

## Supplementary Information for

Rapid experimental evolution of reproductive isolation from a single natural population

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### **This PDF file includes:**

Tables S1 to S10  
Captions for movies S1 to S4

### **Other supplementary materials for this manuscript include the following:**

Movies S1 to S4

**Table S1.**

Principal component analysis (PCA) of louse body size.

		<b>Louse morphology</b>	<b>Principal components</b>		
			PC1	PC2	PC3
Eigenvalues			2.382	0.338	0.280
% variation			79.4%	11.3%	9.3%
Eigenvectors	Body length		0.585	- 0.126	- 0.801
	Metathorax width		0.571	0.765	0.297
	Head width		0.576	- 0.631	0.519
Loadings	Body length		0.903	- 0.073	- 0.424
	Metathorax width		0.882	0.445	0.157
	Head width		0.889	- 0.367	0.275

The PCA is based on the body length, metathorax width, and head width of 3096 lice measured at six-month intervals over the course of the experiment.

**Table S2.**

Linear mixed model (LMM) summary comparing the *overall body size (PC1)* of lice on different size pigeons over the course of the 48-month experiment.

<i>Random effects</i>	<i>Variance</i>	<i>Std. dev.</i>			
Lineage (Individual bird)	0.013	0.116			
Aviary	0.027	0.165			
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. err.</i>	<i>df</i>	<i>t value</i>	<i>Pr(&gt; t )</i>
Intercept (feral pigeons)	1.308	0.123	20.700	10.663	< 0.001*
Host (giant runts)	0.449	0.159	14.800	2.817	0.013*
Time	0.006	0.002	3086.000	2.542	0.011*
Sex (male lice)	-2.437	0.119	3078.000	-20.412	< 0.001*
Host x Time	0.009	0.003	3086.000	3.151	0.002*
Host x Sex	-0.053	0.140	3076.000	-0.377	0.706
Time x Sex	0.001	0.003	3076.000	0.394	0.693
Host x Time x Sex	0.001	0.004	3073.000	0.253	0.800

This LMM is based on the PC1 scores of 3096 lice measured over the 48-month experiment. Data for lice at Time 0 are a random subsample of lice drawn from the starting population. Data for the rest of the experiment (Time 6 mo. - 48 mo.) are for lice sampled from 32 individual birds (16 feral pigeons, 16 giant runt pigeons) housed in 4 aviaries (4 birds per aviary) for each host treatment. \* Indicates significance.

**Table S3.**

Linear mixed model (LMM) summary comparing the *body length* of lice on different size pigeons over the course of the 48-month experiment.

<i>Random effects</i>	<i>Variance</i>	<i>Std. dev.</i>			
Lineage (Individual bird)	115.500	10.750			
Aviary	105.400	10.260			
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. err.</i>	<i>df</i>	<i>t value</i>	<i>Pr(&gt; t )</i>
Intercept (feral pigeons)	2625.854	9.056	29.200	289.953	< 0.001*
Host (giant runts)	49.380	11.548	19.400	4.276	< 0.001*
Time	-0.568	0.186	3086.300	-3.053	0.002*
Sex (male lice)	-405.548	9.623	3077.900	-42.143	< 0.001*
Host x Time	0.929	0.229	3088.600	4.064	< 0.001*
Host x Sex	-9.383	11.311	3075.100	-0.830	0.407
Time x Sex	0.043	0.266	3075.800	0.161	0.872
Host x Time x Sex	-0.111	0.328	3073.200	-0.338	0.735

This LMM is based on the length scores of 3098 lice measured over the 48-month experiment. Data for lice at Time 0 are a random subsample of lice drawn from the starting population. Data for the rest of the experiment (Time 6 mo. - 48 mo.) are for lice sampled from 32 individual birds (16 feral pigeons, 16 giant runt pigeons) housed in 4 aviaries (4 birds per aviary) for each host treatment. \* Indicates significance.

