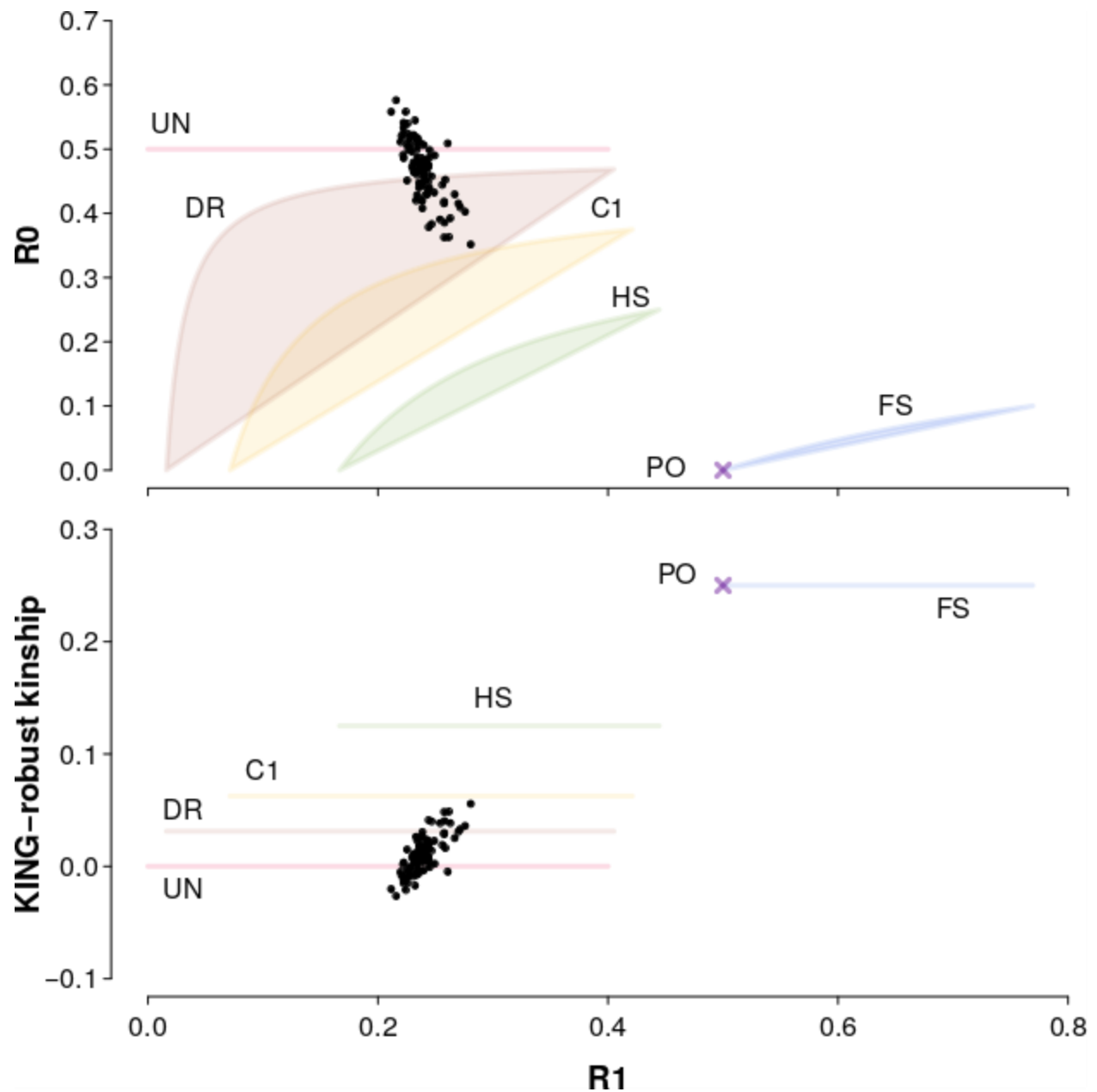


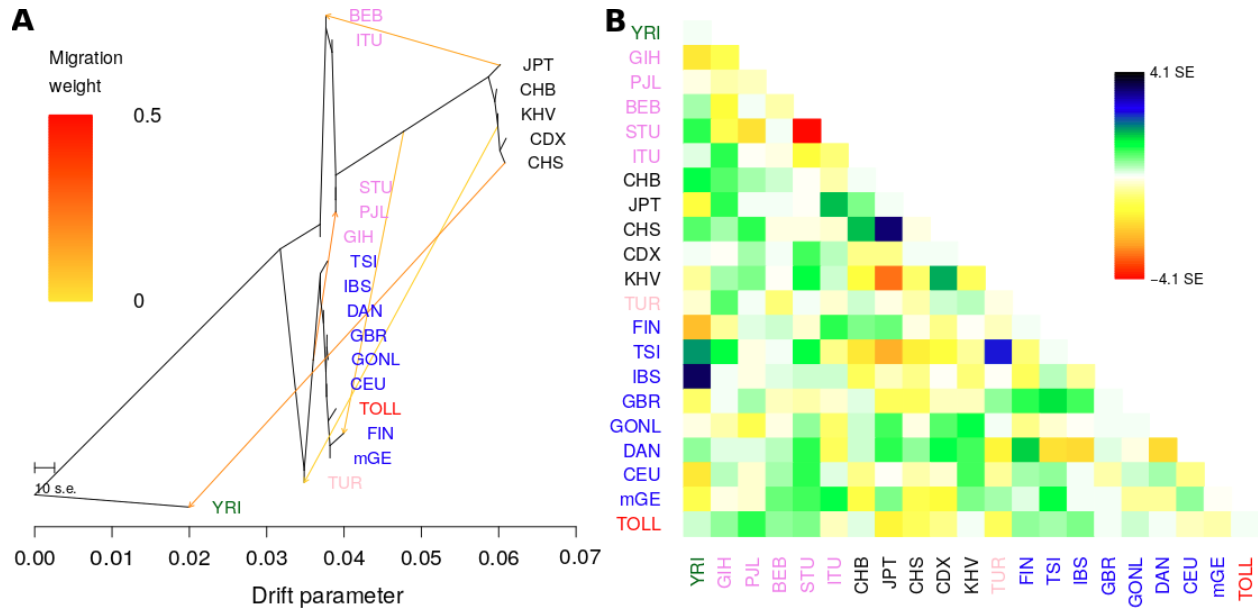
## **Supplemental Information**

### **Low Prevalence of Lactase Persistence in Bronze Age Europe Indicates Ongoing Strong Selection over the Last 3,000 Years**

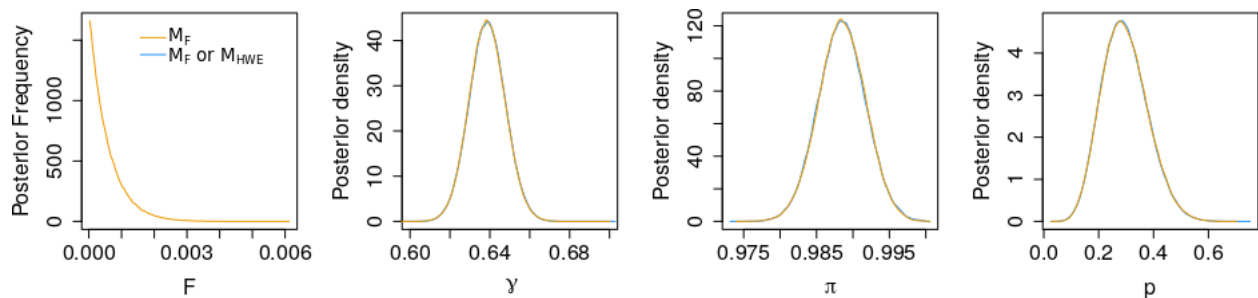
**Joachim Burger, Vivian Link, Jens Blöcher, Anna Schulz, Christian Sell, Zoé Pochon, Yoan Diekmann, Aleksandra Žegarac, Zuzana Hofmanová, Laura Winkelbach, Carlos S. Reyna-Blanco, Vanessa Bieker, Jörg Orschiedt, Ute Brinker, Amelie Scheu, Christoph Leuenberger, Thomas S. Bertino, Ruth Bollongino, Gundula Lidke, Sofija Stefanović, Detlef Jantzen, Elke Kaiser, Thomas Terberger, Mark G. Thomas, Krishna R. Veeramah, and Daniel Wegmann**



