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ARTICLE

The effects of recent changes in breeding preferences on maintaining traditional Dutch chicken genomic diversity

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Abstract

Traditional Dutch chicken breeds are marginalised breeds of ornamental and cultural-historical importance. In the last decades, miniaturising of existing breeds (so called neo-bantam) has become popular and resulted in alternatives to original large breeds. However, while backcrossing is increasing the neo-bantams homozygosity, genetic exchange between breeders may increase their genetic diversity. We use the 60 K SNP array to characterise the genetic diversity, demographic history, and level of inbreeding of Dutch heritage breeds, and particularly of neo-bantams. Commercial white layers are used to contrast the impact of management strategy on genetic diversity and demography. A high proportion of alleles was found to be shared between large fowls and neo-bantams, suggesting gene flow during neo-bantams development. Population admixture analysis supports these findings, in addition to revealing introgression from neo-bantams of the same breed and of phenotypically similar breeds. The prevalence of long runs of homozygosity (ROH) confirms the importance of recent inbreeding. A high diversity in management, carried out in small breeding units explains the high heterogeneity in diversity and ROH profile displayed by traditional breeds compared to commercial lines. Population bottlenecks may explain the long ROHs in large fowls, while repetitive backcrossing for phenotype selection may account for them in neo-bantams. Our results highlight the importance of using markers to inform breeding programmes on potentially harmful homozygosity to prevent loss of genetic diversity. We conclude that bantamisation has generated unique and identifiable genetic diversity. However, this diversity can only be preserved in the near future through structured breeding programmes.

Introduction

Since the time of multiple, independent domestication events in South and Southeast Asia (Liu et al. 2006; Kanginakudru et al. 2008; Miao et al. 2013), domestic chicken (*Gallus gallus domesticus*) populations have experienced intensive human-induced evolution. As a result of domestication and selection for a variety of purposes (Liu et al. 2006), domesticated chicken breeds have developed an exceptional diversity in morphology, physiology, and behaviour (Rubin et al. 2010). However, demographic events, such as population bottlenecks, admixture of populations, founder effects, genetic drift, and inbreeding, have also contributed to shaping most of the novel genetic variation within the domesticated chicken genome (Groeneveld et al. 2010; Dana et al. 2011; Elferink et al. 2012).

Chicken populations began to differentiate into breeds after domestication. Preferential breeding of traditional populations exhibiting subsets of specific morphological variants gave rise to a wide range of standardised fancy breeds fixed for a few morphological traits and subjected to low-selection intensity for diverse purposes (Groeneveld et al. 2010; Tixier-Boichard et al. 2011). However, it was with the increased interest in more efficient selection programmes especially since the second half of the 20th century (Groeneveld et al. 2010; Tixier-Boichard et al. 2011), that a handful of standardised breeds started to be intensively selected for either growth (meat production) or

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reproductive (egg-laying) traits (Burt 2005; Muir et al. 2008; Groeneveld et al. 2010). The development of experimental and commercial lines led to the replacement of local breeds across the world. As a result, numerous traditional breeds have gone extinct (Granevitze et al. 2009), while those that survived are nowadays used for either backyard hobby farming, ornamental and (competitive) fancy breeding (Woelders et al. 2006; Tadano et al. 2008; Wilkinson et al. 2012), or cultural-historical heritage conservation (Woelders et al. 2006; Zanetti et al. 2010; Pham et al. 2013). In rare cases, traditional chicken breeds are used for high-value niche market products (Van Marle-Koster and Nel 2000; Van Marle-Köster et al. 2008; Pham et al. 2013).

The recent history of traditional breeds in The Netherlands provides numerous genotypes to characterise. Furthermore, the results of such genetic characterisation studies can be used as a tool in on-going efforts to preserve chicken diversity nationally. Although the majority of the Dutch breeds have originally been bred for production traits, breeding for egg or meat production has usually ceased. As a result, traditional breeds have become marginalised and have now an almost exclusively ornamental or cultural historical significance. Hobby breeders are the most important stakeholders involved in the conservation of specific varieties or breeds (Woelders et al. 2006), but their number is limited and often getting smaller over the years.

Until recently, Dutch chicken genetic resources comprised mostly large fowls and bantam breeds, whose origin can be dated back to the 16th and 18th century (Dana et al. 2011). Traditional Dutch breeds have their origin in Europe, although some East and Southeast Asian influences have been found in a few breeds, as a result of occasional or repetitive introgression. Introgression from European and Mediterranean breeds has also been observed to a lesser extent (Dana et al. 2011). Of the traditional breeds with past productive significance, the North Holland blue derived from the Belgian Malines, whereas Asian chickens, such as the Cochin, Brahma, and Langshan, were involved in the formation of Barnevelders and Welsummers. Malays, Japanese bantams, and Sumatras were involved in the formation of several old traditional breeds (the so-called country fowls) including Frisian fowls, Dutch bantams, Bread fowls, Dutch booted bantams, Dutch fowls, and, Schijndelaars whereas no recorded history of genetic influence from Asiatic chickens was recorded for other country fowls, including the Assendelft fowls, Drenthe fowls, and Groninger Mews. Ornamental breeds, including the Dutch Owl bearded, Dutch Polish bearded and Dutch Polish non bearded, are thought to derive from Polish bearded chickens firstly introduced in the Italian peninsula from Asia through Greece and, despite their ancient origin, are still kept by hobby breeders for ornamental and (competitive) fancy showing. As for some of the country fowls, also the Lakenvelder does not have a recorded history of genetic influence from Asiatic chickens (Dana et al. 2011).

In the last decades, fancy breeders have become interested in the development of bantam forms of large breeds. Neo-bantams have become popular among hobby breeders for their captivating and petite appearance and because they are more easily housed in a hobby setting. For these reasons, it is likely that neo-bantams will soon replace the large fowl counterparts. The aim of the bantamising trend is to obtain a small-sized individual exhibiting all of the standard (large) breed's characteristics, but in a smaller size. Invariably, the bantam forms of large breeds are made by crossing the original large breed with a small breed, such as the Dutch bantam (a 'true' bantam), or more recently with other more recently created bantam breeds. Breeders have repetitively crossed the first generation of neo-bantams to the parental generation of large fowls. Although backcrossing has contributed to the creation of bantam forms of almost all of the standard large breeds, crossing of related animals may pose a threat to the long-term existence of neo-bantams due to the accumulation of harmful and deleterious variants and inbreeding depression. However, introgression from local and imported stocks from Asia and neighbouring European countries, along with the occasional genetic exchange between farmers, are important sources of increased genetic diversity. Therefore, informing management and conservation programmes based on genetic data may prevent future loss of genetic diversity of traditional breeds.

The study of the genetic diversity harboured by commercial chicken lines may provide insights into and new perspectives on the genetic management and conservation priority of traditional populations. Such insights are possible since the genetic management of populations, along with demographic and selection history, influence the extent of genetic diversity and breed's identity (Granevitze et al. 2007; Muir et al. 2008; Mtileni et al. 2011; Elferink et al. 2012). In particular, effective management has shown to be critically important when the target population shows reduced genetic diversity and high level of inbreeding. In commercial lines, this is due to the decreased number of active breeding stocks, restricted within-line selection, and absence of genetic introgression from non-commercial populations (Muir et al. 2008).

