration of egg-laying visits to deter egg-laying females from attempting major excavations, or provide a cue for males to recognize egg-laying visits in female reactions to the aggression. The switch from attempted sexual coercion probably signaled to an egg-laying female that she could proceed with the final stages of egg-laying without risk to the egg arising from further copulation attempts, but this negative function does not explain why there is male aggression at all during the later stages of egg-laying visits. I therefore suggest that this aggression is a by-product of something else, leading non-exclusive candidates being a stalemate in sexual conflict and a behavioural syndrome.

INTRODUCTION

In species where males compete for access to females and fertilization is internal, males may use coercion against females in an attempt to obtain copulations. This coercion may take two forms: it may be directed specifically at obtaining copulations, often referred to as *sexual coercion*, (Clutton-Brock and Parker, 1995), or it may aim to exert more indirect or long-term influence over females so that the likelihood of copulation and ultimately fertilization are enhanced (Stumpf and Bösch, 2010). Examples of the latter approach include herding of females to form harems by mountain gorillas *Gorilla g. beringei* (Sicotte, 2002), plains zebra *Equus burchelli* (Fischhoff et al., 2009) and Przewalski horses *Equus ferus przewalskii* (King and Gurnell, 2007), and mate-guarding of individual females in a very broad range of taxa including great tits *Parus major* (Hansen et al., 2009), crickets *Gryllodes sigillatus* (Bateman et al., 2001) and octopuses *Abdopus aculeatus* (Huffard et al., 2010). Although harem formation and mate-guarding do not routinely involve explicit coercion of the female, and may protect her from harassment by rival males (Sundaresan et al., 2007), the underlying role of coercion can be observed when the defence threatens to break down. For example, lesser grey shrike *Lanius minor* males retaliate against their mate-guarded partner when paternity uncertainty is experimentally increased (Valera et al., 2003). Similarly, mate-guarding waterfowl (Anatidae) employ forced copulation against their mates after observing apparently successful forced copulation by extra-pair males (McKinney et al., 1983). Although *sexual coercion* can be defined so that harem formation and mate-guarding might appear to be included (Smuts and Smuts, 1993), typical usage restricts the term to specific acts of coercion (Clutton-Brock and Parker, 1995; Smuts and Smuts, 1993).

Where male aggression is not directed specifically at obtaining copulations, and therefore appears to be an indirect or long-term tactic, the objective may not be as straightforward as the formation and maintenance of harems or individual mate-guarding. For example, male mountain gorillas behave aggressively toward dominant females in their harem apparently to minimize female-female conflict and encourage subordinate females to stay (Sicotte, 2002). Male mounting alone, often coercive but without transfer of sperm, is sufficient to inhibit female re-mating in feral fowl *Gallus gallus domesticus* (Lovlie et al., 2005). Much aggression by male spider monkeys *Ateles geoffroyi* (Campbell, 2003) and chimpanzees *Pan troglodytes verus* (Stumpf and Bösch, 2010) may function as a way of confirming male dominance over females, with implications for foraging as much as for mating. Where male aggression does not obviously fit standard models, it is all the more important to seek an explanation.

Previous work (chapter 1) has established that one form of mating aggression by male Australian brush-turkeys *Alectura lathami* is directed specifically at obtaining copulations, and therefore fits a sexual coercion model. A second form of mating aggression, however, was found not to have this function. The two forms of aggression are detailed in Table 1. Whenever used specifically in relation to brush-turkeys, the term *attempted sexual coercion* henceforth refers to the first form of aggression, which is typified by running at the female and making repeated copulation attempts. The second form of aggression involves just two behaviours, maintaining a position close to the female and delivering pecks to her body, henceforth for brevity referred to collectively as *up-close aggression*. If up-close aggression is indeed aimed at longer-term or indirect influence over females, the objective of the influence is unclear, as males are unable to mate-guard or form harems in this species (Jones, 1990b) (D. Wells pers. obs.).

Australian brush-turkeys are members of the megapode clade (Family Megapodidae). Uniquely among birds, megapodes incubate their eggs using environmental sources of heat. In the brush-turkey system, the heat-source is a mound of soil and decomposing vegetation which is always constructed by a male, defended by the current male owner against rival males, and used as a means of attracting females (Jones et al., 1995). Harem formation and individual mate-guarding are not possible for brush-turkey males because, by remaining near their mounds in order to guard them against take-over by other males, males are unable to follow and defend females (Jones, 1990a). Males are polygynous and females polyandrous (Jones et al., 1995). Females are more likely to visit mounds while the male is present, and may or may not copulate or lay an egg while visiting. Egg-laying visits typically include copulation, but always prior to egg-laying itself (Jones 1990b, Birks 1997). As the first phase of an egg-laying visit is not obviously different from a non-laying

visit, it is not clear how soon before egg-laying itself that males are able to distinguish the two visit types, and hence to adjust their behaviour.

Although males employ both sexual coercion and up-close aggression during female egg-laying visits, up-close aggression seems particularly frequent and severe during these visits (Jones, 1987). Having focused on copulation and sexual coercion in chapter 1, I here focus on the possible mechanisms and functions of male aggression (and in particular up-close aggression) during egg-laying visits. More specifically, my aim is to understand why brush-turkey males peck at females during egg-laying visits, when one might expect cooperation with the female to ensure that she safely lays an egg which is most likely his (Birks 1997).

Table 1 Pre-defined behaviours of interest for this study. Definitions of specific behaviours are similar to, but in some cases amplify or simplify, those in Jones 1987. Collective terms, however, are original to this study. *Female mound activity* is a collective term for a group of female behaviours by means of which females appear to assess or prepare a mound for egg-laying. Evidence for the distinction between male aggression and male enticement, and for the distinction between two different forms of male aggression (*attempted sexual coercion* and *up-close* aggression), is presented in chapter 1.

Behaviour	Definition
Female mound activity:	
dig	move substrate with a backward motion of the legs, resulting in commencement or expansion of a hole
probe	insert head into hole, apparently to test temperature
scratch	move substrate with a backward motion of the legs without commencing or expanding a hole
Male attempted sexual coercion:	
toward	move toward female without running; male's intention remains unclear, either because the female avoids him or he suspends action.
failed copulation	move toward female to copulate, but without completing copulation; movement is usually rapid, with neck outstretched
barge	run toward female, resulting in significant physical contact because she fails to move sufficiently quickly
charge	run toward female without making contact, either because she moves or he suspends action
Male up-close aggression:	
hover	maintain a position within one body-length of female
bird-peck	peck with downward stroke to female's head, body or tail; also use bill to grasp female's neck or head
Male enticement:	
maintain	dig, probe or scratch (as for *female mound activity* above)
flattened	lower body to substrate, and lie motionless with wings out and tail spread
motionless	a simplified version of flattened, with emphasis on lack of movement, usually in a crouched position in a hole
ground-peck	peck repeatedly at the substrate but without foraging

Several functional or proximate hypotheses can be developed to explain aggression during egg-laying visits, up-close aggression generally, or both. The first hypothesis is based on the observation that males often respond aggressively to female mound-directed activities, the digging, probing and scratching associated with either preparing a hole for egg-laying, or apparent assessment of the mound's incubation properties with a view to future egg-laying. For brevity, these mound-directed activities will henceforth be referred to as *mound activity* (for details, see Table 1). This leads to the suggestion that males are reluctant to allow females to dig excessively in their mounds (Jones, 1987). As female digging is at its maximum during egg-laying visits, one expects such behaviour to be most obvious during these visits. At least two hypotheses can be distinguished in this suggestion. The first is a proximate hypothesis, henceforth referred to as the *activity-stimulated* hypothesis, namely that female mound activity stimulates male aggression. The second hypothesis, henceforth referred to as *mound-protection*, assumes that the first hypothesis is correct, and asserts that the function of the aggression is to minimize disruption to the sensitive temperature-regime within the mound and/or to minimize the amount of mound-rectification the male must perform after the female leaves the mound. This hypothesis predicts that aggression limits the scope of female mound activity and possibly the duration of female visits. Insofar as visit duration is reduced, visits from alternative females may be enabled, thereby increasing the male's mating opportunities.

A third hypothesis links egg-laying with copulation. It has been suggested that passage of the egg immediately prior to egg-laying may flush sperm from the oviduct, reducing the likelihood of fertilization from copulations prior to egg-laying (Jones, 1990c). As previously explained, all copulations during egg-laying visits occur prior to egg-laying itself. The *visit-extension* hypothesis proposes that the function of aggression during egg-laying visits is to lengthen the visit, thus allowing time for sperm to reach sperm-storage organs before passage of the egg (Jones, 1990c).

Finally, the *visitor-streaming* hypothesis proposes that males distinguish females intent on laying from other females by observing their reaction to up-close aggression, and then expelling the latter (Jones, 1987). Several variants of this idea can be imagined. For example, it might be that up-close aggression directly removes non-laying females, or perhaps the situation is the reverse of this, and up-close aggression, being more drawn-out, is more likely to allow the female to stay. The male might then rely on the accumulated effect of all forms of aggression over time to remove non-layers. The advantage of removing non-layers is presumably that it enables visits by alternative females, especially if the non-layer has already copulated or her visit has already been lengthy, while non-removal of layers protects the male's likely investment in the egg.

I therefore pose the following questions:

1. Does female mound activity stimulate male aggression and, if so, is one mode of aggression (*up-close aggression* as opposed to *attempted sexual coercion*) more likely to be stimulated?
2. Do these two modes of aggression vary in incidence and susceptibility to stimulation according to whether visits involve egg-laying and/or copulation?
3. Do attempted sexual coercion and up-close aggression vary in incidence across visit time during egg-laying visits?
4. Is there a relationship between up-close aggression and the duration of egg-laying visits or the scope of the female's excavation?
5. How do males recognize egg-laying visits prior to egg-laying itself, and how soon are they able to adjust their behaviour?
6. Under what circumstances are females most likely to leave the mound?

It is possible that up-close aggression has no identifiable function, when considered in isolation, and may be a by-product of something else, leading non-exclusive candidates being a stalemate in sexual conflict and a behavioural syndrome. The term *behavioural syndrome* has been applied to behaviours which are consistent across multiple different contexts, and may be dysfunctional when considered in one of these contexts in isolation (Rodgers et al., 2006; Sih et al., 2004).

Table 2 lists each hypothesis developed above, proposes a way of testing it, and links the test to the relevant question. Questions 3 and 5 are not included in Table 2 because they do not relate to specific hypotheses. These questions are nevertheless important for the following reasons: to establish that male behaviour is a response to the egg-laying status of a visit, it is important to confirm that males can recognize these visits (Question 5), which is likely to be reflected in the incidence of male aggression across visit time (Question 3).

Table 2 Hypotheses which attempt to explain the male aggression peculiar to egg-laying visits. Also listed are testable predictions and the questions which test these predictions.

Hypothesis	Type	Testable prediction	Relevant questions
Activity-stimulated	proximate	Female mound activity stimulates male aggression	1,2
Mound-protection	functional	Male aggression minimizes the scope of female mound-excavations and/or the duration of female visits	4
Visit-extension	functional	Male aggression extends visit duration as measured from the last copulation to the commencement of egg-laying	4
Visitor-streaming	functional	Male aggression removes females who do not intend to lay an egg, while allowing egg-laying females to stay	6

METHODS

Behavioural observation, recording and compilation

Field studies were conducted on a free-living, individually colour-banded brush-turkey population at the town of Pearl Beach in New South Wales, Australia. Observation was by five Sony HDR-SR7 video cameras mounted at active mounds and programmed to run daily for 3.5 hours from first light. Unless otherwise specified, data is here drawn from the second half of the 2008-9 breeding season. With some exceptions due to access difficulties or extreme weather, each mound was observed for seven consecutive days. The methods employed here overlap significantly with those of chapter 1, to which the reader is referred for further details. Only pre-defined behaviours were recorded (see Table 1). In particular, there will be repeated reference to the two component behaviours of up-close aggression, defined respectively as: maintain a position within one body-length of the female (*hover*) and peck the female's body (*bird-peck*).

The effect of female mound activity on male aggression

Three approaches were used to assess the effect of female mound activity on male aggression. The first was to identify instances of male aggression and enticement and to determine whether these behaviours were more likely to be preceded by the presence or absence of female mound activity. The strength of this approach was that it tracked male responses to the absence of female mound activity as well as its presence. Its weakness was that it did not track male failures to respond to female actions, hence making it difficult to identify the full range of male responses (including non-responses) to a given female action. The second approach was, therefore, to carry out an explicit female-to-male analysis. In brief, this involved identifying changes in male behaviour (whether aggression commenced or ceased) in response to specified female behaviours (whether or not she was engaged in mound activity). A third approach extended this by also considering delayed male responses occurring within 60 seconds, on the assumption that because female behaviour toward males was non-aggressive, it might elicit a male response which was neither prompt nor overlapping. Paired t-tests were employed for all of these approaches and test assumptions were verified prior to analysis in each case.

All three of these approaches provided evidence on whether female mound activity or the lack of it was more likely to trigger male aggression. Whereas the first approach focused on male behaviour and asked what female behaviour preceded it, the second and third focused on female behaviour and asked what changes in male behaviour succeeded it.

Variation in male aggression by visit type

Ignoring visits while the male was absent, female visits could be grouped into three types: egg-laying; non-egg-laying with copulation; and non-egg-laying without copulation. Two approaches were used to assess variation in male aggression by visit type. Firstly, the mean aggression rate per minute was calculated and compared for each mode of aggression by visit type, using a paired t-test.

The second approach was to compare actual male responses to female mound activity (see above) with expected responses by visit type. The actual male responses used were those which focused on male behaviour and asked what female behaviour preceded it, as this version tracked male responses to the absence of female mound activity as well as to its presence. Expected responses to mound activity were calculated by visit for each mode of aggression by multiplying the total frequency of responses by the rate of female mound activity. For example, if there were 20 bouts of up-close aggression during a visit and female mound activity occupied 40% of visit time, we would expect mound activity by chance to precede eight bouts of up-close aggression. The difference between actual and expected occurrences was then calculated for up-close aggression and compared with that for attempted sexual coercion, using a paired t-test. This provided a measure of whether males were more likely to respond to mound activity with one mode of aggression than the other in specified visit types. Test assumptions were verified prior to analysis.

Variation in male aggression across visit time

Male and female behaviours were plotted against time, searching for patterns in the timing of male aggression during egg-laying visits. To enable both events and states to be shown on the same scale, behaviour was expressed in cumulative percentages. This was an exploratory technique which identified apparent changes in pattern, but was unable to assess statistical significance. To assess statistical significance, each individual visit was split at the point when the pattern changed according to the exploratory technique, and the incidence of aggression before and after the split was compared using a paired t-test, with test assumptions verified prior to analysis. It is important to note that, when addressing a similar question in chapter 1, all visits without distinction were split at the same point, namely when 45% of visit time had elapsed. Here, however, each visit was split according to the timing of specific changes in male behaviour, which were potentially different for each visit.

Relationship between up-close aggression and either the duration of egg-laying visits or the scope of the female's excavation

Thus far I have treated bird-peck as a state with a defined beginning and end, so that temporal sequences can be identified, and thereby the impact of one party's behaviour

on another. Here, however, my question is whether greater incidence of this behaviour correlates with large-scale outcomes such as longer or shorter visit durations. For this purpose, it is more appropriate to treat bird-peck as an event, measured as the number of pecks per minute. This more accurately reflects the severity of the bird-peck than does its duration. Hover continued to be treated as a state, and measured as duration per minute. Rates for both hover and bird-peck were assessed for periods falling between key events as appropriate for the hypothesis being tested, for example between the last copulation and the commencement of egg-laying for the visit-extension hypothesis. Data was summarized at three levels of detail: by individual visit; by unique combination of male and female; and by male. Unless otherwise stated, results are here stated by unique combination of male and female, as both parties seemed to exert an influence. An additional 15 egg-laying visits were included from a 2009-10 dataset. This yielded a total of 24 visits after excluding three visits which did not include copulation (rendering them irrelevant for the purpose of testing the visit-extension hypothesis). A further visit was excluded because both male and female were present at the commencement of filming, and elevated levels of bird-peck from commencement suggested that the visit had started some considerable time earlier. To assess the scope of the female's excavation, points were scored according to the depth of the final egg-hole (between 1 and 3 points), its width at the top (0 – 3) and base (0 – 3), whether multiple holes were commenced (1 for each hole), whether connecting channels were dug between them (1 for each channel) and whether a pre-existing hole was incorporated (1 if not). The width and depth of holes were estimated subjectively when reviewing video footage. Bivariate linear regression was employed in all hypothesis tests, with test assumptions verified prior to analysis.

Male recognition of egg-laying visits
To the extent that male behaviour was different for egg-laying visits, males were presumably able to recognize these visits prior to egg-laying itself. The behavioural record was examined to identify the cues that males might use to distinguish between female visit types, focusing on three areas: the incidence of female events or states; the timing of those events or states; and their rate or consistency over time. Means and standard deviations were assessed for similarity and ranges for degree of overlap.

Female departures from the mound
Here, departures were included only if they brought the female's visit to an end, and hence represented the cessation of mound activity and copulation. Egg-laying visits were also excluded, as the mode of terminating the visit was effectively predetermined. Each departure was reviewed to determine whether it was immediately preceded by male aggression (within ten seconds), and if so which mode of aggression was involved. I also recorded the frequency and types of aggression within the five-minute period preceding

departure, and whether there was male enticement of the female after her departure. Differences were assessed using a t-test, with test assumptions verified prior to analysis.

RESULTS

The effect of female mound activity on male aggresion

Male up-close aggression was significantly more likely to be preceded by female mound activity (t_{paired} = -2.69, *P* = 0.023), while male enticement showed a significant effect in the opposite direction, being preceded by lack of female mound activity (t_{paired} = 3.48, *P* = 0.006). There was no significant difference for male attempted sexual coercion (Fig 1). While these results focus on male behaviour and identify the female behaviour which preceded it, a second approach focused on female behaviour and asked what changes in male behaviour succeeded it. In the great majority of cases (74%), no male response could be identified. Nevertheless, female mound activity was significantly more likely to be succeeded by the commencement of male aggression (t_{paired} = 2.99, *P* = 0.014) and the cessation of mound activity was significantly more likely to be succeeded by the cessation of aggression (t_{paired} = -2.47, *P* = 0.033) (Fig 2). There were no significant differences for arriving at or leaving the mound or entering or leaving a hole.

Male responses to female behaviour were also considered if they were delayed by up to 60 seconds. The female's leaving a mound or a hole was significantly more likely to be followed by the male, on a delayed basis (i.e. within 60 seconds), ceasing aggression or commencing enticement (t_{paired} = -3.57, *P* = 0.005) (Fig 3). Specifically, the association was almost entirely between the female leaving a mound and the male subsequently commencing enticement. All other associations were non-significant.

The most striking results are those in Figure 1, but they need to be checked for consistency with Figure 2. According to Figure 2, female mound activity is significantly more likely to be succeeded by male aggression, which Figure 1 leads us to expect will be predominantly up-close aggression. Of 170 such cases in Figure 2, 117 (69%) were indeed up-close aggression, broadly consistent with the Figure 1 finding. In short, female mound activity is more likely to be followed by male up-close aggression (Figures 1 and 2) and absence of female mound-activity is more likely to be followed by attempted sexual coercion (Figure 1).
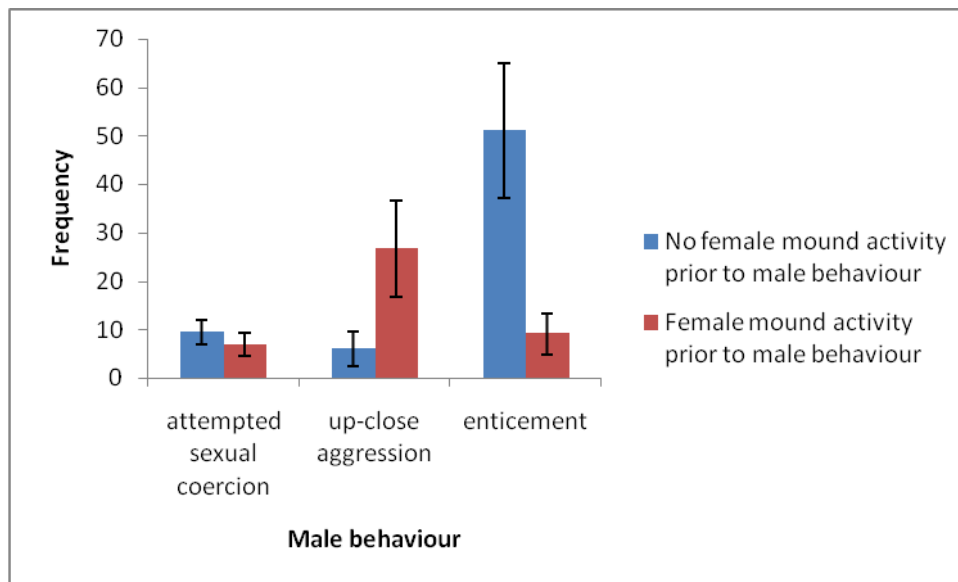
Figure 1 Mean frequency of the presence or absence of female mound activity preceding specified male behaviours (n = 11 males).
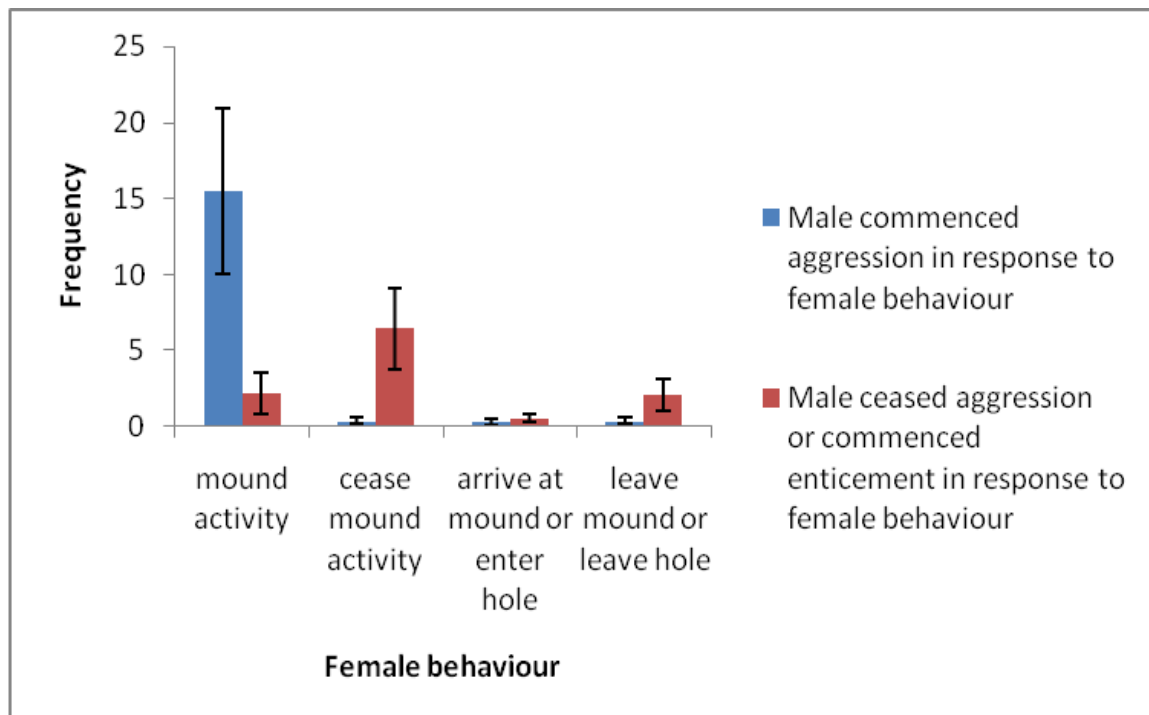

Figure 2 Mean frequency of male behaviours succeeding specified female behaviours, where a change in male behaviour could be identified (n = 11 males).
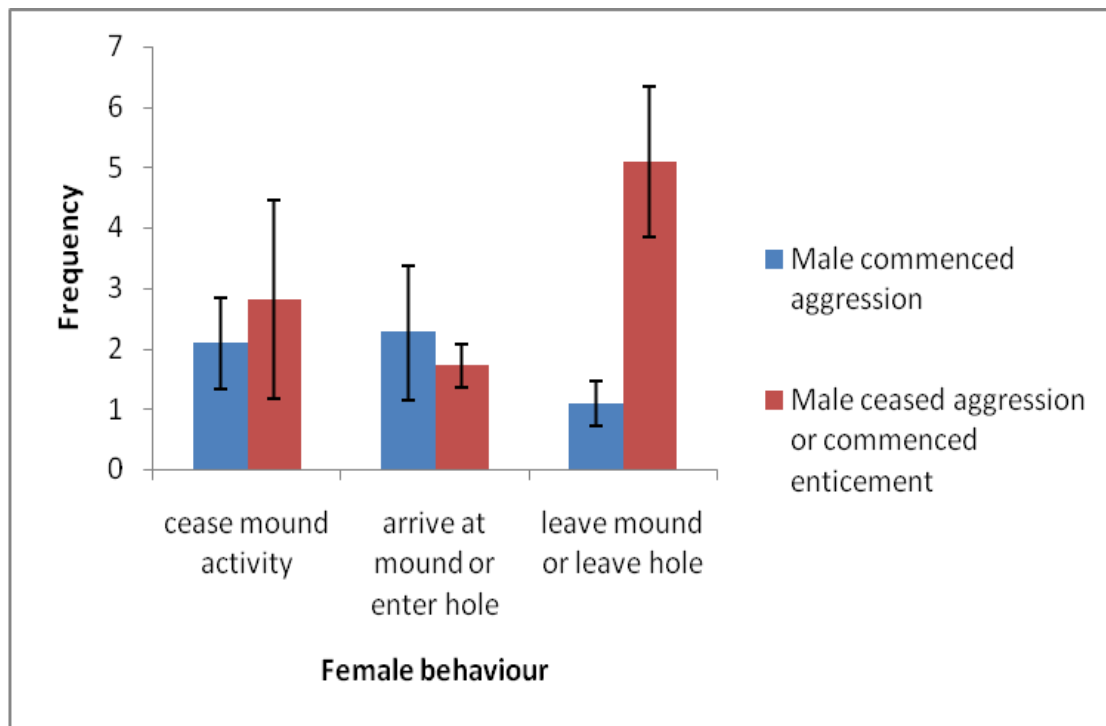
Figure 3 Mean frequency of delayed male behaviours succeeding specified female behaviours, where a change in male behaviour could be identified (n = 11 males). Male behaviours are included if they occurred within 60 seconds of the female behaviour and are not included in Fig 2. As mound activity is a state, it can have no delayed male responses (delayed responses can only be attributed to the cessation of mound activity).

