

Paternal care in the Australian brush-turkey, *Alectura lathami*, a megapode bird with uniparental male care

Sharon M. Birks

Section of Neurobiology and Behavior, Seeley G. Mudd Hall, Cornell University, Ithaca, NY 14853, USA

Male Australian brush-turkeys, *Alectura lathami*, provide all parental care by building and tending large incubation mounds. Females visit and lay eggs in the mounds of several males sequentially, but they provide no parental care after laying. Because males and females meet only briefly at mounds to copulate and lay, males have no obvious means of ensuring paternity. I used DNA fingerprinting techniques to determine paternity for 65 brush-turkey chicks. Eighteen chicks (27.7%) did not match the mound-tending male. Some of these paternity exclusions were evidently caused by females switching rapidly from one mound to another, but the majority (23.1% of eggs) appeared to result from females copulating with males other than the one in whose mound they were currently laying. However, the frequency of these copulations (4.5%) was much lower than the estimated frequency with which they fertilized eggs, perhaps because their timing during the ovulatory cycle differed relative to most other copulations. The percentage of eggs excluded in paternity analyses ranged from 20.0% to 43.8% for individual males but did not appear to affect male parental care. Several factors favor male parental care regardless of paternity. Males can accommodate eggs from several females in one mound, which increases the opportunities for additional matings without increasing the cost of parental care. In addition, paternity appears to be unpredictable and hard to assess, and a facultative reduction in care would be difficult without abandoning a mound entirely. **Key words:** *Alectura lathami*, Australian brush-turkey, DNA fingerprinting, extrapair copulation, male parental care, megapode, paternity. [*Behav Ecol* 8:560–568 (1997)]

Though female animals initially invest more than males in the production of gametes and young, males may contribute significantly to the care of offspring, usually by guarding, incubating, or feeding them. The extent of male parental care varies enormously across taxa (for a review see Clutton-Brock, 1991; Ridley, 1978), but the ecological and social factors underlying this variation are not well understood. When males provide parental care, they often risk investing in young they did not sire, and low confidence of paternity is a common explanation for the difference in male and female tendencies to care for young. While males should evolve adaptations to help guarantee that they invest in their own offspring, these can be hindered by counter-adaptations by other males, and/or by selection on females to mate multiply.

Trivers's (1972) synthesis of the relationship between parental care and sexual selection generated an enormous amount of theoretical and empirical research into the evolution of male parental care. Despite this, the extent to which paternity should affect male care is still unclear. Theorists have produced models that predict no influence of paternity on male care or some influence depending on the costs of parental care and trade-offs with other reproductive opportunities, the variation in paternity, and the opportunity to assess paternity (for reviews and recent discussions, see Owens, 1993; Westneat, 1995; Westneat and Sherman, 1993; Whittingham and Liffield, 1995). Early models (Grafen, 1980; Maynard Smith, 1978) predicted no effect of paternity on male care. However, these models assumed equal paternity of all broods and no cost to care other than lost mating opportunities—assumptions that are usually unrealistic and make the

models difficult to apply to most species. Recently, modelers have attempted to make some of their assumptions more realistic by including mortality costs to parental care, varying paternity among broods, and/or allowing individuals to assess paternity of broods; all models have predicted that paternity should affect parental care in some situations (Werren et al., 1980; Westneat and Sherman, 1993; Whittingham et al., 1992; Winkler, 1987; Xia, 1992).

Empirical studies combining genetic analyses of paternity with behavioral information have produced varied results. Males of some species reduce parental investment when their paternity is likely to be low (Davies et al., 1992; Dixon et al., 1994; Hartley et al., 1995; Møller and Birkhead, 1991, 1993; Weatherhead et al., 1994), while others show no decrease despite low probability of fathering offspring (Liffield et al., 1993; Stutchbury et al., 1994; Westneat, 1995; Westneat et al., 1995; Whittingham and Liffield, 1995). Thus, it is clear from both models and empirical work that many factors influence how and when paternity will influence male care. Good information on ecology and mating systems is needed to make specific predictions about the relationship between paternity and male care, but this information is lacking for most animals (Clutton-Brock, 1991; Westneat and Sherman, 1993). For example, it is often not clear how costly particular forms of paternal care are, how likely males are to be able to control or assess paternity, and what other reproductive options males may have if they reduce parental care.

Bird species with uniparental male care (e.g., some ratites, tinamous, and shorebirds) are rare (Lack, 1968), but they offer excellent opportunities for exploring how paternity influences paternal care. Males of these species typically invest heavily in the care of young and could pay a high cost in terms of wasted reproductive effort if they care for others' offspring, yet little is known about paternity in most of these species (but see Oring et al., 1992). For example, some species with uni-

S. M. Birks is currently at the Department of Zoology, Box 351800, University of Washington, Seattle, WA 98195, USA.

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parental male care are polyandrous and/or polygynous; males may incubate eggs and care for young of several females.

The unusual behavior of these males leads naturally to several questions, such as, Do such species usually have special ecological adaptations or conditions that make paternal care less costly for them than it is for other birds? How often do these males care for unrelated offspring? Do species with uniparental male care generally have paternity levels higher than species with less male care? Do males care for eggs or young regardless of paternity, or do they respond facultatively by decreasing investment when paternity is likely to be low? Here I provide information about paternity in the Australian brush-turkey, *Alectura lathami*, a multiple-mating bird with uniparental male care, and attempt to answer some of these questions for this species.

