The Auk 114(2):291–295, 1997

Problems with Removal Experiments Designed to Test the Relationship between Paternity and Parental Effort in a Socially Polyandrous Bird

IAN G. JAMIESON[1,3] AND JAMES S. QUINN[2]
[1]*Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand; and*
[2]*Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1, Canada*

Aspects of sperm-competition research in birds have progressed from purely descriptive studies that establish multiple paternity (e.g. Gowaty and Karlin 1984, Gavin and Bollinger 1985, Westneat 1987) to a more experimental approach that examines the relationship between paternity and male parental effort (e.g. Møller 1988, Koenig 1990, Davies et al. 1992, Whittingham et al. 1993). These experiments take the form of removing resident males during the female's fertile period as a means of disrupting one of the cues that males presumably use to assess their paternity (Schwagmeyer and Mock 1993), and then returning them back to their territory and observing their behavior. Parental care by a removed male is predicted to decrease continuously with decreasing paternity in species with more than one male breeder and caregiver at the nest (Whittingham et al. 1992). In addition, removed males with low or no paternity have been known to destroy eggs upon being released back onto their territory, forcing females to renest and thus allowing the males a further reproductive opportunity (Koenig 1990, Robertson 1990).

We attempted to test the general prediction that paternal effort should decrease with decreasing paternity using the polyandrous Pukeko (*Porphyrio porphyrio*). Pukeko exhibit a variable mating system, with about half of our study population composed of groups with two to three unrelated, co-breeding males. Alpha males do not guard the female during her fertile period and will tolerate copulations by other males within the group. This results in shared paternity and low reproductive skew among males (as shown by DNA fingerprinting analysis), and equal contribution in parental effort (Jamieson et al. 1994, Jamieson 1997). According to the predictions, Pukeko males removed early in the egg-laying period should show a reduced effort in parental care in response to decreased paternity, whereas control males removed after the laying period should exhibit normal levels of parental care.

As we will show, the results of testing the relationship between paternity and parental effort were inconclusive because residents showed unusually high levels of aggression toward removed males that were released back onto their territories for both experimental and control groups. However, we present the results of the experiment to provide a cautionary note on the possible pitfalls of removal experiments on a highly social bird.

Field methods.—The study was conducted at Otokia Wildlife Reserve and an adjacent wetland area located 30 km south of Dunedin on the South Island of New Zealand. Observations and banding commenced in early spring (September) when Pukeko are establishing territories. Birds were caught using funnel and remote-control traps baited with corn and barley and individually color-marked with plastic leg bands. For DNA fingerprinting, 200 μL of blood from the brachial vein of adults, and a maximum of 150 μL from the femoral vein of newly hatched chicks, was collected and stored in a 1.6-ml lysis buffer (Seutin et al. 1991).

Four-hour observation sessions were conducted twice daily (early morning and afternoon/evening) from blinds overlooking the study area and from vehicles along the roadside using binoculars and spotting scopes. Dominance status was determined through interactions during feeding and posturing between group members (see Craig 1977, Jamieson and Craig 1987). The frequency of incubation bouts was recorded using video cameras placed near the nest, or during regular observation sessions conducted during the incubation period. Provisioning of chicks also was recorded during observation sessions.

Beta males were captured and temporarily held in separate cages (2.1 × 0.8 × 1.0 m) near the study site. The removals were carried out under the approval of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals (Application No. 64–93). Birds were visually isolated from each other using shade cloth on the sides of the cages. Each cage contained two to three large clumps of rushes (*Carex* sp.) that served as cover. The rushes were placed in a large metal tray filled with 3 cm of water, and the rest of the floor was covered with straw. Birds were fed daily with corn, barley, commercial poultry pellets, and fresh grass.

Experimental design.—This experiment is part of a long-term study of Pukeko. Between 1990 and 1992, we collected data on number of copulations and proportion of time spent incubating eggs and feeding chicks by alpha and beta males, and relationships to paternity as determined by DNA fingerprinting (Jamieson et al. 1994). For our experimental work in 1993 and 1994, we compared parental effort of removed males with data collected from unmanipulated groups in previous years.

Thirteen removals were carried out in total; 12 in-

[3] E-mail: ian.jamieson@stonebow.otago.ac.nz

TABLE 1 Summary of results of experimental and control removals of Pukeko.

