TECHNIQUES AND RESULTS OF ARTIFICIAL INSEMINATION WITH GOLDEN EAGLES

by
James W. Grier*
Division of Biological Sciences and Laboratory of Ornithology
Cornell University
Ithaca, NY 14850

Introduction

The techniques and some of the events involved in the successful artificial insemination and incubation of Golden Eagles (*Aquila chrysaetos*) at Cornell University during 1972 are summarized. Cooperative insemination techniques (Berry, 1972) were used primarily, as opposed to the traditional forced, massage methods normally used for poultry (see Smyth, 1968). Cooperative insemination requires birds that are imprinted to human handlers as discussed by Temple (1972). The reproductive behavior of such birds is described by Hamerstrom (1970), Temple (1972) and Berry (1972).

This project was "extracurricular" on my part, as I was busy with other research, and many of the data are not as complete as they might otherwise have been. Thus, in addition to describing techniques that worked, this report is intended to stimulate more thorough future research on some of the questions raised. Additional biological results of the work are being prepared for separate publication.

Source of Birds and Housing Methods

Four "pre-Act" (1962) adult Golden Eagles, all 10 to 12 years old, were borrowed from private owners and transported to Cornell during the summer and late fall of 1971. One of the males and one of the females were obtained originally from the wild as late eyasses. The other male and female were allegedly obtained as a brancher and a passager, respectively.

The two females were housed at the Behavioral Ecology Building in rooms measuring 10x20 feet with a 12- to 18-foot high slanted ceiling. One end of the room was open to the outside with vertical bars (thinwall conduit) to retain the birds. One-way observation windows at the other end of the room allowed the birds to be viewed undisturbed. Each room was furnished with a long perch covered with artificial grass 4½ feet above the floor, one bare tree branch three feet high, a bale of straw on the floor, a bath pan, a nest platform (3x4 feet and three feet high with heavy sticks and straw on top), loose sticks on the floor, and blocks of wood (for the birds to play with).

The two males were kept at my home on outside perches, tied with jesses

*Current address: Department of Zoology, North Dakota State University, Fargo, North Dakota 58102.

and leashes, during the day. They were moved inside to a barn on shelf perches at night. Loose straw was placed on the floor below the shelf perches for catching mutes; the birds also used the straw for building material during the nesting period.

The males were in view of each other at all times. The females could hear but not see each other.

All four birds were fed a variety of road-killed birds and mammals, and whole chickens of various ages. They were allowed as much as they wanted to eat.

I spent sufficient time, including handling and feeding, in the presence of the birds to assure that each was well adjusted to my presence and satisfied with its immediate surroundings prior to the breeding season. Handling did not include feeding on the fist, except for two of the birds when first obtained. Food was placed near or below the perches and the birds were then either left alone or I simply sat down nearby.

All lighting was natural with care taken to insure that none of the birds was accidentally exposed to artificial light after dark.

Behavior of the Eagles and Insemination Techniques

With the onset of the breeding season, all four birds displayed courtship behavior and vocalizations toward me, constructed or attempted to construct nests with the available materials, the males gave viable semen, and the females laid eggs. The male and female showing the most vigorous and complete complements of reproductive behavior were those originally obtained as eyasses. All of the birds showed considerable individual variation in behavior.

The males copulated on my arm when I picked them up to move to a different perch. The birds showed traces of the behavior early in the season (one began 16 December) by giving a "chirpy" call and partially lowering the body on my gloved arm. Movement on my part, such as walking, would stop this behavior so I simply stood still and gave them an opportunity to copulate on my arm or shoulder each time I picked them up (Figures 1, 2). Their behavior gradually advanced (over a period of weeks) to include balling of the feet, spreading of the abdominal feathers, spreading and flapping of the wings, a loud vocalization, swinging and sideways positioning of the tail, and, finally, production of semen of variable amounts and qualities.