**Table S4.**

Linear mixed model (LMM) summary comparing the *metathorax width* of lice on different size pigeons over the course of the 48-month experiment.

<i>Random effects</i>	<i>Variance</i>	<i>Std. dev.</i>			
Lineage (individual bird)	1.003	1.001			
Aviary	0.911	0.954			
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. err.</i>	<i>df</i>	<i>t value</i>	<i>Pr(&gt; t )</i>
Intercept (feral pigeons)	297.60	1.129	70.400	263.636	< 0.001*
Host (giant runts)	4.192	1.388	41.400	3.022	0.004*
Time	0.059	0.027	3067.800	2.209	0.027*
Sex (male lice)	-13.085	1.378	3088.600	-9.495	< 0.001*
Host x Time	0.106	0.033	3082.900	3.231	0.001*
Host x Sex	-0.223	1.620	3085.700	-0.137	0.891
Time x Sex	0.054	0.038	3086.800	1.416	0.157
Host x Time x Sex	0.014	0.047	3083.900	0.304	0.762

This LMM is based on the metathorax scores of 3103 lice measured over the 48-month experiment. Data for lice at Time 0 are a random subsample of lice drawn from the starting population. Data for the rest of the experiment (Time 6 mo. - 48 mo.) are for lice sampled from 32 individual birds (16 feral pigeons, 16 giant runt pigeons) housed in 4 aviaries (4 birds per aviary) for each host treatment. \* Indicates significance

**Table S5.**

Linear mixed model (LMM) summary comparing the *head width* of lice on different size pigeons over the course of the 48-month experiment.

<i>Random effects</i>	<i>Variance</i>	<i>Std. dev.</i>			
Lineage (individual bird)	0.587	0.767			
Aviary	2.654	1.629			
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. err.</i>	<i>df</i>	<i>t value</i>	<i>Pr(&gt; t )</i>
Intercept (feral pigeons)	288.072	1.044	13.600	275.940	< 0.001*
Host (giant runts)	1.821	1.390	10.700	1.310	0.218
Time	0.067	0.017	3091.700	4.105	< 0.001*
Sex (male lice)	-10.962	0.862	3087.800	-12.712	< 0.001*
Host x Time	0.023	0.020	3092.400	1.113	0.266
Host x Sex	-0.306	1.013	3085.200	-0.302	0.763
Time x Sex	-0.014	0.024	3085.500	-0.577	0.564
Host x Time x Sex	0.010	0.029	3083.000	0.344	0.731

This LMM is based on the head scores of 3105 lice measured over the 48-month experiment. Data for lice at Time 0 are a random subsample of lice drawn from the starting population. Data for the rest of the experiment (Time 6 mo. - 48 mo.) are for lice sampled from 32 individual birds (16 feral pigeons, 16 giant runt pigeons) housed in 4 aviaries (4 birds per aviary) for each host treatment. \* Indicates significance.

**Table S6.**

“Typical” variation in size dimorphism of *C. columbae*.

Feral pigeon	Male lice (n)	Female lice (n)	Mean male length (μm)	Mean female length (μm)	Mean body length dimorphism (μm)
1	5	5	2015	2507	-492
2	3	4	2114	2572	-458
3	2	1	2160	2604	-444
4	4	3	2158	2588	-430
5	1	4	2150	2573	-423
6	2	8	2173	2586	-413
7	2	14	2162	2571	-409
8	10	17	2178	2555	-377
9	5	2	2193	2545	-352
10	2	1	2227	2574	-347
11	5	6	2231	2566	-335
12	10	4	2153	2481	-328
13	10	10	2217	2540	-323
14	10	10	2229	2543	-314
15	10	5	2204	2516	-312
16	2	3	2227	2539	-312
17	14	10	2230	2539	-309
18	5	5	2206	2513	-307
19	3	2	2227	2515	-288
20	4	5	2231	2514	-283
21	13	6	2212	2486	-274
22	10	5	2242	2510	-268

Data are based on the length of 262 adult *C. columbae* removed from 22 wild-caught feral pigeons in Salt Lake City, Utah. All of the lice collected from each bird were used. We subtracted the mean *C. columbae* female length from the mean *C. columbae* male length of lice from each bird to generate 22 sexual dimorphism scores. These data were used to represent the natural range in size dimorphism between male and female *C. columbae* (grey shaded regions in Fig. 4A-C). Mean (± SD) length dimorphism =  $-354.4 \pm 65.4 \mu\text{m}$

**Table S7:**

Nonlinear (quadratic) and linear model summaries comparing the relationship between body length dimorphism and copulation time (Fig. 4A).