Informing management of heritage breeds with genetic data has become feasible due to the development of cheap, chicken SNP panels (Groenen et al. 2011). Moreover, by applying SNP genotype data, major questions in conservation genetics can finally be addressed (Bosse et al. 2012). In the absence of pedigree data, which is the norm for non-commercial chicken populations, of immediate importance

Table 1 Summary details of the 37 traditional Dutch chicken breeds and 4 commercial white egg layer lines

Population	Abbreviated name	Management	Cluster	N	Flocks	Types	Sampling country	Sampling year
Assendelft fowl	AssFw	LF	CL3	15	4	4	Netherlands	2011 (10); 1998(5)
Assendelft fowl bantam	AssFwB	NB	CL3	2	2	1	Netherlands	2011 (2)
Barnevelder	Barnev	LF	CL1	24	10	2	Netherlands	2009 (10); 1998 (14)
Barnevelder bantam	BarnevB	NB	CL1	7	6	3	Netherlands	2011 (7)
Brabanter	Brab	LF	CL2	20	4	6	Netherlands	2011 (5); 2009 (5); 1998 (10)
Brabanter bantam	BrabB	NB	CL2	10	3	6	Netherlands	2011 (10)
Breda fowl	BreFw	LF	CL3	20	5	7	Netherlands	2011 (10); 1998 (10)
Breda fowl bantam	BreFwB	NB	CL3	10	3	7	Netherlands	2011 (10)
Chaam fowl	ChaFw	LF	CL3	10	2	3	Netherlands	2011 (29; 2009 (8)
Dutch bantam	DB	B	CL3	20	9	7	Netherlands	2011 (1); 2009 (9); 1998 (10)
Dutch booted bantam	DBdB	B	CL3	19	3	8	Netherlands	2011 (2); 2009 (7); 1998 (10)
Dutch fowl	DFw	LF	CL3	20	3	6	Netherlands	2011 (10); 1998 (10)
Dutch fowl bantam	DFwB	NB	CL3	4	3	4	Netherlands	2011 (4)
Dutch owl bearded	DOwBd	LF	CL2	24	5	8	Netherlands	2011 (5); 2009 (5); 1998 (14)
Dutch owl bearded bantam	DOwBdB	NB	CL2	11	4	6	Netherlands	2011 (11)
Dutch Polish bearded	DPBd	LF	CL2	13	2	2	Netherlands	2011 (1); 2009 (2); 1998 (10)
Dutch Polish bearded bantam	DPBdB	NB	CL2	10	5	7	Netherlands	2011 (9); 2009 (1)
Dutch Polish non bearded	DPnBd	LF	CL2	20	3	8	Netherlands	2011 (4); 2009 (6); 1998 (10)
Dutch Polish non bearded bantam	DPnBdB	NB	CL2	10	3	9	Netherlands	2011 (5); 2009 (5)
Drenthe fowl	DrFw	LF	CL3	20	2	7	Netherlands	2009 (10); 1998 (10)
Drenthe fowl bantam	DrFwB	NB	CL3	2	2	1	Netherlands	2011 (2)
Eikenburger bantam	EikenbB	B	CL3	4	1	1	Netherlands	2011 (4)
Frisian fowl	FriFw	LF	CL3	24	4	6	Netherlands	2011 (4); 2009 (6); 1998 (14)
Frisian fowl bantam	FriFwB	NB	CL3	7	5	6	Netherlands	2011 (7)
Groninger Mew	GrMw	LF	CL3	19	7	3	Netherlands	2009 (9); 1998 (10)
Groninger Mew bantam	GrMwB	NB	CL3	10	7	3	Netherlands	2009 (10)
Kraienkoppe	KraiK	LF	CL2	20	3	7	Netherlands	2011 (2); 2009 (8); 1998 (10)
Kraienkoppe fowl bantam	KraiKFwB	NB	CL2	5	2	4	Netherlands	2011 (5)
Lakenvelder	LakVe	LF	CL4	20	8	–	Netherlands	2011 (10); 1998 (10)
Lakenvelder bantam	LakVeB	NB	CL4	6	4	1	Netherlands	2011 (6)
North Holland Blue	NHBl	LF	CL1	20	4	1	Netherlands	2016 (1); 2011 (5); 2009 (3); 2007 (1); 1998 (10)
North Holland Blue bantam	NHBlB	NB	CL1	1	1	–	Netherlands	2011 (1)
Schijndelaar	Schijd	LF	CL3	10	1	4	Netherlands	2009 (10)
Schijndelaar bantam	SchijdB	NB	CL3	1	1	1	Netherlands	2009 (1)
Sumatra	Sumt	B	CL3	10	–	–	Netherlands	1998 (10)
Welsummer	Welsum	LF	CL1	24	6	1	Netherlands	2011 (10); 1998 (14)
Welsummer bantam	WelsumB	NB	CL1	8	6	1	Netherlands	2011 (8)
White egg layers – line R01	White_R01	C	–	29	1	–	Netherlands	–
White egg layers – line W1	White_W1	C	–	51	1	–	Netherlands	–
White egg layers – line WA	White_WA	C	–	66	1	–	Netherlands	–

Table 1 (continued)

Population	Abbreviated name	Management	Cluster	N	Flocks	Types	Sampling country	Sampling year
White egg layers – line WD	White_WD	C	–	48	1	–	Netherlands	–

N represents the sample size, *Flocks* the number of fancy breeders that contributed to the total sample size of a breed, and *Types* the number of morphological varieties (feather colour) present in the total sample size of a breed. There is no correspondence between *Flocks* and *Types*, as a single breeder can have contributed to the total sample size with different morphological varieties. The number in parenthesis reported after the year in the column *Sampling year* identifies the number of individuals within a breed sampled in that specific year. Abbreviations under the column *Management* represent the subdivision of chicken populations into clusters based on their genetic management (*LF* large fowl, *B* bantam, *NB* neo-bantam, *C* commercial). *Cluster* identifies the group the breed belongs to according to the principal component analysis (*CL1* past-productive, *CL2* ornamental, *CL3* country fowls, *CL4* Lakenvelder)

is the assessment of the degree of relatedness between populations, their genetic uniqueness, and degree of inbreeding. SNP arrays provide an alternative approach to estimate the traditional inbreeding coefficient, F_{ped}, by detecting continuous segments of homozygous SNPs (runs of homozygosity - ROH) (Kim et al. 2013; Szpiech et al. 2013). Studying ROHs provides insights into past and present population history, selection pressure, and management (Bosse et al. 2012; Purfield et al. 2012; Herrero-Medrano et al. 2013; Kim et al. 2013).

Numerous diversity studies of traditional chicken populations from different countries and continents are reported in the literature. However, these studies have been based on a limited number of genetic markers of lower resolution and genome coverage than SNP arrays. Regarding the traditional Dutch chicken breeds, only few studies have focused on the assessment of the breed genetic diversity and contribution to conservation (Eding and Meuwissen 2001; Hillel et al. 2003). Invariably, such characterisation studies were incomplete, since neo-bantam breeds were not considered.

Here, we use the 60 K SNP array to characterise the genetic diversity and inbreeding of all recognised Dutch heritage breeds and most of the bantam forms. In particular, we investigate the process by which the neo-bantams are formed, their degree of inbreeding due to presumed small founder size, and their potential contribution to the total Dutch chicken genetic diversity. Finally, we study the effects of the structured management experienced by commercial lines on their genetic diversity and demographic history to better inform genetic management and conservation of Dutch heritage breeds.

Materials and methods

Chicken populations

A total of 674 individuals from forty-one chicken populations originating from the Netherlands and resulting from different demographies and management strategies were included in the study (Table 1). The complete set of chicken populations included 37 traditional fancy breeds (480 individuals), comprising true bantams, large fowls, and bantam counterparts (neo-bantam), two commercial white egg layer sire lines (R01 and W1) (80 individuals), and two commercial white egg layer dam lines (WA and WD) (114 individuals). Among the traditional breeds, 476 individuals were sampled from part-time, hobby, and fancy breeders of known provenance, while sperm of four individuals of the breed North Holland Blue was provided by the Centre for Genetic Resources (CGN), The Netherlands. The total number of individuals per breed varied from 1 to 66, with a maximum of 24 individuals for the fancy breeds, whereas the total number of fancy breeders contributing to the total sample size ranged from 1 to 10. Sample collection of fancy breeds took place over 13 years (1998–2011) (Table 1). Due to the important variation in sample size over the time frame considered, changes in genetic diversity over time were not analysed. Since pedigrees are generally not recorded by fancy breeders or breeding organisations, this information was not available to this study. In fact, the absence of such information that is vital for effective management was a major incentive for the comprehensive genotyping effort detailed in this study. Phenotypic information was collected in the form of feather colour only for those breeds sampled between 2009 and 2011.

