

# The role of local adaptation in sustainable village chicken production

## Supporting Information

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## Supplementary Note 1. Parasite ecology

Parasites were highly aggregated (i.e. most chickens had few parasites, but a few had many), but the distributions differed significantly between woreda (Kolmogorov-Smirnoff test; ascarids:  $D = 0.105$ ,  $P < 0.01$ ; Lice:  $D = 0.201$ ,  $P \leq 0.001$ ). The prevalence of ascarid infection in Horro was 21.8% (95% CI 18.7 – 25.3%) compared to 11.3% (95% CI 9.0 – 14.2%) in Jarso (Chi Square statistic 8.6,  $P = 0.003$ ). Similarly, lice infection prevalence was much higher in Horro (43%; 95% CI 39.5 – 47.2%) compared to Jarso (23%; 95% CI 20.1 – 26.7%; Chi Square statistic 14.2,  $P < 0.001$ ).-Both the corrected moment estimate of  $k^1$  (Horro = 0.21, Jarso = 0.05) and the index of discrepancy<sup>2</sup> (Horro = 0.81, Jarso = 0.91) demonstrated that lice showed greater aggregation in the Jarso population compared to Horro. Aggregation of ascarid populations was similar between the two woredas (index of discrepancy Horro = 0.950, Jarso = 0.951). These variations in parasite aggregation implies regional differences in impact of parasitism on host population dynamics<sup>3</sup>.

## **Supplementary Note 2. GWAS for parasitic resistance**

Supporting the finding from the multidimensional scaling and the selective sweep analysis, GWAS identified distinct genomic regions controlling parasitic resistance in Horro and Jarso chickens. The Manhattan and Q-Q plots displaying the GWAS results are shown in Fig. 4 and Supplementary Fig. 3, respectively, while details of the significant SNP markers are shown in Supplementary Table 3.

Interestingly, the candidate region on chromosome 1, where 12 SNPs were significantly associated with resistance to ascarid parasitism in Horro chickens (Supplementary Table 3), was within a region that we identified as a site of a selective sweep. The positive selection signal in that region harbours the mastermind-like protein 2 (MAML2) gene and *gga-mir-6570*. The *gga-mir-6570* is a micro RNA with unknown function. The MAML2 gene is involved in the amplification of genes related to transcription factors (HES1) that can interact with cytokine signals and regulate the immune response of intestinal epithelial cells, playing an important role in host resistance to gastrointestinal parasites as has been shown in other species<sup>4,5</sup>. However, further studies are required to determine if MAML2 is the actual causative gene for ascarids resistance in Horro chickens. Collectively, the significant SNP markers in Horro chickens explained a total of 27% and 18% of the phenotypic variance for resistance to ascarids and lice infection, respectively, and 16% and 14%, in Jarso.

### **Supplementary Note 3. Selective sweep analysis**

Supporting the finding from multidimensional scaling that the two populations (Horro and Jarso) were genetically distinct (Supplementary Fig. 4), selective sweep analysis identified considerable variation in the genetic landscape of the two chicken ecotypes. Details of the selective sweep analyses using SweeD and the iHS test results are shown in Supplementary Fig. 2 and Supplementary Table 5. The results of the selective sweep analysis are consistent with known bird characteristics. At the rose comb chromosomal inversion region on Chr7 (57), preferred in Horro, iHS analysis revealed a stronger peak in Horro (Supplementary Fig. 2C).

Similarly, GWAS results for rose versus simple comb (Supplementary Fig. 2C) revealed multiple SNP markers on chromosome 7 overlapping with the region where the selection sweep and the rose comb chromosomal inversion were previously identified (Supplementary Table 2).

## Supplementary Table 1. Characteristics of the two woredas (Horro and Jarso) included in this study.

Data sources provided as footnotes.