The study species

Australian brush-turkeys belong to the family Megapodiidae, birds that use environmental heat sources to incubate their eggs (Jones and Birks, 1992; Jones et al., 1995). They are large (approximately 2.2 kg) galliform birds common in scrub and rainforest along the east coast of Australia. Males of this species are the sole providers of parental care; they build large incubation mounds which they maintain for several months each year (Jones, 1988a). Mound construction takes from 1 to several weeks and involves raking up a considerable amount (usually several tons) of organic material and dirt from the forest floor. Once constructed, mounds gradually reach a stable incubation temperature of about 33°C and are thereafter tended daily by the male, who mixes in fresh material, aerates the decomposing vegetation, and fills holes made during laying (Seymour and Bradford, 1992).

Male brush-turkeys build one to three mounds each year and may usurp mounds of competitors (Jones, 1990a). Females may visit many males during the breeding season and usually lay in the mounds of several (Birks, 1992; 1996). They typically lay one egg every 2–5 days, and some lay > 20 eggs per year (Baltin, 1969; Birks, 1996; Fleay, 1937). Chicks dig their way out of the mound after hatching and receive no further parental care (Jones, 1988b).

Male and female brush-turkeys generally do not associate away from mounds (Jones, 1990b). Though females typically mate with males immediately before laying, they can visit and mate with multiple males (Birks, 1996). Since copulations at the time of laying cannot fertilize the current egg, mound-tending males have no obvious means of ensuring paternity. Here, I report the results from a study of paternity in brush-turkeys in which I used DNA fingerprinting techniques and behavioral observations to determine (1) how often males cared for eggs they did not fertilize and (2) whether the occurrence of such eggs was predictable based on behavioral observations of males and/or females. In addition, I discuss the ecological factors that may favor paternal care by males in this species despite the males' lack of control over paternity.

METHODS

Field methods

I studied a population of brush-turkeys in and near North Tamborine Environmental Park (NTEP), Southeast Queensland, Australia, from July to December 1989–1992. A detailed description of this site is given by Jones (1987, 1988c). NTEP comprises approximately 8 ha of subtropical rainforest containing 9–14 ($\bar{x} \pm \text{SD} = 11.0 \pm 2.2$) active brush-turkey mounds each year. Mounds are scattered throughout the park and on adjacent private properties. I numbered and mapped

active mounds, and inactive mounds were monitored for activity weekly.

Brush-turkeys were free to move within the study site, surrounding residential area, and in several other nearby wildlife preserves. Adult birds were caught in drop-traps baited with cracked corn and were marked with numbered steel leg-bands and sex-specific colored patagial wing-tags (blue for males; yellow or orange for females). All adults in the study population were marked by 1990; juveniles and adults that joined the population were marked each year thereafter. Because many individuals were unmarked in 1989, I used only data from 1990 to 1992 for analyses here. A total of 9 males ($\bar{x}/\text{year}: 8.0 \pm 2.2$) and 19 females ($\bar{x}/\text{year}: 11.5 \pm 1.7$) bred in the study site during at least one of these observation years.

Previous studies at NTEP by Jones (1987) showed that 96.3% of egg-laying, 93.5% of copulations, and 76.2% of females visits occur in the early morning and that sexual activity is confined to incubation mounds or areas immediately adjacent to mounds. Thus, during my study, most behavioral observations were made in the morning (dawn to about 0900 h) from hides and (in 1991 and 1992) with three to four Sony CDF-55 video cameras placed on tripods at active incubation mounds.

All active mounds within the park were observed regularly. Usually three to six mounds were watched simultaneously using multiple observers and/or video cameras; observations were generally more frequent at mounds that were receiving the most female visits. Most mounds were watched for 3–5 days each week; behavioral observations at mounds totaled 898 watches and 2316 h. Detailed information was collected on all male and female interactions at mounds, including copulations, egg laying, and inter- and intrasexual aggression.

In addition to mound observations, behavior was noted during 200 h of afternoon mound measurements, 260 h of trapping brush-turkeys in the afternoon or recording interactions at feeding areas, 10 h of observations at roost sites just before roosting, and many afternoons spent walking through the park to monitor active mounds or dig up eggs for paternity analysis. Two mounds were within sight of the field residence and were monitored casually during the afternoon: no copulations or egg-laying were observed in the afternoon or away from mounds.

Blood collection

I collected about 1 ml of blood from the brachial vein of each adult bird at the time of capture and mixed it either with an equal amount of STE buffer (10 mM Tris, 10 mM NaCl, 2 mM EDTA; pH 8.0) or with a 1:10 ratio of Queen's lysis buffer (Seutin et al., 1991). Blood was stored in liquid nitrogen in the field and was kept frozen on dry ice during transport.

To collect blood samples from chicks, I used the following procedure: Eggs were numbered with a pencil or permanent ink pen within 1–5 min of being laid, and their location within the mound was mapped using a tape measure and several location coordinates (e.g., distances from trees or vines within or near the mound). Both the laying female and mound-tending male usually left the mound during egg-marking but quickly resumed normal behavior after marking. After marking, eggs were partially buried to keep them warm. The tending male always returned and finished burying the egg, usually within a few minutes. Eggs were left in mounds for 30–40 days to incubate under natural conditions, then were dug up. Occasionally unmarked eggs were found and collected, though the female who laid them was unknown. Recovered eggs were placed in large Styrofoam coolers containing fresh mound material kept warm with hot water bottles during transport to nearby (1 h away) Griffith University, where they

were placed in a Linder and May growth cabinet maintained at 35°C and 85–95% humidity. Chicks with similar laying dates were incubated in separate boxes to prevent mix-up at hatching. About 2 days after hatching (when they would normally be emerging from the mound), I took a small blood sample (100 µl) from the brachial vein of each chick. Chicks were then transported back to the study site and released.

