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## Supplemental Information

### Divergence, Convergence, and the Ancestry of Feral Populations in the Domestic Rock Pigeon

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#### Supplemental Inventory

**Figure S1, related to Figure 1. Coefficients of genetic population membership of 361 individuals representing 70 domestic breeds and 2 free-living populations of pigeon.** Complete genetic structure results for  $K = 2-25$ . A subset of these results are shown in the main text due to space constraints.

**Table S1, related to Figure 1. Summary of populations.** Contains a list of population abbreviations used in the paper and their corresponding full names, and well as the number of individuals genotyped, number of alleles, and heterozygosity statistics for each population.

**Table S2, related to Figure 1. Locus information for 32 microsatellite markers.** Contains a list of names, primer sequences, repeat motifs, number of alleles, heterozygosity statistics, and differentiation statistics for the 32 markers used in this study.

**Table S3, related to Figure 1. Pairwise  $D_{est}$  values for breeds with  $n \geq 3$  individuals.** Genetic differentiation statistics for all breeds and populations in the study using a calculation optimized for smaller sample sizes.

**Table S4, related to Figure 1. Pairwise  $F_{ST}$  values for breeds with  $n \geq 3$  individuals.** Genetic differentiation statistics for all breeds and populations in the study using a standard calculation method.

#### Supplemental Experimental Procedures

Microsatellite identification, sample collection, DNA isolation, PCR and genotyping, data set filtering, linkage disequilibrium tests, genetic structure analysis, phylogenetic tree, and genetic differentiation statistics.

