

Reports

Coturnix Quail as a Laboratory Research Animal

Abstract. The *Coturnix* quail is recommended to interested investigators, especially to embryologists and physiologists, for use in research because of its hardiness, ease of handling, precociousness, and great laying ability.

Many investigators in embryology and physiology prefer working with birds because of the accessibility of the embryos, the relative lack of expense and the short breeding cycles. One of the main drawbacks is the difficulty in getting confined birds, other than poultry, to reproduce at an economical rate, if at all. Therefore, we call the attention of interested investigators to the value of the *Coturnix* quail, *Coturnix coturnix japonica* Temminck and Schlegel (1) as a laboratory research animal.

This bird is an ideal laboratory animal for workers in the fields of avian embryology and physiology, especially endocrinology. It is extremely hardy, is easy to raise, reproduces at 6 weeks of age, and is very prolific. The *Coturnix* does not seem to be susceptible to any of the common diseases of the bobwhite quail.

The *Coturnix* was introduced into this country from Japan by the Missouri Conservation Commission (2) in order to determine whether it could become established ecologically and thus become a supplement to other game-bird species. Several states have subsequently been propagating and releasing the bird. To date, however, there is little evidence that the *Coturnix* has been established as a wild-game species.

Coturnix quails for research purposes

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should not repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to one 2-column figure (that is, a figure whose width equals two columns of text) or to one 2-column table or to two 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

can be obtained from some state conservation and wildlife agencies. The birds may be subject to individual state game regulations in accordance with the state laws.

Development of the young *Coturnix* is extremely rapid. The birds more than triple their size and weight during the first week after hatching. The first flight feathers are evident at 3 days of age, and strong flight is possible at 2 weeks. The birds are sexually mature at 6 weeks of age; a few lay as early as 38 days. Fertility is low at first, but by 50 days of age the fertility may be as high as 90 percent.

Sexes are easily recognizable at 3 weeks of age, and with practice reliable determination of sex can be made at 2 weeks. The females have a gray-and-black speckled breast, whereas the males have an even-colored brown breast with only a few speckles.

Interesting and extreme variations occur in the eggs, which may be snow-white, flesh-colored, dark or light brown, blue speckled to blue-violet, or brown mottled with a combination of all these colors. The size variation of the eggs is great; the length ranges from 20 to 35 mm. The eggs are quite large for the size of the adult bird.

The incubation period for the eggs in an incubator at 100°F is 16 days \pm 8 hours. Incubator temperatures of 103°F or above are harmful, and hatchability at these temperatures is low. Humidity should be kept at 60 to 70 percent, and the eggs should be turned at 8-hour intervals throughout incubation. Humidity should be raised to about 95 percent on the 15th day for highest hatchability. The hatchability, based on the number of fertile eggs, is about 60 to 70 percent under these artificial incubation conditions. However, we have found the eggs to be so plentiful that hatchability above 60 percent is usually not necessary for routine laboratory purposes.

For embryological studies, incubation should be timed from approximately 3 hours after the eggs are placed in the incubator, in order to allow them to reach incubator temperature. Eggs may be held as long as 2 weeks at 25°C before incubation without apparent loss of viability.

The young chicks are allowed to dry

partially before being taken out of the hatching incubator. Next they are placed in a brooder with a coarse flooring to prevent their feet from slipping from under them. After 2 weeks in the brooder they are ready to be placed in the larger outdoor pens.

Breeder cages should be made of $\frac{1}{2}$ -in. mesh hardware cloth. A cage of 3 by 6 by 1.5 ft is suitable for housing 20 birds. The ratio of 5 males to 15 females will give 90-percent egg fertility. An excess number of males may lead to severe pecking. Although the top of the cage may be made of hardware cloth, a burlap covering is more suitable, since the birds are excitable and will scalp themselves against the wire top if they are disturbed. One end of the cage is provided with a protective wooden covering to provide shelter for the birds against extreme weather conditions. Freezing temperatures will not harm adult birds, but it is advisable to place a heat lamp at one end of the cage during extreme conditions. The outdoor pens must be protected by a fence, since cats, dogs, and other predators may disturb the birds and upset the laying schedule.

We used the following feeding regimen of Purina feeds with excellent results: From hatching to 2 weeks, the chicks were fed Game Bird Startena; after 2 weeks Game Bird Growena was added to the Startena in increasing amounts until, at 4 weeks, a 100-percent Growena ration was used. Growena was fed from 4 through 8 weeks, and after 8 weeks Game Bird Layena was used. Finely crushed oyster shells were added to the Layena to prevent the occurrence of soft-shelled eggs.

Food and fresh water were available at all times. Scratch feeds, such as cracked corn, should be used in the winter to provide extra carbohydrate. The *Coturnix* evidently has an extremely high rate of metabolism, as can be seen from the large intake of food and water, the great amount of droppings, and the high rate of egg laying.

The normal breeding season for the *Coturnix* starts in April and continues through September. During April and September the birds may become extremely restless, especially at night. This restlessness is apparently caused by a natural migratory instinct (3). Scalping against the top of the cage is greatest at this time.

The minimum amount of light per day required for egg laying is approximately 13½ hours. The game farm of the Alabama Department of Conservation at Prattville uses constant, 24-hour lighting in an indoor unit and obtains eggs throughout the year (4). Even under the most adverse conditions of pecking and scalping, the females continue to lay normally. During molting periods, laying will be greatly reduced.

Many phases of the biology of the *Coturnix* have not been investigated. The bird's response to various types of light stimuli needs to be studied. Hormonal studies conducted on the *Coturnix* would be of great interest. At present, research is being conducted in our laboratory on the normal embryology and the gonadal development of these birds.

