

## INVASION GENETICS: THE BAKER AND STEBBINS LEGACY

# Mixed ancestry and admixture in Kauai's feral chickens: invasion of domestic genes into ancient Red Junglefowl reservoirs

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## Abstract

A major goal of invasion genetics is to determine how establishment histories shape non-native organisms' genotypes and phenotypes. While domesticated species commonly escape cultivation to invade feral habitats, few studies have examined how this process shapes feral gene pools and traits. We collected genomic and phenotypic data from feral chickens (*Gallus gallus*) on the Hawaiian island of Kauai to (i) ascertain their origins and (ii) measure standing variation in feral genomes, morphology and behaviour. Mitochondrial phylogenies (D-loop & whole Mt genome) revealed two divergent clades within our samples. The rare clade also contains sequences from Red Junglefowl (the domestic chicken's progenitor) and ancient DNA sequences from Kauai that predate European contact. This lineage appears to have been dispersed into the east Pacific by ancient Polynesian colonists. The more prevalent MtDNA clade occurs worldwide and includes domesticated breeds developed recently in Europe that are farmed within Hawaii. We hypothesize this lineage originates from recently feralized livestock and found supporting evidence for increased *G. gallus* density on Kauai within the last few decades. SNPs obtained from whole-genome sequencing were consistent with historic admixture between Kauai's divergent (*G. gallus*) lineages. Additionally, analyses of plumage, skin colour and vocalizations revealed that Kauai birds' behaviours and morphologies overlap with those of domestic chickens and Red Junglefowl, suggesting hybrid origins. Together, our data support the hypotheses that (i) Kauai's feral *G. gallus* descend from recent invasion(s) of domestic chickens into an ancient Red Junglefowl reservoir and (ii) feral chickens exhibit greater phenotypic diversity than candidate source populations. These findings complicate management objectives for Pacific feral chickens, while highlighting the potential of this and other feral systems for evolutionary studies of invasions.

**Keywords:** conservation genetics, *Gallus gallus*, hybridization, invasive species

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## Introduction

Humans have dispersed over most of the Earth's surface, and we have not made these journeys alone. Our

migrating ancestors were accompanied by both accidental stowaway species (Mack *et al.* 2000; Lockwood *et al.* 2005; Estoup & Guillemaud 2010) and domesticated taxa that were utilized for food, labour or companionship (Larson *et al.* 2007, 2012). Subsequent to anthropogenic dispersal, many domesticated species have escaped cultivation and colonized new habitats, a process termed feralization. It can be helpful to think about feralization

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as 'domestication in reverse', as it involves the removal of direct anthropogenic control over natural and sexual selection regimes (Price 1984). Thus, feral population persistence requires survival and reproduction within novel social and ecological contexts. While there has been extensive research into what facilitates or hinders invasions of nondomesticated species (e.g. standing genetic and phenotypic variation), the process of feralization is less well understood. Progress in this area will help advance our basic understanding of biodiversification and can help mitigate feral species' impacts on native ecosystems and competitors (Loope 1999).

Feral habitats can potentially exert strong selection on several components of fitness in the wild (e.g. mate acquisition, foraging success, predator avoidance and disease resistance). Evolutionary responses to these selection pressures will depend upon both the genetic variability of feral populations and the genetic architecture of fitness-related traits (Goodwin 2007; Zohary *et al.* 2012). These properties of feral populations result from combined histories of domestication and feralization, each of which can be complex (Verardi *et al.* 2006; Stephens 2011; Feulner *et al.* 2013; McTavish *et al.* 2013; Nussberger *et al.* 2013). By characterizing genetic and phenotypic variation in feral taxa, we can therefore make progress towards the interrelated goals of understanding feral populations' origins and ascertaining their capacities to respond to current and future selection. To date, very few studies have jointly examined genetic and phenotypic variation in feral species using modern tools (although see Hampton *et al.* 2004; Randi 2008).

Here, we examine genotypic and phenotypic variation in feral chickens (*Gallus gallus*) on the Hawaiian island of Kauai. The origins of these birds are presently unclear; they have been alternatively described as either escaped farm pests ('feral domestic fowl') or as a 'legacy species' introduced by Polynesian colonists (i.e. Red Junglefowl), the chicken's closest free-living ancestor (Eriksson *et al.* 2008; see Box 1). Broad-scale studies of Pacific 'chicken' biogeography (using MtDNA markers) have also drawn conflicting conclusions as to whether contemporary populations are of ancient origin (Polynesian Red Junglefowl) or are descended from domestic breeds that originated more recently in Europe (Storey *et al.* 2012; Larson *et al.* 2014; Thomson *et al.* 2014). These uncertainties complicate efforts to use *G. gallus* biogeography to reconstruct Polynesian expansion into the Pacific and, possibly, South America (Storey *et al.* 2007, 2012; Beavan 2014; Bryant 2014; Thomson *et al.* 2014). They also raise important questions about best practices for feral population management. Although chickens and Red Junglefowl (RJF) can interbreed, applied biologists regard the two lineages very differently. Domestic chickens are a globally critical food

resource, vectors of highly lethal pathogens and our planet's most abundant bird (for example, see [http://www.uspoultry.org/economic\\_data/](http://www.uspoultry.org/economic_data/)). In contrast, RJF are poorly suited to commercial food production, are threatened or endangered in their native range and merit stringent conservation effort (Peterson & Brisbin 1998). Thus, ascertaining the history of Kauai's chickens will have important implications for invasion biology, cultural anthropology, and *G. gallus* conservation and management.

Our aim in this study was to characterize the demography, genetics and phenotypes of *G. gallus* on Kauai and thereby elucidate their origins and capacity for evolutionary responses to feral selection pressures. We assessed population substructure and phylogeny using whole-genome sequencing (WGS) of modern samples taken from disparate Kauai sampling localities. We then determined relationships among sampled individuals' nuclear and mitochondrial genomes, including previously published data sets from (i) both ancient (pre-European contact) and modern samples from the Pacific, (ii) RJF and (iii) modern domestic chicken breeds. We also measured phenotypic traits that are known to differ between domesticated chickens and RJF (rooster vocalizations, leg colours and plumage; see Box 2) among free-living *G. gallus* on Kauai. We used these data together to determine (i) whether Kauai's feral chickens are of mixed, Polynesian or European origin, (ii) whether there is evidence of interbreeding between feral lineages and (iii) whether co-ancestry and admixture on Kauai is associated with enhanced phenotypic variation.

## Methods

### *Genetic and phenotypic data collection*

Chickens were donated from private individuals living on Kauai. We preserved blood in RNAlater and extracted DNA using salt extraction techniques (Aljanabi & Martinez 1997) in a Swedish laboratory (samples imported under permit DRN 6.2.18-1361/13). A total of 23 samples were thus procured from eight different regions of the island (see Fig. 1A and Table S1, Supporting information). We also recorded vocalizations, plumage and leg colours from an additional 21 individuals at these and other nearby localities (Table S2, Supporting information).

