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Signals of selection and ancestry in independently feral *Gallus gallus* populations

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Abstract

Recent work indicates that feralisation is not a simple reversal of domestication, and therefore raises questions about the predictability of evolution across replicated feral populations. In the present study we compare genes and traits of two independently established feral populations of chickens (*Gallus gallus*) that inhabit archipelagos within the Pacific and Atlantic regions to test for evolutionary parallelism and/or divergence. We find that feral populations from each region are genetically closer to one another than other domestic breeds, despite their geographical isolation and divergent colonisation histories. Next, we used genome scans to identify genomic regions selected during feralisation (selective sweeps) in two independently feral populations from Bermuda and Hawaii. Three selective sweep regions (each identified by multiple detection methods) were shared between feral populations, and this overlap is inconsistent with a null model in which selection targets are randomly distributed throughout the genome. In the case of the Bermudian population, many of the genes present within the selective sweeps were either not annotated or of unknown function. Of the nine genes that were identifiable, five were related to behaviour, with the remaining genes involved in bone metabolism, eye development and the immune system. Our findings suggest that a subset of feralisation loci (i.e. genomic targets of recent selection in feral populations) are shared across independently established populations, raising the possibility that feralisation involves some degree of parallelism or convergence and the potential for a shared feralisation 'syndrome'.

KEYWORDS
adaptive evolution, feralisation, invasion biology, population genomics

1 | INTRODUCTION

When wild organisms independently colonise ecologically similar environments, their descendants can be studied as replicated evolutionary experiments. This permits us to test for parallel or non-parallel changes in genotypes and phenotypes in nature, and thereby assess the predictability of evolution outside of laboratory settings. Several classic studies that have taken this approach provide

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powerful evidence of parallel evolution (see examples in stickleback and lizards; Colosimo et al., 2005; Mahler et al., 2013). Conversely, some lab experiments have found that even under perfectly replicated conditions, evolution can be highly contingent on population ancestry and/or stochastic processes. For example, minor differences in standing genetic variation can determine whether or not an adaptive trait will evolve under relatively strong selection spanning thousands of generations, when using *E. coli* (Blount et al., 2018). Other theoretical studies also find that population history affects the degree of repeatability and propensity of parallel evolution in different populations (Barghi et al., 2020; Otte et al., 2021).

Feral study systems are well suited for testing fundamental evolutionary questions (Gering, Incorvaia, Henriksen, Conner, et al. 2019; Mabry et al., 2021), including the degree to which responses to recent selection are predictable and reversible in separate populations. When domesticated animals re-colonise the wild, they confront natural selection pressures that were often relaxed in their recent (captive) ancestors; feralisation can therefore be expected to drive rapid evolution in populations that harbour sufficient additive genetic variation in traits undergoing selection (Gering, Incorvaia, Henriksen, Conner, et al., 2019; Gering, Henriksen, Conner, Wright, et al., 2019; Henriksen et al., 2018). Since independently feral populations have often undergone similar environmental change (e.g. increased social competition for territories and/or mates, requirements to seek out food and shelter, and interactions with naturally occurring pathogen communities), the descendent populations can be compared to learn if parallel environmental changes drive the evolution of overlapping sets of genes and/or gene functions. These contrasts are especially informative (with respect to general features of feralisation) where colonisation timelines and invaded habitats are similar among the allopatric populations that are being compared.

Another compelling feature of feral study systems is that focal populations often originate from relatively well-documented starting points. The domesticated sources of feral populations are often well studied and relatively well-known genetically, certainly in comparison to the actual wild progenitors of domesticated species that existed several thousands of years ago. Of course, independent feral populations of a given domesticated species may also be different because of factors such as divergent founder (domesticated) source populations, random genetic drift, and/or localised admixture between feral, domesticated and wild relatives (Gering, Incorvaia, Henriksen, Conner, et al., 2019). This makes feral populations excellent models for studying whether, and how, colonising populations' sources impact contemporary evolution.

A final benefit of studying feralisation is that evolutionary changes accompanying domestication have been the subject of intensive recent study (Wright, 2015; Wright et al., 2020). This background knowledge permits testing, across a wide array of organisms, whether genes and/or functions that were previously modified under artificial selection undergo further change when domesticated taxa recolonise the wild (Johnsson, Williams, et al., 2016).

Prior studies of feralisation have often focused on individual population case studies. For example, genomic analyses of feral chickens (*Gallus gallus*) on Kauai Island, Hawaii, recently found evidence of rapid recent evolution at loci controlling traits that were also modified under domestication (e.g. genes that regulate behaviour, reproduction and growth) (Johnsson, Gering, et al., 2016). Despite this functional overlap, selective sweeps found in Kauai's feral fowl were largely different from known *G. gallus* domestication genes and improvement genes that were identified by selective sweep analyses (Johnsson, Gering, et al., 2016). Strong selective sweeps in modern domesticated birds are more likely to represent 'improvement' genes that have been selected relatively recently during intensive modern breeding for layer and broiler chickens. For example, a coding change in the gene *TSHR* is found in virtually all modern domesticated birds (Rubin et al., 2010), but this mutation is largely lacking in archaeological domesticated chickens (Girdland Flink et al., 2014; Loog et al., 2017). Given this distinction between selective sweep regions found in Kauai's feral chickens versus recent domestication/improvement selective sweeps found in captive *G. gallus*, it is now important to compare additional (independently feral) gene pools. This will help determine how replicable feralisation processes are, and whether they are a model for parallel evolution.

The sources of feral populations are obviously of importance when comparing their gene pools. In the specific case of Kauai's chickens, admixture between wild-living Red Junglefowl and escaped domesticated birds appears to have capacitated recent adaptation (Gering et al., 2015; Johnsson, Gering, et al., 2016). This admixture most likely followed the releases of large numbers of domesticated birds during hurricanes Iniki (in 1992) and Iwa (in 1982) into the wild, as reported by locals and supported by chromosomal painting techniques (Martin Cerezo et al., 2023). This timeframe also coincided with exponential growth of the feral population's density on Kauai Island (Gering et al., 2015). Chickens were first brought to the Hawaiian Islands (including Kauai) by Polynesian settlers (circa 400–1200), and were thought to be Red Junglefowl imported for ritual and/or cockfighting purposes (though may also have been partially domesticated) (Kirch, 2011; Thomson et al., 2014). Subsequent introductions of domesticated *G. gallus* to Hawaii followed European contact that began centuries later (1778) and involved domesticated chicken breeds of largely western origins. For example, large numbers of animals have been imported to the Hawaiian Islands via a local hatchery (Asagi) that was established in 1935 and specialises in Cornish Rocks (a broiler breed), White Leghorns (a layer breed), and smaller numbers of heritage breeds (www.asagihatchery.com). Importation and release of live *G. gallus* from commercial sources also coincided with introductions of non-commercial 'backyard' chickens kept by residents for egg production, meat production and cockfighting which, though illegal in the United States, remains highly popular on Kauai and throughout the Pacific region (Young, 2014). Contemporary genomes of feral Kauai chickens are consistent with recent interbreeding among these diverse and asynchronously introduced source populations (Martin Cerezo et al., 2023).

