Supporting Information Morger et al 2015

Figure S1 Schematic representation of the functional regions of *TLR2*

Schematic representation of the different functional regions of the house mouse *TLR2* based on Jin et al 2007. LRR: Leucine-rich repeat; TIR domain: Toll/interleukin-1 receptor homology domain; Red box: Ligand binding sites; Blue box: *TLR2-TLR1* heterodimerisation sites; Green: sequenced fragment (amino acid 236-618, based on house mouse *TLR2*)

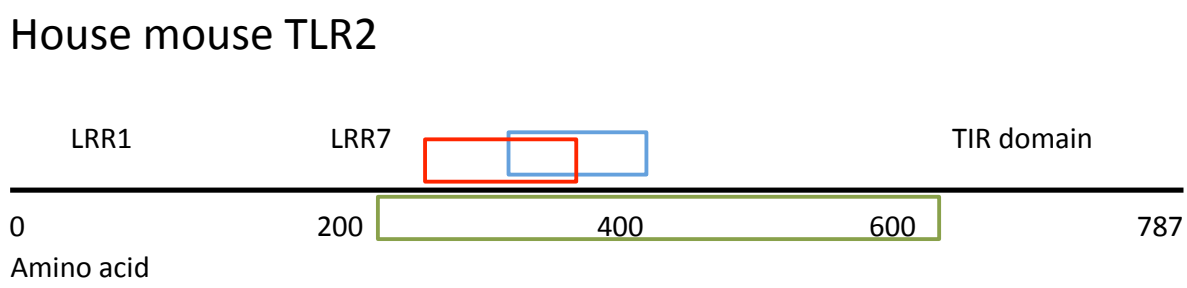

Figure S2 Phylogenetic tree based on *cytb* sequences

Neighbour-joining tree of *cytb* sequences and 14 GenBank reference sequences representing the seven European bank vole lineages. A *cytb* sequence of *Myodes rutilus* was used as an outgroup.

