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An Artificial Hybrid between White Leghorn Cock and Japanese Green Pheasant Hen Produced by Insemination[1)]

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(Plate 1; Text-figs. 1–2; Tables 1–2)

In ornithological literature, the chicken-pheasant hybrids produced by natural matings are not rare, but those obtained by artificial insemination appear to be relatively few (cf. GRAY, 1958). In the course of studies on artificial insemination of poultry, the authors attempted to cross a Single Comb White Leghorn cock on a Japanese Green Pheasant hen (*Phasianus versicolor* VIEILLOT) by employing the technique previously reported by YAMANE, TSUKUNAGA and TAKAHASHI (1962, *a* & *b*). In 1962, a single insemination was performed on a pheasant hen apparrently at the end of her laying period. Nevertheless, one egg was secured on the second day after insemination and proved fertile; the hybrid embryo hatched normally and developed to a male morphologically quite different from the chicken-pheasant hybrids hitherto reported.

The experimental result seemed to show that the technique applied is fairly satisfactory for the purpose of crossing the birds of different genera in which natural matings are hardly possible. This paper preliminarily deals with the procedures of insemination as well as the hybrid produced.

MATERIALS AND METHODS.

The Single Comb White Leghorn cock used for semen collection was raised at the Zootechnical Laboratory, Hiroshima Agricultural College, Saijo; it was 415 days old and weighed 2,100 *g* at that time.

The collection of semen was carried out by abdominal massage employing the "Hiroshima" model bird holder and semen receptacle (YAMANE, TSUKUNAGA and TAKAHASHI, 1962, *b*) at 2: 30 p.m. on June 19, 1962. The ejaculate showed milky white color and very dense consistency, amounting 0.7 *ml* in volume. Immediately

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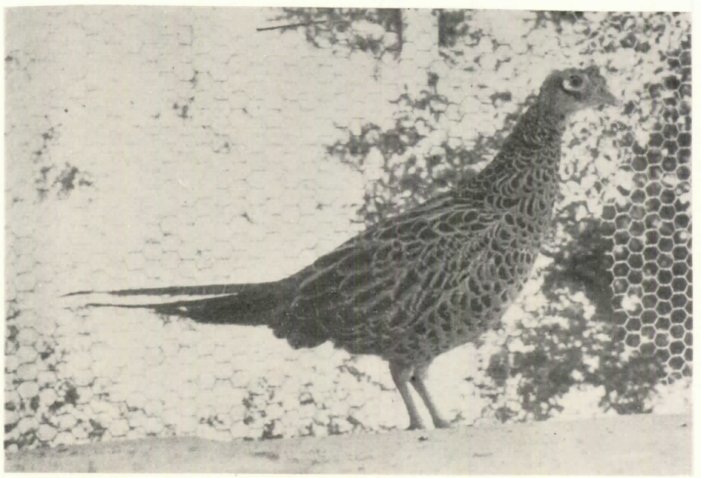
after collection, the ejaculate was diluted with an osmotically adjusted egg yolk-citrate buffer (YAMANE, TSUKUNAGA and TAKAHASHI, 1962, *a*) which was composed of 1 volume 6.5% $Na_3C_6H_5O_7 \cdot 2H_2O$, 1 volume 9.0% glucose solution, and 2 volumes fresh fowl egg yolk. This diluted semen was gradually cooled from 40° to 2°C in $2\frac{1}{2}$ hours and stored at 2°C in a refrigirator.

The Japanese Green Pheasant hen used for insemination was artificially hatched from one of eggs collected in a "wild" nest which was found near the campus of the Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, and reared there to mature age.[1] At the time of insemination the hen was 387 days old and weighed 805 *g*; she was strictly separated from any male bird (Text-fig. 1). In 1962, she produced 6 eggs irregulary during the period from May 22. to the end of that month. From June 2. on, a regular laying occurred, producing 17 eggs until June 20., when the insemination was carried out.



Text-fig. 1. Japanese Green Pheasant female inseminated with S. C. W. Leghorn semen.