Sampling and genotyping

DNA was extracted from blood of 191 samples (1998), sperm of 4 individuals (2007), and from fertile hatching eggs of 287 samples (2007–2011). Genotyping and quality control (QC) were performed separately using the standard protocols for the Illumina Infinium iSelect 60 K BeadChip. Raw data were analysed using the Genome Studio software package (Illumina Inc.) (Groenen et al. 2011). The 60 K SNP chip contained 52,232 SNPs uniformly distributed across the Gallus_gallus5.0 chicken genome, comprising 29 autosomes (Gga 1–28 and Gga 33), two sex chromosomes

(that is, 2577 SNPs on the Z chromosome and 7 on the W chromosome), and one linkage group (that is, 49 SNPs on LGE64). The array also included several variants of unknown mapping position ($n = 507$), whereas no variants were mapped to the mitochondrial genome. Variants on the two sex chromosomes, linkage group, and variants of unknown physical position were all removed from both traditional and commercial breeds, separately. A total of 49,092 variants were retained in both datasets. Genotype filtering was applied to the merged dataset (traditional breeds and commercial lines) after removing individuals mislabelled.

SNP quality control and marker selection

We used PLINK v1.9 (Chang et al. 2015) for genotyping data quality control. Samples genotyped for <90% of markers were excluded, along with SNPs genotyped for <90% of the animals. Monomorphic variants were also discarded. Using these criteria, 2121 SNPs were excluded because of the low genotyping rates (Table S1), while 38 samples were removed due to a low genotyping rate (Table S2). Although only one individual of the Assendelft fowl bantam, Drenthe fowl bantam, North Holland Blue bantam, and Schijndelaar bantam passed the quality control, we excluded these breeds only from the calculation of the population genetic diversity estimates, because the extremely small sample size precluded such calculations. No additional filtering for minor allele frequencies was carried out, because removal of rare alleles could lead to overestimated results in under sampled populations (Toro et al. 2009). Similarly, no filtering for linkage disequilibrium, Mendelian error, and deviation from Hardy–Weinberg equilibrium (HWE) were performed, owing to the lack of pedigree information for the traditional populations and the interest in investigating deviations from HWE. The final data set consisted of 632 individuals from 33 traditional Dutch breeds and 4 commercial lines genotyped for 46,971 SNPs.

Population genetic diversity

Mean expected (H_E) and observed (H_O) heterozygosity, mean minor allele frequency (MAF), and mean inbreeding coefficient (F_{IS}) were averaged across all loci and individuals within a population, respectively. Measures of molecular diversity were estimated using PLINK v1.9. As a result of the wide range of sample size across traditional breeds (from 4 to 23 after QC), we decided to test the influence of a variable sample size on the population genetic diversity estimates by randomly sample a different number of individuals within each breed. Individuals were randomly selected from those that passed the quality control. Mean expected (H_E) and observed (H_O) heterozygosity, mean minor allele frequency (MAF), and mean inbreeding coefficient (F_{IS}) were then estimated for each newly sampled breed. A *t*-test was used to statistically test whether differences in population statistics were attributed to the sample size.

To further investigate the consequences of divergent management practices to the genetic diversity, we followed the genetic cluster analysis carried out by Hillel et al. (2003) dividing our populations into four groups, as follow: (1) LARGE FOWL, including large fowls of fancy breeds selected for specific morphological traits; (2) BANTAM, which included all the true bantams for which no large fowl counterparts exist; (3) NEO BANTAM, consisting of recently established bantams of large fowls; and (4) COMMERCIAL, which included the commercial lines intensively selected for quantitative traits related to egg production.

Genetic relationships between traditional and commercial populations and among fancy breeds were investigated through the Principal Component Analysis (PCA), which was performed on the genotype data using the R package SNPRelate (Zheng et al. 2012) for R v3.2.0 (R Development Core Team 2008). Pairwise genetic distance (D) for all pairwise combinations of individuals of traditional breeds was calculated on unpruned data as $D = 1 - D_{ST}$, where D_{ST} is the average proportion of alleles shared among individuals. The 1-IBS matrix was afterward used for phylogenetic reconstruction producing a Neighbor-Joining (NJ) tree in PHYLIP v3.696 (Felsenstein 2004) with random input order. The unrooted tree was then visualised with Figtree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Population genetic admixture

Population genetic admixture was analysed using the model based clustering method ADMIXTURE v1.3.0 (Alexander and Novembre 2009). Although the software assumes that all populations share the same ancestral group, a K value identifying the number of ancestral components needs to be provided to perform the analysis. Due to the unknown genetic structure of our chicken populations, we decided to perform an unsupervised admixture analysis by carrying out a cross-validation (CV) procedure for K values ranging from 1 to 40. The CV procedure aimed to select the K value exhibiting the lowest cross-validation error estimate, which represents the most parsimonious number of clusters. Following the genetic diversity analysis, we resolved to restrict the admixture analysis to the clusters identified by the PCA of traditional breeds, thus reducing the likelihood of biased results owing to the considerable different sample size. Results were visualised with Pong (Behr et al. 2016).

To formally test whether admixture occurred across our traditional chicken populations, and to additionally measure

Table 2 Molecular diversity statistics of traditional chicken breeds and commercial lines and of populations within the four management-based clusters

