Cryptosporidium parvum IId family: clonal population and dispersal from Western Asia to other geographical regions

Rongjun Wang[1], Longxian Zhang[1*], Charlotte Axén[2], Camilla Bjorkman[3], Fuchun Jian[1], Said Amer[4], Aiqin Liu[5], Yaoyu Feng[6], Guoquan Li[1], Chaochao Lv[1], Zifang Zhao[1], Meng Qi[1], Haiju Dong[1], Helei Wang[1], Yanru Sun[1], Changshen Ning[1] & Lihua Xiao[7*]

[1] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, China. [2] Department for Animal Health and Antimicrobial Strategies, Swedish National Veterinary Institute, Uppsala, Sweden. [3] Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden. [4] Department of Zoology, Faculty of Science, Kafr El sheikh University, Kafe El Sheikh, Egypt. [5] Department of Parasitology, Harbin Medical University, Harbin, China. [6] School of Resource and Environmental Engineering, East China University of Science and Technology, Shanghai, China. [7] Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, USA.

∗Corresponding author. Mailing address for Longxian Zhang: College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, P. R. China. Phone: 86-371-63555689. Fax: 86-371-63558180. E-mail: zhanglx8999@yahoo.com.cn.
Mailing address for Lihua Xiao: Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333. Phone: (404) 718-4161. Fax: (404) 718-4197. E-mail: lxiao@cdc.gov.

Supporting Information

Figure S1 Phylogenetic network analysis of multilocus sequence typing data of 111 *C. parvum* IId isolates. The number labeled beside circle indicates the MLST as shown in table S1. Each circle indicates a unique MLST of *C. parvum* and Circle size represents the number of samples present in each MLST.

Figure S2 Linkage disequilibrium (LD) among different populations by DnaSP analysis of concatenated sequences from 12 genetic loci.

Figure S3 Estimate of the number of ancestral population (K). (A) Plot of the mean likelihood L (K). (B) Plot of the mean difference between successive likelihood values of K, L'(K) = L(K) − L(K − 1). (C) Plot of the mean differences between successive values of L'(K), where |L''(K)| = |L'(K + 1) − L'(K)|. (D) Plot of the delta K (ΔK), ΔK = m|L''(K)|/s[L(K)], where m = mean of the absolute values of L''(K), s = SD of L(K).

Table S1 Specimens used in this study and their subtype identity at the 12 genetic loci.

Table S2 Genetic diversity of *C. parvum* IId DNA sequences.

Table S3 Genetic diversity of *C. parvum* IId DNA sequences at the GP60 locus.

Table S4 Analysis of linkage disequilibrium in *C. parvum* IId isolates.

Table S5 Background information for *C. parvum* IId isolates in Sweden.

Table S1 Specimens used in this study and their subtype identity at the twelve loci

			Subtype at each locus*											
Specimen ID	**Host**	**Country**	**MSC 6-7**	**RPGR**	**MSC 6-5**	**DZ-HRGP**	**ZPT**	**CP56**	**Chom3T**	**CP47**	**HSP70**	**Mucin-1**	**ML2**	**Gp60**	**MLST subtype**
FY4	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY6	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY10	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY23	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY25	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY41	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY42	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY54	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY59	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY67	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY70	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY71	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY72	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY76	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY77	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY78	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY1	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY2	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY3	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY5	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY6	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY14	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY16	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY18	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY19	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY23	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY24	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY26	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY27	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY28	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY29	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY30	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY31	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY32	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
DY33	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1