<b>Quadratic (NLM)</b>				<b>AICc 418.407</b>
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>t value</i>	<i>Pr(&gt; t )</i>
Intercept	9.043	1.288	7.020	< 0.001 *
Poly (Body length dimorphism, 2)1	12.839	9.640	1.332	0.189
Poly (Body length dimorphism, 2)2	-22.163	9.640	-2.299	0.026 *
<b>Linear (LM)</b>				<b>AICc 421.408</b>
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>t value</i>	<i>Pr(&gt; t )</i>
Intercept	15.492	5.206	2.976	0.004 *
Body length dimorphism	0.017	0.013	1.282	0.205

These models are based on 56 pairs of lice and assume a binomial distribution with logit link. AICc scores are provided to aid in model discrimination. \* Indicates significance



**Table S8:**

Generalized nonlinear (quadratic) and linear model summaries comparing the relationship between body length dimorphism and egg production (Fig. 4B).

<b>Quadratic (GNLM)</b>				<b>AICc 65.413</b>
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>Pr(&gt; z )</i>
Intercept	-1.662	0.648	-2.566	0.0100 *
Poly (Body length dimorphism, 2)1	-3.607	4.423	-0.815	0.4150
Poly (Body length dimorphism, 2)2	-17.597	8.967	-1.962	0.0497 *
<b>Linear (GLM)</b>				<b>AICc 74.207</b>
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>t value</i>	<i>Pr(&gt; t )</i>
Intercept	15.492	5.206	2.976	0.004 *
Body length dimorphism	-0.001	0.003	-0.423	0.672

These models are based on 58 pairs of lice and assume a binomial distribution with logit link. AICc scores are provided to aid in model discrimination. \* Indicates significance

**Table S9.**

Number of offspring produced by lice placed on individually caged feral pigeons.

