

# **Wolbachia load variation in *Drosophila* is more likely caused by drift than by host genetic factors**

## **Supporting information**

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**Table s1: Characteristics of oligonucleotide primers** used for the quantification of *Wolbachia* relative density and average Octomom copy number by qPCR.

**Table s2: Statistics associated with linear regression models on data from experiment #1** (relative wMelPop density and average Octomom copy number).

**Table s3: Pairwise comparisons performed from linear regression outputs of the experiment #1** (relative wMelPop density and average Octomom copy number).

**Table s4: Statistics associated with linear regression models on data from experiment #2** (relative wMelPop density and average Octomom copy number).

**Table s5: Pairwise comparisons performed on the linear regression models from the second introgression experiment** (relative wMelPop density and average Octomom copy number).

**Table s6: Statistics associated with linear regression models on data from the reciprocal crosses experiment** (relative wMelPop density and average Octomom copy).

**Table s7: Pairwise comparisons performed on the linear regression models from the reciprocal crosses experiment** (relative wMelPop density and average Octomom copy).

**Figure s1: Relationship between the relative density and the average octomom copy number per *Wolbachia*** in replicate lines at each timepoint of experiment #1.

**Figure s2: Relationship between the relative density and the average octomom copy number per *Wolbachia*** in replicate lines at each timepoint of the new introgression experiment (exp. #2).

**Figure s3: Correlation between the relative density and the average octomom copy number per *Wolbachia*** within each replicate line of the new introgression experiment (exp. #2).

**Figure s4: Correlation between the relative density and the average octomom copy number per *Wolbachia*** in replicate lines at each timepoint of the 'reciprocal crosses' experiment (exp. #3).

**Figure s5: Correlation between the relative density and the average octomom copy number per *Wolbachia*** within each line of the 'reciprocal crosses' experiment (exp. #3).

**Table s1: Characteristics of oligonucleotide primers** used for the quantification of *Wolbachia* relative density and average Octomom copy number by qPCR. The amplification efficiency (mean  $\pm$  SE) was determined by in-run standard curves, using 2 duplicates of a 10-fold dilution series of purified PCR products from  $10^3$  to  $10^8$  copies.

Gene	Sequence	Efficiency	References
RP49dd-R	5'-CGA-TCT-CGC-CGC-AGT-AAC-3'	96.7 % $\pm$ 0.6	Steckel and Boutros, 2005
RP49dd-F	5'-CTG-CCC-ACC-GGA-TTC-AAG-3'		
WD0505-qR	5'-ACG-CGA-GCA-TCT-TCC-ATA-AG-3'	94.6 % $\pm$ 0.7	Chrostek <i>et al.</i> , 2013
WD0505-qF	5'-TGT-TCC-TGG-TGG-ATC-ATC-TG-3'		
WD0513-qR	5'-AGC-ATG-TCC-TCT-GTG-CCA-TC-3'	93.3 % $\pm$ 0.9	Chrostek <i>et al.</i> , 2013
WD0513-qF	5'-TTA-ACC-GGC-CAG-TCT-TAT-CG-3'		

Chrostek, E. *et al.* (2013) 'Wolbachia Variants Induce Differential Protection to Viruses in *Drosophila melanogaster*: A Phenotypic and Phylogenomic Analysis', *PLoS Genetics*, 9(12). doi: 10.1371/journal.pgen.1003896.

Steckel, M. and Boutros, M. (2005) 'Rapid Development of Real-Time RT-PCR Assays Using Universal ProbeLibrary: Applications for Dissecting Signaling Pathways by RNA Interference', *Biochemica*, 3, pp. 17–19.

**Table s2: Statistics associated with linear regression models on data from experiment #1** (relative wMelPop density and average Octomom copy number per *Wolbachia*).

Lines	Mean density	SEM density	Mean Octomom	SEM Octomom	P-value density	P-value Octomom
w1118-MP	1.23	0.21	1.50	0.20	Reference	Reference
Arabia-MP1	2.69	0.81	2.78	0.45	0.047	0.047
Bolivia-MP1	1.14	0.11	1.27	0.08	0.955	0.633
RC-MP1	2.16	0.62	3.17	0.88	0.149	0.030
China-MP1	1.62	0.26	2.29	0.37	0.325	0.169
France-MP1	3.87	1.22	5.02	1.13	0.004	< 0.001
USA-MP1	9.48	1.13	7.40	0.54	< 0.001	< 0.001
w1118-CS	1.20	0.17	1.03	0.14	ND	ND

**Table s3: Pairwise comparisons performed from linear regression outputs of the experiment #1.**

Summary information of the pairwise comparisons with p-values adjusted using the Tukey's method. Analyses from bacterial density (log-transformed data) and Octomom copy numbers (log-transformed data) respectively below and above the black diagonal.