The clearest distinction between the histories of feral chickens on Kauai versus Bermuda is that Red Junglefowl were never imported and released into Bermuda. This is largely because ancient Polynesians did not venture into the Atlantic region. Additionally, the landraces and 'backyard' varieties found in the two regions are likely non-identical, because the European explorers and slaves who colonised Bermuda beginning 1505 were closely connected (via culture and commerce) to European, African and Caribbean locales whereas Hawaii was connected via more global recent trade routes and fashions (including Asian, Pacific, American and European). Finally, there are no large commercial poultry operations in Bermuda and chickens are largely kept by Bermuda residents as fancy pets (vs. for food and cockfighting). The Bermuda Poultry Fancier's Society was formed there more than a century ago, and current members reported to us that diverse domesticated breeds have long been imported to the island, including breeds developed in Europe (e.g. d'Uccles, Orpingtons, Faverolles, Friesians, Old English Game) and the Americas (e.g. Plymouth Rock, Brahma), from diverse stock sources (personal communication Ronnie Lopes, of Bermuda Poultry Fancier's Society and US Master's Cup Poultry Show). Escaped domestic *G. gallus* have been highly successful in colonising the tiny 53.2 km^2 Bermudian archipelago, reaching an estimated size of >30,000 individuals by 2011 and displaying a panoply of phenotypes seen in fancy chickens (e.g. rose, walnut, and duplex combs, polydactyly, barred and silver plumage patterns, and bantam morphology) (Government of Bermuda, 2013). Similar to Kauai, Bermuda's feral *G. gallus* densities have only recently reached their current high densities, with local authorities hypothesising that damage from Hurricane Emily (in 1987) kickstarted exponential growth (Government of Bermuda, 2013).

Feral populations that lack recent Red Junglefowl ancestry (like Bermuda) could be predicted to show evolutionary divergence from Kauai's admixed fowl in their contemporary gene pools. On the other hand, feralisation might filter input sources and/or select for parallel genomic and phenotypic changes if a common feralisation 'syndrome' is adaptive in both regions. To evaluate these predictions, we examined genomic and phenotypic data from feral chickens of Bermuda and compared them to feral chickens from the Hawaiian island of Kauai. Kauai and Bermuda chickens have colonised strikingly similar environments. Both locales have subtropical climates in which feral chickens inhabit a variety of urban, suburban, rural and wild habitats. Both Kauai and Bermuda ecosystems lack native terrestrial carnivores and, perhaps consequentially, have sustained unusually large and dense populations of feral *G. gallus* for decades (average group size for wild Red Junglefowl is around 6–8 birds, whilst groups on these islands can be as large as 30 or more birds (personal observation)). In the present study, feral birds from Bermuda ($n = 21$) and Kauai ($n = 25$) were sampled, phenotyped and genotyped (10× density). They were then used to identify candidate feralisation selective sweeps, with the sweep regions further characterised using chromosomal painting techniques (see Section 4).

2 | RESULTS

2.1 | Summary of sequencing statistics

An average of 262.90 million reads were obtained per sample (min: 208.02 M, max: 312.93 M, std: 25.90 M), generating an average coverage of 36.77× (min: 19.19×, max: 42.89×, std: 3.57×). On average, 98.58% of the reads mapped to the reference genome (min: 97.00%, max: 99.06%, std: 0.52%). Mapping quality was consistent between samples, ranging from 56.00 to 56.90 (average: 56.53, std: 0.22). In total, 98.42% of the genome (min: 97.63%, max: 98.66%, std: 0.28%) had a coverage of at least 10×.

12,819,455 SNPs were found for the Bermudian samples. After filtering, 12,053,478 (94.02%) of those SNPs were kept and used for further analysis of selective sweep regions. For the Hawaiian samples, 11,913,016 SNPs were kept. The Bermudian and Hawaiian samples had 9,734,945 of the analysed SNP locations in common. 7,174,074 SNPs were kept after merging with data from wild and domesticated populations for the Chromopainter analyses, due to missing data in the domesticated and Red Junglefowl populations.

2.2 | Feral chicken ancestry

An Admixture analysis was used to compare the two feral populations with two Red Junglefowl populations (one collected from the wild, the other from a zoo population), two broiler populations, and two layer populations (all from Qanbari et al., 2019). This analysis found that these populations showed the most support for six founder populations (CV-error $n = 6$, 0.79268, next lowest scores were $n = 5$ Cve = 0.79773, $n = 7$ Cve = 0.79791, $n = 8$ Cve = 0.79800, $n = 4$ Cve = 0.80401). The feral chickens clustered with one another, as did the Red Junglefowl populations (though some White Layer introgression appeared in the Indian Red Junglefowl population). The two broiler populations appeared to have different origins, as did the White and Brown layer populations (see Figure 1). Focusing solely on the two feral populations, the Bermudian population had more introgression from Brown layers and Broilers from population A, whilst the Kauai feral population had more introgression from the White layer population. All other Admixture plots are shown in Figure S1.

A PCA plot of the data displays a similar pattern to the Admixture results (see Figure 1b). The two Layer populations were closely related and showed pronounced divergence to the other analysed breeds (most likely due to being the most intensively selected of all the modern domesticated breeds). When these two populations were removed to better visualise the remaining population structure (see Figure 1c), feral, broiler and Red Junglefowl all clustered by themselves with their respective population pairs. A similar pattern was observed when comparing Principal Component 3 with Principal Component 4 (Figure 1d).