DY34	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
SQ5	cattle	China	1	1	1	1	1	1	1	1	1	1	1	5	1
FY52	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
SQ1	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
SQ2	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
SQ3	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
SQ4	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
SQ7	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
SQ8	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
SQ9	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
NX4	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
NX93	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
NX139	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
NX140	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
NX203	cattle	China	1	1	1	2	1	1	1	1	1	1	1	5	2
M120	horse	China	1	1	1	2	1	1	1	1	1	1	1	5	2
M123	horse	China	1	1	1	2	1	1	1	1	1	1	1	5	2
M126	horse	China	1	1	1	2	1	1	1	1	1	1	1	5	2
M218	horse	China	1	1	1	2	1	1	1	1	1	1	1	5	2
M118	horse	China	1	1	1	2	3	1	1	1	1	1	1	5	3
M330	horse	China	1	1	1	2	2	1	1	1	1	1	2	5	4
A18	hamster	China	1	1	1	2	1	1	1	1	1	1	1	1	5
A19	hamster	China	1	1	1	2	1	1	1	1	1	1	1	1	5
ET-1	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-2	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-3	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-4	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-5	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-6	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-7	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-8	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-9	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
ET-10	cattle	Egypt	1	1	1	2	1	1	1	3	1	1	1	7	6
SN-01	cattle	Sweden	3	1	1	7	1	2	1	2	2	3	5	5	7
SN-02	cattle	Sweden	3	1	1	7	1	2	1	2	2	3	5	5	7
SN-03	cattle	Sweden	3	1	1	7	1	2	1	2	2	3	5	9	8
SN-04	cattle	Sweden	3	1	1	7	1	2	1	2	2	3	5	9	8
SN-13	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	4	9	9
SN-42	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	4	9	9
SN-43	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	4	9	9

SN-44	cattle	Sweden	2	1	1	7	1	2	4	2	2	3	4	9	10
SN-10	cattle	Sweden	3	1	1	4	1	2	4	2	2	3	5	8	11
SN-11	cattle	Sweden	3	1	1	7	1	2	5	2	2	3	5	8	12
SN-12	cattle	Sweden	3	1	1	7	1	2	5	2	2	3	5	8	12
SN-28	cattle	Sweden	3	1	1	7	1	2	2	2	2	3	5	8	13
SN-29	cattle	Sweden	3	1	1	7	1	2	2	2	2	3	5	8	13
SN-30	cattle	Sweden	3	1	1	7	1	2	2	2	2	3	5	8	13
SN-31	cattle	Sweden	3	1	1	7	1	2	2	2	2	3	5	8	13
SN-14	cattle	Sweden	3	1	1	4	1	2	4	2	2	4	5	8	14
SN-17	cattle	Sweden	4	1	2	3	1	2	3	3	2	2	3	2	15
SN-18	cattle	Sweden	4	1	2	3	1	2	3	3	2	2	3	2	15
SN-19	cattle	Sweden	4	1	2	3	1	2	3	3	2	2	3	2	15
SN-24	cattle	Sweden	4	1	2	3	1	2	3	3	2	2	3	2	15
SN-39	cattle	Sweden	3	1	1	7	1	2	1	2	2	3	5	3	16
SN-20	cattle	Sweden	5	1	1	5	1	2	4	4	2	3	5	4	17
SN-21	cattle	Sweden	3	1	1	5	1	2	4	4	2	3	5	4	18
SN-22	cattle	Sweden	3	1	1	7	1	2	1	2	2	3	4	11	19
SN-23	cattle	Sweden	3	1	1	7	1	2	2	2	2	3	4	11	20
SN-25	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	5	10	21
SN-26	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	5	10	21
SN-16	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	4	10	22
SN-32	cattle	Sweden	3	1	1	7	1	2	5	2	2	3	5	6	23
SN-33	cattle	Sweden	3	1	1	7	1	2	5	2	2	3	5	6	23
SN-40	cattle	Sweden	3	1	1	7	1	2	5	2	2	3	5	6	23
SN-41	cattle	Sweden	3	1	1	7	1	2	5	2	2	3	5	6	23
SN-34	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	5	6	24
SN-35	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	5	6	24
SN-36	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	5	6	24
SN-37	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	5	6	24
SN-38	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	5	6	24
SN-45	cattle	Sweden	3	1	1	7	1	2	4	2	2	3	5	6	24
SN-05	cattle	Sweden	3	1	1	6	1	2	5	2	2	3	5	6	25
SN-06	cattle	Sweden	3	1	1	6	1	2	5	2	2	3	5	6	25
SN-07	cattle	Sweden	3	1	1	6	1	2	5	2	2	3	5	6	25
SN-08	cattle	Sweden	3	1	1	6	1	2	5	2	2	3	5	6	25
SN-09	cattle	Sweden	3	1	1	6	1	2	5	2	2	3	5	6	25

*Subtypes 1-11 at the gp60 gene respectively represent IIdA15G1, IIdA16G1, IIdA17G1, IIdA17G1, IIdA19G1, IIdA20G1e, IIdA20G1f, IIdA22G1, IIdA23G1, IIdA24G1, and IIdA26G1.