#### Supplemental References

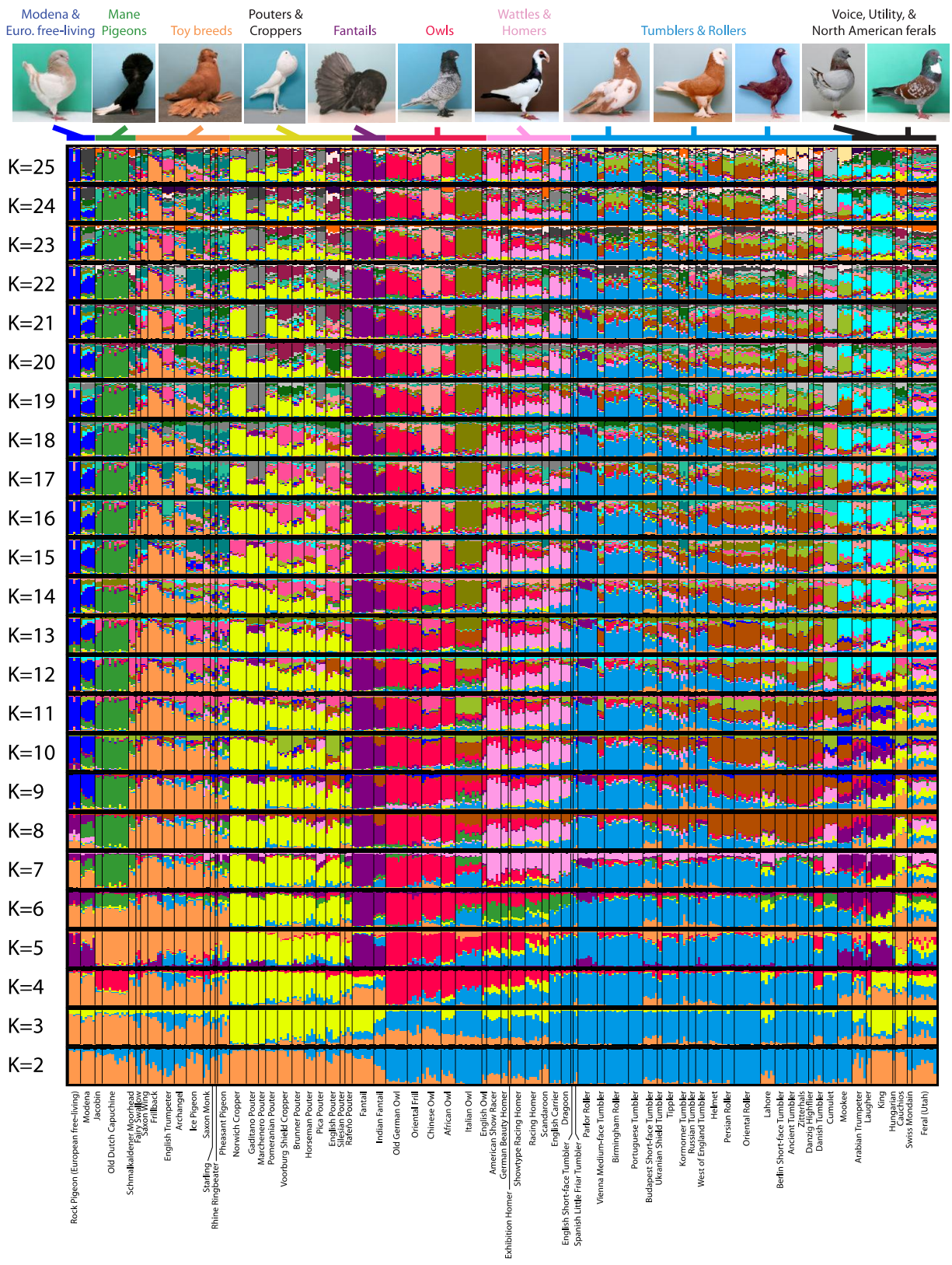


Figure S1.

**Figure S1, Related to Figure 1. Coefficients of Genetic Population Membership of 361 Individuals Representing 70 Domestic Breeds and Two Free-Living Populations of Pigeon**

Results are shown for  $K = 2-25$ . Note that beyond  $K = 9$ , small numbers of breeds (as few as one) from several groups show memberships in new clusters. For example, at  $K = 10$ , three pouter breeds show membership in a new group to the exclusion of other pouters. At  $K = 11$ , the Italian Owl shows membership in a new group to the exclusion of other owls. Breeds pictured (left to right): Modena, Jacobin, English Trumpeter, English Pouter, Fantail, Oriental Frill, Scandaroon, English Short-face Tumber, West of England Tumbler, Zitterhall (Stargard Shaker), Show King, Cauchois. Photos courtesy of Thomas Hellmann and are not to scale.

**Table S1, Related to Figure 1. Summary of Breeds**

<b>Breed/ Population #</b>	<b>Abbreviation</b>	<b>Name</b>	<b>N<sub>Ind</sub></b>	<b>N<sub>A</sub></b>	<b>H<sub>O</sub></b>	<b>H<sub>E</sub></b>
1	AFO	African Owl	6	82	0.410	0.406
2	ANC	Ancient Tumbler	4	73	0.396	0.376
3	ARA	Arabian Trumpeter	6	89	0.357	0.434
4	ARC	Archangel	5	81	0.408	0.398
5	ASR	American Show Racer	6	85	0.402	0.418
6	BIR	Birmingham Roller	10	92	0.351	0.417
7	BST	Berlin Short-face Tumbler	5	79	0.360	0.405
8	BUP	Brunner Pouter	5	84	0.398	0.418
9	BUT	Budapest Short-face Tumbler	6	84	0.379	0.400
10	CAU	Cauchios	5	94	0.426	0.493
11	CHO	Chinese Owl	8	90	0.333	0.391
12	CUM	Cumulet	6	71	0.333	0.337
13	DAG	Dragoon	4	66	0.316	0.332
14	DAH	Danzig Highflier	2	45	0.246	0.168
15	DAT	Danish Tumbler	4	74	0.356	0.392
16	ENC	English Carrier	5	77	0.345	0.350
17	ENO	English Owl	2	62	0.448	0.344
18	ENP	English Pouter	6	51	0.140	0.257
19	ENT	English Trumpeter	5	83	0.414	0.419
20	EST	English Short-face Tumbler	1	36	0.161	0.078
21	EXH	Exhibition Homer	1	39	0.219	0.109
22	FAN	Fantail	9	80	0.321	0.358
23	FAS	Fairy Swallow	2	62	0.311	0.355
24	FER	Feral (Utah)	10	145	0.497	0.573
25	FRL	Frillback	6	93	0.363	0.442
26	GAP	Gaditano Pouter	5	61	0.373	0.303
27	GEB	German Beauty	3	74	0.326	0.378
28	HEL	Helmet	6	83	0.335	0.405
29	HOP	Horseman Pouter	5	89	0.415	0.421
30	HUN	Hungarian	1	41	0.323	0.156
31	ICE	Ice Pigeon	7	107	0.406	0.492
32	INF	Indian Fantail	5	74	0.288	0.378
33	ITO	Italian Owl	11	92	0.372	0.402
34	JAC	Jacobin	3	54	0.250	0.254
35	KIN	King	9	97	0.436	0.442
36	KOT	Kormorner Tumbler	4	71	0.276	0.379
37	LAH	Lahore	6	93	0.320	0.426
38	LAU	Laugher	2	52	0.379	0.246
39	MAP	Marchenero Pouter	3	52	0.181	0.234