Population	Management	Average MAF ± SD	Average H_O ± SD	Average H_E ± SD	F_{IS} ± SD
Assendelft fowl	LF	0.221 ± 0.15	0.272 ± 0.18	0.295 ± 0.17	0.076 ± 0.21
Barnevelder	LF	0.256 ± 0.15	0.204 ± 0.14	0.335 ± 0.16	0.369 ± 0.16
Barnevelder bantam	NB	0.184 ± 0.16	0.222 ± 0.21	0.246 ± 0.19	0.113 ± 0.12
Brabanter	LF	0.252 ± 0.15	0.283 ± 0.17	0.331 ± 0.16	0.083 ± 0.15
Brabanter bantam	NB	0.223 ± 0.16	0.204 ± 0.17	0.294 ± 0.18	0.537 ± 0.12
Breda fowl	LF	0.235 ± 0.15	0.258 ± 0.17	0.309 ± 0.17	0.154 ± 0.15
Breda fowl bantam	NB	0.217 ± 0.15	0.234 ± 0.18	0.289 ± 0.17	0.482 ± 0.12
Chaam fowl	LF	0.240 ± 0.15	0.316 ± 0.20	0.316 ± 0.16	0.332 ± 0.06
Dutch bantam	B	0.222 ± 0.15	0.210 ± 0.14	0.298 ± 0.16	0.263 ± 0.18
Dutch booted bantam	B	0.220 ± 0.15	0.247 ± 0.18	0.296 ± 0.16	0.091 ± 0.21
Dutch fowl	LF	0.213 ± 0.15	0.217 ± 0.16	0.287 ± 0.17	0.104 ± 0.24
Dutch fowl bantam	NB	0.208 ± 0.16	0.216 ± 0.22	0.275 ± 0.19	0.453 ± 0.06
Dutch owl bearded	LF	0.249 ± 0.15	0.293 ± 0.17	0.328 ± 0.16	0.033 ± 0.25
Dutch owl bearded bantam	NB	0.225 ± 0.15	0.280 ± 0.19	0.297 ± 0.17	0.162 ± 0.41
Dutch Polish bearded	LF	0.165 ± 0.16	0.219 ± 0.22	0.222 ± 0.19	−0.067 ± 0.24
Dutch Polish bearded bantam	NB	0.205 ± 0.16	0.181 ± 0.15	0.274 ± 0.18	0.329 ± 0.49
Dutch Polish non bearded	LF	0.166 ± 0.15	0.162 ± 0.15	0.232 ± 0.17	0.237 ± 0.25
Dutch Polish non bearded bantam	NB	0.215 ± 0.15	0.212 ± 0.17	0.288 ± 0.17	0.280 ± 0.25
Drenthe fowl	LF	0.236 ± 0.15	0.250 ± 0.15	0.314 ± 0.16	0.156 ± 0.15
Eikenburger bantam	B	0.083 ± 0.14	0.116 ± 0.22	0.108 ± 0.18	0.613 ± 0.04
Frisian fowl	LF	0.211 ± 0.15	0.245 ± 0.18	0.284 ± 0.17	−0.063 ± 0.17
Frisian fowl bantam	NB	0.229 ± 0.15	0.238 ± 0.19	0.302 ± 0.17	0.208 ± 0.14
Groninger Mew	LF	0.177 ± 0.15	0.197 ± 0.18	0.242 ± 0.18	−0.011 ± 0.15
Groninger Mew bantam	NB	0.203 ± 0.16	0.225 ± 0.19	0.268 ± 0.19	0.498 ± 0.05
Kraienkoppe	LF	0.232 ± 0.15	0.288 ± 0.17	0.311 ± 0.16	0.010 ± 0.22
Kraienkoppe fowl bantam	NB	0.183 ± 0.16	0.188 ± 0.20	0.242 ± 0.19	0.520 ± 0.03
Lakenvelder	LF	0.171 ± 0.16	0.214 ± 0.19	0.230 ± 0.19	0.054 ± 0.17
Lakenvelder bantam	NB	0.207 ± 0.16	0.266 ± 0.22	0.272 ± 0.19	0.008 ± 0.26
North Holland Blue	LF	0.245 ± 0.14	0.317 ± 0.17	0.326 ± 0.15	0.001 ± 0.20
Schijndelaar	LF	0.213 ± 0.15	0.266 ± 0.19	0.287 ± 0.17	0.425 ± 0.12
Sumatra	B	0.179 ± 0.17	0.327 ± 0.33	0.230 ± 0.20	−0.311 ± 0.01
Welsummer	LF	0.198 ± 0.16	0.237 ± 0.18	0.266 ± 0.18	0.036 ± 0.20
Welsummer bantam	NB	0.171 ± 0.16	0.210 ± 0.21	0.228 ± 0.19	0.137 ± 0.48
White egg layers – line R01	C	0.181 ± 0.16	0.250 ± 0.21	0.241 ± 0.19	0.482 ± 0.02
White egg layers – line W1	C	0.183 ± 0.16	0.247 ± 0.20	0.243 ± 0.19	0.495 ± 0.02
White egg layers – line WA	C	0.153 ± 0.16	0.209 ± 0.21	0.209 ± 0.20	0.572 ± 0.02
White egg layers – line WD	C	0.154 ± 0.16	0.212 ± 0.20	0.207 ± 0.19	0.563 ± 0.02
Management group (N. populations)					
LARGE FOWL ($n = 17$)	LF	0.324 ± 0.11	0.247 ± 0.07	0.410 ± 0.10	0.390 ± 0.16
BANTAM ($n = 4$)	B	0.285 ± 0.13	0.224 ± 0.11	0.368 ± 0.13	0.370 ± 0.18
NEO BANTAM ($n = 16$)	NB	0.311 ± 0.12	0.224 ± 0.08	0.397 ± 0.11	0.427 ± 0.14
COMMERCIAL ($n = 4$)	C	0.260 ± 0.15	0.227 ± 0.13	0.337 ± 0.16	0.542 ± 0.04

Population genetic diversity statistics are averaged across loci and individuals within each population

MAF minor allele frequency, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, *SD* standard deviation

Abbreviations under the column Management represent the subdivision of the chicken populations into clusters based on their genetic management (*LF* large fowl, *B* bantam, *NB* neo-bantam, *C* commercial)

its extent, we calculated three-population test estimates (*f3* statistics) (Reich et al. 2009) and their corresponding normalised value (z-score), calling the "threepop" module implemented in the TREEMIX software package v1.13 (Pickrell and Pritchard 2012). We decided to restrict the three-population test to the traditional populations, because we did not expect genetic admixture between commercial and non-commercial breeds. In the *f3* statistics, we considered the triplet of the populations (C; A, B), where C is the target, or test, population, and A and B are the source, or reference, populations. The normalised z-scores were calculated by jack-knifing in blocks of 500 SNPs. A significant negative value of the *f3* statistic ($z \leq -3.80$) indicated an admixture event between the test and the two ancestral populations. We performed all possible triplet combinations, considering only breeds with more than one sample as test population.

Runs of homozygosity

Population demographic history was investigated through the detection of homozygous stretches along an individual's SNP data, as implemented in the -homozyg option in PLINK (Howrigan et al. 2011). We defined a run of homozygosity (ROH) as a tract of homozygous genotypes that was greater than 10 Kb in length, and identified in a genome-sliding window of 30 SNPs. To ensure that the entire observed stretch from the first SNP to the last SNP was homozygous (true ROH), we excluded stretches with a mean tract density > 1 Mb/SNP, and with a maximum gap between two consecutive homozygous SNPs of 1000 Kb. To lower the underestimation of ROHs due to genotyping errors and/or missing genotypes, we allowed only one heterozygous SNP and one missing call per window. The detected ROHs were then classified into three categories intended to correspond to different demographic processes: short ROH (<1000 Kb) reflected homozygosity of ancient haplotypes if not founder effects; medium (1–3 Mb) background relatedness within populations; and long (> 3 Mb) recent parental relatedness (Szpiech et al. 2013). A genomic measure of individual autozygosity, F_{ROH}, was calculated as the proportion (0–1) of the autosomal genome covered by stretches of consecutive homozygous SNPs following McQuillan et al. (2008),

$$F_{ROH} = \frac{\sum L_{ROH}}{L_{auto}},$$

where $\sum L_{ROH}$ is the total length of all of an individual's runs, and L_{auto} is the total genome length across the autosomes covered by SNPs (McQuillan et al. 2008). In calculating L_{auto} the genetic map containing markers not filtered for low genotype calls was used to reduce the likelihood of underestimating the total autosomal genome length. According to our SNP panel, L_{auto} was ~906 Mb. Individual and population mean values of F_{ROH} were estimated for all ROHs and for the three ROH-length threshold classes. The correlation between the genomic measure of autozygosity (F_{ROH}) and the inbreeding coefficient estimated from genotype frequency (F_{IS}) was calculated for all homozygous stretches and for the three ROH classes, respectively. All plots were generated with the R package ggplot2 for R v3.2.0.

Results

Population genetic diversity

Table 2 shows the results of the population and management-based genetic diversity analysis of the traditional breeds and commercial lines that passed genotyping data quality control. The management-based analysis also included the Assendelft fowl bantam, Drenthe fowl bantam, North Holland blue bantam, and Schijndelaar bantam breed, from which the total number of 41 populations. Average minor allele frequency across traditional chicken populations ranged from 0.165 ± 0.16 (Dutch Polish bearded) to 0.256 ± 0.15 (Barnevelder). Average observed (H_O) and expected heterozygosity (H_E) varied between 0.116 ± 0.22 (Eikenburger bantam) and 0.327 ± 0.33 (Sumatra) and between 0.108 ± 0.18 (Eikenburger bantam) and 0.335 ± 0.16 (Barnevelder), respectively. Average inbreeding coefficient (F_{IS}) ranged from −0.311 ± 0.01 (Sumatra) to 0.613 ± 0.04 (Eikenburger bantam) (Table 2). Traditional breeds that showed signatures of outbreeding were also the Dutch Polish bearded (−0.067 ± 0.24), Frisian fowl (−0.063 ± 0.17), and Groninger Mew (−0.011 ± 0.15), whereas high inbreeding coefficient estimates were also reported for the neo-bantams of Brabanter (0.537 ± 012) and Kraienkoppe fowl bantam (0.520 ± 0.03) (Table 2). Overall, higher within-breed inbreeding coefficient estimates were displayed by neo-bantams than large fowl counterparts. Moreover, compared to commercial lines, more heterogeneous molecular diversity estimates were observed for traditional breeds, supporting differences in selective breeding and demographic history. As expected, level of excess homozygosity (F_{IS}) negatively correlated with the observed heterozygote frequencies ($r = -0.54$, p-value = 0.001).

