Playing chicken with red junglefowl: identifying phenotypic markers of genetic purity in Gallus gallus

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Abstract

We report the results of a novel experiment, in which genetically pure male red junglefowl Gallus gallus (Richardson strain) were deliberately crossed with domestic female chickens to create contaminated lines of known purity, reaching as high as 93.75%. Phenotypic characters generally used as indicators of purity (reduced or absent female comb, male eclipse plumage, etc.) all appeared to at least some extent in domestically contaminated progeny and moreso in successively more pure generations of the experiment, suggesting that such phenotypic characters may have little, if any, utility in characterizing red junglefowl stocks as to their genetic purity.

Introduction

Genetic contamination of wild populations via hybridization with domesticated stocks represents a serious, but underappreciated concern in the conservation of biodiversity (Brisbin, 1995; Rhymer & Simberloff, 1996). In spite of widespread lack of concern for the conservation status of red junglefowl Gallus gallus (Delacour, 1951; Johnsgard, 1999; BirdLife-International, 2000), recent studies of phenotypic characters suggest that genomes of most wild populations of the species have been contaminated via widespread hybridization with feral village chickens (Brisbin, 1996; Peterson & Brisbin, 1998; Brisbin et al., 2002). These concerns, as well as the intense research efforts that focus on behavior and molecular evolution of birds that are referred to as ‘junglefowl,’ but lack documentation of the lineage under study (Desjardins & Morais, 1990; Akishinonomiya et al., 1994; Collias, Collias & Jennrich, 1994; Akishinonomiya et al., 1996; Smith et al., 1996; Ligon, Kimball & Merola-Zwartjes, 1998; Zuk et al., 1998; Nishibori et al., 2005; Jensen, 2006; Liu et al., 2006), demand identification of pure versus contaminated junglefowl genomes, a capacity that presently depends on phenotypic characters of uncertain utility and dependability (Peterson & Brisbin, 1998; Brisbin et al., 2002). These concerns are amplified still more when one considers that precisely the characters that are the focus of many behavioral studies (Ligon et al., 1998; Zuk et al., 1998) are those that differ between pure and intermixed junglefowl (Peterson & Brisbin, 1998; Brisbin et al., 2002), creating the possibility of serious misinterpretation and confusion.

Numerous phenotypic characters have been identified as signals of genetically pure junglefowl genomes. These features include (1) the presence of an eclipse plumage in males between June and September, in which the red or yellow neck hackles are replaced with spatulate black feathers, along with other plumage alterations; (2) absence of a comb and wattles in hens; (3) slender, dark legs; (4) horizontal body posture and carriage of the tail in a horizontal position; (5) a simpler and shorter call (Delacour, 1951; Kimball, 1958; Morejohn, 1968, 1974; Crawford, 1990; Peterson & Brisbin, 1998; Brisbin et al., 2002). Although these features were used in a recent effort to assess the genetic status of wild populations (Peterson & Brisbin, 1998), no information is available that guarantees that possession of these traits actually assures that a population is free of domestic genes.

The purpose of the present study was to document phenotypic changes across a sequence of junglefowl-chicken hybrids of varying but known degrees of genetic purity to test the reliability of these phenotypic traits as indicators of pure junglefowl derivation. We hybridized males from the most pure captive red junglefowl line known [the Richardson strain; (Peterson & Brisbin, 1998; Brisbin et al., 2002)] with hens from a domestic strain of bantam chicken (Brisbin, 1993), and then backcrossed the female progeny to pure junglefowl males for four generations. Our rationale for this experimental approach was that the most likely
scenario for contamination of wild genomes would be via pure junglefowl males mating with free-ranging female village chickens, followed by dispersal of the young back into the forest, where they would subsequently backcross to pure wild birds (Brisbin, 1974). Four generations of hybridization and backcrossing produced known hybrids that were 93.75% pure junglefowl, and our evaluation of their phenotypic characteristics is presented here for comparison with those of other current existing captive ‘junglefowl’ lines.