Table S2 Genetic diversity of *C. parvum* IId DNA sequences at 12 loci

Source	Number of sequences	No. of polymorphic (segregating) sites, S	No. of haplotypes, h	Haplotype diversity, Hd	Nucleotide diversity, Pi	Average number of nucleotide differences, k
China	58	27	3	0.06836	0.00014	0.93103
Sweden	43	76	8	0.71429	0.00223	15.27353
Egypt	10	0	1	0.00000	0.00000	0.0000

Table S3 Genetic diversity of *C. parvum* IId DNA sequences at the GP60 locus

Source	Number of sequences*	No. of polymorphic (segregating) sites, S	No. of haplotypes, h	Haplotype diversity, Hd	Nucleotide diversity, Pi	Average number of nucleotide differences, k
Western Asia (Iran, Jordan, and Kuwait)	13	7	8	0.859	0.00267	2.128
Europe (United Kingdom, Spain, Netherland, Belgium, Portugal, Sweden, Ireland, and Romania)	22	6	7	0.697	0.00252	1.255
Other countries in Asia (China, India, Malaysia)	4	0	1	0.000	0.00000	0.000
Egypt	2	1	2	1.000	0.00116	1.000
Australia	2	1	2	1.000	0.00112	1.000

*The 43 GP60 sequences represent 15 *C. parvum* IId subtypes in various countries downloaded from the NCBI database, including IIdA14G1, IIdA15G1, IIdA16G1, IIdA17G1, IIdA18G1, IIdA19G1, IIdA20G1, IIdA21G1, IIdA22G1, IIdA23G1, IIdA24G1, IIdA25G1, IIdA26G1, IIdA28G1, and IIdA29G1. The accession numbers of GP60 sequences used include AB560748, AB560740, AB560742, AB560743, AB560746, AY738185, AY738186, AY738189, AY738194, AY738193, AB560745, GU458803, EU549718, EU549717, EF576976, HQ005740, DQ280495, EF576975, HQ005740, DQ280496, JQ028866, DQ280497, AY166806, FJ917374, FJ917376, HQ005750, EU868625, JQ028865, EU549714, JX183810, JX043492, EU549712, EU549713, JX183811, HQ631423, GQ121027, HQ009809, JF495152, HQ241928, JF727762, FJ839877, AB514086, and AB514086.

Table S4 Analysis of linkage disequilibrium in *C. parvum* IId isolates

Group	No. of completely typed	Standardized index of association (I^S_A)	$V_D > L$	Significance statistic
Overall (China, Sweden and Egypt)	111	0.5591	Yes	P < 0.001
China	58	0.0297	Yes	P = 0.004
Sweden	43	0.2733	Yes	P < 0.001

Note: V_D = the pairwise variance, L = 95% critical value.