I collected the semen in the palm of my free hand. Semen production continued as long as I gave the birds attention and accepted copulation. One male gave viable sperm (checked with microscope) over a period of 111 days; the other male, which started later, produced motile sperm for a period of 70 days. Excluding times of only partial behavior, the birds copulated on my arm a total of 468 times, of which 216 (46%) resulted in "usable" semen (greater than 0.05 cc with motile sperm). Semen volume ranged up to 0.20 cc per ejaculate.

Secondary male sexual behavior included nest building, the typical, melodious call of the species while on the perch, more thorough plucking of prey and increased preference for eating the anterior end, a loud "yelp" call after eating while standing on the remains of the carcass (which I interpreted to be a call to

Figure 1. Male Golden Eagle copulating on arm. Note balled feet and Grier's right arm under tail to collect semen. Photograph by S. A. Temple.

Figure 2. Male Golden Eagle copulating on shoulder. Photograph by L. J. Gaeta.

the "mate" that food is available), and a voluntary release of the food at my approach. Prior to the breeding season the males typically guarded the food with "mantling" behavior at my approach.

Semen was collected from my hand into a narrow-ended blunt glass tube connected to a syringe. Semen held up to 3½ hours in the glass tube showed no visible decrease in quality (estimated percent motility). All actual inseminations with females, however, were conducted within ½ hour of obtaining the semen. Sperm were observed without dilution or staining (owing to lack of time) under a light microscope at 430x with a coverslip. When the semen was to be used, I collected it on a sterile plastic glove to prevent contamination from my hand (which was observed to reduce viability) and to prevent loss of any volume on the irregular surface of the hand. The semen was transported in the glass tube in which it was collected. This tube and syringe were carried in turn upright in a small glass tube to catch any semen that might spill out.

The behavior of the females prior to egg laying went through several stages. Both showed the same behavior but to slightly different degrees and lasting different lengths of time (days to weeks). Normal behavior of the birds during the fall and winter involved quiet perching most of the time with occasional changing of perches, maintenance behavior, and some playing with objects in the room. During early spring and lasting several weeks the birds became noticeably more playful: running about the room, pouncing on and footing objects, and jumping about with the objects with wings flapping. They also began calling melodiously at this time, although one bird (the eyass) called much more frequently and louder than the other. This was followed by a stage of working with sticks. The birds walked around, inspected and picked up sticks in their feet and beaks, and carried them haphazardly about the floor and occasionally to the nest where they would pull at them with their beaks as in plucking and eating. They made little attempt to arrange them on the nest, however.

The next type of activity was very deliberate nest building in which the birds would take sticks to the nest and carefully arrange them around the perimeter. After doing that for a few days they carried and arranged the straw lining. In the final stage they formed a "cup" in the lining both with "scraping" actions by pushing the feet backwards while in a prostrate position, similar to falcons, and by standing, turning, and arranging the straw with the beak. Later on, while incubating eggs, the females maintained the "cup" by reaching out with their beaks from the resting position and pulling straw into a mound surrounding the body.

Initial insemination of both females was with forced massage techniques. As egg laying commenced, however, one of the females (the passage bird) began posturing voluntarily for insemination. I stimulated the voluntary posture by placing one hand under her abdomen, then one hand on her back while standing beside her (Figure 3). She would droop the abdominal feathers, bend forward, lower and spread her wings, raise her tail, and evert the cloaca, into which the semen was then injected. She accepted insemination voluntarily most readily on days *prior* to laying an egg and least readily the day following egg laying.

For forced insemination I held the female the first time we did it. That caused her to be upset at me and she would not allow me to approach her for about a day until she gradually accepted me again. For all remaining inseminations an assistant, strange to the bird, picked the female up. This resulted in the birds' being upset at that person without any interference in my relationship with them.

Forced inseminations were accomplished as follows: The assistant entered the room and picked the bird up on a gloved fist. The bird showed no resistance to that person the first time, but thereafter she had to be caught first. When the bird bated on its own accord it was lowered and placed on the floor on its breast. I then entered the room from behind so that the bird could not see me and took its legs in one hand. The assistant then massaged the abdomen and prolapsed the oviduct. I used my free hand to inject the semen. Then the assistant took the legs again; I left the room; he released her on the floor; and he left the room. The inseminations required a total time, once the bird was caught, of one minute or less.