	w1118-MP	Arabia-MP1	Bolivia-MP1	RC-MP1	China-MP1	USA-MP1	France-MP1	Octomom Copy Number
w1118-MP		0.147	0.993	0.340	0.589	< 0.001	< 0.001	
Arabia-MP1	0.041		0.022	0.998	0.986	0.001	0.186	
Bolivia-MP1	1.000	0.372		0.003	0.185	< 0.001	< 0.001	
RC-MP1	0.767	0.997	0.733		0.834	0.009	0.491	
China-MP1	0.954	0.943	0.940	0.999		< 0.001	0.022	
USA-MP1	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		0.683	
France-MP1	0.005	0.961	0.050	0.728	0.427	0.001		
Bacterial density								

**Table s4: Statistics associated with linear regression models on data from experiment #2 (relative wMelPop density and average Octomom copy number).**

Replicate lines	Mean density	SEM density	Mean Octomom	SEM Octomom	P-value density	P-value Octomom	Generation
w1118-MP	1.01	0.14	1.72	0.23			1
Bolivia-MP1	1.51	0.29	1.07	0.02	> 0.1	> 0.1	1
Bolivia-MP2-L1	0.72	0.24	0.96	0.40			
Bolivia-MP2-L2	0.80	0.63	0.90	0.63			
Bolivia-MP2-L3	1.36	0.22	1.78	0.05			
w1118-MP vs Bolivia-MP2 lines	ND	ND	ND	ND	0.699	0.718	1
Bolivia-MP1	0.95	0.36	0.36	0.22	> 0.1	0.022	13
Bolivia-MP2-L1	5.07	8.20	3.40	1.49			
Bolivia-MP2-L2	1.07	7.32	0.93	1.23			
Bolivia-MP2-L3	1.42	8.40	0.98	1.84			
Bolivia-MP1	0.70	0.05	0.87	0.04	< 0.001	< 0.001	25
Bolivia-MP2-L1	3.49	0.42	4.49	0.66			
Bolivia-MP2-L2	0.91	0.07	0.88	0.03			
Bolivia-MP2-L3	2.75	0.52	0.90	0.23			
USA-MP1	6.17	3.36	4.86	0.98	0.081	0.025	1
USA-MP2-L1	1.35	1.02	1.78	0.44			
USA-MP2-L2	2.06	0.95	2.65	1.88			
USA-MP2-L3	2.44	1.22	1.86	0.36			
w1118-MP vs USA-MP2 lines	ND	ND	ND	ND	0.015	0.345	1
USA-MP1	5.06	0.47	2.51	0.47	> 0.1	0.005	13
USA-MP2-L1	1.95	2.86	2.99	0.51			
USA-MP2-L2	3.18	2.73	2.86	0.65			
USA-MP2-L3	1.04	0.40	1.30	0.33			
USA-MP1	8.57	0.73	6.28	0.47	< 0.001	< 0.001	25
USA-MP2-L1	7.41	1.39	6.01	0.75			
USA-MP2-L2	6.39	0.85	5.53	0.49			
USA-MP2-L3	1.02	0.17	0.84	0.26			
Generation effect in Bolivia-MP2 lines	ND	ND	ND	ND	< 0.001	< 0.001	1 vs. 25
Generation effect in USA-MP2 lines	ND	ND	ND	ND	< 0.001	0.005	1 vs. 25

**Table s5: Pairwise comparisons performed on the linear regression models from the second introgression experiment.**

Summary information of the pairwise comparison with adjusted P-values with Tukey's method (bacterial density and Octomom copy numbers respectively below and above the black diagonal).

Generation 1	Bolivia-MP2-L1	Bolivia-MP2-L2	Bolivia-MP2-L3	USA-MP2-L1	USA-MP2-L2	USA-MP2-L3	
Bolivia-MP2-L1		0.699	0.626	ND	ND	ND	Octomom Copy Number
Bolivia-MP2-L2	0.513		0.999	ND	ND	ND	
Bolivia-MP2-L3	0.548	0.999		ND	ND	ND	
USA-MP2-L1	ND	ND	ND		0.270	0.999	
USA-MP2-L2	ND	ND	ND	0.733		0.304	
USA-MP2-L3	ND	ND	ND	0.727	1.000		
Bacterial density							
Generation 13	Bolivia-MP2-L1	Bolivia-MP2-L2	Bolivia-MP2-L3	USA-MP2-L1	USA-MP2-L2	USA-MP2-L3	
Bolivia-MP2-L1		0.831	0.999	ND	ND	ND	Octomom Copy Number
Bolivia-MP2-L2	0.929		0.799	ND	ND	ND	
Bolivia-MP2-L3	0.999	0.908		ND	ND	ND	
USA-MP2-L1	ND	ND	ND		0.921	0.013	
USA-MP2-L2	ND	ND	ND	0.996		0.044	
USA-MP2-L3	ND	ND	ND	0.510	0.390		
Bacterial density							
Generation 25	Bolivia-MP2-L1	Bolivia-MP2-L2	Bolivia-MP2-L3	USA-MP2-L1	USA-MP2-L2	USA-MP2-L3	
Bolivia-MP2-L1		< 0.001	< 0.001	ND	ND	ND	Octomom Copy Number
Bolivia-MP2-L2	< 0.001		0.943	ND	ND	ND	
Bolivia-MP2-L3	0.011	0.003		ND	ND	ND	
USA-MP2-L1	ND	ND	ND		0.806	< 0.001	
USA-MP2-L2	ND	ND	ND	0.394		< 0.001	
USA-MP2-L3	ND	ND	ND	< 0.001	< 0.001		
Bacterial density							