As a further analysis, we expressed the shared chunk count matrix from Chromopainter as a dissimilarity matrix by subtracting

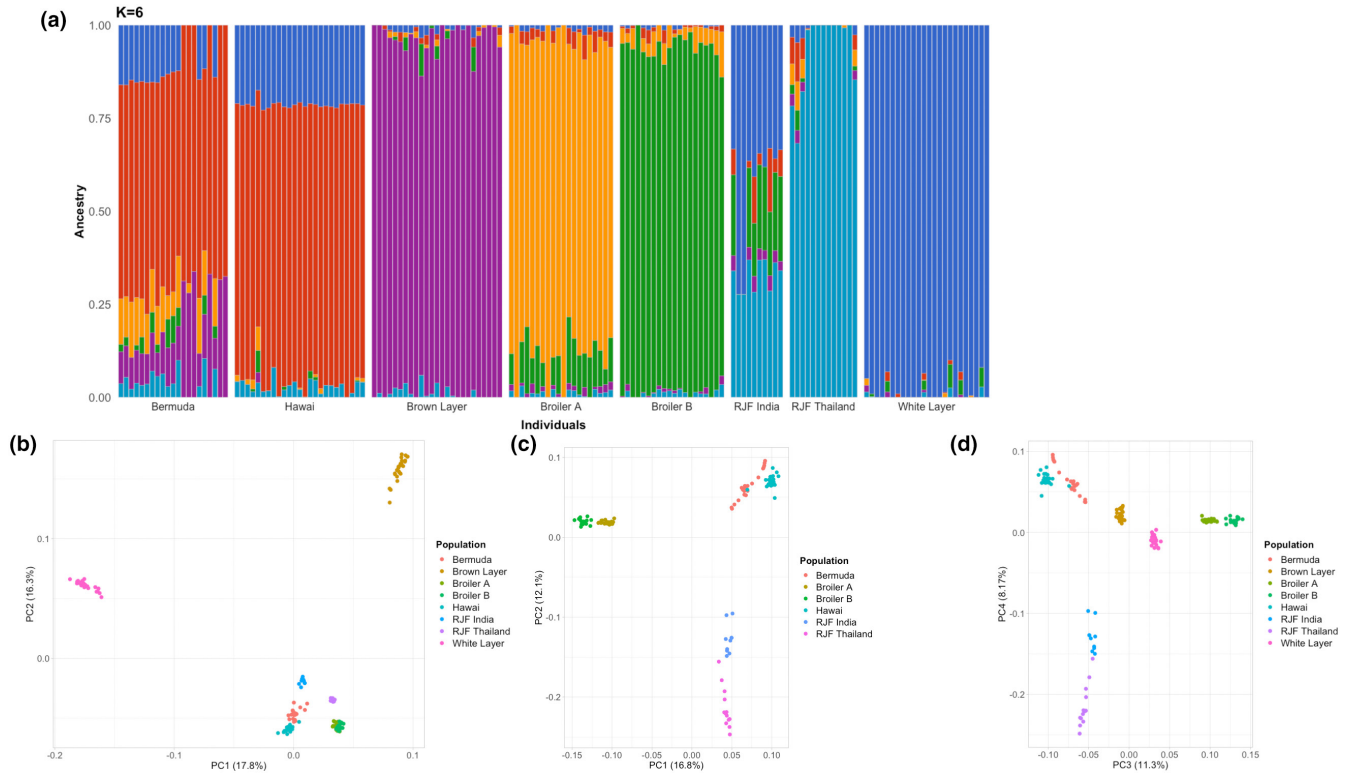

FIGURE 1 (a) Admixture Plot assessing the relatedness of Bermudian chickens with a variety of different domestic breeds and Red Junglefowl. Graph shown for $k=6$. (b) PCA plot of the same data (PC1 vs. PC2) showing the relatedness between the feral, domestic and RJF sub-populations. (c) PCA plot of the same data excluding the layer populations (PC1 vs. PC2). (d) PCA plot of the same data (PC3 vs. PC4).

each element from the maximum shared chunk count using the Splitstree v4 package (Huson & Bryant, 2005). This allowed us to again visualise the relationships between the feral, wild and domestic populations (see Figure S2). Once again, the two feral populations clustered adjacent to one another, whilst the other domestic and wild sub-populations were all distinct from one another.

2.3 | Selective sweep detection

Selective sweep detection was performed to identify genomic regions undergoing selection. Three separate techniques were used: extended haplotype homozygosity mapping, a composite likelihood ratio test (Huber et al., 2016) and a Tajima's *D* approach (see Section 5.4). The Extended Haplotype Homozygosity test identifies genomic regions with high local haplotype homozygosity, considered to be a strong indication of signatures of positive selection (Sabeti et al., 2002). The Composite Likelihood Ratio test calculates the likelihood ratio of the null hypothesis, calculated from the neutral (genome-wide) frequency spectrum, whilst the alternative hypothesis is calculated using a model where neutral selection has been altered by recent selection. This technique can separate out footprints of positive selection from background selection (Charlesworth, 2012). Bermuda ($n=21$) and Hawaii ($n=25$) samples were analysed using each sweep detection technique separately.

2.3.1 | Extended haplotype homozygosity (EHH) mapping

For the Bermuda dataset, a total of 386 putative selective sweep regions were identified within Bermuda *G. gallus* genomes using EHH mapping (Table S1). A threshold of 1% of the genome was selected. The mean selective sweep length was 24 kb bases (median 20 kb, SD 12.2 kb). For the Kauai population, 351 selective sweeps were detected, with a mean selective sweep length of 26 kb (median 20 kb, SD 14.6 kb), see Table S1. Seventeen of the selective sweeps detected in the two feral populations overlapped with one another (permutation test, $p<.001$, see Section 4 and Table S2). To ascertain how many of the selective sweep regions overlapped with domestication selective sweeps, the selective sweeps identified in prior studies that focused on *G. gallus* domestication were compared with the Kauai and Bermuda selective sweeps. Qanbari et al. (2019) identified 304 domestication/improvement selective sweeps in their analysis, with five of these overlapping Bermudian EHH sweeps (permutation test, $p>.05$), and four of these overlapping Kauai EHH selective sweeps (permutation test $p>.05$) (see Table S3).

2.3.2 | Composite likelihood ratio mapping

In the Bermudian population we detected a total of 455 selective sweep regions using composite likelihood ratio (CLR) mapping (mean

TABLE 1 Selective sweep regions shared between Hawaii and Bermuda that were identified by at least two different mapping approaches in each population.

Region_nr	Chr_name	Region_start	Region_end	Gene_start	Gene_end	Biotype	Stable_id	Overlap_with_domestic_sweeps
1	1	17,550,927	17,570,928	17,209,559	17,632,970	protein_coding	ENSGALG00000023584	No
2	1	32,761,411	32,781,411	NULL	NULL	NULL	NULL	Yes
3	2	78,323,570	78,338,404	78,327,060	78,338,570	protein_coding	ENSGALG00000035925	No

32 kb, median 20 kb, SD 24.8 kb; Table S4), with a significance threshold of 1% of the genome used. In the Kauai population a total of 456 selective sweep regions were identified with CLR (mean 32 kb, median 20 kb, SD 29.6 kb). Of the selective sweep regions identified using CLR in the feral populations, 44 of these overlap (permutation test, $p < .001$) (see Table S5). When comparing with the domesticated selective sweeps identified by Qanbari et al. (2019), 10 of the selective sweeps detected in Bermuda overlap (permutation test, $p > .05$) and 21 of the Kauai selective sweeps overlap (permutation test, $p < .001$; see Table S3).