Variation in male aggression by visit type

Not only was up-close aggression significantly more likely to occur than attempted sexual coercion during egg-laying visits (t = -12.57, $P < 0.001$) (Fig 4), it was more likely to be a response to female mound activity than one would expect by chance during egg-laying visits (t= -5.37, $P < 0.001$) (Fig 5). There were no other statistically significant differences.
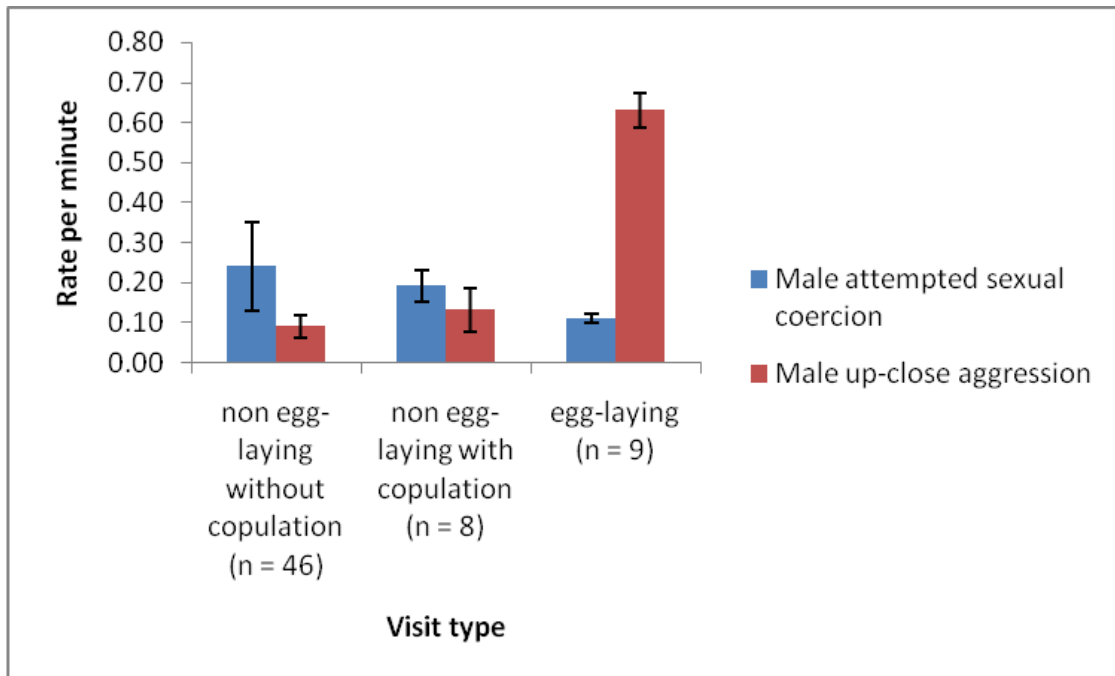
Figure 4 Mean incidence (rate per minute) of two different modes of male aggression for three different types of female visit.
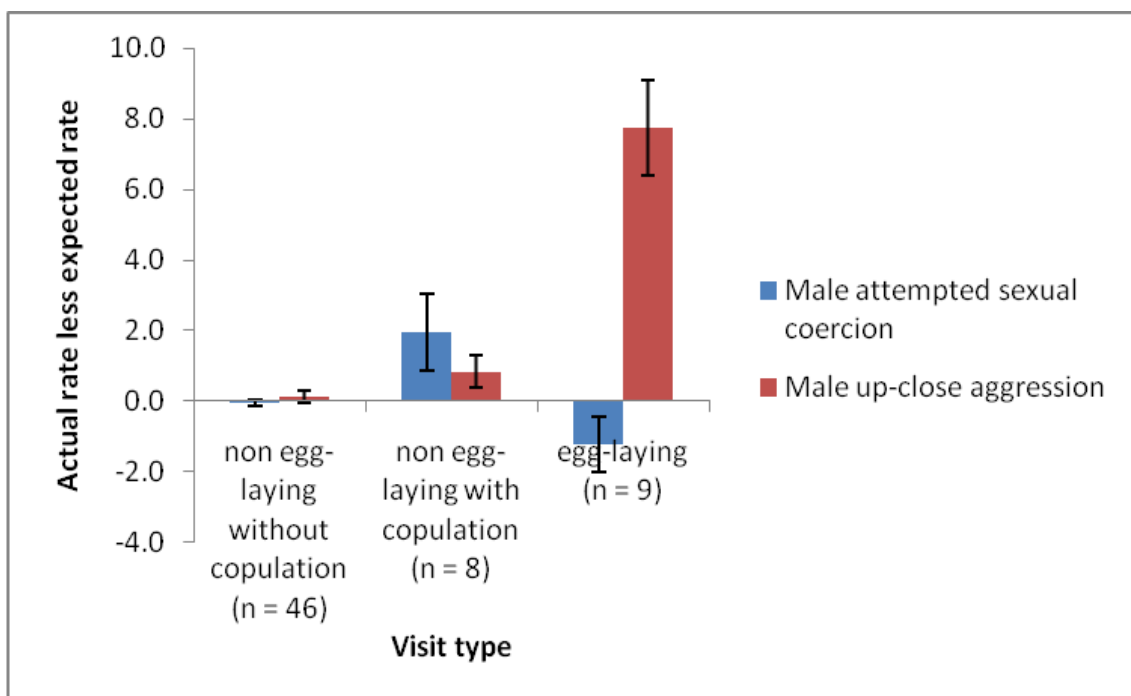

Figure 5 Differences in likelihood that mound activity will stimulate male aggression, by visit type, i.e. mean differences in rates per minute (actual less expected) for two different modes of male aggression and three types of female visit.

Variation in male aggression across visit time

All egg-laying visits from the 2008-9 dataset (n = 9) and the 2009-10 dataset (n = 15) were analyzed. Analysis revealed that males switched from attempted sexual coercion to up-close aggression on all such visits and that the switch was in no case reversed, although males sometimes used the behaviours characteristic of attempted sexual coercion to finally expel females who had completed laying. Elements of the switch were often separated in time, the most frequent pattern being for up-close aggression to switch on before attempted sexual coercion switched off. Switching always preceded egg-laying itself and almost always followed copulation, exceptions being three visits which did not include copulation at all, one visit in which hover and bird-peck commenced immediately, and one in which the female returned to copulate after apparently laying, and then apparently laid again on the following day. Given that successive eggs must be separated by a gap of at least two days (Birks, 1997), this female must have laid on only one of these occasions.

It is possible that switching from attempted sexual coercion to up-close aggression is characteristic of all longer visits, not just egg-laying visits. The above analysis was therefore repeated for non-egg-laying visits with durations in excess of 15 minutes and not interrupted by substantial periods of female absence, all drawn from the 2008-9 dataset. As for egg-laying visits, attempted sexual coercion tended to occur early during visits, but less markedly so, while the hover component of up-close aggression occurred later during visits, but again less markedly so. Bird-peck was not analysed because its total duration was less than one minute spread across 10 non-laying visits. Detailed examination revealed that while all three elements of the switch had been present in each egg-laying visit (hover and bird-peck separately switching on in addition to attempted sexual coercion switching off), there was no non-egg-laying visit for which this was true. Two visits showed evidence of two elements of the switch, seven showed evidence of one element, and one of none (n = 10).

To determine whether the switch from attempted sexual coercion to up-close aggression was statistically significant, each of the 2008-9 egg-laying visits was split at the point where the switch was complete, and each non-laying visit at the latest point at which there was evidence of at least one element of a switch (or at the mid-point if there was no evidence). Before-split and after-split rates were then compared. During egg-laying visits, attempted sexual coercion was significantly elevated prior to the switch (t_{paired} = 5.56, P < 0.001) and both hover and bird-peck significantly depressed (t_{paired} = -10.70, P < 0.001 and t_{paired} = -2.49, P = 0.038 respectively) (Fig. 6). For non-laying visits, bird-peck was not analysed because it was so infrequent. The differences for attempted sexual coercion and

hover were significant, and in the same direction as for egg-laying visits, but less strongly so (t_{paired} = 2.47, P = 0.036 and t_{paired} = -4.04, P = 0.003 respectively).
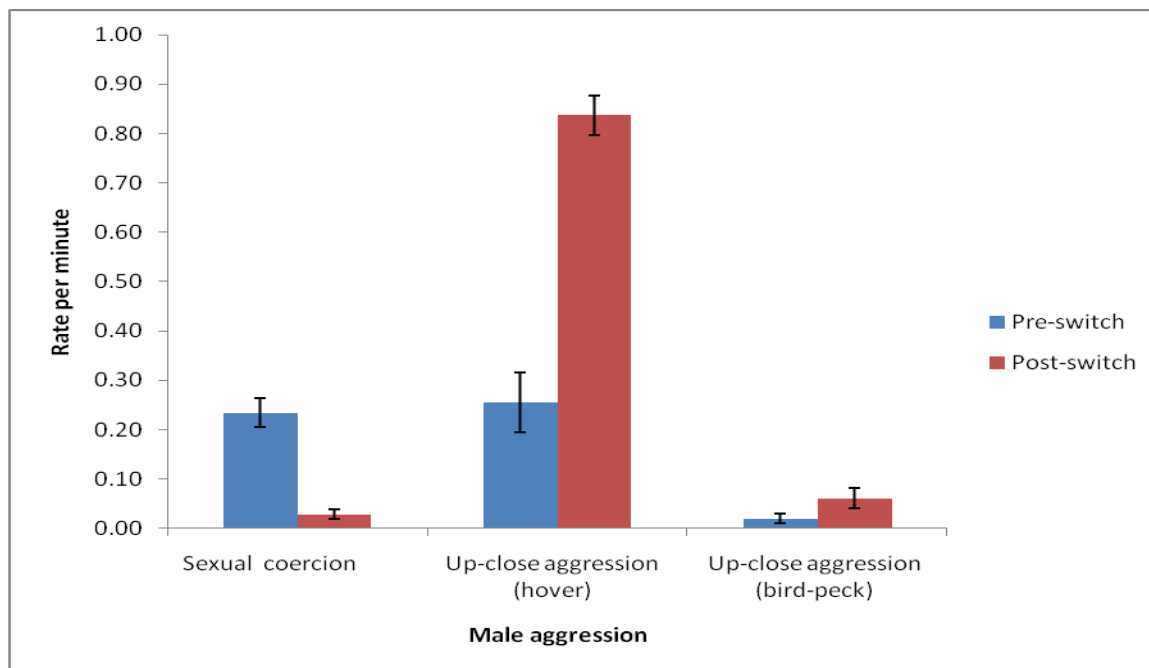

Figure 6 Differences between the mean incidence of male behaviour pre- and post-switching from attempted sexual coercion to up-close aggression for 9 egg-laying visits

Relationship between up-close aggression and either the duration of egg-laying visits or the scope of the female's excavation

Because bird-peck was almost entirely restricted to egg-laying visits (see above), results are here expressed in terms of this behaviour rather than hover. Substantially similar results would have been reported if I had chosen to focus on hover. Contrary to the visit-extension hypothesis, there was no significant relationship between elapsed female visit time and the rate of bird-peck from the last copulation to the commencement of egg-laying (R^2 = 0.11, P = 0.29). The result was not substantially altered if apparent peck severity (peck head as a ratio of total pecks) was substituted for the overall rate of pecking, if this rate was weighted by peck severity subjectively assessed by the observer, or if potential outliers were excluded. In contrast, the mound protection hypothesis leads us to expect a negative association between male aggression and visit elapsed time. There was, however, no significant relationship between elapsed female visit time and rate of bird-peck from the substantial commencement of bird-peck to the commencement of egg-laying (R^2 = 0.034, P = 0.527). Once again, the result was not substantially altered if the approach to measurement was modified as above. Again contrary to the mound-protection hypothesis, there was no significant relationship between the scope of female

excavation and the rate at which males pecked females from the substantial commencement of pecking to the commencement of egg-laying ($R^2 = 0.07$, $P = 0.34$).

There was, however, a significant positive relationship between the base rate of female mound activity, as measured by the number of leg strokes per minute by the female prior to the substantial commencement of pecking, and the rate of the male's subsequent pecking (linear regression, $R^2 = 0.26$, $P = 0.016$) . This relationship disappeared, however, if the male's peck rate was weighted by apparent peck severity (linear regression, $R^2 = 0.02$, $P = 0.536$), suggesting that the overall peck rate did not vary substantially between visits, as males traded off rate and severity against each other. Bird-peck also appeared to have no effect on the female's stroke rate, which if anything increased once pecking commenced. There was no significant relationship between the male's pecking rate and mound activity as a proportion of female visit time (linear regression, $R^2 = 0.003$, $P = 0.853$).

Male recognition of egg-laying visits
The question here is not whether egg-laying visits were different from non-laying visits, but whether there were simple ways for males to recognize individual visits as egg-laying. One possibility is that the passage of time subsequent to key events was sufficient to trigger recognition. If that was the case, the male's complete switch to up-close aggression was a useful reference-point, as it seemed to signal his recognition of the female's egg-laying intentions. An obvious pattern was that, with only one exception, all copulations preceded the male's switch, but there was considerable variability and hence little evidence of coordination between the timing of the switch and the last copulation (mean elapsed time of 13.3 ± 20 minutes St. dev., range 0.2 to 66.8 minutes), and in the three cases where no copulation occurred on egg-laying visits, there was nevertheless a clear switch. Similar variability applied to elapsed time from when an egg-hole was excavated: females might commence substantial digging as opposed to scratching after or before the switch, and selection of the final egg hole often occurred after the switch. Less variability was observed in relation to visit start time, but the pattern was very different from that observed for longer non-laying visits (mean elapsed time of 22.8 ± 12.7 minutes for the former as opposed to 10.0 ± 5.5 for the latter), suggesting that the switch was not triggered by elapsed time from visit commencement. This was also suggested by mean switch-time for egg-laying visits (22.8 minutes), which was less than mean visit duration for the longer non-laying visits (36.3 minutes), indicating that males were not relying on visit duration to exclude the possibility that a visit was non-laying. Timing of the switch in relation to commencement of substantial female mound activity during egg-laying visits was also highly variable (mean elapsed time of 18.5 ± 19.3 minutes), but a clear regularity was nevertheless evident: with the exception of only one visit, no element of the switch

from attempted sexual coercion to up-close aggression preceded the commencement of substantial female mound activity. This suggests that, during egg-laying visits, the male's switch to up-close aggression, and hence his apparent recognition of the female's egg-laying intentions, may be triggered by some aspect of female mound activity.

During egg-laying visits, there was usually a clear point at which the rate of mound activity became elevated, and then continued at this higher level until egg-laying commenced. During this period, female mound activity occupied 92% of elapsed time for egg-laying visits, with a range of individual values from 66% to 100%. For non-laying visits, commencement of substantial mound activity could also be identified, although it was not so clearly marked. Focusing on longer non-laying visits whose duration might cause them to be mistaken for egg-laying visits, it was found that mound activity occupied only 45% of elapsed time from substantial commencement to visit end, with a range of individual values from 8% to 81%. If males were relying on these ranges, they would have been able to distinguish most egg-laying visits from non-laying visits, but not all, as there was substantial overlap (Fig. 7).

Figure 7 Three measure of female mound activity which might enable males to distinguish egg-laying visits from non-laying visits. All measures are expressed as ranges of values. If using the rate of female mound activity (A) or the consistency of the rate over time (B), males would not have been able to identify visit type for all visits in the current sample (nine egg-laying visits and 10 longer non-laying visits), as the ranges overlap. However, if using female resistance to male attempts to interrupt mound activity (C), males might have been able to identify visit type for all visits, as the ranges do not overlap.

Egg-laying visits were also marked by consistency in the rate of female mound activity over visit time, regardless of the rate itself. The distribution of female mound activity during the period from substantial commencement to the start of egg-laying (for egg-laying visits) or visit end (for longer non-laying visits) was compared with a uniform distribution. Highly consistent mound activity was inferred from a close match between the two distributions. Using differences in cumulative percentage values as a criterion,

rates of mound activity for egg-laying visits were more consistent within visits than were rates for longer non-laying visits (mean difference of 2.51 as opposed to 9.58, t = -3.85, *P* = 0.001). Expressed as a percentage of the highest difference, the range for non-laying visits once again overlapped with the range for egg-laying visits (Fig. 7).

Finally, females were more resistant to male attempts to interrupt their mound activity during egg-laying visits. Rather than ceasing mound activity, females responded to male aggression by continuing mound activity or switching its form (e.g. from digging to scratching) 80% of the time on egg-laying visits as opposed to 36% for other visits where the male was present. If males were relying on the range of female resistance rates, they might have been able to distinguish all non-laying visits from egg-laying visits in the present sample, as the ranges did not overlap (Fig. 7).Greater female resistance to male aggression during egg-laying visits applied equally to attempted sexual coercion and to up-close aggression.

In summary, males were probably not relying on elapsed time subsequent to key events to recognize egg-laying visits, but on certain aspects of female mound activity, namely its rate, the consistency of that rate over time, and most particularly the female's resistance to interruption by the male.

Female departures from the mound
Of 54 departures, 85% were followed by male enticement. Where this was not the case, it was often because the male was concentrating his attention on a second female who was simultaneously present. Departures were significantly more likely than expected by chance to be immediately preceded by attempted sexual coercion (t = 2.74, *P* = 0.008). There was some evidence that departures were also more likely to be immediately preceded by up-close aggression, but not significantly so (t = 1.72, *P* = 0.09). During the five-minute period preceding departure, attempted sexual coercion occurred significantly less often than expected by chance (t = -3.52, *P* < 0.001), and there was no difference in up-close aggression (t = .61, *P* = 0.55). In only two of 54 visits was it possible to identify the following sequence during the five-minute period: up-close aggression followed by the aggression characteristic of attempted sexual coercion, immediately followed by the female's departure.

DISCUSSION

Our analysis enables us to provide a clear account of the proximate mechanisms underlying male aggression. Up-close aggression dominated egg-laying visits (Fig. 4), was generally stimulated by female mound activity (Figs 1 and 2), and was most likely to be stimulated specifically during egg-laying visits, out of proportion to the mound activity which occurred (Fig. 5). The activity-stimulated hypothesis was therefore strongly supported, but only for male up-close aggression as opposed to attempted sexual coercion. Continuing our analysis in terms of proximate mechanisms, there was a clear switch from attempted sexual coercion to up-close aggression during egg-laying visits, the most frequent pattern being an increase in up-close aggression, followed some time later by the cessation of attempted sexual coercion. This switch always preceded egg-laying itself and almost always followed copulation. Elements of a switch also occurred during longer non-laying visits, but less markedly so. During egg-laying visits, the switch seemed to signal male recognition that the female intended to lay, and apparent acceptance that further copulations were unlikely.

Males probably recognized egg-laying visits by the high rate and consistency of female mound activity, but more particularly by the female's determination to continue with mound activity despite interruption by the male's aggression. Within a restricted time-window, females appeared to be driven by the powerful imperative to lay an egg which represented 10% of their body weight (Jones and Birks, 1992). One researcher reports an egg beginning to emerge from the cloaca while a female was still in the process of digging (Birks, 1996). Females were, therefore, prepared to incur a cost in order to continue mound activity, thus apparently 'honestly' signaling their intentions to the male, who switched the form of his aggression in response. Arguably, the male's commencement of substantial up-close aggression already indicated his awareness of the female's intentions, and the delay in ceasing attempted sexual coercion merely represented a reluctance to give up on copulations. The abandonment of copulation prospects, however, seemed a significant decision and was retained as a criterion of complete switching.

An obvious explanation for this temporal pattern is that, as visits proceed, females become less tentative, perform mound activity in longer bouts and (especially during egg-laying visits) dig in one area of the mound. All of these changes make the female increasingly a fixed target, thereby making up-close aggression more possible because the male can more easily maintain close company with her. As with all approaches thus far discussed, however, this does not take us beyond description and mechanism. We do not

yet have a functional explanation for male up-close aggression, in particular why it is stimulated by female mound activity.

As framed here, the mound-protection hypothesis proposes that female mound activity stimulates male up-close aggression because males wish to minimize disruption of the mound temperature regime or to minimize the rectification work they must perform if females dig excessively. Although there was some evidence that a higher bird-peck rate reduced the female's mound activity rate during egg-laying visits, there was no evidence that it reduced the scope of the female's excavation. There was similarly no evidence that male aggression reduced the duration of female visits, there being no significant relationship between elapsed visit time and rate of bird-peck from the substantial commencement of pecking to the commencement of egg-laying. It is appropriate to focus on bird-peck here because female mound activity stimulates up-close aggression, is most likely to stimulate it specifically during egg-laying visits, and bird-peck is the form of up-close aggression almost entirely restricted to egg-laying visits.

With the exception of enhancing opportunities for alternative females to visit, it is difficult to perceive an advantage for the male in minimizing female mound activity, which closely resembles the male's own maintenance activities. By mixing material, males are thought to enhance heat production by thermophylic bacteria and fungi, reduce the likelihood of compaction, and make oxygen more available to any eggs already in the mound (Jones et al., 1995; Seymour, 1985). As females presumably have the same if not more interest in these outcomes, it is difficult to avoid the conclusion that their mound activity not only has a similar effect, but is to a degree directed toward these outcomes. Females excavate holes which are wide as well as deep, apparently in order to ensure that the material which covers the egg is as friable and aerated as possible. By advertising the popularity of the male's mound to potential visitors, female mound activity would seem to have greater advantages for the male than the male's own efforts in this regard, the trade-off being some rectification of the mound once the female has departed. Moreover, when rectifying the mound, males often took advantage of the hole dug by the female, and instead of merely filling it, dug a series of new holes, depositing the freshly-excavated material in the previous hole. This suggests that large holes of the type excavated by females are not disadvantageous to males. We cannot exclude the possibility that the mound-protection hypothesis is correct, but if so it draws attention to an apparently dysfunctional behaviour.

A subsidiary functional explanation for male hostility to mound activity might be assertion of male dominance, with the male insisting that mound activity should always be with his consent. Certainly visits to the mound were with the male's consent, the female almost

always observing a protocol of briefly leaving and then returning whenever the male was absent and found her present on his arrival. Males not only invited females onto their mounds, however, they also appeared to invite mound activity. By pacing around an existing hole, rhythmically pecking at the ground and moving back and forth into and out of the hole, the male appeared to signal to the female his desire for her to enter the hole. Females typically took up this invitation, the male then allowing a period of uninterrupted access. Sometimes he appeared to dig a 'demonstration hole' specifically for this purpose. These observations are consistent with the finding that male enticement was more likely to be triggered by the absence of mound activity than its presence (Fig. 1). Males were, therefore, not unconditionally opposed to mound activity, and at least some of it seemed to happen on their specific invitation. Moreover, if male dominance is the underlying function, one would expect males to more readily interrupt females who dug in areas of the mound to which they had not been invited, and no such pattern was observed. One would also expect greater interruption to mound activity in the initial stages of a visit, when the male was seeking copulations and presumably emphasizing his control of an essential resource and not, as here, when he had given up on copulations. Attempted sexual coercion, the aggression more likely to occur earlier during visits, however, was more likely to be a response to the lack of female mound activity than to its presence (Fig. 1).

Finally, it might be suggested that the male is hostile to excavation because it may damage eggs already in the mound. This also seems unlikely. However deep and wide an egg-hole might be, a further small hole was generally dug at the base of the egg-hole, into which the egg was deposited. Excavation therefore predominantly took place above any existing eggs. Moreover, females were no doubt as concerned as the male for eggs of their own already in the mound. It might be argued that females have less knowledge than males of where all existing eggs are located, but this suggests that males would be more hostile to excavation in some locations than in others, and no such pattern was observed.

These attempts to provide a function for male hostility to mound activity also fail to explain why this hostility is so much greater during egg-laying visits, out of proportion to the mound activity which occurs. In contrast, the visit-extension hypothesis does not suffer from this defect. It proposes that the function of aggression during egg-laying visits is to lengthen the visit, thus allowing time for sperm to reach sperm-storage organs before passage of the egg. It predicts a positive relationship between the number of male bird-pecks per minute and visit duration, i.e. more pecks per minute interfere with the female and induce her to take longer. There was no significant relationship, however,

between elapsed visit time and rate of bird-peck from the last copulation to the commencement of egg-laying.

Finally, the visitor-streaming hypothesis proposes that up-close aggression functions in various possible ways to remove non-layers who have over-stayed their welcome, while allowing egg-laying females to stay. There was no evidence for the first component of this hypothesis, namely that males wanted to remove non-layers. Overwhelmingly, female departures at the conclusion of non-laying visits were followed by male ground-peck or mound maintenance, in short enticement, suggesting that the male preferred the female to return. It could be argued that maintenance was not necessarily enticement in this case, as the male might have been rectifying the mound, but the male practice of using maintenance as enticement was very clear: a sudden commencement of maintenance or a sudden increase in its rate was a sure sign that a female was nearby (see chapter 4), and it seems likely that maintenance had the same significance when it followed the female's departure. With minimal exceptions, the male presumably had no way of distinguishing temporary from visit-terminating departures. Male aggression immediately preceding the termination of non-laying visits was probably therefore attempted sexual coercion. There was also no evidence of males routinely employing up-close aggression to test the female's response, and then employing the aggression characteristic of attempted sexual coercion in order to eject her. Similarly, there was no evidence that females who had not previously copulated were more likely to be chased away (Birks, 1999), regardless of whether 'chased' was interpreted strictly to mean a determined pursuit, or loosely to include charging or otherwise moving toward the female. In summary, contrary to the visitor-streaming hypothesis, males seemed to want virtually all females to stay. The only substantial exceptions were egg-laying females who had completed laying, although even here, males allowed time for females to tamp down around the egg and to perform initial hole-filling, apparently in the interests of the egg. Such females even managed to take advantage of this by sometimes undertaking new mound investigations, for example by commencing a new hole.

As to the second component of the visitor-streaming hypothesis, males probably did want prospective egg-layers to stay, as they had a likely interest in the welfare of the egg, the only genetic study on paternity having found that 55-80% (mean 72%) of the eggs in any given mound were fathered by the male owner of that mound (Birks, 1997). Certainly the only period when males were consistently non-aggressive during female visits was when the egg was being deposited, and the male's prior abandonment of copulation prospects arguably recognized the threat to the egg if copulation occurred shortly before egg-laying. Although it could be argued that the male should not care whether the female lays the egg in his mound, as an expelled female can lay in an adjacent mound, this does not take

account of the female's need to establish visiting rights at the alternative mound, in competition with its regular female visitors (chapter 5). It therefore seems likely that males wanted egg-laying females to stay.

It was found that female resistance to male aggression was probably a cue for males to recognize egg-laying visits, suggesting this as a possible function for up-close aggression. If the male's switch from attempted sexual coercion to up-close aggression marked his recognition of the female's egg-laying intentions, however, then most of the female's response to up-close aggression was after the fact. It was primarily her response to the aggression preceding the switch, namely attempted sexual coercion, which provided the cue. The only substantial role for up-close aggression would then be to confirm that the female's behaviour did not subsequently change, and possibly to allow her greater freedom to resist. There was no difference, however, between female resistance to up-close aggression as opposed to attempted sexual coercion, suggesting that the former was no easier to resist, although it is possible that females are less likely to depart in response to up-close aggression, as discussed immediately above.

In summary, we now have considerable understanding of male aggression during the later stages of female egg-laying visits: what triggers it, why it takes a particular form, and how the male recognizes egg-laying visits, enabling him to switch his mode of aggression. What we do not yet understand is the function of this aggression. The male's objective cannot be copulation, at least not directly, as he abandons the mode of aggression specifically associated with copulation. Indeed, the significance of up-close aggression may partly consist in the fact that it is clearly not attempted sexual coercion, hence signaling to the female a change in the male's intentions. The information content of the signal may be that she can proceed with the final stages of egg-laying without risk to the egg arising from further copulation attempts, and without the need to leave. If so, we have identified a function for up-close aggression, but it is a merely negative one. We still do not understand why there is aggression at all during the later stages of egg-laying visits.

A behavioural pattern may be an adaptation, i.e. it evolved because it is functional; it may acquire a different or additional function over time; or it may have no apparent adaptive function, considered in isolation (Fox and Westneat, 2010), as appears to be the case here. If so, it may be a by-product of something else. Three non-exclusive possibilities are at least initially plausible. The first, namely that females prefer more aggressive males, will be rejected when female choice is analyzed (chapter 4). The second possibility is a stalemate in sexual conflict, producing a no-winners outcome (Parker, 2006). Males may be attempting to achieve an outcome, but this has not been identified in my study because females are equally determined to frustrate it. Sexual conflict in this species is

characterized by stereotyped interactions in a war of attrition which will be examined in more detail in chapter 3. For the present, note the way in which male and female behaviours oppose and apparently cancel each other: the female commences mound activity with a given stroke-rate, which the male responds to with a correspondingly higher or lower bird-peck rate, while adjusting peck severity to apparently conform to an overall budget for bird-peck effort; bird-peck does not reduce the female's stroke rate (if anything, she speeds up, suggesting stereotyped opposition, or possibly urgency to complete the task); there is some evidence that she spends a lower proportion of her time on mound activity, but there is no impact on the scope of her excavation or the length of her visit.

A third possibility is that males are aggressive toward females because they cannot 'switch off' the aggression required to defend their mound against rival males, just as male aggression toward gravid females seems to correlate with male aggression toward rival males in sticklebacks *Gasterosteus aculeatus* (Bakker, 1986). The term *behavioural syndrome* has been applied to behaviours which are consistent across multiple different contexts, and may be dysfunctional when considered in one of these contexts in isolation (Rodgers et al., 2006; Sih et al., 2004). Some evidence for the relevance of this approach to brush-turkeys can be found in the male tendency to eject all intruders from the mound, not just rival males. During this study, males were observed responding aggressively to dogs, cats, wallabies, monitor lizards and even mound-excavating humans, within constraints imposed by the male's own safety (see also Jones 1987). This approach might explain the male's strong reaction to female mound activity, although it is not clear why he reacts especially strongly during egg-laying visits, out of proportion to the mound activity which occurs. Perhaps his underlying attitude is hostility, which is initially obscured by his focus on copulations, and is only fully released during the later stages of egg-laying visits, which are the only visits during which all prospect of copulation is eventually abandoned.

The prevalence of agonistic relations in brush-turkey social life generally (Jones, 1987) may also provide the basis for a behavioural syndrome. This was previously suggested as an explanation for attempted sexual coercion as a primary male mating tactic (chapter 1), but it may also account for aggression as a default behaviour once copulation prospects have been abandoned. Additionally, when combined with sexual conflict, it might explain why mound activity stimulates aggression: having given up on his primary objective (copulation), the male continues the conflict by other means, by opposing the female's primary objective (mound activity).

In summary, we have identified several proximate mechanisms for male aggression during the later stages of female egg-laying visits. There is strong evidence that aggression was triggered by female mound activity, it probably took the form of up-close aggression because the female tended to focus on one area of the mound as all longer visits proceeded, hence making it easier for the male to maintain close company with her, and the male probably decided when to switch to up-close aggression based on the rate and consistency of the female's mound activity, and the degree of her resistance to attempted sexual coercion. Considered in isolation, however, no positive function could be identified for up-close aggression: no evidence could be found for the visit-extension or mound-protection hypotheses, and only negative evidence could be found for the visitor-streaming hypothesis. I therefore suggest that this aggression is a by-product of something else, leading non-exclusive candidates being a stalemate in sexual conflict or a behavioural syndrome.