Methods

Two pure red junglefowl males were used as the paternal stock. They were descended from a line (the Richardson strain) collected in eastern India in the vicinity of Dehra Dun in the 1960s (Brisbin et al., 2002). The population has been maintained in genetic isolation in numbers of 10–50 individuals since the 1960s. Because of the small size of the captive population of this line and its key role in the genetic conservation of the species, only two individuals could be spared for this experiment (the initial parent male died during the experiment, and is now preserved as specimen KUMNH 110223; other examples of this junglefowl stock are KUNHM 88896, 110221–110222, 110224).

The domestic bantams used as initial maternal stock were composed of four hens chosen randomly from a breed known as the Carolina bantam chicken. As explained elsewhere (Brisbin, 1993), the Carolina bantam chicken was developed over a period of >40 years by allowing free-choice mating within both captive and free-ranging (barnyard) habitats, of the descendents of a mixed flock of several standard bantam breeds or varieties (Latimer, 1976; Latimer & Brisbin, 1987). As such, this breed has developed over many years under conditions of both natural and free-choice sexual selection similar to those experienced by free-ranging domestic village chickens within the native range of the red junglefowl. This breed, although originally diverse in terms of plumage and comb phenotype (KUNHM uncataloged), has now, through generations of natural and sexual selection, become fairly uniform in phenotype: black or red plumage in males, dark brown or black plumage in hens, red or gold hackles in males, and tail held erect (Brisbin, 1993).

The experimental design was simple: the first pure male and four feral hens were penned together, whereas a control line composed of one to three male and four female Carolina bantam chickens (KUNHM 90607, 90609, 110209–110220) was kept in an adjacent pen. The result of this first experimental cross is referred to as the F1 generation, and is 50% pure junglefowl (KUNHM 110178–110191). In successive generations, we backcrossed the F1 hens to the same pure male, resulting in 75% pure individuals (KUNHM 110166–110177); third and fourth generations in the experiment crossed the female offspring of this generation and the next (respectively) against the second pure male to produce individuals that were 87.5% pure (KUNHM 110192–110202) and 93.75% pure (KUNHM 110203–110208). The hybridized and control lines were thus maintained in the same environment (adjacent pens) during their entire development. In each generation, as indicated by the KUNHM catalog numbers listed above, voucher study skin specimens were prepared of three to 14 individuals per generation after their participation in the production of the next generation, being sacrificed at the ages of 9–36 months. (After initial concern about the young age of some six of the specimens – 9–12 months – we repeated all analyses omitting them, and found no qualitative differences, and so we report on the full dataset herein).

After preparation and full drying of study-skin vouchers, all museum specimens in the experiment were measured: bill length from the anterior edge of nostril to tip, tarsus length from the proximal end of the tarsometatarsus to the lowest undivided scute above foot, leg cross-sectional area (indexed as the product of diameter front-to-back × diameter side-to-side at the midpoint of the tarsometatarsus), straight-line spur length (males only), middle toe length (without claw) from the base of the phalanx to the base of the toenail, wing chord (unflattened), tail length (from the base to the tip of the longest rectrix), comb size (indexed as the product of approximate length along the dorsal midline × approximate height at the tallest point) and wattle size (indexed as the product of approximate length × approximate breadth). These measurements were imported into S-Plus for statistical analysis.

Results

Males of the two parental lines (0 and 100% purity) did not differ in most of the characters assessed (tarsus length, spur length, middle toe length, wing chord and tail length; Table 1). The other characters (bill length, leg cross-section, comb size and wattle size) showed statistically significant differences between male parental lines (Mann–Whitney U-tests, all P < 0.05). Although these patterns appear robust, it should be borne in mind that our sample sizes of pure junglefowl males are small (n = 4).

Similarly, using simple linear regression to assess trends of character values with respect to genetic purity, we found the regression slopes to be significantly different from zero in the same suite of variables (bill length, leg cross-section, comb size and wattle size; Fig. 1). For example, female comb area was reduced from large combs present in control females (364–465 mm²) to 3–6% (i.e. 9–21 mm²) in the 93.75% pure generation. In general, the declines were smooth and more or less linear (Fig. 1).