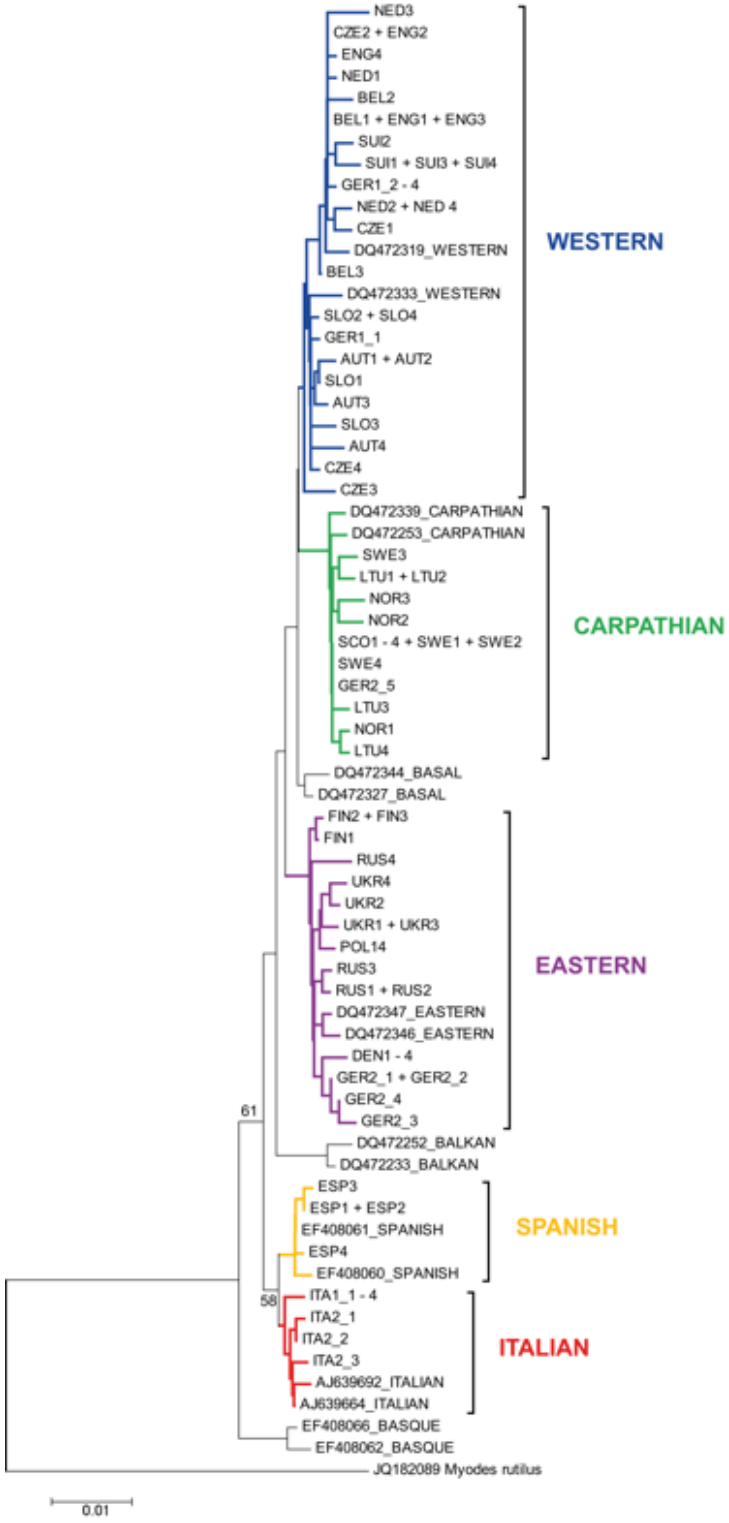

Figure S3 *cytb* genealogy

Network based on *cytb* sequences. Each line indicates a change at one nucleotide. Blue: Western lineage; Green: Carpathian lineage; Purple: Eastern lineage; Red: Italian lineage; Yellow: Spanish lineage. The network was constructed in the program TCS 1.21. The origin of the sequences is indicated in the bubble. If a sequence was observed several times we give the number of observations by 'x number of observations' (e.g. x2 indicates that the sequence was observed twice in the population).