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Chapter 3 – Outlasting the enemy: Sexual conflict in Australian brush-turkeys

A male brush-turkey attempts to copulate with a female, but she resists. Although she has braced her back in order to take his weight, she refuses to tilt the anterior portion of her body any closer to the ground.

ABSTRACT

Sexual conflict arises where male-female interactions have an optimum outcome for males which is different from the optimum outcome for females, and both optima cannot be achieved simultaneously. Sexual conflict seems to characterize male-female interactions in Australian brush-turkeys, *Alectura lathami*, because females resist male attempts to mate coercively, while males resist female mound activity. My analysis established that females responded to male aggression both tactically during the visit and strategically via their visiting pattern. Female responses to male aggression during the visit enabled an increase in mound activity, but this benefit was traded off against costs which varied according to timescale. For example female refusal to copulate incurred a short-term increase in one form of male aggression, but the cost shifted to a higher copulation rate over the course of the visit as a whole. The most frequent female visiting pattern was to visit while the male was present rather than when he was absent, to extend visit duration beyond what appeared to be strictly necessary, and to lay a series of eggs at the same mound for periods which in some cases exceeded 45 days. The probable advantage of visiting in this way was that it maximized her time together with the same male, and hence

habituated him to her company. Two forms of male aggression therefore fell significantly over a sequence of visits by the same female at the same mound. Female mound activity also fell over a sequence of visits, while there was some evidence that the copulation rate increased, suggesting a closer alignment of male-female objectives over time. In general, however, the behaviour of both sexes was characterized by unvarying and cyclical perseverance in the pursuit of conflicting objectives. The appropriate game-theory model is probably the asymmetric war of attrition. It is likely that the female's cost of continuing a contest is lower than the male's, while the male's benefit from winning is higher than the female's, hence producing the observed drawn-out contests.

INTRODUCTION

Sexual conflict arises where male-female interactions have an optimum outcome for males which is different from the optimum outcome for females, and both optima cannot be achieved simultaneously (Chapman, 2006; Parker, 2006). Sexual conflict is typically about mating/fertilization or parental investment, but other points of conflict are also possible, for example reproductive resources, infanticide or mate cannibalism (Chapman, 2006). Sexual conflict does not require an overt contest between males and females. For example, a higher optimum mating rate for one sex may result in the opposite sex rejecting mating opportunities more frequently, independent of whether fights erupt over the issue (Bro-Jorgensen, 2007). On the other hand, an overt contest between males and females does strongly suggest sexual conflict. If males attempt to mate coercively, and females clearly resist them, it is likely that the optimum male mating rate is to some degree against the interest of females, and sexual conflict can be inferred with reasonable confidence. Sexual conflict may lead to antagonistic co-evolution between the sexes, in a cyclic process of adaptation and counter-adaptation, or it may lead to a mutualistic outcome more consistent with traditional models of sexual selection (Pizzari and Snook, 2003). The former is more likely if the female's direct costs of mating outweigh indirect benefits, such as sons capable of inflicting similar mating costs, and there is sex-limited expression of genes at different loci in males and females (Parker, 2006; Pizzari and Snook, 2003; Shaw and Wiley, 2010).

Previous work has shown that male brush-turkeys *Alectura lathami* attempt to enhance their copulation rate by sexual harassment, specifically by a combination of aggression and enticement, and that females resist copulation attempts by a variety of behavioural means (chapter 1). Having concentrated on male aggression and its role in attempted sexual coercion (chapter 1) and during female egg-laying visits (chapter 2), here I shift focus to the two-way interaction between male and female, asking whether female

responses to male aggression modify either the aggression or its consequences. Where males employ sexual harassment, male-female conflict often takes the form of a contest over which party can persevere the longest in a given behaviour (Clutton-Brock and Parker, 1995). This pattern does appear to characterize male-female interactions in this species.

When brush-turkey females visit males at their mounds, sexual conflict appears to focus on two key issues: whether mating will occur, but also to what degree the female will be allowed to dig, probe and scratch in the mound. . This activity, henceforth referred to as *female mound activity,* appears to be a key female objective, firstly because sexually active females must eventually lay an egg, and hence dig a substantial hole in the male's mound, and secondly because females appear to explore the incubation properties of the mound during non-laying visits (Birks, 1996). If sexual conflict provides an appropriate framework, then females are presumably attempting to maximize mound activity while minimizing male aggression and/or its consequences, copulation and possible injury being the most obvious. Although clear evidence of female injury was not observed during this study (D. Wells pers. obs.), females can be killed by males in captivity (Jones et al., 1995).

Female responses to male aggression take many different forms across a wide range of taxa. Primate females, for example, may signal sexual receptivity when a new male takes over a harem, preferentially associate with dominant males, emigrate from harems controlled by especially aggressive males, form female coalitions, and may even fight males (Smuts and Smuts, 1993). These responses appear to have no brush-turkey equivalents. Harems and individual mate-guarding are entirely absent (Jones, 1990b), females who are bystanders during attempted sexual coercion of other females were not observed intervening on their behalf, and females were not observed overtly fighting males (D. Wells pers. obs.). Responses by female brush-turkeys include acceptance or rejection of male copulation attempts, temporary absence from the mound, and temporary retreat to the side of the mound. It is likely that these responses represent different trade-offs between conflicting female objectives, for example the decision to copulate may be a trade-off between maximizing mound activity on the one hand and minimizing copulations on the other.

Fertilization does not necessarily follow from mating and may also be a point of conflict, distinct from mating itself. It is important to note that all copulations during egg-laying visits occur prior to egg-laying itself (Birks, 1997; Jones, 1990c). It has been suggested that passage of the egg immediately prior to egg-laying may flush sperm from the oviduct, reducing the likelihood of fertilization from copulations prior to egg-laying (Jones, 1990c), and hence that the function of at least some male aggression during egg-laying visits is to

lengthen the visit, thus allowing time for sperm to reach sperm-storage organs before passage of the egg (Jones, 1990c). Previous research, however, found no evidence for this hypothesis (chapter 2). The current chapter will, therefore, assume that fertilization is not a separate point of conflict, distinct from conflict over mating.

Thus far we have focused on what might be termed within-visit conflict, i.e. on male and female interactions during female visits to mounds. Conflict may also be reflected in the female visiting pattern, for example females may be more likely to visit mounds while males are absent, or they may visit in a way which reduces the incidence or cost of male aggression over time. For example, females appear to make repeated visits to only one mound, in some cases switching to a different mound after an extended period (Birks, 1996; Jones, 1994). It is possible that females adopt this pattern because male aggression reduces over time with repeated visits, or females are better able to predict the male's behaviour with repeated experience.

To summarize, it is possible to identify female responses to male aggression, both tactical (acceptance or rejection of male copulation attempts, temporary absence from the mound, and temporary retreat to the side of the mound) and strategic (visiting while the male is absent or making repeated visits to the same mound). It is also possible to identify female objectives, namely to minimize copulations, maximize mound activity and reduce the severity of male aggression. Given that all of these variables are measurable, it may also be possible to assess the degree to which each female response achieves female objectives, and hence to determine whether there are trade-offs between objectives.

 I therefore addressed the following questions:

1. Do female within-visit responses help females to minimize copulations, maximize mound activity or reduce the severity of male aggression, and is there evidence of trade-offs between these objectives?
2. To what degree are interactions between males and females a contest over which party can persevere the longest in a given pattern of behaviour?
3. What is the female visiting pattern and does it help her to minimize copulations, maximize mound activity or reduce the severity of male aggression?

 Game theory appears an appropriate analytical tool for male-female conflict because success depends on the opponent's behaviour and is therefore frequency-dependent (Briffa and Sneddon, 2010). Although behavioural ecologists typically apply game theory to male-male contests, where both parties to the contest are potentially capable of dangerous fighting, this does not prevent its application to male-female contests, in which females merely resist male aggression (Clutton-Brock and Parker, 1995; Parker, 2006).

Some models were initially developed specifically for sexual conflict, and were only subsequently applied to male-male conflict (Parker, 2006). In an extended Discussion, I also attempt to identify the most appropriate game-theory model.

METHOD

Behavioural observation, recording and data compilation
Field studies were conducted on a free-living, individually colour-banded brush-turkey population at the town of Pearl Beach in New South Wales, Australia (33.54°S, 151.30°E). Observations were recorded remotely by five Sony HDR-SR7 video cameras mounted at active mounds and programmed to run daily for 3.5 hours from first light. Unless otherwise specified, data is here drawn from the second half of the 2008-9 breeding season and the first half of the 2009-10 breeding season. With some exceptions due to access difficulties or extreme weather, each mound was remotely observed for seven consecutive days. Seventeen mounds were observed during 2008-9 for a total of 355.3 hours and sixteen in 2009-10 for a total of 361.3 hours.

In order for a female mound visit to be recorded, it was necessary for the female to be physically present on the mound at some point, even though she might be temporarily absent during the visit. Absences lasting up to 22 minutes were allowed, during which the female was typically in the vicinity of the mound (for full details, see chapter 1). Only pre-defined events or states were recorded (see Chapter 2, Table 1). Particular attention is drawn to the distinction between two forms of male aggression: *attempted sexual coercion*, which is typified by the male running at the female or making repeated copulation attempts, and *up-close aggression*, which consists in the male maintaining a position close to the female, and in some cases pecking her. Attempted sexual coercion was analyzed in detail in chapter 1 and up-close aggression in chapter 2.

Behaviour was treated as a state if it tended to occur in bouts, and as an event if it did not. To reduce the manual effort required to record behaviour on an all-occurrences basis accurate to the nearest second, a behavioural state could be recorded as an uninterrupted bout so long as there had been no interruption longer than 15 seconds, and the behaviour occupied at least 75% of the elapsed time recorded. It follows that a recorded bout of female digging, for example, was typically interrupted by periods of standing, walking or foraging, but the interruptions were brief, and did not invalidate the observation recorded, namely that the female was predominantly engaged in digging. These remarks are particularly relevant when interpreting the results for Question 2.

Female within-visit responses to male aggression

For each female response to male aggression, the incidence of relevant behaviours for a five-minute period prior to the response was compared with their incidence for a five-minute period subsequent to the response using a paired t-test. Test assumptions were verified prior to analysis. The period was reduced if certain events occurred within the five-minute period (visit commencement, visit termination, egg-laying and any other female response of the type under consideration). Because of this variability in period length, the incidence of behaviours was expressed as a rate per minute. Responses were excluded from the analysis altogether if they occurred within two minutes of the above events or if they occurred during a visit whose total duration was less than five minutes.

Temporary female absences from the mound were distinguished from temporary retreats to the side of the mound, while female rejection of copulation opportunities were separated into two groups: those in which the male succeeded in making physical contact with the female and those in which he did not. The duration of temporary female absences and temporary retreats to the side of the mound did not affect the analysis, as the period prior to the absence or retreat was measured up to the commencement of this state, and the period subsequent to the absence or retreat from its conclusion.

Female within-visit responses were also investigated using regression techniques, with test assumptions verified prior to analysis. Several multivariate regressions were run, treating rates for each of the female responses as simultaneous independent variables, and treating rates of female mound activity, male aggression and copulation in turn as dependent variables. As the focus was on male-female interactions, each visit by a uniquely identifiable female was treated as a data point, so long as the male mound-owner was present. For the same reason, rates were expressed relative to the duration of the female's presence at the mound, excluding periods of temporary absence or temporary retreat to the side of the mound. As this approach potentially inflated sample size and biased the result toward mounds receiving more female visitors, the analysis was repeated by treating each unique combination of male and a female as a data point. As opposed to treating each visit as a data point, this second method came at a cost, namely an inability to include visit sequence in the model, and was therefore used only as a check on the reported results.

As female responses are here defined in relation to male aggression, and hence thought to be caused by this aggression, there is necessarily a positive association between female responses and male aggression, even though the former is also thought to be an attempt to minimize the latter. It was therefore necessary to distinguish between a male's mean aggression rate for all female visits, and the aggression rate specific to each visit. The difference between these two rates was treated as evidence of the effect of a female response on male aggression. For example a visit-specific aggression rate which was lower than the mean aggression rate was treated as evidence that female responses during the visit had reduced male aggression. This logic was applied only to male aggression. Where the dependent variable was rate of copulation or female mound activity, only the visit-specific rate was used.

Perseverance contests
Only visits exceeding 10 minutes in duration and involving the presence of both male and female were considered when analyzing perseverance. A contest over perseverance can take several forms. Perseverance was said to be *unvarying* if individuals persisted with a specific behaviour for long periods of time, despite the other party's behaviour, and *cyclical* if they ceased the behaviour, often in response to the other party's behaviour, only to resume it in a repetitive cease-resume cycle. Where the behaviour in question was a state, it was recorded as an example of unvarying perseverance only if it exceeded 10 minutes in duration. If it was an event, it was recorded if there were at least 20 iterations without interruption. Where behaviour was cyclical, it was recorded as an example of cyclical perseverance if there were at least two iterations of the complete cycle. Although the distinction between perseverance and non-perseverance was a continuum, it was necessary to select a minimum standard for unvarying perseverance in order to measure it. Detailed and repeated video observation of behaviour at mounds suggested a natural break at 10 minutes for states and 20 iterations for events

Male unvarying perseverance could coincide with female unvarying perseverance, and could coincide with other male behaviours classifiable as unvarying perseverance so long as the definition of the behaviours supported this treatment. For example, male hover (maintaining a position close to the female) could co-occur with many other male behaviours. Because female mound activity was defined to include both digging (excavating a hole) and scratching (moving mound material without excavating), a bout of mound activity could be classified as unvarying perseverance and simultaneously as cyclical perseverance, if there was repeated alternation between digging and scratching.

Unlike unvarying perseverance, which could be exhibited by either individual in isolation from the other, cyclical perseverance involved interaction between the two parties, with one responding to the actual or anticipated behaviour of the other. Where there were arguably two cycles occurring simultaneously, only one was recorded, namely the cycle with greater duration. When interpreting results for unvarying perseverance in particular, it is important to note the remarks above regarding interruptions to a behavioural state: interruptions of up to 15 seconds were allowed when recording, so long as the relevant behaviour occupied at least 75% of the recorded period.

To determine the degree to which male-female conflict took the form of perseverance contests, the elapsed time of perseverance as defined above was measured as a percentage of visit elapsed time. To confirm that bouts of unvarying perseverance were distinguishable from behaviour by the same sex at other times during visits, the rate of the relevant behaviour during the bout was compared with the overall visit rate using a paired t-test, with test assumptions verified prior to analysis.

Female visiting pattern
Where females were color-banded and hence could be uniquely identified regardless of the mound at which they were observed, a paired t-test was used to compare the frequency and duration of their visits to a primary mound with the frequency and duration of their visits to all other mounds. Duration here included periods of temporary absence, as females were generally in the vicinity of the mound during those periods. This approach was of limited utility for the two study datasets, as each mound was generally observed for only seven days, and observation periods included at maximum three mounds simultaneously. Moreover, mounds observed simultaneously were not necessarily physically adjacent to each other. Equivalent summaries were therefore prepared for two additional datasets where adjacent mounds had been observed simultaneously for much longer periods, thereby increasing the chance of observing female visits to multiple mounds in a similar timeframe. Paired t-tests were run for each of the four datasets. If females concentrated on a primary mound, an explanation might be found in any differences between earlier and later visits in the temporal sequence of visits. To determine whether visits later in the sequence were associated with reduced male aggression, visit sequence was included as an independent variable in the multivariate regressions outlined above for Question 1.

To determine whether females were more likely to visit mounds while the male was absent, the duration of visits while the male was absent was expressed as a ratio of the time available for such visits to occur and compared with the corresponding ratio for visits while the male was present, using a paired t-test. To test the impact of female exposure to the male, visit duration excluded periods of temporary female absence or retreat to the

side of the mound. As egg-laying females were obliged to visit, egg-laying visits were excluded from the analysis. The analysis was performed for each combination of male and uniquely identifiable female. Where females could not be identified, they were included in miscellaneous groupings of banded, un-banded and unknown females for each male. Test assumptions were verified for all paired t-tests prior to analysis.

RESULTS

Female within-visit responses to male aggression

When pre- and post-event behaviours were compared, female rejection of copulation attempts followed a consistent pattern, regardless of whether the male succeeded in making physical contact with the female. Rejection was followed by an increase in female mound activity (t_{paired} = -2.559, P = 0.015, n = 37 where physical contact occurred and t_{paired} = -4.677, P < 0.001, n = 75 where physical contact did not occur). Rejection was also followed by significant increases in both male hover and male bird-peck, again regardless of whether physical contact occurred, the weakest result being the increase in hover where physical contact occurred during the copulation attempt (t_{paired} = -2.254, P = 0.03). During egg-laying visits, there was usually a clear point at which substantial mound activity commenced, and then continued at a high rate until egg-laying itself (chapter 2). To exclude the possibility that the increase in mound activity reported here merely reflected this long-term temporal pattern, rejections were excluded from the analysis where this sustained rise in mound activity occurred within the review period either prior to or subsequent to the rejection. After these exclusions, there was still a significant increase in female mound activity subsequent to rejections generally (t_{paired} = -4.514, P < 0.001, n = 100) and rejections specifically where the male failed to make physical contact (t_{paired} = -4.289, P < 0.001, n = 68), although the increase was non-significant where the male succeeded in making physical contact, possibly because of a lower sample size (t_{paired} = -1.622, P = 0.115, n = 32). Incidences of male attempted sexual coercion and copulation were not significantly different pre- as opposed to post-rejection.

The necessary corollary of increasing female mound activity subsequent to an event is relatively lower activity prior to it. Which tendency is dominant is best determined by analyzing behaviour over the entire course of visits generally, for example in a multiple regression (in the following results, β values represent standardized correlation coefficients). When controlling for visit sequence, the distinction between egg-laying and non-laying visits, as well as other female responses to male aggression, a higher incidence of copulation rejection predicted higher female mound activity, consistent with the post-

event finding above (strongly significant in 198 cases where the male failed to make physical contact but only a non-significant trend in 52 cases where he did make physical contact, Table 1). A higher incidence of copulation rejection, however, made no significant difference to male hover and bird-peck (Table 1), indicating that these last two elements of the post-response result were short-term only. A higher incidence of copulation rejection also predicted a higher incidence of copulation, regardless of whether the male made physical contact, and a higher incidence of male attempted sexual coercion in the case of rejections where the male failed to make physical contact (Table 1). The latter association may be autocorrelation, however, as failed copulation is itself a form of attempted sexual coercion. In summary, copulation rejection seemed to involve a trade-off between increased female mound activity on the one hand and costs which varied according to the timescale. In the short term, the female incurred an increase in male hover and bird-peck. Viewed from the perspective of the entire visit, however, the cost seemed to shift to increased copulations.

Table 1 Female responses to male aggression as predictors of male aggression, female mound activity and copulation. Several multivariate regressions were run, treating rates for each of the female responses to aggression as simultaneous independent variables, and treating rates of female mound activity, male aggression and copulation in turn as dependent variables. Visit sequence and the distinction between egg-laying and non-laying visits were also included as simultaneous independent variables. As the focus was on male-female interactions, each visit by a uniquely identifiable female was treated as a data point, so long as the male mound-owner was present. In general, rates were expressed relative to the duration of the female's presence at the mound. Male aggression, however, required a special treatment as female responses were defined in relation to male aggression, and therefore necessarily had a positive association with aggression. Rates for male aggression were expressed as the visit-specific rate less the mean rate for the relevant male-female combination, a lower visit-specific rate being treated as evidence that female responses during the visit reduced male aggression.

	Dependent variables									
	Male aggression						Female mound activity (duration/min)		Copulation (frequency/min)	
	Hover (duration/min)		Bird-peck (frequency/min)		Attempted sexual coercion (frequency/min)					
Independent variables	β	P	β	P	β	P	β	P	β	P
Female responses:										
Copulation rejection (frequency/min)	.055	.461	.078	.285	.063	.355	.121	.088	.233	.006*
Copulation rejection, no contact (frequency/min)	.095	.150	-.083	.200	.582	<.001*	.245	<.001*	.147	.048*
Copulation (frequency/min)	.256	<.001*	.158	.017*	.095	.128	.249	<.001*	n/a	n/a
Temporary retreat/absence (duration/min)	-.037	.584	-.092	.164	.141	.024*	.072	.265	.200	.009*
Other key influences:										
Visit sequence	.166	.013*	-.174	.008*	-.279	<.001*	-.104	.100	.131	.078
Egg-laying vs. non-laying visit[†]	.386	<.001*	.435	<.001*	.028	.690	.378	<.001*	.188	.031*

* significant result   †egg-laying visit =1; non-laying visit = 0

When comparing pre- and post-event behaviours, the results for copulation were a more extreme version of those for copulation rejection. As previously reported (Chapter 1), copulation was followed by a strongly significant increase in hover, a significant increase in bird-peck and a strongly significant decline in attempted sexual coercion. It was also followed by a strongly significant increase in female mound activity ($t_{matched}$ = -5.418, $P <$ 0.001). As for copulation rejection, copulations were excluded from the analysis where a sustained rise in mound activity occurred within the review period either prior to or subsequent to the copulation. In addition, copulations often occurred early during visits (chapter 1), when females were tentative and mound activity was therefore less likely. Although copulations occurring within two minutes of visit commencement had already been excluded, those occurring within five minutes of visit commencement were also excluded. After these exclusions, there was still a significant increase in female mound activity post-copulation (t_{paired} = -4.07, $P <$ 0.001, n = 44).

When behaviour was measured over the entire course of visits generally, with other female responses, visit sequence, and the distinction between egg-laying and non-laying visits controlled for in a multiple regression, a higher incidence of copulation predicted a significantly higher incidence of female mound activity, male hover and male bird-peck (Table 1), consistent with the post-copulation results above. Rather than a reduction in male attempted sexual coercion, however, there was evidence of an increase, albeit non-significant (Table 1). In summary, copulation involved a consistent trade-off between increased female mound activity on the one hand and increased male hover and bird-peck on the other. Although a reduction in attempted sexual coercion was a clear short-term benefit of copulation for females, it was not a long-term benefit, and there was some evidence of an increase in this form of aggression.

When pre- and post-event behaviours were compared for temporary female retreats and absences combined, the result was in one respect similar to that for both acceptance and rejection of copulation attempts, namely an increase in female mound activity in the post-event period (t_{paired} = -1.954, P = 0.054, n = 89). Moreover, it is possible to discount the suggestion that this result merely reflected a long-term increase in mound activity during egg-laying visits, as temporary retreats and absences were overwhelmingly a feature of non-laying visits. In contrast to acceptance and rejection of copulation attempts, however, there was no evidence of a corresponding increase in male hover and bird-peck (P values of 0.951 and 0.908 respectively). The incidence of copulation was not significantly different. There was evidence of a reduction in attempted sexual coercion post-response, although non-significant (t_{paired} = 1.834, P = 0.07).

As for female rejection of copulation attempts, a somewhat different picture emerged if one considered the entire course of visits generally in a multiple regression which controlled for other influences. There was no association between the duration of temporary retreats/absences and mound activity (Table 1). Meanwhile, the duration of temporary retreats and absences was positively rather than negatively associated with attempted sexual coercion and with copulation (Table 1). In summary, temporary retreats and absences seemed to reap a short-term benefit in increased mound activity at no discernible cost. Over the course of the visit, however, the benefit was reduced, and increased costs were incurred in the form of a greater likelihood of attempted sexual coercion and copulation.

When the above regressions were repeated with data points defined as each unique combination of male and female rather than each visit, the sample size was reduced, and any bias toward frequently-visited males was removed. Excluding the visit sequence data, 13 results in Table 1 are significant, of which four became non-significant under this alternative treatment. Of these, only one represented a major change: there was no longer any association between temporary retreats/absences and attempted sexual coercion.

Behavioural perseverance

There were four types of unvarying perseverance: uninterrupted female mound activity and uninterrupted male hover, ground-peck and bird-peck. During bouts of female mound activity exceeding 10 minutes in duration, the female's activity occurred at a significantly higher rate than for the overall visit (t_{paired} = 18.278, *P* < 0.001). The same was true for bouts of male hover and ground-peck exceeding 10 minutes in duration (t_{paired} = 10.72, *P* < 0.001 and t_{paired} = 3.365, *P* = 0.002 respectively) and for male bird-peck with more than 20 iterations (t_{paired} = 6.756, *P* < 0.001). Bouts of female mound activity exceeding 10 minutes in duration accounted for 41% of visit elapsed time for visits during which the bouts occurred. The equivalent ratios for uninterrupted bouts of male hover, ground-peck and bird-peck were 37%, 40% and 33% respectively (Table 2). Visit elapsed time here excluded periods of temporary female absence or retreat to the side of the mound, as the relevant behaviours were only included in the analysis when both male and female were present on the mound. When these distinct bouts were considered together, after allowing for periods of overlap between male bouts, and occasions when male and female behaviour occurred simultaneously, they accounted for 60% of visit elapsed time for visits during which the bouts occurred and 30% for all visits exceeding 10 minutes (Table 2).

Table 2 Unvarying perseverance, i.e. mean duration of bouts of behaviour exceeding 10 minutes in duration (for behavioural states) or exceeding 20 iterations (for events), where the behaviour persisted despite the other party's behaviour. Mean duration is also expressed as a ratio of the female's presence at the mound. Only visits exceeding 10 minutes in duration were considered. The final row shows the effect of considering distinct bouts together, after allowing for periods of overlap between male bouts, and occasions when male and female behaviour occurred simultaneously.

Behaviour	Male/ Female	Mean duration of long-lasting bouts (mins)	Mean duration as proportion of female presence at the mound	
			For visits during which the bouts occurred	For 143 visits with elapsed time exceeding 10 mins
Behavioural states:				
Mound activity	Female	19.02	0.41 (n = 20)	0.14
Hover	Male	18.00	0.37 (n = 17)	0.10
Ground-peck	Male	13.37	0.40 (n = 26)	0.10
Behavioural events:				
Bird-peck	Male	11.32	0.33 (n = 22)	0.06
States & events combined	Both	18.38	0.60 (n = 42)	0.30

Two main types of cyclical perseverance were identified, both involving a female stop-restart pattern. In the first cycle, the female left the mound (or retreated to the side of the mound), often in response to male aggression; the male usually switched to or intensified enticing behaviour; the female then returned to the mound (or returned to the centre of the mound). The second cycle was similar, except that the female left a hole in which she had typically been digging, only to subsequently return to the same or an alternative hole. Cyclical perseverance of the first type involving at least two iterations of the cycle accounted for 74% of visit elapsed time. The equivalent figure for the second type was 60% (Table 3). Visit elapsed time here included periods of temporary female absence or retreat, as the first cycle included such periods. When these distinct cycles were considered together, they accounted for 69% of visit elapsed time for visits during which the bouts occurred and 62% for all visits exceeding 10 minutes (Table 3).

Table 3 Cyclical perseverance, i.e. mean duration of behavioural cycles with at least two iterations of the cycle. Mean duration is also expressed as a ratio of visit elapsed time including periods of temporary female absence because one of the cycles includes female departure and return. Only visits exceeding 10 minutes in duration were included in the analysis. The final row shows the effect of considering these cycles together.