40	MOD	Modena	6	81	0.285	0.411
41	MOO	Mookee	6	89	0.407	0.442
42	NOC	Norwich Cropper	7	76	0.296	0.357
43	ODC	Old Dutch Capuchine	11	94	0.336	0.423
44	OGO	Old German Owl	9	88	0.353	0.383
45	ORF	Oriental Frill	6	84	0.303	0.388
46	ORR	Oriental Roller	11	96	0.334	0.438
47	PAT	Parlor Roller	8	76	0.329	0.358
48	PER	Persian Roller	5	61	0.257	0.312
49	PHP	Pheasant Pigeon	5	82	0.302	0.425
50	PIC	Pica Pouter	4	77	0.424	0.403
51	POM	Pomeranian Pouter	5	82	0.396	0.434
52	POT	Portuguese Tumbler	6	80	0.392	0.368
53	RAF	Rafeño Pouter	3	67	0.368	0.345
54	RAH	Racing Homer	7	105	0.493	0.485
55	RHR	Rhine Ringbeater	1	44	0.467	0.219
56	ROD	Rock Pigeon (European free-living)	5	74	0.640	0.416
57	RUS	Russian Tumbler	3	82	0.483	0.450
58	SAM	Saxon Monk	3	72	0.419	0.365
59	SAW	Saxon Wing	3	72	0.355	0.393
60	SCA	Scandaroon	3	56	0.279	0.260
61	SCM	Schmalkaldener Moorhead	3	60	0.241	0.296
62	SHH	Showtype Racing Homer	6	100	0.492	0.502
63	SIP	Silesian Pouter	2	57	0.452	0.309
64	SLF	Spanish Little Friar Tumbler	2	60	0.350	0.328
65	STA	Starling	2	74	0.516	0.441
66	SWM	Swiss Mondain	2	56	0.459	0.289
67	TIP	Tippler	7	102	0.436	0.466
68	UKS	Ukranian Shield	2	47	0.250	0.199
69	VIE	Vienna Medium-face Tumbler	3	58	0.286	0.264
70	VOS	Voorburg Shield Cropper	6	75	0.311	0.382
71	WOE	West of England Tumbler	5	86	0.326	0.395
72	ZIT	Zitterhals (Stargard Shaker)	5	64	0.208	0.315
<b>Mean</b>			5.0	75.5	0.354	0.365
<b>Standard deviation</b>			2.5	18.6	0.087	0.091

**N**<sub>ind</sub>, number of individuals; **N**<sub>A</sub>, total number of alleles; **H**<sub>o</sub>, observed heterozygosity, **H**<sub>E</sub>, expected heterozygosity.