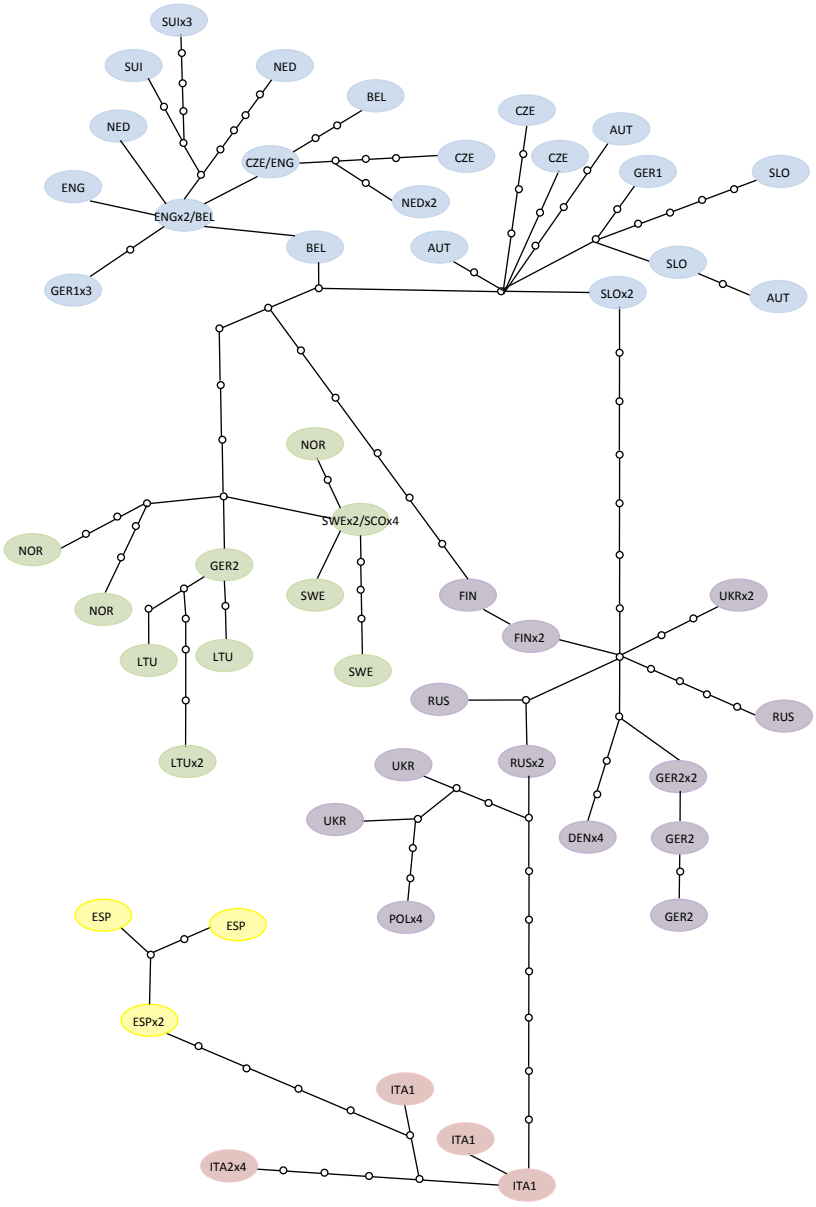

Figure S4 *TLR2* haplotype frequencies within populations

Populations are coloured according to their *cytb* linage (yellow = Spanish, red = Italian, blue = Western, purple = Eastern, green = Carpathian) and haplotypes are coloured according to clusters (blue = c_1, orange = c_2, green = c_3; grey = c_4).