### *Sequencing and variant calling*

DNA samples were sequenced using the SOLiD 5500xl platform at Uppsala Genome Center, part of the National Genomics Infrastructure, and were analysed

**Box 1.** Biogeographic history of Hawaiian *Gallus gallus***Potential sources of Kauai's *Gallus gallus***

Archaeological evidence indicates that chickens were first introduced to the Hawaiian island chain by AD1200 (including Kauai, see Fig. 1a) via human migration into the eastern Pacific (Wilmshurst *et al.* 2011; Thomson *et al.* 2014). Their sources were most likely Red Junglefowl (RJF) transported from the western Pacific by Polynesian settlers (Thomson *et al.* 2014). An additional 857 Pacific RJF were introduced to Kauai in 1939 in a state-sponsored effort to maintain game bird populations in the islands (Pyle & Pyle 2009). Therefore, it is possible that wild RJF persisted on these islands for over 1000 years, although this reservoir population may also be more recently derived (most likely from other Polynesian-dispersed sources in the Pacific). In this manuscript, we consider *G. gallus* from both ancient and historic (1939) RJF introductions as 'heritage' animals because (i) both were dispersed from their native range without experiencing modern, artificial selection for food production, and (ii) modern and ancient samples from Kauai share MtDNA genotypes (see Results); thus, if RJF re-introductions contributed to feral gene pools, then both ancient and historic introductions originated from closely related source populations.

In the light of anecdotal claims from Kauai residents that contemporary *G. gallus* originated within the last few decades, it is also possible that RJF were extirpated from Hawaii and/or have been replaced by escaped domestics. In the recent past, multiple European-derived, modern breeds have been cultivated in Hawaii for food production and cockfighting (personal communication from Kauai residents to D. Wright and E. Gering; and online sales from Asagi hatchery, Oahu, see <http://www.asagihatchery.com/>). In the 1980s and 1990, Tropical storm Iwa and Hurricane Iniki destroyed many of the coops containing Kauai's domestic birds, released their occupants into local forests and potentially spurred large-scale species invasions. Consistent with this possibility, our analysis of *G. gallus* point counts revealed marked increases in population densities during the last few decades (see Fig. 1). Nonetheless, this expansion of domestic genes into Polynesian-derived reservoirs may have been preceded by earlier episodes of introgression, as morphological analyses of five skins that were sampled on Kauai in 1895 also showed evidence of 'genetic pollution' from domesticated chickens (Peterson & Brisbin 2005).

In summary, the gene pool of feral Kauai's *G. gallus* may descend from ancient Polynesian RJF introductions, from historic (1930s) RJF re-introductions and/or from domestic chickens of recent European origins.

**History of Pacific *G. gallus***

The domestication of the chicken is believed to have occurred up to 8000 years ago in China, South Asia and South-East Asia (West & Zhou 1988). Much more recently, domestic breeds have undergone a range of phenotypic and genotypic changes. Domestic breeds show a loss of nuclear genetic diversity (Muir *et al.* 2008) yet still exhibit a high degree of structure and variability in mitochondrial (Mt) sequences (Fumihito *et al.* 1996; Kanginakudru *et al.* 2008; Silva *et al.* 2009; Thomson *et al.* 2014), with nine major Mt clusters identified worldwide (Liu *et al.* 2006). MtDNA sequences from several ancient Hawaiian specimens fall solely within haplogroup D, a clade restricted to Asia-Pacific areas (Thomson *et al.* 2014; but see also Beavan 2014; Bryant 2014; Storey *et al.* 2007). In contrast, a small modern sample ( $n = 10$ ) taken from the Koke'e region of Kauai was solely comprised of haplogroup E (Thomson *et al.* 2014). The E haplogroup currently occurs worldwide and, together with haplogroups A and B, is the source of European-derived domestic food production breeds (Liu *et al.* 2006). Both D and E clades have been found within modern Pacific samples, with the majority of samples outside of Hawaii being haplogroup D (Thomson *et al.* 2014; see Fig. 1).

using computational resources provided by the Uppsala Multidisciplinary Center for Advanced Computational Science (Lampa *et al.* 2013). Fragment reads of 75 bp were sequenced with one individual run per lane. In addition to the Kauai samples, we also sequenced two RJF males, one in the same manner as the Kauai samples and one to approximately 20× coverage with paired reads of 50 bp plus 35 bp. Reads were aligned to the chicken reference genome version GALGAL4 with

LIFESCOPE GENOMIC ANALYSIS Software version 2.5.1. For mitochondrial (Mt) genome analyses, we used the consensus sequence generated by LIFESCOPE. For the nuclear genome, we called variants as follows: first, we marked and removed duplicate reads with Picard (<http://picard.sourceforge.net>). We then performed local realignment around potential indels and base quality score recalibration with GATK, followed by variant calling with the GATK Unified Genotyper

**Box 2.** Phenotypic signatures of *Gallus gallus* domestication

Domestication has induced a multitude of heritable changes in *G. gallus* phenotypes, including changes in behavioural, reproductive and physiological traits (Wright *et al.* 2006, 2008, 2010; Johnsson *et al.* 2012, 2014). Perhaps some of the most striking alterations are in the plumage, with the classic red, black and green feather pattern of the RJF giving way to far more variable coloration in domestic and fancy chicken breeds. Broiler and layer birds (selected for meat and egg production, respectively) have been bred to display a range of coloration, although the vast majority of broiler breeds are white (of the Aviagen, Cobb and Grimaud breeds available, only the Rowan Ranger, Cobb Sasso and Hubbard Color breeds are brown or black, see [www.aviagen.com](http://www.aviagen.com), [www.cobb-vantress.com](http://www.cobb-vantress.com), [www.hubbardbreeders.com](http://www.hubbardbreeders.com)). Most commercial layer breeds are either white or reddish brown (e.g. the Hy-Line W36, CV22, Silver-Brown, Brown and White Leghorn breeds), while heritage breeds of layer chickens tend to exhibit far greater plumage diversity (see [www.hpbaa.com](http://www.hpbaa.com)).

The genetics of plumage colour is fairly well understood in the chicken. For example, the major locus causing white coloration in the chicken is the *Dominant White* mutation, occurring at the *PMEL17* gene (Kerje *et al.* 2004); other color mutations at *MC1R* are also known (Kerje *et al.* 2003). Yellow legs are another characteristic that distinguished many domestic chickens from RJF (which are fixed for grey legs); the locus controlling this polymorphism has also been previously identified (Eriksson *et al.* 2008).

The extensive variation in plumage and coloration introduced by domestication can be helpful in determining whether an RJF gene pool has been 'contaminated' by the introgression of domesticated alleles (e.g. Brisbin & Peterson 2007). However, captive intercross studies also show that it is difficult to infer the degree of introgression within individuals based on plumage or other phenotypic characters (Condon 2012). This is perhaps unsurprising, given that poultry breeders have long understood the inheritance of most *G. gallus* phenotypes (including plumage traits) to be subject to epistasis.

While RJF and domestic chickens bear many similarities in vocal repertoires, they are reported to differ consistently in the length of the last syllable of the rooster crow (Collias 1987), a trait that is considered diagnostic of domestic vs. RJF ancestry (Miller 1978). Evidence of genetic effects on call phenotypes is further supported by enhanced call variation following hybridization between domestic *G. gallus* breeds (Marler *et al.* 1962). To our knowledge, the present study is the first to compare vocalizations from numerous chicken breeds and from individuals sampled in multiple (worldwide) localities. It therefore offers new insights into the relative roles of genes and environments in *G. gallus* vocalizations. Our results confirm a significant difference between calls recorded from chickens and RJF (see Results). Thus, plumage colour, skin colour and vocalizations of Kauai birds comprise three genetically controlled traits that can be compared with *G. gallus*' ancestral (RJF) and derived (domesticated) states.