**Table s6: Statistics associated with linear regression models on data from the reciprocal crosses experiment (relative wMelPop density and average Octomom copy).**

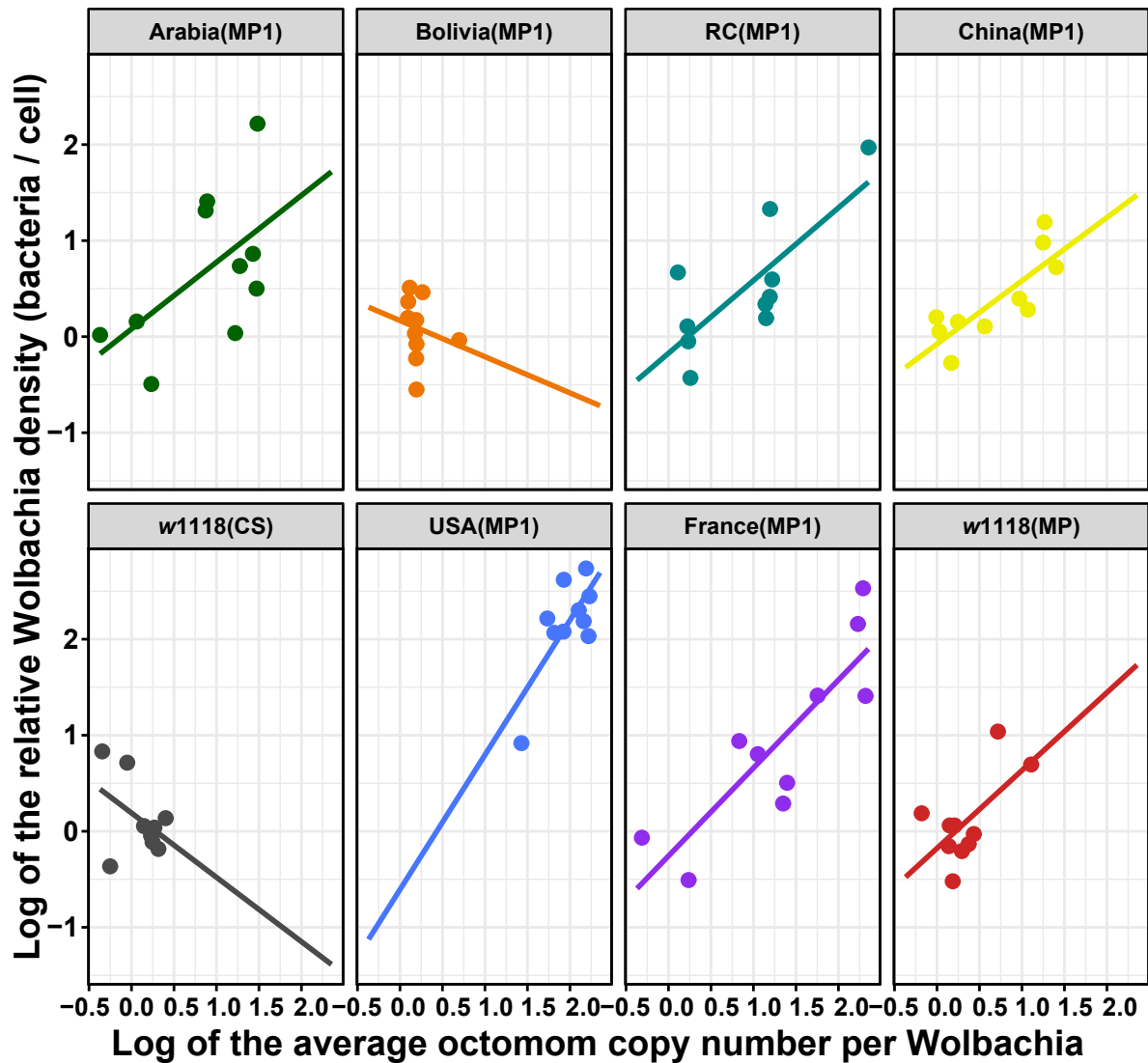
Replicate lines	Mean density	SEM density	Mean Octomom	SEM Octomom	P-value density	P-value Octomom	Generation
Bolivia-MP1	1.23	0.28	1.09	0.02	0.047	< 0.001	1
Bolivia(USA-MP1)L1	5.39	1.79	5.34	0.74			
Bolivia(USA-MP1)L2	5.80	2.83	5.53	1.80			
Bolivia(USA-MP1)L3	3.75	1.37	4.74	1.36			
Bolivia-MP1	1.24	0.36	0.53	0.22	< 0.001	< 0.001	13
Bolivia(USA-MP1)L1	9.20	2.01	3.37	0.67			
Bolivia(USA-MP1)L2	2.26	0.64	1.91	0.17			
Bolivia(USA-MP1)L3	3.85	1.19	2.34	0.26			
Bolivia-MP1	0.73	0.04	0.87	0.04	< 0.001	< 0.001	25
Bolivia(USA-MP1)L1	1.51	0.14	0.83	0.08			
Bolivia(USA-MP1)L2	16.58	2.64	7.99	1.38			
Bolivia(USA-MP1)L3	10.99	4.54	4.47	0.93			
USA-MP1	6.46	3.09	3.79	1.08	0.018	< 0.001	1
USA(Bolivia-MP1)L1	1.74	0.49	1.27	0.26			
USA(Bolivia-MP1)L2	1.37	0.37	1.38	0.26			
USA(Bolivia-MP1)L3	1.01	0.08	1.21	0.09			
USA-MP1	4.80	0.48	2.52	0.47	< 0.001	< 0.001	13
USA(Bolivia-MP1)L1	0.79	0.20	0.88	0.05			
USA(Bolivia-MP1)L2	1.01	0.17	1.07	0.15			
USA(Bolivia-MP1)L3	0.36	0.05	0.56	0.02			
USA-MP1	8.50	0.73	6.20	0.47	< 0.001	< 0.001	25
USA(Bolivia-MP1)L1	1.05	0.15	0.96	0.05			
USA(Bolivia-MP1)L2	0.94	0.08	0.99	0.04			
USA(Bolivia-MP1)L3	1.99	0.29	0.99	0.03			
Generation effect in Bolivia(USA-MP1) lines	ND	ND	ND	ND	0.015	< 0.001	1 vs. 25
Generation effect in USA(Bolivia-MP1) lines	ND	ND	ND	ND	0.052	0.059	1 vs. 25
Bolivia(USA-MP1) lines vs USA-MP1	ND	ND	ND	ND	0.798	0.586	1
USA(Bolivia-MP1)lines vs Bolivia-MP1	ND	ND	ND	ND	0.416	0.559	1