Cycle	Cycle elements	Mean duration of long-lasting cycles (mins)	Mean duration as proportion of visit elapsed time incl. periods of temporary female retirement/absence		Mean no of iterations
			For visits during which the cycles occurred	For 143 visits with elapsed time exceeding 10 mins	
Mound leave & return	female leaves mound (or retires to side of mound), often in response to male aggression; male usually entices; female returns to mound (or returns to centre of mound)	29.90	0.74 (n = 68)	0.38	5.19
Hole leave & return	female leaves hole, often in response to male aggression; male usually entices; female re-enters hole	26.92	0.60 (n = 47)	0.24	6.62
above cycles combined		28.68	0.69 (n = 113)	0.62	5.77

Female visiting pattern

Of the four datasets reviewed, the most relevant was an early-season 2008-9 dataset which summarized observations at three mounds for 45 days consecutively. The mounds were adjacent to each other, and were the only mounds active during the relevant period at the northern end of the study area, the most active area for female visitation. Although observation was in principle for 135 mound-days, this was reduced to 122 mound-days as a result of equipment failure or human error. Most females concentrated on visiting a single mound, henceforth referred to as the *primary mound*, with visits to other mounds (*secondary mounds*) almost always involving no copulation or egg-laying. Some females visited a single primary mound for a period of at least 45 days, with no more than occasional visits to alternative mounds. Three of 13 females switched primary mounds during the study period. In one case, the switch represented a clean break, in a second there was a transition period during which the female visited both mounds, and in a third the switch represented only a shift in emphasis, this female exhibiting the least focus in her visiting pattern. Females who switched primary mounds could be treated in three different ways: they could be excluded from the analysis, treated as though they had only one primary mound throughout the study period, or assigned to two different primary mounds in succession. Regardless of treatment, visits to primary mounds by each female were significantly more frequent and of significantly greater duration than visits by the same female to all other mounds combined. For example, when mound-switching females were assigned to two different primary mounds in succession, primary mounds received significantly more visits (t_{paired} = 3.35, P = 0.006) and visits of significantly greater duration (t_{paired} = 3.04, P = 0.01) (Fig. 1).
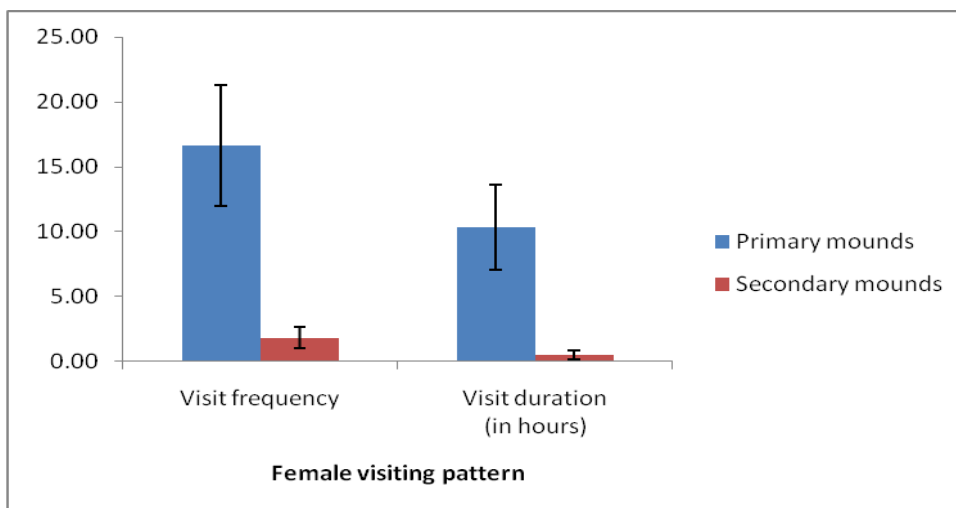

Figure 1 Differences between the incidence of female visits to primary and secondary mounds, as measured by mean visit frequency and duration. Three adjacent mounds were observed for 45 days consecutively (n = 13 females).

Regardless of the treatment of females who switched, primary mounds received significantly more and longer visits by the same female than all other mounds combined in all four datasets, the only exception being visit duration for a late-season 2007-8 dataset. This dataset included the clearest example contrary to the pattern identified here, namely a female who made four egg-laying visits to a secondary mound without suspending visits to the primary mound. With this exception, no visits to secondary mounds in any of the datasets involved egg-laying. With the same exception, plus a single visit involving a different female, none involved copulation. Using the two study datasets only, which included all known active mounds at the study site for two consecutive breeding seasons, no evidence was found of females visiting mounds substantially distant from each other. The mean distance between all active Pearl Beach mounds (587.8 metres) exceeded both the mean distance between mounds visited by the same female (202.7 metres, t = 15.529, $P < 0.001$) and the maximum for any one female (568.5 metres). This confirmed the approach adopted here, namely to focus on physically adjacent mounds when attempting to identify female visiting patterns.

A likely explanation for this distinction between primary and secondary mounds was a decrease over time in the incidence of male aggression during a sequence of primary-mound visits. Controlling for female responses to male aggression and for the distinction between egg-laying and non-laying visits, later visits in a sequence were associated with a significant increase in male hover, but a significant decrease in bird-peck and a strongly significant decrease in attempted sexual coercion (see Table 1). The decrease in attempted sexual coercion is illustrated in Figure 2. In summary, males harassed the female less and pecked her less over the course of a series of visits, despite maintaining close company with her for longer periods.
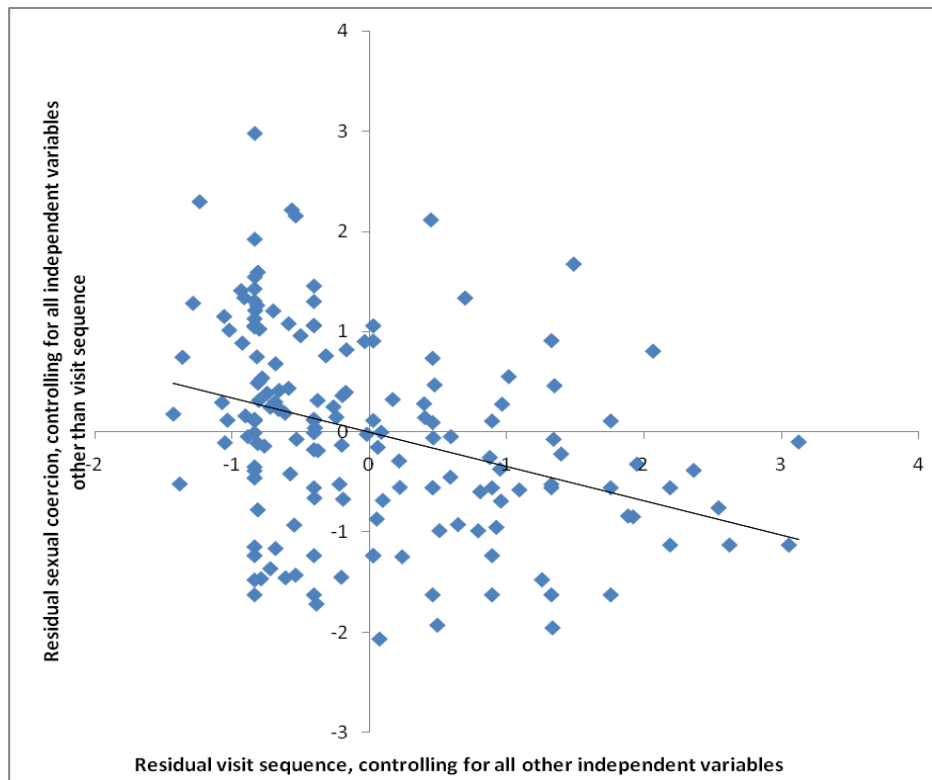
Figure 2 Partial regression plot showing the specific contribution of visit sequence to predicting attempted sexual coercion, holding all other independent variables constant. This illustrates one of the key findings summarized in Table 1, namely that later visits in a sequence were associated with a significant decrease in attempted sexual coercion ($P < 0.001$)

Rather than visiting while the male was absent, in general females were more likely to visit while he was present. When visit duration was expressed as a ratio of the opportunity for visits of the relevant type to occur, females visited more frequently when the male was present ($t_{paired} = -4.471$, $P < .001$, n = 71). Within the sample taken, a distinction could nevertheless be drawn between those females who visited only when the male was absent (32% of uniquely identifiable females) and females who visited only when he was present (48%). For a further 10% of females, visits while the male was present represented at least 75% of all visits by the relevant female. For the last two categories treated as a single group, namely females who overwhelmingly visited while the male was present, the visit ratio was significantly different from the ratio for all other females ($t_{paired} = -5.769$, $P < 0.001$, n = 41). For females who visited only when the male was absent, the difference was also significant, but in the opposite direction ($t_{paired} = 3.206$, $P = 0.004$, n = 23). Males who simultaneously maintained two active mounds are included in the above results, but could be considered a special case, as their presence at one mound necessarily offered greater opportunities for females to visit during their absence at the other. For such males, the proportion of females visiting only when the

male was absent increased to 55%, and the proportion visiting only when he was present dropped to 25%. When visit duration was expressed as a ratio of the opportunity for visits of the relevant type to occur, neither visit type was significantly more likely for two-mound males, although a divergence between the two visiting strategies was nevertheless evident.

DISCUSSION

My analysis established that female responses to male aggression achieved short-term female gains in the form of increased mound activity while incurring costs which varied according to the time-scale. The gain in increased mound activity was sometimes lost over the full length of the visit. The behaviour of both sexes was characterized by unvarying and cyclical perseverance. Females concentrated at any one time on visiting a single primary mound, were more likely to visit while the male was present than when he was absent, and experienced reduced male aggression later in a sequence of visits.

Male aggression at brush-turkey incubation mounds influenced the pattern of female visits to those mounds and the behaviour of females when they visited. Some females (32% of uniquely identifiable visitors) avoided the aggression by visiting only when the male was absent. Such females were presumably not breeding during the observation period, but may have bred at other times. The majority of females (58%), however, overwhelmingly visited only when the male was present. Visiting pattern therefore seemed to be a disruptive trait, the clear divergence suggesting that visiting was not a passive response to chance circumstances, but females were actively seeking out the male's absence in one case and his presence in the other. Where males maintained two mounds simultaneously, and females therefore had more opportunity to visit while the male was absent, more females did so, but a divergence between the two patterns was nevertheless evident. Breeding females obviously required the male's presence for copulation, but the more general advantage of his presence only became clear in the context of other aspects of the visiting pattern: by repeatedly visiting the same male, females benefitted from a reduction in male aggression over time.

The reduction in male aggression over a series of female visits applied specifically to attempted sexual coercion and the bird-peck component of up-close aggression, although the hover component of up-close aggression increased (*visit sequence* in Table 1). Despite the reduction in attempted sexual coercion, there was some evidence that females were more rather than less likely to copulate (non-significant trend, Table 1), indicating a shift

in female priorities from resisting to accepting copulations later in a sequence of visits, when egg-laying became the primary focus. Females were significantly more likely to copulate during egg-laying visits (*egg-laying vs. non-laying visit*, Table 1), probably because of the need to protect an unusually thin-shelled egg (Birks, 1999), but the increase in copulations reported here was an additional effect, after controlling for the distinction between egg-laying and non-laying visits. There was also evidence of reduced female mound activity later in a sequence of visits (non-significant trend, *visit sequence*, Table 1), possibly because the female was by now very familiar with the mound. Reduced female mound activity was consistent with the reduction in male bird-peck, mound activity being a stimulant of bird-peck (chapter 2). Mound activity by females, however, is also a stimulant of hover behaviour in males (chapter 2). Increasing hover despite decreasing mound activity suggested a greater female willingness to tolerate the male's close presence, perhaps because he was less likely to peck her. These changes in male and female behaviour over a series of female visits were recorded during an observation period of typically one week and at maximum two weeks at each mound, and were therefore unlikely to be explained by longer-term trends, such as a possible gradual reduction in male aggression or female resistance to copulation over the course of a season.

In short, the function of the female's visiting pattern became apparent. Given that a breeding female would eventually make egg-laying visits, which exposed her to male aggression for longer periods (chapter 2), it was advantageous for the female to visit while the male was present, thereby maximizing their time together on the mound, and habituating him to her company. For the same reason, it was also advantageous for the female to visit a single primary mound, and hence male, at the expense of any secondary mounds. Copulation and egg-laying overwhelmingly occurred at primary mounds in Pearl Beach, although this contrast was less extreme in a non-urban population (Jones, 1987). The advantage for females of maximizing time with one male also helps to explain an otherwise puzzling feature of female behaviour, namely a tendency to extend visit duration beyond what appeared to be necessary, often soaking up time with preening, foraging or standing motionless, behaviours which did not require access to the mound, and often occurred during temporary retreats to the side of the mound (D. Wells unpublished data).

When females visited mounds while the male owner was present, there was a clear contrast in male and female objectives: males concentrated on obtaining copulations and females on mound activity (chapter 1). Although contrasting, these objectives were not necessarily in conflict, and might conceivably have enabled cooperative behaviour. Unambiguous cooperation, however, was restricted to two brief episodes: copulation,

when the female raised her tail and moved it to one side in order to facilitate cloacal contact (chapter 1) and egg-laying, when the male almost always suspended his pecking of the female (chapter 2). Outside of these few seconds in the former case or minutes in the latter, and notwithstanding the diminution in hostilities noted immediately above, male-female interactions were either agonistic or potentially so. Male enticement of the female onto the mound did not itself involve conflict, but it could be replaced by attempted sexual coercion suddenly and unpredictably. For example, the male flattened display (lying outstretched on the mound in a defenseless posture, usually during the female's arrival) was often followed by a copulation attempt, and even in some cases by a vigorous charge directly out of the flattened posture. It is likely that much of the coercive effect of attempted sexual coercion derived from its unpredictability. As a series of visits proceeded, there was no discernible increase in cooperation, but there was a reduction in male hostility, apparently associated with increasing familiarity and a closer alignment of objectives. The female had achieved much of what she wanted, and therefore required less time for mound activity, while the male was more likely to obtain what he wanted, namely a higher copulation rate.

This assumes that there are two points of conflict, namely copulations and female mound activity, but there can also be sexual conflict over the degree of parental investment by each sex (Chapman et al., 2003; Parker, 2006). Parental investment in this species is entirely pre-hatching. Females contribute resource-rich eggs which support super-precocial chicks, while males contribute a mound and the maintenance activity required to ensure it functions effectively as an incubator. During female mound-visits, however, both sexes interacted with the mound in ways which potentially enhanced its qualities as an incubator. If parental investment was a point of conflict, it was not clear whether each sex was attempting to shift responsibility to the other, or conversely was assuming responsibility in spite of the other. Female choice is negatively associated with male maintenance during female visits (see chapter 4), suggesting that females were not interested in male parental investment during visits. It could be that males were nevertheless interested in providing it, but the more plausible interpretation is that they were using it as a form of enticement, maintenance often appearing to function as a default behaviour for periods when the female performed little mound activity. If this interpretation is correct, it was low mound activity which caused male maintenance, rather than male maintenance which caused low mound activity. I have assumed that females are strongly interested in mound activity, and because mound activity presumably has its basis in the welfare of the egg, I am assuming that females are thereby making a parental investment. It was difficult to interpret male responses to mound activity, however, as conflict over this investment. In enticement mode, males invited mound activity (chapter 2), apparently because this made the female more likely to stay.

In aggression mode, they resisted it, but this neither shifted responsibility to the female nor wrested it from her. Parental investment therefore seems an unlikely point of conflict.

During their visits, females employed various tactics which involved different trade-offs between a benefit (mound activity) and two costs (copulation and male aggression). Whether or not to copulate was predominantly under female control (chapter 1), as was the decision to temporarily leave or retreat to the side of the mound. It is therefore likely that the incidence of behaviour post-response was closer to the female's preferred outcome, relative to its incidence pre-response, which was closer to the male's preferred outcome. On this criterion, the clearest female trade-off was between the benefit of mound activity and the cost of male up-close aggression. Females obtained a substantial benefit at a substantial cost when they copulated, a lower benefit at a lower cost when they refused to copulate, and an even lower benefit at no discernible cost when they temporarily left or retreated to the side of the mound. It was very clear that females obtained greater access to the mound immediately after they had copulated, as most proceeded directly to a hole in order to dig. Females were also able to obtain increased access after refusing to copulate, perhaps because they judged that one copulation attempt would not be immediately followed by more attempted sexual coercion. Some females appeared to invite copulation and then refuse it, in order to engineer this situation. Females returning from a temporary absence or retirement also obtained a short-term benefit in mound access, but less marked than in the above cases. The benefit of increased female mound activity incurred a cost in increased male up-close aggression because the former stimulated the latter (chapter 2), and could be expected to do so most markedly subsequent to copulation, where mound activity increased most strongly. Temporary absences and retirements seemed to escape this consequence by interrupting the typical sequence of events, and encouraging the male to switch to enticing behaviour.

Female willingness to incur the cost of increased up-close aggression may be viewed as an unavoidable consequence of increased mound activity, but it may also indicate that up-close aggression is less costly to females, possibly because it is more predictable and hence less stressful. As suggested above, it may be that the form of aggression males employed to obtain copulations was effective in this regard because it was less predictable and hence more stressful. None of the female responses was succeeded by a reduction in up-close aggression, but two were succeeded by a reduction in attempted sexual coercion, strongly significant in the case of copulations and almost significant for temporary absences and retirements. In the latter case, females appeared to be anticipating attempted sexual coercion, and sometimes triggered it when they began to leave or move away from the centre of the mound. When triggered in this way, however, the aggression became more predictable.

When behaviour was measured over the entire course of visits generally, it was possible to identify the overall effect of each female response to aggression while controlling for other female responses, visit sequence and the distinction between egg-laying and non-laying visits. For each response, a higher incidence predicted a higher incidence of mound activity, significantly so in two of four cases, and close to significant in a third (Table 1). This was consistent with evidence above that mound activity was the benefit sought by females in trade-offs. Inconsistent with up-close aggression being the associated cost, this form of aggression recorded only a modest increase (in one of four cases for hover and similarly for bird-peck, Table 1). Meanwhile, a higher incidence of each female response also predicted a significantly higher incidence of copulation (three significant increases in Table 1, noting the *not applicable* result for the copulation response, it being impossible to regress a copulation rate on a copulation response).

This raises two issues. Firstly, how was it possible for the cost of the female's trade-off to shift from increased up-close aggression in the short term to increased copulation over the entire course of the visit? The answer appeared to be that, rather than focusing on behaviour immediately subsequent to the female's response, and comparing it with behaviour prior to the response, I was now including both sets of behaviour without distinction, plus any behaviour outside either of the five-minute periods of record. In response to females obtaining increased access to the mound, males probably increased attempted sexual coercion in the pursuit of increased copulation. This interpretation is consistent with the two significant increases in attempted sexual coercion (Table 1). If correct, it implies that, although males had maximum copulations as an objective, they were also sensitive to the female's success in obtaining her objective: if female mound activity was high, males demanded copulations even more insistently, apparently as a quid-pro-quo.

Secondly, with increases in both copulation and mound activity, both sexes achieved their objectives to some degree. This might mean that male and female objectives were not in conflict after all, or alternatively that both sexes achieved a compromise outcome which reflected near equality in their competitive strengths. Given the agonistic character of male-female interactions, the latter interpretation seemed more likely. It appeared that the male wanted more copulations than he received and the female wanted more mound activity than she was allowed, but both were forced to compromise, probably because of near equality in competitive strength. Except in unusual circumstances, the male could not force the female to copulate (chapter 1), and he could pursue a departing female for any substantial period only at the risk of being absent for the visits of alternative females and interloping rivals. Meanwhile, the female could not force the male to provide access to his mound.

Consistent with near equality in competitive strength, both sexes were prepared to repeat a behaviour many times in order to achieve their objective. When bouts of either male or female unvarying perseverance were considered together, after allowing for occasions when they occurred simultaneously, they accounted for 60% of visit elapsed time for visits during which the bouts occurred and 30% for all visits exceeding 10 minutes (Table 2). For behaviours defined as events, the most extreme case was a bout of bird-peck lasting 17 minutes, during which the male pecked the female at a mean rate of 21 pecks per minute. When bouts of cyclical perseverance were considered together, they accounted for 69% of visit elapsed time for visits during which the bouts occurred and 62% for all visits exceeding 10 minutes (Table 3). Cyclical perseverance could involve many iterations of the cycle (maximum 25) and could last for an extended period (maximum 111 minutes).

Such perseverance might suggest that the appropriate game-theory model is some version of war of attrition, but repetition is also characteristic of the sequential assessment model (Clutton-Brock and Parker, 1995). Any attempt to identify the appropriate model must address the difficulty that the conflicting objectives of males and females are not always clear in the brush-turkey case. Where attempted sexual coercion is involved, it is clear that the male objective is to obtain copulations and that the female objective is to avoid them (chapter 1). In contrast, no function could be identified for up-close aggression (chapter 2). It is nevertheless clear that the objective of females is to maximize mound activity, while the immediate objective of males is to impose costs on that activity, even if the function of doing so is unclear.

The sequential assessment model assumes that each party to the contest assesses its opponent, using behaviour during the contest as the basis for assessment. Given that assessment accuracy increases with increasing sample size, it pays contestants not only to repeat agonistic behaviour, but to repeat at a consistent level of intensity, to allow an accurate rolling average (Briffa and Sneddon, 2010). Previous research has shown that once significant female mound activity commences during egg-laying visits, its rate does not substantially vary until egg-laying itself (chapter 2). A similar pattern was also observed for male up-close aggression during the same period (D. Wells unpublished data). Once males have switched from attempted sexual coercion to up-close aggression during egg-laying visits, therefore, the behaviour of both male and female brush-turkeys seems to fit the sequential assessment model (Briffa and Sneddon, 2010). In any contest in which assessment is mutual, and made during the contest itself, it is unclear how resolution will occur if neither contestant gives up, perhaps because they are closely-matched in competitive ability. For reasons stated above, there are grounds for considering male and female brush-turkeys as closely-matched antagonists. Moreover, long-lasting contests, typical of this species, are generally considered evidence of a close

match in competitive ability (Clutton-Brock and Parker, 1995). An extended version of the sequential assessment model assumes that, where opponents are closely matched, the contest will go through a series of phases, each phase being marked by new behaviours with increasing intensity (Briffa and Sneddon, 2010). One might consider the switch to up-close aggression during egg-laying visits as such a phase change, but any switch away from attempted sexual coercion is more likely to represent reduction rather than escalation (see above). Moreover, when the rates of all types of male aggression are graphed against visit time for all visit types, there is little evidence of escalation during visits (D. Wells unpublished data). Lack of evidence for escalation therefore counts as evidence against the sequential assessment game as an appropriate model.

Simple versions of the war of attrition assume that contestants self-assess only, and do not assess each other's competitive abilities. Contestants give up when contest costs have exceeded an individual threshold determined prior to the contest by their own competitive abilities and their own value of winning. The evolutionarily stable strategy is to be unpredictable, so that opponents cannot identify this threshold, and win simply by persisting for a slightly longer period. As for the sequential assessment game, contestants monitor each other's behaviour, but in this case only to ensure that the opponent's energy expenditure is equal to their own, and hence that they are not cheating (Briffa and Sneddon, 2010). These points are broadly consistent with the brush-turkey case. Despite the tendency for male aggression to follow a declining trajectory over a series of visits, the level of aggression was quite variable between individual visits, as was the female's rate of mound activity (D. Wells unpublished data). Although females were often inactive on the mound while the male was highly active, in such cases the male's activity overwhelmingly took the form of enticement; by contrast, when they occurred together, male aggression was broadly matched in intensity with female mound activity (chapter 2). An extended version of the game, the asymmetric war of attrition, has the additional advantage of allowing for differences between the contestants' estimates of the value of winning and the value of time allocated (Parker, 2006), appropriate for a male-female as opposed to a male-male conflict. This version also allows for mutual assessment (Briffa and Sneddon, 2010), and is therefore compatible with the highly uniform behavioural rates referred to above in the context of sequential assessment.

The asymmetric war of attrition may also provide a mechanism for the declining trajectory of male aggression over a series of visits. Where contestants are unfamiliar with each other, they are more likely to make mistakes in mutual assessment, with the result that both may assess themselves in the likely winning role and hence commit greater time and energy to the contest (Clutton-Brock and Parker, 1995). This reasoning has been used to explain the 'dear enemy' effect, the tendency for territory-defending males in a wide

range of taxa to respond more aggressively to an intruder who is unknown as opposed to a familiar neighbour (Ydenberg et al., 1988). In the brush-turkey case, similar reasoning predicts not only a decline in male aggression over a series of visits by the same female, but also a decline in female mound activity. A decline in mound activity was noted above, and attributed to the female's reduced need to investigate the mound, but it may also reflect a reduced tendency to respond agonistically to the male.

Both war of attrition and sequential assessment assume that the benefit of winning and the cost of continuing the contest are key parameters, and that long-lasting contests may reflect near equality in the value of these variables for each participant (Clutton-Brock and Parker, 1995). Both games also assume that the cost of continuing the contest is measured in the currency of elapsed time (Clutton-Brock and Parker, 1995). It was argued above that the female strategy of habituating the male to her company is expected to increase visit duration. One consequence is that the female's cost of continuing the contest will be much less than might be expected, allowing her to persevere even when the value of winning is quite low. The male's cost of continuing may be higher, but so too might be his value of winning, hence producing the drawn-out contests which are observed. It should also be noted that females who spend extended periods on the mound thereby deny access to their female rivals, a point which will be considered in more detail in chapter 5.

Male-female interactions in Australian brush-turkeys appear to be characterized by sexual conflict. Females responded to male aggression both tactically during the visit and strategically via their visiting pattern. Females appeared to trade-off a benefit in increased mound activity against costs in the form of increased copulation or male aggression. In general, however, the behaviour of both sexes was characterized by unvarying and cyclical perseverance in the pursuit of conflicting objectives, the appropriate game-theory model probably being the asymmetric war of attrition.

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Chapter 4 - Mate choice by female Australian brush-turkeys: remote assessment of male-resource cues

A male brush-turkey maintains his mound in the absence of female visitors. Females may, however, be observing from a distance.

ABSTRACT

Where males defend resources which are essential for female reproduction and females are the more choosy sex, females require information about both males and resources, and therefore probably rely on multiple cues. To reduce the effect of male aggression, female Australian brush-turkeys *Alectura lathami* appear to make initial assessments of both males and mounds from a distance, but then visit and apparently assess a mound. Investment in the initial remote assessment is probably substantial, as females tend to visit a single primary mound for extended periods, with only minor visits to alternative mounds. It is therefore likely that the principal cue for female choice in this species is male-mound information such as the degree to which the male maintains his mound, this being the best available source of information about mound quality from a distance. Maintenance by the male is thought to enhance heat production by thermophylic bacteria and fungi, reduce the likelihood of compaction, and make oxygen more available to eggs in the mound. Results from sequential multiple regressions indicated that male-mound cues were

better predictors of female choice than male, mound or climatic cues such as rainfall. Male-mound cues did not represent a combination of male and mound elements which were independently assessed, but could also be jointly assessed. Rather, what appeared to be happening was that females were using observations of the male-mound relationship to provide information about both the male and the mound, and in particular the latter. Within the male-mound category, results from simultaneous multiple regression indicated that the key variables were male maintenance and attendance, as well as male enticement of females onto the mound. By examining the distribution of arrival times of females seeking to visit while the male was absent, it became clear that females had been present nearby prior to the male's departure. Together with additional evidence, this confirmed that females were probably assessing males and mounds from a distance, and were, therefore, able to observe male maintenance effort while no female visits were occurring.

INTRODUCTION

Where males defend resources which are essential for female reproduction and females are the more choosy sex, it is often unclear whether females are choosing males or the resources which the males control. In the likely event that females are to some degree choosing both (Eckert and Weatherhead, 1987), females are probably responding to a combination of male cues and resource cues. The male vs. resource question, therefore, intersects with another question which has received increased research attention, namely the role of multiple cues in female choice (Candolin, 2003; Lehtonen and Wong, 2009). Female European bitterlings (*Rhodeus sericeus*), for example, are initially influenced to inspect a male's spawning site by the male's courtship behavior and coloration, but make their decision to spawn based on close inspection of the spawning site itself (Candolin and Reynolds, 2001). In this case, male cues can be clearly distinguished from resource cues, with the former dominant during the early stage of courtship when the female is some distance from the resource, while the latter dominate once the female draws closer.

Where females respond to a male's behavior in relation to a resource, a third type of cue is present. In many fish species, males ensure that oxygen is supplied to developing embryos by fanning eggs laid in their nests. This behavior may be a cue for female choice, as has been established for sand gobies *Pomatoschistus minutus* (Lindström et al., 2006) for example. In that case, females are responding not merely to male cues and resource cues, but also to the male's behavior in relation to the resource, i.e. male-resource cues. Such cues likely provide indirect information about both the male (vigour, parental care abilities) and the resource (eggs laid in this nest will be well aerated).

Female Australian brush-turkeys, *Alectura lathami*, may take similar advantage of multiple cues when assessing males and the incubation mounds which males control. Females are not subject to mate-guarding, and therefore have considerable freedom to observe males and their mounds from a distance. Once they move onto a mound, however, females are subject to significant male aggression, including attempted sexual coercion (chapters 1 and 2). This combination of female freedom to visit and potentially high visit costs suggests that initial female assessment of males and mounds is likely to be undertaken at a distance from the mound, using cues which are available at that distance. These will include male-mound cues in addition to male cues and mound cues.

Brush-turkeys are members of the megapode clade (Family Megapodidae). Uniquely among birds, megapodes incubate their eggs using environmental sources of heat, usually

microbial decomposition in a mound of soil and rotting vegetation. On the basis of this ancestral pattern, variations have evolved. Although most megapodes are monogamous, brush-turkeys are both polygynous and polyandrous. In the brush-turkey mating system, males construct mounds, defend them against rival males, control their temperature via ongoing maintenance and mate with multiple females each season. Females visit mounds, often copulate with the male and periodically dig large holes in the mound in order to bury their eggs. There is no post-hatching care at all. Chicks are entirely left to their own devices, including having to dig their own way out of the mound. Females are not subject to mate-guarding, do not pair-bond with the male and also mate multiply, although apparently not as multiply as the male.