Experimental group	No. of co-breeding males/females	Removed male	Duration of removal (days)	No. of eggs on release	Proportion of offspring fathered by removed male[a]	Aggression towards removed male	Incubated eggs	Fed chicks	Behavior of removed male
Experimentals (male removed after first one to two eggs laid)									
1	2/2	GA–YY	11	11	0/8	Yes	No	2 days	Chased away from nest and eggs several times; fed chicks infrequently when they first hatched then left territory.
2	2/2	WA–RO	9	6	0/5	??	No	No	Left 3 days after release and joined nonterritorial flock.
3	2/2	OA–GR	10	5	2/4	Yes	No	No	Aggression shown by group initially; male remained in territory but away from nest and subsequently bred in later nesting attempt.
4	2/2	OA–WGW	13	7	0/6	Yes	No	Not initially	Aggression by alpha male and females; initially stayed away from nest and chicks; attempted copulations with yearling helper then after a week started feeding chicks; bred in later nesting attempt.
5	3/1	GA–YRY	7	5	—	??	??	—	Stayed away from other group members; 3 days after release 3 eggs destroyed; all eggs destroyed after 6 days; unknown whether group renested.
Controls (male removed after completion of clutch)									
1	2/1	YA–OW	10	5	—	No	Yes	Yes	First seen incubating 4 days after release and regularly thereafter.
2	2/1	WA–ORO	10	5	—	Yes	No	—	Aggression by alpha male; stayed on edge of territory; 3 days after release eggs destroyed; unknown whether group renested.
3	3/2	GA–WO	9	13	—	Yes	No	No	Persistent aggression by alpha male and females; male remains apart from group and leaves territory after 3 days.

TABLE 1. Continued.

Experimental group	No. of co-breeding males/females	Removed male	Duration of removal (days)	No. of eggs on release	Proportion of offspring fathered by removed male[a]	Aggression towards removed male	Incubated eggs	Fed chicks	Behavior of removed male
Controls (male removed after completion of clutch)									
4	3/1	WA-WR	8	7	—	No	No	—	2 days after release male started courting female; after 5 days 1 egg destroyed; after 7 days 2 more eggs destroyed, then remaining eggs; male renested with group.
5	2/1	YA-OG	9	6	—	??	No	No	Upon release, bird was weak and lethargic; appeared fully recovered next day but not seen back on territory and joined nonterritorial flock.

[a] Number of offspring does not equal number of eggs on release because some eggs were lost/damaged or failed to hatch.

volved beta males and one involved a gamma male (a control group). For the experimental groups ($n = 6$), beta males were caught and removed the day the first egg was laid or at the latest, the day the second egg was laid (eggs generally are laid two days apart). The male was kept in the aviary until the clutch was complete, which took nine days on average (range 7 to 13 days). All experimental removals were carried out during the 1993 field season. For control groups ($n = 7$), all males had been observed incubating the eggs before being removed 0 to 8 days after the last egg was laid and held for nine days in the aviary (the average of the experimental groups). Control removals were conducted in the 1994 field season. The two years did not differ with respect to time of laying, number of territories, or density of birds (Jamieson unpubl. data).

DNA fingerprinting.—Fingerprinting analyses were conducted on broods from experimental groups to determine the extent to which paternity had been reduced for removed beta males relative to that of unmanipulated groups from our previous studies (Jamieson et al. 1994). We had intended to examine paternity for control groups, but poor hatching success and egg destruction in four of five groups precluded analyses from being carried out. Fingerprinting methods for Pukeko are described in detail in Jamieson et al. (1994).

Results.—One experimental and two control groups lost nests to predation by Swamp Harriers (*Circus approximans*) while the removed male was in captivity and thus are not included in the analysis below. This reduced the number of experimental and control groups to five each. Experimental and control birds lost similar amounts of body mass (15.2 ± SE of 2.4% and 14.8 ± 3.6%, respectively) in captivity (Wilcoxon rank-sum test, $W = 29.5$, $P = 0.75$). Although high, mass loss was not deemed excessive for a relatively large ground bird (1,100 g) that only rarely flies. Upon release, all but one male (see below) appeared healthy and behaved normally.

DNA results indicated that the removed beta males in experimental groups fathered fewer offspring relative to beta males in unmanipulated groups. Fingerprinting data from 1990–1992 showed that on average, beta males fathered 40 ± 4% (range 18 to 60, $n = 10$) of offspring in a brood (Jamieson et al. 1994) compared with only 12 ± 12% (range 0 to 50, $n = 4$) when beta males were removed (Table 1), although the difference is not significant ($W = 87.0$, $P = 0.09$), presumably because of the small number of removed males. Removed males exhibited little or no parental care (Table 1) relative to beta males in unmanipulated groups, which contributed, on average, 44 ± 4% ($n = 8$) of male incubation bouts and 44 ± 1% ($n = 6$) of male feeding bouts, in groups with two males, and 32 ± 2% ($n = 2$) and 23% ($n = 1$) of incubation and feeding bouts, respectively, in groups with three males.