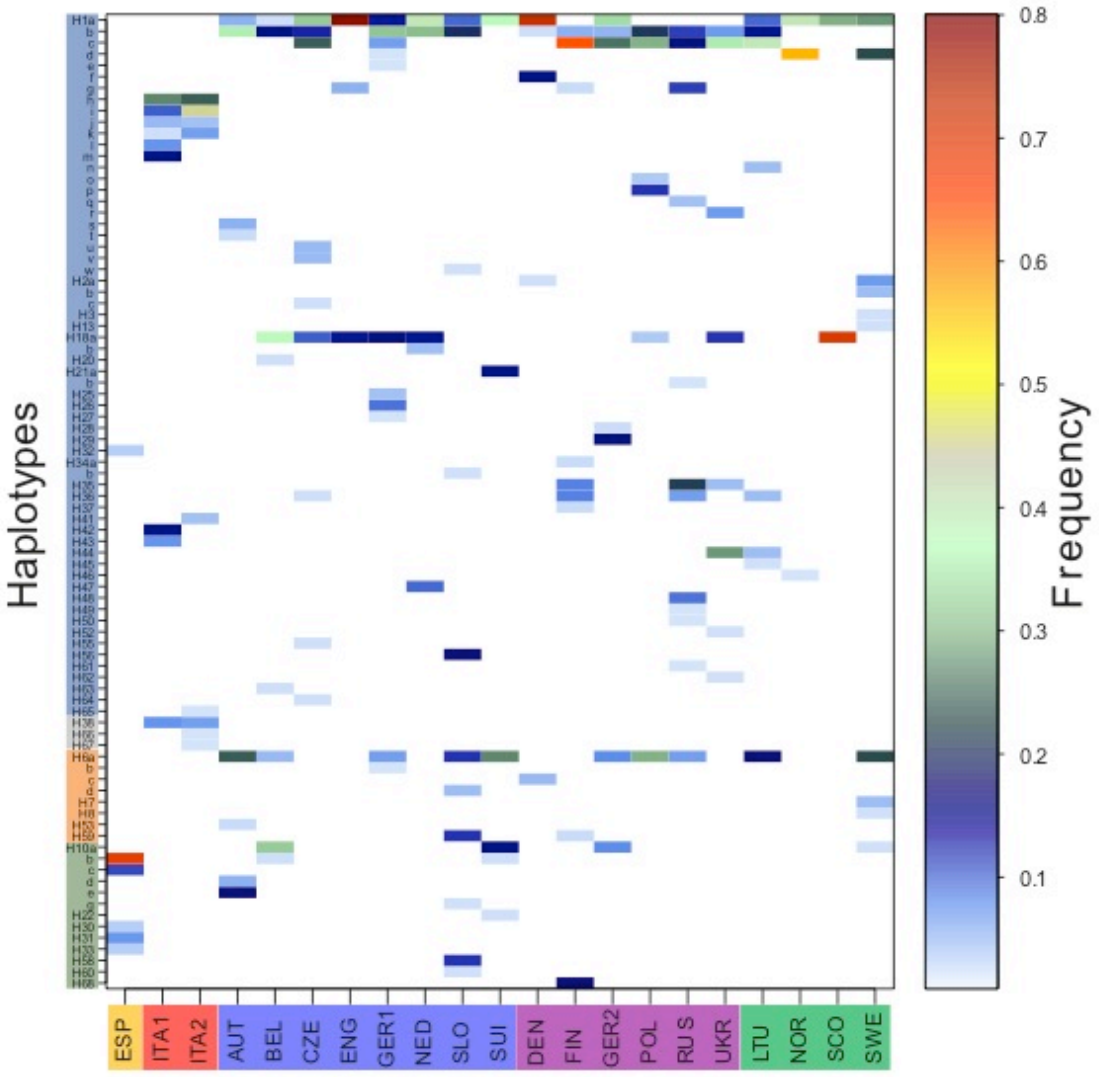

Figure S5 Amino acid polymorphisms in the four bank vole *TLR2* clusters

Amino acid differences (in red) between the most common haplotypes of the four *TLR2* clusters.

```
                    ....|....| ....|....| ....|....| ....|....| ....|....|
                   236      245        255        265        275        285
Cluster 1, Hap 1    NFRFSELSAE EINSPMKKLA FRNADLTDES FNELLKLLRY TPELLQAEFD
Cluster 2, Hap 6    NFRFSELSAE EINSPMKKLA FRNADLTDES FNELLKLLRY APELLQAEFD
Cluster 3, Hap 10   NFRFSELSAE EINAPMKKLA FRNADLTDES FNELLKLLRY TPELLQAEFD
Cluster 4, Hap 38   NFRFSELSAE EINSPMKKLA FRNADLTDES FNELLKLLRY TPELLQAEFD

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           295        305        315        325        335
Cluster 1, Hap 1    DCALDGVGDF QPSESDIVRE LGKVETLIIR RLHIPRFYSF NDLRSVYSLL
Cluster 2, Hap 6    DCALDGVGDF QPSESDIVRE LGKVETLIIR RLHIPRFYSF NDLRSVYSLL
Cluster 3, Hap 10   DCALDGVGDF QPSESDIVRE LGKVETLIIR RLHIPRFYSF NDLSSVYSLL
Cluster 4, Hap 38   DCALDGVGDF QPSESDIVRE LGKVETLIIR RLHIPRFYSF NDLRSVYSLL

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           345        355        365        375        385
Cluster 1, Hap 1    EKVKRITVEN SKVFLVPCEF SQRLRSLEFL DLSENLMVEE YLRNSACEGA
Cluster 2, Hap 6    EKVKRITVEN SKVFLVPCEF SQRLRSLEFL DLSENLMVEE YLRNSACEGA
Cluster 3, Hap 10   EKVKRITVES SKVFLVPCEF SQRLRSLEFL DLSENLMVEE YLRNSACEGA
Cluster 4, Hap 38   EKVKHITVEN SKVFLVPCEF SQRLRSLEFL DLSENLMVEE YLRNSACEGA

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           395        405        415        425        435
Cluster 1, Hap 1    WPSLQTLILS QNYLKSIERT GEILLTLKNL TDLDISRNSF RPMPDTCRWP
Cluster 2, Hap 6    WPSLQTLILS QNYLKSIERT GEILLTLKNL TGLDISRNSF RPMPDTCRWP
Cluster 3, Hap 10   WPSLQTLILS QNYLKSIERT GEILLTLKNL TGLDISRNSF RPMPDTCRWP
Cluster 4, Hap 38   WPSLQTLILS QNYLKSIERT GEILLTLKNL TDLDISRNSF RPMPDTCRWP

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           445        455        465        475        485
Cluster 1, Hap 1    PKMRFLNLSS TGIWAVKMCI PQTLEVLDVS NNNLDSFSLF LPRLRELYIS
Cluster 2, Hap 6    PKMRFLNLSS TGIWAVKTCI PQTLEVLDVS NNNLDSFSLF LPRLRELYLS
Cluster 3, Hap 10   PKMRFLNLSS TGIWAVKTCI PQTLEVLDVS NNNLDSFSLF LPRLRELYLS
Cluster 4, Hap 38   PKMRFLNLSS TGIWAVKTCI PQTLEVLDVS NNNLDSFSLF LPRLRELYLS

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           495        505        515        525        535
Cluster 1, Hap 1    RNKLKTLPDG SLFPVLLVMK IRENALSTFS KDQLGSFPKL ETLEAGDNHF
Cluster 2, Hap 6    RNKLKTLPDG SLFPVLLVMK IRENALSTFS KDQLGSFPKL ETLEAGDNHF
Cluster 3, Hap 10   RNKLKTLPDG SLFPVLLVMK IRENALSTFS KDQLGSFPKL ETLEAGDNHF
Cluster 4, Hap 38   RNKLKTLPDG SLFPVLLVMK IRENALSTFS KDQLGSFPKL ETLEAGDNHF

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                           545        555        565        575        585
Cluster 1, Hap 1    VCSCELLSFT LGQPALSQVL PDWPDSYLCN SPPRLRGQRV QEARPSVLEC
Cluster 2, Hap 6    ICSCELLSFT LGQPALSQVL PDWPDSYLCD SPPRLRGQRV QEARPSVLEC
Cluster 3, Hap 10   ICSCELLSFT LGQPALSQVL PDWPDSYLCN SPPRLRGQRV QEARPSVLEC
Cluster 4, Hap 38   VCSCELLSFT LGQPALSQVL PDWPDSYLCD SPPRLRGQRV QEARPSVLEC

                    ....|....| ....|....| ....|....| ...
                           595        605        615     Amino acid (Mus musculus)
Cluster 1, Hap 1    HQALLVSGVC CALLLLILLI AGLCHHFHGL WYL
Cluster 2, Hap 6    HQALLVSGVC CALLLLILLI AGLCHHFHGL WYL
Cluster 3, Hap 10   HQALLVSGVC CALLLLILLI AGLCHHFHGL WYL
Cluster 4, Hap 38   HQALLVSGVC CALLLLILLI AGLCHHFHGL WYL
```