The birds were generally inseminated the day following the laying of each egg except for the one female that began accepting inseminations voluntarily; she was inseminated almost daily. One forced insemination after a nine-day interval without laying was done (unintentionally) just seven hours prior to laying an egg. The egg was not damaged in the handing, although the pigment of that egg was much more blurred than on others, possibly resulting from movement of the egg during the time pigment was being added. Only one of the forced inseminations occurred at a time when I estimated that ovulation might be occurring. This was followed by the nine-day gap in laying which might indicate an interference with ovulation by the handling (cf. last sentence of paragraph three paragraphs prior to this one). The long gap may, however, have simply resulted from possible recycling processes which are not yet understood in these birds. On the basis of these data I would recommend inseminating once a week, as is done with poultry, unless the bird accepts insemination voluntarily and one wants to do it more often.

To obtain more than the normal number of eggs, I removed eggs as soon as they were laid until I felt enough had been taken. One female laid four eggs, on 19 and 22 March, and 1 and 5 April; the other laid five, on 1, 5, 12, late 15 or early 16, and 19 April. No more eggs were laid after the females were allowed to incubate.

The last two eggs of the one female and the first four of the other were fertile. The fifth egg of the second female was either infertile or development stopped in the very early stages. Of the two first infertile eggs, one was laid unexpectedly before we inseminated and the other was laid 51 hours following the first insemination. One of the fertile eggs was laid nine days after the last previous potential insemination. (She was also inseminated seven hours before oviposition, as described above.) The minimum determined interval between laying was 72 hours. From these data and information from other species I suspect that the egg may spend approximately 70 hours in the oviduct before laying in Golden Eagles.

The prolonged egg laying appeared to cause progressively thinner eggshells (Table 1). However, the fifth egg from the one female appeared to have a thicker shell after calcium (powdered milk) was added to the food following the fourth and very thin-appearing egg. These results have not been compared with normal variation for the species because of the small sample size.

Table 1. Shell thickness[1] of successive eggs.

Female	Egg No.	1	2	3	4	5
Chrys		0.50	0.56	0.48	0.47	
Furious		0.61	0.59	0.58	0.55	0.59

[1]Shell and membrane, dried three months at room temperature. Measured to 0.01 mm at girth with Starrett micrometer.

Incubation and Hatching of Eggs

All but one of the fertile eggs were incubated in a forced-air incubator under standard poultry conditions (temperature 99.2-100.0 F; relative humidity 55-60%; turned 90 degrees automatically every two hours) but with cooling for 10 to 30 minutes twice a day in a room at 82 F and additional turning of 180 degrees once a day. The remaining fertile egg was left under one of the females (the eyass) which was known to incubate well. The other female incubated poorly, including this year with a substitute turkey egg.

To allow the incubating female to eat, stretch, and exercise, I exchanged incubation duties one to three times a day with her. I entered her room, she stood up slowly and left the egg, and I covered it with a cloth-covered hot-water bottle warmed to 99-101 F while I sat nearby. She ate occasionally, stretched, roused, muted, bathed occasionally, and was active in the room, including much play and footing of perches and other objects. After a variable length of time (mean of 16.5 minutes, range 4-61, with an increase toward the end) she came back to the nest (Figure 4), I removed the hot-water bottle, and she settled onto the egg.

Development of the eggs during incubation could be partially assessed by candling, but changes in weight revealed little. Under a good candling light and with practice and careful turning, the eggs initially showed a light green glow with a diffuse and indistinct shadow from the yolk in the center of the egg. By the fifth day the yolks floated higher in the egg, casting a more distinct shadow and, in the fertile eggs, showed some enlargement. The infertile egg showed no further change except for a gradual over-all darkening of the egg as it lost moisture until the fourth week when the distinct shadow of the yolk could no longer be seen. In the fertile eggs, however, the shadow approximately doubled in

Figure 3. Stimulating female Golden Eagle into copulatory posture prior to cooperative insemination. Photograph by S. A. Temple.