	<b>Horro</b>	<b>Jarso</b>
Population estimate (2011)*	87,505	134,426
Population density per sq. km	Moderate <sup>±</sup>	Moderately dense <sup>¶</sup>
Predominant faiths*	Christianity (Ethiopian Orthodox or Protestant Christian)	Islam
Topography	Undulating, good vegetation cover with varied grasslands <sup>±</sup>	Steeply undulating, bushes, shrubs and forest vegetation <sup>¶</sup>
Annual rainfall	1200-1800 mm <sup>±</sup>	700-800 mm
Main rainy season	Early May to late September <sup>±</sup>	July to September <sup>¶</sup>
Agriculture	High potential, producing an annual food surplus <sup>±</sup>	Annual food deficit <sup>¶</sup>
Main agricultural products	Teff, niger seed (as a cash crop), cattle <sup>±</sup>	Khat, vegetables, sorghum and maize <sup>¶</sup>

\*Unpublished data from the local Woreda Offices of Agriculture

<sup>±</sup> Food Economy Group (2009) <sup>6</sup>

<sup>¶</sup> Food Economy Group (2008) <sup>7</sup>

**Supplementary Table 2. The most significant SNPs associated with switch from single to rose comb mutation in Horro and Jarso chickens in Ethiopia from genome-wide association study.**

Associated genes were scanned within a window of 50 kb upstream and 50 kb downstream of the SNP showing statistically high significant association.

Chromosome	Position	P value	Q value	Associated genes
7	21583378	7.27E-18	2.33E-12	STK11IP, OBSL1, INHA, CHPF, ENSGALG00000011233, TMEM198, ASIC4, GMPPA, ENSGALG00000011252, ENSGALG00000026721
7	20413623	8.60E-18	2.33E-12	KCNH7
7	21754266	1.61E-17	2.90E-12	GLB1L, STK16, ANKZF1, ATG9A, ABCB6, ZFAND2B, ENSGALG00000000433, IHH, FAM134A, SLC23A3, NHEJ1
7	16110177	4.10E-17	4.49E-12	ATF2, ATP5G3
7	21383967	4.71E-17	4.49E-12	PLA2R1, LY75, ENSGALG00000027744, ENSGALG00000011172
7	18512158	5.97E-17	4.49E-12	CERS6
7	17730249	8.42E-17	4.49E-12	ENSGALG00000021856, GAD67, SP5, MYO3B
7	21464697	9.44E-17	4.49E-12	LY75, ENSGALG00000011172, SLC4A3
7	15743377	9.46E-17	4.49E-12	MTX2, HOXD4, HOXD8, ENSGALG00000023420, ggamir-6624, gga-mir-1713, ggamir-10b
7	21260451	9.46E-17	4.49E-12	RBMS1, ITGB6

### Supplementary Table 3. Single Nucleotide Polymorphism (SNP) markers associated with *ascarids* and lice resistance in Horro and Jarso chickens.

Data were collected from 760 indigenous village chickens (384 from Horro and 376 from Jarso) during 4 cross-sectional surveys in May/June and October/November of 2011 and 2012.