A study of anomalies occurring in the embryos and of the genetic and environmental factors affecting them would be of interest. We have found a number of cases of incomplete twinning. These twins, with one head and two bodies, have been found to be alive in the eggs as late as the 13th day of incubation. Several cases of pectocephaly, such as have been recorded for the chick by Landauer (5), have been found, including cases where the cranium completely failed to form over the cerebrum.

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References and Notes

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Hypoxanthine in Rosy and Maroon-like Mutants of *Drosophila melanogaster*

Hadorn and Schwink (1, 2), in an examination of the pleiotropic effects of the rosy² (*ry*²; location, 3: 51 ±) mutant of *Drosophila melanogaster*, demonstrated that the mutant is deficient in isoxanthopterin and contains an excessive quantity of 2-amino-4-hydroxypteridine. At the same time, Forrest, Glassman, and Mitchell (3) observed that the maroon-like (*ma-l*; location, 1: near *beadex*) mutant of *Drosophila melanogaster* is deficient in an enzyme capable of catalyzing the oxidation of 2-amino-4-hydroxypteridine to isoxanthopterin.

Subsequent work has provided quantitative data on pteridine concentrations in the *ry* mutants (4), evidence that both the *ry* and *ma-l* mutants are deficient in the pteridine-oxidizing enzyme (5), and evidence that the enzyme involved is

Table 1. Absorbing compounds in mutants and wild-type *Drosophila melanogaster*. The presence or absence of substances corresponding to hypoxanthine and uric acid was consistent in all five solvents. Approximately 5 percent of maximum quantities would have been detected by the methods used.

Extract	Uric acid position	Hypoxanthine position
Wild (Oregon)	+	-
<i>ry</i> ¹	-	+
<i>ry</i> ²	-	+
<i>ma-l</i>	-	+

xanthine dehydrogenase (5). As indicated elsewhere (5, 6), this enzyme deficiency should lead to an accumulation of hypoxanthine as well as 2-amino-4-hydroxypteridine. This expectation has been found to be correct by observations of chromatograms from the mutants and wild-type *Drosophila* and by the isolation and identification of hypoxanthine from extracts of *ry*² adult flies. It has also been observed that hypoxanthine rather than uric acid is a major nitrogenous excretion product of the mutant flies.

Chromatograms of squashed animals (7) or boiled extracts, and of known purines as controls, were developed in the following solvents: (i) *n*-propanol (2 parts): 1 percent aqueous NH₃ (1 part); (ii) isopropanol (3 parts): 1M formic acid (1 part); (iii) *n*-propanol (3 parts): 2N HCl (1 part); (iv) isopropanol (7 parts): 1 percent aqueous NH₃ (3 parts); (v) 5 percent (by volume) acetic acid in water. Positions of all absorbing materials on the chromatograms were noted with the aid of a Mineral Light ultraviolet lamp (principal emission, 2537 Å). Results pertinent to this discussion are summarized in Table 1. By extraction of absorbing materials, followed by spectrophotometric measurements, it was determined that the mutants that accumulate hypoxanthine contain from 1 to 3 µg of the purine per animal (approximately 1 mg wet weight).

Chromatograms of excretion products scraped from the walls of culture bottles yielded a picture similar to that indicated in Table 1.

For the isolation of hypoxanthine, 50 g of washed adult *ry*² flies were extracted with 350 ml of boiling water. After filtration and addition of a concentrated solution of lead acetate (3 g), the solution was adjusted to pH 9 with NH₄OH. After centrifugation, the precipitate was discarded and the supernatant was adjusted to pH 10. The resulting precipitate was removed by centrifugation, resuspended in water and treated with

H₂S. The supernatant solution, after removal of lead sulfide, was evaporated to dryness and then extracted with 2 ml of hot water. This extract was streaked on a sheet of Whatman No. 4 paper and chromatographed in solvent i (Table 1). Absorbing material (2537 Å) at the hypoxanthine position was eluted with water, and chromatography was repeated with solvent v (Table 1). The eluate from the second chromatogram was evaporated to dryness, and the product was dissolved in 1 ml of hot water. Following treatment with a small amount of charcoal, the purine was crystallized from hot water with addition of ethanol to a slight turbidity. The yield after two recrystallizations was 5.5 mg. The product was chromatographically identical with hypoxanthine in the five solvents given above. It was also identical with hypoxanthine with respect to absorption spectra in 0.1N NaCl ($\lambda_{\text{max.}} = 248 \text{ m}\mu$; $\log E_{\text{max.}} = 4.03$) and in 0.1N NaOH ($\lambda_{\text{max.}} = 261 \text{ m}\mu$; $\log E_{\text{max.}} = 4.06$).

Although hypoxanthine has been isolated in a pure form only from *ry*² mutant, chromatographic and spectral characteristics of corresponding materials from the other mutants are in excellent agreement with the conclusion that the mutants tested accumulate and excrete hypoxanthine in the place of uric acid. It should also be noted that *ry*¹ and *ry*² may be genetic duplicates but that *ma-l* represents quite a different genetic locus. Nevertheless, the morphological and biochemical phenotypes are surprisingly similar. It is also of interest to note that in *Drosophila* the degradation of purines and their excretion as uric acid are not essential, nor is hypoxanthine particularly toxic to the animal even in the large quantities accumulated. However, at higher temperatures the *ry*² mutant behaves as a late pupal or early imaginal semilethal (1), and it is possible that the purine accumulation is a contributory factor to this phenomenon (8).

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