DNA fingerprinting

I conducted all molecular analyses in the laboratory of C. F. Aquadro at Cornell University, using standard techniques detailed elsewhere (Birks, 1996; Westneat, 1990). I extracted nuclear DNA from blood and digested it with the restriction enzyme *Hinf*I. The resulting fragments were size-sorted by gel electrophoresis, blot-transferred to a nylon membrane, and probed using radioactively labeled 18.15 probe (provided by A. Jeffreys; see Jeffreys et al., 1985a,b).

I scored bands following Westneat's (1990) procedures. The identity of most chicks' mothers was known from observing eggs being laid; the putative father was the mound-holding male. Fingerprint gels were arranged so that chicks were run alongside their mothers and putative fathers, and comparisons were usually between individuals separated by ≤ 2 gel lanes. Some individuals (e.g., mound-holding males) were run multiple times per gel to facilitate band alignment. Most gels contained all the putative offspring and their mothers from one to two males' mounds during 1 year. Bands were drawn with colored pens on an acetate overlay marked with a fine grid, and their position was compared to that of bands in neighboring lanes. Individuals were not scored blindly; I knew whether they were males, females, or chicks.

I scored bands as identical if they were no more than 0.5 mm apart (vertical migration distance) and were of similar intensity (< 2 -fold difference). Bands were scored as "novel" if they were not shared with either the mother or putative father. Novel bands were scored conservatively: only those that were clearly different from the mother and putative father were considered novel. Some bands could not be scored either because (1) they were opposite a band of greater intensity or an imperfection in the fingerprint that would have obscured their presence or (2) the sample from the individual in the comparison lane did not have enough DNA for a light band to show up. The percentage of band-sharing between any two individuals was calculated as two times the number of shared bands divided by the total number of bands scored in both lanes (Wetton et al., 1987). Number of scorable bands/lane varied with the individual scored, the individual to whom it was being compared, and quality of the DNA samples and fingerprint. I scored an average of 27.4 (± 6.34 SD; $n = 58$) bands for females, 26.2 (± 6.26 SD; $n = 123$) for chicks, and 26.2 (± 5.94 SD; $n = 65$) for males. Sample sizes refer to the number of dyads, which are comparisons of females to offspring, offspring to mothers and putative fathers, and males to putative offspring, respectively.

I determined paternity using data from novel fragment and band-sharing analyses. Novel bands can occur because of mutation, extrapair fertilization, or through error in assigning social parents. Misassignment of putative male parents in this study was unlikely because the identity of the mound-tending male was obvious and did not usually change during the breeding season. Because I and my assistants watched eggs being laid, misassignment of the female was also unlikely. However, if eggs were partially buried by females before we marked them, and if there were many eggs already present in the mound, there was a small possibility the wrong egg could be marked. Another possible source of error was misassigning chicks to eggs after hatching. To control for this, I matched

Table 1

Distribution of collected eggs and paternity among individual males

Male ID	Year	Mound	Female ID ^a	Eggs	Eggs aired	Eggs excluded	% Excluded ^b
519	90	103	306	3	0	3	25.0%
			228	3	3	0	
	91	103	232	4	4	0	
			?	2	2	0	
521	90	115	221	5	4	1	23.5%
			13+	4	4	0	
			191	6	3	3	
			226	1	1	0	
			?	1	1	0	
526	90	107	229	1	0	1	
527	90	2	227	1	1	0	
			?	1	1	0	
532	91	39	231	3	1	2	43.8%
			227	2	1	1	
	92	109	+11	5	5	0	
			15+	3	0	3	
			306	2	2	0	
			?	1	0	1	
536	90	108	306	2	2	0	
537	90	13	191	2	1	1	20.0%
			112	3	3	0	
	92	8	191	1	1	0	
			229	1	1	0	
			230	1	0	1	
			319	3	2	1	
			13+	2	2	0	
			?	2	2	0	

^a ? = Female was unknown.

^b The percentage of eggs excluded was calculated only for males from whom ≥ 10 eggs were analyzed for paternity.

known mothers independently to chicks using band-sharing coefficients to estimate relatedness. One chick could not be matched to its mother and was omitted from further analyses.

RESULTS

Egg distribution

I successfully analyzed 65 chicks for paternity. These chicks were hatched from eggs collected during 3 years from 7 males at 10 mounds (Table 1). Fourteen females laid at these mounds, for a total of 20 different male/female pairings. The number of chicks analyzed from each mound averaged 6.7 (± 4.85 SD; range: 1–17) and, for each male, 9.57 (± 6.47 SD; range: 1–17).

Genetic parentage

For 58 of the 65 chicks for whom scorable fingerprints were generated, I knew the identity of both the mother and mound-tending male. Seven chicks hatched from unmarked eggs, and for one comparison the mother was mistakenly omitted from the gel; for these eight comparisons only the male was known, and thus novel band analysis was not possible. For these chicks, I determined paternity from band-sharing coefficients (see below).

