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Genetic endangerment of wild Red Junglefowl *Gallus gallus*?

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Summary

Domestic chickens were derived from the wild Red Junglefowl *Gallus gallus*. A survey of 745 museum specimens of Red Junglefowl suggests that most wild populations have been contaminated genetically by introgression of genes from domestic or feral chickens. A male eclipse plumage, which appears to be an indicator of pure wild genotypes, was found in populations in the western and central portions of the species's range, but not in the easternmost populations. Eclipse plumages probably disappeared from extreme south-eastern Asia and the Philippines prior to the advent of intensive scientific collecting (about 1860) and have not been observed in Malaysia and neighbouring countries since the 1920s. Populations exhibiting eclipse plumages were found in north-eastern India as late as the 1960s, but the dense human populations there make their continuing genetic integrity uncertain. These data suggest that surveys of wild and captive populations should be undertaken to assess the genetic integrity of this species. A re-evaluation of the conservation status of Red Junglefowl might then follow.

Introduction

The evolution of chickens can be conceived of as a three-step process: (1) evolution and speciation of wild ancestors, (2) domestication, and (3) diversification into numerous varieties under artificial selection by humans (Stevens 1991). We suggest an additional process: subsequent replacement of wild genes through hybridization with feral or free-ranging domestic stock. This process may have caused, or may now be causing, the genetic extinction of wild populations.

The genus *Gallus* includes four species distributed across southern and south-eastern Asia: Green Junglefowl *G. varius* of Java and associated islands, Ceylon Junglefowl *G. lafayettii* of Sri Lanka, Grey Junglefowl *G. sonneratii* of central and western India, and Red Junglefowl *G. gallus*. The last-named species is broadly distributed from western India and throughout South-East Asia, in the Philippines, and on numerous Pacific islands, to which it was evidently introduced by humans. Although discussion regarding the origin of chickens has raged since Darwin's time, domestication from Red Junglefowl is not disputed (Stevens 1991). Domestication was long thought to have taken place in India for cockfighting (by 4000 years BP), but more recent archaeological evidence indicates domestic chickens in China as early as 8000 yr BP (West and Zhou 1989). Hence, domestication may have taken place further east in South-East Asia or southern China.

Many observers have noted that most modern captive "Red Junglefowl" lack a male eclipse moult and other plumage characteristics generally considered to typify wild populations (Morejohn 1968). Because individuals from eastern wild populations appear more similar to domestic chickens in these characters, we considered two alternative hypotheses: (1) that two lineages with distinct ontogenetic sequences are present across the species range, domestic stocks deriving from the easternmost populations; and (2) that genetic contamination of wild populations by domestic genes has led to the disappearance of wild-type characters, particularly in the eastern part of the range. We present evidence favouring the latter hypothesis, and therefore suggest that introgression of domestic genes into the wild Red Junglefowl gene pool threatens the integrity of the species, thereby raising serious conservation concerns.

Characters and methods

Specimens of Red Junglefowl housed in 19 museum collections in the United States, Canada, and Europe were inspected, or data were provided by curators. Museums included the American Museum of Natural History (AMNH); British Museum (Natural History) (BMNH; selected specimens); Canadian Museums of Nature; Cincinnati Museum of Natural History & Science; Denver Museum of Natural History; Field Museum of Natural History (FMNH); Los Angeles County Natural History Museum; Museum of Comparative Zoology, Harvard University (distributional data only); Museum of Vertebrate Zoology, University of California, Berkeley; Museum of Zoology, University of California, Los Angeles; Musée d'Histoire Naturelle, Bruxelles; Musée de Zoologie de l'Université de Liège; Muséum d'Histoire Naturelle, Geneva; Muséum National D'Histoire Naturelle, Paris (MNHNP); U.S. National Museum of Natural History; University of Florida Museum of Natural History; University of Kansas Natural History Museum (KUNHM); University of Michigan Museum of Zoology; and Yale Peabody Museum. Information was collected from a total of 745 specimens.

