Table S1. *Wolbachia,* SGHV and *Trypanosoma* prevalence in *Gff* populations (divided by mtDNA haplogroup and sex as well as total) belonging to:

(a) the northern microsatellite group: AR, BK, BN, DK, MY, OS and PT

	mtDNA	Sex	Sample Size	% *Wolbachia*	% SGHV	% *Trypanosoma*
AR	N	♀	7	28.6	28.6	14.3
	N	♂	3	0	0	0
	Total		10	20	20	10
BK	N	♀	38	50	7.9	7.9
	N	♂	31	58.1	6.5	9.7
	Total		69	53.6	7.2	8.7
BN	N	♀	8	12.5	25	25
	N	♂	2	0	50	0
	S	♀	2	0	0	50
	S	♂	3	0	0	33.3
	Total		15	6.7	20	26.7
DK	N	♀	14	57.1	0	7.1
	N	♂	14	71.4	35.7	21.4
	Total		28	64.3	17.9	14.3
MY	N	♀	0	-	-	-
	N	♂	16	31.3	25	12.5
	Total		37	51.4	10.8	5.4
OS	N	♀	7	14.3	14.3	57.1
	N	♂	13	23.1	23.1	30.8
	Total		20	20	20	40
PT	N	♀	10	20	20	20
	N	♂	7	0	0	0
	Total		17	11.8	11.8	11.8

(b) the southern microsatellite group: BU, BV, JN*, NA, KL and SS

	mtDNA	**Sex**	**Sample Size**	**% *Wolbachia***	**% SGHV**	**% *Trypanosoma***
BU	S	♀	19	31.6	0	26.3
	S	♂	15	26.7	0	20
	Total		34	29.4	0	23.5
BV	S	♀	20	75	0	0
	S	♂	15	40	13.3	0
	Total		35	60	5.7	0
JN	N	♀	19	57.9	5.3	26.3
	N	♂	21	71.4	23.8	42.9
	Total		43	65.1	14	34.9
KL	S	♀	5	100	0	0
	S	♂	5	100	0	0
	Total		10	100	0	0
NA	S	♀	19	5.3	15.8	10.5
	S	♂	20	10	20	25
	Total		40	7.5	17.5	17.5
SS	S	♀	12	83.3	0	16.7
	S	♂	29	89.7	13.8	44.8
	Total		52	86.5	7.7	28.8

*Both northern (N) and southern (S) mtDNA haplogroups co-occur in JN individuals

(J. S. Beadell et al., PLoS Negl. Trop. Dis. 4:e636, 2010), but the individuals we have infection data for are all assigned to the northern haplogroup.

(c) the western microsatellite group: KB, KF, KK, KR and MS

	mtDNA	Sex	Sample Size	% *Wolbachia*	% SGHV	% *Trypanosoma*
KB	S	♀	9	44.4	0	22.2
	S	♂	15	53.3	0	33.3
	Total		24	50	0	29.2
KF	N	♀	8	37.5	62.5	12.5
	N	♂	7	0	28.6	57.1
	S	♀	1	0	100	0
	S	♂	1	0	0	0
	Total		17	17.6	47.1	29.4
KK	S	♀	14	50	21.4	21.4
	S	♂	14	21.4	14.3	7.1
	Total		28	35.7	17.9	14.3
KR	N	♀	8	0	12.5	0
	N	♂	4	0	25	25
	S	♀	0	-	-	-
	S	♂	3	0	33.3	0
	Total		15	0	20	6.7
MS	N	♀	4	0	0	25
	N	♂	5	20	0	20
	S	♀	5	0	0	20
	S	♂	2	0	0	0
	Total		16	6.3	0	18.8

Table S2. (a) *Wolbachia* (*Wol.*), SGHV and *Trypanosoma* (*Tryp.*) individual infection comparisons. Group means were compared by simple logistic regression contrast. Tsetse flies were assigned to groups based on sampling locality (abbreviations from Table 1), microsatellite data (n=north, s=south, w=west) and mtDNA data (N=north, S=south). We also compared sexes (F=female, M=male) within these groups. The table only contains comparisons with significant p-values (Pr(>|z|)). P-values have also been corrected (Adj(Pr(>|z|))) using logically constrained multiplicity adjustment (P. H. Westfall, J. Am. Stat. Assoc. 92:299-306, 1997), except in the case of comparisons involving sampling locality where 'free combination' adjustment was used (Westfall and SAS Institute, Cary, NC 1999). Comparisons significant after adjustment are shown in bold type (Adj(Pr(>|z|))<0.05).