Previous work has identified a female pattern of laying a series of eggs in the mound of a single primary male for an extended period, and making few if any visits to other males (chapter 3). Such limited visiting of alternative males involves the risk of a sub-optimal choice. Given frequent male attempts at sexual coercion, even a small number of visits involve a copulation risk if the primary male is sub-optimal. It is therefore likely that females invest substantially in initial assessment from a distance, probably making repeated observations of several potential mates and returning repeatedly to males previously sampled before visiting. Various models have been developed to explain sequential mate choice, but it was not possible to test them in this study because females were generally only observable when on or close to the mound. Remote assessment has been recorded in other taxa, for example female damselfly *Calopteryx splendens xanthostoma* perform 'flybys' over oviposition sites guarded by territorial males before selecting a site at which to lay (Hooper and Sivajothy, 1997).

As with European bitterlings, initial assessment by female brush-turkeys is followed by a visit to the incubation mound, enabling direct inspection of the resource under male control. In the case of the bitterling, direct assessment appears to be the key determinant of whether the female decides to spawn (Candolin and Reynolds, 2001). In the brush-turkey case, however, the position is less clear. The female pattern of overwhelmingly visiting only one male during an extended period suggests that information gathered during visits has little feedback effect on subsequent visiting behavior, and that the key decision is made prior to the first visit. It can be assumed that females were assessing the incubation properties of a mound when they dug, probed and scratched in a mound during the non-laying visits which preceded egg-laying visits, and were interspersed among these visits. However they may have been equally concerned with habituating the male to their company (chapter 3), preventing rival females from visiting (to be considered in chapter 5), searching for the best location in the mound for egg-laying, having already chosen the mound, or supplementing the male's own maintenance effort

in an attempt to ensure mound quality. It is therefore possible that assessment from a distance is the most important phase in the choice process, with direct inspection merely confirming the choice. To the extent that direct inspection provides additional, more accurate information, it might influence the size of the eggs laid by the female (Göth, 2007), rather than whether or not she lays at all.

The degree to which female choice is influenced by male cues, resource cues and male-resource cues is difficult to determine, as high-quality males often control high-quality resources. Experimental manipulation, however, is sometimes possible in the field. For example in pied flycatchers, *Ficedula hypoleuca*, territory quality has been distinguished from male arrival time in the breeding grounds by ensuring that, as each male arrives, there is only one randomly-selected territory available (Alatalo et al., 1986; Sirkia and Laaksonen, 2009). An alternative approach is to manipulate the quality of nest boxes after males have settled but before females arrive (Slagsvold, 1986). In some cases, field observation allows discrimination non-experimentally. For example, the influence of male size has been assessed while holding nesting burrow location (elevation) constant in fiddler crabs, *Uca pugilator*, by measuring male mating success only in the context of zones with similar elevations, hence taking advantage of the gradual increase in elevation from shoreline to the rear of a beach (Christy, 1983). In other cases, however, the researcher must attempt to distinguish the influence of males and resources statistically, as for example in red bishops, *Euplectes orix* (Friedl and Klump, 2000) or puku and topi antelopes, *Kobus vardoni* and *Damaliscus lunatus* (Balmford et al., 1992).

There are limited opportunities to experimentally separate the influence of males and resources in brush-turkeys. Provision of artificial mounds would be extremely difficult, given the size of mounds, and adjustments to mound quality would be promptly rectified by the male owner, as evidenced by the male owner's response to mound excavation (D Wells personal observation). It would be possible to remove brush-turkey males from their mounds and observe the impact on female visitation, but such manipulation is unlikely to reveal much about female choice in a natural setting, particularly as breeding females prefer to visit while the male is present (chapter 3). If the male has been receiving female visitors, his sudden disappearance would be a highly unusual event, unless immediately succeeded by a replacement male. The replacement male, selected on phenotypic criteria to contrast with the original owner, and captured and transported from elsewhere in the study site, would probably flee on release, and eventually be succeeded after some elapsed time by a different male of unknown phenotype. Furthermore, relevant phenotypic criteria are largely unknown, as female choice of male phenotypes has hardly been studied at all in this species. Given these difficulties, no attempt was made to decouple male from mound experimentally, and I relied instead on

statistical methods to attempt to disentangle the influence of male cues, mound cues and male-mound cues on female brush-turkey choice. This approach may be particularly appropriate, given the likely importance of male-mound cues, and hence the limited utility of examining female choice of males in isolation from mounds, or vice-versa.

If the objective is to distinguish the influence of male, mound and male-mound cues, and a female is at least initially making assessments from a distance, it is important to consider which cues are more available to her from that distance. With respect to the male, she can observe various physical attributes such as overall body size and wattle size. If able to individually identify the male, she probably knows something about his past mound-owning activities, and his current position in the dominance hierarchy. She can also observe how aggressive he is. An assessment of parasite load or of olfactory cues, on the other hand, would require a mound visit in order to observe the male from close quarters. With respect to the mound, relevant information is much more limited. She knows its location and size, but direct information about its quality as an incubator (temperature, temperature stability, moisture level, degree of compaction) can only be obtained by physically accessing the mound. These seem to be the key pieces of information for incubation success (Seymour, 1985), and none of them is readily apparent.

By concentrating on the male and the mound in isolation, however, we have potentially neglected male-mound cues. From a distance, females can also observe the time spent by a male in attendance at his mound, his maintenance effort on the mound, how long he has owned the mound, how securely he owns it and whether he constructed it, refurbished it, or acquired it by force from another male. The answer to our question therefore appears to be that the female has least information about the mound, more about the male, but most about the male-mound relationship, i.e. the male's behavior in relation to the mound. As the mound is an essential reproductive resource for the female, and this is the element she knows least about, it is likely that she will focus on those aspects of the male-mound relationship which indirectly indicate mound quality. Of these, the most likely candidate is the degree to which the male maintains the mound. Male maintenance effort has, therefore, been measured with considerable care in this study, to ensure that genuine effort is distinguished from window-dressing. Where males were aware of a female's presence, a sudden upsurge in maintenance effort was effectively discounted if there were frequent interruptions to peck at the substrate or to observe the approach of the female (see chapter 1 for recording rules).

A simple causal model can identify the key influences on female choice (Fig. 1). Female choice is operationally defined as the number of copulations received by a male or

alternatively the number of eggs laid in his mound. These are the most appropriate surrogates of choice, as they are directly linked to reproductive success and they do not occur on every visit. It has been plausibly argued that egg size is also important as an indicator of female choice (Göth, 2007), but the required excavations would have been prohibitively time-consuming for this project. Exerting a direct influence on female choice are a set of influences which will henceforth be referred to as *fundamental influences*, namely male, mound and male-mound cues, together with possible climatic influences such as rainfall or time of breeding season (early or late). Given that females appear to assess the mound before copulating and especially before laying eggs, and this assessment probably influences female choice, I also recognize female visiting behavior as a *precursor to female choice*. Relevant visiting behavior includes the degree to which females dig, probe and scratch in the mound (henceforth referred to as *female mound activity*), the duration of female visits to the mound and the number of females visiting. Fundamental influences exert their effect on female choice directly and also indirectly via precursors to female choice.

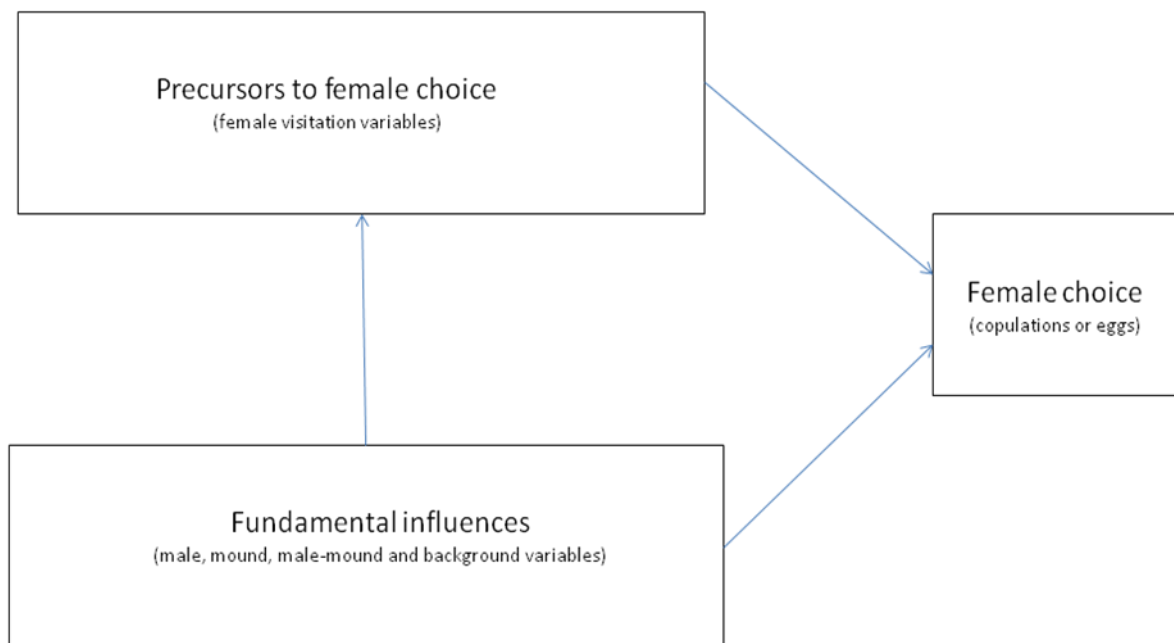
Figure 1 A model of causal influences on female choice. Fundamental influences have both a direct effect on female choice and an indirect effect via female visiting behavior, which functions as a precursor to female choice.

Male aggression toward females is an especially strong confounding influence when attempting to analyze female choice in this species. Males use sexual harassment as a

means of obtaining copulations (chapter 1). As a response to female mound activity, especially during egg-laying visits, males stand close to the female and periodically deliver pecks to her body (henceforth referred to as *up-close aggression*, chapter 2). It is therefore likely that any association between sexual harassment and copulation reflects coercion rather than female choice, and any association between up-close aggression and egg-laying or between up-close aggression and female mound activity does not reflect female choice of aggressive males. Male aggression toward females has, therefore, been excluded from the analysis. It is nevertheless important to ask whether females do prefer aggressive males. One precursor to female choice, namely the number of females visiting, is less open to these difficulties, and has been used to assess female preference for aggressive males.

Given that female choice may rely on a range of cues, the following questions are posed:

1. What is the relative importance of male cues, mound cues and male-mound cues in brush-turkey female choice?
2. For each of these categories, which specific variables are most influential?
3. Are females able to make their assessments from a distance?
4. Do females choose more aggressive males?

METHODS

Field studies were conducted on a free-living, individually colour-banded brush-turkey population in the town of Pearl Beach in New South Wales, Australia (33.54°S, 151.30°E). Observations were recorded remotely by five Sony HDR-SR7 video cameras mounted at active mounds and programmed to run daily for 3.5 hours from first light. Although males were often present at their mounds outside these hours, my observations confirm a previous finding (Jones, 1987) that female visits overwhelmingly occur early in the morning. With some exceptions due to access difficulties or extreme weather, each mound was observed for seven consecutive mornings. Data presented here is drawn from the second half of the 2008-9 breeding season and the first half of the 2009-10 season. Each unique combination of male, mound and season was defined as a data point, except that a male simultaneously maintaining two mounds was treated as a single data point. This definition yielded a total of 30 data points corresponding to 21 distinct males.

Only pre-defined behavioural events and states were recorded. For females, these were eggs laid, copulations, and mound activity; for males they were attendance at the mound, mound maintenance and attempts to entice females onto the mound. Recording

accuracy was to the nearest second during female visits and during pre- and post-visit periods, defined as periods of up to four minutes prior to and subsequent to the visit. Male enticement (including maintenance) was often most intense during pre- and post-visit periods, and experience showed that four minutes was almost always long enough to capture this behaviour. See chapter 1 for details of how behavioural states were distinguished from each other, how visits were defined and how females were individually identified. When recording male attendance and male maintenance effort outside of female pre-visit, visit and post-visit periods, timings were rounded down to the nearest minute.

To ensure fair comparison between data points, all behavior was expressed relative to the opportunity for it to occur and be recorded. For example, male maintenance during female visits was expressed relative to the total duration of visit, pre-visit and post-visit periods, while male maintenance outside of female visit periods was expressed relative to observation duration less visit, pre-visit and post-visit durations. Female visits were highly variable in duration. So too were pre- and post-visit periods, being limited by how long the male was present prior to a visit, whether the visit coincided with another visit or followed on immediately after it, and how close visit commencement was to the commencement of filming. Female behavior, in contrast, was always expressed relative to observation time as females could potentially visit at any time.

Male attendance at the mound was measured in the first instance as the elapsed time during which the male was in camera shot or his presence could be inferred from mound material being moved by scratching or raking activity. This understated his presence, however, as a male who was not detectable on this basis was nevertheless often close to the mound. Males were treated as truly absent only for the elapsed time until their first arrival in the morning and for out-of-shot periods of 17 minutes or longer. These two measures were found to be good predictors of true absence when observations were made from hides. Two mounds under the common ownership of a single male were observed from hides for the first three hours of daylight every day, with only minor exceptions, from 25 Nov 2007 to 9 Jan 2008. True absence was recorded when the male owner could no longer be observed, even when the observer left the hide and searched for him within sight of both mounds. A surrogate for true absence was calculated as any opening period of absence plus absences n minutes or longer, where n could be any whole number from 8 to 20 inclusive. It was found that the best surrogate was any opening absence plus absences 17 minutes or longer.

Knowledge of a male's mound-owning experience was incomplete because the pre-study period was unknown and males apparently new to ownership may have had experience in

the prior season at mounds not immediately discovered or only active for part of the season. One year was added to all elapsed times to reflect this unknown.

Steel walk-in traps were regularly used to capture un-banded adults, who were then measured, banded and released. Capture and banding occurred during the breeding season, by which time birds which hatched in the previous season were 6-18 months old. On the assumption that birds were on average captured during the second breeding season after hatching, two years were added to the capture date to derive estimated age. Twelve standardized site transects were performed between 6th and 31st December 2007, the observer moving at a steady pace in order to minimize the chance of double-counting un-banded individuals. The transect route included all streets in Pearl Beach in a pre-specified sequence. Further transects were performed in subsequent seasons to confirm that the geographical distribution of females over the study site did not vary substantially by season. Locations of mounds and other sites of interest were established using a GPS receiver (GPS 76, Garmin).The variable *potential female visitors* represented the mean distance between each mound and the nearest 30% of sightings of females during transects of the study site. Previous work has established that females do not visit mounds substantially distant from each other, the mean distance between mounds visited by the same female being 202.7 metres (chapter 3). Mounds very distant from females are therefore probably irrelevant when estimating potential female visitors. The nearest 30% of sightings was chosen as a reasonable estimate of potential female visitors because the mean of this value across all mounds was 228.61 metres, close to 202.7 metres.

Measurements of male weight, head-bill length, wing length, tarsus length and tail length were taken during capture. Because extension of the male wattle is under voluntary control, and is at its maximum when females are actually or potentially present, measurements were made on the basis of individual video frames during or just prior to female visits. Two male images were selected, one lateral and one anterior, both at the same location on the mound. Using Image J software, the following measurements were recorded: head bill length and wattle length (both lateral view); wattle area (anterior view, using the Image J polygon tool). To minimize the impact of variable distances from the camera and variable camera angles at different mounds, wattle length and area were expressed relative to head bill length per the lateral view, adjusted to actual head bill length obtained from captive measurement (Fig. 2). Each measurement was taken on three occasions and then averaged, and the process was repeated for a second image. In the event of substantial discrepancies, new images were obtained.

Figure 2 Lateral and anterior images of a male at approximately the same location on a mound. Head bill length and wattle length have been superimposed on the lateral image, and wattle area on the anterior image. Both images are enlarged and cropped versions of individual video frames.

Mound temperature was measured using a probe thermometer (HD-500, HLP Controls) inserted to a depth of 35 cm at four locations each approximately 10 cm inside the crater-like rim which is characteristic of active mounds. To minimize the risk of damage to eggs, the insertion depth was almost certainly shallower than the depth at which eggs had been laid. Although the temperature in the immediate proximity of eggs was therefore probably higher, significant distortion was unlikely as all mounds were subject to the same treatment. Mound moisture level was measured using a moisture probe (MP 406 with MPM 160, ICT International) inserted to a depth of 20 cm.

Rainfall was recorded by a Pearl Beach resident at 9 AM daily. Where days were missing from the record, the first subsequent measure was allocated to individual days using Bureau of Meteorology (Narara station) records as a basis for apportionment. For relevant dates, the two rainfall records were strongly correlated (r = 0.916, P < 0.001). Air temperature was as reported by the Bureau of Meteorology.

Variable reduction

As explained above, fundamental influences on female choice were assumed to be male, mound and male-mound cues, together with possible climatic influences such as rainfall or time of season (early or late). Fundamental variables used in the analysis represent only a sub-set of those available, the number being reduced, usually by combining variables based on manual inspection of correlation tables. For example, the rate of male maintenance during female visits and immediately pre-visit and immediately post-visit were combined by averaging because they were strongly correlated and were easy to interpret when combined. The number of variables was reduced to no more than six for each of the categories of interest (male, mound, male-mound and climatic, see Table 1). For variables characterizing female behavior during visits and hence thought to represent precursors to female choice (Table 2), variable reduction was achieved by principal components analysis.

Table 1 Fundamental variables, being possible cues for female choice, grouped according to whether they are attributes of males, mounds, or male-mound relationships, or represent climatic influences. These variables are a sub-set of those available, the number being reduced by manual inspection of correlation tables. Male aggression is also included as a potential cue for female choice.

Variable	Explanation	How expressed
Male variables		
Overall body size	weight (gm.), head bill length (mm.), wing length (mm.), tarsus length (mm.), tail length (mm.)	each measure converted to a Z score; find the mean of Z scores
Relative wattle size	wattle length and wattle area (per Image J analysis of video frame)	Each measure as ratio of known head bill length; find the mean of the ratios
Age	Elapsed time from the earliest of banding date or first sighting, plus two years	Absolute value
Mound variables		
Mound temperature mean	Between two and five samples of temperature on separate observation days from each of four locations in the mound	Mean of the samples
Mound temperature standard deviation	As for temperature mean	Standard deviation of the samples
Mound moisture level mean	Between two and five samples of moisture level on separate observation days from each of four locations in the mound	Mean of the samples
Mound moisture level standard deviation	As for moisture level mean	Standard deviation of the samples
Potential female visitors	Distance between mound and locations at which females were sighted during full transects of the study site	Mean distance in metres to closest 30% of sightings
Male-mound variables		
Visit-related male maintenance	Duration of male digging, scratching and probing during female visits including periods immediately pre- and post-visit, when maintenance is often most intense	As ratio of male presence during female visits, including pre- and post-visit periods
Visit-related male enticement	Duration of male ground-peck, motionless and flattened during female visits including periods immediately pre- and post-visit	As ratio of male presence during female visits, including pre- and post-visit periods
Male attendance plus maintenance outside of female visits	Elapsed time male was present overall at the mound plus duration of male digging, scratching and probing outside of female visit, pre-visit and post-visit periods	Attendance as ratio of observation time; maintenance as ratio of observation time not including visit, pre-visit and post-visit periods; find the mean of the two ratios
Mound-owning experience	Elapsed time from the date focal male was first known to own a mound, plus one year	Absolute value
Number of mounds maintained	Number of mounds actively and simultaneously maintained by a male during the observation period	Absolute value (1 or 2)
Climatic variables		
Time of season	First vs. second half of season	First = 1
Rainfall	Daily rainfall (mm.) to 9am during observation period and 5 days prior	Mean value
Also relevant		
Attempted sexual coercion	Frequency of male charge, barge, attempted copulation and 'toward'; this form of aggression is associated with copulations (chapter 1)	As ratio of male presence during female visits, excluding pre- and post-visit periods
Up-close aggression	Duration of 'hover' and frequency of 'bird-peck'; this form of aggression is associated with egg-laying visits (chapter 2)	As ratio of male presence during female visits, excluding pre- and post-visit periods

Table 2 Precursor variables, being aspects of female visitation which are thought to foreshadow female choice. All are expressed as a ratio of observation duration. Using principal components analysis, these variables were subsequently combined into a single precursor variable.

Variable	Explanation
Female mound activity	Duration of female digging, scratching and probing during visits
Visit time-in	Duration of visit excluding periods of temporary absence and periods when the female withdrew to the side of the mound
Visit elapsed time	All-inclusive duration of visit
Number of female visitors	Number of distinct females visiting, assessed on a per day basis
Female in-shot	Duration of female being in camera shot

The relative importance of variable categories and the most influential variables in each category

These questions were taken together and answered in reverse order. Fundamental variables were assumed to have a causal influence on female choice both directly and indirectly via female precursors to choice (see Fig. 1). Each of these causal influences was investigated by running linear regressions to identify the best predictors of female choice or precursors to choice. Prior to analysis, test assumptions were verified in all cases. The procedure used was as follows.

To answer Question 2 on the most influential variables in each category:

1. A simultaneous regression was run for each category of fundamental variables (male, mound, male-mound and climatic), assessing the relevant variables as predictors of female choice and precursors to choice
2. As a measure of relative predictive power, variables were ranked within each category in descending order by absolute Beta value (standardized correlation coefficient)

To answer Question 1 on the relative importance of categories:

3. The top-ranking variables from each category were selected; there being only 30 data points, a total of no more than six variables were allowed to go forward to Step 4, in conformity with the principle of no more than one variable for every five data points
4. Using the reduced list from Step 3, four sequential regressions were run, each time with a different category (as represented by the top-ranking variable for that category) in the last position
5. As a measure of relative ability to predict female choice or precursors to choice, categories were ranked in descending order by change in R^2 (variance explained) when in the last position; this effectively partitioned the variance so that change in R^2 for the variable in the last position represented the variance solely attributable to that variable, after allocating any shared variance to other variables.

Residuals were not always normally distributed when the objective was to predict the frequency of copulations and (especially) eggs, these being relatively infrequent events. Step 1 and Step 2 of the above procedure were therefore repeated by converting copulation frequency and egg frequency into dichotomous yes/no variables and using multivariate analysis of variance (MANOVA) to rank fundamental variables within categories. Although the conventions of MANOVA assume that the distinction between dichotomous groups has a predictive role, the mathematics is indifferent to this convention, and is equally valid if one assumes the reverse. For example, if males receiving eggs are characterized, say, by larger body size and wattle size than are males who do not receive eggs, it is valid to treat the presence of eggs as a predictor of body size and wattle size, or equally (as here) to treat body size and wattle size as predictors of eggs. The ranking of variables by this means was then compared to the ranking derived from simultaneous linear regression.

Mound temperatures and moisture levels were only collected for the 2009-10 season, necessitating the use of mean values for the 2008-9 data. This removed all mound temperature and moisture variance in the 2008-9 values, and may have resulted in an under-estimate of the relative influence of mound variables. In addition to performing Steps 1 to 5 for the complete dataset, therefore, these steps were separately repeated for the 2009-10 data alone. Procedures were modified to allow for a reduction in the number of data points from 30 to 16.

In each of the standard linear regressions, checks were carried out for normal distribution of residuals, multicollinearity problems and outliers. Variables found to be multicollinear in the linear regressions were not co-included in the MANOVAs. All regressions and MANOVAs were conducted in SPSS. Regression techniques equivalent to those outlined above were also used to assess precursor variables as predictors of female choice (see Fig. 1).

Prediction and causality
Where fundamental variables or variable categories were strong predictors of female choice, specific evidence was sought on the direction of causality (see Discussion).

Are females in a position to watch from a distance?
Where possible, females in the vicinity of the mound were directly observed, and sighting frequencies compared with visit frequencies. Females were directly observed either by an observer in a hide or by video observation of an open space adjacent to a specific mound. In the former case, two mounds under the common ownership of a single male were observed from hides for the first three hours of daylight every day, with only minor exceptions, from 25 Nov 2007 to 9 Jan 2008. While in either hide, each situated

approximately 20 metres from the relevant mound, observers did not appear to affect behaviour, as evidenced by the fact that male-female interactions on the mound were in general indistinguishable from those observed by video at other mounds. Observers left the hide only to confirm that the male had left the vicinity, or when he moved between mounds.

Those females who visited while the male was absent often seemed to arrive soon after he departed, suggesting that they had been present nearby, awaiting his departure. For the mean duration of male absence, the number of female visit commencements was compared to the number expected by chance, both variables being expressed relative to the opportunity to visit in the male's absence, i.e. the number of males still absent. A Kolmogorov-Smirnov test was used to assess degree of deviation between an expected uniform distribution and the distribution of female arrivals. Test assumptions were verified prior to analysis.

Do females choose more aggressive males?
Any association between male aggression and either copulation or egg-laying is likely to reflect attempted sexual coercion in the former case or up-close aggression in response to egg-laying and female mound activity in the latter (chapters 1 and 2). Whether it also reflects female preference for aggressive males was examined by focusing on the number of female visitors to a mound. Unlike other precursor variables, which are primarily ways of measuring the duration of female visits to the mound and are likely to be positively associated with the opportunity for male aggression, this variable may capture female interest in a less problematic way. If a male receives female visitors on average for two hours per day, he can afford to be aggressive at a higher rate per minute of female visit time than a male who only receives visitors for thirty minutes per day. If the former male has on average three visitors during the two-hour period, however, there is no obvious reason to suppose that his aggression rate will be higher than if he received on average one visitor during the same period. Attempted sexual coercion was therefore assessed as a predictor of the number of females visiting, while controlling for the duration of female presence at the mound (a key stimulant of attempted sexual coercion) and the most influential variables for each of the four fundamental variable categories. Using similar logic, up-close aggression was assessed as a predictor of the number of females visiting, while controlling for the duration of female mound activity (a key stimulant of up-close aggression) and the most influential variables for each of the four fundamental variable categories. The regression technique employed for this purpose was sequential regression, with test assumptions verified prior to analysis.

RESULTS

Variable reduction

Principal components analysis yielded very clear results when applied to the five precursor variables representing female visitation thought to be preliminary to female choice, a single factor accounting for 90.8% of the variance. Factor loadings were applied to the data for each data point in order to generate a single precursor variable (*visit time-in* 0.993, *female in-shot* 0.991, *visit elapsed time* 0.976, *female mound activity* 0.959, *number of female visitors* 0.835; Eigen value 4.538; see Table 2 for variable definitions). This composite variable can be interpreted as the degree of female interest in males and/or mounds, but will continue to be referred to, albeit in the singular, as **the** *precursor variable*.

Principle components analysis was less successful when applied to fundamental variables. Six factors were extracted with Eigen values in excess of one, the first factor explaining only 15.5% of the variance. The extracted factors also made little conceptual sense. An alternative technique was therefore used, namely manual scrutiny of a correlation table prepared for each category (male, mound, male-mound and climatic). A correlation table was prepared for each category, ensuring that all variables for that category were included. Where variables were strongly correlated (Pearson correlation coefficient in excess of 0.3) and the correlation was significant, the possibility of combining variables was considered. This action was taken only if the combined variable could be easily interpreted and it did not defeat any of the aims of the study, e.g. some behaviours correlated with certain measures of male aggression, but were not combined because aggression was excluded from the analysis of female choice. Almost all reduction was achieved by combining rather than culling variables. The fundamental variables remaining after this reduction process are listed in Table 1.

The relative importance of male cues, mound cues and male-mound cues in brush-turkey female choice

For the full regression sample (n = 30), the male-mound category was the best predictor of copulations, eggs laid and the precursor variable, significantly so in the third case, and close to significant in the remaining two cases (Table 3). The male-mound category was also the best predictor for the 2009-10 sample (n = 16) in two of three tests, the exception being the prediction of eggs. In contrast, there was no pattern in the rank assigned to other categories using either sample. Use of the 2009-10 data with comprehensive mound data did not elevate the rank of the mound category, after taking into account the non-inclusion of the climatic category. Exclusion of the climatic category from the 2009-10 analysis was necessitated by a small sample size, but probably resulted in little distortion, as all data points in the smaller sample shared the same time of season, and the only remaining climatic variable (rainfall) recorded a low Beta value in simultaneous regression.

For each category, which specific variables are most influential?

For the full regression sample (n = 30), the highest ranking variable for the male-mound category was *Male attendance plus maintenance outside of female visits* (Table 4). This variable was also the best predictor for the 2009-10 sample and for the full sample using MANOVA. These results were statistically significant in six of eight tests. Although residuals were not always normally distributed when predicting copulations or eggs, the multiple regression results do therefore appear to be reliable, as they are consistent with the MANOVA results. Relative wattle size was consistently the highest-ranking variable in the male category for the full regression sample, but this was not true for the 2009-10 sample or for MANOVA. No single variable or pair of variables consistently ranked highest in other categories.

Table 3 Fundamental variable categories as predictors of copulations (A), eggs (B) and the precursor variable (C). For the full sample (n = 30) variable categories are ranked in descending order as predictors of frequency of copulations, frequency of eggs and value of the precursor variable, using change in R Squared (variance explained) as the ranking criterion. Variables are also ranked by change in R Squared for the 2009-10 sample only (with comprehensive mound data).