Several phenotypic features might be used to distinguish pure Red Junglefowl from domestic stocks or hybrids, including the following (Lucas and Stettenheim 1972, Delacour 1977):

(1) *Eclipse plumage*. Some Red Junglefowl moult into an eclipse plumage (Lucas and Stettenheim 1972). The only portions of the plumage visibly affected are the hackles in the middle of the male's back, which are black and not elongated in the eclipse plumage, in contrast to the elongated, red-orange plumes of the main plumage. Females pass through parallel moults, but resulting plumages are indistinguishable from each other (Morejohn 1968). The eclipse plumage typically occurs in the months of June to September, with moult into the basic plumage as late as October (Johnsgard 1986). Domestic stocks appear to lack this plumage entirely (Morejohn 1968).

(2) *Leg colour*. Most Red Junglefowl have slender, dusky blackish legs, whereas domestic stocks have thick, warty-skinned, often yellowish legs. Many apparently wild individuals have light brown legs, and a few wild-caught birds show legs typical of domestic stocks.

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(3) *Hen comb.* Red Junglefowl hens lack combs almost completely, with nubs occasionally visible only when crown feathers are parted. Domestic hens, however, frequently have prominent combs and wattles.

Additional differences are believed to exist in other characters not as easily studied: the cock's crow in domestic stocks has an added terminal syllable (Ali and Ripley 1987), and domestic stocks hold the tail vertically instead of horizontally (Beebe 1926, Delacour 1977). These characters, however, were not included in the present study. Data were collected from a total of 745 apparently adult specimens (351 males, 394 females).

The three characters described above were evaluated on each specimen, either by one of the authors or by the curators of the respective collections. Eclipse plumages were noted as present or absent; leg colour was noted on a four-point scale (0 = black, 3 = yellow or pearl-white, on dried study skins); and combs were scored as to the number of "teeth" present (length and width of comb would have been preferable, but such measurements were not possible on dried study skins. All this information was organized in a data base, with fields for museum, catalogue number, date, sex, age and the three characters described above. Localities were keyed to maps to assess geographic distributions of characteristics.

Results

Character variation

Eclipse plumages were noted in 10 specimens out of 132 males collected in the appropriate season (June–October). In general, leg colour ranged from black to corn yellow. Combs were either absent or much reduced in hens.

Eclipse-plumaged birds tended to have blackish legs (four of five individuals for which data were available). No relationship was apparent for comb structure, possibly because comb size was not correlated with number of comb teeth. Other individuals showed extreme values for several characters in combination: for example, specimen FMNH 77565 had swollen, warty yellow legs, a large comb with seven points, and arched tail streamers. Nonetheless, relationships among these characters were not straightforward. For instance, no relationship existed between number of comb points and leg color ($n = 351$, $r^2 = 0.002$).

Evidence for swamping

A critical first question is whether the character variation described above results from hybridization and introgression from domestic stocks, or whether it simply represents natural variation in wild populations. Although subjective and anecdotal, specimen evidence indicates that hybridization is likely to have been involved: for instance, the tag for specimen KUNHM 43655 reads "going into forest with domestic hen." The tag for specimen FMNH 77565 reads "the domestic form most common here", and indeed that specimen shows ample evidence of influence of domestic genes (see above).