The insemination took place at 1: 30 p.m. by use of semen which had been stored 24 hours including the shipping time of 2 hours by train and half an hour by bus from Saijo to Fukuyama. At insemination, the pheasant hen was brought up on the same bird holder which had been used for the domestic cock. When the hen was pressed at her abdomen beneath the ilio-pubal parts from caudal to oral with an operator's hand, the bird responsed its vagina everting. This enabled an inseminator to be introduced into the uterus with great ease. The inseminator consisted of a glass syringe of 1 *ml* content and a polyethlene catheter of 10 *cm* length. The volume of semen introduced into the uterus amounted 0.4 *ml*. Microscopic examination of semen just before insemination revealed that practically 80 percent sperms

1) Typical form of the Japanese pheasant is found in the Main Island and Shikoku; other three subspecies are known each from Hokuriku, Kyushu, and Izu and Oshima. The hen here described belonged to the typical subspecies of the Main Island.

were very active and the other 20 percent, active. It has been previously reported that in fowl semen, when undiluted or inadequately diluted, bending of sperm cell occurs at its midpiece which causes significantly the decrease of fertility. In the present experiment, however, the use of the above dilutor completely prevented from neck-bending of sperms even after 24 hours storage.

On the second day following the insemination, the hen produced an egg but no further laying occurred, probably due to the termination of her breeding season. This one and only egg was placed as usual into incubator on June 24. and a sound hybrid chick hatched on the 25th day of incubation, strictly speaking, after 24 days and 10 hours. The chick was further raised in a cage, supplying with ordinary poultry rations; it is full grown and still living.

CHARACTERISTICS OF CHICKEN-PHEASANT HYBRID OBTAINED.

Since the chicken-pheasant hybrid obtained resembles more pheasant than domestic fowl, the description of its characteristics will be here made comparing with the pheasant male. Table 1 shows the data of observations on the adult hybrid as well as the adult pheasant, while for the measures of size of the hybrid both parent birds are parallely considered as seen in Table 2.

Among the characteristics of the hybrid bird, the most striking fact is that the head is bilaterally decorated with lobe like-developed areas of velvety feathers, the areas being deep red with black dots (Pl. 1). Another marked character is the predominance of green, in part greenish violet, coloration of throat, neck and forehand. It is pecurious that the whole feature of the hybrid more resembles the "English Pheasant"[1] (Text-fig. 2) than the two hybrids from the reciprocal crosses described by YAMASHINA (1942), one from Green Pheasant ♂ × Japanese Game ♀ and the other, from Green Pheasant ♂ × Japanese Bantam ♀. It is evident that the chicken-pheasant hybrids show a great variation in their characteristics according to the genotypes of parent fowl and pheasant crossed as well as the type of matings. It is no wonder, therefore, that the new matings as S. C. W. Leghorn ♂ × Japanese Pheasant ♀ in the present experiment could produce a type quite new to ornithological literature. The comparison in detail of the hybrid presented here with those obtained naturally and artificially by previous authors shall be postponed until the production of a certain number of hybrids by the same matings as well as reciprocal crosses, since the experiments are being continued.

As for the body measures, the hybrid seems to deviate more toward the pheasant from the intermediate between the parents as shown in Table 2, but a single case does not permit to generalize such a conclusion.

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1) "English Pheasant" is said to be a breed fixed from the cross between English native pheasant and Common pheasant (*Phasianus colchicus*).