**Figure S1: Relatedness between the 14 retained Tollense samples.** PO: parent-offspring, FS: full-siblings, HS: half-siblings/avuncular/grandparent-grandchild, C1: first cousin, UR: unrelated, DR: distantly related. All Tollense samples are not more than distantly related. **Related to STAR Methods, Kinship analysis.**



**Figure S2: TreeMix results allowing for 5 migration events.** A) Full unrooted tree with modern samples from 1000 Genomes, GONL and Turkey data sets as well as 5th century Bavarians. The Tollense branch is in red, Europeans in blue, South Asians in pink, Turkish in brown, East Asians in black, Yoruba in green. No migration events include Tollense. B) Standardized Residuals of TreeMix analysis with the same color code for the populations. All comparisons involving Tollense have small residuals. **Related to Figure 2B, 2C and STAR Methods,  $F_{ST}$  and TreeMix.**



**Figure S3: Posterior densities on inbreeding.** In yellow, posterior densities estimated under the inbreeding model are shown for the inbreeding coefficient  $F$ , the shape parameter  $\gamma$  on the distribution of minor allele frequencies, the fraction of polymorphic loci  $\pi$  and the allele frequency  $p$  on a randomly chosen locus for illustration. The posterior densities shown in blue were obtained by averaging those estimated under the inbreeding model ( $M_F$ ) and a model of strict Hardy-Weinberg equilibrium ( $M_{HWE}$ ) according to their posterior probabilities of 0.994 and 0.006, respectively. Note that no posterior on  $F$  is available under  $M_{HWE}$ . **Related to Figure 2A.**

Lab.Code	Inv.-Nr.	find situation	Strontium	RAW	Unique Mapped	On target [X]	Authentic [%]	MT	Y	R_y	95% CI	Assignment	LP Genotype
WEZ15	ALM 2000/1382-0001	stray find	0.71162	138927958	7421870	3.2	99.32	U2e1a1	I2a2a1a2a1a1	0.0992	0.0974-0.101	XY	0/0
WEZ16	ALM 2008/0459-0001	dive find	0.71293	50211044	649639	0.52	99.91	T2b		0.004	0.0031-0.0049	XX	0/0
WEZ24	ALM 2011/1145-0428	excavation	0.7128	74214214	1969375	6.85	99.9	H27	I2a2	0.0795	0.0765-0.0825	XY	0/0
WEZ35	ALM 2008/0460-0223	dive find	0.70922	92005736	3303834	13.26	99.93	K1c1	R1b1a2a1a2	0.0901	0.0876-0.0925	XY	0/0
WEZ39	ALM 1996/0855-0055	excavation	0.70918	100464498	1751036	4.65	99.18	J2b1a1	I2a2	0.0839	0.0803-0.0876	XY	0/0
WEZ40	ALM 1996/0855-0141	excavation	0.71501	80400056	3336088	11.54	97.67	T1a1	R1b1a2a1	0.0996	0.0968-0.1024	XY	0/0
WEZ48	ALM 2010/1093-1022	excavation	0.71425	199798822	644943	2.61	94.06	J1c	I2a2	0.0836	0.0787-0.0885	XY	0/0
WEZ53	ALM 2013/0463-2000	excavation	0.70972	66332680	1891290	2.96	99.84	U2e2a1a2	R1b1a2	0.0999	0.0964-0.1034	XY	0/0
WEZ54	ALM 2013/0463-1056	excavation	0.71016	75329652	2789209	12.97	96.41	V3a	R1b1a2a1a2	0.0902	0.0878-0.0926	XY	0/0
WEZ56	ALM 2013/0463-1009	excavation	0.70933	254400764	4884607	24.94	99.81	T2b	R1	0.0881	0.0859-0.0903	XY	0/1