Cluster 1: Haplotype 1 (GenBank accession: JN674535.1); Cluster 2: Haplotye 6 (GenBank accession JN674538.1); Cluster 3: Haplotype 10 (GenBank accession JN674541.1); Cluster 4: Haployte 38 (GenBank accession KJ612441.1)

Table S1 Sampling locations

Population ID, country, sampling location, altitude, coordinates of the sampled bank vole populations and permit numbers under which samples were collected.

ID	Country	Location	Altitude	Coordinates	Ethical permit number and office
ESP	Spain	Legazpi	550m	N 43.0322°, E -	10219 / 2003. Gipuzkoako Foru
		Arantzazu	800m	2.3232°	Aldundia
		Lastur	200m	N 42.9869°, E -	10219 / 2003. Gipuzkoako Foru
		Izki	750m	2.4039°	Aldundia
		Oiz	680m	N 43.2522°, E -	10219 / 2003. Gipuzkoako Foru
		Urkiola	590m	2.3372°	Aldundia
				N 42.7153°, E -	810 / 2003. Arabako Foru Aldundia
				2.4614°	14411 / 2003. Bizkaiko Foru
				N 43.2221°, E -	Aldundia
				2.6094°	14411 / 2003. Bizkaiko Foru
				N 43.0709°, E -	Aldundia
				2.6821°	
ITA1	Italy	La Selva	350-	N 43.2167°, E	D.D. 5493,16 November 2010,
		Forest	700m	11.0667°	Regione Toscana, Italy
ITA2	Italy	Viterbo	300m	N 42.5168°, E	Permit Number 0024822, Ministry of
				12.1204°	Environment, Rome, Italy.
AUT	Austria	Ötscher	855-	N 47.8721°, E	BD2-N-200/071_2007;NÖ UA-
		Mountain	1240m	15.1944°	180000/159
					Amt der Niederösterreichischen
					Landesregierung
BEL	Belgium	Beaumont	221m	N 50.2784°, E 4.2496°	2012-14 DNF: Département de la
					Nature et des Forêts .
CZE	Czech	Stružná	675m	N 50.1853°, E	27873/ENV/11 Ministry of the
	Republic			13.0091°	Environment of the Czech Republic
ENG	England	Manor Wood	56m	N 53.3301°, E -	UK Home Office project licence
				3.0516°	number 40/3235
GER1	Germany	Elsenthal	578m	N 48.8474°, E	Trapping was performed in a project
				13.3779°	that was embedded in the “Vector in
					climate change investigations”
					(VICCI) network in Bavaria granted