Table S5. Background information for *C. parvum* IId specimens in Sweden

Specimen ID	City (Province)	Farm No.	GP60 subtype	MLST subtype
SN5	Falkoping (Vastergotland)	1	IIdA20G1	25
SN6	Falkoping (Vastergotland)	1	IIdA20G1	25
SN7	Falkoping (Vastergotland)	1	IIdA20G1	25
SN8	Falkoping (Vastergotland)	1	IIdA20G1	25
SN9	Falkoping (Vastergotland)	1	IIdA20G1	25
SN10	Falkoping (Vastergotland)	2	IIdA22G1	11
SN14	Skara (Vastergotland)	2	IIdA22G1	14
SN1	Skara (Vastergotland)	3	IIdA19G1	7
SN2	Skara (Vastergotland)	3	IIdA19G1	7
SN39	Toreboda (Vastergotland)	4	IIdA16G1	16
SN11	Angelholm (Skane)	5	IIdA22G1	12
SN12	Angelholm (Skane)	5	IIdA22G1	12
SN13	Angelholm (Skane)	6	IIdA23G1	9
SN17	Angelholm (Skane)	7	IIdA16G1	15
SN18	Angelholm (Skane)	7	IIdA16G1	15
SN19	Angelholm (Skane)	7	IIdA16G1	15
SN24	Angelholm (Skane)	8	IIdA16G1	15
SN36	Angelholm (Skane)	9	IIdA20G1	24
SN37	Angelholm (Skane)	9	IIdA20G1	24
SN38	Angelholm (Skane)	9	IIdA20G1	24
SN45	Angelholm (Skane)	9	IIdA20G1	24
SN28	Laholm (Skane)	10	IIdA22G1	13
SN29	Laholm (Skane)	11	IIdA22G1	13
SN30	Laholm (Skane)	11	IIdA22G1	13
SN31	Laholm (Skane)	11	IIdA22G1	13
SN40	Degeberga (Skane)	12	IIdA20G1	23
SN41	Degeberga (Skane)	12	IIdA20G1	23
SN42	Vinslov (Skane)	6	IIdA23G1	9
SN43	Vinslov (Skane)	6	IIdA23G1	9
SN44	Vinslov (Skane)	6	IIdA23G1	10
SN3	Vaxjo (Smaland)	13	IIdA23G1	8
SN4	Vaxjo (Smaland)	13	IIdA23G1	8
SN16	Kalmar (Smaland)	14	IIdA24G1	22
SN25	Kalmar (Smaland)	15	IIdA24G1	21
SN26	Kalmar (Smaland)	15	IIdA24G1	21
SN32	Forsheda (Smaland)	16	IIdA20G1	23
SN33	Forsheda (Smaland)	16	IIdA20G1	23
SN20	Linkoping (Ostergotland)	17	IIdA17G1	17
SN21	Linkoping (Ostergotland)	17	IIdA17G1	18
SN22	Uppsala (Uppland)	18	IIdA26G1	19
SN23	Uppsala (Uppland)	18	IIdA26G1	20
SN34	Falkenberg (Halland)	19	IIdA20G1	24
SN35	Falkenberg (Halland)	19	IIdA20G1	24