At the management level, breeds selected for a specific morphological standard (LARGE FOWL) were the most polymorphic, followed by neo-bantams, which showed slightly similar genetic diversity estimates, and true bantams (that are, Dutch bantam, Dutch booted bantam, Eikenburger bantam, and Sumatra). Polymorphism measures of the

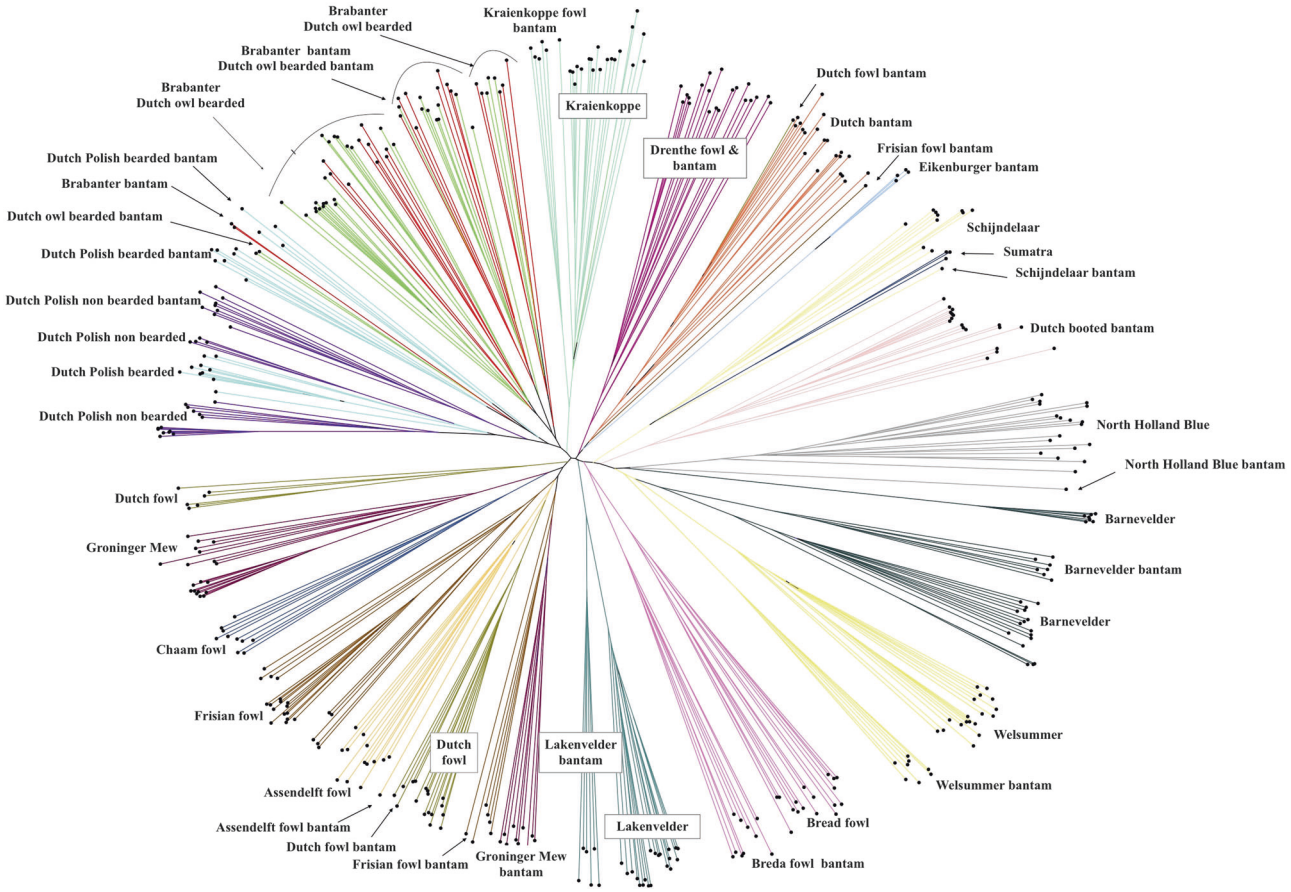

Fig. 1 Unrooted phylogenetic tree showing genetic relatedness of the 37 traditional chicken breeds. The phylogeny tree was constructed using the Neighbor-Joining method with random input orders and the pairwise 1-IBS-distance matrix. Large fowls and bantam counterparts of each breed are reported with the same colour. The name of each breed is indicated in the figure with, in some cases, the use of an arrow. For abbreviations, refer to Table 1

COMMERCIAL cluster displayed intermediate values, supporting the reduced genetic diversity reported in previous studies (Granevitze et al. 2007; Muir et al. 2008). Average inbreeding coefficient showed an opposite pattern, with the NEO-BANTAM cluster being the most inbred of the traditional breeds cluster, followed by LARGE FOWL and BANTAM (Table 2).

Population genetic estimates calculated for each population after randomly select a different number of individuals that passed the quality control are reported in Table S3 of the Supplementary Material. Population genetic diversity estimates of the same population calculated on a different sample size did not significantly differ from those reported in Table 2, except for the expected heterozygosity, whose estimates were significantly different in both random sampling scenarios (Table S4). The standard deviation of all genetic estimates were considerably high, especially that of the inbreeding coefficient, which decreased when increasing the sample size, except for the Frisian fowl bantam and Dutch Polish bearded bantam (Table S3).

Population genetic structure and admixture

Results on the breed genetic differentiation were consistent in the Neighbor-Joining (NJ) tree and principal component analysis. Moreover, the principal component analysis of traditional breeds revealed a more complex population structure and higher genetic similarities between traditional breeds than with commercial lines (Figure S1). The NJ tree showed an average high proportion of alleles identical-by-state (IBS) shared between the neo-bantams and large fowl counterparts. As a result, large fowls and neo-bantams were separately grouped within the same cluster, as shown, for example, by the Breda fowl and Breda fowl bantam (Fig. 1). The NJ tree also identified several subdivided breeds, including the Barnevelder, Frisian fowl, Groninger Mew, and Dutch bantam, for which some individuals were separately grouped within the same cluster, and the Dutch fowl, for which individuals were grouped in two separate clusters, one closer to the Groninger Mew while the other between the Assendelft fowl and Frisian fowl bantam (Fig. 1). The