					by the Bavarian Ministry of Environment and Health. Trapping was performed as prognostic trapping during investigations for human hantavirus and Rickettsia infections in collaboration with Bavarian Forest National Park
NED	Netherlands	Renkum	51m	N 51.5997°, E 5.4515°	Alterra, ALT06.2004
SLO	Slovenia	Rakovník	326m	N 46.1412°, E 14.3853°	35701-21/2002 Slovenian Environmental Agency (ARSO)
SUI	Switzerland	Zürichberg	580m	N 47.3983°, E 8.5561°	2012_17; Amt für Lebensmittelsicherheit und Tiergesundheit
DEN	Denmark	Furesø	25m	N 55.7919°, E 12.3960°	Order No. 330 of 19.03.2013. Ministry of Environment
FIN	Finland	Konnevesi	100m	N 62.6164°, E 26.3460°	ESLH-2008-04660/Ym-23, Animal Experiment Board
GER2	Germany	Rothenklempenow	6m	N 53.5174°, E 14.2029°	Forest protection team of Landesforst Mecklenburg-Vorpommern led by Margrit Bemmann performed the monitoring trapping in frame of their responsibilities. We thank Ulrike M. Rosenfeld, Dörte Kaufmann, Ulrike Duve, Kati Sevke, Mathias Schlegel and Thomas Büchner for help with trapping and dissection. Funding was provided by the German Federal Ministry of Education and Research (BMBF) through the National Research Platform for Zoonoses (project codes 01KI1018 and 01KI1303).
POL	Poland	Mazury lake district (Pisz, Ryn, Mikolajki)	127m	N 53.8266°, E 21.4673°	No. 73/2010 First Warsaw Local Ethics Committee for Animal Experimentation
UKR	Ukraine	Oranoe Field Station	132m	N 51.0333° E 30.1167°	No permit is required for trapping small mammals

RUS	Russia	Shigaevo	289m	N 57.3530°, E 58.7083°	License number N 212/647-249 Administration of Rosprirodnadzor in Sverdlovsk region; Russian Foundation for Basic Research – 14_04_00614
LTU	Lithuania	Žagarė Regional Park	40m	N 56.3261°, E 23.2186°	No permit is required for trapping small mammals in Lithuania
NOR	Norway	Telemark	67m	N 59.3920°, E 8.9044°	2007/11612 ART-VI-ID, Direktoratet for naturforvaltning
SCO	Scotland	Loch Lomond	15m	N 56.0776°, E -4.6381°	UK Home Office license PPL 60 / 3977
SWE	Sweden	Kalvs mosse	22m	N 55.7078°, E 13.4869°	M101-06 and M141-10 issued by the Malmö / Lund, Sweden ethical board for animal experiments

Table S2 Inferring *TLR* haplotypes using the program PHASE

Proportion of homozygotes (*Homozygotes*) in the different study populations and the proportion of heterozygous individuals carrying at least one of the common haplotypes occurring in homozygous state (i.e. a verified haplotype) within a population (*Heterozygotes with known*). Haplotypes from all clusters occurred in homozygous state. To further confirm the accuracy of the inference of haplotypes using the program PHASE we have cloned *TLR2* for a subset of Swedish bank voles. The obtained sequences were 100% identical to the inferred (PHASEd) haplotypes. Results of the phasing procedure were very similar when analyzing populations separately (as presented in the main text), all samples together, or when including verified haplotypes as a reference. Importantly, all procedures led to the same haplotype network structure and to identical estimates of *TLR2* cluster frequencies within populations.