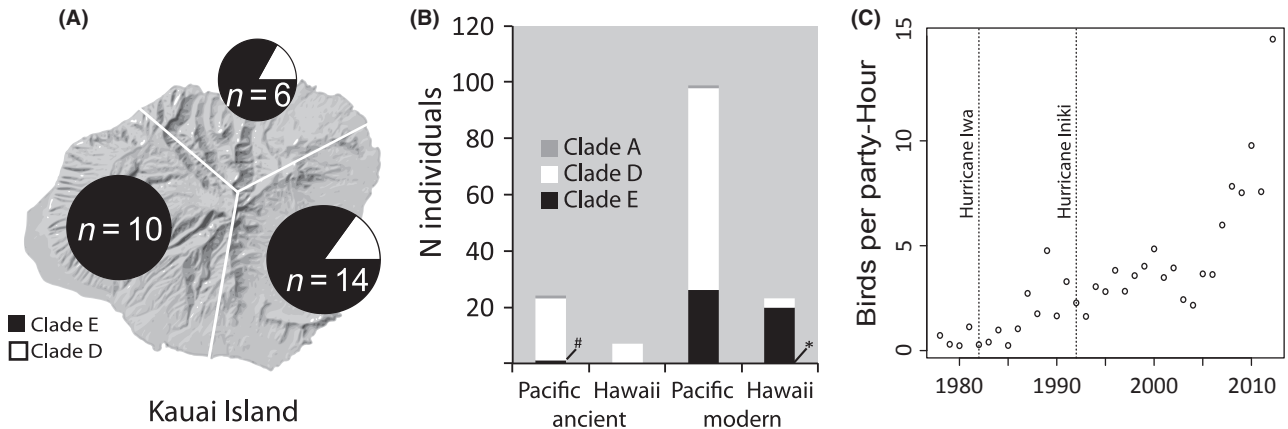
(DePristo *et al.* 2011). Finally, we took a random subset of markers from each chromosome (1-28 and Z) for use in the PCA and STRUCTURE analyses (see details below).

*Mitochondrial DNA phylogeny*

For the whole Mt genome phylogeny, we aligned our 23 Mt sequences with 61 whole mitochondrial genomes available in GenBank (using haplogroup designations established by Miao *et al.* (2013); see their Supplementary data set 3). We also included the Mt sequence of the chicken reference genome (also RJF) and used the duck Mt genome (BGI duck version 1.0) as an out-group. For the Mt control region phylogenies, we aligned our samples with the Mt sequences from Hawaii chickens collated by Thomson *et al.* (2014), see their Supplementary data set 6 (10 modern Hawaiian samples and seven ancient Hawaiian samples were

used, along with others from eastern Polynesia). We constructed two mid-point-rooted control region phylogenies: one including only D and E haplogroup sequences from Hawaii and one including all D and E haplogroup sequences from Pacific islands. Sequences were aligned with CLUSTALW version 2.1.0 (Larkin *et al.* 2007) and the phylogeny was constructed with MRBAYES version 3.2.3 (Ronquist & Huelsenbeck 2003; Altekar *et al.* 2004) using the general time reversible model allowing for a proportion of invariable sites and a gamma distribution for the evolutionary rates at the other sites. We ran two metropolis-coupled Markov chain Monte Carlo simulations for one million iterations, saving every tenth iteration and discarding the first 25 000 samples as burn-in. The estimated sample size was above 100 and the potential scale reduction factor was close to 1.0 for all parameters, suggesting convergence. Trees were drawn with FIGTREE version 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>).





**Fig. 1** (A) Map of Kauai showing MtDNA haplogroup frequencies from sampling localities in the western, central and northern areas of the island (details provided in Table S1, Supporting information). (B) Data from modern and ancient MtDNA sequences show a recent increase in frequency of clade E, which is associated with domestic chickens of European origin, which are now farmed worldwide. Data shown consist of western and eastern Polynesian samples taken from Thomson *et al.* (2014) and Dancause *et al.* (2011). \*E haplogroup samples that are disputed as potential contamination (see text), #Hawaii samples from the current study only. (C) Data from Christmas bird counts in Kapa'a and Waimea (Kauai). Increased densities of feral *G. gallus* coincided with two major storm events (indicated by dashed lines) that damaged island infrastructure and may have facilitated the feralization of escaped live-stock.

### Population structure analysis

Population structure was analysed using three different approaches. First, we used principal component analysis (PCA) (Wu *et al.* 2011). The genotype data consisted of 2900 single nucleotide and indel variants spanning the chicken genome, where we selected 100 markers each from chromosomes 1-28 and Z. Principal component analysis was performed in the R statistical computation environment (R Core Team 2012) using the `prcomp` function. The first and second principal components explained 5% and 4% of the variance, respectively. Individual scores on the first and second principal components were displayed in scatterplots created with the `ggplot2` package in R (see `ggplot2.org`). Of the above-mentioned 2900 variants, we used the 1042 that were biallelic and had complete genotypes for all 23 samples for principal component analysis within the Kauai population.

Next, we analysed the same variants using the Bayesian clustering approach implemented in the program `STRUCTURE` (Pritchard *et al.* 2000). We used a model run burn-in procedure of 100k replicates, followed by 100k MCMC simulations, repeating parameters for 20 runs at each value of *K* (*K* = 1 through *K* = 5). We extracted assignment proportions for the best-supported value of *K* (following guidelines from the author; see 'Results') using `STRUCTURE HARVESTER` (Earl 2012) and plotted these results using the package `pophelper` in R (available at <https://github.com/royfrancis/pophelper>).

Finally, we used `ADMIXTURE` software (Alexander *et al.* 2009; Alexander & Lange 2011), which fits the same model as `STRUCTURE` but with a faster maximum-likelihood

algorithm (suitable for genomewide data sets) to assess Kauai population genetics in the context of worldwide *G. gallus* biogeography. We included the 60k chicken SNP chip genotypes published by Wragg *et al.* (2012) for comparison. Specifically, we extracted the 13 412 SNP markers that were typed both on the 60k chip and in our sequencing data, that did not contain private alleles and that could be mapped to the `GALGAL4` version of the chicken reference genome in Ensembl version 76. The Japanese Totenko chickens formed an extreme isolated group in a preliminary principal component analysis (data not shown) and were excluded from further analysis, as well as the White Star chickens of unknown geographical origin. We fit models with *K* ranging from 1 to 6 and chose a value of *K* = 3 based on fivefold cross-validation. Cross-validation means partitioning the data, in this case into fifths, and repeating the analysis one time without each subset. Each time, the excluded subset is predicted based on the fitted model. The model with *K* = 3 had the lowest cross-validation error. We also performed principal component analysis on this data set. For principal component analysis of the worldwide chicken data set, we used the 3860 markers that had complete data. Four individuals were excluded from our `ADMIXTURE`, `STRUCTURE` and PCA analyses as they were known siblings.

### Vocalization and colour traits

Recordings of crowing roosters were made in the field using a Pocketrak 2G recorder (Yamaha). We simultaneously collected field observations and digital

photographs to determine individuals' plumage features and leg colours. The most common plumage phenotypes observed approximated 'classic' RJF types for both males and females (see Fig. 2A–C). We also observed multiple individuals with plumage phenotypes that are not observed in RJF, including several individuals with white marking flecks and a smaller number of individuals with other plumage patterns (e.g. black or mostly white; Fig. 2D–F). To compare call traits among individuals that differed in morphology, we categorized individuals with non-RJF phenotypes (alternative plumage and/or yellow legs) as 'Kauai chickens' and those with classic RJF morphology as 'Kauai RJF'.