Population-Trait	SNP	Location Chr (bp)	GWAS P-value	Additive Effect (P-value)	Dominance Effect (P-value)	Phenotypic variance Explained (%)
Horro-Ascarids	<b>Affx-51395569</b>	<b>4(26433072)</b>	<b>9.86E-10</b>	<b>0.69(3E-08)</b>	<b>0.017(1E01)</b>	<b>6.35</b>
	<b>Affx-50290757</b>	<b>1(184473669)</b>	<b>9.16E-08</b>	<b>0.43(5E-04)</b>	<b>-0.11(2E-01)</b>	<b>5.76</b>
	<b>Affx-50290761</b>	<b>1(84476389)</b>	<b>9.16E-08</b>	<b>0.42(5E-04)</b>	<b>-0.11(2E-01)</b>	<b>5.7</b>
	Affx-50602732	1(2970697)	1.41E-07	0.24(5E-02)	0.029(3E-01)	2.6
	Affx-50291280	1(184716656)	1.99E-07	0.42(5E-04)	-0.13(2E-01)	5.34
	Affx-50290798	1(184494014)	4.51E-07	0.42(5E-04)	-0.15(2E-01)	4.97
	Affx-50290806	1(184498373)	4.51E-07	0.42(5E-04)	-0.15(2E-01)	4.97
	Affx-50290833	1(184512886)	4.51E-07	0.42(5E-04)	-0.15(2E-01)	4.97
	Affx-50290920	1(184557389)	4.51E-07	0.42(5E-04)	-0.15(2E-01)	4.97
	Affx-50290878	1(184536319)	4.75E-07	0.42(5E-04)	-0.15(2E-01)	4.97
	Affx-51803998	8(28420210)	5.85E-07	0.40(1E-08)	-0.29(2E-04)	7.51
	Affx-51886011	Z(17454128)	6.09E-07	0.32(5E-03)	-0.17(2E-01)	4.88
	Affx-50437894	1(86530715)	6.90E-07	0.25(2E-07)	-0.16(7E-02)	4.63
	Affx-50290861	1(184526541)	9.60E-07	0.42(5E-04)	-0.17(2E-01)	4.68
	Affx-51474491	4(72987651)	9.93E-07	0.14(1E-05)	-0.03(3E-01)	2.52
	Affx-50297800	1(187630516)	1.02E-06	-0.06(4E-01)	0.36(4E-02)	6.34
	Affx-51499617	4(86883228)	1.05E-06	0.33(2E-02)	-0.11(3E-01)	2.71
	Affx-50715211	17(1678508)	1.07E-06	0.15(1E-07)	-0.10(1E-02)	1.65
	Affx-50293847	1(185855549)	1.12E-06	0.42(5E-04)	-0.16(2E-01)	4.52
	Affx-51742393	7(32782523)	1.27E-06	0.43(4E-08)	-0.31(9E-04)	2.55
Affx-51439929	4(53159983)	1.28E-06	0.12(1E-01)	0.21(7E-02)	1.91	
Affx-51423075	4(43062611)	1.90E-06	0.28(1E-03)	-0.01(1E-02)	2.63	
Affx-50715209	17(1677754)	1.93E-06	0.16(2E-07)	-0.10(1E-02)	2.37	
Affx-50602707	12(9129213)	2.01E-06	0.23(7E-02)	-0.01(3E-01)	2.57	
Horro-Lice	<b>Affx-50247966</b>	<b>1(163438917)</b>	<b>8.75E-09</b>	<b>0.14(0.02)</b>	<b>-0.08(0.08)</b>	<b>4.86</b>
	<b>Affx-51920244</b>	<b>Z(54899528)</b>	<b>1.10E-07</b>	<b>0.25(4.44E-07)</b>	<b>NA</b>	<b>4.65</b>
	Affx-51396079	4(26712172)	1.65E-07	0.14(0.12)	0.12(0.31)	0.45
	Affx-51027527	20(3275819)	6.34E-06	0.50(0.01)	-0.25(0.17)	5.91
Jarso-Ascarids	<b>Affx-50191853</b>	<b>1(131923500)</b>	<b>1.13E-07</b>	<b>0.34(3E-04)</b>	<b>-0.16(9E-02)</b>	<b>6.38</b>
	<b>Affx-50191852</b>	<b>1(12934262)</b>	<b>1.18E-07</b>	<b>0.36(1E-11)</b>	<b>-1.0(5E-06)</b>	<b>5.45</b>
	Affx-51821322	8(9142732)	1.93E-07	0.36(1E-11)	-0.32(7E-07)	2.00
	Affx-50202170	1(131425)	3.84E-07	0.36(1E-11)	-0.32(6E-07)	1.84
	Affx-51292557	3(59183002)	7.43E-07	0.34(2E-10)	-0.29(1E-05)	1.88
	Affx-50208640	1(140680362)	1.45E-06	0.20(8E-02)	-0.02(3E-01)	1.64
Jarso-Lice	Affx-51792475	8(23623021)	1.85E-06	0.31(3E-06)	-0.20(7E-02)	4.38
	Affx-50177942	1(124154574)	1.90E-07	0.12(0.23)	0.28(0.04)	1.28
	Affx-50180709	1(125664425)	2.84E-07	0.30(4.E-05)	-0.12(0.27)	2.51
	Affx-50186093	1(128920164)	6.90E-07	0.61(3E-04)	-0.33(7E-02)	2.12
	Affx-50662749	14(4250721)	7.31E-07	0.33(7E-03)	-0.08(3E-01)	2.11
	Affx-51237344	3(29295130)	1.05E-06	0.36(7E-11)	-0.14(0.27)	1.55
	Affx-51445157	4(56180250)	1.20E-06	0.13(1E-02)	0.10(1E-01)	0.43
	Affx-51758008	7(6562703)	1.46E-06	0.52(1E-07)	-0.38(6E-04)	1.65