**Table S2, Related to Figure 1. Locus Information for 32 Microsatellite Markers**

Loc	Marker	Fwd primer	Rev primer	Repeat motif	N <sub>A</sub>	H <sub>O</sub>	H <sub>E</sub>	D <sub>est</sub>	Source
L01	ClijT17	AGTTTTAATGAAGGCACCTCT	GTTTGATGGAGTTGCTATTTTGTCT	GGAT	10	0.441	0.782	0.545	Traxler et al. (1999)
L02	ClijD32	GAGCCATTTTCAGTGAGTGACA	GTTTGCAGGAGCGTGTAGAGAAGT	GT	12	0.553	0.858	0.660	Traxler et al. (1999)
L03	ClijD01	GATTTCTCAAGCTGTAGGACT	GTTTGATTTGGTTGGGCCATC	CA	25	0.607	0.877	0.644	Traxler et al. (1999)
L04	ClijD17	TCTTACACACTCTCGACAAG	GTTTCCACCCAAATGAGCAAG	CA	10	0.507	0.737	0.432	Traxler et al. (1999)
L05	UU-ClI10	CCCTCCAATTTGGCTAAACA	GCAGAAAGCAAGGAAACACC	GT	6	0.427	0.690	0.409	This study
L06	UU-ClI11	CCTTCAAAGGTCACCTAGTCC	TTCCTGAACACCTCAGTAAAAGG	CAAA	7	0.258	0.336	0.083	This study
L07	UU-ClI12	CGCCAGACTGTATTGTGAGC	AGCATGGCTGTTCTTTGAGG	CA	11	0.513	0.767	0.467	This study
L08	UU-ClI13	TGTGGAACCACACAATCAGG	CTTGGGATCAATTTGAAAAATAC	GT	14	0.457	0.741	0.408	This study
L09	UU-ClI16	CGAGTGGACTCAGCCTTAGC	TGTGCACTGCTTTATGACAGG	CA	4	0.386	0.598	0.299	This study
L10	ClijT02	AGTTTTAATGAAGGCACCTCT	TGTAGCATGTCAGAAATTGG	CATC	12	0.501	0.686	0.322	Genbank G73189.1 (Achmann et al., unpublished)
L11	UU-ClI03	CAAACAGAAAACCAACCAACC	CTGGGTCAGTGTGTTTGAAT	CA	4	0.070	0.111	0.027	This study
L12	UU-ClI04	TCCCAGAAATCTTCGTAACCTGA	ATTCCAGGTGACAAAGAACCAT	CA	5	0.223	0.380	0.114	This study
L13	UU-ClI09	CCAAATCACATCTGTCAAGTGC	AGCAGAGGTGCTGTTTGAGG	GT	7	0.099	0.130	0.046	This study
L14	UU-ClI14	CAGAACGTTTTGTTCTGTTTGG	TCTTGCTGCAGTCTTCATCC	GT	20	0.509	0.816	0.561	This study
L15	UU-ClI15	AGACGCCTTCAGGTTAGAGC	TGAGGGTGACAGAACACTGG	CA	7	0.191	0.356	0.179	This study
L16	UU-ClI17	TTGGGATCCTGACATTTATCC	TAGGTCCTGGATGGAACAGC	GT	11	0.249	0.753	0.576	This study
L17	UU-ClI05	TCCATGCGTCTGTCTGTCC	AGCTGTTGATTGCAGACTGG	GT	12	0.295	0.646	0.398	This study
L18	UU-ClI06	TTTGAAAAACATGGATTGTGC	AATTTGCAGAGGGTGAGTGG	CA	5	0.351	0.494	0.190	This study
L19	UU-ClI07	GCTGCCTGTTACTACCTGAGC	CTGGCCATGAAATGAACTCC	GT	10	0.276	0.448	0.191	This study
L20	UU-ClI08	GGCAGAATGAGCTATGTGACC	CAGCTCAGGGTAATATCAAACCG	CA	9	0.418	0.679	0.353	This study
L21	ClijT24	CCAGCCTAAGTGAAACTGTC	CCTTCCAACCCACATTATT	TGGA	9	0.601	0.812	0.471	Genbank G73196 (Achmann et al., unpublished)
L22	ClijT47	ATGTGTGTTTGTGCATGAAG	ATGAAAGCCTGTTAGTGGA	TATC	9	0.457	0.658	0.356	Genbank G73190.1 (Achmann et al., unpublished)
L23	ClijD28	AAACCATCACTTATGCCAAC	ACTGATTCTGGTACTCTGG	CA	3	0.044	0.129	0.092	Genbank G73192.1 (Achmann et al., unpublished)
L24	ClijD35	GGGAGCTTAAGGGATTATTG	ATTCCTTGCATGCCTACTTA	GT	7	0.262	0.413	0.136	Genbank G73199.1 (Achmann et al., unpublished)
L25	ClijD16	GCAGTGATAAAGTTCTGGAACA	GTTTGCCTCACCGTGACATCA	GT	21	0.472	0.730	0.398	Traxler et al. (1999)