Of the 65 chicks analyzed, 17 had ≥ 3 novel bands ($\bar{x} = 5 \pm 1.3$ SD; Figure 1). An additional chick whose mother was

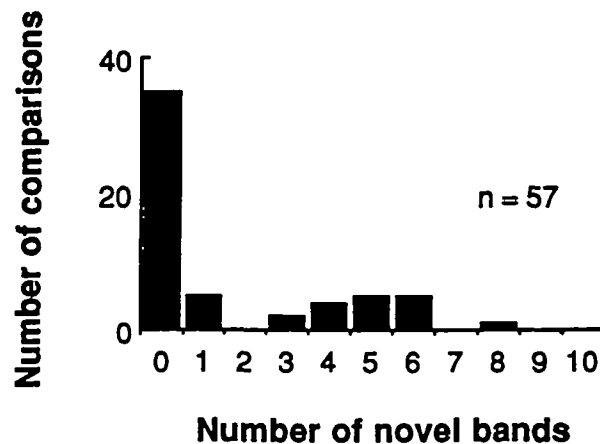


Figure 1
Novel band distribution for all male-chick comparisons, when the mother was known. Comparisons with ≤ 1 novel band probably result from mutation, whereas the presence of ≥ 3 novel bands indicates that a different male sired the egg (see text).

unknown was excluded because of its low band-sharing coefficient (Figure 2a), giving a total of 18 chicks (27.7%) that did not match the mound-tending male. Forty-seven chicks (72.3%) had ≤ 1 novel band and/or a high band-sharing coefficient with the mound-tending male.

Mutation is expected to produce small numbers of novel bands. Single novel bands occurred in 5 chicks (8.8% of those chicks with both parents assigned), giving a band mutation rate of about 1 in 300, which is comparable to mutation rates found in previous studies of birds (Hartley et al., 1993; Jeffreys, et al., 1985a; Westneat, 1993).

The number of novel bands should be higher when a parent has been misassigned, and an estimate of the number of novel bands expected for a parental misassignment can be derived given the average number of bands scored for each individual and the background proportion of bands shared in the population (Westneat, 1993). In the NTEP brush-turkey population, 21 apparently unrelated individuals shared $0.41 (\pm 0.078 \text{ SD})$ of their bands (number of bands scored = $28.43 \pm 4.4 \text{ SD}$; shared = $11.62 \pm 2.64 \text{ SD}$). The average allele frequency (q) was 0.232 ($x = 2q - q^2$) (Jeffreys, et al., 1985b). A parent would be expected to share $(1 + q - q^2) / (2 - q)$ bands with its offspring (Jeffreys et al., 1985a) or 0.666, leaving 0.334 of the offspring's bands (about 9 bands) that should have been shared with the other parent. If that parent is mismatched, then 0.41 of these bands (about 4) would be expected to be shared between the two parents due to the background level of band-sharing in this population, leaving about 5 novel bands (Westneat, 1993).

The average number of novel fragments for chicks with > 1 novel fragment was $5 (\pm 1.27 \text{ SD})$, as predicted (Figure 1). Some variation is expected due to physical differences among fingerprints and in the number of bands inherited from each parent. In this case, the number of novel fragments (for chicks with > 1) ranged from 3 to 8. The distribution for novel bands was discontinuous, as expected (unless mutation rate is high). Thus, I assumed any chick with ≤ 1 novel fragment was sired by the male tending the mound where the egg was collected, while chicks with ≥ 3 novel fragments were sired by other males.

I used band-sharing coefficients to confirm these conclusions and to assign paternity to the eight chicks whose mother was unknown or not present on the gel (Figure 2a). Chicks shared 0.73 ± 0.082 of their bands with their mothers (Figure

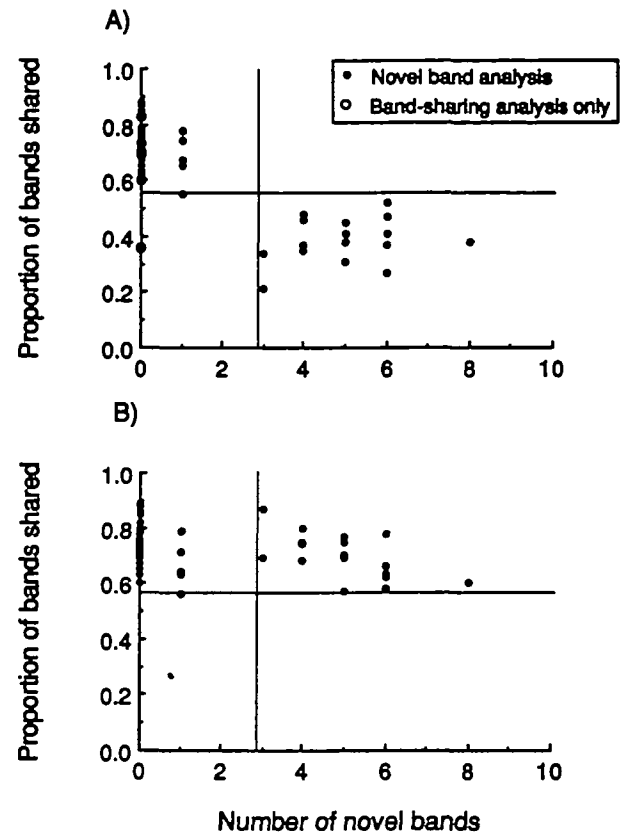


Figure 2
Relationship between band-sharing proportions and number of novel bands for (A) putative fathers and chicks hatched from eggs in their mounds and (B) known mothers and their chicks. Comparisons between males and chicks with known mothers ("novel band analysis") and without known mothers ("band-sharing analysis only") are shown separately panel A. Dashed lines indicate the criteria for excluding parentage (see text).