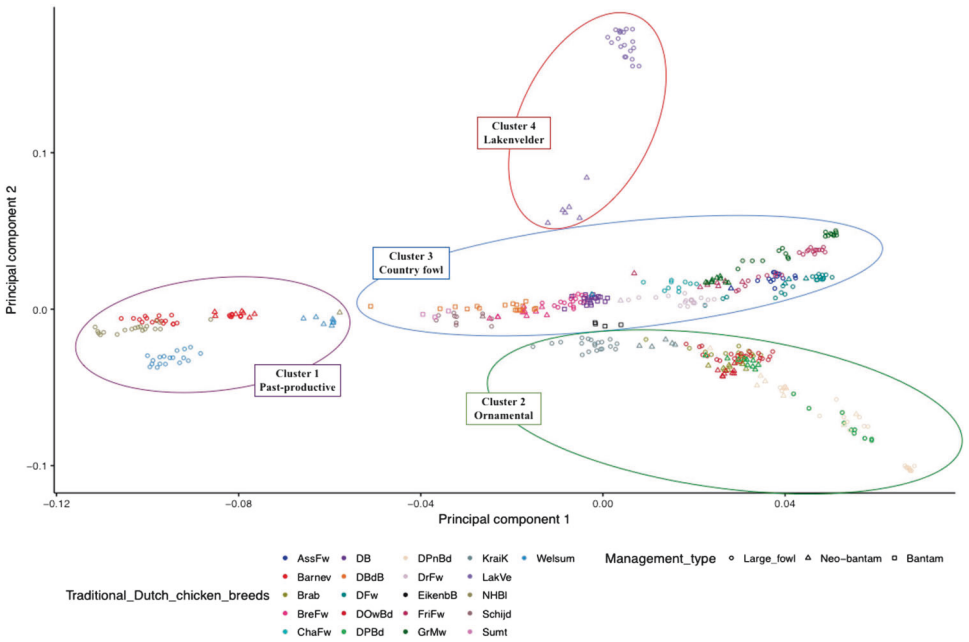

Fig. 2 Principal component analysis plot for PC1 and PC2 of traditional Dutch chicken breeds. The principal component analysis was performed on all individuals that passed the genotyping data quality control, for a total of 442 samples. Large fowls and bantam counterparts are represented with the same colour but in two different shapes (circle and triangle, respectively, as reported in the legend *Management_type*). For simplicity, only the abbreviated name of the large fowl is reported in the *Traditional_Dutch_chicken_breeds* legend. True bantams, that are the Dutch bantam, Dutch booted bantam, Eikenburger bantam, and Sumatra, are represented with the same square shape *Management_type*), since they do not have any large fowl counterpart, but have different colours since they are distinctive breeds. The four coloured circles represent the first (purple), second (green), third (light blue), and fourth (red) cluster described in the main text. For abbreviations, refer to Table 1

NJ tree also captured recent gene flow, as shown by the Dutch fowl bantam and Frisian fowl bantam (indicated with an arrow in Fig. 1), which clustered together with the Dutch bantam, a breed that has been used in the bantamisation of the large fowl counterparts. Similar pattern was observed for the Schijndelaar and Schijndelaar bantam, which both clustered together with their source population represented by the Sumatra (indicated with an arrow in Fig. 1). The heterogeneity showed by the subdivided breeds and the recent gene flow reported for some individuals were well captured in the PCA under *cluster 1*, which identified large fowls and neo-bantams of breeds with past productive significance, and *cluster 3*, which was defined by the oldest breeds of The Netherlands, the so-called country fowls (Fig. 2). In both PCA and NJ tree, the ornamental breeds (*cluster 2* in the PCA) showed a distinctive clustering pattern, as displayed by the intermingled breeds, including the Brabanter, Dutch Owl bearded, Dutch Polish bearded, Dutch Polish non-bearded, and bantam counterparts. The NJ tree and PCA identified two main sub-clusters: the first represented by the Dutch Owl bearded and Brabanter, and the second by the Dutch Polish bearded and Dutch Polish non-bearded (Figs. 1 and 2). We did not observe a clear separation between the large fowls and neo-bantams, which may indicate a complex on-going gene flow among the ornamental breeds. The high similarity in phenotypes displayed by the fancy breeds may also support the genetic exchange, as well as question their genetic identity.

The results of the ADMIXTURE analysis (Figure S2–S4) performed on the clusters identified by the PCA (except for the Lakenvelder cluster which was combined with *cluster 3*) were consistent with what reported in the PCA and NJ tree and complied with the breeds' development history. However, we also observed divergent admixture patterns across samples based on their year of sampling, with the recently sampled individuals showing a less unique genetic make-up, as a result of on-going gene flow. The genetic origin of the neo-bantams already captured in the NJ and PCA were better represented in the ADMIXTURE analysis, in which neo-bantams are the result of introgression from original, large-sized fowls and true bantams of morphologically analogous breeds, and, more recently, of neo-bantams of the same or of different breeds. For instance, the Dutch owl bearded bantam showed introgression from the large fowl counterpart, along with the Dutch Polish bearded bantam, Dutch Polish non bearded, and Dutch Polish non bearded bantam (Figure S3).

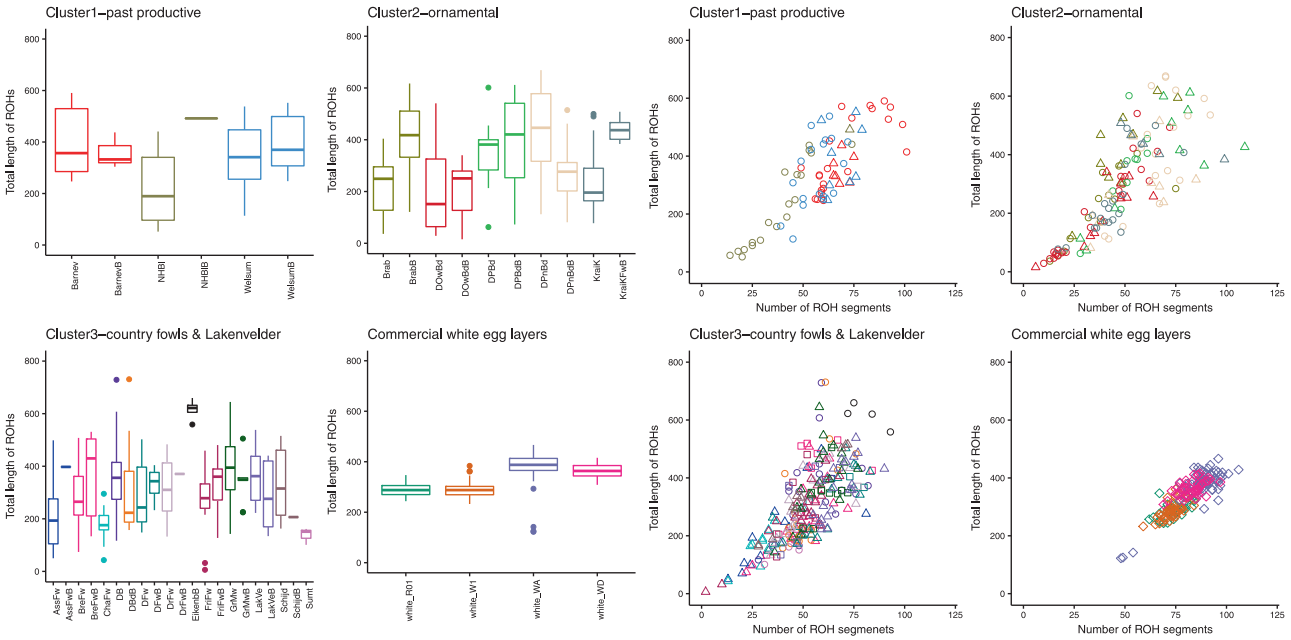

Fig. 3 Distribution of ROHs across the genome and ROH profile. **a** The total length of homozygous segments (ROHs) detected across the genome is reported for each breed and expressed in megabases (Mb). **b** ROH profile given by the total number of homozygous segments and total segment size in megabases of all 37 traditional chicken breeds. In both figures, breeds were grouped according to the clusters identified in the principal component analysis, except for cluster 3, which also included the Lakenvelder and Lakenvelder bantam. Commercial lines where grouped in another cluster called *Commercial white egg layers*. For the North Holland blue bantam, Assendelft fowl bantam, Drenthe fowl bantam, and Schijndelaar bantam, the distribution of ROH segments across the genome is represented by a straight line, because only one individual was left after quality control. Colours and shapes were the same of Fig. 1 (cycle: large fowl; triangle: bantam counterpart; square: true bantam), except for the commercial white egg layers cluster, which were represented with a different shape

A high number of significant negative *f3* statistics (Table S5) was observed with the Dutch fowl bantam as admixed population and most of the remaining breeds as source populations, confirming introgression from varying populations. Of the 27,282 *f3* statistics, 1125 were found to be significant (z-score < −3.80)