Population	***Homozygotes***	***Heterozygotes with known***
Overall	0.32	0.90
ESP	0.54	1
ITA1	0.11	0.35
ITA2	0.43	0.67
AUT	0.35	0.91
BEL	0.32	0.92
CZE	0.37	0.92
ENG	0.59	1
GER1	0.19	0.65
NED	0.10	0.56
SLO	0.05	0.11
SUI	0.32	1
DEN	0.74	1
FIN	0.30	0.57
GER2	0.44	1
POL	0.25	0.78
RUS	0.10	0.16
UKR	0.15	0.77
LTU	0.30	0.93
NOR	0.52	1
SCO	0.42	1
SWE	0.15	0.77

Table S3 Origin of *TLR2* haplotypes

Haplotype, cluster, GenBank accession number and haplotype origin.

Haplotype	Cluster	GenBank accession	Origin
1	1	JN674535.1	all except ESP
2	1	JN674536.1	CZE, DEN, SWE
13	1	JN674544.1	SWE
18	1	KJ612421.1	BEL, CZE, GER1, ENG, SCO, NED, POL, UKR
20	1	KJ612423.1	BEL
21	1	KJ612424.1	SUI, RUS
25	1	KJ612428.1	GER1
26	1	KJ612429.1	GER1
27	1	KJ612430.1	GER1
28	1	KJ612431.1	GER2
29	1	KJ612432.1	GER2
32	1	KJ612435.1	ESP
34	1	KJ612437.1	FIN, SLO
35	1	KJ612438.1	FIN, RUS, UKR
36	1	KJ612439.1	CZE, FIN, LTU, RUS
37	1	KJ612440.1	FIN
41	1	KJ612444.1	ITA2
42	1	KJ612445.1	ITA1
43	1	KJ612446.1	ITA1
44	1	KJ612447.1	LTU, UKR
45	1	KJ612448.1	LTU
46	1	KJ612449.1	NOR
47	1	KJ612450.1	NED
48	1	KJ612451.1	RUS
49	1	KJ612452.1	RUS
50	1	KJ612453.1	RUS
52	1	KJ612455.1	UKR
55	1	KJ612458.1	CZE
56	1	KJ612459.1	SLO
61	1	KM979279.1	RUS
62	1	KM979280.1	UKR
63	1	KM979281.1	BEL
64	1	KM979282.1	CZE
65	1	KM979283.1	ITA2
6	2	JN674538.1	AUT, BEL, SUI, GER1, GER2, DEN, SWE, LTU, POL, RUS, SLO
7	2	JN674539.1	SWE
8	2	JN674540.1	SWE
53	2	KJ612456.1	AUT
59	2	KJ612462.1	FIN, SLO
10	3	JN674541.1	AUT, BEL, SUI, GER2, ESP, SWE, SLO
22	3	KJ612425.1	SUI
30	3	KJ612433.1	ESP
31	3	KJ612434.1	ESP
33	3	KJ612436.1	ESP
58	3	KJ612461.1	SLO
60	3	KM979278.1	SLO
68	3	KM979286.1	FIN
38	4	KJ612441.1	ITA1, ITA2
66	4	KM979284.1	ITA2
67	4	KM979285.1	ITA2

Table S4 *TLR2* population divergence

Pairwise genetic distance (F_{ST}) between populations based on TLR2 haplotypes. Values that were statistically significant after correction for multiple testing are highlighted in bold.