In unmanipulated groups, males act as a cohesive unit, jointly defending the territory and rarely showing signs of intragroup aggression (Jamieson et al. 1994). By contrast, the removed male in three of five experimental groups was continuously chased away from the nest and eggs by both male and female group members (Table 1). In the other two groups, aggression was not seen, but these particular territories were difficult to observe, and one of the males left the territory only three days after being released. Overall, removed males from two experimental groups left the territory and two remained away from the nest but bred in a subsequent nesting attempt. In one group, three days after the male's release broken eggs were found outside the nest but were not typical of predation by harriers or stoats (*Mustela erminea;* Jamieson pers. obs.). It is not known whether the group renested.

Contrary to expectations, in only one of five control groups did the released male incubate eggs and feed offspring (Table 1). In two groups, aggression was directed toward the removed male, as occurred in the experimental groups. Control males either left the territory or, in two cases, eggs were found destroyed in a similar manner to that described for the experimental group soon after the male's release.

The responses to removals revealed no particular pattern with respect to the number of co-breeding males or females within a group for either experimental or control groups, although all three cases of egg destruction occurred in groups with only one female (Table 1). There also was no pattern in the male's response (e.g. leave, destroy eggs) and whether it was relatively early or late in the breeding season.

Discussion.—The lack of parental behavior shown by removed males, and the high level of aggression and incidents of egg destruction seen within groups, have not been recorded previously in groups of Pukeko. However, there were no clear differences in the pattern of response between control and experimental groups. Removal of the beta male from his group and the time spent in the aviary appeared to have two main effects. First, removals may have caused some males to behave as if they had low paternity irrespective of whether they had been removed during or after the egg-laying period. Aggression from the resident birds may have prevented beta males from providing parental care, but this by itself cannot explain why control males apparently destroyed eggs that they had potentially fathered. Perhaps the extended period away from the nest and territory and/or the aggressive behavior the resident birds directed toward them meant that even control males had difficulty reliably assessing their paternity. Alternatively, the relatively long period in captivity may have reduced prolactin levels in control birds to levels similar to that of experimental birds. High levels of prolactin are associated with incubation in many bird species, but these can decline when incubation is experimentally disrupted (Goldsmith 1991). Hence, the two groups of birds tended to behave similarly when released. It may be that removal experiments of this type are not suitable for species in which the males perform a significant proportion of the incubation.

The second effect was on the resident birds who acted aggressively toward the removed birds after they had been released back on their territories. Even females who are normally submissive to males within their group (Craig and Jamieson 1990) persistently chased off removed males. Much evidence suggests that Pukeko readily distinguish individuals with whom they engage in regular interactions, both within groups and between adjacent groups (Jamieson pers. obs.). Although it is possible that the extended period away during the removal meant that group members did not initially recognize the beta male upon his release, it is unlikely that such "mistaken identity" would have persisted for many days.

The group's behavior might be interpreted as a form of punishment to beta males for leaving the territory and not assisting with parental duties. However, the idea that parental care is a form of payment for residency on a territory, and punishment and eviction a penalty for not helping, is more appropriate in explaining the behavior of nonbreeding helpers who are subordinate to adult breeders and have no opportunity for dispersal (Gaston 1978, Mulder and Langmore 1993). Harassment of one of the possible fathers would not benefit the other co-breeders, especially if the bird ends up leaving the territory or not contributing to care of the offspring. In addition, aggression by group members appeared to be spontaneous and occurred before any of the eggs had been damaged and thus could not have been a response to the removed male destroying eggs.

It is more likely that group members were aggressive toward the removed male because he was a potential threat to the nest and eggs. Several experimental studies have indicated that removal or replacement birds with low probability of parentage will damage eggs or evict nestlings (e.g. Emlen et al. 1989, Koenig 1990, Robertson 1990). The unusual circumstances in which eggs were damaged in three of our study nests suggest that removed males destroyed eggs, causing the female to renest, although we have no direct observation of males tampering with nests. Furthermore, group members chased control males, ones who presumably had high probability of paternity and who had already incubated the eggs prior to removal. Therefore, we suggest that because of the relatively long period away from the territory, some co-breeders treated removed males as they would intruders, and they did not differentiate between males removed during egg laying (experimentals) versus after egg laying was completed (controls).

In retrospect, this problem may have been circumvented if experimental and control males had been removed for a shorter period of time (e.g. three to four days). However, shorter removals would have minimized the effect of reducing paternity because the egg-laying period ranges from 8 to 10 days (and possibly longer for nests with two females). Hence, target males would have been present for most of the egg-laying period. In conclusion, our results suggest that a cautionary approach should be taken in interpreting removal experiments, especially if captivity has subtle effects on behavior (even during short-term removals). This may be particularly relevant to highly sociable species such as the Pukeko.

