Moyers et al. Supplementary Materials

Materials and Methods

Field Captures and Pre-Experiment Housing

All birds were caught using wire traps suspended around tube shaped bird feeders or mist nets placed in close proximity of bird feeders. Following processing and preceding the experiment, all birds were temporarily housed within an indoor animal care facility on the campus of Virginia Tech. For the first 19-24 hours, birds were held individually in small cages (46 × 46 × 76 cm) in the same indoor room at a constant light cycle (12L:12D) and temperature (22-24°C), and provided with *ad libitum* food and water. After ~24 hours, birds were held in pairs in small cages (46 × 46 × 76 cm), or in groups of four in larger cages (46 × 76 × 84 cm), but all other housing conditions remained unchanged.

Birds were monitored every 3-4 days during a 14-day quarantine period. Pre-experiment, any individuals that showed clinical signs of mycoplasmal conjunctivitis (swelling, redness, or exudate in either eye), or were ever housed with birds that showed visual signs of pathology were isolated and held in a separate room and were not used in experimental groups. Additionally, the week before establishing experimental flocks, we screened all birds for Mg-specific antibodies (see below) and only birds seronegative for Mg exposure were used in the experimental groups.

Experimental housing

The aviary facility had a solid roof providing the birds protection from precipitation, as well as a cement foundation reaching approximately 1 m in height. The internal half of the aviary

compartments were enclosed with waterproof walls, while the external half of the aviaries were enclosed with heavy-duty zoological grade mesh exposing the compartments to ambient temperatures and natural light-dark cycles. Radio-frequency identification antennas recorded from 06:00 to 19:00 EST, ranging from 59-91 mins before sunrise to 107-113 mins after sunset throughout the study. Thus, readers were active during all periods of daylight.

Quantifying Mg-specific Antibodies

We collected ~100 µL of blood by puncturing the brachial vein using a 26-gauge sterile needle, and collecting blood in heparinized micro-capillary tubes. Blood samples were immediately stored on ice for up to 4 hours after collection. Capillary tubes with clay plugs were centrifuged at 12000 rpm for 8 minutes in a microhematocrit centrifuge (Model M24 from LW Scientific, Lawrenceville, GA, USA), at which point the plasma was extracted using a Hamilton syringe (with three deionized water rinses in between each extraction). Extracted plasma was then stored at -20°C until processing.

We used the IDEXX FlockChek *M. gallisepticum* antibody ELISA kit (IDEXX, Westbrook, ME) to measure Mg-specific antibodies, following the manufacturer's protocol with modifications. The blocking step consisted of adding 300 µl of 1% BSA in PBS (Pierce 10X BSA, Thermo Fisher Scientific, Rockford, IL, USA) to room temperature plates and incubated for forty minutes at room temperature. All washing steps consisted of washing the plate three times with 350 uL of PBS with 0.05% Tween20 using a TriContinent MultiWash III System (VWR International, Bridgeport, NJ, USA). Plasma samples were diluted 1:50 in sample buffer, and incubated for one hour at room temperature. Pre-diluted kit antibody was also incubated for one hour at room temperature. Absorbance was measured at 630 nm using a SpectraMax 190

Microplate Reader (VWR International, Bridgeport, NJ, USA). Samples were run in duplicate and the average was calculated for further analysis. To control for inter-assay variation, we calculated all output values as the ratio of the sample absorbance to that of the positive control using the following equation: (sample mean – negative control)/(positive control – negative control).

Tracking Transmission

Deoxyribonucleic acid was extracted from a subset of conjunctival swabs (days 0, 6, 12, 18) using Qiagen DNeasy 96 Blood and Tissue kits (Qiagen, Valencia, CA). We then used quantitative polymerase chain reaction (qPCR) to estimate numbers of MG in the conjunctiva using primers and a probe that target the *mgc2* gene of Mg [1,2]. Standard curves of 2.98×10^{1} to 2.98×10^{8} copy numbers produced using a plasmid containing a 303 bp *mgc2* insert were included in each run [1].

Table S1. Descriptions of behavioral metrics extracted from RFID data. All variables except relative feeder preference were averaged for each week within individual for statistical analyses.