SNPs highlighted in bold were significant at genome-wide threshold; SNPs not in bold were significant at suggestive genome-wide threshold; phenotypic variance ~% proportion of phenotypic variance explained by SNP;

NA Not applicable

## Supplementary Table 4. Flock sizes

Data collected from 320 households per region recruited over two consecutive years as part of the chicken genomics and health survey. Participants were required to own at least two adult birds.

	Horro			Jarso			Regional differences (P value <sup>¶</sup> )	
	May/Jun	Oct/Nov	P value <sup>¶</sup>	May/Jun	Oct/Nov	P value <sup>¶</sup>	May/Jun	Oct/Nov
<b>Hens</b>	2 [2-3]	2 [2 -3]		3 [2 -4]	3 [2 -4]		0.001	<0.001
<b>Cocks</b>	1 [0.5-1]	1 [1-2]		1 [0 - 1]	1 [0 - 1]		0.224	0.03
<b>Young stock</b>	7 [2-12]	2 [0-6]		0 [0-2]	1 [0 - 4]		<0.001	0.09
<b>Total flock size</b>	11[5.5-16.5]	6 [3-10]	<0.01	4 [3-6.5]	6 [3-8]	<0.01	<0.001	0.8

Bird numbers given as median [inter-quartile range]

<sup>¶</sup>Mann-Whitney-Wilcoxon test



**Supplementary Table 5. The most significant ten SNPs showing a recent positive selection in Horro chickens using integrated haplotype score (iHS) analysis.**

Associated genes were mapped within a window of 50 kb upstream and 50 kb downstream of the SNPs showing statistically high significant association ( $P \leq 0.0001$ ).

Chromosome	Position	iHS score	$-\log_{10}(P)$	Associated genes
7	19991308	5.443488	7.281936587	
7	19779756	5.314097	6.969855189	GRB14
7	21583378	5.220369	6.748199091	SLC4A3, STK11IP, INHA, OBSL1, ENSGALG00000011233, TMEM198, CHPF, ASIC4
7	16311522	5.17897	6.651472284	CHN1, CHRNA1, WIPF1
6	21637946	4.989241	6.217405545	CPN1, DNMBP, ABCC2, CUTC, COX15, ATP6V0E2, ENTPD7, SLC25A28, GOT1, ENSGALG00000020753
7	15711977	4.759045	5.711055654	MTX2
7	19801640	-4.75002	5.691666197	
7	19894616	-4.71645	5.619788195	
7	20286636	4.707024	5.599704148	KCNH7
7	17038749	4.649004	5.476849601	