Runs of homozygosity

The average proportion of the genome covered by ROHs reflected the genetic diversity and demographic history of traditional breeds, along with the degree of inbreeding. Compared to the homogenous values reported for the commercial lines (Fig. 3a, *cluster commercial white egg layers*), traditional breeds showed important variation in the total length of ROHs (*cluster 1–3*, Fig. 3a). The ROH profile (*cluster 1–3*, Fig. 3b) of the traditional breeds displayed more differences in pattern compared to the commercial lines (Fig. 3b), which exhibited similar average cumulative size as well as average ROH number. Although white egg layers displayed a similar ROH profile, individuals from the dam line (WA, WD) had a higher average cumulative size and average ROH number compared to the sire line (R01, W1), confirming the higher homozygosity reported in Table 2. To investigate the effects of specific demographic processes on the distribution of ROHs across the genome, we divided the detected segments into three ROH-length threshold classes: short (< 1 Mb), medium (1–3 Mb), and long (> 3 Mb). Medium and long ROHs were the most abundant classes in both traditional breeds and commercial lines (Fig. 4a). Compared to the large fowl counterparts, neo-bantams showed a higher number of medium and long ROHs, with the highest number found in the Eikenburger bantam, followed by Barnevelder bantam and Dutch Polish bearded bantam (Fig. 4a). Although traditional breeds had a lower number of ROHs than commercial lines (Fig. 4a), homozygous segments covered a significant proportion of their genome, and particularly of neo-bantams, as shown by the Brabanter bantam and Kraienkoppe fowl bantam (Fig. 4b).

To assess ROH as an indicator of inbreeding, we compared frequency-based estimates of inbreeding with genomic measures of individual autozygosity. Results confirmed the higher degree of inbreeding of neo-bantams compared to the large fowl counterparts for all three ROH-length threshold classes (Table S6). Also based on the genomic measure of autozygosity, the Eikenburger bantam was the most inbred breed, with a genome-wide F_{ROH} of 0.66. Moreover, we reported a positive, significant correlation ($r = 0.57$, p-value = <2.2e–6) between F_{ROH} and F_{IS} estimates.

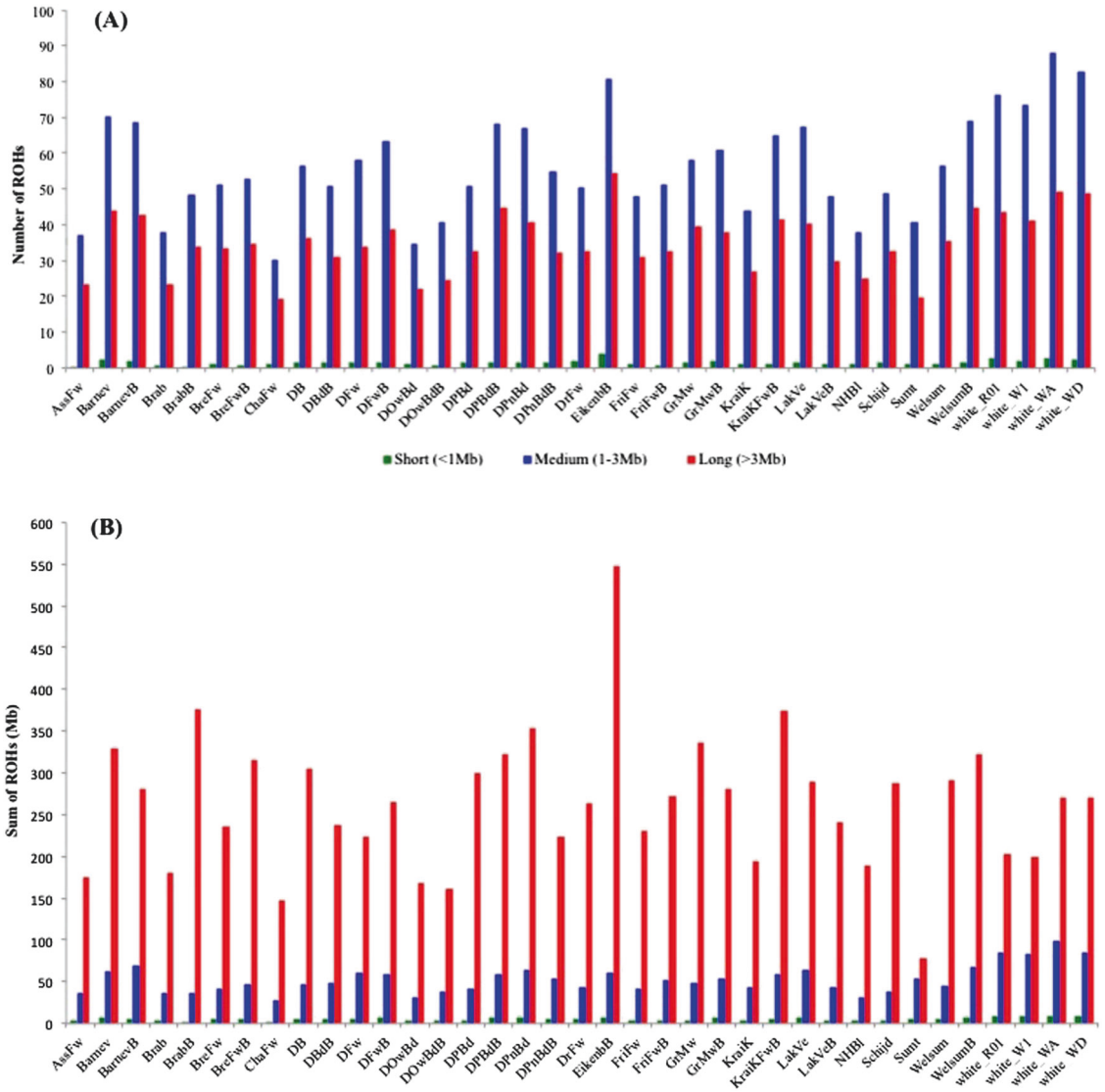

Fig. 4 Total number of ROHs and proportion of the genome covered by ROHs. **a** The average number of ROHs belonging to the three size classes short (< 1 Mb), medium (1–3 Mb), and long (> 3 Mb) for the 33 traditional breeds and 4 commercial lines. **b** The total size of the genome covered by a particular class of ROH in one individual averaged per breed. In both figures, the Assendelft fowl bantam, Drenthe fowl bantam, North Holland Blue bantam, and Schijndelaar bantam were excluded because of the extremely small sample size ($N = 1$), which precludes the calculation of the breed-averaged parameters represented in the figure. For abbreviations, refer to Table 1

Discussion

The availability of a large number of SNPs resulting from the development of high-density SNP assays has considerably improved the accuracy to assess population structure and relationship among populations, along with the genetic diversity either within or between populations (Herrero-Medrano et al. 2013). High-density SNP arrays are also used to assess the effects of inbreeding through the occurrence of runs of homozygosity (ROH), which are increasingly used to infer past and present demography (Bosse et al. 2012; Purfield et al. 2012; Herrero-Medrano et al. 2013). The application of SNP chip data to assess population genetic diversity and genetic management of traditional breeds is, although still scarce, improving. Here we present the first comprehensive study on the population genetic diversity, population relationship, and demography of all traditional chicken breeds of the Netherlands recognised by the poultry community, to provide recommendations on their conservation and genetic management. The breeds studied are part of the Dutch poultry genetic resources and are also included in the FAO Domestic Animal Diversity Information System (DAD-IS). All traditional breeds of chicken are described by breed-specific morphological standards, including, among others, plumage colour and pattern, feather structure and pigmentation,

comb morphology, skin colour, and eggshell pigmentation. Despite the exceptional diversity both in qualitative and quantitative traits, most of the traditional breeds are rare breeds or varieties having the status of endangered or critically endangered (Woelders et al. 2006).