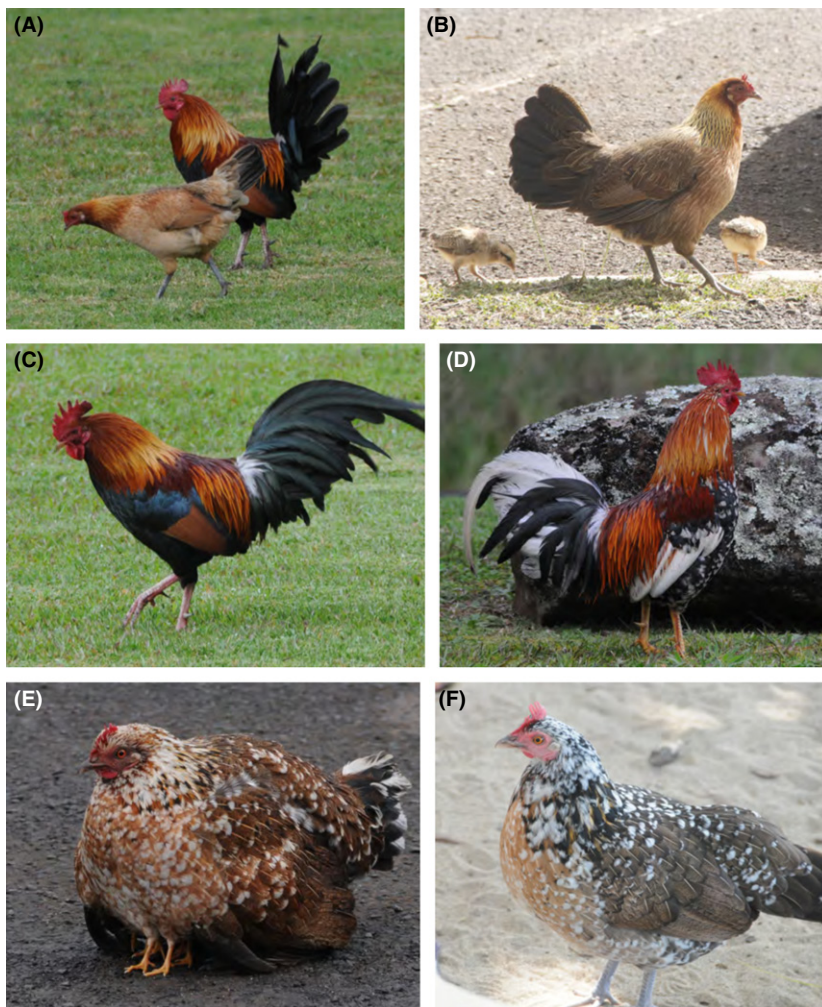
Recordings of localities were selected to span the same major regions of the island as genetic analyses (Fig. S1, Supporting information). We supplemented field-collected vocalizations with published measurements and recordings collected from public databases (Table S2, Supporting information). We deliberately selected both RJF and chickens from a range of localities worldwide to reduce any contribution of environmental

variation on our analyses. Vocalizations were quantified using RAVEN PRO software (Cornell). We limited analyses to durations of previously described call syllables (Collias 1987) because (i) inspections of sonograms suggested that the recording equipment and conditions might confound call frequency analyses and (ii) call frequencies, unlike syllable durations, are known to be influenced by hormones and the social environment (Leonard & Horn 1995). We discarded measurements whenever recording quality and/or call properties made it difficult to isolate syllable onset/offset, which was occasionally the case for the third syllable of calls. This was not an issue for the acoustically distinctive last syllable (see Table S2, Supporting information).

## Results

### *Mt DNA Phylogenies*

Whole Mt genome analyses revealed a total of 3 'D' haplogroup individuals and 20 'E' haplogroup individuals.



**Fig. 2** Sample *G. gallus* phenotypes from Kauai. Panes A–C depict the standard Red Junglefowl (RJF) plumage. Panes D–F illustrate white coloration (D, E, F) and yellow legs (D, E), two genetically regulated traits that do not occur in native RJF.

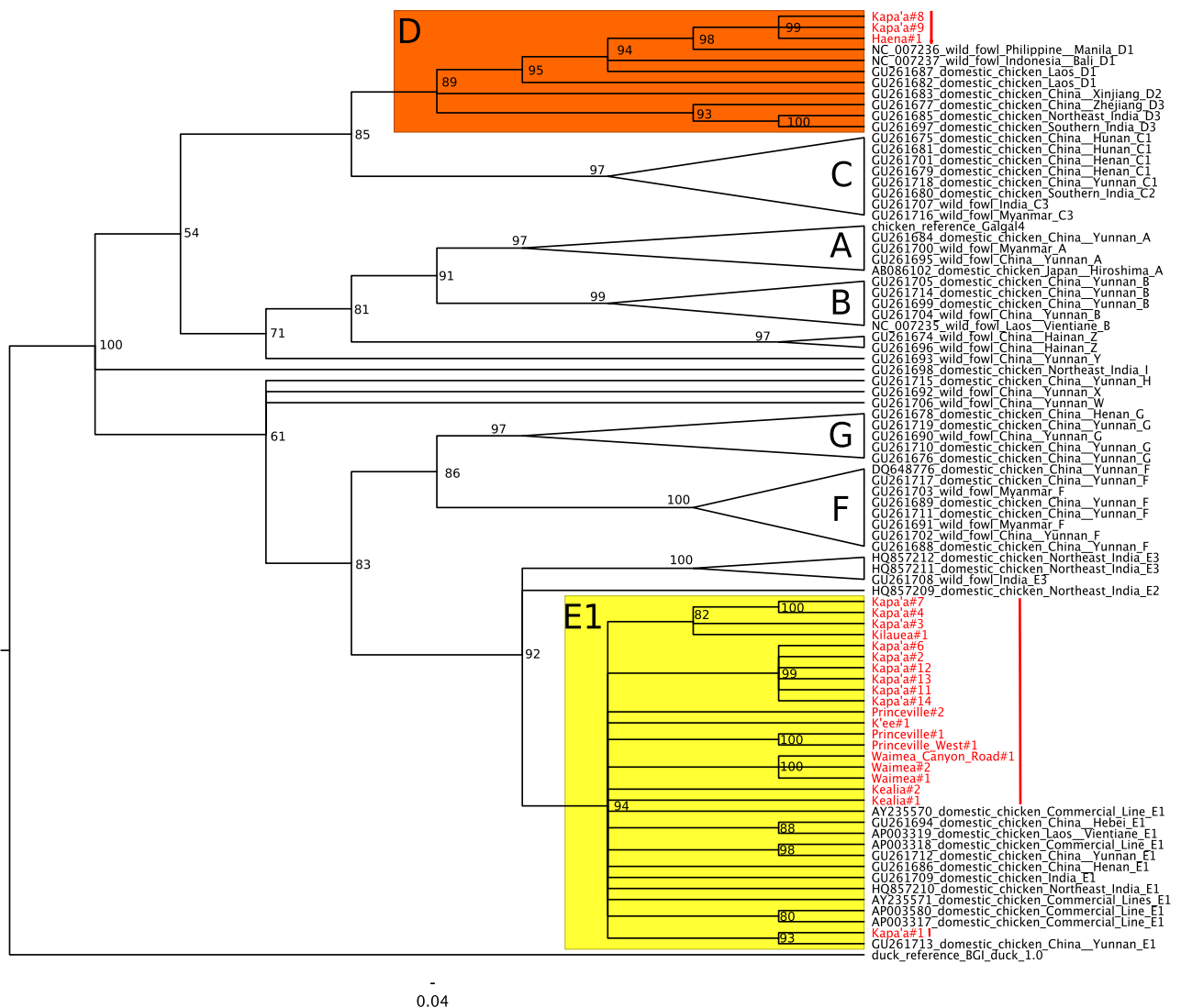
'E' haplogroup sequences from Kauai clustered with those from domestic chickens of recent European origin (notably, various commercial line chickens; see Fig. 3). In contrast, the three 'D' haplogroup sequences were affiliated with sequences from wild fowl collected in Manila, Bali, India and Myanmar and from domestic chickens sampled in China. Within Hawaii, the co-occurrence of divergent Mt clades (with different, although overlapping, geographical ranges) suggests the possibility of multiple geographical origins.