Figure 4. Female Golden Eagle returning to nest to resume incubation. Hot-water bottle is covering egg. Photograph by J. W. Grier.

size between the fifth and sixth days and the shadow covered the top one-third of the egg by the seventh day. From the eighth day on all that could be seen in most of the fertile eggs was a clear, distinct, and gradually enlarging air cell at the broad end of the egg with the remainder opaque. In one egg (F4) which was white and had a relatively thin shell for that female, the development of embryo and vessels was visible for several days, until that egg also became opaque.

The weight changes during incubation (Figure 5) of two eggs that hatched, one that developed until hatching, and an infertile egg, were nearly identical; the developing eggs lost weight slightly faster after about the third week. Three eggs in which the embryos died earlier in development appeared to stop losing weight after the time when the embryos were estimated to have died. The one which may not have been fertile in the first place (F5) lost the small air cell it had and appeared to gain 0.5 g over a period of seven days; a spring scale (150 g capacity) was used and the 0.5 difference may have been due to the inaccuracy of that type of scale.

The causes of death of the three embryos that died in mid to late stages of development are not known. Artificial incubation rarely achieves a complete hatch even under the best conditions; the conditions used may not have been optimal for eagle eggs; and turning, in particular, may have been inappropriate or excessive at the later stages (cf. Baerends and Drent, 1970, and remarks by W. Nelson at 1971 and 1972 RRF Raptor Breeding Conferences, e.g. Nelson, 1972).

The first two fertile eggs in the incubator and the one under the female hatched after six weeks of incubation. The egg under the female "hatched" prematurely after it had just pipped and the female accidentally stepped on it. That chick's yolk sac was not completely absorbed and the blood was still circulating in the chorionic vessels. Hence, in the absence of the accident, it probably would have hatched the following day. The bleeding was stopped by clamping the umbilical cord, the yolk sac was pushed into the abdominal cavity, and the umbilicus was sutured closed (Figure 6). Development of the other two was complete at hatching but they had to be helped from the shells after signs of weakening at 43 and 34 hours after pipping. The problem in hatching appeared to result from dried membranes and, in one case, may have been complicated by a very small egg (C3) in which the chick was unbelievably packed. The latter chick's head was pressed out of normal symmetry (which gradually became normal after hatching) and it appeared unable to rotate in the egg.

I used a large, essentially empty, forced air hatcher for the first hatching. The second was transferred to a still-air hatcher full of other hatching eggs but after it had already pipped and possibly so late that the membranes may have already started drying. The chicks appeared to be stimulated into vigorous activity and cheeping whenever the hatching eggs were moved or the door was opened to add wet sponges for increased humidity. Part of the drying problem may have been due to inadequate external stimulation during active hatching, resulting in a prolonged hatching period and greater opportunity for drying than would