Acknowledgments.—We thank A. Donald, S. Hellier, S. Morgan, and N. Patenaude for assistance in the field and C. Chong for assistance with the fingerprinting analysis. M. McKenzie, I. Dickson, P. Simpson, and V. Allen provided equipment and technical assistance, and P. Barnard and R. Simmons provided office space during manuscript revision. We also thank Otago Fish and Game Council, New Zealand Department of Conservation, and G. L. and J. L. Adam for access to the study site and use of their property for the aviary facility. L. Gibbs and R. Montgomerie provided helpful comments on earlier drafts of the manuscript. This research is supported by the University of Otago (I. G. J.) and Natural Science and Engineering Council of Canada (J. S. Q.).

Literature Cited

CRAIG, J. L. 1977. The behaviour of the Pukeko, *Porphyrio porphyrio*. New Zealand Journal of Zoology 4:413–433.

CRAIG, J. L., AND I. G. JAMIESON. 1990. Pukeko: Different approaches and some different answers. Pages 385–412 *in* Cooperative breeding in birds: Long-term studies of ecology and behavior (P. B. Stacey and W. D. Koenig, Eds.). Cambridge University Press, Cambridge, United Kingdom.

DAVIES N. B., B. J. HATCHWELL, T. ROBSON, AND T. BURKE. 1992. Paternity and parental effort in Dunnocks *Prunella modularis*: How good are male chick-feeding rules? Animal Behaviour 43:729–745.

EMLEN, S. T., N. J. DEMONG, AND D. J. EMLEN. 1989. Experimental induction of infanticide in female Wattled Jacanas. Auk 106:1–7.

GASTON, A. J. 1978. The evolution of group territorial behavior and cooperative breeding. American Naturalist 112:1091–1100.

GAVIN, T. A., AND E. K. BOLLINGER. 1985. Multiple paternity in a territorial passerine: The Bobolink. Auk 102:550–555.

GOLDSMITH, A. R. 1991. Prolactin and avian reproductive strategies. Pages 2063–2071 *in* Acta XX Congressus Internationalis Ornithologici (B. D. Bell, Ed.). Christchurch, New Zealand, 1990. New Zealand Ornithological Congress Trust Board, Wellington.

GOWATY, P. A., AND A. A. KARLIN. 1984. Multiple maternity and paternity in single broods of apparently monogamous Eastern Bluebirds (*Sialia sialis*). Behavioral Ecology Sociobiology 15:91–95.

JAMIESON, I. G. 1997. Testing reproductive skew models in a communally breeding bird, the Pukeko *Porphyrio porphyrio*. Proceedings of the Royal Society of London Series B 264:335–340.

JAMIESON, I. G., AND J. L. CRAIG. 1987. Dominance and mating in a communal polygynandrous bird: Cooperation or indifference toward mating competitors? Ethology 75:317–327.

JAMIESON, I. G., J. S. QUINN, P. A. ROSE, AND B. N. WHITE. 1994. Shared paternity among non-relatives is a result of an egalitarian mating system in a communally breeding bird, the Pukeko. Proceedings of the Royal Society of London Series B 257:271–277.

KOENIG, W. D. 1990. Opportunity of parentage and nest destruction in polygynandrous Acorn Woodpeckers, *Melanerpes formicivorus*. Behavioral Ecology 1:55–61.

MØLLER, A. P. 1988. Paternity and paternal care in the swallow, *Hirundo rustica*. Animal Behaviour 36:996–1005.

MULDER, R. A., AND N. E. LANGMORE. 1993. Dominant males punish helpers for temporary defection in Superb Fairy-Wrens. Animal Behaviour 45:830–833.

ROBERTSON, R. J. 1990. Tactics and counter-tactics of sexually selected infanticide in Tree Swallows. Pages 381–390 *in* Population biology of passerine birds: An integrated approach (J. Blondel, A. Gosler, J.-D. Lebreton, and R. McCleary, Eds.). Springer-Verlag, Berlin.

SCHWAGMEYER, P. L., AND D. W. MOCK. 1993. Shaken confidence of paternity. Animal Behaviour 46:1020–1022.

SEUTIN, G., B. N. WHITE, AND P. T. BOAG. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82–90.

WESTNEAT, D. F. 1987. Extra-pair fertilizations in a predominantly monogamous bird: Genetic evidence. Animal Behaviour 35:877–886.

WHITTINGHAM, L. A., P. O. DUNN, AND R. J. ROBERTSON. 1993. Confidence of paternity and male parental care: An experimental study in Tree Swallows. Animal Behaviour 46:139–147.

WHITTINGHAM, L. A., P. D. TAYLOR, AND R. J. ROBERTSON. 1992. Confidence of paternity and male parental care. American Naturalist 139:1115–1125.

Received 17 June 1996, accepted 14 November 1996.
Associate Editor: T. E. Martin