**Table s7: Pairwise comparisons performed on the linear regression models from the ‘reciprocal crosses’ experiment.**

Summary information of the pairwise comparison with adjusted P-values with Tukey’s method (bacterial density and Octomom copy numbers respectively below and above the black diagonal).

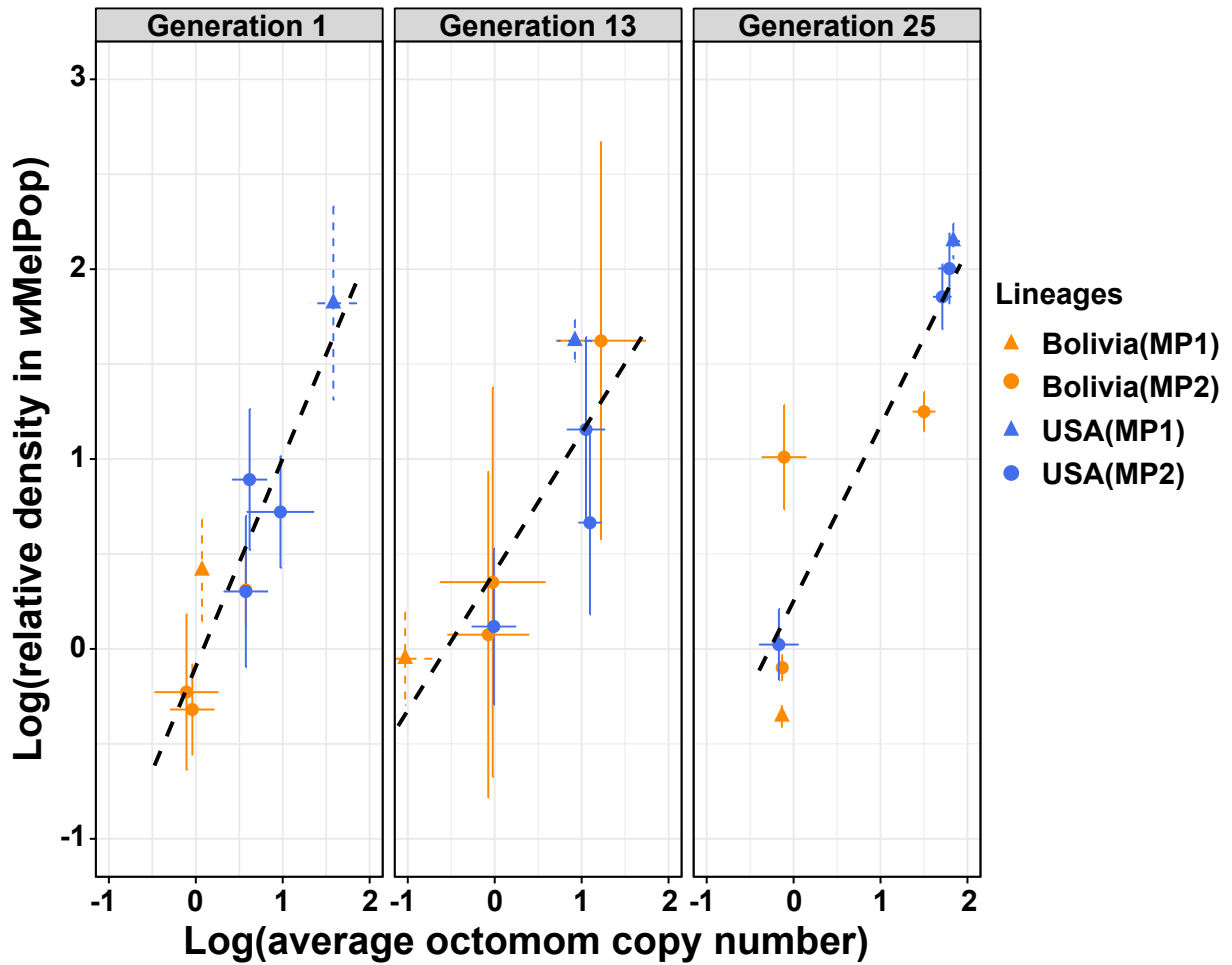
Generation 1	Bolivia(USA-MP1)L1	Bolivia(USA-MP1)L2	Bolivia(USA-MP1)L3	USA(Bolivia-MP1)L1	USA(Bolivia-MP1)L2	USA(Bolivia-MP1)L3	
Bolivia(USA-MP1)L1		0.999	0.966	ND	ND	ND	Octomom Copy Number
Bolivia(USA-MP1)L2	0.989		0.933	ND	ND	ND	
Bolivia(USA-MP1)L3	0.664	0.832		ND	ND	ND	
USA(Bolivia-MP1)L1	ND	ND	ND		0.988	0.998	
USA(Bolivia-MP1)L2	ND	ND	ND	0.962		0.957	
USA(Bolivia-MP1)L3	ND	ND	ND	0.744	0.949		
Bacterial density							
Generation 13	Bolivia(USA-MP1)L1	Bolivia(USA-MP1)L2	Bolivia(USA-MP1)L3	USA(Bolivia-MP1)L1	USA(Bolivia-MP1)L2	USA(Bolivia-MP1)L3	