Behavior	**Definition**
Time spent on feeder	Sum of time spent on the feeder per day
Bout length	Time difference between an individual arriving at, and departing from, a given feeder port. To account for potential gaps in PIT-tag "reads" by the RFID antennae, we assumed a 4 s or longer gap in detection of the same individual indicated distinct feeding bouts
Relative Feeder Preference	The total amount of time an index bird spent on the preferred feeder divided by the proportion of time expected if equal time was spent on each available feeder
Aggressive Interaction	Any instance where one individual left a feeder port and a different individual arrived at the same feeder port within 2 s. This "rule" was shown to be 100% predictive of an aggressive displacement in [3].
Following Latency	The duration of time between an index bird leaving a feeder port and a group member arriving at the same feeder port

Table S2. Results of linear mixed models examining how treatment (feeder density), sex, and time (week or day post-inoculation) predicted feeding behaviors and at-feeder social interactions for group members in the study. All non-significant pairwise interactions were removed from the final model. Bird identity was included as a random effect in all models, as was flock identity for all models regarding flockmate behavior. (Test-statistics are t-staistics, and P-values are Pr (>|t|)

with the exception of the final model which uses Z-Statistic[a], and Pr (>|Z|)[b].) Significant values ($p \leq 0.05$) are bolded.

Dependent variable	Variables in Final Model	Class	Parameter Estimate	S.E.	DF	test-stat	P-value
Average time on feeders per day log_{10} transformed (index birds)	Intercept	N/A	3.41	0.13	14.03	26.03	**<0.0001**
	Sex	Female	-0.23	0.13	65.85	-1.78	0.080
	Treatment	High Density	-0.23	0.17	8.25	4.34	0.190
	Week	Week 0	0.19	0.044	300	3.33	**<0.0001**
		Week 1	0.15	0.044	300	2.21	**0.001**
		Week 2	-0.10	0.044	300	2.21	**0.028**
		Week 3	-0.23	0.044	300	-1.42	0.190
Average time on feeders per day log_{10} transformed (index birds)	Intercept	N/A	3.49	0.2	16.68	17.83	**<0.0001**
	Sex	Female	-0.39	0.23	7	-1.70	0.130
	Treatment	High Density	-0.12	0.27	17.16	-0.46	0.650
	Week	Week 0	0.15	0.19	32	0.80	0.430
		Week 1	0.20	0.19	32	1.08	0.300
		Week 2	-0.23	0.19	32	-1.21	0.240
		Week 3	-0.43	0.19	32	-2.30	**0.030**
	Treatment*Week	High Density*Week 0	0.10	0.27	32	0.35	0.730
		High Density*Week 1	0.16	0.27	32	0.59	0.560
		High Density*Week 2	0.47	0.27	32	1.76	0.089
		High Density*Week 3	0.76	0.27	32	2.84	**0.008**
Average feeding bout length log_{10} transformed (flockmates)	Intercept	N/A	1.41	0.073	14.5	19.39	**<0.0001**
	Sex	Female	-0.06	0.064	65.45	-0.99	0.330
	Treatment	High Density	0.09	0.095	10.46	0.91	0.380
	Week	Week 0	0.17	0.038	294.2	4.37	**<0.0001**
		Week 1	0.16	0.038	294.2	4.13	**<0.0001**
		Week 2	0.00	0.038	294.2	0.09	0.930
		Week 3	0.16	0.038	294.2	4.14	**<0.0001**
	Treatment*Week	High Density*Week 0	-0.12	0.54	294.2	-2.14	0.330
		High Density*Week 1	-0.16	0.54	294.2	-2.99	**0.003**
		High Density*Week 2	-0.10	0.54	294.2	-1.82	0.070
		High Density*Week 3	-0.18	0.54	294.2	-3.36	**<0.001**
Average feeding bout length log_{10} transformed (index birds)	Intercept	N/A	1.33	0.11	13.91	11.65	**<0.0001**
	Sex	Female	0.02	0.14	7	0.16	0.880
	Treatment	High Density	0.01	0.13	7	0.05	0.960
	Week	Week 0	0.33	0.1	36	3.29	**0.002**
		Week 1	0.57	0.1	36	5.72	**<0.0001**
		Week 2	0.09	0.1	36	0.88	0.380
		Week 3	0.01	0.1	36	2.02	0.051
Aggressive interactions (flockmates)	Intercept	N/A	75.03	13.39	10.84	5.60	**<0.001**
	Sex	Female	-0.04	5.64	80.4	-0.01	0.990
	Treatment	High Density	-51.16	18.7	10.29	-2.74	**0.020**
	Week	Week 0	69.66	7.21	288.2	9.65	**<0.0001**
		Week 1	36.45	7.21	288.2	5.05	**<0.0001**
		Week 2	-15.58	7.21	288.2	-2.16	**0.032**
		Week 3	-32.34	7.21	288.2	-4.78	**<0.0001**
	Treatment*Week	High Density*Week 0	-22.35	10.34	288.1	-2.16	**0.032**
		High Density*Week 1	-12.01	10.35	288.4	-1.16	0.250
		High Density*Week 2	25.94	10.35	288.4	2.51	**0.013**
		High Density*Week 3	58.95	10.35	288.4	5.70	**<0.0001**
Aggressive interactions (index birds)	Intercept	N/A	49.81	12.12	17.99	4.11	**<0.001**
	Sex	Female	10.93	14.08	7.02	0.78	0.460
	Treatment	High Density	-29.19	16.91	18.52	-1.73	0.100
	Week	Week 0	49.40	12.25	31.04	4.03	**<0.001**
		Week 1	3.00	12.25	31.04	0.25	0.810
		Week 2	-30.80	12.25	31.04	-2.51	**0.017**
		Week 3	-23.40	12.25	31.04	-1.91	0.065
	Treatment*Week	High Density*Week 0	-22.60	17.33	31.04	-1.30	0.200
		High Density*Week 1	-1.66	17.97	31.22	-0.09	0.930
		High Density*Week 2	23.80	17.33	31.04	1.37	0.180
		High Density*Week 3	33.00	17.33	31.04	1.90	0.066
Following latency (flockmates)	Intercept	N/A	4.48	0.2	N/A	22.85[a]	**<0.0001[b]**
	Sex	Female	0.11	0.079	N/A	1.42[a]	0.16[b]
	Treatment	High Density	0.89	0.27	N/A	3.27[a]	**0.0011[b]**
	Week	Week 0	0.11	0.027	N/A	4.19[a]	**<0.0001[b]**
		Week 1	0.47	0.033	N/A	14.43[a]	**<0.0001[b]**
		Week 2	0.40	0.033	N/A	12.13[a]	**<0.0001[b]**
		Week 3	0.06	0.03	N/A	2.00[a]	**0.045[b]**