Wolbachia				
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
s	n	2.49	0.013	**0.013**
n	w	2.73	0.006	**0.006**
s	w	4.63	0.000	**0.000**
BV (F)	BV (M)	2.04	0.042	0.514
BK	BN	2.62	0.009	0.285
BK	BU	2.28	0.022	0.501
BK	KF	2.48	0.013	0.374
BK	MS	2.69	0.007	0.251
BK	NA	4.11	0.000	**0.003**
BK	OS	2.52	0.012	0.352
BK	PT	2.73	0.006	0.233
BU	NA	2.31	0.021	0.482

BV	AR	2.08	0.038	0.645
BV	BN	2.79	0.005	0.204
BV	BU	2.51	0.012	0.355
BV	KF	2.69	0.007	0.251
BV	MS	2.86	0.004	0.172
BV	NA	4.21	0.000	**0.002**
BV	OS	2.73	0.006	0.233
BV	PT	2.92	0.003	0.149
DK	AR	2.23	0.025	0.525
DK	BN	2.91	0.004	0.152
DK	BU	2.68	0.007	0.251
DK	KF	2.84	0.004	0.181
DK	KK	2.11	0.035	0.624
DK	MS	2.98	0.003	0.128
DK	NA	4.32	0.000	**0.001**
DK	OS	2.89	0.004	0.163
DK	PT	3.06	0.002	0.103
JN	AR	2.36	0.018	0.449
JN	BN	3.01	0.003	0.119
JN	BU	3.04	0.002	0.111
JN	KF	3.04	0.002	0.111
JN	KK	2.39	0.017	0.434
JN	MS	3.08	0.002	0.100
JN	NA	4.61	0.000	**0.000**
JN	OS	3.12	0.002	0.090

JN	PT	3.23	0.001	0.066
KB	BN	2.37	0.018	0.439
KB	KF	2.04	0.042	0.673
KB	MS	2.44	0.015	0.401
KB	NA	3.46	0.001	**0.032**
KB	OS	2.00	0.045	0.698
KB	PT	2.35	0.019	0.452
KK	NA	2.68	0.007	0.253
SS	AR	3.65	0.000	**0.016**
SS	BK	3.63	0.000	**0.018**
SS	BN	4.05	0.000	**0.004**
SS	BU	4.94	0.000	**0.000**
SS	BV	2.73	0.006	0.233
SS	DK	2.25	0.025	0.522
SS	JN	2.39	0.017	0.434
SS	KB	3.23	0.001	0.066
SS	KF	4.51	0.000	**0.000**
SS	KK	4.32	0.000	**0.001**
SS	MS	4.12	0.000	**0.003**
SS	NA	6.03	0.000	**0.000**
SS	OS	4.70	0.000	**0.000**
SS	PT	4.53	0.000	**0.000**
		SGHV		
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
s (M)	s (F)	2.25	0.024	0.071

N	S	2.28	0.022	**0.022**
KF	BK	3.62	0.000	**0.019**
KF	BV	3.07	0.002	0.111
KF	DK	2.03	0.042	0.756
KF	JN	2.60	0.009	0.343
KF	KK	2.03	0.042	0.756
KF	NA	2.24	0.025	0.608
KF	PT	2.12	0.034	0.701
KF	SS	3.33	0.001	**0.051**
Trypanosoma				
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
s (M)	s (F)	2.39	0.017	**0.050**
BU	BK	1.99	0.046	0.796
JN	BK	3.24	0.001	0.074
KB	BK	2.36	0.018	0.527
KF	BK	2.16	0.031	0.681
OS	BK	3.11	0.002	0.105
OS	DK	1.96	0.050	0.813
OS	KK	1.96	0.050	0.813
OS	KR	1.97	0.048	0.805
SS	BK	2.76	0.006	0.255