Phylogenetic analyses of the Mt control region (CR) allowed us to compare ancient and contemporary sequences from the Hawaiian Islands (Fig. S2, Supporting information). The CR sequences of three 'D'

haplogroup individuals we sampled on Kauai were highly similar to sequences from archaeological specimens that predate European contact with Hawaii. These sequences were obtained from multiple, geographically distant Kauai localities (Kapa'a and Haena). Thus, the 'ancient-like' clade D is present on the island today, is not restricted to one region and is likely to be recovered from additional localities upon further sampling.

*Coloration and vocalizations*

Most birds on Kauai exhibited the RJF plumage phenotype, but in several cases, we observed moderate amounts of white and/or brown feathers and/or yellow



**Fig. 3** Bayesian whole MtDNA genome phylogeny for birds from Kauai in relation to domestic chickens and RJF. Subtrees representing haplogroups other than D and E have been collapsed. Posterior probabilities (expressed as percentages) are indicated at nodes. Kauai samples are highlighted with red brackets.

legs (see Fig. 2). These traits indicate the presence of domestication-specific alleles and thus further support mixed and/or admixed ancestry for the island's feral *G. gallus*. As previously described in *G. gallus* (e.g. Miller 1978), individual roosters on Kauai produced highly repeatable crows in the field (results not shown). ANOVA tests of differences in crowing traits among individuals found no differences in first and second syllable durations (first syllable  $F_{3,32}=1.98$ ,  $P = 0.14$ , second syllable  $F_{3,32}=0.671$ ;  $P = 0.58$ ). In contrast, we found highly significant differences in durations of third ( $F_{3,31}=6.074$ ;  $P = 0.0022$ ) and fourth syllables of rooster crows ( $F_{3,43}=10.85$ ;  $P = 1.97e-05$ ). Post hoc (Tukey HSD) tests revealed a difference between calls of domestic chickens and all other groups in the third syllable (Fig. 4). The fourth syllable duration found significant differences in each pairwise comparison, with the exceptions of chicken  $\times$  Kauai chicken and RJF  $\times$  Kauai RJF. In other words (i) fourth syllable durations differed between domestic chickens and RJF, and (ii) feral *G. gallus* with chicken-like coloration produced chicken-like calls, whereas feral *G. gallus* with RJF-like coloration produced RJF-like calls (Fig. 4). Finally, a Levene's test indicated that the different Kauai phenotypes (Kauai RJF and Kauai chicken) had significantly more variation than the RJF and domestic groups analysed for fourth syllable duration ( $F_{3,43} = 3.2$ ,  $P = 0.03$ ), although not for third syllable duration.

To test whether vocalizations of birds on Kauai were predicted by location, birds were classified into three regions (north, east and west), with third and fourth syllable duration then tested between pairs of Kauai locations (see Fig. S3, Supporting information). Tukey HSD tests revealed no differences with either third syllable (smallest  $P > 0.9$ ) or fourth syllable (smallest  $P > 0.29$ ).

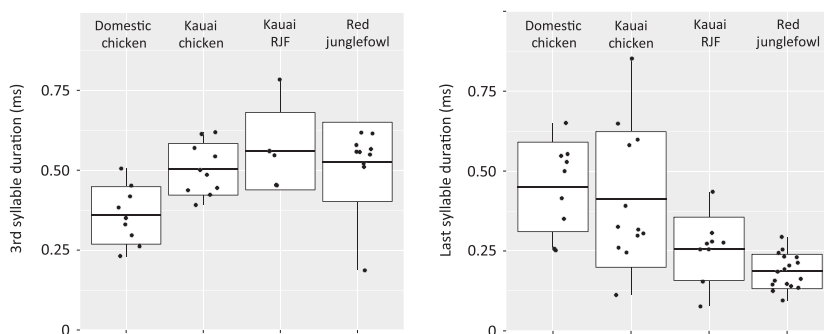
### Population genetic structure

PCA analysis of nuclear genotypes from Kauai revealed a variable, yet continuous, single population (see

Fig. 5A). When plotted among genotypes from worldwide chicken breeds, genotypes from Kauai exhibited considerable variation, particularly given the island's diminutive size in relation to other sampling areas. As a group, the Kauai sample was largely nested among those from both RJF and European *G. gallus domesticus* (see Fig. 5A). It is therefore difficult to determine from nuclear data alone whether Kauai birds stem from RJF, European or hybrid origin. In contrast, Kauai genotypes were easily distinguished from those of Africa, Asia, North America and all but one South American breed.

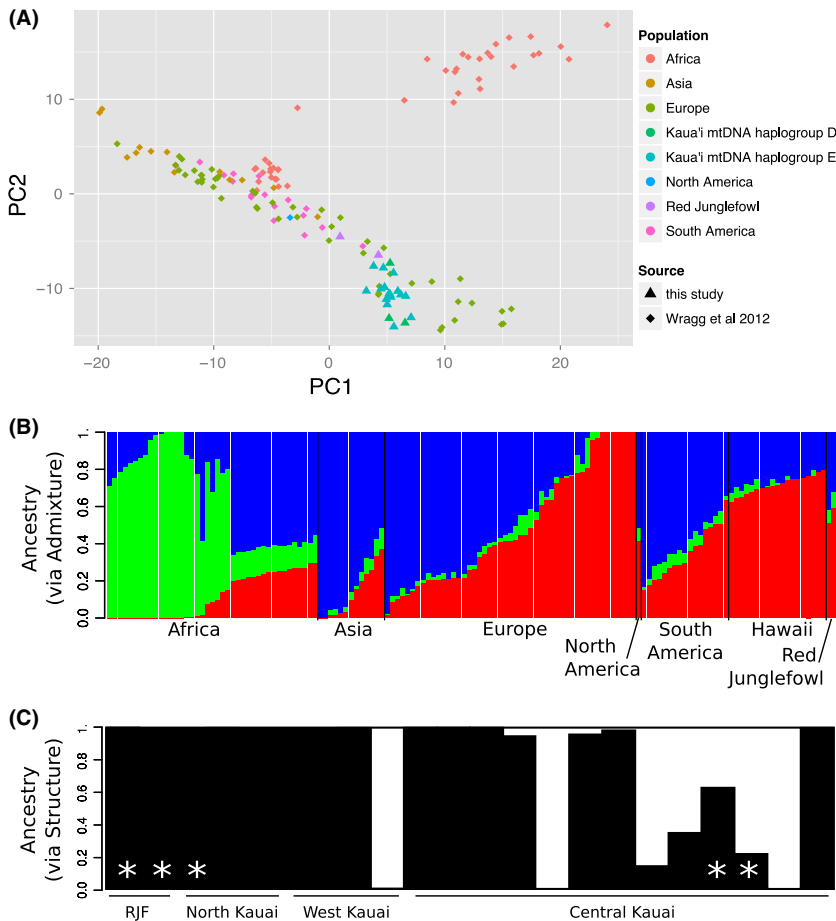
Our ADMIXTURE analysis supported three origins for worldwide *G. gallus* genotypes (see Fig. 5B). Kauai genotypes were most similar to RJF (with a mostly 'red' origin in Fig. 5B, in addition to some 'blue' and a small amount of 'green') and distinct from most other *G. gallus* genotypes. In an exception to this pattern, 4–6 of the European samples showed the same population components as these RJF individuals. STRUCTURE analyses of Kauai genotypes suggested  $k = 2$  founder populations, indicative of multiple origins (see Fig. 5C). This finding adds support for a joint European and RJF ancestry for these birds, with the caveat that European genotypes were highly variable. Thus, further samples from both RJF and Europe will be highly informative, particularly for elucidating whether one Kauai subpopulation truly corresponds to RJF according to nuclear genetic markers. At  $k = 2$ , STRUCTURE also revealed (i) the presence of admixed individuals in Kauai and (ii) that Mt and nuclear genotypes do not cosegregate (i.e. Mt clades 'E' and 'D' are not associated with divergent nuclear backgrounds).