2.3.3 | Tajimas's *D* mapping

In the Bermuda population, 168 selective sweep regions were identified (mean 57 kb, median 40 kb, SD 51.9 kb), whilst in the Kauai population 165 selective sweep regions were identified (mean 58 kb, median 40 kb, SD 38.1 kb), see Table S6. Between these two feral populations, 39 selective sweep regions were shared (permutation test, $p < .001$) (Table S7). In contrast, Bermuda had 18 overlaps (permutation test, $p < .001$) with the domestication selective sweeps from Qanbari et al., and Kauai had 17 overlaps (permutation test, $p < .001$) (see Table S3).

Consensus feral sweeps and overlaps

For the feral populations, we considered that if a selective sweep was detected by at least two selective sweep finding methods it represented stronger evidence of a true selective sweep. Note that this may sometimes not be the case, as any given detection technique may be better tuned to detecting a specific sweep type that is 'missed' by other methods. Nonetheless, we chose to minimalise false positives by focusing on consensus selective sweeps (though individual selective sweep types are also presented in Tables S1–S10). In the case of the Bermudian population, 59 regions were detected in such a manner (Table S8), whilst in the case of the Kauai population 106 selective sweep regions were detected (Table S9). When we check for overlaps between these two sets of selective sweep regions, three are detected in both population samples (Table 1), with this being a significant enrichment over the null distribution (permutation test, $p < .001$). Of these, one selective sweep also overlapped with the domestication sweeps identified by Qanbari et al. (2019) (see Table 1). An overview figure of the locations of selective sweeps identified by the different methods is presented in Figure S3.

2.4 | Selective sweeps origin identification

To identify the origin of the three shared Hawaiian and Bermudian feral selective sweeps (i.e. are they domesticated haplotypes that have become fixed vs. wild Red Junglefowl haplotypes) Chromopainter software was used (Lawson et al., 2012). This takes the feral genomes and 'paints' on the domesticated and wild haplotypes to ascertain which are most likely to be the donor population

of a given sweep haplotype. Two separate layer and broiler populations were used as donors, as well as two Red Junglefowl populations (from Thailand and India respectively). These were then used as donors for the Bermuda and Hawaii populations, whilst an additional run with Bermuda was also performed, also including Hawaii as an additional donor. In the case of the Bermudian feral selective sweeps, domesticated haplotypes were the most likely donor populations, with broilers and layers donating similar amounts for two of the shared selective sweeps (chr1@17.5Mb, chr1@32.8Mb). In the case of the third sweep (chr2@78.3 Mb), the broiler donors were the strongest donor for the Bermudian population. A similar pattern was also seen for the Hawaiian population, though in this case the broiler population was the largest donor for each selective sweep (see Figure 2a–d). The degree of donorship from Red Junglefowl alleles was broadly similar for the two feral populations.

By further breaking down the domesticated populations into the individual donor sub-populations, we once again see a similar pattern, but also that it is often only one of the two candidate donor sub-populations that contributes the most to a particular sweep. In particular, the layer donors are actually more prevalent when sub-populations are used, with the brown layer donorship high for Hawaii at chr1@17.5Mb, the white layer a high donor for Hawaii at chr2@78.3Mb, and the brown layer a high donor for Bermuda at chr1@32.8 Mb (see Figure 2b). When focusing on the Bermuda population, with the addition of Hawaii as a donor, we can see a strong overlap between the two feral populations, with Hawaii the largest donor for the sweep at chr2@78.3 and the joint largest for the sweep at chr1@17.5Mb (see Figure 2c,d).

Of the three shared sweeps, one also overlapped with a domestication sweep (chr1@32.8 Mb). In this case, the domesticated donor is much more evident than either the Hawaiian or Red Junglefowl donors.

2.5 | Gene annotation and function

The 59 selective sweeps regions detected by both methods for the Bermudian population contain a total of 61 genes when using the Ensembl genome annotation browser, though a large percentage are long non-coding RNA and other gene-free regions (see Table S8 and below). A total of nine fully annotated genes were identified in selective sweeps (*ADCY1*, *CALU*, *CRAMP1*, *DRD3*, *MYST/Esa1-associated*, *PTPRB*, *TAFA5*, *TSNARE1* and *ZC3H12A*). These genes are involved in anxiety, schizophrenia, depression and related behaviours (*ADCY1*, *DRD3*, *PTRB*, *TAFA5*, and *tSNARE1*) bone remodelling (*MYST/Esa1-assctd*), eye development/vision (*PTRB*), the immune system (*ZCH3H12a*) and metabolism (*CALU*). There was no enrichment for these types of gene functions,