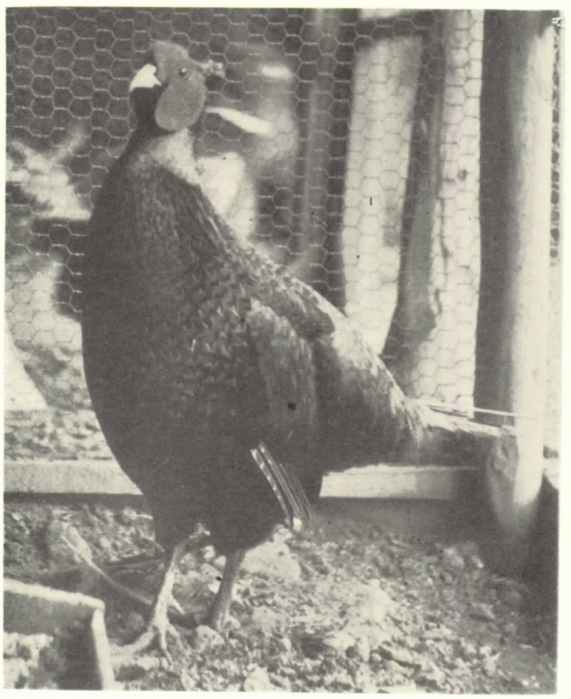
Table 1. Description of hybrid male between S. C. W. Leghorn ♂ and Japanese Pheasant ♀, comparing with Japanese Pheasant male.

	Japanese Pheasant male	Chicken ♂ × Pheasant ♀ hybrid male
Head	Crown, covered with greenish black feathers, possessing a pair of tufts of 15 *mm* length; above and beneath the eye on each side of the face, a well-defined area of scarlet velvety feathers present; the area is dotted with black feathers; besides, a narrow bare ridge runs from the eye to ear-tufts; beak, light grey or horny; iris, brownish yellow; no comb, wattles, nor ear-lobes, present.	Crown, greenish black, possessing a pair of ear-tufts of 6 *mm* length; on each side of the face, above and beneath the eye a scarlet velvety area more strongly developed than in the pheasant, being more markedly dotted with black feathers; both the upper and under velvety areas so well developed as to build each a lobe at its upper and under margin, respectively; both the upper lobes, erectile and contact each other above the crown, but very liable to shrink when the bird frightened. Between the areas, a bare velvety-feathered stripe of skin present reaching the occipital region. Upper mandible, bluish white; under mandible, yellowish white; iris bluish.
Throat and neck	Greenish violet with metalic luster; no ring around the neck present.	Greenish violet, lustered; no ring around the neck present.
Body	Breast, brilliant greenish black; interscapular region, back and saddle, covered with greenish black feathers crescented brownish; belly, greenish black; flanks, dark or blackish brown; upper tail-coverts, silky and olive grey.	Interscapular region and back, brilliant green, feathers being crescented with light brown; breast, deep greenish black; flank, brownish black; saddle and upper tail coverts, silky and greenish grey, in part olive green.
Wings	Wing-coverts, greenish grey; primaries consisting of 8–10 feathers, brownish black with 7–9 whitish bars; secondaries, brownish, pepper-and-salt; tertiaries, brownish crescented.	Wing-coverts, grey; primaries consisting of 9 feathers, brown with about 8 bars, though not well defined; seconderies and tertiaries, almost identical with those of the pheasant male.
Tail	Consisted of 16 greenish grey feathers, carried low; the middle tail feathers show 24–25 black bars bilaterally lamellated with brown silky feathers.	Consisted of 17 feathers; feathers, black-barred; their bilateral brownish lamellated parts, wider than in the pheasant.
Legs	Shank, slate-like, covered with 15–16 scales in a row; spur, slender and sharp, directing rather upward, deep grey in coloration but paler than claws.	Shank, paler than in the pheasant; spur, short, thick and horizontally directed.
Style	Forehand, more strongly developed than rear, carried low or horizontal; the height on feet, 29–30 *cm* at most.	Rear, more strongly developed than forehand, body, carried rather upright; the height standing on feet, 44 *cm*.
Call	Characteristic crow with piercing tone.	Usually silent; sporadical uttering something like 'co-co-co'.
Temperament	Wild.	Tamable only by attendant.

Table 2. Measures of size in parent birds and hybrid male obtained by S. C. W. Leghorn ♂ × Japanese Pheasant ♀.