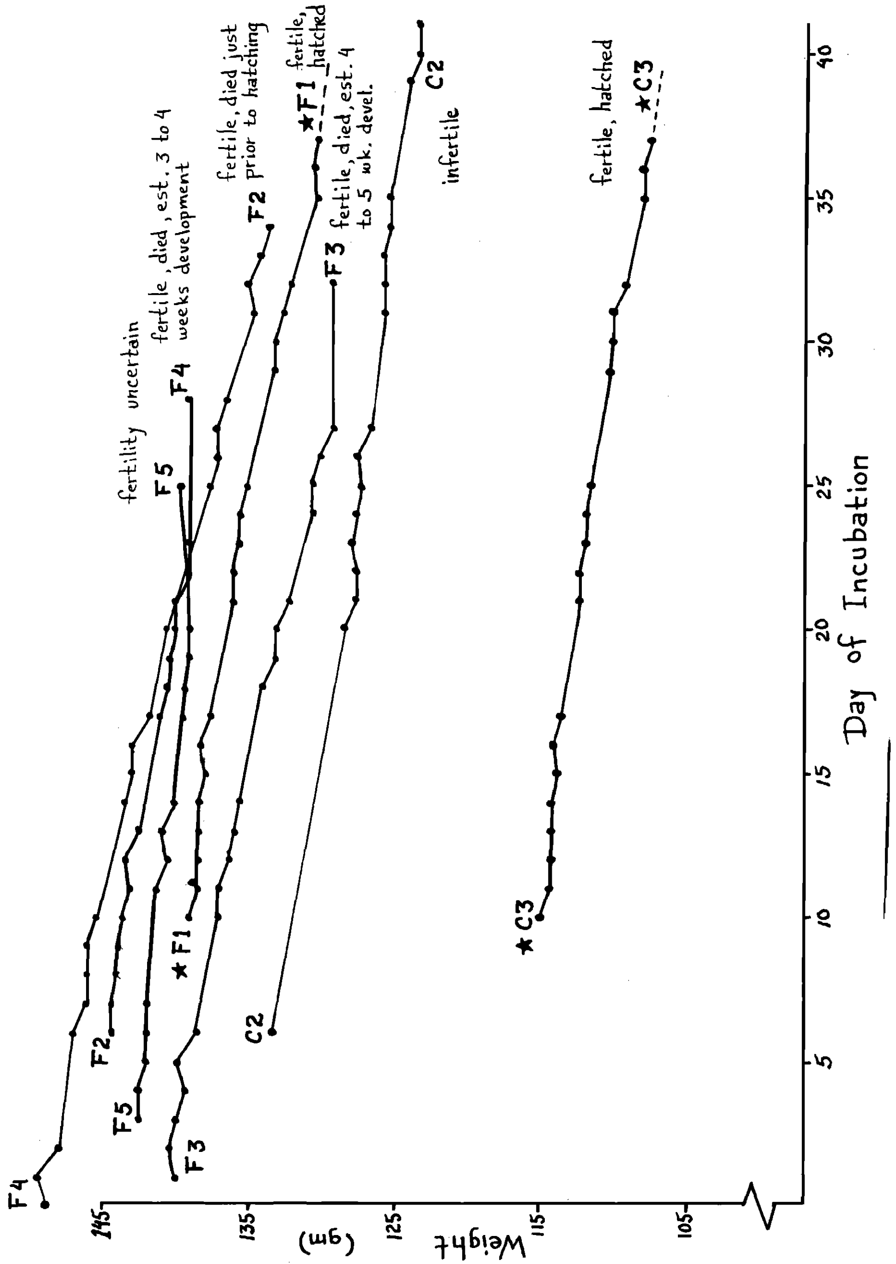

Figure 5. Weight changes of eggs during artificial incubation.

occur under a bird. Other potential factors may have been sub-optimal incubating conditions resulting in weaker chicks and lack of possible direct help by the parent bird. For comments on hatching-help behavior by adult birds see Buhler (1971).

Initial Rearing of Chicks

After hatching, the chicks were kept in the hatcher until the down was fluffed up (aided by rubbing with cotton) and they had rested. The chicks were then taken to my home where they were given constant surveillance. Feeding was determined by their food-begging and by watching the quality of their mutes. Since hatching had been assisted in all three cases and the chicks were considered weak, initial feedings were assisted by force feeding. The first feedings were given eight, five, and 17 hours, respectively, after hatching. The latter, premature chick still had much yolk and was not ready to eat as soon after hatching as the others.

"Brooding" temperatures for the chicks were determined by their behavior (see below) and varied slightly between the different chicks. Their preferred ambient temperature was approximately 32 C initially and decreased almost daily. I used a light bulb adjusted to different heights over an open box for heat control. This worked well for controlling the temperature but the lowered humidity and or direct radiation resulted in gradual dehydration of the chicks. I would recommend instead a more closed container initially with a heating wire or covered light bulb for heat and a pan of water for increased humidity (cf. Snelling, 1972).