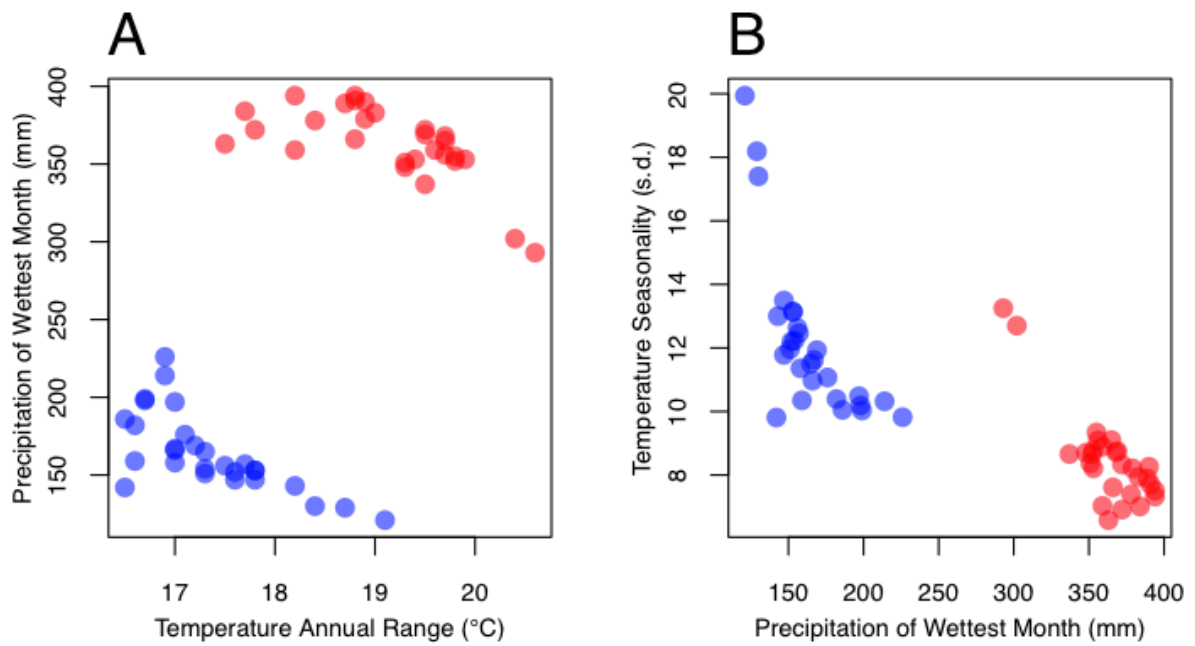
**Supplementary Table 6. Frequency count of plumage colour variants in hens in the two study areas.**

Plumage colour	Horro N (%)	Jarso N (%)	Total N (%)	$\chi^2$	P Value
Brown	139 (62.05)	133 (57.33)	272 (59.65)	0.1866	0.7
Wheaten	35 (15.63)	48 (20.69)	83 (18.20)	0.7049	0.4
Black	9 (4.02)	34 (14.66)	43 (9.43)	6.0605	0.01
Red	26 (11.61)	7 (3.02)	33 (7.24)	5.0436	0.02
White	15 (6.70)	9 (3.88)	24 (5.26)	0.7516	0.4
Lavender	0 (0.00)	1 (0.43)	1 (0.22)	ND	

**Supplementary Table 7. Ownership of adult chickens and usage of chickens (within past 12 months) in the two study regions, Horro and Jarso, Ethiopia, for different categories of household income.**

Data from 200 households in Horro and Jarso woreda in Ethiopia (total n = 400).