A As predictors of copulations				
	Sequential linear regression full sample (n = 30)			Sequential linear regression 2009-10 (n = 16)
Category	Rank by ΔR^2	ΔR^2	P	Rank by ΔR^2
Male-mound	1	.134	.100	1
Mound	2	.080	.094	3
Climatic	3	.005	.671	n/a
Male	4	.003	.747	2

B As predictors of eggs laid				
	Sequential linear regression full sample (n = 30)			Sequential linear regression 2009-10 (n = 16)
Category	Rank by ΔR^2	ΔR^2	P	Rank by ΔR^2
Male-mound	1	.169	.072	3
Male	2	.043	.483	1
Mound	3	.000	.915	2
Climatic	4	000	.935	n/a

C As predictors of precursor variable				
	Sequential linear regression full sample (n = 30)			Sequential linear regression 2009-10 (n = 16)
Category	Rank by ΔR^2	ΔR^2	p	Rank by ΔR^2
Male-mound	1	.233*	.010	1*
Climatic	2	.075	.069	n/a
Male	3	.046	.336	2
Mound	4	.015	.396	3

*Statistically significant value

Table 4 Fundamental variables as predictors of copulations (A), eggs (B) and the precursor variable (C). For the full sample (n = 30) fundamental variables are ranked in descending order within category as predictors of frequency of copulations, frequency of eggs and value of the precursor variable, using absolute Beta value as the ranking criterion. One simultaneous regression was run for each category. Variables are also ranked by Beta for the 2009-10 sample only (with comprehensive mound data) and by Partial Eta Squared for the full sample using MANOVA.

A As predictors of copulations					
	Linear regression full sample (n = 30)			Linear regression 2009-10 (n = 16)	MANOVA full sample (n = 30)
Variable	Rank by β within category	β	P	Rank by β within category	Rank by Partial Eta Squared within category
Male-mound category					
Male attendance plus maintenance outside of female visits	1	.317	.087	1*	1*
Mound-owning experience	2	.296	.097	2	3
No of mounds maintained	3	.174	.328	n/a	2
Male category					
Relative wattle size	1	.302	.109	3†	1*
Overall body size	2	-.241	.197	1	2*
Age	3	.138	.457	2†	3†
Mound category					
Mound temperature mean	1	.403	.065	5	3
Mound moisture level mean	2	-.184	.408	3	1
Potential visitors	3	.184	.397	4	4
Mound temperature std. dev.	4	.067	.791	2	2†
Mound moisture level std. dev.	5	.002	.993	1	5†
Climatic category					
Time of season	1	-.296	.126	n/a	2*
Rainfall	2	-.265	.169	1	1*

B As predictors of eggs laid					
	Linear regression full sample (n = 30)			Linear regression 2009-10 (n = 16)	MANOVA full sample (n = 30)
Variable	Rank by β within category	β	P	Rank by β within category	Rank by Partial Eta Squared within category
Male-mound category					
Male attendance plus maintenance outside of female visits	1	.466*	.015	1	1*
Mound-owning experience	3	-.225	.206	2	4
Male maintenance during female visits	4	-.204	.250	3	3
No of mounds maintained	5	.165	.350	n/a	2*
Male category					
Relative wattle size	1	.297	.121	3	1
Overall body size	2	-.272	.152	1	2
Age	3	-.169	.368	2	3
Mound category					
Mound moisture level std. dev.	1	-.274	.273	1	4
Potential visitors	2	.172	.441	4	5
Mound moisture level mean	3	-.153	.468	2	3
Mound temperature mean	4	.075	.787	3	2
Mound temperature std. dev.	5	.018	.943	5†	1†
Climatic category					
Rainfall	1	-.602*	.005	1	1
Time of season	2	.100	.610	n/a	2†

C As predictors of precursor variable			
	Linear regression full sample (n = 30)		Linear regression 2009-10 (n = 16)
Variable	Rank by β within category	β	p	Rank by β within category
Male-mound category				
Male attendance plus maintenance outside of female visits	1	.608*	.001	1*
No of mounds maintained	4	.074	.648	n/a
Mound-owning experience	5	-.041	.796	2
Male category				
Relative wattle size	1	.427*	.022	3
Overall body size	2	-.292	.106	2
Age	3	-.135	.446	1
Mound category				
Mound temperature mean	1	.275	.173	2
Mound moisture level mean	2	-.188	.339	1
Potential visitors	3	.104	.609	4
Mound temperature std. dev.	4	-.047	.819	5
Mound moisture level std. dev.		n/a	n/a	3
Climatic category				
Time of season	1	-.502*	.015	n/a
Rainfall	2	.116	.554	1

*Statistically significant value

†Direction of the relationship (positive or negative β value) has changed

The precursor variable as a predictor of copulations and eggs
For completeness, the precursor variable was assessed as a predictor for each of the female choice variables, namely copulations and eggs. In both cases, the association was positive and statistically significant (for the full sample, $\beta = 0.548$, $P = 0.002$ for copulations and $\beta = 0.725$, $P < 0.001$ for eggs).

Are females in a position to watch from a distance?
Observation of females in the vicinity of mounds suggested that the ratio of off-mound to on-mound females was somewhere between 0.4 and in excess of 4. The lower ratio was based on sightings of individually identifiable females from a hide, and was almost certainly an under-estimate because of identification difficulties in natural vegetation. The higher ratio resulted from video observation of an open space adjacent to a mound, and was almost certainly an over-estimate because it was impossible to determine how many sightings were of the same individual.

An indirect approach was therefore also employed. The minority of females who visited while the male was absent often seemed to arrive soon after he departed, suggesting that they had been present nearby, awaiting his departure. When assessed over the mean duration of male absence (41 minutes), the distribution of female visit commencements was skewed to the left during the first 20 minutes (Fig. 3). The distribution was significantly different from an expected uniform distribution (Kolmogorov-Smirnov test, $P = 0.021$). Both actual and expected distributions were expressed relative to the opportunity to visit in the male's absence, i.e. the number of males still absent.

Do females choose more aggressive males?
For the full regression sample (n = 30), male attempted sexual coercion had zero effect as a predictor of the number of female visitors, after controlling for female time at the mound and the most influential variables in each of the fundamental categories ($\Delta R^2 = 0.00$, $P = 0.982$). For the same sample, male up-close aggression was a slightly better predictor of the number of female visitors, after controlling for female mound activity and the most influential variables in each of the fundamental categories ($\Delta R^2 = 0.018$). The relationship between up-close aggression and the number of female visitors was positive ($\beta = 0.162$) but far from significant ($P = 0.324$). No evidence was therefore found that females chose more aggressive males.
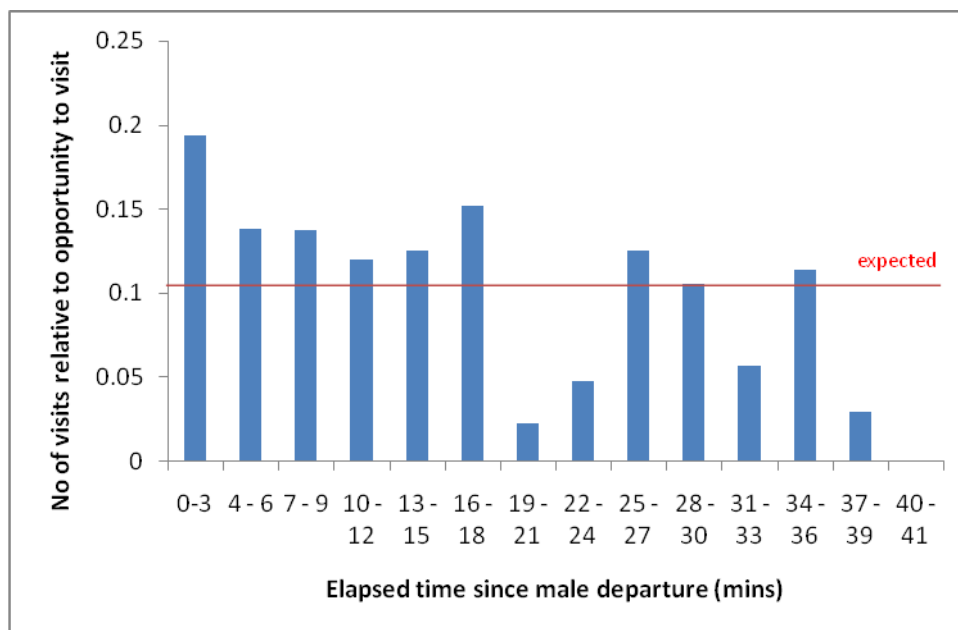
Figure 3 Frequency distribution of 65 female visit commencements occurring within the mean period of male absence (41 minutes), where both the male's prior departure time and subsequent re-arrival time were known. As these are female visits in the male's absence, the number of visit commencements is expressed relative to the opportunity to visit in the male's absence, i.e. the number of males still absent. The distribution is significantly different from a uniform distribution of 0.11 visit commencements relative to the opportunity to visit (Kolmogorov-Smirnov test, P = 0.021). Females were able to visit soon after the male's departure apparently because they had been nearby, observing the male.

DISCUSSION

Where males defend resources which are essential for female reproduction, a perennial question is whether choosy females place more reliance on male cues or resource cues. My analysis suggests that brush-turkey females do not principally rely on either, the principal cue for female choice being the male-resource relationship. As hypothesized, the degree to which the male maintains his mound is a key predictor of female choice. Given that the maintenance in question occurs when females are not visiting, these findings are consistent with the view that remote assessment is critical for female choice in this species. Also consistent with this view is evidence that females are able to observe the male while he is present at the mound, in some cases without his knowledge. Females probably employ remote assessment because it minimizes the costs of male aggression. No evidence was found that females prefer more aggressive males.

Male-resource cues provide indirect mate choice information
While controlling for other categories, the male-mound category was consistently the best predictor of copulations received by a male, the number of eggs laid in his mound, and female visitation as represented by the precursor variable (Table 3). It is important to stress that the male-mound category does not represent a combination of male and mound elements which are independently assessed by females, but can also be jointly assessed, with non-additive results. Such independent and combined assessment was found to occur when male size, nest size and male size x nest size interaction were assessed as cues for female choice in sand gobies *Pomatoschistus minutus*. In isolation, male size and nest size had little influence on female choice, but together they had a significant impact (Lehtonen et al., 2007). This approach is unlikely to provide a model for the brush-turkey case, as information about the mound is initially much less available to the female than information about the male. Rather, what appears to be happening is that females are using observations of the male-mound relationship to provide indirect information about both the male and the mound, and in particular the latter.

The male-mound relationship consists in the male's behavior toward the mound. If he vigorously maintains the mound, for example, he is thought to be enhancing heat production by thermophylic bacteria and fungi, reducing the likelihood of compaction, and making oxygen more available to eggs in the mound (Jones et al., 1995; Seymour, 1985, 1995). He is also providing evidence of vigour, and hence of his own quality. The male-mound relationship is a cue which is more available to the female and possibly more reliable than alternative cues which provide independent information about male and

mound. The male-resource relationship may also play a role in the sand goby case: Lehtonen et al. suggest that female rejection of small gobies in large nests, for example, may reflect the likelihood that the former are unable to perform the amount of egg-fanning required by the latter (Lehtonen et al., 2007).

Specific male-resource cues and evidence of causality

If brush-turkey females principally use male-mound cues, it is important to consider which specific male-mound variables are the best predictors of female choice. While controlling for other variables in the male-mound category, the highest rank was consistently occupied by the variable *Male attendance plus maintenance outside of female visits* (see Table 4). In order to make inferences about female choice, however, it is necessary to identify possible causal mechanisms and to ensure that, as far as possible, all common causes have been included in the model (Keith, 2006). There is no temporal sequence which can be used as evidence of causality, and as is frequently the case when behavior by one individual is thought to influence the behavior of another, the relationship may be reciprocal (Takahashi et al., 2008). Females may respond to high attendance and maintenance rates by visiting more, or conversely, males may respond to high visit rates by increasing their attendance and maintenance effort. It seems likely, however, that the former influence is primary.

Firstly, the hypothesis that female visits encourage males to intensify their maintenance and attendance effort assumes that this effort is effective in attracting females, i.e. that causality must also be operating in reverse. If this were not the case, there would be no advantage to males in responding in this fashion. Secondly, if enhanced maintenance effort is principally a male's response to his own success, one would expect a strong positive association between male success and male maintenance while the female is present, i.e. during female visit, pre-visit and post-visit periods. Maintenance during these periods, however, was such a poor predictor of female choice that in most cases Beta was negative. A plausible explanation of this outcome is that females discount maintenance effort while they are present, and treat maintenance effort while they are not explicitly present as a more 'honest' indicator of the male's behavior and hence of the quality of the mound. If this interpretation is correct, eavesdropping in this case is focused, not on male-male encounters (Wong and Candolin, 2005), but on male parental investment. At the commencement of the breeding season, moreover, there is inevitably a period when males have not yet succeeded in obtaining copulations or eggs, but are nevertheless investing heavily in both maintenance and attendance. Such effort cannot be explained by previous success and is the prelude to the most active part of the season (83% of copulations and 63% of eggs occurred in the first half of the season). It is therefore likely that females are responding to male maintenance more than males are responding to

female visits. Meanwhile, male attendance at the mound may function as a proxy for maintenance, as males who are frequently present at the mound are more likely to maintain the mound, producing a strong correlation between these two variables and necessitating their combination in a single variable.

Having established that a variable assessable from a distance predicts female choice, and identified a causal mechanism which might explain its predictive force, it is further necessary to consider whether, as far as possible, all common causes have been included in the model. Without this assurance, we may be missing the key causal relationship. Male-mound variables are distilled from a list which originally included 60 variables. Male variables represent a reasonable sample of conventional morphometrics, although the inclusion of wattle and head UV reflectance (Jones and Göth, 2008) would have been a definite advantage. Many more mound variables might have been included, such as mound aspect, shade cover, surrounding vegetation etc., but these are understood to be important to incubation via the effect they have on the mound variables which have been measured, namely mound temperature mean and standard deviation and mound moisture level mean and standard deviation (Seymour, 1985). An obvious addition to mound variables is mound size, which was assessable at a distance. Unfortunately, mounds were often removed by human action after filming, and before they could be measured. An obvious addition to climatic variables is air temperature, but this was strongly correlated with time of season, and had to be omitted in order to avoid multicollinearity. It is also possible that complete categories of variables have been missed. For example, it may be that females are assessing male parasite load or using olfactory cues when visiting the mound, perhaps to exclude closely-related individuals. It could also be that influential females determine the choices of other females. The variables included, however, do seem to include the most likely influences on female choice, and therefore provide reasonable assurance that male-mound variables are indeed the most important.

The association found here between female choice and elevated maintenance implies little about causation, however, if brush-turkey females are not present to observe male maintenance and attendance outside of visit periods. Females who visited the mound during the male's absence often arrived soon after his departure, suggesting that they had observed his departure, and had therefore been present nearby. This result also indicated that males were not always aware of a female's presence in the vicinity. Direct observation suggested that the ratio of off-mound to on-mound females was somewhere between 0.4 and in excess of 4.The latter finding in particular is consistent with a study of female home ranges, which found that females generally did not visit mounds when nearby (76.4% of the time) and that the minimum average number of mounds in a

female's breeding-season home range was 7.62 (Jones, 1987). In this case comparative data must be treated with caution, as it is drawn from a Queensland non-urban population which does not always match the Pearl Beach population, but it does strongly indicate that females are able to regularly assess males and mounds from a distance, without physically accessing the mound. Male behaviours such as charges, ground-peck and maintenance are visually conspicuous and are unlikely to be missed by females in the vicinity. If she is reasonably close, a female does not even have to have the mound in her line of sight, as the sound of a male maintaining his mound is presumably sufficient. Female presence near mounds is also indicated by the tendency of visiting females to briefly depart, apparently to eject rival females in the vicinity, and by the ability of a rival female to appear on the mound at the moment that an egg-laying female commences egg-laying itself, and is therefore unable to respond to her presence. It is also indicated by the female habit of foraging in small groups, so that the visit of one individual will be observed by others, by increased male enticement effort without a subsequent visit, indicating a nearby female, and by the previous finding that, depending on their visiting strategy, females either seek out the male's absence or seek out his presence (chapter 3), suggesting that they are observing until the appropriate opportunity arises.

An obvious objection to the argument presented here is that female choice correlates with male maintenance and attendance because there is a common cause, namely the quality of the mound. Females are choosing high quality mounds, it could be argued, and these mounds are owned by high-maintaining males. If these were the causal links, however, one would expect male maintenance and attendance to become poor predictors of female choice once one controlled for mound quality. Not only are male maintenance and attendance strong predictors even when mound quality is controlled for, mound quality is itself only a modest predictor of female choice, suggesting that females are employing male-mound cues in preference to mound cues. Apparently contrary to these conclusions, a non-urban Queensland study reported that females made frequent 'sampling visits' to mounds before commencing to lay a series of eggs (Birks, 1996). The term 'visit', however, was defined to include merely being within sight of the mound for more than one minute, which is treated in the present study as remote assessment rather than visiting. More information is required to determine whether the two sets of results are actually in conflict.

Another possible objection is that females can simply visit any mound while the male is absent, thereby acquiring mound information directly, without being subject to male aggression. Females are presumably most interested in the stability of mound temperature and moisture over the long term, however, and would therefore need to make a series of visits over time. This may be more costly (and offer no advantage in

information quality) than relying on male-mound information, especially given the male's tactic of maximizing his presence during the hours of major female activity. A breeding female also needs to habituate the male to her presence and females who predominantly visited while the male was absent did not appear to be breeding (chapter 3).

Male-resource cues in other taxa
Female preference for elevated levels of mound maintenance has parallels in other species. In sand gobies *Pomatoschistus minutus*, for example, females prefer males whose parental effort (nest-fanning) is experimentally elevated by reduced oxygen levels (Lindström et al., 2006). As exclusive male care occurs in more than 60% of the fish families which exhibit parental care, and females often have the opportunity to observe males caring for eggs already deposited in their nests by other females, it is likely that male parental behavior is widely used as a cue for female choice in fish (Hale and St. Mary, 2007; Ostlund et al., 1998; Pampoulie et al., 2004). Behaviors such as fanning, anti-predator guarding, cleaning, and removing dead eggs and debris (Pampoulie et al., 2004) are equivalent to mound maintenance and attendance by male brush-turkeys, even though eggs are open to view in the former case, but not in the latter. In both cases, male behavior provides male-resource cues which are likely to be useful to choosy females.

By a similar logic, the male-resource relationship is less likely to provide cues for female choice where male birds construct nests, as there is generally no need for ongoing maintenance by the male, and females can verify nest quality by a single close inspection, as for example in baya weavers, *Ploceus philippinus* (Quader, 2005). In contrast, where male anti-predator defence is important, observation of a male bird's behavior in relation to the resource is likely to be a more reliable indicator than inspection of the resource itself, especially if time is short. Relevant male behavior could include behavior which is likely to correlate with anti-predator defence, such as territorial defence against rival males. A study of polygynous red-winged blackbirds, *Agelaius phoeniceus* found that 41% of broods were taken by predators, anti-predator defence varied among males, and defensive capability was potentially predictable by females using dominance status, epaulet size as a morphological correlate of dominance status, response to conspecific male intruders or, by implication, direct observation of male anti-predator defence (Eckert and Weatherhead, 1987). Male nest defence intensity was one of four variables found to predict nesting success, although it did so only weakly (Milks and Picman, 1994). Females who mated with extra-pair males on adjoining territories experienced reduced predation rates, probably because the extra-pair male was providing additional anti-predator defence (Gray, 1997). In a polygynous species such as red-winged blackbirds, anti-predator defence is likely to be an important influence on female choice because predators usually threaten only one nest at any one time, and the resident male (or the

male on an adjoining territory) is therefore able to provide defence to any relevant nest, regardless of the order in which females settled. In this respect, paternal feeding is a much less shareable form of care (Weatherhead, 1990). Although none of these findings directly establishes that females use resource-defence behavior as a cue for mate choice, they do indicate motive and opportunity. Such motive and opportunity is likely to apply to other bird species, nest predation being the most important cause of nesting failure among birds generally (Gray, 1997).

Male-resource cues may also appear in unexpected guises. Song rate in birds is a male attribute, but it can function as a male-resource cue if it varies according to territory quality. Female blackcaps, *Sylvia atricapilla*, prefer males with high song rates. It has been argued that song rate in this species operates partly as a signal of intrinsic male quality, but more importantly as an indication that the male controls a territory with denser vegetation and hence reduced predation risk (Hoi-Leitner, 1995).

Other variables assessable at a distance
In addition to the most influential cue for brush-turkey female choice (*male attendance plus maintenance outside of female visits*), some male morphological characters were probably also assessable at a distance from the mound. The direction of causation is less at issue here, as female behavior cannot conceivably influence male morphology in the relevant timescale. Relative wattle size was positively associated with female choice or the precursor variable in seven of eight tests (Table 4), and the association was statistically significant in two of the tests. These results are from regressions and MANOVAs while controlling only for other variables in the same male category. Relative wattle size, however, correlated strongly with a variable in a climatic category, namely time of season (r = -0.725, p < 0.001), indicating that males have larger wattles in the first half of the season. When tested using only the 2009-10 data, drawn entirely from the first half of the season and therefore effectively controlling for time of season, the association between relative wattle size and female choice was either weak or negative in all three relevant tests. There is therefore no evidence that females chose males with larger wattles. Nevertheless, wattles do seem to be sexually selected, and further research is required, preferably experimental. Meanwhile overall body size was negatively associated with female choice in all eight tests (Table 4), although the association was statistically significant in only one case. Females may therefore choose smaller males, perhaps because smaller males are less likely to inflict damage when they are aggressive. Male age was also negatively associated with female choice in seven of eight tests, although only weakly.

The number of mounds a male maintained simultaneously was an additional male-mound variable assessable from a distance. This was positively associated with female choice in

all five tests (Table 4), although the association was statistically significant in only one of the tests (only five tests were relevant as there were no two-mound males in the 2009-10 sample). Although previous research found that two-mound males received almost twice as many eggs (Jones, 1994), the association here was weaker, perhaps because the number of such males in the sample was low. All other variables assessable from a distance gave inconsistent or weak results. Notably, the proximity of a mound to potential female visitors was not only a poor predictor of female choice (all eight tests non-significant, Table 4), but the relationship, if any, was the reverse of what one might expect. The further a mound was from potential female visitors, the more likely it was to be chosen (positive association in all eight tests). This suggests that females were actively choosing which mounds to visit, and not merely visiting mounds on the basis that they were conveniently located. It also suggests that females were avoiding competition from other females.

Variables not assessable at a distance
For categories which were predominantly not assessable at a distance, mound variables ranked between second and fourth out of four categories, and only third out of four categories as predictors of the number of eggs laid in a mound (Table 3). One would expect that both mound temperature and moisture level standard deviation would be negatively associated with choice, as females would seek to avoid variation. *Mound temperature standard deviation*, however, was positively associated with female choice in three of eight tests (Table 4).*Mound moisture level standard deviation* was negatively associated with female choice in five of seven tests, and not associated at all in a sixth. None of these standard deviation results was statistically significant (Table 4).

Mound temperature mean also played a surprising role. This variable was positively associated with female choice in all eight tests (Table 4), despite the sample-wide mean being 33.7^o, somewhat above mean mound temperatures estimated by three separate studies (Jones and Göth, 2008). Given the likely underestimation of temperature when using a probe thermometer (see Methods), it therefore appears that females preferred to lay their eggs in warmer mounds. This result was consistent with a female-biased adult sex ratio (chapter 5) together with the finding that female embryos are more likely to survive in warmer mounds (Göth and Booth, 2005). For completeness, it should also be noted that *mound moisture level mean* was negatively associated with female choice in all eight tests, a result which was generally consistent with *rainfall* also being negatively associated in six of eight tests (Table 4).During artificial incubation of eggs, some chicks died from the fungal infection aspergillosis. Elevated moisture levels may present a similar risk in the field.

Males or mounds

Although male-mound information is the most important cue for female choice, its primary function, as suggested above, is probably as an indirect indicator of male or mound quality, with mound information likely to be more important because it is less available during remote assessment. This does not mean, however, that mound information is more important overall. Females restrict their need for male information by choosing to mate only at mounds, thereby ensuring that they mate only with mound-owning and hence competitive males (see chapter 1). Moreover, by mating only during the first three hours of daylight, females reinforce this effect because male owners know when to be present and subordinate males therefore have little opportunity to successfully interlope at mounds they do not own (see chapter 1). Male-male competition similarly facilitates female choice in a wide range of taxa (Candolin, 1999; Friedl and Klump, 2000). Although mounds are obviously important for female choice because of their mandatory role in incubation, and they seem more important than males during remote assessment, it is impossible to say whether they are more important overall, on the evidence available here.

Conclusion

It has been argued the brush-turkey females may make the most informed mate decisions of any vertebrate (D Jones, personal communication). As shown by the positive rather than negative association between female choice and the distance between a mound and potential female visitors, females are actively choosing, and not visiting mounds merely because they are conveniently located. Females are able to make a separate decision about each individual egg, and appear able to make detailed, daily and iterative assessments of males and mounds throughout an unusually long breeding season. Nevertheless, male aggression at incubation mounds imposes a cost on information-gathering at the mound itself, and forces females to invest heavily in remote assessment prior to visiting. Females predominantly visit only one male during an extended period, suggesting a significant restriction on the information available to them, and probably in some cases sub-optimal choice. Constrained in this way, females appear to rely mainly on male-mound cues which are assessable at a distance, such as male maintenance effort while no female visits are occurring. Evidence that females are present outside of visit periods confirms that such assessments are both possible and likely. Evidence from both fish and bird taxa suggests that male-resource cues may be more widely used in female mate choice.

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Chapter 5 - Competition, choosiness and parental investment: insights from the Australian brush-turkey example

Two females fight for access to a mound while the male owner is absent. Although competition does not necessarily imply aggressive behaviour such as fighting, aggression is a good indicator of competition.

ABSTRACT

Explanations of male-female differences in competition and choosiness have traditionally assumed that competition in one sex is driven by the limited availability of the opposite sex, that parental investment by the opposite sex is the key limiting factor, and that choosiness is the mirror image of competition. Female brush-turkeys, *Alectura lathami*, appear to be both competitive and choosy, consistent with a mating system in which male-female differences in parental investment are minor, and both sexes therefore limit each other. Males, however, appear to be exclusively competitive, consistent with a mating system in which female parental investment is substantially larger than that of males. My analysis confirmed that females were competitive, that males were non-choosy and that the operational sex ratio was female-biased. Females appeared to compete with each other, not because of male parental investment, but because the supply of active mounds was limited by male competition. Meanwhile, males appeared to compete with each other, not because their access to females was limited, but because there has always been strong selection on males to compete for control of mounds,

resource defence polygyny being a shared derived character of the brush-turkey clade. Despite their very large investment in the mound, males were non-choosy, apparently because the marginal cost of an additional female visit, copulation or egg was low relative to the marginal benefit. Females meanwhile were choosy because the marginal cost of an additional egg was high relative to the marginal benefit. Generalizing these results, it is suggested that Sex A is likely to be competitive if it has limited access to sex B, but the limit may be imposed by various ecological and historical factors, not just the cost of parental investment by sex B. Sex A is likely to be choosy if its own marginal cost of reproductive events is high relative to marginal benefits, regardless of its total or average reproductive costs, and regardless of whether sex B is competitive or choosy.

INTRODUCTION

Male-female differences in competition and choosiness have traditionally been explained in terms of relative parental investment. First clearly formulated by Robert Trivers, the core insight in the traditional explanation relies on a distinction between the limiting sex and the limited sex. If one sex is limiting in the sense that it is less available for mating, then the opposite sex will be limited by this restriction and will compete for access to the limiting sex. The limited sex is therefore competitive and, equally important, the limiting sex is assumed to be choosy (Kokko et al., 2006; Trivers, 1972). When Trivers developed this approach, he assumed that parental investment was the key factor driving limitation, arguing that females who invest more in offspring must spend more time out of the mating pool, thereby forcing males to compete. Hence the common pattern of competitive males and choosy females, most notably in polygynous species, as for example in elephant seals, *Mirounga angustirostris* (Cox and Le Boeuf, 1977) and tungara frogs, *Physalaemus pustulosus* (Ryan and Rand, 1990). The traditional explanation ascribes sex-role reversal to greater parental investment by males than females, as for example in pipefish, *Nerophis ophidian* (Berglund and Rosenqvist, 2003) and food-limited zaprochiline katydids (Gwynne and Simmons, 1990). In social monogamy, where there is at least a rough equality in parental investment, it predicts that males and females will limit each other and will therefore be both competitive and choosy, although not equally so. Examples include zebra finches, *Taeniopygia guttata* (Holveck et al., 2011) and great spotted woodpeckers, *Picoides major* (Michalek and Winkler, 2001).

Regardless of the mating system, we do not expect to find a pattern in which females are both competitive and choosy, consistent with monogamy, but males are exclusively competitive, consistent with polygyny. There is good evidence, however, that the sex roles of Australian brush-turkeys conform to this pattern. In order to explain this

unexpected pattern, it is first necessary to review this species' unusual mode of reproduction and mating system.