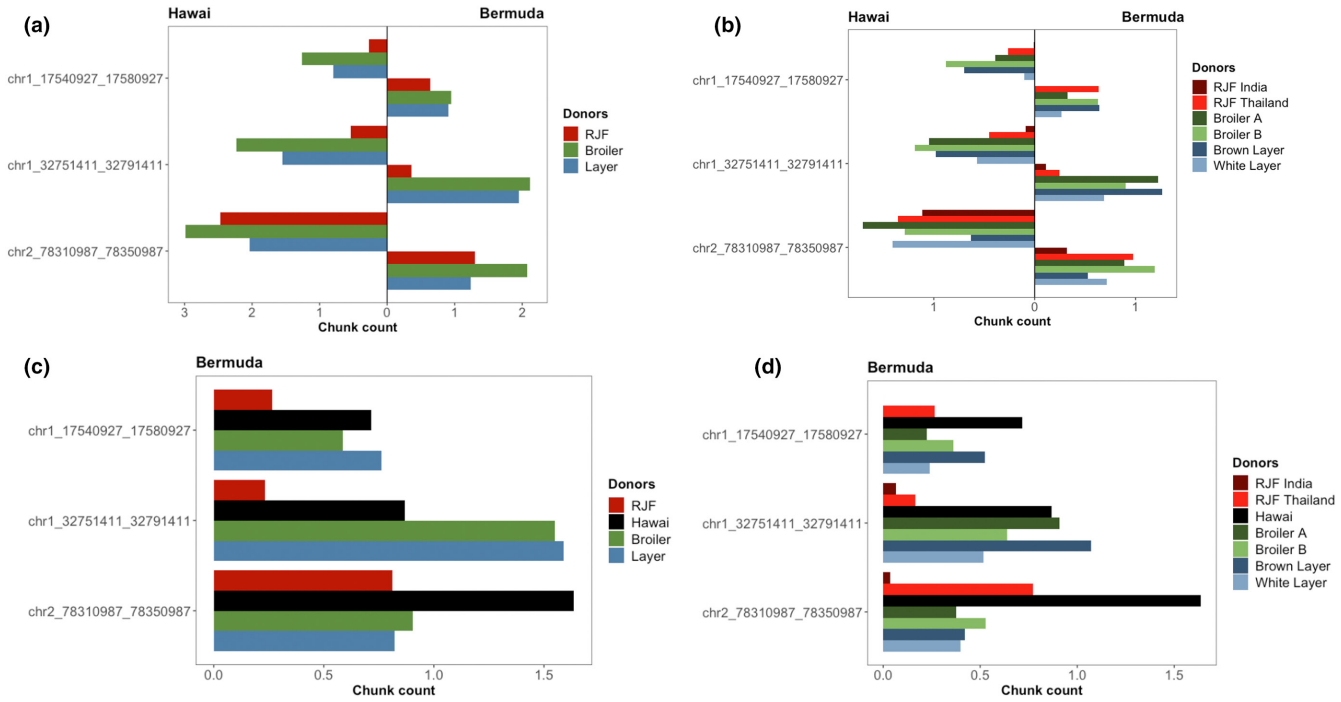

FIGURE 2 Chromopainter analysis for sweeps origins. (a) Chromopainter analysis showing Hawaiian (left side) and Bermudian (right side) populations at the three shared feral sweeps locations sweeps (*y*-axis, labelled chr1_17340927, chr1_32751411 and chr2_78310987). For the two feral populations, domestic (DOM, grey bar) and wild RJF (RJF, red bar) birds were used as donors, with the size of the red and grey bars indicating the number of chunks that were similar from each donor. Therefore, the larger the bar, the greater the donor similarity to the feral population. (b) This is the same analysis as (a), however the donor populations have now been further subdivided into sub-populations (Brown Layer, White Layer, Broiler A, Broiler B, Red Junglefowl Thailand and Red Junglefowl India). (c) Chromopainter analysis of Bermudian sweeps, with this analysis also including Hawaii as a donor population, as well as domestic and RJF birds. The same *y*-axis notation as in (a) is used. (d) The same analysis type as in (c) however donor populations are once again subdivided as in (b).

however. Of the 61 genes, only 21 were able to be assessed in the PANTHER over-representation analysis (using complete GO processes setting), with the abovementioned nine genes plus a further 12 unannotated genes still included. The only enrichment was for unclassified processes/genes in this set ($p < .0001$). Of the 61 genes, 40 were long non-coding RNAs (lncRNA). Similar results were found with the Hawaii consensus sweep regions – in this case 171 genes were present in these sweeps, with 131 being useable by Gene ontology software PANTHER (Protein Analysis Through Evolutionary Relationships) (Mi et al., 2012). The only enriched GO process was once again unclassified ($p < .0001$), whilst 25 of the genes were lncRNAs, 10 were micro, miscellaneous or sno RNAs, 25 were unknown and the remainder protein coding (see Table S9). Gene function was very diverse in the case of the Hawaiian gene set, though of note is the presence of *SEMA3A* (chr1@9.4Mb), which was one of the three most significant improvement/domestication-related genes detected by Rubin et al. (2010). Similarly, the gene *tSNARE1* was also found in a selective sweep identified by Rubin et al. (2010).

2.6 | Recent improvement/domestication signals in feral populations

Very little overlap existed between the selective sweeps detected in modern domesticated populations, and those detected in the two feral populations. Several genes have been previously identified as likely being highly important in modern domestication, by dint of being present in selective sweeps detected in multiple domesticated breeds (Rubin et al., 2010). In particular, the genes *TSHR*, *BCDO2* and *SEMA3A* were the three genes present in the strongest domestic sweep regions in the Rubin et al. study, and in the case of *TSHR* and *BCDO2* also contained exonic non-synonymous mutations. It is important to note that these selective sweeps identified in Rubin et al. (2010) and Qanbari et al. (2019) almost certainly represent modern improvement-related domestication genes, whereby very strong recent selection is occurring in modern broiler and layer breeds, as opposed to early domestication-related genes that were selected during the initial phases of chicken domestication.

In the case of *TSHR*, the gene is located at 40.97 Mb–41.02 Mb on chromosome 5. A sweep covering 40.4–40.8 Mb was detected in both feral populations using Tajima's *D* (see Table S6), whilst a sweep was detected using CLR in the Bermudian population at 40.17–40.19 Mb and at 41.3 Mb in the Kauai population (see Table S4). None of these actually overlap the *TSHR* gene itself, and the allele frequency of the exonic mutation locus (present at 41020256-61 bp) was found to be variable. In the case of the Bermudian population, the SNP present had a frequency of three homozygote reference individuals, 11 heterozygote individuals and seven homozygote variant individuals, whilst the Hawaiian population had an allele frequency of seven variant homozygotes, four heterozygotes and 12 homozygous alternate individuals. When assessing the Tajima's *D* result for the specific sweep region in the Kauai population we get a Tajima's *D* statistic of 1.4, with the 1% cut-off threshold being 3.4. In the case of the Bermudian population, two Tajima's *D* statistics were calculated (for 40,960,000–41,000,000 and 41,000,000–41,040,000) with values of 2.9 and 3.5, with one being above the 1% threshold of 3.3.

Similarly, only one sweep was present within the relative vicinity of *BCDO2* (located at 6,140,660–6,152,816 bp), with this sweep being detected at 6.5 Mb in the Bermudian population using CLR (see Table S4). The Tajima's *D* statistic for this specific sweep region in the Bermudian population was 1.3 (with the cut-off being 3.3) and for the Kauai population this value was 1.1 (with the cut-off being 3.4), showing no significant fixation.

In contrast, a consensus sweep (found using both CLR and Tajimas'*D*) was detected in the Kauai population at 9.4–9.44 Mb on chromosome 1, that fully overlapped the gene *SEMA3A* (9.34–9.51 Mb), and is therefore the only one of these three improvement/domestication genes that has reliable support for being present in a feral population (see Table S8). In this case it appears that *SEMA3A* is either undergoing further positive selection within feral populations, or it retains a signal of earlier artificial selection (e.g. due to selective neutrality and/or loss of non-domesticated alleles as the population became feral). One further point is the gene *tSNARE1*, which was identified in the feralisation-related selective sweeps, is also identified (albeit not as strongly as the above-mentioned genes) in an earlier analysis of domestication-related *G. gallus* loci (Rubin et al., 2010). This once again highlights the possibility that some of the polymorphisms selected during domestication may still be advantageous beyond captive environments, regardless of whether they serve the same functional role(s) in feral habitats.