Bolivia(USA-MP1)L1		0.206	0.562	ND	ND	ND	Octomom Copy Number
Bolivia(USA-MP1)L2	0.002		0.882	ND	ND	ND	
Bolivia(USA-MP1)L3	0.037	0.482		ND	ND	ND	
USA(Bolivia-MP1)L1	ND	ND	ND		0.537	0.010	
USA(Bolivia-MP1)L2	ND	ND	ND	0.535		< 0.001	
USA(Bolivia-MP1)L3	ND	ND	ND	0.018	0.001		
Bacterial density							
Generation 25	Bolivia(USA-MP1)L1	Bolivia(USA-MP1)L2	Bolivia(USA-MP1)L3	USA(Bolivia-MP1)L1	USA(Bolivia-MP1)L2	USA(Bolivia-MP1)L3	
Bolivia(USA-MP1)L1		< 0.001	< 0.001	ND	ND	ND	Octomom Copy Number
Bolivia(USA-MP1)L2	< 0.001		0.006	ND	ND	ND	
Bolivia(USA-MP1)L3	< 0.001	0.145		ND	ND	ND	
USA(Bolivia-MP1)L1	ND	ND	ND		0.981	0.956	
USA(Bolivia-MP1)L2	ND	ND	ND	0.904		0.999	
USA(Bolivia-MP1)L3	ND	ND	ND	0.001	< 0.001		
Bacterial density							

**Figure s1:** Relationship between the relative density and the average octomom copy number per *Wolbachia* in replicate lines at each timepoint of experiment #1.