Finally, it should also be noted that geographical separation could play a role in the population structure based on nuclear genetic markers, with individuals from central regions appearing to be more variable and distinct from those from northern and western regions. Therefore, in summary, the nuclear genetic markers, although potentially indicating a hybridized population between European and RJF chickens, cannot rule out other possibilities.



**Fig. 4** Durations of third and fourth syllables of rooster crows sampled in the field (Kauai) and mined from public databases and literature (worldwide). For sampling details, see Table S2 (Supporting information).





**Fig. 5** (A) PCA plot of genetic data showing PC1 vs. PC2 for samples from Kauai in relation to various other chicken breeds (taken from Wragg *et al.* 2012). (B) ADMIXTURE plot showing probable ancestry of Kauai samples in relation to other chicken breeds (using data from Wragg *et al.* 2012). (C) STRUCTURE plot indicating assignment proportions for individuals sampled on Kauai. \*Individuals with D-clade mitochondrial sequences. RJF = Red Junglefowl sequences sampled from a captive population (see Table S1, Supporting information).

## Discussion

### *Evidence of mixed ancestry in Kauai's feral G. gallus*

We discovered several intriguing patterns of genetic variation within Kauai's feral chickens. First, our analyses of whole Mt genomes revealed that two divergent Mt lineages co-occur on the island (clades 'E' and 'D'). The E haplogroup includes sequences found in modern European breeds that are cultivated worldwide for food. In contrast, the D haplogroup is overwhelmingly restricted to Asia and the Pacific and (based on ancient DNA sequences) was already present on Kauai nearly 1000 years ago. Ancient and modern sequences from other Pacific Islands suggest that this lineage was dispersed by Polynesian settlers long before European exploration. Thus, clade D either persisted on Kauai into the present day or was subsequently repopulated from a closely related source population.

Within Kauai, the historic displacement of clade D by clade E may have accompanied the feralization of domestic animals, a possibility that is supported by evidence of a rapid increase in *G. gallus* density within

Kauai's recent past (Fig. 1C). Among Kauai residents, this change is typically attributed to the damage of island infrastructure following tropical storms Iwa and Iniki, which potentially released farm birds into local forests. Alternatively (or additionally), increased tourist activity since the 1970s may have contributed to the feralization of Kauai domestics by providing habitat, food or other key resources to escaped animals (Pyle & Pyle 2009). Further study is needed to ascertain the contributions of these biotic, abiotic and anthropogenic facilitators of invasion and to assess their potential role(s) in the recent expansion of clade E.

### *Evidence of admixture from pacific G. gallus*

This is the first study to jointly examine Mt and nuclear genotypes from Pacific feral chickens. Our nuclear (PCA) analyses reveal that some genotypes found in Kauai are distinct from other populations, although they are similar to European samples (see Fig. 3 and Fig. S2, Supporting information). ADMIXTURE analyses of nuclear data indicated mixed ancestry of Kauai individuals, which share source populations with candidate

European and RJF founders (Fig. 5A,B), although more RJF samples are required to verify this pattern. *STRUCTURE* analyses of Kauai birds indicated that (i) population substructure exists within Kauai, (ii) admixed genotypes occur within some Kauai individuals (Fig. 5C), and (iii) the subpopulations delimited by *STRUCTURE* (using nuclear genotypes) are not restricted to individuals with either 'D' or 'E' mitotypes. Our nuclear genetic analyses are thus consistent with the hypothesis that the descendants of Polynesian-introduced birds (RJF) and feral domestics are interbreeding within the Pacific, although they cannot rule out alternative hypotheses without additional (Mt and phenotypic) evidence.

#### *Phenotypic variation among feral G. gallus*

Despite extensive knowledge of the genetic underpinnings of *G. gallus* phenotypes, very few studies have measured both genetic and phenotypic variation in non-captive populations of this species. Our analyses of coloration (plumage) and behaviour (vocalizations) of Kauai chickens give insight to their history and evolutionary potential as follows: (i) 'classic' RJF traits (plumage, call characters) are prevalent within modern Kauai. (ii) Phenotypes on Kauai are both intermediate between, and more variable than, those of RJF and domestics (see Figs 2 and 4). In fact, the minima and maxima of the calls of Kauai birds are more extreme than the RJF or domestic birds sampled and also display greater variation. (iii) Coloration and behaviour phenotypes are apparently correlated within individuals (i.e. individuals exhibiting colour phenotypes associated with domestic genes had domestic-like calls; see Fig. 4), although this result is largely driven by four individuals in the domestic-like Kauai group.

Both increased population density and transitions to feral habitats are likely to involve radical changes in social and natural selection regimes. The phenotypic and genetic variabilities we report suggest the potential for evolutionary responses, but these may be constrained by antagonistic pleiotropy and/or epistatic interactions between the loci that control selected traits. It is hoped that future studies of these possibilities (including analysis of genetic and phenotypic data from a single pool of individuals) can help determine whether or not observed trait correlations are indicative of evolutionary constraint.

In combination, our findings suggest that Pacific feral chickens present excellent opportunities for studies of postinvasive evolution. *ADMIXTURE* and *STRUCTURE* results potentially indicated that the Kauai population is distinct from other standard breeds of chicken and resembles RJF. However, the number of RJF samples was

very low in this study, and the Kauai samples also overlapped with certain European samples in the *ADMIXTURE* results.

Very few individuals exhibited mitotypes associated with RJF dispersed by early Polynesians. In contrast, despite evidence of a recent population expansion (causing a tripling in the population size since 1992; see Fig. 1C), the plumage of individuals closely resembled the classic RJF phenotype. This is unusual given that RJF phenotypes are not typically observed following hybridization between RJF and domestic layers (personal observations of RJF x White Leghorn hybrids by D. Wright, but see Condon 2012). The contrast between both the nuclear and phenotypic data and the Mt sequence variation implies that natural or sexual selection may favour ancestral, RJF-like traits. Additional studies of the Kauai population should therefore test for genomic signatures of feralization in the form of selective sweeps (Storz 2005) and could reveal, for the first time, the types of genetic changes that occur with feralization. Further studies from both RJF and other Polynesian islands would increase the power of such analyses while providing more definitive insight into *G. gallus* invasion(s) of the Pacific.

#### *Conservation implications*

Our findings complicate management priorities for Kauai's feral flocks, which defy simple classification as RJFs (which are considered threatened) or as domestic chickens (the world's most abundant bird). A morphological study of RJF suggested that they are threatened by genetic pollution (i.e. domestic introgression) throughout their native range (Peterson & Brisbin 1998). Our genetic data indicate the possibilities of mixed and admixed ancestry on Kauai, confirming the threat admixture poses to native RJF. At the same time, our study highlights the potential of feral birds as reservoirs of genetic variation that might one day abet RJF conservation. Hawaii State law currently protects 'wild chickens' found in natural areas. State agencies also sponsor efforts to eradicate 'free-flying domestic chickens' found in developed areas, which are considered alien pests. Based on the small sample presented here, these conservation and control efforts appear to target a common gene pool derived from both 'heritage' (Polynesian RJF) and feralized *G. gallus* founders. Ecological effects of Hawaiian *G. gallus* have not been studied but probably include deleterious impacts on the islands' natives and endemics. Thus, genetic, cultural and environmental considerations present both ethical and applied challenges for feral chicken management.