3 | DISCUSSION

3.1 | Summary of feral gene pool comparisons

Analyses of Bermuda's feral chickens (and comparisons to counterparts on Kauai) revealed both unique and common features of independently feral *G. gallus* in the Atlantic versus Pacific regions. Admixture analyses suggest that the two feral populations originate from genetically similar colonisation source(s), yet they also show asymmetrical signals of recent gene flow and selection. Bermudian feral chickens appeared to have more similarity to broiler and brown layer populations, whilst Hawaiian feral chickens had introgression from White Layer birds (or their ancestors). Hawaiian feral birds also had more introgression from Red Junglefowl (the source of domesticated chickens) in comparison to their feral Bermuda counterparts. These findings mirror what we know of the two feral population histories, with large numbers of White Layers released by the Asagi hatchery in Hawaii following tropical storms and attendant opportunity for introgression from the Red Junglefowl already present in the Hawaiian islands.

TABLE 2 Morphometric comparison of feral Bermudian chickens with Red Junglefowl and White Leghorn chickens. Bermudian data taken in the field from 40 female adults, 55 male adults. Kauai data taken in the field from 36 male adults, 36 female adults.

Chicken population	♂ Bodyweight (+SD)	♂ Comb mass (+SD)	♀ Bodyweight (+SD)	♀ Comb mass (+SD)	Duplex comb (%)	Pea comb (%)	Rose comb (%)	Leg colour
Red Junglefowl (wild)	1119 (+138)	NA	799 (+130)	1.1 (+0.3)	0	0	0	Grey
Kauai feral (feral hybrid)	1106 (+232)	2.73 (+2.38)	833.6 (+157)	0.31 (+0.28)	0	0	0	Grey + yellow
Bermudian feral (feral domestic)	1153 (+340)	3.4 (+2.9)	799 (+187)	0.34 (+0.29)	3	3	1	Grey + yellow
White Leghorn (domestic)	1900 (+138)	7.15 (+1.38)*	1629 (+110)	7.3 (+0.6)	0	0	0	Yellow

Note: Red Junglefowl data and White Leghorn female data taken from captive adults (230 days old) as presented in Wright et al. (2008). Data for male White Leghorn males taken from 29-week-old subadults (von Schantz et al., 1995), which are nearly at adult body mass but below well below their final/adult comb mass.

3.2 | Summary of selective sweep analyses

Common selective sweeps were detected among Kauai and Bermuda feral populations, at least two of which involved loci and variants that were not found in earlier studies of domestication or breed improvement selection. Haplotypes in this set of shared feralisation sweeps chiefly exhibit domesticated origins, though Red Junglefowl donorship is also evident in at least one case. However, in the case of Bermuda, the comparison with the highest number of shared regions was consistently shown to be the Hawaiian population. This highlights the genetic similarity between these two distinct feral populations, and a feasible role for shared recent selection in the filtering and/or allele-frequency shifts of contemporary feral gene pools.

When the panel of candidate sweeps from Bermuda chickens is considered alone, very little overlap is found with recent improvement/domestication-related selective sweeps. This pattern has previously reported in Kauai feral chickens. Thus, the available data suggest that feral populations are more likely to share selective sweep loci with each other than with contemporary domesticated breeds. This confirms that feralisation is not a mere reversal of artificially selected genomic changes. Additionally, the majority of Bermudian selective sweeps involved genomic loci different to those found in the Kauai gene pool. Thus, despite a significant number of overlapping selective sweep regions, Bermuda and Kauai feral chickens' local environments, ancestries and/or stochastic processes have still resulted in many differences between the two populations. Nonetheless, further study may reveal functional overlap in selected polymorphisms of these, and other, feral populations (e.g. via changes at divergent loci controlling shared biological pathway/s and traits).

Despite the overarching differences in feral sweep loci between Kauai and Bermuda, the significant overlap of selective sweeps between these two populations offers intriguing evidence of potential evolutionary parallelism. Future genotype–phenotype studies, both for traits involving shared feral sweeps and those produced by potentially convergent ones, can help determine if feral populations consistently exhibit similar outcomes to feralisation-related selection.

3.3 | On the phenotypes of independently feral populations

Given the similarity in phenotypes observed in the two feral populations, this opens up the possibility that feralisation results in consistent changes. This would then represent a 'feralisation syndrome', mirroring the domestication syndrome. Among the traits comprising a shared putative 'feralisation syndrome' are reduced body mass and comb size (as seen in Red Junglefowl), and plumage colour and patterning that resemble the Red Junglefowl's to varying degrees. In a similar manner to domestication, there is the potential that feralisation is driven by a set of alleles that are common between populations. However, even with the commonality

of this feralisation syndrome, certain heritable, domestication-related phenotypes were observed in Bermudian chickens, but not Hawaiian chickens. These include polydactyly (additional toes), yellow leg-skin and comb variants (e.g. rose and duplex combs (see Table 2)).

3.4 | Functional implications of outlier loci in Bermuda's feral chickens

Exploring gene function and annotation within sweep loci from feral gene pools offer clues as to which traits are selected in feral chickens. Unfortunately, the large number of unannotated genes found in the Bermudian sweeps limits our ability to test for enrichments of particular gene functions. Recognising this limitation, it is still of interest to examine gene functions in those genes that are annotated. In the case of the nine genes within Bermuda sweeps that were annotated, several had previously been identified as affecting anxiety-related behaviours (see below). Additional roles for annotated genes included bone remodelling, eye development or vision, and the immune system, suggesting possible connections to life histories, sensory ecology and/or pathogen resistance. In the case of the Hawaiian sweeps, function was highly variable.

Concerning behavioural genetics, five of the nine genes from Bermuda sweeps were strongly associated with anxiety, schizophrenia, depression and their related behaviours (*ADCY1* (Chen et al., 2022, 2023; Sundararajan et al., 2018), *DRD3* (Liu et al., 2022; Sofronov et al., 2022; Staal, 2015), *PTRB* (Ishiguro et al., 2008), *TAFA5* (Huang et al., 2021) (Li, Li, et al., 2020), and *tSNARE1* (Fromer et al., 2016; Gu et al., 2015; Li et al., 2019; Li, Shen, et al., 2020; Plooster et al., 2021; Schrode et al., 2019; Sleiman et al., 2013; Whelan et al., 2018)). Although the studies linking these genes to behaviour come mostly from human studies, we have previously demonstrated a strong cross-species replication for anxiety-related genes in chickens, humans and mice (Johnsson, Williams, et al., 2016). Thus, the relationship between anxiety-related traits and adaptive (e.g. anti-predator) behaviour may explain the presence of such behavioural genes in the Bermudian population. In particular, Red Junglefowl display much more anxiety-related/anti-predator behaviour than their domesticated counterparts (Johnsson, Williams, et al., 2016), and hence the feral chickens appear to be reverting to ancestral behaviour in wild habitats where feral cats, humans and other wild animals present credible mortality risks. It is also possible that these genes are influenced by other sources of behavioural selection in wild settings (such as high-stakes social interactions, and the demands of successful foraging in complex and changing environments).