Arabia(MP1):  $0.696x + 0.079$ ;  $R^2 = 0.36$ ;  $P = 0.085$   
 Bolivia(MP1):  $-0.377x + 0.168$ ;  $R^2 = 0.04$ ;  $P = 0.5753$   
**RC(MP1):  $0.758x - 0.173$ ;  $R^2 = 0.59$ ;  $P = 0.009$**   
**China(MP1):  $0.659x - 0.077$ ;  $R^2 = 0.66$ ;  $P = 0.005$**   
 W1118(wMelCS):  $-0.670x - 0.191$ ;  $R^2 = 0.19$ ;  $P = 0.236$   
**USA(MP1):  $1.4022x - 0.607$ ;  $R^2 = 0.54$ ;  $P = 0.001$**   
**France(MP1):  $0.916x - 0.257$ ;  $R^2 = 0.73$ ;  $P = 0.002$**   
**W1118(MP):  $0.811x - 0.177$ ;  $R^2 = 0.40$ ;  $P = 0.049$**

**Figure s2:** Relationship between the relative density and the average octomom copy number per *Wolbachia* in replicate lines at each timepoint of the new introgression experiment (exp. #2).



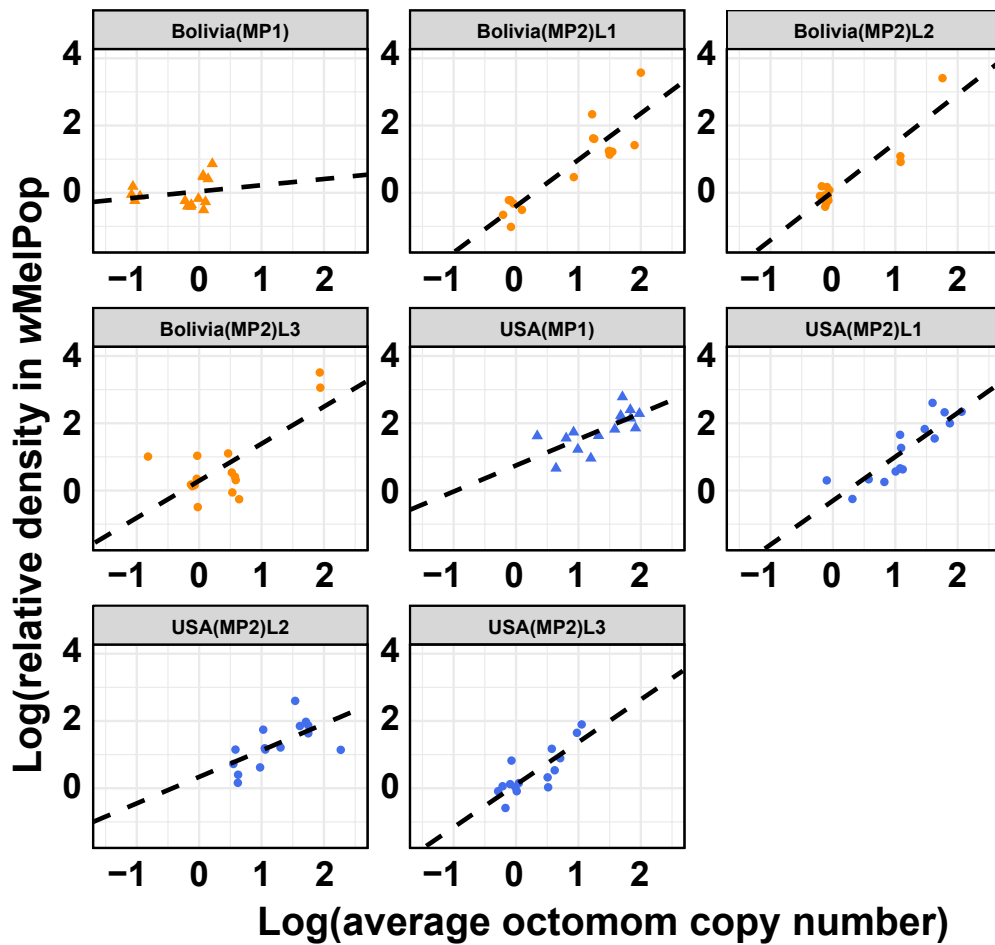
Generation #1:  $1.096x - 0.093$ ;  $R^2 = 0.84$ ;  $P = 0.001$