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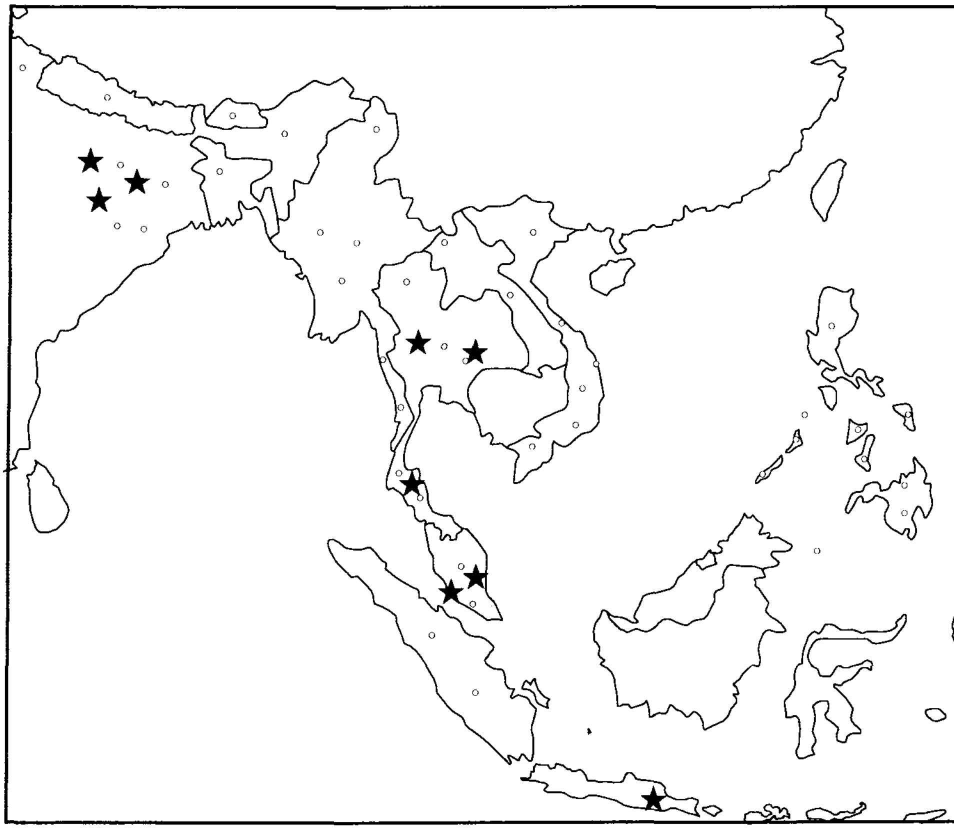

Figure 1. Map of southern and south-eastern Asia, showing the geographic distribution of Red Junglefowl specimens examined in this study. Collecting localities for specimens in eclipse plumage are indicated with stars; other localities are indicated with open circles.

Geographic distribution of "clean" genes

The presence of eclipse plumage is the most reliable guide available to the geographic distribution of wild-type Red Junglefowl, although the persistence of eclipse plumage after initial hybridization cannot be ruled out. It is clear, however, that this trait is lost after repeated hybrid crossing (Morejohn 1968). We found such pure stocks from across southern Asia from western India to Thailand and Malaysia (Figure 1, Table 1). No eclipse-plumaged birds were found in the furthermost south-east areas of Asia (Vietnam, Laos, Cambodia), or on any of the islands where the species is possibly (Philippines) or certainly introduced (e.g. New Hebrides, Celebes, Fiji Islands).

Because eclipse-plumaged individuals are rare, however, the degree to which these apparent geographic patterns are the result of sampling error is open to question. In India and Nepal, 4 of 22 (18.2%) males collected in the appropriate season showed eclipse plumages; in Myanmar, Thailand and Malaysia, 6 of 31 (19.4%) males were in eclipse; these two proportions do not indicate significant variation in encounter probabilities (Fisher exact test, $P > 0.05$). However, none

Table 1. Details of eclipse-plumaged Red Junglefowl specimens encountered in this study

Catalogue number	Locality	Date	Notes
KUNHM uncat.	India, north-central portion, Dehra Dun	1960s	Member of captive colony taken from wild stock in late 1960s
FMNH 80556	Thailand, Nong Dorn	7 July 1927	—
FMNH 20741	India, Central Province, Belwani-Kisli	28 July 1946	—
FMNH 20743	India, Central Province, Belwani-Kisli	23 Sept. 1946	Orange-red feathers in pin; back otherwise black
AMNH 203747	Malaysia, Pahang	no date	—
BMNH 1881.5.1.5311	India	Before 1881	—
BMNH uncat.	Malaysia, Malacca	no date	—
BMNH 1955.1.503	Thailand, south-western portion, Koh Lak	29 June 1917	—
BMNH 1955.1.509	Thailand, eastern portion, Pak Jonk	3 June 1916	—
BMNH 1955.1.510	Thailand, eastern portion, Pak Jonk	3 June 1916	—
Robinson and Kloss (1920)	Java, eastern portion, Idjen Volcano, Banjoewangi	—	Cite series including males in eclipse plumage

of 13 males from the appropriate season in Vietnam and Cambodia, or 7 males collected in the appropriate season from the Philippines showed evidence of eclipse plumage. This absence is unexpected, based on a statistical comparison with birds from India to Malaysia (Fisher exact test, $P = 0.05$). Hence, frequencies of eclipse plumages vary geographically across the species's geographic distribution, with individuals from the western portion showing eclipse plumages more frequently.