Brush-turkeys are members of the megapode clade (Family Megapodidae). Uniquely among birds, megapodes incubate their eggs using environmental sources of heat, usually microbial decomposition in a mound of soil and decomposing vegetation. On the basis of this ancestral pattern, variations have evolved. Although most megapodes are monogamous, brush-turkeys are both polygynous and polyandrous. In the brush-turkey mating system, males construct mounds, defend them against rival males, control their temperature via ongoing maintenance and mate with multiple females each season. Females visit mounds, often copulate with the male and periodically dig large holes in the mound in order to bury their eggs. There is no post-hatching care at all. Chicks are entirely left to their own devices, including having to dig their own way out of the mound. Females are not subject to mate-guarding, do not pair-bond with the male and also mate multiply, although apparently not as multiply as the male.

To establish that brush-turkeys sex roles are indeed as described above, it is first necessary to clearly distinguish competition from choice, and subsequently from choosiness. Individuals can be said to be competing for a resource, for example a member of the opposite sex, when access by one makes access by the other(s) more difficult (Andersson, 1994). For competition to occur, therefore, the prospective mate must have limited availability. Individuals can be said to be exercising choice if they discriminate, showing evidence that they prefer some mates over others. Two elements can be distinguished in mate choice: an individual's preference function is the order in which prospective mates are ranked, while choosiness is the degree to which the individual is prepared to invest effort in exercising a preference (Jennions and Petrie, 1997). Clearly, evidence for the existence of a preference will not be found unless choosiness is present to some degree. Consequently, use of the term 'choosiness' usually implies that the effort invested is substantial, or at least more than trivial.

There is clearly some basis for the view that choosiness is the mirror image of competition. The most minimal preference function in one sex, even without choosiness, may be sufficient to favour competition among members of the opposite sex (Andersson, 1994), if only because any preference further limits the availability of the choosing sex. Conversely, competition among members of one sex is arguably sufficient to generate a preference function among members of the opposite sex. Whether it is sufficient to generate choosiness is open to doubt, however. Contrary to the view that competition and choosiness mirror each other across the sex divide, it is also possible for choosiness to foster competition in the same sex. If multiple choosy individuals prefer the same mating

partner whose availability is limited in some way, then the choosy individuals are likely to compete, for example female lek-breeding peacocks (*Pavo cristatus*) attempt to monopolize preferred males by repeatedly courting them (Jennions and Petrie, 1997).

Previous work has indicated that female brush-turkeys are choosy, while males are not. When copulation opportunities were rejected, it was almost invariably the female who rejected the male's copulation attempt (chapter 1). At some cost to themselves, females also endured other forms of male sexual harassment, nevertheless either refusing to copulate or copulating less frequently than desired by the male (chapter 3). Males occasionally failed to respond to female solicitation, but this may have been because females often solicited and then resisted the resulting copulation attempt (chapter 3). The female visiting pattern also indicated choosiness. Although females were probably visiting mounds only in their foraging range, there being no evidence that they visited mounds at substantial distances from each other (chapter 3), the distance between mounds and locations at which females were habitually present during study-site transects was a poor predictor of female visits to mounds (chapter 4). In short, females appeared to actively choose a mound rather than passively accepting it merely because it was conveniently located. Various lines of evidence suggested that females were assessing mounds and males from a distance, probably on a detailed, daily and iterative basis (chapter 4). Once a specific male and mound were chosen, there appeared to be a confirmation process, during which the female made repeated visits, carried out extensive digging, probing and scratching in the mound, and copulated in preparation for egg-laying (chapter 4).

As indicated above, male eagerness to copulate suggests non-choosiness. This was partly obscured by the fact that males drove females away, as well as attempting to entice them onto the mound. With the ability to uniquely identify females, however, it was clear that males were not selectively enticing some females and driving others away, but were both enticing and repelling each female, in an attempt at sexual coercion (chapter 1). This interpretation assumes that males can recognize individual females, an assumption which is confirmed by the finding that male aggression reduces over a series of visits by the same female (chapter 3) and is consistent with low dispersal and a relatively long lifespan for this species. Further investigation of male non-choosiness is nevertheless required, as it is possible that males discriminated between females when two or more visited simultaneously.

The evidence for male competition is unequivocal. Any rival male in the immediate vicinity of a mound was ejected promptly and vigorously by the male owner of the mound (Jones, 1994) except in the very rare event that the male owner did not recognize the visitor as a male or was himself ejected, resulting in an expulsion or usurpation. Where two or more

females visited simultaneously, there was also evidence of female competition, in that one female would generally obtain exclusive access, in some cases aggressively (Birks, 1996). Further investigation of female competition is nevertheless required, as female-female aggression was considerably less frequent and intense than male-male aggression. Although competition does not necessarily imply aggression, aggression is a good indicator of competition.

Given that males are clearly competitive and females are clearly choosy, the traditional explanation predicts that females are the limiting sex. It also predicts that if females are found to be competitive as well as choosy, then males also place some form of limitation on females. If male non-choosiness is confirmed, however, it would be at odds with the assumption that if a sex is limiting it also tends to be choosy. This in turn suggests that some confounding factor suppresses male choosiness or alternatively that there are problems with the traditional explanation.

Evidence from species with unusual sex roles suggests that there are indeed problems with the traditional explanation. The limited sex is assumed to be subject to stronger sexual selection both because it is forced to compete and because it is subject to choice by the choosy sex. In the polygynous and polyandrous eclectus parrot, *Eclectus roratus*, the bright red and blue coloration of the females appears to function in female-female competition over nest hollows, while the iridescent green of males appears to function in male-male competition over access to females at nest hollows (Heinsohn, 2008). Both females and males appear to be choosy, but there is strong evidence that male choosiness is not the mirror image of female competition in this species because the limiting factor driving the latter appears to be ecological, namely the limited supply of suitable naturally-occurring nest hollows, plus variation in the quality (principally dryness) of those which are available. Resource control in this species gives females fecundity benefits, while the resulting variation in female reproductive potential encourages choosiness in the males (Heinsohn, 2008). Parental care in the Eurasian dotterel, *Charadrius morinellus*, is entirely provided by males. Although females are competitive, consistent with sex-role reversal, no evidence has been found for male choosiness (Owens et al., 1994), contrary to the traditional explanation.

Key to understanding brush-turkey sex-roles is to determine whether males or females or both are the limiting sex, which in turn requires establishing the sex ratio, in particular the operational sex ratio, namely the number of reproductively active females relative to reproductively active males. In the present chapter, therefore, I address the following questions:

1. Are females competitive?

2. Are males non-choosy?
3. What is the sex ratio, in particular the operational sex ratio?

Possible explanations for brush-turkey sex-roles are explored in an extended discussion.

METHODS

Field studies were conducted on a free-living, individually colour-banded brush-turkey population in and close to the town of Pearl Beach in New South Wales, Australia (33.54°S, 151.30°E). Observations were recorded remotely by five Sony HDR-SR7 video cameras mounted at active mounds and programmed to run daily for 3.5 hours from first light. Unless otherwise specified, data is here drawn from the second half of the 2008-9 breeding season and the first half of the 2009-10 breeding season. With some exceptions due to access difficulties or extreme weather, each mound was remotely observed for seven consecutive days. Seventeen mounds were observed during 2008-9 for a total of 355.3 hours and sixteen in 2009-10 for a total of 361.3 hours.

In order for a female mound visit to be recorded, it was necessary for the female to be physically present on the mound at some point, even though she might be temporarily absent during the visit. Visits could be interrupted by periods of absence lasting up to 22 minutes. Direct observation of these absences has shown that in most instances the female was in the vicinity of the mound (for full details, see chapter 1).

Are females competitive?
For this purpose, competition between females was inferred if one female's use of the mound made usage by another female less likely, a zero-sum approach which is widely used in the ecology and behavioural ecology literature (Andersson, 1994). Where the presence of one female at the mound coincided with or followed on immediately after that of another, the detailed behavior record was examined for evidence of competitive interactions, for example one female might dig and scratch in the mound while the other merely stood on the side of the mound, or one female might remove another from the mound by charging or chasing her (see Results below for details of other interactions). Only two-female (dyadic) interactions were considered, as it was difficult to determine who was influencing whom where three or more females were involved. Interactions between three or more females were relatively rare in the datasets used in this study. For each interaction, a distinction could generally be made between the female in occupation of the mound and the female who arrived later, although this distinction was not essential. If one female was in occupation, and a second female arrived and then left on

several occasions, each occasion was treated as a separate case. Where multiple competitive interactions occurred in a given case, only the most extreme interaction was recorded. Given that mounds were almost always large enough to accommodate two females simultaneously, the appropriate null hypothesis was that both females would exhibit the same behaviour toward the mound, assessed using a binomial test.

Are males non-choosy?

Using the same dataset as that used for Question 1, but excluding cases where the male was absent, it was possible to record male responses to the simultaneous presence of two females. As females appeared to compete with each other, simultaneous presence was not generally an opportunity for males to copulate with both females. The null hypothesis was therefore that males would demonstrate choice (or choosiness) by preferring one female over the other, assessed using a binomial test. Preliminary observation suggested that males tended to avoid intervening in female-female interactions, but if they did intervene, they were more likely to favour the female in occupation. Where males intervened against the female in occupation, the behavior record was examined in an attempt to identify possible explanations.

What is the sex ratio?

The ratio of females to males in a population can be expressed in at least three ways: the operational sex ratio (sexually active females relative to males), adult sex ratio (adult females relative to males) and hatching sex ratio (number of female hatchlings relative to males). The operational sex ratio was measured by estimating the mean number of distinct female visitors per day to each active mound (or pair of mounds where a male owned two) according to the video record.

The adult sex ratio was measured by using the mark-recapture method. Banding commenced in 2004 under the auspices of the NSW National Parks and Wildlife Service, and continued as part of this project. During the 2007-8 breeding season, an estimate of banded adults known to be present was compiled from all sources (bandings performed during the year plus sightings made opportunistically, during mound observations or during walking transects of the site). The ratio of banded to un-banded individuals was estimated, based exclusively on sightings during standardized site transects, where the observer moved at a steady pace through the study site, thereby minimizing the chance of double-counting un-banded individuals. Twelve transects were performed between 6th and 31st December 2007, the transect route including all streets in Pearl Beach in a pre-specified sequence. The number of un-banded adults in the population was estimated by applying this ratio to the number of banded adults known to be present, yielding an estimate of population size. As these calculations were analyzed by sex, they also yielded an estimated adult sex ratio. All attribution of sex for bandings and sightings was based on

appearance of sexually dimorphic traits during the breeding season (males having a more developed neck wattle). DNA from blood samples taken at the time of banding was also subsequently analyzed, to confirm the attribution of sex.

Finally, an attempt was made to determine the hatching sex ratio. During 2007-8 and 2008-9, eggs were excavated from mounds, artificially incubated, and a feather was plucked from the chick. DNA from the feather was then analyzed. All DNA extraction used a salting out technique. Polymerase chain reaction (PCR) employed 2250F and 2718R primers (Fridolfsson and Ellegren, 1999), optimized for brush-turkeys by M. Gillings. The difference between numbers of male and females was assessed for statistical significance using a binomial test.

RESULTS

Are females competitive?
Where the presence of one female at the mound coincided with or followed on immediately after that of another, 96 interactions were observed, although one was excluded because the result was indeterminate and twelve were excluded because one of the females was engaged in egg-laying, and could not respond to the second female. Of the remaining interactions (n = 83), females charged at or chased other females in an attempt to remove them from the mound (18.07% of cases), fought with them (6.02%), walked toward them with the result that they left (12.05%) or walked toward them without this result (3.61%). In 12.05% of cases, the female in occupation of the mound left on the arrival of a second female. Digging and scratching in the mound by one female but not the other (30.12% of cases) indicated that use of the mound by one was incompatible with a similar use by the other, and was therefore also treated as evidence of competition.

On the other hand, there was mound activity by both females in 4.82% of cases, indicating that unambiguously non-exclusive access to the mound was sometimes possible, though rare. Lack of mound activity by either female (13.25% of cases) could be interpreted as non-exclusive access or as a stand-off with neither party prepared to retreat. Even when mound activity by neither female was grouped together with activity by both, and treated as non-competitive behavior, competitive interactions between females were significantly more likely than expected, the null hypothesis being that both females would behave similarly toward the mound (binomial test, $P < 0.001$, n = 83). There is therefore strong evidence for competition between females over mound access.

Are males non-choosy?

Using the same dataset as that used for Question 1 (n = 96), but excluding cases where the male was absent (n = 6), it was found that males did not intervene in female-female interactions in 64.44% of cases, significantly more often than expected, the null hypothesis being that males would demonstrate a preference for one female (binomial test, $P = 0.004$). Where the male did intervene, he intervened on behalf of the female in occupation in 65.26% of cases. Although this was not significantly more often than expected by chance, it was close to being so (binomial test, $P = 0.055$). There was therefore a significant pattern of males not intervening in female-female interactions, and a non-significant trend for them to support the female in occupation when they did so (Fig. 1). Moreover, where the male intervened on behalf of the newly-arrived female (n = 11), it was found that the female in occupation had completed the egg-laying process and appeared to be engaged in supplementary mound activity (three cases), or had indicated subordinate status by moving aside and allowing the newly-arrived female greater access to the mound (two cases). In other cases the newly-arrived female was resuming an extended visit or was the most frequent visitor over the recent past (two cases), or had copulated with the male on the same day (two cases). In only two cases was there no evident explanation for the male's departure from his typical pattern.

What is the sex ratio?

The adult sex ratio for 2007-8 was 3 females for every male (n = 126). Where visual sex attribution could be checked using DNA methods, such attribution was confirmed to be correct in 27 of 30 cases (90%), suggesting that distinguishing the sex of adults based on morphology was broadly reliable. The operational sex ratio (mean number of distinct female visitors per day to each active mound) was 1.65 females for every male in the first half of the 2008-9 season, and 1.15 females for every male in the second half of the 2007-8 season. Overall ratios for individual males were as high as 2.67.

Owing to difficulties associated with DNA extraction, it was not possible to estimate the hatching sex ratio, DNA being obtained from only 20 chick feathers, of which 13 were found to be female. Although this result was not significantly female-biased (binomial test, $P = 0.13$), suggesting an even sex ratio, more females were nevertheless found in the sample, suggesting that a larger sample size might have indicated a female-biased hatching ratio.
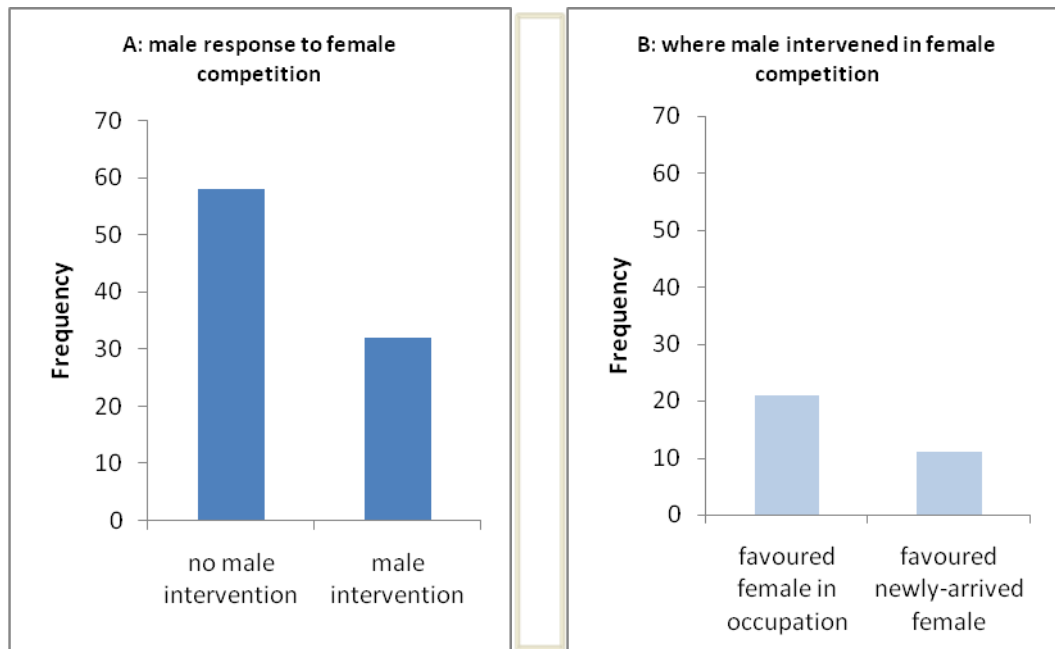
Figure 1 When two females were present on the mound, (A) the frequency of male intervention vs. non-intervention in response to female competition and (B) where the male intervened, the frequency of intervention in favour of the female in occupation vs. the newly-arrived female. The difference in A is statistically significant (binomial test, $P = 0.004$, n = 90). The difference in B is close to significant (binomial test, $P = 0.055$, n = 32).

DISCUSSION

My analysis supports the hypothesis that females are competitive and males are non-choosy. When combined with previous findings, it provides strong evidence that brush-turkeys conform to the unusual sex roles suggested above: females are both competitive and choosy, but males are exclusively competitive. This pattern occurs against the background of a female-biased operational sex ratio, consistent with males being the limiting sex. In the following extended discussion, an attempt is made to explain this allocation of sex roles. I start, however, with further evidence for competition between females over mound access.

Female competition
Females were significantly more likely to compete with each other than to tolerate mound access by other females, a result which was consistent with the findings of previous researchers (Birks, 1996; Jones, 1987). Although competition does not necessarily imply aggression, aggression is a good indicator of competition. When

observations were made from a hide, females were heard to make grunting vocalizations when confronting each other, even on occasion appearing to mimic the body movements used by males to produce their characteristic 'booming' signal, although the resulting sound was halfway between a boom and a grunt. Female wattle inflation and vocalization during competition over mounds has been previously reported (Birks, 1996). It was also clear that agonistic interactions between females occurred more frequently than could be directly observed by a video camera trained on the mound, although much could be inferred from the behavior of the female in occupation of the mound. Females on mounds visually scanned the surrounding area, while males did so only when they were alone on the mound. This suggested that the scanning was not to detect predators but to detect approaching females (visitors from the perspective of males but rivals from the perspective of females in occupation). An especially abrupt departure by the female in occupation, particularly if she returned promptly, suggested that her mission was to remove a rival female. In a previous study, it was found that females were more likely to prevail over rival females if their dominance rank was high (as measured during aggressive interactions at a feeding site), they had previously laid in the contested mound, they arrived at the mound before their rival, and they intended to lay during the current visit (Birks, 1996).

The most aggressive female observed from the vantage-point of a hide during 2007-8 effectively succeeded in monopolizing access to a mound in the early part of the following season, when observations were made via video (not included in the two main datasets for this study). Over a period of 31 days during the height of the breeding season (16 August to 15 September), this individual accounted for 84% of visit duration at the mound (2,014 of 2,402 minutes including periods of temporary absence), 82% of copulations (68 of 83) and 87.5% of eggs laid (7 of 8). At this mound, she was responsible for the most extreme example of female aggression captured on video, namely running at speed onto the mound and, without interacting with the male, charging another female digging in the mound, apparently biting her on the head. The attacked female was eventually responsible for bringing the effective monopoly to an end by laying an egg on the day following the above period. The duration of that egg-laying visit was the shortest recorded, at only 16 minutes, probably because of the threat of aggression from the dominant female (unpublished data).

Female competition amplifies the effects of male-female sexual conflict
Previous work has suggested that because a breeding female eventually makes egg-laying visits, which expose her to male aggression for longer periods, it is advantageous for her to focus her visits on a single male, thereby maximizing their time together on the mound, and habituating him to her company (chapter 3). This hypothesis was supported by the

finding that males did indeed reduce two forms of aggression during later visits in a series of visits (chapter 3). By extension, it is also advantageous for the female to extend the duration of her visits, beyond what was otherwise necessary (chapter 3). Both of these effects are probably amplified by female-female competition. Increasing the duration of a visit not only habituates the male to the female's company, it also denies rival females access to the mound, a function which has been previously suggested (Birks, 1996). Similarly, repeatedly visiting the same mound not only allows the female to become familiar with the male, it also allows her to become familiar with her female rivals, potentially enhancing her chances of either dominating them or identifying opportunities to visit despite being subordinate to them.

Previous work has also suggested that females interrupt their mound visits with periods of temporary absence which appear to reap a short-term benefit for the female in increased mound activity at no discernible cost, but in the longer term incur increased costs (chapter 3). As females generally remain in the vicinity of the mound, these absences also allow females to repel potential rivals, while avoiding uninterrupted exposure to the male's aggression. In effect, they represent a way of extending visit duration. It is notable that the aggressive female mentioned above also exhibited a strong tendency to interrupt her visits with temporary absences. It is therefore likely that female competition works in the same direction as male-female conflict, by extending the duration of visits and focusing them on a single primary male and mound.

In some circumstances, however, female competition can disrupt the focus on a primary male and mound. The especially aggressive female mentioned above was also responsible for the clearest deviation from this pattern. During observation from a hide in 2007-8, she was observed in repeated conflict with a rival female who had commenced laying a series of eggs. Apparently as a response to this competition, the especially aggressive female laid a series of four eggs in an adjacent mound under video observation, without any preliminary non-laying visits, while continuing to make non-laying visits to the original mound. She then re-commenced egg-laying in the original mound, even though her competitor was still laying there.

Sex ratios and the competition vs. choosiness principle revisited
Site transect and tag-recapture information suggested that the adult sex ratio was strongly female-biased at approximately 3 : 1. As male-male aggression is intense, it is likely that young males avoid established breeding populations dominated by mature males, probably suffering high mortality rates and isolation, but eventually forming male-biased satellite pioneering groups in urban areas (D Jones, pers. comm.). Meanwhile, observations at mounds suggested that the operational sex ratio was also female-biased at approximately 1.65 : 1 during the first half of the season, when females were most

likely to visit. Whether the hatching ratio at Pearl Beach was female-biased was difficult to determine because of a low sample size. It has been found that female embryos are more likely to survive in warmer mounds (Göth and Booth, 2005), and previous research has established that the mean temperature in Pearl Beach mounds was higher than mean temperatures estimated by three separate studies (chapter 4). Although a statistically-significant hatching bias could not be established in this case because of low sample size, if we assume that such a bias was nevertheless present, differential survival must apply early during incubation, because the average egg in this case was incubated at least half of the time at an artificially-controlled ideal temperature.

The most relevant finding here is that the operational sex ratio was female-biased. If fewer males are available in the mating pool, then females are expected to compete for access to them, regardless of the explanation for their restricted availability. It seems reasonable, therefore, to start with the hypothesis that males are the limiting sex. The traditional approach assumes that this will be because of greater parental investment by males, but it is difficult to test this assumption because the traditional approach also insists on a distinction between parental investment and mating effort, a distinction which is problematic in this case. The mound represents mating effort because the male uses it to attract females, but he succeeds in doing so only because it also represents parental investment (Birks, 1997; Jones, 1992). Only mounds which can potentially function as incubators are likely to attract substantial numbers of female visitors. It is therefore useful to review the male's reproductive costs generally, regardless of the degree to which they involve parental investment, and consider how they might lead to restrictions on the number of males in the mating pool.

Do male reproductive costs explain the female-biased operational sex ratio and hence female competition?

A mound must initially be constructed, typically near the commencement of the breeding season. If it already exists, it may be refurbished. Construction takes between 21 and 77 days at five to seven hours per day, involves the raking of between two and four tons of material, and results in the loss of up to 20% of bodyweight in captive male birds (Jones, 1990b). The mound functions most efficiently as an incubator if it is maintained on a daily basis, and it must be defended against rival males. These three costs, namely construction (or refurbishment), maintenance and mound defence are by far the largest of the male's costs, and they are costs of securing access to the mating pool. Only males who own a functioning mound are in the mating pool, as it is only they who appear able to obtain copulations (for discussion see chapter 4). Additionally, there are costs associated with each reproductive event, namely each female visit, copulation or egg. While not trivial, these costs are small in comparison to the three major costs, which are therefore much

more likely to impose limitations on the availability of males, hence causing females to compete.

The male costs of being in the mating pool relate entirely to mounds, and lead us to expect a relative shortage of mounds. Consistent with this expectation, there was on average one inactive mound for every active mound each season at the study site. If all existing mounds had been refurbished and rendered active, the operational sex ratio would have become male-biased and males would no longer have been the limiting sex, assuming all other relevant factors were held constant. This interpretation assumes that males who did not own a mound (on average 56% of all males in the population) were effectively excluded from the mating pool, and hence from the operational sex ratio, because their short-term prospects of mating success were zero, despite presumably being capable of mating (chapter 4). The bottleneck in the supply of mounds, however, was unlikely to be caused by a shortage of males able and willing to refurbish and maintain them. Immature males are known to construct mounds as early as four to five months of age. Critically, however, they do so only in the absence of older males (Jones, 1987). The probable cause of the shortfall was dominant males owning mounds in the vicinity who kept rival males away, thereby enhancing the competitive advantage of their own mounds (Jones, 1990b, 1994). Moreover, it was not obvious why a smaller number of mounds should limit the breeding opportunities of females, causing them to compete. The physical size of the mound did not appear to limit females because, as revealed by excavation, eggs could be buried within a few centimetres of each other, suggesting a very high carrying capacity. The largest number of eggs reported in a mound is 58, although these were not all present simultaneously (D. Jones pers. comm.). There was also a potential problem with gas exchange between the egg and the mound substrate, as developing embryos competed for oxygen with the microbes responsible for heat generation, but the number of eggs in the mound was unlikely to be a limiting factor so long as there was ongoing maintenance to aerate the mound substrate (Jones et al., 1995).

We are left with one obvious limiting factor, namely time. During the first half of the season, female visits while the male was present (within camera view, as opposed to assumed to be in the vicinity) consumed on average 35.2% of the time available for such visits to occur during a standard 3.5 hours of observation per day, making no deduction for periods when female visits overlapped (the equivalent figure during the second half of the season was 12.6%). Because females were generally in the vicinity of the mound during the temporary absences which punctuated their visits, and were therefore in a position to influence other visiting females, visit duration here included temporary absences. For the period of greatest daily activity, namely the first two hours of

observation time, and for the three most popular mounds during the first half of the season, female visits consumed 57.9%, 65.1% and 65.4% of the time available respectively. On specific days, these ratios exceeded 100% because of overlapping visits. It could be objected that we are not explaining female competition here because, as we have seen, female competition is itself one of the influences tending to increase visit duration. While this is true for each female, it may be that competition reduces rather than increases visit duration for all females considered together, as evidenced by the example above, where one female managed to effectively prevent other females from visiting.

Females therefore appeared to compete with each other firstly because the supply of mounds was limited by male competition, and secondly because access to the mounds which were available was limited by the time required by females to perform the digging, probing and scratching which they typically undertook. Another important limiting factor was the female tendency to visit mounds overwhelmingly in the early morning (chapter 4), possibly an evolutionary constraint on egg-laying for all birds (Birks, 1996), with non-laying visits also following this strongly-conserved pattern. We set out to explain female competition in terms of male parental investment, or at least reproductive costs, but we have ended up apparently explaining it in terms of male competition and the female visiting strategy, which has been interpreted as a response to male aggression during mound visits (chapter 3). These conclusions support the general principal stated above that any restraint on the availability of one sex may generate competition among members of the opposite sex. Ecologists have long recognized that a wide range of resource constraints can drive competition (Wiley, 1998), and there seems no compelling reason to appeal to parental investment as the only constraint in this case.

It could be objected that the argument here is circular, as we have failed to distinguish parental investment from 'mating effort', a distinction which Trivers in particular insists on. Clearly, searching for a mate does not increase the survival chances of offspring, and is therefore not parental investment, but it is not immediately clear why this distinction is considered so important for competition and choosiness. The reasoning is probably that efforts to acquire a mate are strongly marked by either competition or choosiness as the case may be, depending on the species under discussion. In attempting to explain why males in a particular species compete for mates, for example, any appeal to the costs which males incur in that competition is circular because it assumes the very competition which is to be explained.

Circularity is not involved, however, if we explain female competition, as above, by appealing to other factors, namely male competition and male-female conflict. Moreover,

insisting on the distinction between parental investment and mating effort assumes that each component comes in a separate parcel distinct from the other, potentially ruling out of scope those cases, such as the brush-turkey, where males merge the two by using control over a reproductive resource as leverage for mating. In some instances of resource defence polygyny, males merely take control of a resource, contribute nothing to it which benefits potential offspring, and their costs can therefore be described as entirely arising from mating effort, as for example in dung flies (Blanckenhorn et al., 2002). In other cases, such as nest-building fish, however, both nest-construction and tail-fanning are simultaneously mating effort and parental investment (Lindström et al., 2006). Even where males take control of a resource without enhancing it, their efforts arguably represent parental investment as well as mating effort if there is stronger male-male conflict for control over the best resources. Males who present nuptial gifts to potential partners are not involved in resource defence, but their behavior is similarly difficult to classify as either mating effort or parental investment, despite considerable effort by researchers to do so (Vahed, 1998). More generally, mating effort is increasingly recognized as an important influence on the evolution of male mate choice, regardless of whether it is distinguishable from parental investment (Edward and Chapman, 2011).