An unexpected finding of our experimental crosses was the extremely wild, wary and flighty behavior of the hybrid progeny. Although hand-raised in brooder boxes and handled regularly from hatching, these birds showed all the wild and flighty behavior of the pure junglefowl parental line and none of the tameness of their bantam chicken mothers. Even at ages of only a few days, hybrid chicks would often dash themselves against the walls of their brooder boxes during routine care operations. The extreme care required to prevent birds from injuring themselves complicated
Table 1 Summary of statistical comparisons among chickens and red junglefowl *Gallus gallus* of different levels of purity

<table>
<thead>
<tr>
<th></th>
<th>Pure versus control males</th>
<th>Male regression</th>
<th>Female regression</th>
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<tbody>
<tr>
<td>Bill length</td>
<td>*</td>
<td>–0.0206x+16.858*</td>
<td>–0.0190x+15.291*</td>
</tr>
<tr>
<td>Tarsus length</td>
<td>–</td>
<td>–0.0254x+71.067</td>
<td>–0.0325x+59.844</td>
</tr>
<tr>
<td>Leg cross section</td>
<td>*</td>
<td>–0.300x+85.025*</td>
<td>–0.115x+49.89*</td>
</tr>
<tr>
<td>Spur length</td>
<td>–</td>
<td>–0.0189x+26.846</td>
<td>–</td>
</tr>
<tr>
<td>Middle toe length</td>
<td>–</td>
<td>–0.001x+45.721</td>
<td>–0.0043x+40.806</td>
</tr>
<tr>
<td>Wing chord</td>
<td>–</td>
<td>–0.0450x+220.4</td>
<td>–0.0053x+194.0</td>
</tr>
<tr>
<td>Tail length</td>
<td>–</td>
<td>–0.0860x+256.5</td>
<td>–0.0682x+135.5</td>
</tr>
<tr>
<td>Comb size</td>
<td>*</td>
<td>–10.363x+2012.46*</td>
<td>–4.256x+379.5*</td>
</tr>
<tr>
<td>Wattle size</td>
<td>*</td>
<td>–4.188x+614.7*</td>
<td>–0.393x+38200*</td>
</tr>
</tbody>
</table>

Pure versus control provides the results of a Mann–Whitney *U*-test comparison of pure junglefowl (*n*=4) and control Carolina bantam chicken males (*n*=10) (sample sizes for pure junglefowl females were insufficient to permit parallel analyses). Simple linear regression results of character value on purity level are presented within sexes; asterisks indicate regressions for which slopes were significantly different from zero; sample sizes were 36 for males and 29 for females. Boldface type indicates variables considered in further analyses (Fig. 1).

Discussion

Our previous work with red junglefowl (Peterson & Brisbin, 1998) assumed that a suite of phenotypic characters existed that could be used to detect genetically pure individuals. Based on this premise, we argued that most of the wild population of the species is likely contaminated by crossing with feral chickens, and that it may effectively have been extirpated across much or all of its distribution (Peterson & Brisbin, 1998, 2005; Brisbin et al., 2002). The present study reassesses those characters as reliable indicators, and our results make the picture considerably more bleak.

Our results indicate significant trends in the direction of the pure junglefowl parental stock in all characters that differed between the two parental lines. What is more, only in wattle and comb size (males only; Fig. 1) are pure junglefowl outside of the range of the 93.75% pure individuals. If genetic contamination events were far into the past, as is suggested by ample specimen evidence (Peterson & Brisbin, 1998, 2005; Brisbin et al., 2002), then some wild stocks may be more than 92% pure, and yet still have domestic chicken influence, and these contamination events would be undetectable using phenotypic markers. Hen combs had been perhaps the most promising of possible indicator traits; yet, the near-disappearance of combs after just a single backcrossing (75% pure; Fig. 1) is disappointing, suggesting that hen combs are not reliable indicators of purity either. As such, our results indicate that the phenotypic markers assessed here do not serve as reliable indicators of full purity – only of relative purity, that is better than 50–75% pure, which is a pretty poor indicator and helps the situation very little.