Temporal distribution of wild characteristics

Specimens collected in the appropriate season for detection of the eclipse plumage are dated 1847–1981. These records can be taken as a sample of the presence of wild versus contaminated phenotypes over that period.

Of the 10 records of eclipse-plumaged junglefowl (Table 1), eight wild-caught individuals were identified at least as to year of origin. These individuals included records from 1881, 1916 (2) 1917, 1927, 1946 (2), and 1966. Compared against the entire sample of males collected in the appropriate season, these specimens were not distinguishable from a random sample (bootstrap comparison based on 1,000 randomly sampled octets, $P = 0.464$). Hence it can be concluded that the eclipse-plumaged specimens are not significantly older than other individuals in the study.

Nevertheless, the complete absence of eclipse plumage in birds from the Philippines and extreme south-eastern Asia suggests that these characters disappeared from those regions prior to most specimen sampling. The last specimens showing this plumage from the Malaysian region were from the 1920s, whereas

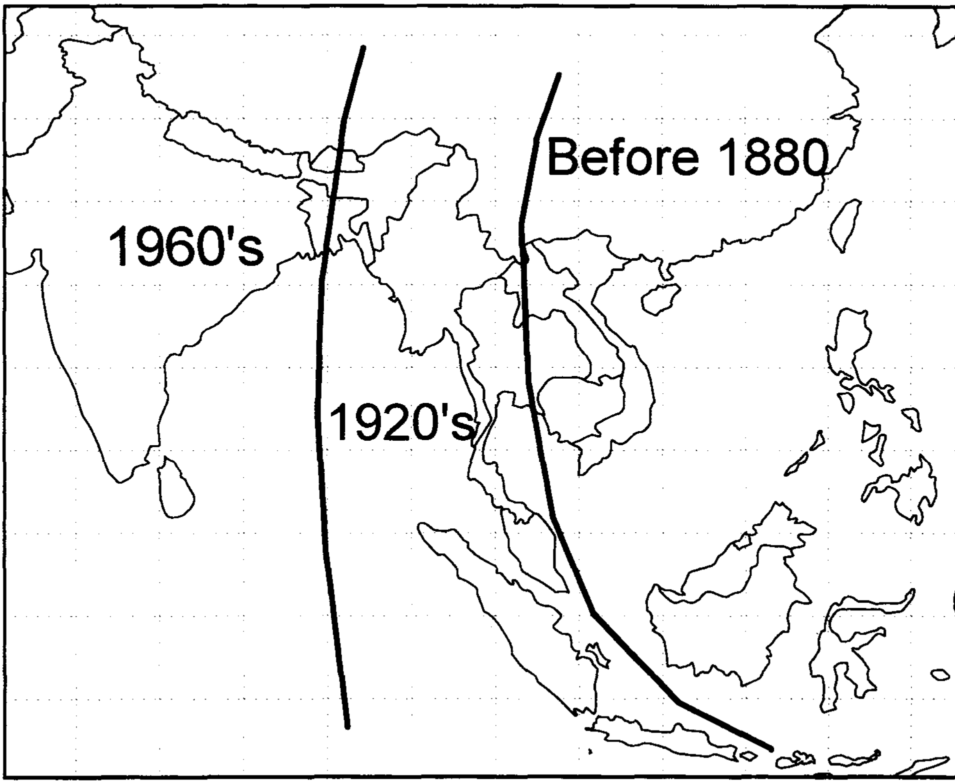

Figure 2. Map of southern and south-eastern Asia, showing a schema for times of last detection (probable extinction) of eclipse plumages across the geographic distribution of Red Junglefowl.

the trait persisted in India and neighbouring countries until at least the 1960s (Figure 2). Whether or not populations showing eclipse plumage still persist in South Asia will remain unknown until further museum or field studies are possible.