L26	CljD19	CCGTTTCTTCTAATGCAC	GTTTGGATTTCTGGGAGTGTATG	CA	9	0.099	0.653	0.401	Traxler et al. (1999)
L27	PG4	CCCATCTCCTGCCTGATGC	CACAGCAGGATGCTGCCTGC	TCCA	7	0.466	0.730	0.444	Lee et al. (2007)
L28	PG5	GTTCTTGGTGTTCATGGATGC	AGTTACGAAATGATTGCCAGAAG	TTTG	3	0.139	0.234	0.083	Lee et al. (2007)
L29	UU-ClI02	TGGGCAAGGTACACTTTTAGGT	CTTTATGCTCCCCCTTGAGAT	CA	9	0.450	0.746	0.505	This study
L30	PG7	CATTGGTCAGGAGGAGGTGGTGGG	TCTGCCACTCACTCGCCCTC	TTG	6	0.420	0.703	0.432	Lee et al. (2007)
L31	UU-ClI01	TCCTTACTGCGTTTCTCTCCTC	AAAGAGAGGGCACTGATTTGAA	CA	4	0.370	0.555	0.262	This study
L32	CljD11	CCAATCCCAAAGAGGATTAT	ACTGTCCTATGGCTGAAGTG	CA	12	0.485	0.783	0.434	Genbank G73194.1 (Achmann et al., unpublished)
<b>Mean</b>					<b>9.4</b>	<b>0.362</b>	<b>0.595</b>	<b>0.342</b>	
<b>SD</b>					<b>5.1</b>	<b>0.159</b>	<b>0.224</b>	<b>0.177</b>	

$N_A$ , number of alleles per locus;  $H_O$ , observed heterozygosity;  $H_E$ , expected heterozygosity;  $D_{est}$ , estimator of actual differentiation [1].







## **Supplemental Experimental Procedures**

### **Microsatellite Identification**

Seventeen new microsatellite loci were identified by enriching genomic DNA for (CA)<sub>n</sub> dinucleotide repeats [2]. We purified 10 µg DNA from feral pigeon muscle tissue and digested with MboI. We enriched for repeats in the digest fragments using streptavidin beads and a biotinylated (GT)<sub>15</sub> probe [2], and the recovered fragments were cloned using a TOPO TA Cloning Kit (Invitrogen) and sequenced. Primers flanking microsatellites in the resulting sequences were designed using Primer 3 [3]. The new markers were deposited in Genbank, accession numbers GF111523 – GF111539. Nine additional published microsatellite markers [4, 5] and six unpublished markers deposited in Genbank (accessions in Table S2) were also included, for a total of 32 markers. An M13 sequence tag (5' CAC GAC GTT GTA AAA CGA C 3') was added to the 5' end of all forward primers to allow annealing of a fluorescently labeled oligonucleotide during PCR reactions [6, 7].

### **Sample Collection**

Blood samples were collected at local pigeon shows including the Utah Pigeon Club Premier Show (2009), the National Pigeon Association Grand National Pigeon Show (Salt Lake City, 2010), and at the homes of local pigeon fanciers. Additionally, breeders in the USA and elsewhere were contacted using online databases of pigeon organizations and submitted feather samples. Breeders interested in submitting samples were sent feather collection kits and detailed instructions, and samples were returned to us by mail. To increase the geographic scope of our sample, additional feather samples were collected in person at the Bund Deutscher Rasseflügelzüchter annual show (Dortmund, Germany) in 2009. Collection protocols were approved by the University of Utah Institutional Animal Care and Use Committee, protocol 09-04015, and importation of samples from outside the USA was approved under USDA APHIS permit 110106 to MDS.