Figure S1

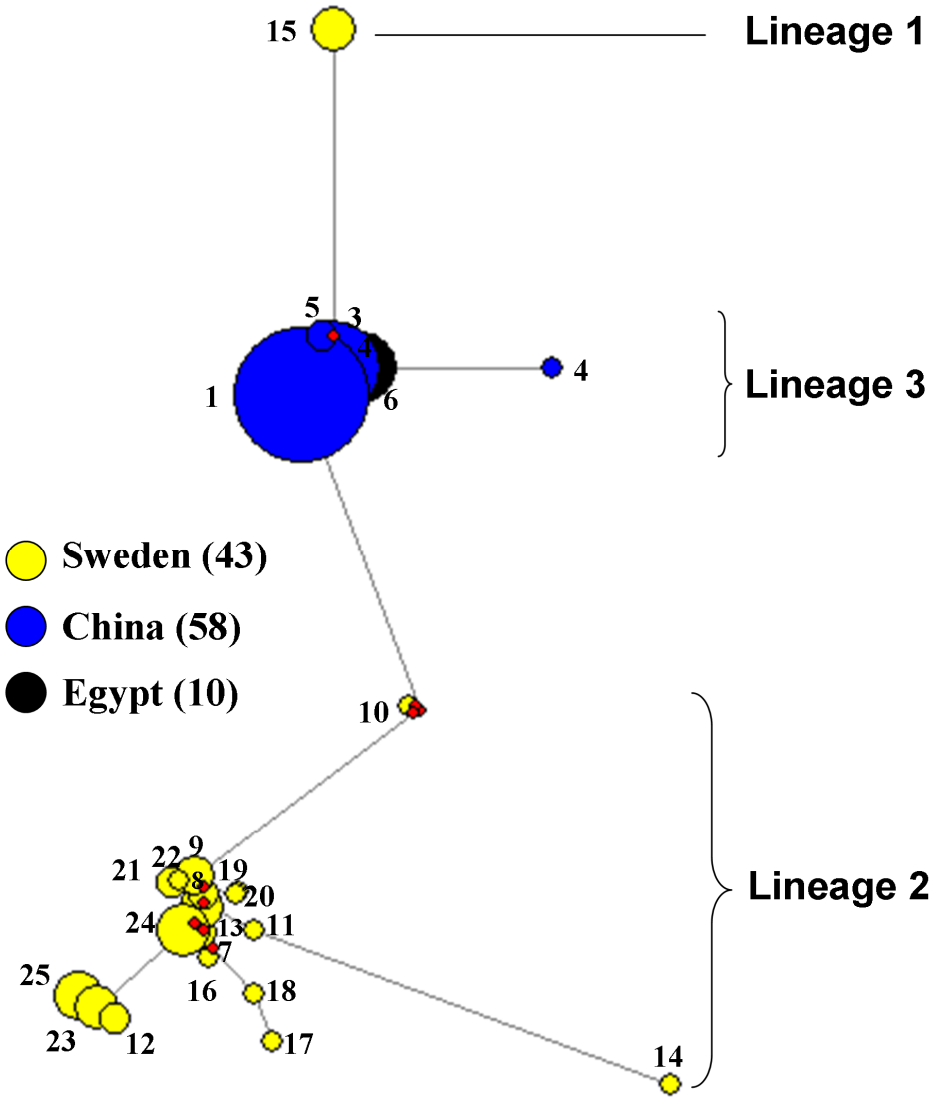

15
Lineage 1
5
3
4
1
6
4
Lineage 3
Sweden (43)
China (58)
Egypt (10)
10
21
22
9
19
8
20
24
13
11
7
25
16
18
23
12
17
14
Lineage 2