2b). Since these comparisons are between known related individuals, band-sharing coefficients between chicks and their fathers should also fall within this distribution. Assuming a normal distribution (Snedecor and Cochran, 1989) and a mean and variance estimated from mother/chick comparisons, the lower one-tailed 99% confidence limit for related individuals is 0.56. Because all comparisons between chicks and putative fathers that generated ≥ 3 novel bands fell below this level, and nearly all comparisons generating ≤ 1 novel band fell above it, my conclusion that chicks with ≥ 3 novel bands were fertilized by another male was confirmed. In one comparison, a chick with one novel band had a band-sharing coefficient of 0.55. Given that this chick was right on the edge of the distribution and had only one novel band, I concluded it was sired by its putative father. For the eight comparisons without novel band analysis, seven fell within the distribution for unrelated individuals generated from known mother/offspring comparisons, and one fell well outside this distribution and was thus assumed to be unrelated to the putative father (Figure 2).

Three of four males had similar percentages of excluded offspring (20–25%), but one male had 43.8% (Table 1). While these differences are not statistically significant with this small sample size ($\chi^2 = 1.95$, $p = .58$, testing the null hypothesis that the number of excluded offspring should be equally distributed among males), they nonetheless indicate the poten-

Female I.D.

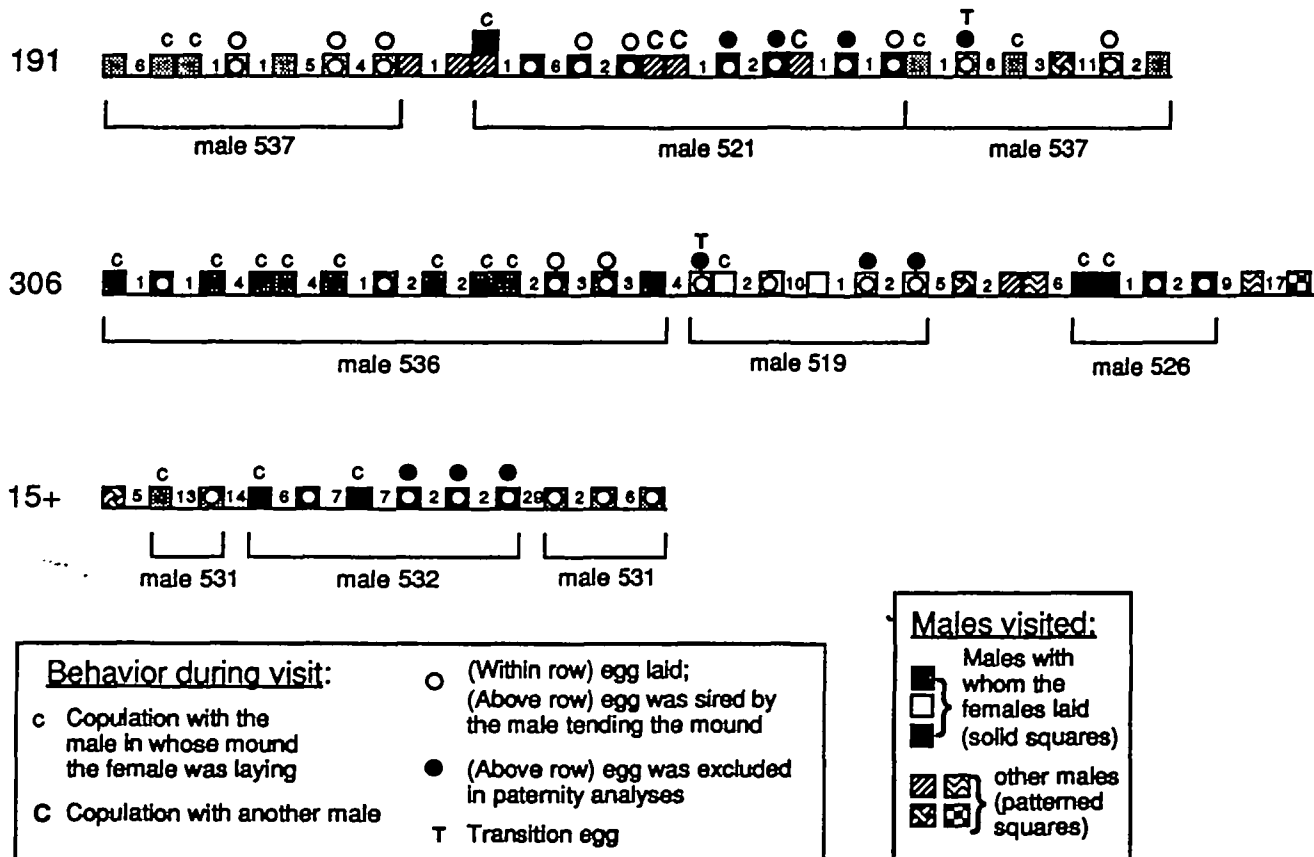


Figure 3

Egg series for the females who laid 10 of the 18 eggs excluded in paternity analyses. Each square represents a separate mound visit on 1 day; different shades or patterns indicate which males were visited. Males in whose mound the females laid are indicated below each line and are represented by solid-filled squares; other males visited are represented by patterned squares. Visits are in chronological order and occurred within one breeding season for each female, but not necessarily on consecutive days. The number of days that had passed between observed visits is indicated in the space between those visits. Egg symbols *within* rows include all eggs observed laid. Females always copulated before laying during these visits. Egg symbols *above* rows indicate eggs that were analyzed for paternity. Only the transition eggs analyzed for paternity are marked with a "T."

tial for large differences in paternity for different males, either because of random variation or because of female preferences for particular males or mounds.