### **DNA Isolation**

Blood and feather samples from 735 individuals were selected for DNA extraction based on breed and geographical origin. DNA extraction from feathers was carried out using methods described by Bayer de Volo et al. [8]. This protocol was optimized for higher DNA purity with the following modification: after the addition of ammonium acetate and removal of supernatant, two additional spins were performed to remove additional keratin and protein. DNA extractions using blood were performed using 10 µL of blood and either standard phenol-chloroform methods or a DNeasy Blood and Tissue kit (Qiagen).

### **PCR and Genotyping**

PCR reactions contained 0.01 µM forward primer with an M13 tag on the 5' end, 0.4 µM each of reverse primer and M13 forward primer with a fluorescent label (FAM, VIC, NED, or PET) on the 5' end, 0.25 U Taq DNA polymerase, and 10 ng genomic DNA in a final volume of 10 µL. Thermal cycling was performed as described by Schuelke et al. [7] and Protas et al. [6]. PCR products were analyzed on an ABI 3100 and allele sizes were determined using GeneMapper v3.7 (Applied Biosystems) using the allele binning function. Each genotype call was also checked manually for accuracy.

To test for sex linkage, 478 samples with sex information were used in a chi-squared test to identify markers with differential overrepresentation of alleles between males and females. Although one marker, CliμD35, showed a statistically significant difference between males and females ( $p = 0.02$  after Bonferroni correction) it is probably not located in the sex-determining region of the genome. Only 3 of the 7 alleles exhibit this sex bias and both males and females are heterozygous at this locus ( $H_{o(\text{males})}=0.182$ ,  $H_{o(\text{females})}=0.289$ ,  $H_{o(\text{all birds})}=0.236$ ).

### **Data Set Filtering**

We excluded individuals with missing genotypes at more than 12 markers, resulting in the retention of 581 of the 735 individuals. We also excluded multiple, related birds of the same breed from the same breeder to avoid overrepresentation of close relatives. Pedigree information was obtained directly from breeders either in person at shows, by phone, or by email. Multiple birds from the same breeder were excluded from the data set if: (1) they were confirmed siblings or parent-offspring pairs, (2) breeders could not positively rule out that birds were siblings or parent-offspring pairs, or (3) we could not contact breeders to establish relationships among their birds. Nearly all individuals in the data set are unrelated by grandparent. The only exceptions are confirmed first cousins in the following four breeds: Marchenero Pouter (2 individuals are cousins), Rafeño Pouter (3), Cumulet (2), and Spanish Little Friar Tumbler (2). The minimum allelic difference between cousins within these breeds is 26%. These samples were included in the final data set because seventeen other pairs of birds in the final data set have <26% allelic differences, including some pairwise comparisons between birds of different breeds. These filters resulted in a final data set of 361 birds from 70 domestic breeds and 2 free-living populations (Salt Lake City, UT, and Isle of Skye, Scotland), with 90.7% of genotypes represented and a mean sample size of 5.0 individuals per breed.

### **Linkage Disequilibrium Tests**

We used Arlequin v3.11 [9] to test for pairwise linkage disequilibrium (LD) between markers within breeds and the two free-living populations (number of permutations = 1000, number of initial conditions = 2). A mean of 8.2% of all within-breed pairwise comparisons (2914 of 35,712 overall) showed evidence of LD, but patterns of LD were inconsistent among breeds and were likely artifacts of small sample sizes and/or genetic structure in each breed. No pair of markers showed evidence of LD across all breeds. We also used the web interface of GENEPOP 4.0.10 [10] to test for LD between pairs of markers across *all* breeds simultaneously, which should circumvent LD due to genetic structure within breeds and potentially reveal real genomic linkage among markers. Using this approach, LD was not detected for any locus pair across all breeds. A contingency table could not be constructed for the CliμD28-PG5 pair in the all-breed analysis due to missing data, but these two markers were not in LD in any within-breed pairwise comparison.