Generation #13:  $0.731x + 0.407$ ;  $R^2 = 0.72$ ;  $P = 0.008$

Generation #25:  $0.993x + 0.252$ ;  $R^2 = 0.83$ ;  $P = 0.002$

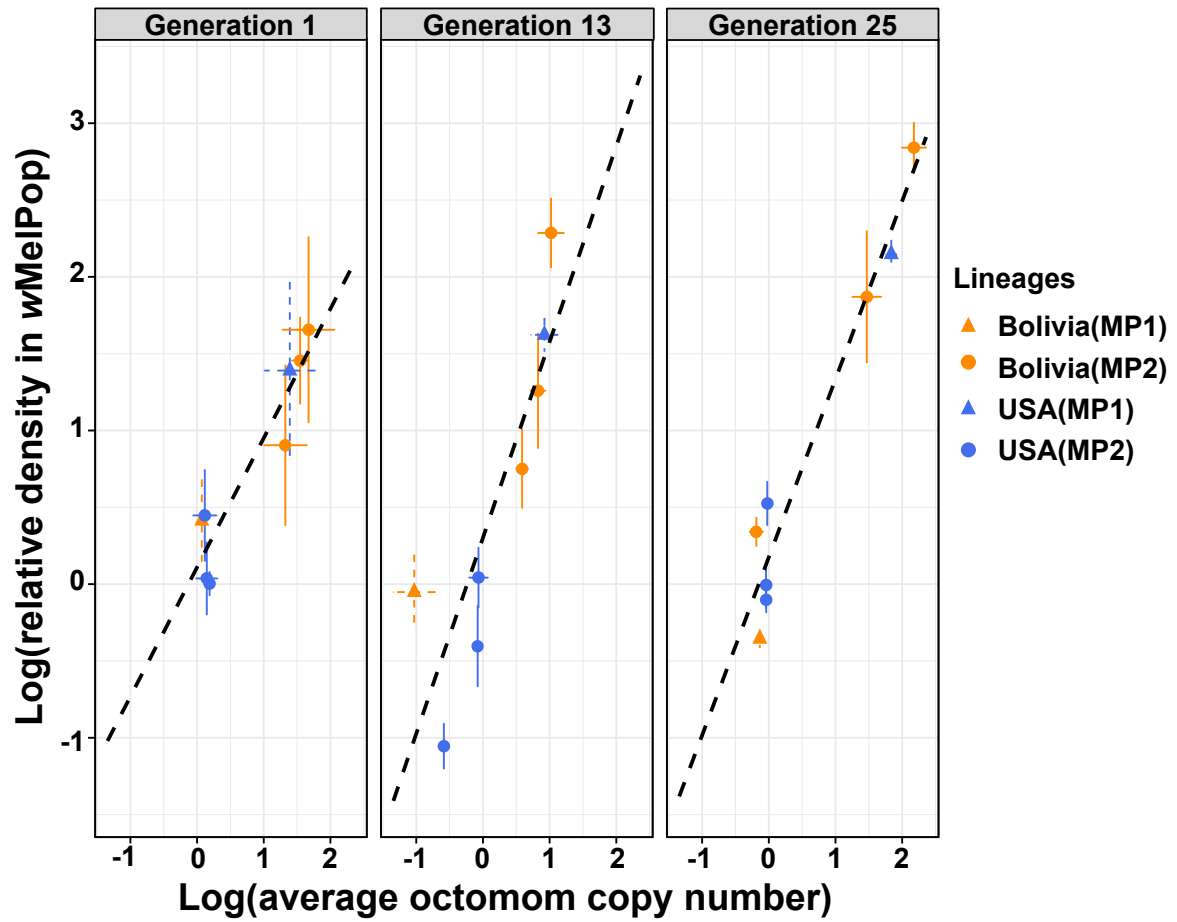


**Figure s3:** Correlation between the relative density and the average octomom copy number per *Wolbachia* within each replicate line of the new introgression experiment (exp. #2).



Bolivia(MP1):  $0.176x + 0.030$ ;  $R^2 = 0.044$ ;  $P = 0.455$   
 Bolivia(MP2)L1:  $1.390x - 0.392$ ;  $R^2 = 0.79$ ;  $P < 0.001$   
 Bolivia(MP2)L2:  $1.460x + 0.042$ ;  $R^2 = 0.86$ ;  $P < 0.001$   
 Bolivia(MP2)L3:  $1.098x + 0.296$ ;  $R^2 = 0.52$ ;  $P = 0.003$   
 USA(MP1):  $0.773x - 0.751$ ;  $R^2 = 0.50$ ;  $P = 0.003$   
 USA(MP2)L1:  $1.312x - 0.317$ ;  $R^2 = 0.75$ ;  $P < 0.001$   
 USA(MP2)L2:  $0.796x + 0.317$ ;  $R^2 = 0.41$ ;  $P = 0.011$   
 USA(MP2)L3:  $1.260x + 0.112$ ;  $R^2 = 0.66$ ;  $P < 0.001$

**Figure s4:** Correlation between the relative density and the average octomom copy number per *Wolbachia* in replicate lines at each timepoint of the 'reciprocal crosses' experiment (exp. #3).