	AUT	BEL	SUI	CZE	GER1	DEN	GER2	ESP	FIN	ENG	SCO	ITA1	ITA2	SWE	LTU	NOR	NED	POL	RUS	SLO
AUT																				
BEL	**0.1650**																			
SUI	**0.1468**	**0.1804**																		
CZE	**0.1284**	**0.1508**	0.0985																	
GER1	0.0449	**0.0892**	**0.1292**	0.0440																
DEN	**0.3500**	**0.3889**	**0.1741**	**0.1694**	**0.2677**															
GER2	**0.1481**	**0.1822**	**0.0697**	0.0144	**0.0914**	**0.1762**														
ESP	**0.3340**	**0.3438**	**0.3385**	**0.3160**	**0.3013**	**0.5259**	**0.3310**													
FIN	**0.2543**	**0.2838**	**0.2845**	**0.1311**	**0.1952**	**0.4441**	**0.1452**	**0.4003**												
ENG	**0.3942**	**0.3954**	**0.2053**	**0.1889**	**0.2896**	0.0422	**0.2103**	**0.5709**	**0.4813**											
SCO	**0.3826**	**0.1949**	**0.3360**	**0.2494**	**0.2377**	**0.4521**	**0.3238**	**0.5355**	**0.4532**	**0.4149**										
ITA1	**0.2308**	**0.2532**	**0.2483**	**0.2160**	**0.2038**	**0.4070**	**0.2292**	**0.3608**	**0.2958**	**0.4429**	**0.4152**									
ITA2	**0.1641**	**0.1878**	**0.1828**	**0.1501**	**0.1386**	**0.3450**	**0.1629**	**0.2946**	**0.2315**	**0.3812**	**0.3535**	**0.0852**								
SWE	**0.1237**	**0.1855**	0.0456	**0.0910**	**0.1039**	**0.1999**	**0.0794**	**0.3139**	**0.2506**	**0.2346**	**0.3171**	**0.2150**	**0.1494**							
LTU	**0.1174**	**0.2003**	**0.1536**	0.0514	**0.0864**	**0.3325**	0.0569	**0.3361**	0.0527	**0.3712**	**0.3745**	**0.2360**	**0.1707**	**0.1324**						
NOR	**0.3286**	**0.3578**	**0.2468**	**0.2280**	**0.2602**	**0.3142**	**0.2379**	**0.4805**	**0.4061**	**0.3493**	**0.4640**	**0.3693**	**0.3070**	**0.1113**	**0.3186**					
NED	**0.1068**	**0.1506**	**0.1103**	0.0320	0.0251	**0.1614**	0.0835	**0.3721**	**0.2839**	**0.1814**	**0.2664**	**0.2545**	**0.1838**	**0.1078**	**0.1564**	**0.2555**				
POL	0.0668	**0.1712**	**0.1537**	**0.0930**	**0.0669**	**0.3949**	**0.1001**	**0.3435**	**0.1180**	**0.4378**	**0.3893**	**0.2321**	**0.1626**	**0.1262**	0.0082	**0.3545**	**0.1654**			
RUS	**0.0983**	**0.1555**	**0.1538**	**0.0852**	**0.0763**	**0.3262**	**0.1014**	**0.2790**	**0.1092**	**0.3589**	**0.3366**	**0.1824**	**0.1169**	**0.1227**	**0.0637**	**0.2915**	**0.1399**	**0.0619**		
SLO	0.0544	**0.1429**	**0.1109**	**0.0875**	**0.0479**	**0.2764**	**0.1040**	**0.2833**	**0.1955**	**0.3156**	**0.3221**	**0.1856**	**0.1198**	**0.0891**	**0.1056**	**0.2661**	**0.0836**	**0.0814**	**0.0772**	
UKR	**0.1828**	**0.1800**	**0.2225**	**0.0939**	**0.1172**	**0.3816**	**0.1225**	**0.3347**	**0.0881**	**0.4083**	**0.3322**	**0.2347**	**0.1694**	**0.1891**	**0.0542**	**0.3450**	**0.1932**	**0.0928**	**0.0853**	**0.1468**

Table S5 Fay & Wu's neutrality test

An excess of high-frequency derived haplotypes (negative Fay & Wu's *H)* was observed in the majority of populations, indicating that positive selection has shaped *TLR2* in most bank vole populations. *Mus musculus* was used as an outgroup.

Population	*H*	*P*
ESP	-15.532	0.000
ITA1	-6.378	0.034
ITA2	-3.596	0.089
AUT	-5.604	0.095
BEL	-12.928	0.034
CZE	-5.252	0.011
ENG	0.535	0.890
GER1	-8.246	0.015
NED	-0.905	0.157
SLO	-8.577	0.048
SUI	-5.485	0.088
DEN	-9.735	0.001
FIN	-15.249	0.011
GER2	-13.140	0.012
POL	-2.181	0.170
RUS	-11.842	0.006
UKR	-2.844	0.035
LTU	-10.380	0.016
NOR	-0.167	0.176
SCO	-1.229	0.072
SWE	-10.390	0.032

Table S6 Analysis of molecular variance (AMOVA) of *cytb*

Level of comparison	**DF**	**Sum of squares**	**Variance component**	**% Variation**	***P***
Among lineages	4	257.48	3.909	55.70	<0.001
Among populations, within lineages	16	115.94	1.450	20.66	<0.001
Within populations	60	99.53	1.659	23.64	<0.001