Figure S2

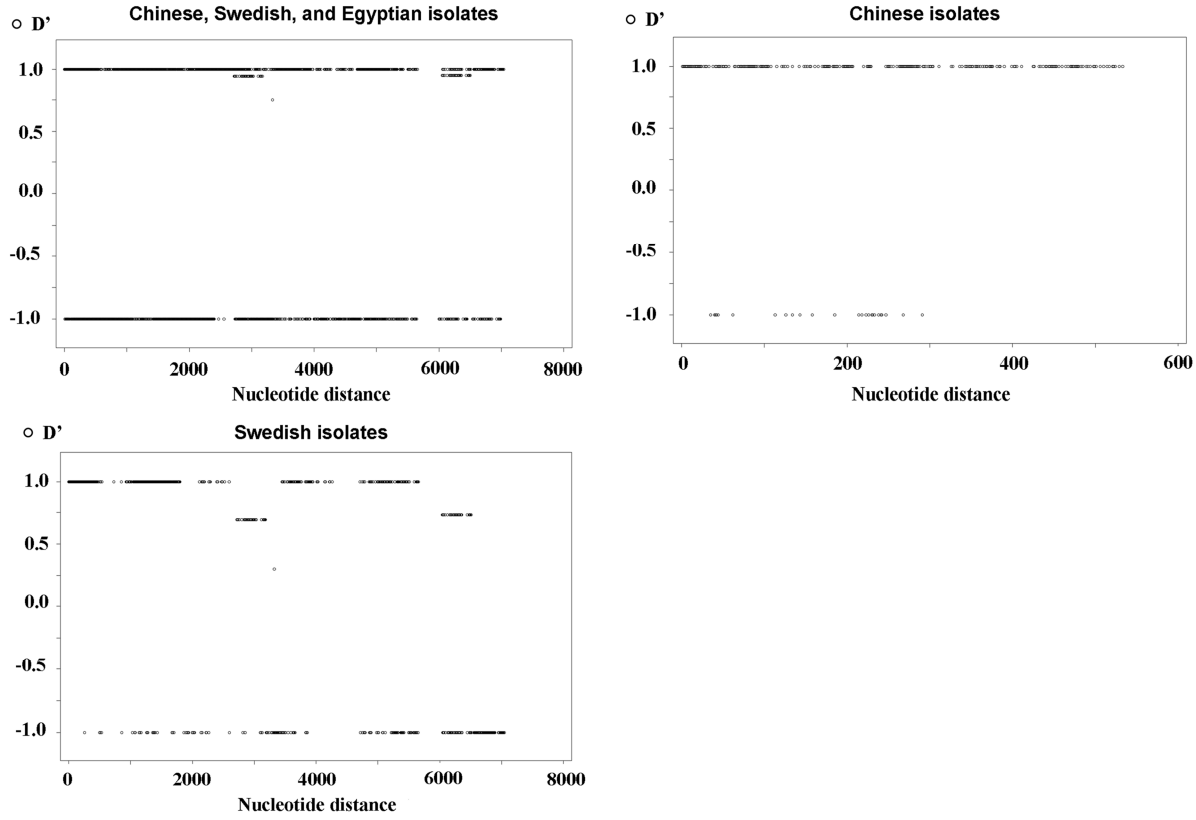
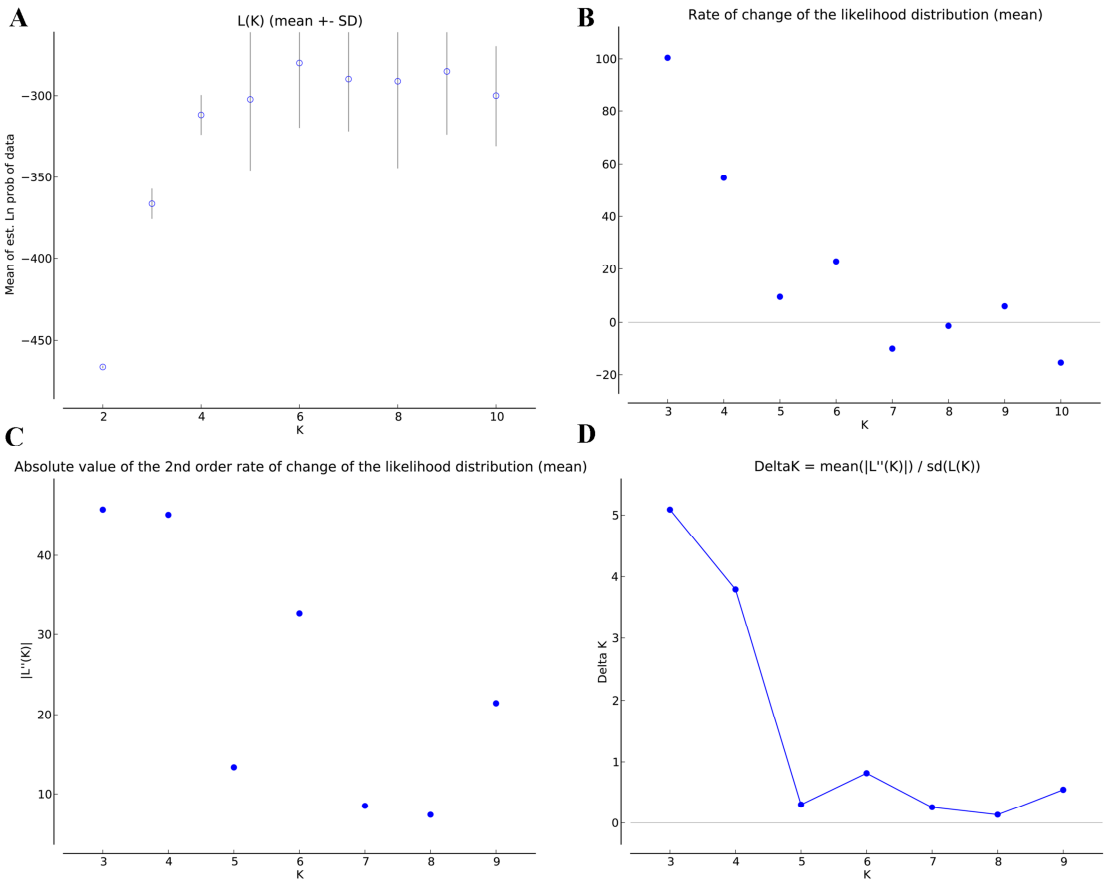

Chinese, Swedish, and Egyptian isolates
D'
1.0
0.5
0.0
-0.5
-1.0
0
2000
4000
6000
8000
Nucleotide distance
Chinese isolates
D'
1.0
0.5
0.0
-0.5
-1.0
0
200
400
600
Nucleotide distance
Swedish isolates
D'
1.0
0.5
0.0
-0.5
-1.0
0
2000
4000
6000
8000
Nucleotide distance

Figure S3


A
L(K) (mean +- SD)
Mean of est. Ln prob of data
−300
−350
−400
−450
2
4
6
8
10
K
B
Rate of change of the likelihood distribution (mean)
100
80
60
40
20
0
−20
3
4
5
6
7
8
9
10
K
C
Absolute value of the 2nd order rate of change of the likelihood distribution (mean)
|L''(K)|
40
30
20
10
3
4
5
6
7
8
9
K
D
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
5
4
3
2
1
0
3
4
5
6
7
8
9
K