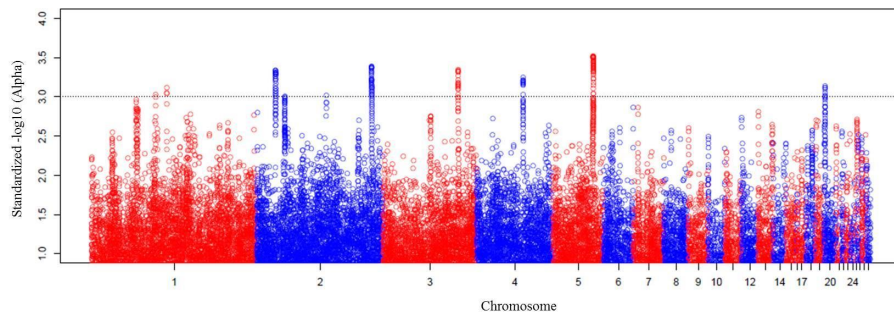
Region	Category	Household income	n	Minimum	1st Quartile	Median	Mean	3rd Quartile	Maximum
Horro	Owned	<2500	30	0.00	1.00	2.00	2.60	3.00	8.00
		2500-5000	39	1.00	2.00	2.00	3.15	3.00	10.00
		5001-7500	37	0.00	2.00	3.00	3.00	4.00	7.00
		7501-10000	35	1.00	3.00	4.00	3.83	5.00	8.00
		10001-15000	32	1.00	2.00	4.00	3.97	5.00	8.00
		>15000	27	1.00	3.00	4.00	4.52	6.00	14.00
	Eaten	<2500	30	0.00	0.00	1.00	1.07	2.00	4.00
		2500-5000	39	0.00	0.00	1.00	1.44	2.00	6.00
		5001-7500	37	0.00	0.00	1.00	4.38	2.00	110.00
		7501-10000	35	0.00	0.25	2.00	2.09	2.00	12.00
		10001-15000	32	0.00	0.75	2.00	2.69	4.00	15.00
		>15000	27	0.00	0.00	2.00	3.07	0.50	26.00
	Sold	<2500	30	0.00	0.00	0.00	1.60	0.50	0.00
		2500-5000	39	0.00	0.00	1.00	2.08	3.00	10.00
		5001-7500	37	0.00	0.00	1.00	1.43	2.00	12.00
		7501-10000	35	0.00	0.00	1.00	2.86	5.00	12.00
		10001-15000	32	0.00	0.00	3.00	4.06	5.25	20.00
		>15000	27	0.00	0.00	4.00	3.85	6.00	13.00
Jarso	Owned	<2500	40	0.00	1.00	2.50	2.80	4.00	9.00
		2500-5000	41	0.00	2.00	2.00	2.90	4.00	9.00
		5001-7500	38	0.00	2.00	3.50	4.21	5.00	17.00
		7501-10000	25	1.00	3.00	4.00	3.80	4.00	9.00
		10001-15000	24	1.00	2.75	4.00	5.17	6.00	22.00
		>15000	30	1.00	3.00	4.00	4.43	5.00	19.00
	Eaten	<2500	40	0.00	0.00	0.00	0.08	0.00	3.00
		2500-5000	41	0.00	0.00	0.00	1.07	0.00	25.00
		5001-7500	38	0.00	0.00	0.00	0.29	0.00	2.00
		7501-10000	25	0.00	0.00	0.00	1.16	0.00	25.00
		10001-15000	24	0.00	0.00	0.00	0.75	0.00	8.00
		>15000	30	0.00	0.00	0.00	1.40	3.50	5.00
	Sold	<2500	40	0.00	0.00	0.00	0.40	0.00	4.00
		2500-5000	41	0.00	0.00	0.00	1.37	2.00	13.00
		5001-7500	38	0.00	0.00	0.00	0.79	1.00	6.00
		7501-10000	25	0.00	0.00	0.00	0.76	1.00	5.00
		10001-15000	24	0.00	0.00	0.00	1.29	1.00	10.00
		>15000	30	0.00	0.00	1.50	2.83	3.00	25.00



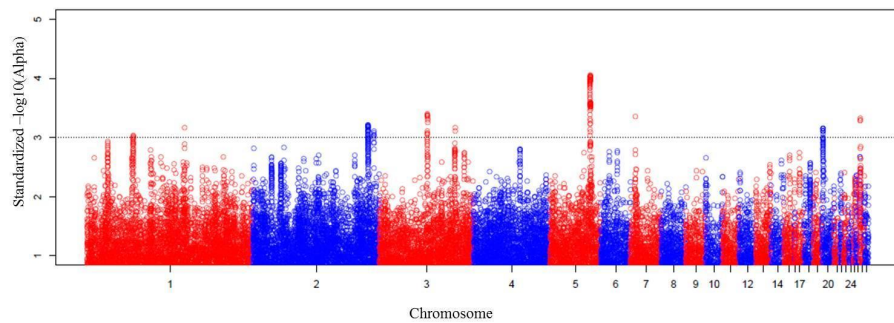
**Supplementary Figure 1. Bivariate plots of climatic differences between study areas.**

Compared to Jarso (blue), Horro (red) is kept in areas with relatively less temperature variation and a higher precipitation during the wettest month.

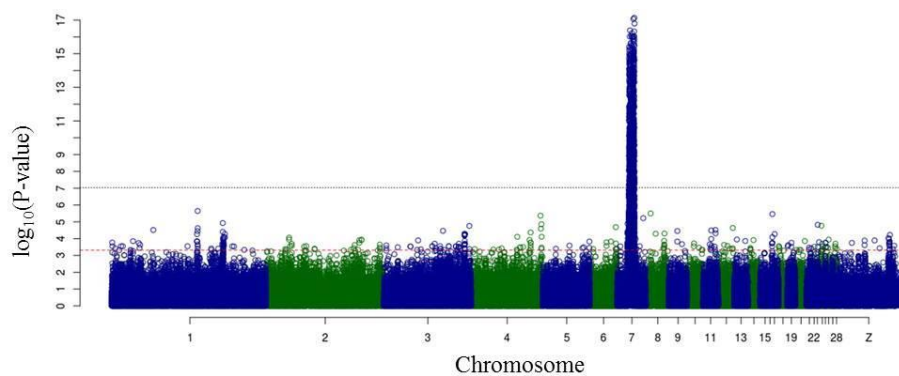
(A)