Combs in pure red junglefowl hens have been the subject of conflicting statements, including some by the present authors. Beebe stated that the female comb is a minute fleshy knob or inconspicuous notched ridge (Beebe, 1926a,b). Baker (1928) stated that the comb and wattles are small, and sometimes absent. Delacour (1947), however, created confusion: in the same publication, he stated that the comb is reduced to a small fold, and that pure hens show neither combs nor wattles. Worse still, we stated that hens lack combs almost completely, with nubs occasionally visible only when crown feathers are parted (Peterson & Brisbin, 1998), but later indicated complete absence of female combs (Brisbin et al., 2002). To resolve these differences, we describe the single pure Richardson hen that has been preserved as a specimen (KUNHM 110221): at first glance, the bird has no comb; however, upon close inspection and parting feathers, indeed a small thickened ridgeline corresponding to the comb was present. This ridgeline has 3 or 4 minute nubs, the largest of which might be 0.5 × 0.5 × 1 mm. Hence, in life, this comb would not have been observable without detailed examination, which may account at least for Delacour’s dual opinion.

Although our evidence is based on relatively small numbers of individuals, eclipse plumages also appear to fit this same profile. Indeed, of the only 12 male individuals in this study that were sacrificed in the correct period of the year (June–September) for detection of a male eclipse plumage (Kimball, 1958; Peterson & Brisbin, 1998), one clearly (KUNHM 110167) and two likely (KUNHM 110175, 110176) show evidence of molt into an eclipse plumage – yet, these individuals were only 75% pure! As such, and given that out of necessity our generations of higher genetic purity were sacrificed in other seasons and were difficult to observe in detail while alive, we are provisionally confident that eclipse plumages had ‘reappeared’ in the experimental line after only a single backcrossing to the pure parent. The broad geographic disappearance of this trait from wild populations (Peterson & Brisbin, 1998) suggests strongly that many wild junglefowl populations are also contaminated – indeed, our results suggest that even populations retaining this trait may nonetheless still be contaminated.
The phenotypic traits discussed above have been used widely in detecting contaminated stocks of captive red junglefowl, which, at least by these criteria, are often in our experience highly contaminated. However, among relatively pure stocks—and those most critical for conservation—these traits are now seen by our results to be of relatively little utility. Perhaps the only real hope for an absolute indicator of purity is among molecular characters, for which sequencing experiments are already underway (Peterson et al., in prep.).

The results of our hybridization experiments have ominous implications for conservation of pure wild red

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**Figure 1** Summary of trends in four phenotypic characters (those for which trends were significant; Table 1) as related to the genetic purity of hybrid chickens/red junglefowl *Gallus gallus*. The two parental stocks are represented as 0% purity (Carolina bantam chickens) and 100% purity (Richardson strain, red junglefowl). Hybrid generations (backcrosses to pure male red junglefowl) are in between: first-generation backcross = 50%, second-generation backcross = 75%, third generation backcross = 87.5%, fourth-generation backcross = 93.75% pure).
junglefowl genomes in the wild. Our behavioral observations suggest that the extremely skittish hybrid chicks that result from hybridization would likely, once independent of their village domestic hen mother, flee to the forest, just as Brisbin’s earlier experiments with cross-fostering had suggested (Brisbin, 1969). In this way, even if village chickens themselves do not stray far from human settlements, hybrid offspring would be expected to move far from these areas into the forest, where backcrossing to pure junglefowl, such as that mimicked by our experiments, would occur.

Given the lack of reliable phenotypic characters, development of effective molecular markers for purity will be critical to any management strategy being developed for wild populations of red junglefowl. Important steps will include (1) conducting immediate field surveys of remote areas to detect any last intact wild populations isolated (if possible) from village poultry, particularly at the western extreme of the species’ distribution where the last relatively pure wild individuals were captured (Brisbin et al., 2002); (2) development of reliable molecular genetic markers by which to screen any possibly pure wild populations that are encountered; (3) management of genetically pure stocks both in captivity and in the wild; (4) making arrangements and agreements to limit and control the use of free-ranging chickens in villages near critical areas of habitat with possibly pure populations; (5) propagate pure captive birds for release in areas of suitable natural habitats that are – or can be made – free of genetic contact with village chickens.