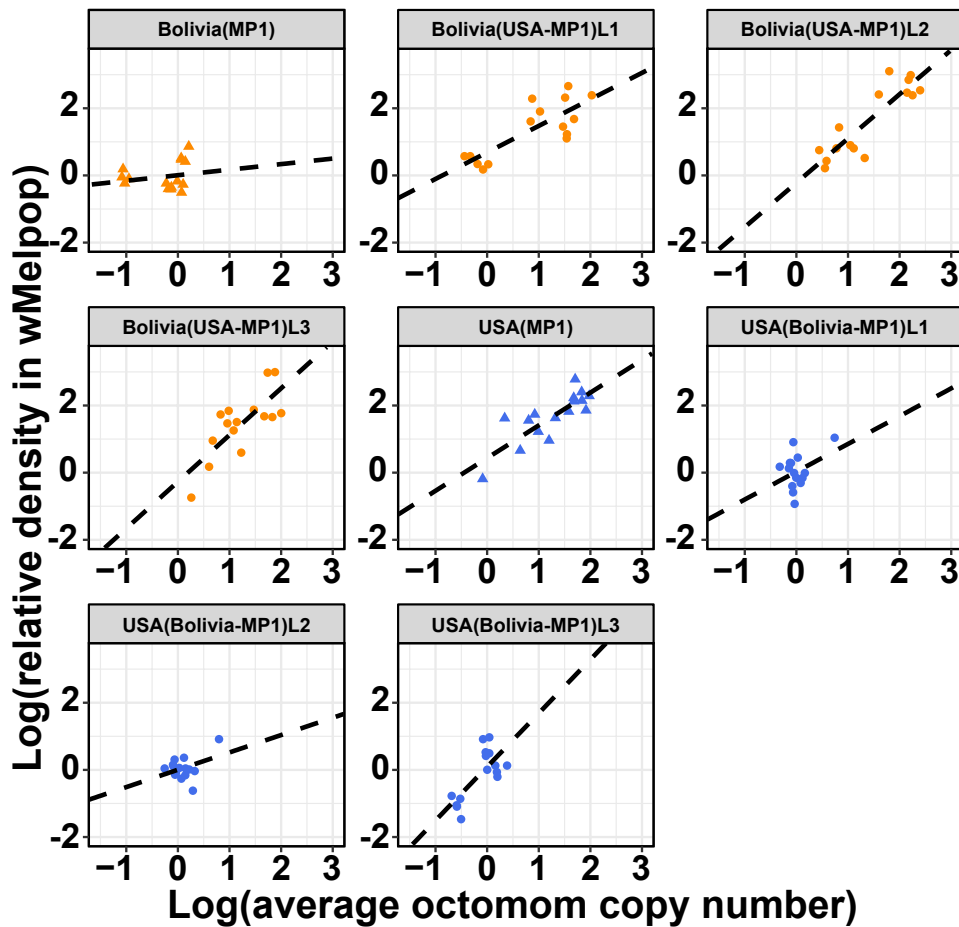


Generation #1:  $0.854x + 0.109$ ;  $R^2 = 0.88$   $P < 0.001$

Generation #13:  $1.275x + 0.302$ ;  $R^2 = 0.74$ ;  $P = 0.006$

Generation #25:  $1.83x - 0.763$ ;  $R^2 = 0.96$ ;  $P < 0.001$

**Figure s5:** Correlation between the relative density and the average octomom copy number per *Wolbachia* within each line of the 'reciprocal crosses' experiment (exp. #3).



Bolivia(MP1):  $0.176x + 0.030$ ;  $R^2 = 0.04$ ;  $P = 0.455$   
 Bolivia(USA-MP1)L1:  $0.783 + 0.692x$ ;  $R^2 = 0.54$ ;  $P < 0.001$   
 Bolivia(USA-MP1)L2:  $1.319x - 0.229$ ;  $R^2 = 0.78$ ;  $P < 0.001$   
 Bolivia(USA-MP1)L3:  $1.400x - 0.025$ ;  $R^2 = 0.58$ ;  $P < 0.001$   
 USA(Bolivia-MP1):  $0.773x - 0.751$ ;  $R^2 = 0.50$ ;  $P = 0.003$   
 USA(Bolivia-MP1)L1:  $0.827x + 0.039$ ;  $R^2 = 0.14$ ;  $P = 0.173$   
 USA(Bolivia-MP1)L2:  $0.510x - 0.009$ ;  $R^2 = 0.14$ ;  $P = 0.167$   
 USA(Bolivia-MP1)L3:  $1.612x + 0.084$ ;  $R^2 = 0.54$ ;  $P = 0.002$