Table S2. (b) *Wolbachia* (*Wol.*), SGHV and *Trypanosoma* (*Tryp.*) co-infection comparisons. Group means were compared by multiple logistic regression contrast. For two-infection comparisons, the groups compared were microsatellites, mtDNA and sampling site (only: AR, BK, BN, DK, JN, KF, KK, NA, OS, PT and SS). For three-infection comparisons, the groups compared were microsatellites (n=north, s=south, w=west) and mtDNA (N=north, S=south). In two-infection comparisons, one infection was compared for differences between groups when the other infection was present (+) or absent (-). In three-infection comparisons, one infection was compared for differences between groups when the other two infections were present (+) or absent (-). Only comparisons with significant p-values (Pr(>|z|)) are reported. Multiple comparison correction was done using logically constrained multiplicity adjustment (Westfall, J. Am. Stat. Assoc. 92:299-306, 1997), except in the case of comparisons involving sampling site where 'free combination' adjustment was used (Westfall and SAS Institute, Cary, NC, 1999). Adjusted p-values (Adj(Pr(>|z|))) smaller than 0.05 are shown in bold type.

Wol-				
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
SGHV (N)	SGHV (S)	2.36	0.018	**0.037**
SGHV (KF)	SGHV (BK)	2.04	0.041	0.828
Tryp (BN)	*Tryp* (BK)	2.14	0.032	0.763
Tryp (JN)	*Tryp* (BK)	2.89	0.004	0.208
Tryp (JN)	*Tryp* (KK)	2.34	0.019	0.605
Tryp (JN)	*Tryp* (NA)	1.98	0.047	0.860
Tryp (JN)	*Tryp* (PT)	2.17	0.030	0.746
Tryp (KF)	*Tryp* (BK)	2.46	0.014	0.512

Tryp (KF)	*Tryp* (KK)	1.92	0.055	0.892
Tryp (OS)	*Tryp* (BK)	2.56	0.010	0.423
Tryp (OS)	*Tryp* (KK)	2.02	0.044	0.844
		SGHV-		
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
Wol (s)	*Wol* (w)	4.2	0.000	**0.000**
Wol (n)	*Wol* (w)	2.44	0.015	**0.053**
Wol (s)	*Wol* (n)	2.31	0.021	0.074
Wol (BK)	*Wol* (AR)	1.94	0.052	0.837
Wol (BK)	*Wol* (BN)	2.41	0.016	0.491
Wol (BK)	*Wol* (NA)	3.8	0.000	**0.010**
Wol (BK)	*Wol* (OS)	2.4	0.016	0.494
Wol (BK)	*Wol* (PT)	2.57	0.010	0.366
Wol (DK)	*Wol* (AR)	2.07	0.038	0.748
Wol (DK)	*Wol* (BN)	2.52	0.012	0.410
Wol (DK)	*Wol* (NA)	3.7	0.000	**0.013**
Wol (DK)	*Wol* (OS)	2.48	0.013	0.437
Wol (DK)	*Wol* (PT)	2.65	0.008	0.311
Wol (JN)	*Wol* (AR)	2.28	0.023	0.599
Wol (JN)	*Wol* (BN)	2.74	0.006	0.259
Wol (JN)	*Wol* (NA)	4.19	0.000	**0.002**
Wol (JN)	*Wol* (OS)	2.86	0.004	0.192
Wol (JN)	*Wol* (PT)	2.98	0.003	0.144
Wol (KK)	*Wol* (NA)	2.77	0.006	0.243
Wol (SS)	*Wol* (AR)	3.24	0.001	0.066