(B)

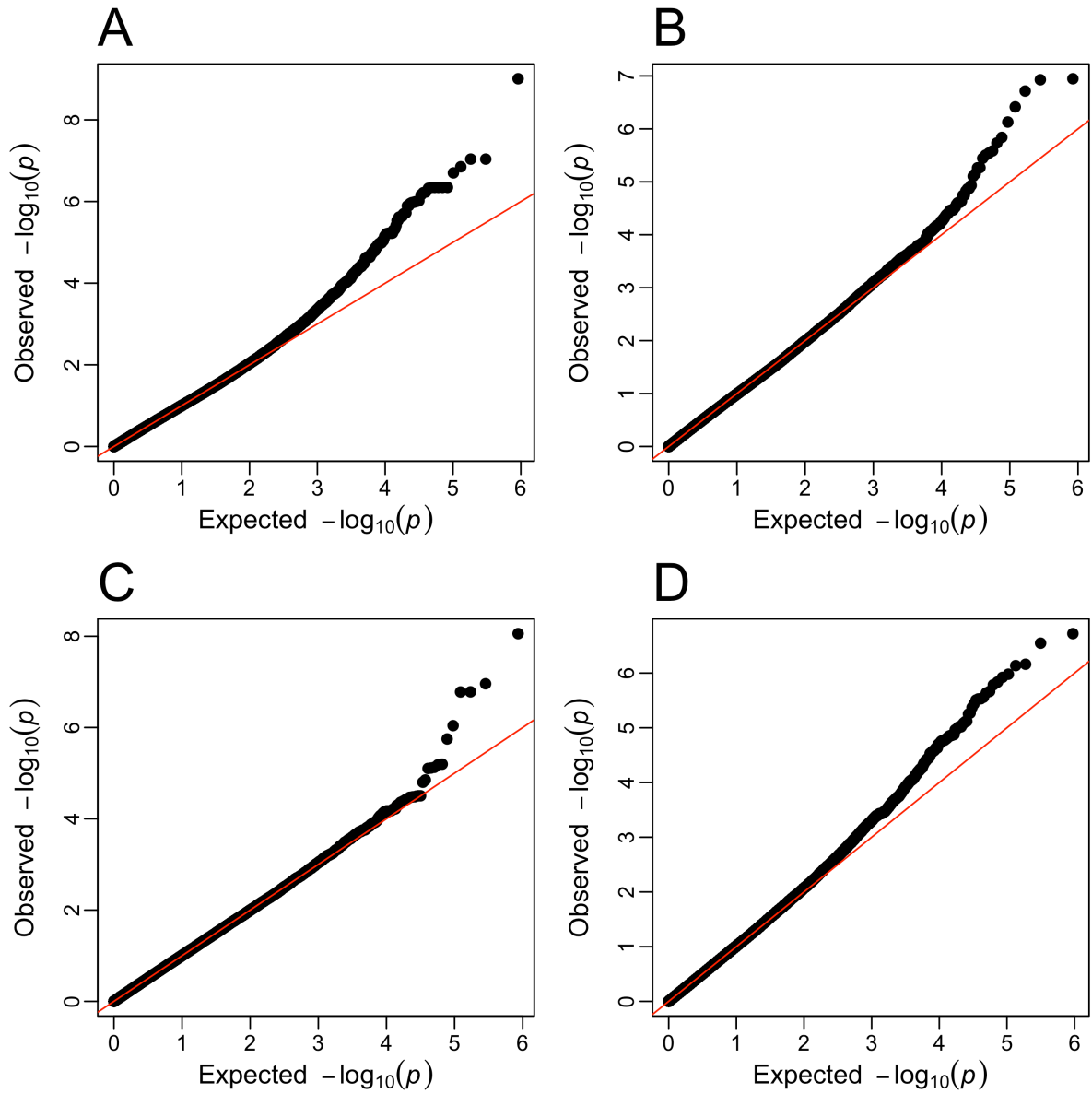


(C)