	S.C.W. Leghorn	Japanese Pheasant		F_1-hybrid
		♂	♀	♂
1. Live weight *g*	2, 100	1, 054	950	1, 090
2. Length of beak *mm*				
a. Linear length from mouth angle to tip of upper mandible.	37	22	26	32
b. Curve length from base to tip of upper mandible.	25	32	24	35
3. Linear length of the longest feather in wings *mm*	290	156	155	162
4. Length of the longest feather in tail *mm*	415	295	225	267
5. Length of shank *mm*	105	65	57	67
6. Length of spur *mm*	35	13	—	5

Remarks: Of the birds in the table, Leghorn male and pheasant hen were actually used for insemination; the pheasant male, though not used for the present experiment, was the one reared together with the above hen in the aviary of Hiroshima University.



Text-fig. 2. English Pheasant male.
(By courtesy of Mr. S. MOCHITSUKI)

DISCUSSION.

Although a relatively large number of chicken-pheasant hybrids have been reported, the pheasant used for crossing, either male or female, has been in a majority of cases Ringneck pheasant, *Phasianus colchicus* L., or its local varieties (GRAY, 1958). As for the hybridization between Japanese Green Pheasant, *Phasianus versicolor* VIEILLOT, and domestic fowl, there seems to be only two cases in literature communicated by YAMASHINA (1942), except some observations of presumed hybrids illustrated by KURODA (1934).

In crossing of chicken on pheasant or reciprocal crossing, it is known that matings between these birds occur very rarely in nature, and also, that, even in successful matings, the fertility and hatchability of the hybrid eggs are exceedingly low. In view of this, attempts have been made by several authors to mate chicken and pheasant by use of artificial insemination.

At the dawning of studies on the artificial insemination, IVANOV (1913) tried the hybridization of chicken and pheasant by applying this technique: the author inseminated 16 domestic hens, each four times, with semen collected *post mortem* from the Vasa deferentia of Caucasian Pheasants but no fertile eggs were obtained. Much later, the first successful insemination was carried out by SANDNES and LANDAUER (1938) though the main objective of the experiments was to determine the sex ratio of the hybrid eggs produced. The authors applied the method of BURROWS and QUINN for collecting semen and obtained in the cross of chicken ♂♂ × pheasant ♀♀ 135 hybrids consisting of 66 males and 69 females whereas in reciprocal cross 29 hybrids consisting of 23 males and 6 females were produced.

In a larger extent, the crossing experiments of Ringneck Pheasant ♂♂ × Dark and Silver Cornish ♀♀, naturally and artificially, were made by SHAKLEE and KNOX (1954) and described about the technique as well as the hybrid birds in detail. Insemination was performed by use of 0.1 *ml* of undiluted or diluted semen in 1 : 2 with modified Ringer solution, yet the fertile eggs were secured only with diluted semen. With 1,409 hybrid eggs from natural and artificial matings, it resulted 3.48 percent "fertility" and 6.12 percent hatchability of "fertile" eggs. When only the artificial insemination is considered, "fertility" was 1.50–3.85 percent and hatchability of "fertile" eggs was 0.00–33.33 percent.[1] One female and four male hybrids developed sufficiently to pip the shell; one female and two male hybrids hatched and were raised to maturity. Subsequently ASMUNDSON and LORENZ (1957) applied exclusively artificial insemination for crossing of Ringneck Pheasant with Cornish and New Hampshire; 0.03–0.05 *ml* undiluted semen was used. Fertility was higher from chicken ♂♂ × pheasant ♀♀ than from the reciprocal cross (22.0–29.3% against 5.1–15.8%) while hatchability of fertile eggs showed 9.8–21.7 percent in the former cross and 25.0–53.6 percent in the latter.