The surprisingly flighty behavior of the hybrid progeny raised in this study, appearing in full intensity even in the first-generation crosses, was not affected by conditions of rearing or attempts to acclimate the birds to handling, similar to our previous experience (Brisbin, 1969). These observations raise questions concerning the extreme difficulty that must have accompanied the original domestication of junglefowl. Peoples in earlier cultures, with less refined husbandry techniques than are available today, would likely have encountered significant challenges to raise and breed such birds in captivity, even when hatched from eggs and hand-reared, probably taking generations of (unconscious) artificial selection for the calmest and most approachable individuals (Trut, 1999). This observation flies in the face of recent arguments based on molecular studies for multiple independent domestication events (Nishibori et al., 2005; Liu et al., 2006). An alternative conclusion is that the birds of the Richardson strain are not representative of the junglefowl that actually got domesticated, as the Richardson birds are wilder and less manageable than any other captive galliform of which we are aware; experienced aviculturists report, for example, that chicks of at least some other junglefowl species can usually be handled without the extreme stress we note with the Richardson birds (I. L. Brisbin, pers. obs.).

Captive ‘red junglefowl’ have been subjects of numerous studies of evolutionary issues such as the role of head ornaments as signals of mates quality in sexual selection (Ligon et al., 1990; Zuk et al., 1990; Ligon & Zwartjes, 1995). However, descriptions of the birds used in such studies frequently indicate that they had larger ornaments than our pure Richardson red junglefowl or even the hybrids. Behavioral studies of ‘red junglefowl’ (Collias et al., 1966; Hakansson & Jensen, 2005) have also used subjects that may be suspect as to genetic purity. Rather, the birds used in such studies seem to be what would be expected of tame village chickens (to be sure, collected from within the range of wild red junglefowl) with a long history of human association and relaxation of natural selection pressures.

The data reported here in the present study thus raise questions as to whether the sizes of combs and wattles have really been subject to selection in pure wild junglefowl or whether selection for larger combs and wattles might not rather be an artifact of domestication and the relaxation of natural selection for smaller head ornaments, as suggested by the data presented.

In the most general sense, hybridization and introgression are phenomena that can threaten the genetic integrity of many wildlife species (Frankham, Bullou & Briscoe, 2004). The case of the red junglefowl described herein, however, involves the specific situation in which the threatened population is either the wild ancestor or a unique primitive form of a species that has since become a common and widely distributed domesticate. In such cases, ubiquitous free-ranging feral or pariah (semi-domesticated) counterparts may invade the threatened population’s range and can compromise the latter’s genetic integrity in important – but often difficult-to-detect – ways. Among birds, similar situations include the wild muscovy duck Cairina moschata of the Neotropics (Hoffman, 1992) and other waterfowl threatened by hybridization with free-ranging feral or domestic mallard ducks Anas platyrhynchos (Haig & Avise, 1996).

Similar examples among mammals include canids (Gottelli et al., 1994; Koler-Matznick et al., 2003; Sillero-Zubiri, Hoffman & Macdonald, 2004) and suids (Oliver, 1993), again with the wild population being threatened with hybridization by free-ranging counterparts of a widespread common domesticate.

The red junglefowl is a wide-ranging species that is not formally considered to be either threatened or endangered in any part of its range (BirdLife-International, 2000). If the threat to red junglefowl from genetic introgression with free-ranging village chickens could now be much more serious and widespread than had been thought, as is suggested by the information presented herein, the conservation status of this species is clearly in need of reassessment. Such a reassessment could be made with respect to the IUCN threat criterion 8.4, which deals with changes in native species population dynamics leading to hybridization. In reality, however, changes in the geometry of human habitation and thus the intrusion by village chickens into previously pristine red junglefowl habitat are now creating an urgent need for reassessment of conservation status. Currently, 48 species are listed as threatened under criterion 8.4, and the addition of red junglefowl to this list would certainly seem to be in order. The prudence of such a reassessment is reinforced for this bird, which, as the wild ancestor of the domestic...
chicken, has certainly had the greatest impact upon human culture, civilization and economic development of any bird species globally.

Acknowledgements

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References