Wol (SS)	*Wol* (BK)	3.29	0.001	0.056
Wol (SS)	*Wol* (BN)	3.71	0.000	**0.013**
Wol (SS)	*Wol* (DK)	2.24	0.025	0.623
Wol (SS)	*Wol* (JN)	2.16	0.031	0.689
Wol (SS)	*Wol* (KK)	3.46	0.001	**0.033**
Wol (SS)	*Wol* (NA)	5.57	0.000	**0.000**
Wol (SS)	*Wol* (OS)	4.26	0.000	**0.002**
Wol (SS)	*Wol* (PT)	4.22	0.000	**0.002**
Tryp (BN)	*Tryp* (BK)	2.11	0.035	0.790
Tryp (JN)	*Tryp* (BK)	2.77	0.006	0.285
Tryp (JN)	*Tryp* (NA)	1.95	0.051	0.879
Tryp (OS)	*Tryp* (BK)	2.62	0.009	0.389
Tryp (OS)	*Tryp* (NA)	1.98	0.048	0.866
Tryp (SS)	*Tryp* (BK)	2.38	0.018	0.581
SGHV+				
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
Tryp (s)	*Tryp* (n)	2.17	0.030	0.146
Tryp-				
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
Wol (s)	*Wol* (w)	4.24	0.000	**0.000**
Wol (n)	*Wol* (w)	2.57	0.010	**0.036**
Wol (s)	*Wol* (n)	2.28	0.023	0.079
Wol (BK)	*Wol* (BN)	2.16	0.031	0.713
Wol (BK)	*Wol* (NA)	3.56	0.000	**0.026**
Wol (BK)	*Wol* (OS)	2.01	0.044	0.815

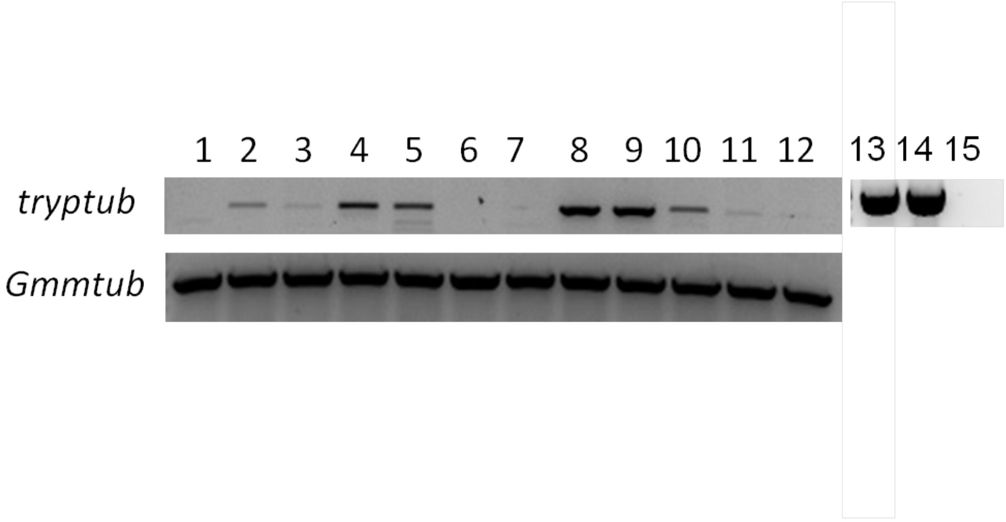
Wol (BK)	*Wol* (PT)	2.51	0.012	0.436
Wol (DK)	*Wol* (AR)	1.95	0.052	0.852
Wol (DK)	*Wol* (BN)	2.49	0.013	0.448
Wol (DK)	*Wol* (KF)	2.04	0.041	0.800
Wol (DK)	*Wol* (KK)	2.27	0.023	0.629
Wol (DK)	*Wol* (NA)	3.81	0.000	**0.011**
Wol (DK)	*Wol* (OS)	2.4	0.016	0.518
Wol (DK)	*Wol* (PT)	2.82	0.005	0.226
Wol (JN)	*Wol* (AR)	2.4	0.017	0.520
Wol (JN)	*Wol* (BN)	2.85	0.004	0.211
Wol (JN)	*Wol* (KF)	2.56	0.011	0.395
Wol (JN)	*Wol* (KK)	2.94	0.003	0.172
Wol (JN)	*Wol* (NA)	4.37	0.000	**0.001**
Wol (JN)	*Wol* (OS)	2.87	0.004	0.201
Wol (JN)	*Wol* (PT)	3.18	0.001	0.086
Wol (SS)	*Wol* (AR)	3.16	0.002	0.095
Wol (SS)	*Wol* (BK)	3.14	0.002	0.098
Wol (SS)	*Wol* (BN)	3.46	0.001	**0.036**
Wol (SS)	*Wol* (KF)	3.42	0.001	**0.041**
Wol (SS)	*Wol* (KK)	4	0.000	**0.005**
Wol (SS)	*Wol* (NA)	5.25	0.000	**0.000**
Wol (SS)	*Wol* (OS)	3.64	0.000	**0.020**
Wol (SS)	*Wol* (PT)	3.8	0.000	**0.011**
SGHV (w)	SGHV (s)	2.1	0.035	0.168
SGHV (n)	SGHV (s)	1.98	0.048	0.168