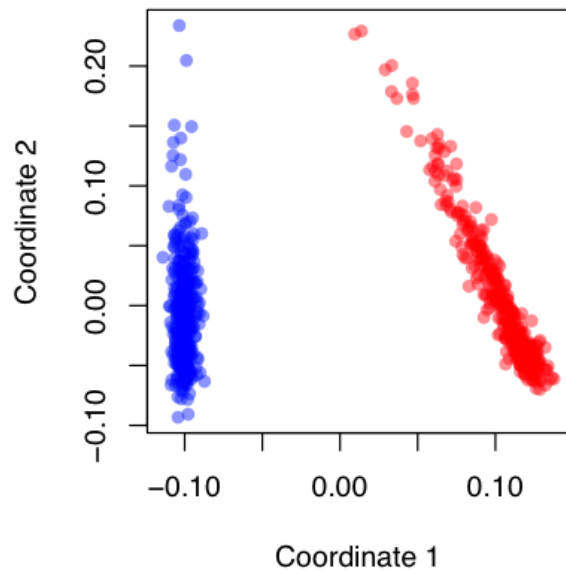
## Supplementary Figure 2. Manhattan plots for SweeD analysis and GWAS for rose comb

Manhattan plots displaying (A) the standardized SweeD plot for Horro chickens, (B) the standardized SweeD plot for Jarso chickens, and (C) genome-wide association analysis results for single comb versus rose comb in Jarso and Horro chickens.



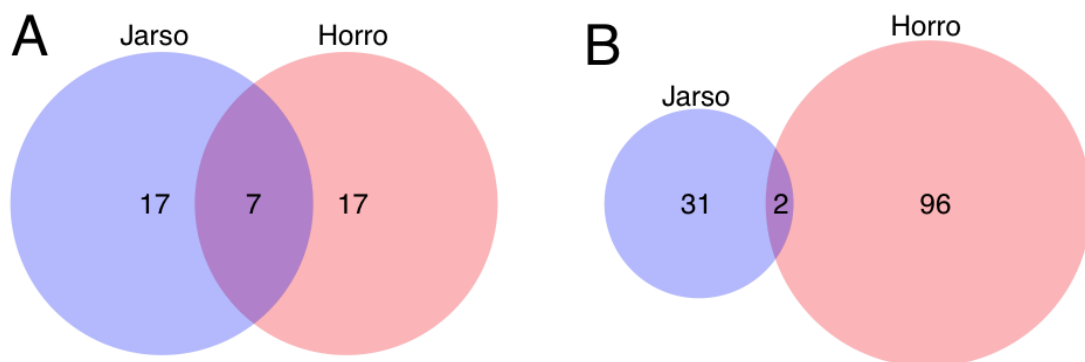
**Supplementary Figure 3. Q–Q plots displaying the GWAS results for ascarid and lice infection**

(A) Ascarids in Horro chickens; (B) Ascarids in Jarso chickens; (C) Lice in Horro chickens; (D) Lice in Jarso chickens Observed  $P$ -values are plotted against the expected  $P$ -values.



**Supplementary Figure 4. Two-dimensional multidimensional scaling plot of genotype data from 760 village chickens.**

Genotype data included 391,384 SNPs that passed quality control from 384 birds from Horro (shown in red) and 376 from Jarso (shown in blue). The two populations demonstrate a clear pattern of differentiation.



**Supplementary Figure 5. Venn Diagrams of loci under strong and recent selection**

**(A) Venn diagram of loci that have been subjected to strong selection pressure in the Ethiopian Horro and Jarso chickens.** The heavily selected genes have been mapped using SweeD software across the autosomal genome (GGA1–28). **(B) Venn diagram for loci under a recent positive selection in Horro and Jarso chickens of Ethiopia.** Loci under moderate selection pressure have been mapped using iHS test of rehh package of the R.



## Supplementary References

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