The remaining known annotated genes affected bone remodelling via osteoblast differentiation (MYST/Esa1) (Hu et al., 2021), the immune system via the regulation of inflammation (Zc3h12a/MCPIP1) (Lin et al., 2013; Matsushita et al., 2009; Miao et al., 2013), and cancer metastasis and development (Nasri Nasrabadi et al., 2020). Once again, these raise exciting questions about how pathogens and life history selection might influence feral gene pools that merit targeted future study – particularly since feral G. gallus are known to host zoonotic diseases (Dubey, 2010).

Altogether, the annotated genes present in selective sweeps conform with our predictions as to how feral birds should adapt to the natural environment. The return of natural and sexual selection pressures appear to drive adaptive responses within the sufficiently diverse gene pools present in Kauai and Bermuda chickens. The fact that anxiety-related genes are present in selected regions of the feral gene pools' genomes provides a tantalising suggestion of selection on behaviour that requires further study. We have found previously that domesticated birds are less anxious than their wild counterparts the Red Junglefowl (Fogelholm et al., 2019; Johnsson et al., 2018; Johnsson, Williams, et al., 2016). The observation that the feral sweeps involve anxiety-related genes, and also harbour alleles of domesticated origin, indicates that such polymorphisms are still potentially circulating in domesticated populations but are not fixed or are maintained at a low frequency.

3.5 | Broader implications for feralisation

In addition to the genetic similarities that we observe between our two feral chicken populations, with a significant number of overlapping selective sweep regions, we also see a return to the 'wild' phenotype in both cases. Other examples of such reversion in coat colour in feral populations come from feral boar populations in the United States and dingo populations in Australia. In the case of the feral boar, one longitudinal study found that domestic coat colour individuals showed a mark decrease over a 13-year period, with a commensurate increase in the black (wild type) coloration over the same period (Gipson et al., 2006), with another study finding the same black coloration to be the dominant adult phenotype in Texan populations (Mapston, 2007). However, another longitudinal study found the reverse, with an increase in spotted individuals at the expense of black individuals (Mayer et al., 1989), though they ascribe this to human hunting preferences. Similarly, dingo populations show an excess of classical ginger coat colour phenotypes over white domestic ones, even when a high degree of domesticated introgression/hybridisation is present (Newsome & Corbett, 1985; Tatler et al., 2021).

It appears that feralisation involves strong selection against the novelty traits produced through domestication, but that domestication also has many advantageous polymorphisms for feral populations. However, these are at low/intermediate frequencies in normal domesticated populations. The importance of domestication-related alleles controlling other, less visible phenotypes during feralisation is also shown by the location of a sweep at a well-known domestication-related gene (*SEMA3A*) that participates in nervous system development, and the fact that chromopainter indicates that many of the alleles in feralisation-related sweeps appear to be domestic in origin.

4 | CONCLUSION

This study demonstrates that feralisation is partly repeatable at the genetic and phenotypic level in chickens, and involves significant enrichment of shared sweeps between independently feral populations. These sweeps also show little overlap to those produced by recent improvement/domestication in modern domestic birds, with a single exception that shows how artificially selected genome regions can either retain signals of selection, or be continuously selected, during the recolonisation of wild habitats. It is important to note that most known improvement selective sweeps may not be representative of the earliest domestication-related genes (i.e. those polymorphisms/mutations selected during the initial domestication process), as pointed out by Girdland Flink et al. (2014). Unfortunately, unless (or until) all domestication genes are known, we cannot fully exclude the possibility of their involvement in feralisation. Nonetheless, our findings suggest that further studies of feralisation genomics will continue to produce novel and complex discoveries. For example, the two populations we compared herein exhibit both parallels and differences in their recent evolutionary trajectories, and have recruited haplotypes to high frequency that stem from both domesticated and wild sources. This complexity is perhaps unsurprising, given that feral populations consist of novel and uniquely human-impacted organisms, and that these organisms exhibit, to remarkable and often vexing degrees, a capacity to thrive in an increasingly human impacted world.

5 | METHODS

5.1 | Field sampling of Bermuda's feral chickens (*G. Gallus*)

Field samples of fresh blood were collected on Whatman Filter cards from chickens that were culled by the Bermudian government in 2015. Individuals were chosen for sequencing (see below) in order to include (1) a range of sublocalities spanning the small archipelago's main islands and (2) a range of microhabitats including developed and undeveloped areas. In total, $n = 15$ males and $n = 6$ females were selected for sequencing. Phenotypic data were not available for the individuals that were used for Whole Genome Sequencing, and was instead collected from individuals sampled in the field in Bermuda during 2018 (Table 2). This consisted of body weight and comb weight measures taken post-mortem (collected from 95 Bermudian feral individuals, 55 males and 40 females), as well as comb morphology recordings (presence of pea comb, rose comb and duplex comb morphs), and the number of different leg colourations present in the population (yellow and/or grey leg colours), taken from 134 Bermudian feral individuals. In addition, comb and body weight, and morphological measures were also taken from 36 male and 36 female Kauai feral birds. For the Kauai birds, 25 birds were blood sampled and sequenced, with samples once again taken from a variety of sublocalities.

5.2 | Publicly available sequence data for domestic and wild *G. gallus*

Whole-genome sequence data for domestic and Red Junglefowl contrasts were obtained by from data published by Qanbari et al. (2019). We downloaded the reads from the European Nucleotide Archive (ENA, http://ebi.ac.uk/ena), and called variants using the same workflow as for the Bermuda samples. The accession numbers and sample labels used are listed in Table S10.

5.3 | DNA sampling and whole genome sequencing

We extracted genomic DNA using the DNEasy Blood and Tissue Kit (Qiagen), according to the manufacturer's protocol. We sequenced 21 Bermuda chicken samples at 30× coverage on the Illumina HiSeq X platform at SciLifeLab, Stockholm, and 25 Kauai chicken samples on an Illumina novoseq X6000. In the Bermuda samples, we trimmed residual adapter sequences with Trimmomatic version 0.36 (Bolger et al., 2014), aligned the sequences to the chicken genome (version Galgal6) using bwa mem version 0.7.17 (Li, 2013) and processed the alignments using a workflow inspired by GATK best practices (DePristo et al., 2011; McKenna et al., 2010; Van der Auwera et al., 2013), including the use of the functions MergeSamFiles, SortSam, BaseRecalibrator, ApplyBQSR, GenomicsDBImport and GenotypeGVCFs from GATK version 4.3.0.0. Sequencing duplicates were removed with the MarkDuplicates function from picard versions 2.27.5 (https://broadinstitute.github.io/picard/index.html). In the Kauai samples, poly-G tails were trimmed using fastp (Chen et al., 2018), after which the workflow mentioned above was followed, but with bwa mem version 0.7.15, GATK version 3.8.1.0 and picard version 1.118. Finally, we filtered the resulting SNPs with the GATK VariantFiltration tool. We retained variants that passed filters QD < 2.0, FS > 60.0, MQ < 40.0, MQRankSum < −12.5 and ReadPosRankSum < −8.0. In a second filtering step using bcftools version 1.14 (Danecek et al., 2021), SNPs with up to 40% of missingness and a minor allele frequency larger than 5% per population were kept. All SNPs for the selective sweep analyses were phased using 50 iterations in Beagle 5.4 (Browning et al., 2021).