SGHV (KF)	SGHV (BK)	2.83	0.005	0.255
SGHV (KF)	SGHV (JN)	2.11	0.035	0.801
SGHV (KF)	SGHV (NA)	2.08	0.038	0.820
SGHV (KF)	SGHV (SS)	2.71	0.007	0.331
Tryp+				
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
Wol (BK)	*Wol* (OS)	1.98	0.048	0.834
Wol (SS)	*Wol* (JN)	2.17	0.030	0.713
Wol (SS)	*Wol* (OS)	2.84	0.005	0.220
SGHV (KF)	SGHV (SS)	1.92	0.055	0.904
SGHV- & Tryp.-				
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
Wol. (s)	*Wol*. (w)	4.04	0.000	**0.000**
Wol. (n)	*Wol*. (w)	2.57	0.010	0.081
Wol. (s)	*Wol*. (n)	2.01	0.045	0.309
Wol.+ & Tryp.-				
Comparison		**z**	**Pr(>|z|)**	**Adj(Pr(>|z|))**
SGHV (N)	SGHV (S)	2.16	0.031	0.117

Table S3. Simple and multiple logistic regression models. Multiple models include two-pathogen (one pathogen as an independent variable and another as a dependent variable) and three-pathogen regression (two pathogens as independent variables and the third as a dependent variable).

Logistic Regression Models				**Dependent Variables**		
				Wol.	**SGHV**	**Tryp.**
Regression	**Simple**	**Independent Variables**	**Host Groups**	Pop	Pop	Pop
				msat	msat	msat
				mtDNA	mtDNA	mtDNA
				Sex	Sex	Sex
	Multiple		**Host Groups**	Sex*Pop	Sex*Pop	Sex*Pop
				Sex*msat	Sex*msat	Sex*msat
				Sex*mtDNA	Sex*mtDNA	Sex*mtDNA
			Two Pathogens	SGHV*Pop	Wol*Pop	SGHV*Pop
				Tryp*Pop	Tryp*Pop	Wol*Pop
				SGHV*msat	Wol*msat	SGHV*msat
				Tryp*msat	Tryp*msat	Wol*msat
				SGHV*mtDNA	Wol*mtDNA	SGHV*mtDNA
				Tryp*mtDNA	Tryp*mtDNA	Wol*mtDNA
			Three Pathogens	SGHV*Tryp*msat	Wol*Tryp*msat	SGHV*Wol*msat
				SGHV*Tryp*mtDNA	Wol*Tryp*mtDNA	SGHV*Wol*mtDNA

Figure S1. Trypanosome infection detection in challenged flies



In order to confirm that the parasites detected in field flies represent true infections, we performed a laboratory experiment where 12 newly eclosed G*mm* males were given an infectious bloodmeal containing 10^5 parasites/ml. Subsequently DNA was prepared from whole flies 48h post challenge. PCR amplification was performed on fly DNA using trypanosome *tubulin* primer set (Lanes 1-12). Trypanosome DNA (Lane 13) and midgut DNA from a fly, which was confirmed to be trypanosome infected by dissection and microscopy two weeks after exposure (Lane 14) were used as positive controls. No DNA control is shown in Lane 15 and served as the negative control. Tsetse *β-tubulin (Gmmtub)* was used as an internal control. All PCR conditions were the same as those performed with the *Gff* field samples (40 cycles of 94ºC for 1 min., 55ºC for 30 sec. and 72ºC for 30 sec., with a final 72ºC). The PCR experiment was independently repeated twice.