Discussion and conclusions

This study provides an excellent example of how systematic collections can be used as a long-term record of biological diversity. In this case the existence of such a storehouse of information provides the critical dimension of time to a study of human interactions with biological diversity. Unanticipated by the collectors and ornithologists who obtained and preserved the specimens over the course of two centuries, they have served to signal a cryptic threat to the single wild bird species arguably of the greatest importance to humans.

We believe that wild Red Junglefowl were originally characterized by a number of features that distinguished them from their domesticated cousins. However, in the course of domestication, artificial selection affecting some characters caused them to either disappear (eclipse plumage) or change (legs, combs and calls), most changes tending to accentuate male secondary sexual characteristics. Subsequently, the ubiquity of human populations in southern and south-eastern Asia provided ample opportunity for contact between domestic chickens and local wild populations, and extensive interbreeding has apparently taken place.

Human influence and contact with domestic stocks appear to have been most intense, or to have occurred over the longest period of time, in South-East Asia. A cline for age of disappearance of eclipse moult extends from India (1960s) through Malaysia (1920s or 1930s) to Vietnam and the Philippines (before 1860). The Philippines and other islands may even have been "seeded" with junglefowl previously modified by early human colonists, and hence hold junglefowl populations that were genetically contaminated from the outset. These findings clearly refute our first hypothesis (two lineages), and point convincingly to the pervasive effects of genetic contamination from domesticated stocks.

Considering Red Junglefowl as a wild bird species, our evidence suggests that contamination with domestic genes may have been widespread if not ubiquitous. This situation parallels closely that of the wild Rock Dove *Columba livia*, which has been introduced worldwide, but is gravely endangered in terms of truly wild populations (Johnston *et al.* 1988). The stronghold of the wild Red Junglefowl is, or more likely was, in western India, where eclipse plumages were present until at least the late 1960s. However given that human populations in the region are extremely dense and still increasing, genetic influence from domestic chickens is probably now strong throughout the region, so that genetically pure wild-type populations may be severely threatened. An important priority now is to conduct field surveys to find populations still unaffected by domestic genes. Promising areas would seem to be western India and Nepal, in the period June to September, when males in eclipse plumage would be encountered, if present.

Another implication of this study is that captive "Red Junglefowl" stocks are likely to be tainted with domestic genes. Recent molecular genetic studies of these stocks intended to infer the geographic origins of domestic chickens (e.g. Fumihito *et al.* 1994) are therefore suspect, because inferred antecedent stocks may be those most heavily contaminated genetically. These studies may have to be repeated based on DNA recovered from the oldest museum specimens and/or populations in remote regions not yet affected by the presence of domestic chickens, if any can be found. Once a DNA profile for pure wild junglefowl is developed from historical specimens, much more objective assessments of the genetic purity of wild and captive-bred Red Junglefowl populations will be possible, based on samples or specimens collected at any time of year.

The Red Junglefowl, so important economically and culturally to humans, is apparently in danger of genetic extinction, so measures should be taken to assure its long-term survival. Fortunately, a colony of apparently pure, wild stock collected in the Dehra Dun region of northern India in the late 1960s has been *maintained in genetic isolation by one of us (ILB)*. This colony appears free of domestic influence: males undergo a yearly moult to a complete eclipse plumage, legs are slender and blackish, combs are relatively simple in males and lacking in hens, and tail streamers are not arched.

A number of priorities emerge. First, regarding captive stocks, uncontaminated lines should be identified using methods presently available (e.g. presence of eclipse plumage), documentation of breeding lines should be undertaken, and pure stocks should be safeguarded by distribution among captive breeding facilities. Another goal, initially based on studies of captive individuals and of old museum specimens, is the development of a DNA profile for the "clean" junglefowl genome. In addition, rapid surveys of populations on the Indian subcontinent and in South-East Asia are required to assess morphological characteristics of, and to collect blood or tissue samples from, individuals in the wild. Only with these additional initiatives will the true extent of the problem highlighted in this paper be understood.

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