Defined as the sex which contributes mobile but nutrient-free gametes, it is not surprising that males seek to employ any available leverage to reproduce. It might be suggested that where male parental investment is combined with mating effort, the 'real' function is mating effort because parental investment is only indirectly involved in reproductive events, and males therefore do not have it in mind. This ascribes function on the basis of intention, however. In the brush-turkey case, the mound is both essential for offspring survival, therefore functioning as parental investment, and essential for acquiring a mate, therefore functioning as mating effort.

A second potential difficulty arises from the way in which, following Trivers, the costs of a parental investment are typically measured, namely 'by reference to its negative effect on the parent's ability to invest in other offspring' (Trivers, 1972). This approach derives from two sources. The first source is a widely-accepted concept of costs as opportunities foregone. The second source is much narrower: Trivers appears to be making a simplifying assumption that parental investment is the only relevant opportunity foregone, when other opportunities may also be relevant in the real world. The potential difficulty this poses in the brush-turkey case is that, because of the effectively unlimited carrying capacity of the mound, referred to above, a male receiving an egg in his mound incurs no cost, measured in the currency of parental investment foregone. It could therefore be argued that the brush-turkey case is out of scope, and hence irrelevant to the merits or otherwise of the traditional explanation of competition and choosiness. Because brush-

turkey mounds are shareable resources (Jones, 1992), brush-turkeys are arguably mere curiosities who establish the outer limits of the argument. It is clear, however, that the male does incur a cost in opportunities foregone, even if not parental investment opportunities. By allowing a female to lay, for example, he loses copulation opportunities with other females, and the opportunity to expend energy for alternative purposes such as foraging or maintaining his mound, rather than conducting an ongoing war of attrition with the laying female. This cost is relatively small, as already pointed out, but it is finite, both in absolute terms and at the margin. The brush-turkey case therefore does seem relevant to the merits of the traditional explanation.

Do female reproductive costs explain male competition?
We seem to have a reasonable explanation for female competition, although one which conflicts with the assumption that male parental investment is the key limiting factor. Can we nevertheless find evidence that female reproductive costs limit males, and hence find an explanation for male competition? Despite the female-biased operational sex ratio, such an idea may not be so outlandish, as the operational sex ratio is considerably less female-biased than the adult sex ratio. In contrast to those of males, female reproductive costs are predominantly attributable to reproductive events, namely visits, copulations and eggs. Given that the egg provides nutrients to the most precocial of all bird young, represents 10% of the female's body weight, and has a yolk which is relatively the largest of any bird egg excepting the Brown Kiwi (Dekker and Brom, 1992), it is the production of the egg which probably represents the greatest cost, and is the most likely to impose limitations on the availability of females, hence causing males to compete.

There is little evidence, however, that females go through periods of unavailability in a mating season lasting up to six months, during which they lay an average of 18-24 eggs in the wild, and a range of 17-56 in captivity (Jones and Göth, 2008). Not only do females copulate during egg-laying visits, they do so more frequently during such visits (chapter 3). Females who are sexually active at the beginning of a season are often active at its conclusion. During the 2008-9 season, all seven individually-identifiable females who were observed visiting mounds in August were still visiting mounds in November and in one case December, the range being 80 to 107 days. Together with the female-biased sex ratio, this suggests that female reproductive costs do not provide an explanation for male competition.

It is more likely that male competition in this system is explained by prior evolution, specifically the sequence of steps leading to male resource defence polygyny in certain species within the megapode group. As external incubation evolved in the common ancestor of megapodes, and the selective pressure for precocial offspring increased, female provision of nutrients to the egg became critical (Jones, 1992). The most

parsimonious interpretation of megapode phylogeny (Fig. 2) is that males in the ancestral species responded by following and guarding females within a system of female-defence monogamy. Resource-defence polygyny subsequently evolved within the brush-turkey clade, comprising the study species (*Alectura lathami*) and species in the genus *Aepipodius*. Resource-defence polygyny may have evolved earlier, in the common ancestor of the brush-turkeys and the malleefowl (*Leipoa ocellata*), as the malleefowl's unique combination of resource defence and monogamy can be interpreted as the brush-turkey system in an arid environment, where monogamy may be imposed by low male-female encounter rates (Jones, 1992). At least one instance of polygyny has been recorded for malleefowl (Weathers et al., 1990). If resource-defence polygyny is a shared derived character of brush-turkeys (and possibly the ancestral malleefowl), then there has always been strong selection on male brush-turkeys to compete for control of mounds, regardless of whether access to females is limited. Having opted to remain with and defend the mound, males are unable to control females, and are left with control over the mound as the only reproductive avenue available.
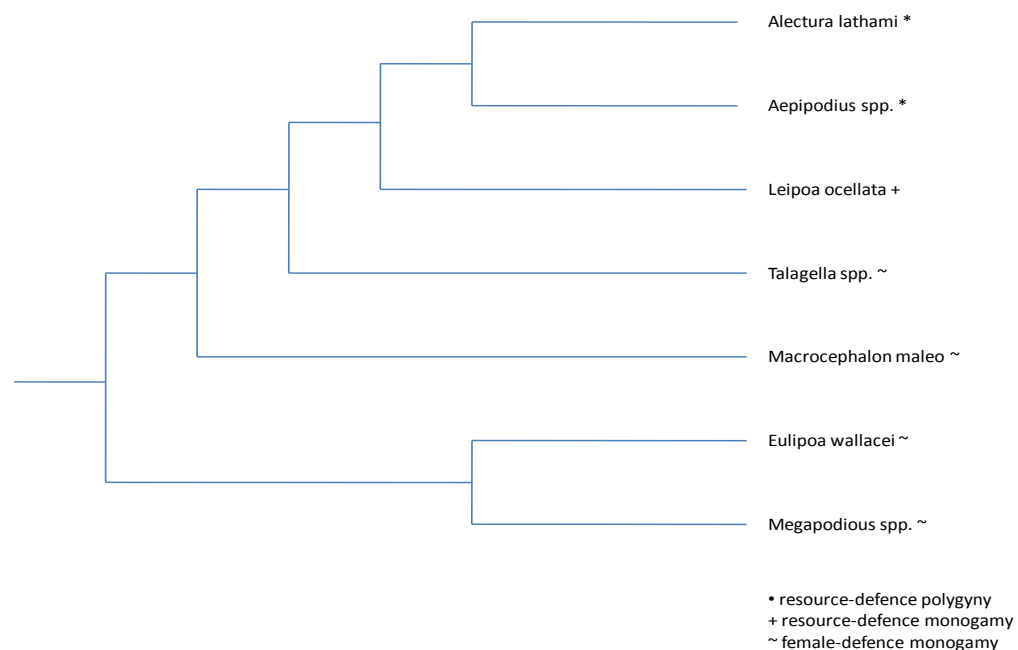
Alectura lathami *

Aepipodius spp. *

Leipoa ocellata +

Talagella spp. ~

Macrocephalon maleo ~

Eulipoa wallacei ~

Megapodious spp. ~

* resource-defence polygyny
+ resource-defence monogamy
~ female-defence monogamy

Figure 2 Maximum-likelihood phylogeny of extant megapodes using a combined dataset of the nuclear gene RDP1 and the mitochondrial gene ND2 (Birks and Edwards, 2002). Assignment of mating systems is based on (Jones, 1990a), resource-defence monogamy in Leipoa being interpreted as resource-defence polygyny modified by low male-female encounter rates in an arid environment.

A proposed explanation for choosiness

Focusing on the largest cost, as we have so far done, may tell us that the sex which incurs this cost is limiting, hence forcing the opposite sex to compete, but it tells us little about the limiting sex itself. One might conclude that the limiting sex will be non-competitive, but it is possible for the limiting sex as well as the limited sex to be competitive, as we saw above in the case of brush-turkey males, who apparently limit females as a consequence of their own competition. Even less can be concluded about the choosiness of the limiting sex. If choosiness is defined as a willingness to incur significant costs in order to identify and mate with a preferred partner, it is not obviously the mirror image of competition, and may vary independently of competition, as noted above. Assessing a potential partner against an absolute or relative standard implies sensitivity to the costs and benefits of choice; making a significant investment in this assessment implies sensitivity to the costs and benefits of choice at a fine scale. To draw conclusions about choosiness, therefore, we need to think at a fine scale, by considering the change in total cost and benefit arising from an individual reproductive event. In short, we need to think in terms of marginal value. The Marginal Value Theorem has been widely applied in behavioural ecology, for example to patch foraging, the timing of cell lysis by phages and the duration of copula by dung flies (Ydenberg, 2010) but apparently not to explaining sex roles.

As we have seen, brush-turkey males are non-choosy. When two female visits overlap or nearly overlap, and female-female competition makes copulation with both females unlikely, one would expect choosy males to intervene in favor of one visitor over the other, and one would not expect the female in occupation of the mound to be more strongly favored when intervention occurred. Brush-turkey males, however, followed a behavioural rule of either not intervening, or intervening on behalf of the female in occupation. Non-intervention resulted in the commencement or continuation of mound visits by females who were dominant over other females, and hence were less likely to be removed by rivals. This outcome was consistent with the hypothesis that males were attempting to maximize copulations. Where males intervened in favor of females currently in occupation, mound activity by this female during the current visit had in all cases demonstrated her interest in the mound, an outcome which was also consistent with the hypothesis that males were attempting to maximize copulations. Even when favoring the female not in occupation, males appeared to focus on the likelihood of copulation, favoring females who had previously copulated on the same day or were more frequent visitors in the recent past, and discriminating against egg-layers who had completed laying or females who had indicated subordinate status to a rival female, and were therefore eventually likely to leave under pressure from the rival. The male's reluctance to intervene might be interpreted as a form of choice in favour of dominant females, but even if this is so, not intervening does not seem to qualify as a significant

investment in choice, and hence to qualify as choosiness. Male intervention on behalf of the female in occupation might be interpreted as an attempt to limit disruption to the mound by newly-arrived females who are likely to start mound activity afresh. Non-intervention in most cases, however, seems to count against this interpretation.

The non-choosiness of brush-turkeys males requires explanation. For brush-turkey males, an additional female visit, copulation or egg has no effect on mound construction costs, which are in the past, and no effect on maintenance or mound-defence costs, which the male is already committed to by his prior decision to be in the mating pool. These costs have been, and will continue to be incurred, regardless of the male's decision, at any specific point in time, whether to seek an additional female visit or copulation, or accept an additional egg. The only costs which are relevant for the male are those attributable to reproductive events. If the event is a female visit, he must devote time and energy to enticement and aggression on average for 21 minutes, and he may need to rectify the mound after the visit has occurred. These costs are non-trivial, but they are small in comparison with the benefits of copulations and eggs and hence the potential benefits of visits. For males, therefore, marginal benefits are in general so clearly greater than marginal costs that there is little advantage in being choosy. Having gained access to the mating pool, males might as well get on with mating. Support for this view can be found in the fact that males were uniformly non-choosy, regardless of the cost of the mound. If they constructed the mound themselves, the cost was clearly greater than if they merely usurped a rival male, but this seemed to have no impact on their choosiness. Greater investment in ongoing maintenance was clearly more costly than less investment, but choosiness seemed unaffected. To suppose otherwise would be to commit the 'Concorde fallacy' of assuming that costs which are either incurred or committed in the past will affect present decisions (Jennions and Kokko, 2010), although it should be noted that past investment is sometimes an indicator of future benefits (Coleman and Gross, 1991).

In one respect, brush-turkey females are in a similar position to males. When making a series of visits focused on a single primary male and mound (chapter 3), females have to a degree committed themselves, and are making decisions which prospectively apply to more than one egg. In species constrained by an optimum 'brood size' for post-hatching care, eggs are laid in quick succession to form a 'clutch'. This does not apply to brush-turkey females (Jones, 1992), but one might almost speak of a 'quasi clutch'. There is in effect a fixed cost of mound access for females, imposed by both male aggression and female competition. Females have to both establish a relationship with the male and establish visiting rights in competition with rival females. Once that cost has been incurred by commencing a series of visits, and the female begins to accumulate direct

knowledge of the incubation properties of the mound, marginal costs fall in relation to marginal benefits, and there is an advantage for the female in continuing her visits.

In another respect, however, brush-turkey females are in a different position from males. Females appear able to switch at any time to a different primary male and mound or to cease breeding entirely. Each egg is potentially a separate investment decision, even if this potential is not generally realized. Each egg carries with it its own distinct parcel of costs in the form of high yolk content relative to egg-size and high egg-size relative to egg-sizes of comparable species (Jones, 1992). Each egg must be separately laid, and therefore requires its own excavation of the mound by the female, together with the male aggression which must be endured, and the copulations which may be traded while the excavation proceeds. For females, therefore, the marginal cost of each egg is close to the average of all of her costs, and is unlikely to be obviously greater than the marginal benefit. It is therefore clear that the female will attend closely to the balance of costs and benefits; in short she will be choosy. Females even appear to adjust their costs to match the benefit, laying larger eggs in superior locations in the mound (Göth, 2007). Such matching is possible only because the female's costs, unlike the male's, are principally tied to individual reproductive events.

A possible objection here is that there are superior explanations of choosiness. One approach is provided by Jennions and Kokko (2010), although it should be noted that these authors are principally focused on explaining relative parental investment by males and females, which is a given and hence out of scope for the discussion here. They define choosiness negatively as a greater willingness to reject mating opportunities, and argue that it is more likely to evolve in the sex which has less to lose by rejecting mating opportunities. This will be the sex which has the lower Bateman gradient, i.e. the sex which has less to gain in offspring production by increasing its mating rate. As the authors themselves point out, however, a low gradient minimizes an obstacle to the evolution of choosiness, but does not explain why choosiness actually evolves in some cases, and not in others (Jennions and Kokko, 2010). In this respect, it is less ambitious than the explanation proposed here. Moreover, the Bateman gradient is not clearly relevant to brush-turkeys. Given that females do not spend extended periods out of the mating pool, lay many eggs over many months, and need to ensure fertilization, it is not clear that they have a low Bateman gradient. Female brush-turkeys may resist copulation as much because resistance gives them a bargaining chip in sexual conflict. Given that males can only mate with the females who visit their mound, and these are a small number of females who visit repeatedly, it is not clear that males have a higher Bateman gradient. The fact that brush-turkey females are choosy, and males are not, seems to require a different explanation.

Summary and further hypotheses on choosiness

Two principles can be distilled from the above:

1. Sex A is likely to be competitive if it has limited access to sex B, but the limit may be imposed by various ecological and historical factors, not just the cost of parental investment by sex B.
2. Sex A is likely to be choosy if its own marginal cost of reproductive events is high relative to marginal benefits, regardless of its total or average reproductive costs, and regardless of whether sex B is competitive or choosy.

It is not suggested that competition or choosiness will always evolve in the above circumstances. For example, despite marginal costs being high relative to the marginal benefits of reproductive events, choosiness may not evolve because of low mate quality variance in the opposite sex (Kokko et al., 2006) or a low encounter rate between the sexes (Parker, 2006).

The traditional explanation appeals to the costs of the limiting sex in an attempt to explain both competition and choosiness. It is suggested here, however, that two different aspects of cost are involved: whether the total cost is high drives competition in the opposite sex; whether the marginal cost is high drives choosiness in the sex which bears the cost. When allied to the assumption that the only relevant cost is parental investment, the traditional approach is reasonably successful at explaining competition because, in many cases, parental investment is by far the largest cost of the limiting sex. It only appears to explain choosiness because parental investment in many species is tied to individual reproductive events, with the result that total (or average) cost correlates with marginal cost. Females in many mammalian species, for example, are thought to be choosy because the total cost of their parental investment is larger than that of the male. If the second principle above is correct, however, what really matters is that their marginal costs are higher. Conversely, their male counterparts are thought to be non-choosy because the total cost of their parental investment is less than that of the female. What may be more relevant, however, is that male reproductive costs are skewed toward the cost of competing for a place in the mating pool, resulting in a low marginal cost of reproductive events for those males who gain access to the pool.

The second principle is more precise than the first, and generates some hypotheses on male choosiness. Firstly, males are expected to be choosy if their costs are tied to reproductive events and these costs are substantial relative to the benefits they derive from the events. For example, sex-role reversal occurs in pipefish, where males care for offspring via a costly form of 'pregnancy' (Berglund and Rosenqvist, 2003). As indicated by the loss of up to 27% of their body weight, male Mormon crickets, *Anabrus simplex*, incur

substantial costs when producing large spermatophores which are consumed by females after mating. These males tend to be choosy, preferring larger more fecund females (Gwynne, 1981). Similarly, males of the southern bottletail squid, *Sepiadarium austrinum*, discriminate between females according to their reproductive maturity and fecundity. Males have approximately 50 spermatophores when virgin, transfer approximately 30 at mating, and are therefore sperm-depleted after mating twice (Benjamin Wegener, personal communication). It is currently unknown whether the spermatophore represents parental investment, perhaps because the female acquires nutrients from it, but this information would make no difference to the proposed explanation for male choosiness. More generally, despite the traditional view that spermatogenesis is effectively costless, males in a wide range of vertebrate and invertebrate taxa have been shown to allocate more sperm to fecund females, typically larger or older individuals, a pattern which is modulated by the probability of sperm competition (Byrne and Rice, 2006; Wedell et al., 2002). In a manner similar to female brush-turkeys who allocate more resources to eggs laid in favorable locations in a mound (see above), males allocate more resources where reproductive potential is higher. Although male behavior here represents mating effort rather than parental investment, this once again makes no difference to the proposed explanation for male discrimination, and probably choosiness. Choosiness potentially applies to any aspect of mating behavior, indeed to any behavior whatever, not merely to parental investment.

Secondly, where males defend resources in order to obtain access to females, nevertheless incurring substantial costs associated with reproductive events, male choosiness is expected to correlate with the marginal cost of reproductive events rather than the cost of acquiring, constructing or defending the resource. Nest-defending male fish, for example, are expected to be choosy to the extent that the nest has limited capacity to hold eggs, there is limited capacity to fan a large number of eggs, or the activity of attracting a potential mate is costly, independent of the effort required to acquire, construct or defend the nest. Male two-spotted gobies (*Gobiusculus flavescens*) defend nests, and fan and clean eggs laid by visiting females. Males prefer females with brighter orange-yellow coloration in the belly, a trait which probably indicates egg or offspring viability. In this case, male choosiness has been attributed to restrictions on the male's capacity to care for eggs and the nest's capacity to contain eggs (Amundsen and Forsgren, 2001). Territory-defending male birds are expected to be choosy to the extent that they contribute to post-hatching care and the territory has limited capacity to support females, regardless of the effort required to acquire or defend the territory. This hypothesis could be tested in the wide range of avian taxa where there is conspicuous female coloration, possibly in part the result of male choice (Amundsen, 2000).

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Conclusion

When the above chapters are considered together, certain patterns emerge. Male aggression toward females is ubiquitous in the brush-turkey mating system. Males attempt to obtain copulations by harassing females (chapter 1). By standing close to the female and periodically delivering pecks to her body (up-close aggression), males also respond aggressively to female digging, probing and scratching in the mound (mound activity), especially during egg-laying visits, where there is a clear shift from attempted sexual coercion to up-close aggression in the later phase of visits (chapter 2). Sexual conflict is intense and prolonged during female visits to mounds, with females resisting coercive mating attempts and males resisting female digging, probing and scratching in the mound (chapter 3). Male aggression also appears to underlie the female pattern of assessing males and mounds remotely, at a distance from aggressive male mound-owners (chapter 4). Female brush-turkeys compete with each other for access to mounds partly because access is limited by the duration of female visits to mounds, extended duration being a key strategy used by females to reduce male aggression over a series of visits (chapter 5). Male aggression toward females therefore influences copulation (chapter 1), female choice (chapter 4) and sex roles (chapter 5) in this species. It is also a key component in sexual conflict (chapter 3) and constitutes the primary male response to females during egg-laying visits (chapter 2).

Despite understanding the consequences of male aggression toward females, we have limited understanding of its functions. The intended function of attempted sexual coercion is clear, but it is unclear why it is used as a primary tactic, i.e. by males who own mounds (chapter 1). A negative function could be identified for male up-close aggression, namely that it is not attempted sexual coercion, and therefore signals to the female that she can proceed without risk of damage to the egg during the later phase of egg-laying visits, but this does not explain why there is aggression at all during this phase (chapter 2). Similarly, the battleground for sexual conflict is clear: females attempt mound activity and males resist them; males attempt to copulate coercively and females resist them (chapter 3). Male opposition to female mound activity appears counter-productive, however, given that, in addition to advertising the popularity of the mound, mound activity probably has beneficial impacts on incubation similar to those of male maintenance (chapter 2). Similarly, female opposition to male copulation attempts appears exaggerated, given that females need their eggs to be fertilized and they are overwhelmingly visiting only one male over an extended period (chapter 3). Addled (and presumably unfertilized) eggs are

encountered quite frequently during mound excavations (6 of 46 eggs, i.e. 13% for the largest excavation in the current study, D Wells unpublished data).

A behavioural pattern may be an adaptation, i.e. it evolved because it is functional; it may acquire a different or additional function over time; or it may have no apparent adaptive function, considered in isolation (Fox and Westneat, 2010), as appears to be the case here. Any proposed by-product explanation must be consistent with what is known about brush-turkeys and will be most parsimonious if it simultaneously addresses all of the above unknowns. The hypothesis that females prefer more aggressive males can be rejected, as no evidence was found for it (chapter 4). Male use of attempted sexual coercion as a primary tactic might be partly explained by limited male control over females combined with exclusive control over the mound, but this pattern applies generally to resource-defence polygyny, where attempted sexual coercion is nevertheless infrequent (chapter 1), and it does not appear to provide an explanation for male up-close aggression. The extended duration of female visits provides opportunities for male aggression to occur, but causality may operate more in reverse here, extended visit duration being identified as a female response to male aggression (chapter 3). Phylogenetic inertia may also play a role, but is unlikely to account for the ubiquity of male aggression in this species (chapter 1). In attempting to explain male up-close aggression, we previously hypothesized that male motivation to defend a mound against rival males may be so strong that defensive behavior spills over into other contexts, and males defend their mound against all intruders, including females, especially if females interfere with the mound by digging, probing and scratching in it (chapter 2). Such a behavioural syndrome, however, does not seem relevant to attempted sexual coercion as a primary tactic, the incidence of attempted sexual coercion being independent of female behavior toward the mound (chapter 2). It is moreover difficult to interpret attempted sexual coercion as directed at defending the mound, when its object is to obtain something from the female herself.

The most parsimonious by-product explanation proposes that, for life-history reasons, brush-turkeys are almost universally agonistic. As for all megapodes, brush-turkey chicks lead an independent existence from the time of hatching and generally do not aggregate on an ongoing basis until they have become juveniles (Göth and Jones, 2003). In contrast to most other megapodes, which are monogamous (Jones et al., 1995), no subsequent experience provides a countervailing influence, as no pair-bond ever develops. To an unusual degree, therefore, brush-turkey social life is marked by the absence of cooperation and a bias toward aggression and resistance to aggression as the default solutions to social conflicts of interest, presumably including conflict over mating. The strength of this suggestion is that it potentially explains not only male aggression

generally toward females, but also the antagonistic female response. A potential weakness is that it does not provide an obvious account of why male up-close aggression occurs specifically in response to female mound activity (chapter 2). Such an explanation can nevertheless be suggested.

During female visits, the male's objective is to obtain copulations while the female's objective is mound activity. The male directs his efforts, not only to achieving his own objective, but also to frustrating the female's objective. Although, for reasons outlined above, the frustration of female mound activity is probably against his own interest, the male's focus seems to lie elsewhere, on the fact that it is against the female's interest. The female similarly directs her efforts to frustrating the male's objective as much as to achieving her own. For both parties, the conflict seems to have imperatives which override other considerations, as you would expect if both treat aggression and resistance to aggression as the default solutions to social conflicts of interest. Although male up-close aggression is in general stimulated by female mound activity, it is most likely to be stimulated specifically during egg-laying visits, out of proportion to the mound activity which occurs (chapter 2). This may be because, during the later phase of egg-laying visits, the male is obliged to give up all prospect of copulation. An aggressive response to this circumstance might be interpreted as spite, with both the actor and the recipient of the act suffering a loss (Hauser et al., 2009), but we have insufficient understanding of the balance of costs and benefits to draw this conclusion. For example it is possible that males are accepting a short-term loss in order to make a longer–term gain, i.e. up-close aggression may be inflicting costs on females for some benefit which is not evident in the noise generated by the conflict.

In summary, brush-turkey life history generates a behavioural syndrome in which aggression and resistance to aggression become the default solutions to social conflicts of interest. Sexual conflict is intense and prolonged, with both sexes engaging in unvarying and cyclical perseverance to a degree which appears dysfunctional. Males seek copulations coercively, even though they control a resource which is essential for female reproduction, and they employ a different form of aggression (up-close aggression) when they are not being sexually coercive. On this interpretation, we failed to identify a functional explanation for up-close aggression because no significant function exists, apart from any acquired as a by-product of other behaviours. For example, prior to egg-laying, up-close aggression does have the negative function of not being attempted sexual coercion, thereby signaling to the female that she can proceed with egg-laying without risk of damage to the egg arising from copulation attempts. A behavioural syndrome of near universal agonism re-states our original problem. Rather than enquiring why the

male is so aggressive, we enquire in what circumstances he is predictably non-aggressive, the answer being during the few minutes around egg-laying.

Male aggression toward females has one further important consequence which we have only briefly touched upon. One can imagine a version of the brush-turkey mating system in which females separate mate choice from mound choice by copulating with males judged to be high quality on some basis which is independent of mound quality, but then laying their eggs in the highest quality mound (Jones, 1987). Although genetic confirmation is lacking, the female visiting pattern strongly suggests that this is unlikely to happen. Females treat the male and his mound as a package by overwhelmingly laying eggs in the mounds of males with whom they copulate (chapter 3). Females also lay a series of eggs with a single male, as though constrained by an optimum 'clutch size', when no such consideration is expected to be relevant. Females may treat the male and his mound as a package because independent assessment of each component finds the highest quality males to own the highest quality mounds. This is unlikely, however. It is more likely that females treat the male and his mound as a package because male aggression forces them to make their assessments remotely, from which distance male-mound (i.e. package) information is most informative, in particular the degree to which the male maintains his mound. Viewed in this light, females have limited capacity to assess males independently of mounds, and even less capacity to assess mounds independently of males. If this interpretation is correct, and mound building and maintenance skills are heritable, then male aggression is at least partly responsible for the survival of the brush-turkey mating system. If females copulated with the highest quality male, but then laid their eggs in the highest quality mound, mound building and maintenance skills would become less common in the population, even if females continued to insist on only mating with mound-owners.

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Ethics Approval

ANIMAL RESEARCH AUTHORITY

MACQUARIE UNIVERSITY ~ SYDNEY

AEC Reference No.: 2007/014
Approval Duration: 16 August 2007 to 15 August 2010

To: **Dr David Alan Wells (PI)**
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       Turramurra NSW 2074
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Other participant (s)
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Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

Title of the project: BRUSH-TURKEY MATING SYSTEM

Type of animal research and description of project:
Research (Wildlife) – The project aims to understand the brush-turkey mating system, specifically: female mound-visiting and copulation patterns, fertilization resulting from copulations at the time of egg-laying compared to copulations not associated with egg laying, female choice and male strategies in response to female behaviour. The study will be carried out by banding and attaching radio collars to selected adult birds, observation and camera monitoring of mounds and then careful excavation of mounds identified as likely to contain eggs. Eggs from identified mounds will be transport to Macquarie University Central Animal House for incubation and hatching. Chicks that hatch will be measured and weighed and a tail feather extracted for DNA analysis. Chicks will then be transported back to the original site of egg collection and released.

Species of animal: Australian brush-turkey *(Alectura lathami)*

Number:
Banding: up to 130 birds may be banded in accordance with the Australian Bird and Bat Banding Scheme
Radio-collaring: up to sixty (60) birds may have an appropriate radio collar device attached
Mounds: up to six (6) mounds may be excavated each breeding season for two breeding seasons
Eggs: up to sixty (60) eggs may be excavated and hatched per breeding season for two seasons

Location:
Pearl Beach and adjacent areas of Brisbane Waters National Park
Central Animal House Macquarie University (incubation and hatching)

Amendments considered by the AEC during last period: N/A

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

Approval was granted subject to compliance with the following conditions: N/A

(This authority has been issued as the above conditions have been addressed to the satisfaction of the AEC)

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **16 August 2007** to **15 August 2008**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a progress report at the end of this period.**

Date: 29/08/07

Assoc. Prof. R Harcourt
Chair of AEC, Macquarie University

Form C (issued under part IV of the Animal Research Act, 1985)