5.4 | Detecting selective sweeps

We detected selective sweeps in the Bermuda and Kauai populations by calculating three relevant statistics, keeping the top 1% regions per statistic, and then selecting regions that were recognised as a sweep by at least two out of the three methods, using the R package GenomicRanges version 1.46.1 (Lawrence et al., 2013). Only autosomal regions were used. First, we estimated pooled Tajima's *D*. Each statistic was calculated in 40 kb sliding windows along the autosomal genome. We standardised

the Tajima's *D* values by subtracting the mean and dividing by the standard deviation. VCFTools version 0.1.16 was used (Danecek et al., 2011).

As a second method, we ran a composite likelihood ratio test for positive selection (Kim & Stephan, 2002). With this, the likelihood ratio of the null hypothesis is calculated from the neutral (genome-wide) frequency spectrum, whilst the alternative hypothesis is calculated using a model where neutral selection has been altered by recent selection. This was calculated by using SweepFinder2 version 1.0 (DeGiorgio et al., 2016). In particular, this technique can separate out footprints of positive selection from background selection (with this being a loss of neutral variation due to a purging of linked deleterious alleles via negative selection (Charlesworth, 2012)). To conduct this, we first computed an empirically derived allele frequency file based on all chromosomal data. This file serves as a null hypothesis in order to calculate a likelihood ratio to detect positive selection. A whole genome scan for selective sweeps was then conducted as per the recommendations given in DeGiorgio et al. (2016). A 20-kb window was used to detect selective sweeps.

The third method used the concept of 'Extended Haplotype Homozygosity'. Where genomic regions with high local haplotype homozygosity are detected, this can be an excellent indication of signatures of positive selection, with such haplotype structure useful in detecting selective sweeps (Sabeti et al., 2002). Strong selection with commensurate Linkage Disequilibrium should lead to an expansion of such haplotypes in the population, prior to them being slowly broken down by recombination. This premise led Sabati et al. to develop the Extended Haplotype Homozygosity test, with this later expanded upon by Voight et al. (2006), Sabati et al (2007) and Tang et al. (2007). This test measures the extent to which an extended haplotype has been transmitted without recombination. Firstly, an allele-specific integrated Haplotype Homozygosity (iHH) is calculated, with this then used to calculate the iHS (a ratio of the iHH for its ancestral and derived alleles). We used the R package rehh version 3.2.2 (Gautier et al., 2017) to calculate the iHS statistic for each individual SNP, by running the data2haplohh, scan_hh and ihh2ihs functions. We then used the per SNP iHS statistic to calculate the maximum iHS statistic for each 20 kb window using the R package tidyverse version 2.0.0 (Wickham et al., 2019).

5.5 | Gene annotation

We downloaded the Gallus gallus Biomart files from the Ensembl ftp server (https://ftp.ensembl.org/pub/release-104/mysql/gallus_gallus_core_104_6/) and added them to a local PostgreSQL v15 database. Earlier detected selective sweep regions were added to the same database. Custom SQL queries were written to select all the known genes that were found in these regions.

5.6 | Overlap tests

We used a simulation test to determine the number of overlaps observed between sweep regions on Bermuda and Kauai was greater than expected by random chance. The test consisted of placing two sets of regions uniformly at random on an interval the size of the autosomal sequenced chicken genome, and counting the overlaps. The two sets had numbers and lengths equal to the number and average length of sweeps observed on Bermuda and Kauai. A permutation procedure was used to calculate the significance, with 5000 replicates used and the number of observed overlaps compared to the probability of obtaining the same number of overlaps by chance (https://github.com/mrtnj/bermuda_overlaps).

5.7 | Chromosome painting

We used CHROMOPAINTERV2 (Lawson et al., 2012) to compare the Bermuda sweep regions to the other populations (Kauai, Red Junglefowl and Domestic chickens). First we combined the vcf files from the separate populations into one vcf file per chromosome using bcftools v1.14 (Danecek et al., 2021). Then, we lifted an earlier Gallus gallus recombination map (Elferink et al., 2010) to Galgal6 using LiftOver(https://genome.ucsc.edu/cgi-bin/hgLiftOver). Then we converted our vcf files and the newly acquired recombination map to an accepted chromopainter format by using the vcf2cp.pl and convertracfile.pl scripts include in the fineSTRUCTURE version 4.1.1 library (Lawson et al., 2012). SNPs were then phased using SHAPEIT v5.1 (Hofmeister et al., 2023). Then we ran ChromopainterV2, using the default parameters, in each selective sweep regions flanked with 20 kb on each side. We painted Kauai and Bermudian populations using Red Junglefowl and domestic sequences as donors. Images were created with the R package tidyverse version 2.0.0 (Wickham et al., 2019).

5.8 | Principal component analysis

The principal components were calculated after linkage pruning the SNPs – using window size 50, step size 10 and – pairwise r^2 threshold of 0.1-, both with PLINK 1.9 (Chang et al., 2015). Images were created with the R package tidyverse version 2.0.0 (Wickham et al., 2019).

5.9 | Admixture

Individual ancestries were estimated by running ADMIXTURE v1.3.0 for K ranging from 1 to 10 and with cross validation enabled. CV-errors for all values of K were taken directly from the ADMIXTURE output, with the .bed file used for the analysis taken from PLINK

output used for the principal component analysis above. Images were created with the R package tidyverse version 2.0.0 (Wickham et al., 2019).

5.10 | SplitsTree analysis

We expressed the shared chunk count matrix from Chromopainter as a dissimilarity matrix by subtracting each element from the maximum shared chunk count. We visualised this distance matrix as a Neighbour-Net (Grünewald et al., 2007) using Splitstree version 4 (Huson & Bryant, 2006).

AUTHOR CONTRIBUTIONS

DW, EG, RH, TG designed the study. EG, DW, RH, MJ, MLMC, DT, AS performed the research. MJ, MLMC, DT, AS analyzed data. DW, EG, MJ, RH wrote the paper.

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CONFLICT OF INTEREST STATEMENT

The authors had no conflict of interest with this study.

DATA AVAILABILITY STATEMENT

Bam files are deposited in the ENA, with general accession ID PRJEB67702 (see also Table S10).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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