### **Genetic Structure Analysis**

We used STRUCTURE v.2.3.2 software [11] to determine genetic clusters in the entire set of 361 birds, and in a subset of breeds for comparison with Darwin's morphological classification. Both analyses used a 100,000-run burnin followed by 100,000 repetitions, and we used pilot runs to ensure that the burnin period was adequate to achieve stability of  $F_{ST}$ , Alpha, and  $r$ . Each value of  $K$  was run 5 times using the admixture model and breed/population assignments as priors (LOCPRIOR model). Default settings were used for all other parameters. The Darwin data set

was run from  $K = 1-15$  (one more than the number of breeds), and the complete data set was run from  $K = 1-25$ . The number of  $K$  values simulated on the full data set was fewer than the number of breeds because our objective was to determine clusters of major breed groups, rather than to examine the structure of individual breeds. We used the web interface of STRUCTURE HARVESTER v0.6.8 [12] to generate concatenated individual and population Q-matrices from the five runs, and these files were used to align the runs using CLUMPP [13] (Greedy algorithm for  $K = 1-6$ , LargeKGreedy algorithm from  $K = 7-25$ , with 30,000 random input orders for both algorithms). Results of the five averaged runs for each value of  $K$  were plotted using DISTRUCT [14].

Determining the “true” value of  $K$  is difficult in STRUCTURE analyses, and many studies rely on biological relevance of the results to determine an appropriate value. Based on the expected number of breed groups,  $K = 9$  is appropriate for our data set. We also used the Evanno method [15] for determining  $K$  as implemented by STRUCTURE HARVESTER [12]. This method determines the most likely value of  $K$  using the rate of change between the log probabilities of the data between successive  $K$  values. STRUCTURE HARVESTER determined that  $K = 2$  is most likely for the complete data set and for the 40 breeds and one free-living population with >50% membership at  $K = 9$  (used to construct the tree in Fig. 2; see below). For the more limited Darwin data set in Fig. 3, we examined genetic structure at  $K = 2$ ,  $K = 3$  (the value suggested by the Evanno method in STRUCTURE HARVESTER [12]),  $K = 4$  (the same number as Darwin’s morphological groups) and  $K = 5$ .

### Phylogenetic Tree

Using STRUCTURE, we first identified all breeds that have >50% membership in a given ancestral cluster at  $K = 9$ . Our goal was to determine relationships among major breed groups, so using a filtered data set could help reduce noise from breeds with complex hybrid ancestry spread across multiple genetic clusters. Individuals from this reduced data set were then grouped into their corresponding breeds and allele frequencies were calculated for each marker. Median allele values were filled in for markers without genotypes for the following breeds and markers (in parentheses): DAT (PG5), ENP (UU-Cli05, UU-Cli06, UU-Cli13, UU-Cli14, UU-Cli15), EST (UU-Cli01), HUN (Cli $\mu$ D19), JAC (Cli $\mu$ T24), LAU (PG5), PIC (PG5), RHR (Cli $\mu$ T17, Cli $\mu$ D28), and VIE (Cli $\mu$ D19). The added allele values account for less than 0.4% of genotypes in the data set and allow the inclusion of these breeds in the calculations of expected heterozygosity, genetic distance, and differentiation statistics. Pairwise Cavalli-Sforza chord genetic distances were calculated among all breeds using the *gendist* program in PHYLIP [16]. A neighbor-joining tree was then constructed using the *neighbor* program in PHYLIP. To assess the confidence of the tree, we generated a 1000-bootstrap data set and constructed a consensus tree using the *consense* program in PHYLIP. A tree graphic was generated using FigTree [17].

### Genetic Differentiation Statistics

Estimated differentiation parameters for markers and populations were calculated using the SMOGD web interface [18]. The  $D_{est}$  statistic is especially well suited for genetic differentiation analysis without very large sample sizes in each population [1]. Nei’s  $F_{ST}$  and heterozygosity statistics were calculated using the *adegenet* module [19] in R [20].

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