Effect of female behavior on paternity

To determine whether paternity was behaviorally predictable, I examined the behavior of females during the time when they laid eggs that were analyzed for paternity. Female brush-turkeys normally mated with and laid eggs in the mound of one male for a period of several weeks before changing rapidly (within a few days) to a new mound (and thus usually to a new male; Figure 3). Females were considered to have "switched" mounds when they laid their first egg at the new mound (Birks, 1992, 1996). Out of 32 cases of mound-switching that occurred, females were observed copulating with new males 47% of the time (on 15 occasions) before laying in their mounds; these females made on average 1.5 (± 1.1 SD) visits and copulated 2.1 (± 1.4 SD) times before laying began. However, on 17 occasions (53%), females laid at a new mound apparently without copulating with the tending male beforehand.

Unless a female copulated with the new male before laying

in his mound, her first egg (and possibly subsequent eggs) laid there would probably be fertilized by the previous male. Because these first eggs were laid in a different behavioral context than most others (i.e., just after switching mounds), they were classified as "transition" eggs. Transition eggs were defined as the first egg seen laid with male B, if the female had mated with a previous mound-owner (male A), with whom she had been laying, within the past 14 days. Fourteen days was chosen as a reasonable estimate of sperm viability in a gallinaceous bird that mates frequently (every few days) during the breeding season (Birkhead and Møller, 1992). It is possible that some eggs classified as transition were not actually the first egg laid with male B (e.g., if the mound was not observed on the day the female laid her first egg there), and it is also possible that more than one egg laid with male B could be fertilized by sperm remaining from male A, but this classification would still predict roughly the minimum proportion of all eggs that were likely to be fertilized by male A due to females switching mounds.

On one occasion, a female interrupted a series with one male to lay one egg with another male, then she returned to the previous male. In this case both the egg laid with male B

and the first laid upon returning to male A were classified as transition eggs. Females occasionally were not seen laying for several weeks between switching mounds. Since their breeding status was unknown, their next egg was labeled "unclassified." Out of 214 eggs seen laid over 4 years, 10.3% ($n = 22$) were transition, 5.6% ($n = 19$) were unclassified, and 84.1% ($n = 180$) were laid as part of a series with one male (including the first egg of each breeding season). However, for various reasons (e.g., several transition eggs failed to hatch, and many others were observed by video camera only and thus were not marked), transition eggs were underrepresented ($n = 3$) in the sample of eggs analyzed for paternity.

Of 670 copulations observed during 712 female visits to mounds, the majority (87.0%; $n = 583$) were with the male in whose mound the female was currently laying; only 4.5% ($n = 30$) were with other males (Birks, 1996). Fifty-seven copulations (8.5%) could not be placed in either of these categories because the female's breeding status at the time of the copulation was unknown. Since these females were occasionally seen at roost sites or other mounds nearby, they were assumed to be laying elsewhere.

If mound-switching resulted in loss of paternity for the first egg of each new series, and if copulations with other males had an equal chance of fertilizing eggs as copulations with the male in whose mound a female was laying, then the frequency of these two behaviors should have been a good predictor of paternity. However, the frequency of excluded young predicted from known copulations with other males and from mound-switching was lower (13.8%) than the actual frequency (27.7%) based on the genetic results. In most cases there was no behavioral evidence to indicate whether excluded young were the result of mound-switching or copulations with other males. In only one case was a female seen soliciting copulations from a male other than the one in whose mound she was laying, before laying three eggs that produced offspring that were excluded in the paternity analysis (female 191; Figure 3). In two cases females laid transition eggs that were sired by a male other than the owner of the mound where the eggs were laid (females 191 and 306; Figure 3).

In general, paternity was higher for males in whose mounds females laid at the beginning of the breeding season than for males that females switched to later on ($\chi^2 = 3.98$, $df = 1$, $p = .05$); on average, 16.1% of eggs (5 of 31) laid with first males were excluded in the paternity analysis, versus 44.4% (12 of 27) laid with later males. This could indicate that sperm from matings with early males continued to fertilize eggs long after mound-switching. However, of the 15 nontransition eggs that were excluded and where the female was known, 4 were laid by females (221, 228, and 319) who had had no previous mate. Thus, these exclusions could not result from mound-switching. For the 11 remaining eggs, it had been a minimum of 11–38 ($x = 24.0 \pm 9.4$ SD) days since the female had last been seen mating with the male at the previous mound, and at least 1–8 ($x = 3.8 \pm 2.4$ SD) copulations with the new male had occurred in the interim (Figure 3). For these eggs to have been fertilized by previous mates, females would have had to store the sperm for unusually long periods of time and use this preferentially over sperm from recent matings. This seems unlikely given current knowledge indicating last-male sperm precedence, possibly via passive sperm loss, in at least some birds (Birkhead and Møller, 1992). Thus, most paternity exclusions (15 of 17) appear to result from females copulating with additional males rather than switching mounds, though the proportion of total eggs evidently fertilized by these males (23.1%) was much higher than that predicted from behavioral observations of copulations with them (4.5%).