WEZ57	ALM 2013/0463- 0967	excavation	0.72016	141863588	3108619	20.35	99.84	H2a1	R1b1a2a1	0.0876	0.0854- 0.0898	XY	0/0
WEZ58	ALM 2013/0463- 0277	excavation	0.71404	137049522	2736722	8.49	99.84	T2b	I2	0.0796	0.0772- 0.0819	XY	0/0
WEZ59	ALM 2013/0463- 1980	excavation	0.7123	90026084	3891124	7.68	99.81	U5a2b1a	R1b1a2a1a2	0.0948	0.0923- 0.0972	XY	0/0
WEZ61	ALM 2013/0463- 1584	excavation	0.71056	152511672	4127941	17.14	99.13	U4b1b1		0.0031	0.0028- 0.0034	XX	0/0
WEZ63	ALM 2013/0463- 1534	excavation	0.70813	74581940	5111733	53.15	90.3			0.1326	0.1291- 0.136	XY	0/0
WEZ64	ALM 2013/0463- 1729	excavation	0.71293	104311626	3827717	2.21	99.92	I1a1a	I2a2a	0.0993	0.0965- 0.1022	XY	0/0
WEZ71	ALM 2013/0463- 0408	excavation	0.71457	189583972	2407538	20.43	99.86	J1c	I2a2	0.0902	0.0869- 0.0935	XY	0/0
WEZ74	ALM 2013/0463- 1588	excavation	0.70939	76112132	1258628	9.68	98.65	T2b		0.0022	0.0017- 0.0027	XX	0/0
WEZ77	ALM 2013/0463- 0658	excavation	0.70952	100925626	2925969	12.66	88.96			0.0551	0.0527- 0.0575	NA	0/0
WEZ51	ALM 2013/0463- 1946	excavation	0.71076	113241302	3247667	22.93	99.77	H1c	I2a2a	0.0927	0.0906- 0.0949	XY	0/0
WEZ83	ALM 2013/0463- 0932	excavation	0.7126	75812954	2351911	15.62	99.79	I4a	I2a2a	0.0971	0.0936- 0.1007	XY	0/1

**Table S1: List of samples sequenced for this study.** Includes sequencing information, on-target depth, mitochondrial based contamination estimations, molecular sexing results, MT and Y haplogroups and Strontium isotope ratios (taken from [S1]) reported for each sample. The local baseline range of  $^{87}\text{Sr}/^{86}\text{Sr}$  at Tollense is 0.709 - 0.712. Samples shown in *italics* were excluded from most analyses because they were highly contaminated, had an on-target depth <

4x, or were dated to a different period. **Related to Table 1.**

Sample 1	Sample 2	Population	Outgroup	D-statistic	Z-score	number of SNPs
WEZ35	WEZ71	LV	YRI	-0.049	-3.56	12292
WEZ74	WEZ24	LT	YRI	-0.119	-3.293	4293
WEZ35	WEZ40	NL	YRI	-0.044	-3.144	15641
WEZ35	WEZ71	PL	YRI	-0.043	-3.123	12334
WEZ35	WEZ54	HR	YRI	-0.049	-3.032	14878
WEZ35	WEZ56	SE	YRI	-0.047	-3.023	17168
WEZ35	WEZ71	IT	YRI	-0.053	-3.023	11410

**Table S2: D-statistics of type D(Tollense 1, Tollense 1; Population,YRI) ordered according to significance.** The 7 significant Tests out of 1638 are shown. LT: Lithuania, NL: Netherlands, HR: Croatia, SE: Sweden, LV: Latvia, PL: Poland, IT: Italy. **Related to STAR Methods, D-statistics with ancient individuals.**

Group	Sample	Site	Country	uncalBP	calBC	Method	Replication	Further Data
Eneolithic	MOB1	Molyukhov Bugor	Ukraine	4810 ± 80	3567 ± 99	2	1	[S3]
	VIN 1	Vinogradnoe		5230 ± 60	4028 ± 87			[S2]
Usatovo	MAJ 9	Mayaki	Ukraine		3500-3000	2	3A from 2E	[S2]
Yamnaya	MAJ 3	Mayaki	Ukraine		3000-2500	2	1	[S3]
	MAJ 4			4175 ± 28	2783 ± 75		3A from 2E	
	MAJ 5				3000-2500	1, 2	1	[S2]
	OVI 2	Ovcharts	Bulgaria	4391 ± 29	3009 ± 60	2		3A from 2E
	OVI 3				3000-2500			
	PEJ 1	Peschanyi V	Russia	4312 ± 94	2949 ± 149		1	
	POP 1	Popovo	Bulgaria		3000-2500	1, 2	1	[S2]
	POP 3							[S3]
	POP 4							[S2]
	RIL 3	Riltsi				2		[S3]
	SUG 2	Kirovograd (Sugokleya)	Ukraine	4226 ± 40 4090 ± 60	2816 ± 73 2697 ± 124			1, 2
	VIN 12	Vinogradnoe		4020 ± 70 3970 ± 70	2589 ± 108 2471 ± 103	5A from 2E (1x C/T)		
	VIN 2						[S2]	
Early	LIS 1	Lisichansk	Ukraine		2700-2500	2	1	[S3]