The variation we report from Kauai could also contribute to the future sustainability of chickens, a

globally critical food resource. There is evidence of losses of genetic diversity during *G. gallus* domestication (Muir *et al.* 2008), which may limit its resilience to future environmental challenges (e.g. pathogens, extreme temperatures, drought). Our case study corroborates the idea that selective and neutral processes might promote genetic variation in feral taxa, which could therefore (theoretically) assist evolutionary rescues of genetically depleted domestic populations (Price 1984).

Regardless of whether Kauai's feral *G. gallus* merit conservation or eradication, they offer a valuable but potentially fleeting opportunity to study evolution in action. Feral chickens are far less abundant on all other Hawaiian Islands, perhaps owing to the presence of natural predators (e.g. mongoose) outside of Kauai. Mongoose became extremely abundant on other islands following deliberate introduction to Hawaii for rodent biocontrol (Mooney & Drake 1986). Considering the success of many other exotic and biocontrol species in interisland colonization, mongoose may soon arrive on Kauai. Given the potential for future population decline or extirpation, we advocate further study of the island's feral chickens. This work will both further document standing diversity and provide baseline data for assessing the impacts of mongoose or other invasions.

Further studies of Pacific feral chickens can help to illuminate how the genetics of colonizing species (e.g. admixture and recombination) can promote biotic invasions (e.g. Hovick & Whitney 2014). Prior work on feral *G. gallus* has principally focused on reconstructing human migration into the Pacific (e.g. Storey *et al.* 2007). As a result, little is presently known about the distribution or consequences of ancestral and derived (domesticated) traits within feral populations. Because domestication commonly involves the elaboration of key life history traits such as growth and fecundity, we might expect the introgression of domesticated alleles into ancestral reservoirs to facilitate population expansion and persistence. Recent work indicates that such introgression has occurred within diverse taxa (e.g. Grossen *et al.* 2014), but its ecological and evolutionary consequences are presently unclear. A rich literature on the domestication process makes studies of 'reverse domestication' well poised to enhance our understanding of colonizing species' genetics, which has remained an active area of biological research over the last 50 years (e.g. other articles in this issue, Whitney & Gering 2015).

## Conclusions

In summary, the chickens present on Kauai represent an incredibly valuable resource for conservation and scientific study, allowing examinations of causes and

consequences of admixture and feralization. We have shown that birds inhabiting Kauai today exhibit characteristics of both original RJF founders and more recently derived European domestics; these characteristics may be involved in adaptation to feral environments. Changes in social and ecological environments attending feralization are likely to promote evolutionary changes, offering exciting possibilities to study adaptation under complex selection regimes. From a conservation perspective, Kauai's *G. gallus* now present something of a conundrum, as they exhibit genetic and phenotypic signatures of RJF ancestry, reflecting possible 'heritage' origins, as well as traits and alleles from invasive domesticated breeds. This complexity presents many challenges and possibilities for further evolutionary studies of 'reverse-domestication' processes.

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## References

- Alexander DH, Lange K (2011) Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics*, **12**, 246.
- Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, **19**, 1655–1664.
- Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, **25**, 4692–4693.
- Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F (2004) Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. *Bioinformatics*, **20**, 407–415.
- Beavan N (2014) No evidence for sample contamination or diet offset for pre-Columbian chicken dates from El Arenal. *Proceedings of the National Academy of Sciences*, **111**, E3582.
- Brisbin IL, Peterson AT (2007) Playing chicken with red junglefowl: identifying phenotypic markers of genetic purity in *Gallus gallus*. *Animal Conservation*, **10**, 429–435.
- Bryant D (2014) Statistical flaws undermine pre-Columbian chicken debate. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, E3584.

- Collias NE (1987) The vocal repertoire of the Red Junglefowl: a spectrographic classification and the code of communication. *The Condor*, **89**, 510–524.
- Condon T (2012) *Morphological detection of genetic introgression in red junglefowl (Gallus gallus)*. MS Thesis, Georgia Southern University.
- Dancause KN, Vilar MG, Steffy R, Lum JK (2011) Characterizing genetic diversity of contemporary pacific chickens using mitochondrial DNA analyses. *PLoS ONE*, **6**, e16843.
- DePristo MA, Banks E, Poplin R *et al.* (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, **43**, 491–498.
- Earl DA (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.
- Eriksson J, Larson G, Gunnarsson U *et al.* (2008) Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. *PLoS Genetics*, **4**, e1000010.
- Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology*, **19**, 4113–4130.
- Feulner PGD, Gratten J, Kijas JW *et al.* (2013) Introgression and the fate of domesticated genes in a wild mammal population. *Molecular Ecology*, **22**, 4210–4221.
- Fumihito A, Miyake T, Takada M *et al.* (1996) Monophyletic origin and unique dispersal patterns of domestic fowls. *Proceedings of the National Academy of Sciences*, **93**, 6792–6795.
- Goodwin D (2007) Horse behaviour: evolution, domestication and feralisation. In: *The welfare of horses* (ed. Waran N), pp. 1–18. Springer, Netherlands.
- Grossen C, Keller L, Biebach I, The International Goat Genome Consortium, Croll D (2014) Introgression from domestic goat generated variation at the major histocompatibility complex of Alpine ibex. *PLoS Genetics*, **10**, e1004438.
- Hampton JO, Spencer P, Alpers DL *et al.* (2004) Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. *Journal of Applied Ecology*, **41**, 735–743.
- Hovick SM, Whitney KD (2014) Hybridisation is associated with increased fecundity and size in invasive taxa: meta-analytic support for the hybridisation-invasion hypothesis. *Ecology Letters*, **17**, 1464–1477.
- Johnsson M, Gustafson I, Rubin C-J *et al.* (2012) A sexual ornament in chickens is affected by pleiotropic alleles at *HAO1* and *BMP2*. Selected during domestication. *PLoS Genetics*, **8**, e1002914.
- Johnsson M, Rubin CJ, Höglund A *et al.* (2014) The role of pleiotropy and linkage in genes affecting a sexual ornament and bone allocation in the chicken. *Molecular Ecology*, **23**, 2275–2286.
- Kanginakudru S, Metta M, Jakati R, Nagaraju J (2008) Genetic evidence from Indian red jungle fowl corroborates multiple domestication of modern day chicken. *BMC evolutionary biology*, **8**, 174.
- Kerje S, Lind J, Schütz K, Jensen P, Andersson L (2003) Melanocortin 1-receptor (MC1R) mutations are associated with plumage colour in chicken. *Animal Genetics*, **34**, 241–248.
- Kerje S, Sharma P, Gunnarsson U *et al.* (2004) The Dominant white, Dun and Smoky color variants in chicken are associated with insertion/deletion polymorphisms in the *PMEL17* gene. *Genetics*, **168**, 1507–1518.
- Lampa S, Dahlo M, Olason P, Hagberg J, Spjuth O (2013) Lessons learned from implementing a national infrastructure in Sweden for storage and analysis of next-generation sequencing data. *Gigascience*, **2**, 9.
- Larkin MA, Blackshields G, Brown NP *et al.* (2007) CLUSTAL W and CLUSTAL X version 2.0. *Bioinformatics*, **23**, 2947–2948.
- Larson G, Cucchi T, Fujita M *et al.* (2007) Phylogeny and ancient DNA of Sus provides insights into neolithic expansion in Island Southeast Asia and Oceania. *Proceedings of the National Academy of Sciences*, **104**, 4834–4839.
- Larson G, Karlsson EK, Perri A *et al.* (2012) Rethinking dog domestication by integrating genetics, archeology, and biogeography. *Proceedings of the National Academy of Sciences*, **109**, 8878–8883.
- Larson G, Piperno DR, Allaby RG *et al.* (2014) Current perspectives and the future of domestication studies. *Proceedings of the National Academy of Sciences*, **111**, 6139–6146.
- Leonard ML, Horn AG (1995) Crowing in relation to status in roosters. *Animal Behaviour*, **49**, 1283–1290.
- Liu Y-P, Wu G-S, Yao Y-G *et al.* (2006) Multiple maternal origins of chickens: out of the Asian jungles. *Molecular Phylogenetics and Evolution*, **38**, 12–19.
- Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution*, **20**, 223–228.
- Loope LL (1999) Hawai'i and the Pacific Islands. In: *Status and Trends of the Nation's Biological Resources*, pp. 747–774. USGS, Washington, D.C.
- Mack RN, Simberloff D, Mark Lonsdale W *et al.* (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological applications*, **10**, 689–710.
- Marler P, Kreith M, Willis E (1962) An analysis of testosterone-induced crowing in young domestic cockerels. *Animal Behaviour*, **10**, 48–54.
- McTavish EJ, Decker JE, Schnabel RD, Taylor JF, Hillis DM (2013) New World cattle show ancestry from multiple independent domestication events. *Proceedings of the National Academy of Sciences*, **110**, E1398–E1406.
- Miao YW, Peng MS, Wu GS *et al.* (2013) Chicken domestication: an updated perspective based on mitochondrial genomes. *Heredity*, **110**, 277–282.
- Miller DB (1978) Species-typical and individually distinctive acoustic features of crow calls of Red Jungle Fowl. *Zeitschrift für Tierpsychologie*, **47**, 182–193.
- Mooney HA, Drake JA (1986) *Ecology of Biological Invasions of North America and Hawaii*. Springer, New York.
- Muir W, Wong G, Zhang Y *et al.* (2008) Review of the initial validation and characterization of a 3K chicken SNP array. *World's Poultry Science Journal*, **64**, 219–226.
- Nussberger B, Greminger MP, Grossen C, Keller LF, Wandeler P (2013) Development of SNP markers identifying European wildcats, domestic cats, and their admixed progeny. *Molecular Ecology Resources*, **13**, 447–460.
- Peterson AT, Brisbin IL (1998) Genetic endangerment of wild Red Junglefowl *Gallus gallus*? *Bird Conservation International*, **8**, 387–394.
- Peterson AT, Brisbin IL (2005) Phenotypic status of Red Junglefowl *Gallus gallus* populations introduced on Pacific Islands. *Bulletin of the British Ornithologists' Club*, **125**, 59–61.
- Price EO (1984) Behavioral aspects of animal domestication. *Quarterly Reviews in Biology*, **59**, 1–32.



- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Pyle R, Pyle P (2009) *The Birds of the Hawaiian Islands: Occurrence, History, Distribution, and Status*. BP Bishop Museum, Honolulu, HI, USA.
- R Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Randi E (2008) Detecting hybridization between wild species and their domesticated relatives. *Molecular Ecology*, **17**, 285–293.
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.
- Silva P, Guan X, Ho-Shing O *et al.* (2009) Mitochondrial DNA-based analysis of genetic variation and relatedness among Sri Lankan indigenous chickens and the Ceylon junglefowl (*Gallus lafayetti*). *Animal Genetics*, **40**, 1–9.
- Stephens D (2011) *The molecular ecology of Australian wild dogs: hybridisation, gene flow and genetic structure at multiple geographic scales*. PhD Thesis, The University of Western Australia, Perth.
- Storey AA, Ramirez JM, Quiroz D *et al.* (2007) Radiocarbon and DNA evidence for a pre-Columbian introduction of Polynesian chickens to Chile. *Proceedings of the National Academy of Sciences*, **104**, 10335–10339.
- Storey AA, Athens JS, Bryant D *et al.* (2012) Investigating the global dispersal of chickens in prehistory using ancient mitochondrial DNA signatures. *PLoS ONE*, **7**, e39171.
- Storz JF (2005) INVITED REVIEW: using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology*, **14**, 671–688.
- Thomson VA, Lebrasseur O, Austin JJ *et al.* (2014) Using ancient DNA to study the origins and dispersal of ancestral Polynesian chickens across the Pacific. *Proceedings of the National Academy of Sciences*, **111**, 4826–4831.
- Verardi A, Lucchini V, Randi E (2006) Detecting introgressive hybridization between free-ranging domestic dogs and wild wolves (*Canis lupus*) by admixture linkage disequilibrium analysis. *Molecular Ecology*, **15**, 2845–2855.
- West B, Zhou B-X (1988) Did chickens go north? New evidence for domestication. *Journal of Archaeological Science*, **15**, 515–533.
- Whitney KD, Gering E (2015) Five decades of invasion genetics. *New Phytologist*, **205**, 472–475.
- Wilmshurst JM, Hunt TL, Lipo CP, Anderson AJ (2011) High-precision radiocarbon dating shows recent and rapid initial human colonization of East Polynesia. *Proceedings of the National Academy of Sciences*, **108**, 1815–1820.
- Wragg D, Mwacharo J, Alcalde J, Hocking P, Hanotte O (2012) Analysis of genome-wide structure, diversity and fine mapping of Mendelian traits in traditional and village chickens. *Heredity*, **109**, 6–18.
- Wright D, Kerje S, Lundström K *et al.* (2006) Quantitative trait loci analysis of egg and meat production traits in a red junglefowl x White Leghorn cross. *Animal genetics*, **37**, 529–534.
- Wright D, Kerje S, Brändström H *et al.* (2008) The genetic architecture of a female sexual ornament. *Evolution*, **62**, 86–98.
- Wright D, Rubin CJ, Martinez Barrio A *et al.* (2010) The genetic architecture of domestication in the chicken: effects of pleiotropy and linkage. *Molecular Ecology*, **19**, 5140–5156.
- Wu C, DeWan A, Hoh J, Wang Z (2011) A comparison of association methods correcting for population stratification in case-control studies. *Annals of Human Genetics*, **75**, 418–427.
- Zohary D, Hopf M, Weiss E (2012) *Domestication of Plants in the Old World: The origin and spread of domesticated plants in South-west Asia, Europe, and the Mediterranean Basin*. Oxford University Press, UK.

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### Data accessibility

All supplementary tables and figures, SNP data, aligned DNA sequences, and tree files referenced in this article have been uploaded to Dryad, along with a summary of included files (<http://dx.doi.org/10.5061/dryad.nv3qs>); sequence data have been submitted to NCBI (BioProject Accession no. PRJNA272379, SRA Accession no. SRP052017).

### Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1** Individuals sampled for mitochondrial and nuclear genomic analyses.

**Table S2** Summary table of vocalizations, plumage and leg color of birds observed in the field on Kauai.

**Fig. S1** Feral *G. gallus* sampling localities. Color coding corresponds to areas sampled for DNA analyses, presented in Figure 5A. The areas labelled North, West, and Central delineate areas used to obtain regional mtDNA haplotype frequencies (depicted in figure 1A). For each of these regions, we also show the number of individuals falling into two categorical phenotypes (chicken, RJF) for which vocalizations were recorded. Phenotype classifications are described in the methods section, and detailed sampling localities are provided in Table S1.

**Fig. S2** Bayesian mitochondrial Control Region (CR) phylogeny of ancient and modern Pacific island birds.