DISCUSSION

The results from this study show that in some situations males may provide substantial parental care even when they have little control over paternity and when paternity is not uniformly high. In this population of Australian brush-turkeys, 27.7% of 65 chicks analyzed for paternity were sired by a male other than the one tending the incubation mound in which the chick's egg was laid ("excluded" eggs or chicks), and in the most extreme case, 43.8% of the chicks analyzed from one male's mounds were sired by other males.

Some females apparently switched mounds without copulating with the new male before laying, and those that did copulate before switching probably still stored sperm from the previous male. Two of the three transition eggs analyzed for paternity were excluded, indicating that most transition eggs were probably sired by previous males. Thus, most males that started their first mound late in the breeding season, and all males building second mounds, had a high probability of caring for some eggs they did not fertilize.

Although brush-turkeys do not have any pair-bonds in the traditional sense (Jones, 1990b), male brush-turkeys would be expected to incur a cost in lost reproductive effort if they cared for many eggs they did not sire. Only 30 (4.5%) of 670 observed copulations were with males other than the one in whose mound the female was currently laying (Birks, 1996), yet up to 23.1% of eggs were apparently fertilized by these infrequent copulations. Because females were acting against the interests of the male whose mound they were using if they visited and copulated with another male during that period, I refer to these copulations as "extrapair" copulations (EPCs), whereas I refer to copulations with the male in whose mound the female was laying as "within-pair" copulations (WPCs). To simplify discussions of copulation behavior, I use these terms here and elsewhere (Birks, 1996) though they differ slightly from some definitions (Westneat et al., 1990) and their use for a promiscuous species is unconventional. However, while these terms are conceptually and semantically useful, it must be kept in mind that in no sense do female brush-turkeys show any loyalty to males, and they may change mounds abruptly at any time. For example, in one case a male died and was replaced by a rival male the next day, but the female who had been visiting that mound continued to copulate and lay there as if no change in ownership had occurred. In addition, male brush-turkeys conspicuously lack some of the typical means of ensuring behavioral compliance from females: there is no mate-guarding, and to secure copulations, males must rely on females to come to their mound.

Effect of the timing of copulations on paternity

Avian eggs are usually fertilized shortly after ovulation, and many birds apparently have last-male sperm precedence (Birkhead and Møller, 1992). Thus, one determinant of copulation success might be the timing of copulations relative to ovulation. If copulations with different males occurred at different times, this might give one male an advantage over the other. Elsewhere (Birks, 1996), I show that most WPCs (63%) in brush-turkeys occurred immediately before laying; studies of other species have shown that copulations during this time period usually have markedly decreased fertilization success (Birkhead et al., 1995). In contrast, all EPCs (by definition) occurred on nonlaying visits, which may have made EPCs more likely to fertilize eggs. Evidence from the paternity analysis is consistent with this idea. For example, two females (15+ and 306; Figure 3) laid a series of excluded eggs (three and two, respectively) despite regular mating with males in whose mounds they were laying, and one female (191) laid a series

three excluded eggs despite equal numbers of visits (three) and copulations (four) with the pair and extrapair males.

Extrapair copulations may be a poor estimator of paternity if they are difficult to observe (Dunn and Liffield, 1994). In Australian brush-turkeys, EPCs were not more difficult to observe than WPCs, nor did they differ behaviorally. Because this study and a previous one at the same site revealed no sign of copulation behavior away from mounds (Jones, 1987) despite hundreds of hours of observations of birds near feeding sites, roosts, and in the forest, I would expect the observed rate of EPCs to be very close to the actual rate. However, the possibility that some copulations occurred away from mounds cannot be ruled out.

The trend for females to lay more excluded eggs at mounds later in the breeding season indicates either that females were using sperm stored from previous matings (e.g., Oring, et al., 1992) or that females engaged in EPCs more often as the breeding season progressed, perhaps in response to increasing availability of males at other mounds. For most paternity exclusions, either the lack of a previous mate or the long period of time between the last observed copulation with the previous mate and the excluded egg(s) ($x = 24.0 \pm 9.4$ days) and the number of observed copulations in the interim ($x = 3.8 \pm 2.4$), make the former explanation seem less likely. Future studies that use larger samples and identify actual fathers could help distinguish between these two possibilities.

Why do male brush-turkeys care for eggs sired by other males?

Incubation mounds reduce the cost of care

The cost of male parental care involves both the immediate effort involved in the care of offspring and the trade-offs involved in providing care versus pursuing other reproductive options. Like other animals, the effort male brush-turkeys put into reproduction may be divided into mating effort (ME; effort expended in acquiring fertilizations), parental effort (PE; effort expended on parental behavior), and somatic effort (SE; effort that increases an individual's chances of surviving until another breeding attempt; Alexander and Borgia, 1979; Low, 1978; Westneat and Sherman, 1993). However, unlike the parental behavior of males of many other species, the effort expended in building and maintaining incubation mounds cannot easily be designated as PE or ME. Instead, this behavior has elements of both PE and ME and is thus similar to other forms of male parental care such as nuptial feeding by insects (Gwynne, 1984) and brood-tending in territorial fishes (Gross and Sargent, 1985; Ridley, 1978). Male brush-turkeys increase the chance of their offspring's survival by guarding the mound and maintaining proper incubation conditions, but their behavior also attracts females for additional matings, because females prefer to lay eggs in mounds that are well maintained (i.e., with particular temperatures and water contents) and are evidently attracted to mounds that have other females visiting and laying (Birks, 1996). In addition, male brush-turkeys apparently allow females to use to their mounds on a *quid pro quo* basis: they are more likely to chase away visiting females who have not previously copulated with them than females who have (Birks, 1996).