The chicks gave three different basic vocalizations: one for food-begging, one for discomfort (too hot or cool), and a mad chitter when more distressed. When comfortable the chicks would sleep quietly and relaxed. When too warm they would extend their wings and neck, then start to cheep, move about, and pant. When too cool they would tuck all extremities close to the body then start to cheep and shiver. They became too warm very easily.

After the incubating female broke the pipped egg but while she was still incubating a substitute egg, one of the other chicks was given to her, at five days of age. She essentially ignored the chick, however, and it had to be removed. The problem may have been due to a number of factors. The female may have lost interest in incubating/brooding; the chick may not have behaved appropriately as a result of not being hatched and initially brooded under a bird. In addition, the female may have been upset by the presence of a strange assistant at the nest to help substitute the egg (while I was working with the chick from the broken egg), even though the female showed no outward signs of being upset by the assistant.

Outcome of 1972 Insemination Work

Two of the chicks were scheduled for fostering out to wild eyries in Colorado but they died in transit from accidental overheating. The remaining chick was nearly lost in the same mishap but survived, recovered, and is alive and well in

Figure 6. Newly-hatched Golden Eagle chick resulting from artificial insemination. This particular chick was premature (see text). Note sutures in umbilicus. Photograph by J. W. Grier.

Figure 7. Surviving immature Golden Eagle resulting from 1972 artificial insemination at Cornell University. Photograph by J. B. (Jerry) Grier.

my care at the time this report was prepared (Figure 7). The chick was hand reared, tame hacked (cf. Hamerstrom, 1971), and is imprinted to humans.

As a concluding remark on the light side, I might add that, although I have not yet been charged with polygamy, I am probably the only individual, man or bird, to have successfully mated with four eagles in one season.

Acknowledgments

I would like to thank L. Gaeta, Fran Hamerstrom, and M. Schweig for the loan of the adult eagles. Dr. T. J. Cade generously provided housing space at the Behavioral Ecology Building, some of the food, and some of the funds (through the Peregrine Fund and N.S.F. Grant GB-31547), as well as encouragement throughout the course of the work. Assistance with insemination and other handling of the birds was provided by J. D. Weaver, S. A. Temple, Phyllis Dague, M. MacLeod, and my wife, Joyce Grier. Dr. A. Bensadoun, Onalee and C. B. Ganong, R. E. Reynolds, J. Skinner, and F. Ward provided valuable advice and assistance on incubation and hatching. Incubation and hatching were conducted in facilities of the Cornell Poultry Science Department and the New York Department of Environmental Conservation. Dr. T. J. Cade, Fran Hamerstrom, and S. Temple provided valuable discussions and comments during the preparation of this manuscript.

Literature Cited

Baerends, G. P., and R. H. Drent. 1970. The herring gull and its eggs. *Behavior* Suppl. No. 17.

Berry, R. B. 1972. Reproduction by artificial insemination in captive American goshawks. *J. Wildlife Manage.* 36:1283-1288.

Buhler, P. 1971. Schluphilfe-Verhalten bei der Scheiereule, *Tyto alba. Vogelwelt* 91:121-130.

Hamerstrom, F. 1970. *An Eagle to the Sky.* Ames: Iowa State University Press. 143 pp.

Hamerstrom, F. 1971. Hacking to the glove. *J. N. Amer. Falconers' Ass.* 10: 34-36.

Nelson, W. 1972. Comments in: R. R. Olendorff (ed.). Special conference on captivity breeding of raptors, 1971. Panel 8, incubation, natural and artificial. *Raptor Res.* 6(Suppl.):B12-B13.

Smyth, J.R., Jr. 1968. Poultry. In: E. J. Perry (ed.). *The Artificial Insemination of Farm Animals.* New Brunswick: Rutgers University Press. pp. 258-300.

Snelling, J. 1972. Comments in: Olendorff (op. cit.), pp. B20-B24. (See Nelson 1972).

Temple, S. A. 1972. Artificial insemination with imprinted birds of prey. *Nature* 237(5353):287-288.

Manuscript received January 20, 1973