Catacomb Culture	LIS 2			4061 ± 31	2592 ± 70		3A from 2E	
	PEJ 4	Peschanyi V	Russia	3908 ± 117	2391 ± 167		1	
	PEJ 5			3908 ± 117	2391 ± 167		3A from 2E	
	TEM 2	Temrta III		4100 ± 50	2710 ± 115	1, 2	1	[S2]
	TEM 4	Temrta V		4110 ± 45	2719 ± 109	2		
	TEM 5			4110 ± 45	2719 ± 109			
	TEM 7			4110 ± 35	2724 ± 104			[S3]
Developed Catacomb Culture	SUG 5			Kirovograd (Sugokleya)	Ukraine		3840 ± 90	2300 ± 132
	VIN 3	Vinogradnoe	4020 ± 70 4060 ± 50	2589 ± 108 2639 ± 116		1, 2	5A from 2E (1x C/T)	[S2]
Yamnaya-Poltavkinskaja	KAL 1	Kalinovka I	Russia		3000-2000	1, 2	1	[S2]
	KAL 2					2	3A from 2E	[S3]
	NIK 1	Nikolaevka III				1, 2	1	[S2]
	NIK 7							
	POD 1	Podlesnyi		4260 ± 50 (GIN 13206) 4290 ± 40 (GIN 13207) 4390 ± 50 (GIN 13208)	2844 ± 71 2931 ± 36 3027 ± 79	2		
	POD 2				3000-2000			
	Late Catacomb Culture	KNO 4		Krasnorechensk	Ukraine		2500-2000	1, 2
LIS 3		Lisichansk	4146 ± 30	2756 ± 83		1, 2	[S1]	
NEV 1		Nevskoe	3640 ± 70	2025 ± 96		2	3A from 2E	[S3]
NEV 3				2500-2000				
NOZ 1		Novozvanovka 2	3929 ± 30	2417 ± 52			1	[S1]

**Table S3: Sample information for Figure 3A.** Method: 1 = Sanger sequencing; 2 = 454 Sequencing of Multiplex PCRs. Replication: Results obtained by Sanger-sequencing were replicated from at least two DNA extracts and 4 PCR amplifications (code: 1) if not indicated differently (e.g. 3A from 2E means three amplifications from 2 DNA extractions). In two cases, a trace-like T allele was found in a PCR, which however could not be reproduced in 4 replications each, and is therefore regarded as an artifact. Authentication criteria for the 454 method are as described in [S2]. Using these methods and authentication criteria, all individuals in the list turned out to be homozygous for the ancestral allele at the major LP associated polymorphisms 13.910 C/T. **Related to Figure 3A.**



**Supplemental References:**

- S1 Price, T., D., Frei, R., Brinker, U., Lidke, G., Terberger, T., Frei, K., M., and Jantzen, D. (2019). Multi-isotope proveniencing of human remains from a bronze age battlefield in the Tollense valley in northeast Germany. *Archaeological and Anthropological Sciences*, 11(1):33–49.
- S2 Wilde, S., Timpson, A., Kirsanow, K., Kaiser, E., Kayser, M., Unterländer, M., Hollfelder, N., Potekhina, I., D., Schier, W., Thomas, M., G., et al. Direct evidence for positive selection of skin, hair, and eye pigmentation in Europeans during the last 5,000 years. *Proceedings of the National Academy of Sciences*, page 201316513, 2014.
- S3 Wilde, S., 2015. *Populationsgenetik kupfer-und bronzezeitlicher Bevölkerungen der osteuropäischen Steppe* (Doctoral dissertation, Universitätsbibliothek Mainz).