Males of most animal species are incapable of caring for more than one female's offspring at a time, which may tend to make parental care less valuable to them than seeking copulations with extra females (Williams, 1975). However, when paternal care evolves as a by-product of male territoriality or resource defense, as it may have in brush-turkeys, males can often accommodate the eggs or young of several females and at the same time attract other females for additional matings, thereby reducing the cost of parental care (Clutton-Brock and

Vincent, 1991; Williams, 1975). Thus, although providing parental care and pursuing mating opportunities are mutually exclusive activities for males of most species (Westneat, 1988), male brush-turkeys can pursue both simultaneously.

In addition, the use of an external heat source for incubation and absence of any parental care after hatching decreases the direct cost of parental care to males and thus reduces the likelihood that males would respond very strongly to variations in paternity. Incubation mounds clearly allow brush-turkey males to care for many more eggs than the males of most other bird species can: the remains of up to 58 eggs have been found in a single brush-turkey mound at the end of a breeding season (Jones, 1987), and I estimated that several mounds received ≥ 40 eggs during my study.

Paternity is unpredictable and difficult to assess

Recent theory predicts that whether and how paternity should affect male parental care will depend not only on the cost of paternal care, but also on (1) the variability in paternity among matings, (2) the predictability of this variability, (3) the degree to which males can assess their paternity, and (4) what options are available to males if they reduce care (Westneat and Sherman, 1993). Paternity should affect paternal care only if it is variable among matings (Grafen, 1980; Maynard Smith, 1978; Westneat and Sherman, 1993), and if it is either predictable in some way (e.g., due to the age of the male, timing of the brood) such that males may evolve a behavioral response over evolutionary time, or if paternity can be assessed directly, such that males may adjust their parental effort within a given breeding attempt (Westneat and Sherman, 1993).

In this study, paternity clearly varied among matings: for some females, all the eggs analyzed from one mound were sired by the male tending that mound, while in other cases, all the eggs were excluded (Table 1). Most of the excluded young appeared to be the result of EPCs. However, most females appeared to engage in EPCs mainly as a means of gaining access to other mounds for evaluation (Birks, 1996). As a result, the distribution of EPCs is probably unpredictable, and in this study no consistent pattern of EPCs was found (Birks, 1996). Thus, there is no reason to expect that males who abandoned mounds with low paternity would have higher paternity at future mounds.

The only predictable source of reduced paternity in this mating system was the exclusions that apparently resulted from females switching mounds rapidly and storing sperm from previous mates. As this behavior seemed to account for only a small part of the lost paternity, and as all males who built mounds later in the breeding season would be affected about equally, it seems unlikely this loss of paternity would have much of an effect on parental care. However, it could result in some selective pressure to build mounds early in the breeding season.

Even if paternity were unpredictable over the long term, it could still affect parental care if male brush-turkeys had some means of assessing paternity of the eggs in their mounds and could then respond facultatively to increase care to those they had sired and decrease care to others. However, males almost certainly lack accurate information about paternity of the eggs within their mounds. Males of bird species with social pair-bonds could potentially evaluate their risk of being cuckolded by gauging the proximity of competitors, the amount of time spent with their mate, or by observing their mates engaging in EPCs (Davies, et al., 1992; Møller, 1991; Whittingham et al., 1993). It is also likely that subtle behaviors associated with extrapair activity would be more noticeable to males who guarded or spent large amounts of time with mates.

However, male brush-turkeys apparently had few if any

means of assessing whether females had engaged in copulations with other males. Males remained in the vicinity of their mounds for most of the day and were chased violently if they approached another male's territory; they were clearly unable to monitor the copulation behavior of females. Other behavioral cues (e.g., the number of copulations they had had with a given female) would probably have been relatively unhelpful given the variation in female behavior, irregular egg-laying intervals, and level of control females had over copulations (Birks, 1996). Even if they could use behavioral cues to assess paternity, male brush-turkeys lack the ability to discriminate among young without abandoning the entire mound and building a new one because eggs from several females are usually distributed throughout incubation mounds, and mounds function essentially as a single unit.

Given these constraints, it was not surprising to find evidence suggesting that male brush-turkeys provide parental care regardless of paternity. For the three mounds with the most complete paternity data, there was no apparent relationship between the length of time a male tended his mound (e.g., before switching to a new one) and paternity in that mound. For example, the male at the mound with the lowest paternity (mound 109 in 1992) tended his mound for the longest period of time (152 days). In addition, in one instance a male died and another male took over his mound, but the latter did not destroy any eggs present.

In summary, the low cost of caring for eggs from several females, unpredictable distribution of paternity, and problems in assessing paternity or facultatively reducing care to some young make it unlikely that male parental care in Australian brush-turkeys would be strongly dependent on paternity. A unique ecological adaptation (the use of incubation mounds) and unusual mating system (promiscuity) have resulted in a relatively weak relationship between paternity and male parental care in this species. This is consistent with recent models of the relationship between paternity and paternal care, which stress the importance of information about paternity, the predictability of paternity, and the costs of caring for young.

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