Assessing the genetic diversity and understanding the relationships among and within populations are the first necessary steps to establish conservation priorities and strategies (Berthouly et al. 2010; Druml et al. 2012). Large sample sizes are usually recommended to accurately estimate population statistics. However, large sample sizes are often difficult or impossible to achieve in genetic diversity studies of threatened and endangered populations (Miyamoto et al. 2008; Pruett et al. 2008). In this study, sample size was a major limitation, as shown by the wide range of sample size across traditional breeds. Such limitation was mainly caused by the small effective population size of most of the breeds here considered and by the limited number of breeders involved in their conservation. For instance, at the time of sampling, the Eikenburger bantam was kept by only one breeder, leading to a sample size of only 4 individuals, while the Barnevelder was kept by roughly 10 farmers, which contributed to a sample size of 24 individuals (Table 1). It is therefore clear that in this study the number of hobby breeders and the popularity of the breed played a major role. We showed that, although genetic diversity estimates showed a varying degree of bias (Table S3-S4), population statistics can nevertheless provide revealing insights into the genetic diversity and, more importantly, the inbreeding history of a breed. Therefore, by keeping in mind potential bias in the results, conclusions and recommendations on the conservation and genetic management of traditional breeds can, but above all, should be attempted; genomic data is the only reliable source to estimate inbreeding and relatedness of marginalised populations in absence of other data sources such as pedigree data.

Practical conservation breeding should also be aware that the ascertainment of the SNP chip results in some bias in terms of detectability of unique genetic variation. Such bias is particularly important if the breed of interest was not involved in the development of the SNP array. Although the traditional Dutch breeds here considered were not used in the development of the 60 K SNP chip, we did not observe a systematic difference between the neo-bantams and the other breeds. Therefore, we expect the ascertainment bias to be minimal. Moreover, we focused on the relatedness and runs of homozygosity (ROHs), both statistics that are less sensitive to missing such breed-specific variation.

A metapopulation-like structure can explain the variable genetic diversity estimates observed across traditional breeds, which are therefore subdivided into small breeder-based breeding units. The genetic diversity within each sub-population is strongly influenced by the breeder's breeding practices and selection preferences. However, results also show that a more or less restricted gene flow and a small local flock size have also divergent consequences on the breeds' genetic diversity, leading to the distinction between large fowls and neo-bantams.

The low genetic diversity observed in some of the large fowls, such as the Groninger Mew, may be explained by the drastic reduction in size of the breeding population occurred in the last century, which further suggests that some of the large fowls may have been close to extinction at some point or points in their history. Despite such population bottleneck, genetic data suggest that diversity was usually restored by crossing surviving individuals with other breeds showing complementary traits. However, the incorporation of genes from phenotypically similar breeds has decreased in popularity, because breeders prefer to use their prized cocks within their own farm. Currently, breeders use backcrossing to obtain a new generation of individuals sharing the same number of traits of the parental generation. As a result, the degree of inbreeding of the sub-population has increased, although the consequences on the long-term genetic diversity vary depending on the farmer's selection preferences and intensity, and flock size. Compared with the large fowls, neo-bantams have a recent historical origin and because of that, the demographic history and within-population genetic relationships are largely unknown. According to our analysis, different evolutionary and human-induced processes have and are now contributing to their metapopulation structure. First of all, the creation of miniaturised fowls has been achieved using breeding strategies different from those of large fowl counterparts. And secondly, breeding strategies to create neo-bantams have rapidly changed over the past decades.

Neo-bantams initially resulted from a cross between large fowls of the same breed and true bantams of a distinct breed, such as Dutch bantams and Sumatras. However, our results indicate that, in the last decades, the bantamising trend has seen a significant change in the number of breed/varieties used. Such changes may have been driven either by the development of new phenotypes in large fowls that breeders want to have in a smaller sized individual or by the breeder's initiative to develop new small size varieties. Changes in the bantamising trend were well captured in the admixture analysis, which highlighted the use of even neo-bantams of the same sub-population and of phenotypically similar breeds to bantamise large-sized individuals. Although the genetic exchange between breeders is at the basis of the neo-bantam genetic diversity, backcrossing pursued for phenotype selection has, over time, considerably increased the degree of inbreeding, which, in our analysis, was the highest across the traditional breed clusters. However, the higher inbreeding coefficient may also result from the small effective flock size.

The analysis of runs of homozygosity (ROHs) can be used to address major concerns in conservation genetics, including inbreeding and population demography (Bosse et al. 2012; Purfield et al. 2012; Herrero-Medrano et al. 2013). Although the 60 K SNP panel allows an appropriate estimation of ROHs, ascertainment bias may underestimate the number of small ROHs (Bosse et al. 2012), while amplifying the total length of the medium and longest ROHs (Purfield et al. 2012). Our results confirm the accuracy of the SNP panel for the analysis of medium and large ROHs and the ability of ROHs to reflect past and present population history, validating previous studies (Bosse et al. 2012).

The analysis of ROH highlights the importance of novel marker-based information to prevent future loss of diversity. The prevalence of long ROHs across traditional chicken breeds is consistent with the limits to effective genetic management resulting from the absence of pedigree data and breed registry, and clearly shows the importance of recent inbreeding for the long-term viability of the populations. The ROH profile confirms such conclusions, in addition to suggest a major effect of individual breeders and breed associations‘ practices on inbreeding. Historic and severe bottlenecks reported in some of the traditional large fowl breeds may further explain the greater proportion of long ROHs, since populations that have already experienced a drastic reduction in the effective population size tend to show a slow recovery, despite the potential increase in population size following the bottleneck (Charlesworth 2009). On the other hand, the higher proportion of the genome covered by long ROHs displayed by neo-bantams can be explained by repetitive (sequential) backcrossing pursued for phenotype selection, since strong selection for breed-specific morphological standards or novel phenotypes acts to maintain long homozygous tracts (Purfield et al. 2012). However, the limited number of founders from which neo-bantams originated also supports their ROH content. The ROH profile of traditional breeds significantly differs from that of modern white-egg layers. White-egg layers experienced a strong population bottleneck in the early second half of the 20th century, which makes them interesting models of inbred populations. In this study, the white-egg layers demonstrate the strong influence of management strategy on inbreeding level. In fact, despite the high number of ROHs, the relative low proportion of genome covered by homozygous segments supports effective genetic management, which is meant to pursue intense, directional selection allowing recessive deleterious alleles to be purged with inbreeding. Furthermore, the higher number of long ROHs confirms the closed population history resulting from the absence of genetic exchange with other breeds/lines, resulting in continuous stretches of homozygosity. In commercial lines we also observed a relative small number of short ROHs (Fig. 4a), which may indicate founder effects and distant inbreeding. In fact, it is likely that some relatedness was already present in the founders. However, recombination deriving from directional selection may also have contributed to break down ROHs in short segments.

The analysis presented here confirms the importance of using genotype data to set up structured breeding programmes and inform genetic conservation of traditional breeds of chicken of the Netherlands. The selection and demographic history that we here reconstructed allow us to provide recommendations on how to effectively conserve genetic diversity of traditional breeds. According to our results, we recommend the national gene bank to consider traditional breeds separately. Moreover, to capture the available genetic diversity, a sufficient number of representative individuals within each breed should be sampled to preferably embrace all breed-specific morphological standards. A major drawback of current conservation efforts of traditional Dutch chicken breeds is the lack of inventories of their genetic resources. The present study demonstrates the use of high-density genotype data to investigate diversity in marginalised populations that can be used to guide sampling for in situ and ex situ conservation.

Genotype data can, in part, make up for the lack of traditional sources of information that inform breeding programs, such as pedigree data and phenotype information, It can also provide insight in the origin and consequences of strong demographic discontinuities, such as population bottlenecks and introgression, as in the case of the bantamised breeds. As such, the Dutch chicken breeds are a good model for other marginalised populations, and a good example for how genomic data can guide conservation efforts in populations that have little other information to go from.

We conclude that bantamisation has generated novel genetic diversity. However, this outstanding diversity can only be preserved in the near future by applying structured breeding programmes that are either informed by pedigree data or genomic variation information.

Data archiving

Genotype data available from Dryad: https://doi.org/10.5061/dryad.1d832h3

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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