Throughout the reports above referred, it may be seen that artificial insemina-

1) The authors are of opinion that some of the "fertility" considered here might be due to forces other than true fertilization by sperm.

tion is useful for producing the chicken-pheasant hybrid but the fertility and hatchability of produced eggs, are remarkably low. As the consequence, the characteristics of adult hybrids experimentally produced have been rarely described. The causal factor of this low fertility is not yet fully explained. Undoubtedly, the difference of chromosomes in number between the parent birds should not be left out of account. The data compiled by MAKINO's monograph (1956) show the chromosome number of the Japanese Green Pheasant to be 2n=82 ♂, 81 ♀ whereas that of the domestic fowl is 2n=78 ♂, 77 ♀; further, in the hybrid from *Gallus gallus* × *Phasianus colchicus*, YAMASHINA (1934) has determined the resultant number of chromosomes as 2n=80 ♂, 79 ♀. There is reason, therefore, to believe that low fertility and early embryonic mortality as well as decrease in hatchability of "hybrid" eggs might be in part due to this chromosomal difference. Nevertheless, considering the fact that the infertility in chicken × pheasant matings, natural or artificial, is not absolutely sterile but conditionally fertile, there seems to be much room for promoting the fertility of "hybrid" eggs by improving the insemination technique, specially by use of appropriate semen dilutor. As a matter of fact, the result of the present experiment indicates to a certain degree this possibility by protecting sperms from unfavorable physiological conditions in the oviduct of genus- or species-different hen.

SUMMARY.

An attempt was made to test the availability of the "Hiroshima" method of artificial insemination for producing the intergeneric hybrid between Single Comb White Leghorn cock and Japanese Green Pheasant hen (*Phasianus versicolor* VIEILLOT).

1. The method was composed of (1) semen collection by abdominal massage with use of a bird-holder, (2) dilution of semen with osmotically adjusted egg yolk-citrate buffer at rate of 1 : 4 and (3) insemination into the uterus.

2. The constituents of the buffer was: one volume 6.5 % $Na_3C_6H_5O_7 \cdot 2H_2O$ solution + one volume 9.0% glucose solution + two volumes fowl egg yolk.

3. Having laid one egg on the second day after a single insemination, the pheasant hen stopped further laying. Nevertheless, this one and only egg was fertile and a male hybrid has developed to adult.

4. It seems to show that the technique here used is fairly available with success for interspecific or intergeneric hybridization of birds.

5. The type of the hybrid produced thereby was quite different from that of chicken-pheasant hybrids known in the ornithological literature, rather resembling the English Pheasant.

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授精法で作出した白レグ雄とニホンキジ雌の人工雑種について

渡辺守之・山根甚信・実永　悟・高橋利男

広島方式家禽人工授精技術の利用価値を試験するために白レグ♂×ニホンキジ♀の属間雑種作出を試みた．この報告は予報的のものであるが次のように要約される．

1. 広島方式授精法は次の3点から成る．
   (1) 精液採取に当っては創案した鳥類保定器を利用して雄鶏の腹部マッサージを行った．
   (2) 原精液は滲透圧を調節した卵ク液で1：4の割合で稀釈した．
   (3) 精液は子宮内に注入した．
2. 用いた卵ク液の成分は次の通りである．6.5%$Na_3C_6H_5O_7 \cdot 2H_2O$液1容量＋9.0%ブドウ糖液1容量＋鶏卵黄2容量．
3. キジ♀は一回授精を行った翌々日一卵を産したのみで，その後産卵を停止したが，この唯一の卵は受精し，順調に孵化育成することができ，現在完全に生長している．
4. 本実験はただ一回のみの施術であり，一卵を生産したのみであるに拘らず，これが完全に受精し且従来孵化育成に困難視されていたニワトリ×キジ雑種が成鳥にまで生育したことは，応用した広島方式家禽人工授精術が鳥類の種間又は属間雑種の作出に有力な手段であることを示すものである．
5. この実験で作出した雑種は，交配した両親が，前研究者のものと因子型を異にしているために，鳥学文献上未知の形態を表わし，その頭部の装飾は寧ろスミレキジに酷似するものとなつて現われた．

Explanation of Plate 1

Head and neck in life of Japanese Green Pheasant cock (left), Single Comb White Leghorn cock (right), and their intergeneric hybrid male (center). Note the remarkable differences in the appendages of the face; the hybrid more resembles English Pheasant than its parents.