Louse pair	Host of male louse	Host of female louse	Male length ( $\mu\text{m}$ )	Female length ( $\mu\text{m}$ )	Body length dimorphism ( $\mu\text{m}$ )	Number of offspring
1	Feral Pigeon	Giant Runt	2147	2870	-723	0
2	Feral Pigeon	Giant Runt	2114	2828	-714	0
3	Feral Pigeon	Giant Runt	2154	2715	-561	0
4	Feral Pigeon	Giant Runt	2189	2739	-550	0
5	Feral Pigeon	Giant Runt	2179	2714	-535	0
6	Feral Pigeon	Giant Runt	2190	2720	-530	0
7	Feral Pigeon	Giant Runt	2218	2705	<b>-487</b>	1
8	Feral Pigeon	Giant Runt	2275	2649	<b>-374</b>	0
9	Feral Pigeon	Giant Runt	2322	2690	<b>-368</b>	2
10	Feral Pigeon	Giant Runt	2220	2587	<b>-367</b>	3
11	Feral Pigeon	Giant Runt	2306	2643	<b>-337</b>	1
12	Feral Pigeon	Giant Runt	2243	2510	-267	0
13	Giant Runt	Feral Pigeon	2229	2704	<b>-475</b>	0
14	Giant Runt	Feral Pigeon	2179	2620	<b>-441</b>	0
15	Giant Runt	Feral Pigeon	2197	2615	<b>-418</b>	1
16	Giant Runt	Feral Pigeon	2202	2583	<b>-381</b>	3
17	Giant Runt	Feral Pigeon	2308	2650	<b>-342</b>	0
18	Giant Runt	Feral Pigeon	2298	2600	<b>-302</b>	0
19	Giant Runt	Feral Pigeon	2196	2458	-262	0
20	Giant Runt	Feral Pigeon	2213	2426	-213	1
21	Giant Runt	Feral Pigeon	2248	2457	-209	0
22	Giant Runt	Feral Pigeon	2362	2552	-190	0
23	Giant Runt	Feral Pigeon	2318	2442	-124	0
24	Giant Runt	Feral Pigeon	2372	2465	-93	0
25	Feral Pigeon	Feral Pigeon	2157	2604	<b>-447</b>	0
26	Feral Pigeon	Feral Pigeon	2315	2733	<b>-418</b>	0
27	Feral Pigeon	Feral Pigeon	2312	2728	<b>-416</b>	1
28	Feral Pigeon	Feral Pigeon	2271	2682	<b>-411</b>	2
29	Feral Pigeon	Feral Pigeon	2220	2631	<b>-411</b>	4
30	Feral Pigeon	Feral Pigeon	2191	2567	<b>-376</b>	0
31	Feral Pigeon	Feral Pigeon	2194	2539	<b>-345</b>	5
32	Feral Pigeon	Feral Pigeon	2159	2495	<b>-336</b>	0
33	Feral Pigeon	Feral Pigeon	2211	2545	<b>-334</b>	3
34	Feral Pigeon	Feral Pigeon	2262	2564	<b>-302</b>	0
35	Feral Pigeon	Feral Pigeon	2281	2577	<b>-296</b>	4
36	Feral Pigeon	Feral Pigeon	2281	2559	<b>-278</b>	0

Body length dimorphism is the difference in length between the male and female louse of each pair. Boldface values are pairs that fall within the typical range of dimorphism (Fig. 4C, Table S6).

**Table S10:**

Generalized nonlinear (quadratic) and linear mixed model summaries comparing the relationship between body length dimorphism and number of offspring produced (Fig. 4C):

<b>Quadratic (GNLMM)</b>			<b>AICc 91.684</b>	
<i>Random effects</i>	<i>Variance</i>	<i>Std. dev.</i>		
Host	0.000	0.000		
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>Pr(&gt; z )</i>
Intercept	-1.791	0.760	-2.358	0.018 *
Poly (Body length dimorphism, 2)1	9.445	4.410	2.142	0.032 *
Poly (Body length dimorphism, 2)2	-21.140	0.027	2.209	0.027 *
<b>Linear (GLMM)</b>			<b>AICc 109.115</b>	
<i>Random effects</i>	<i>Variance</i>	<i>Std. dev.</i>		
Host	0.273	0.522		
<i>Fixed effects</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>Pr(&gt; z )</i>
Intercept	0.275	0.725	0.380	0.704
Body length dimorphism	0.002	0.002	0.849	0.396

These mixed models are based on the reproductive success of 36 pairs of lice from feral or giant runt pigeons transferred to 36 louse-free feral pigeons: 12 pairs included a male giant runt louse and a female feral pigeon louse; twelve pairs included a male feral pigeon louse and a female giant runt louse; twelve (control) pairs included a male feral pigeon louse and a female feral pigeon louse. Models assume a Poisson distribution with a logarithmic link. AICc scores are provided to aid in model discrimination.

\* Indicates significance

**Movie S1.**

“Typical” sized male and female *C. columbae* copulating. Female on top, male on bottom. Dimorphism score = -346 $\mu$ m.

**Movie S2.**

Relatively large male attempting to copulate. Female on top, male on bottom. The male’s abdomen is too long for sustained copulation. Dimorphism score = -197 $\mu$ m.

**Movie S3.**

Relatively small male attempting to copulate. Female on top, male on bottom. The abdomen of the male is too short for copulation. Dimorphism score = -561 $\mu$ m.

**Movie S4.**

Male-male competition in *C. columbae*. One male displaces another male that is already copulating with the female.