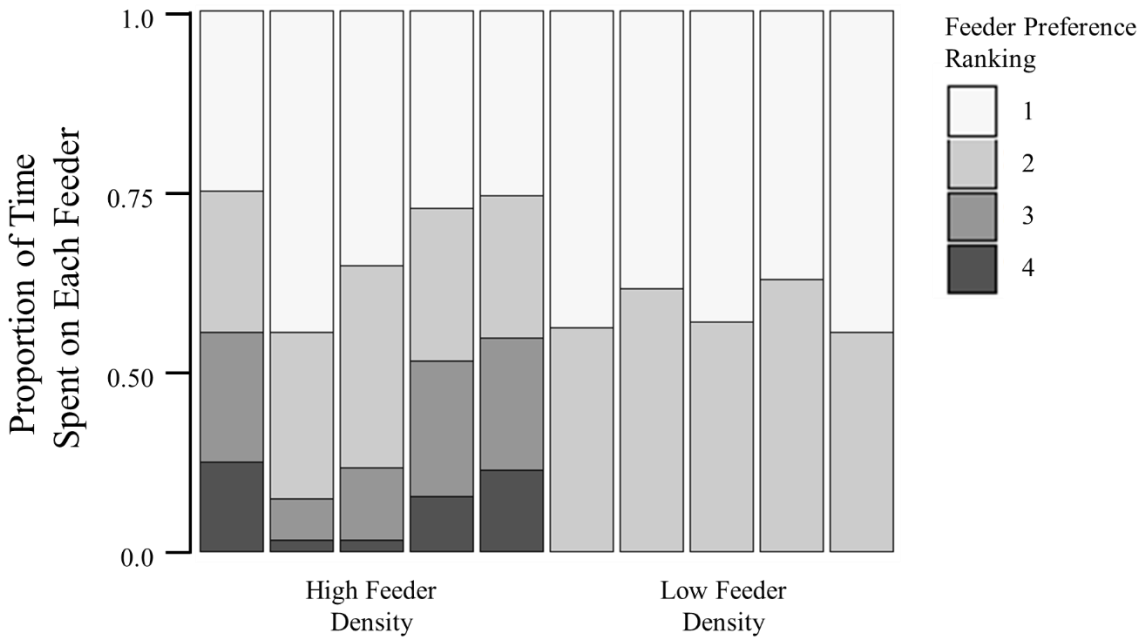

Figure S1. Proportion of time index birds (one column per individual) spent on each available feeder over the course of the study. Index birds at high feeder density (n=5; lefthand columns) had four feeders available, while index birds at low feeder density (n=5; righthand columns) only had two feeders available. Feeders are ordered by preference (top to bottom) from highest (most time spent; lightest gray) to lowest (least time spent; darkest gray) preference based on each individuals's proportion of time spent on that feeder, and not the specific location of that feeder.

References

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2. Hawley DM, Osnas EE, Dobson AP, Hochachka WM, Ley DH, Dhondt A a. 2013 Parallel patterns of increased virulence in a recently emerged wildlife pathogen. *PLoS Biol.* **11**, e1001570. (doi:10.1371/journal.pbio.1001570)

3. Moyers SC, Adelman JS, Farine DR, Moore IT, Thomason CA, Hawley DM. Exploratory behavior is linked to stress physiology and social network centrality in free-living house finches (*Haemorhous mexicanus*). (In Review)