Figure S1, Related to Figure 3



Β Syllable repertoire: C57 N140 83 84 111 112 140 139 20 121 122 123 131 132 133 134 135 1

С

Syllable Measures (CSV A)
Start and end time (sec)
Duration (msec)
Inter-syllable interval (msec)
Starting, final, minimum, maximum, and mean frequency (kHz)
Frequency bandwidth (kHz)
Total syllable amplitude (dB)
Peak syllable amplitude (dB)

Dataset Measures (CSV B)	Syllable Repertoire Modeling Scores (CSV D)	Similarity Boxplot Correlations (CSV G)						
Power spectral density - mean & std (kHz)	Overall repertoire modeling score	Pearson's correlations for the top 5, 25, 50, 75 and						
Frequency bandwidth (kHz)	Average log-likelihood	95% most irequently used RU pairs						
Syllables/second - mean, median, std (counts)	Bayesian information criterion	Pearson's correlations for RU pairs in 1% increments of use from 1-100%						
Inter-syllable interval - mean, median, std (msec)								
Syllable duration - mean, median, std (msec)		RU-Cluster Syllable Counts (CSV H)						
Total syllable number (counts)		RU-cluster designation number						
Total syllable activity (total syllable duration, sec)		Number of RUs within RU-cluster						
Total recording activity (sec)		RU-to-centroid correlation - mean & std						
		Number of syllables in RU-cluster						

Syllable & RU Sequence (CSV E)
Dataset name
Audio file name
Syllable number
Syllable start and end time (sec)
RU designation number

Pearson's correlations for each RU pair
Similarity Boxplot Correlations (CSV G)
earson's correlations for the top 5, 25, 50, 75 a

Similarity Matrix Correlations (CSV F)

Figure S1. Overview of MUPET's graphical user interface, noise refinement functionality, and exportable datasets.

(A) Image of MUPET's graphical user interface. (B) Initial (unrefined) C57BL/6 repertoire (size 140). Repertoire units (RUs) learned from full-spectrum background noise are highlighted in blue and RUs reflecting spectrally impure units learned from noisy, complex, and/or low frequency events are highlighted in red. We suggest removing all full-spectrum noise events. Depending upon user objectives, highly spectrally-impure units can either be removed or larger repertoires can be built to attempt to identify more spectrally pure syllables types. (C) Summary of the measures available in each exportable CSV file.



Figure S2. Spectral basis functions found by non-negative matrix factorization.

(A-E) Spectral basis functions found by non-negative matrix factorization (NMF) (basis vectors) applied on the power spectra (normalized to unit energy and computed from a 512 point FFT using 2 msec frames with a frame shift of 1.6 msec) of the audio recordings of the (A) DBA/2 and (B) C57BL/6 parental strains, (C) F1 cross (B6D2F1) and (D-E) two example offspring strains (BXD42 and BXD62). The columns correspond to the NMF basis vectors and were sorted by peak frequency of spectral amplitude. NMF factorization obtains a set of spectral envelope functions with peak amplitudes lying on a curve that can be approximated mathematically by a logistic function (dashed blue curve) with a center frequency equal to the mean of the Gaussian fit function of the PSD curves in Figure 4 A-C. The dashed red lines indicate the mean frequencies.

Figure S3, Related to Figure 4





Figure S3. Power spectral densities for each strain.

(A-L) Power spectral density (PSD) plots for each strain, listed in order from lowest to highest mean frequency.

Figure S4, Related to Figure 5



Figure S4. Syllable repertoire modeling measures and Pearson correlations for different syllable repertoire build sizes.

(A-C) Pearson correlations for different syllable repertoire sizes as a percentage of the total number of syllables in the repertoire units (RUs). Boxes highlight the optimal syllable repertoire sizes determined here and in **Figure 5**. Optimal syllable repertoire build sizes generally maximize Pearson correlations for each RU as well as the the proportion of (D) RUs and (E) syllables in RUs that contain a user-defined minimum number of syllables (here > 10 syllables per RU learned from datasets of ~5-50K syllables).

Figure S5, Related to Figure 5

Α	B
CS/BL/S repertorse CS/BL/S repertorse 1 2 3 4 5 6 7 6 0 10 11 12 13 14 15 16 7 10 10 10 10 11<	IDBA/2 repretore 1 2 3 4 5 6 7 6 9 5 1
B2F reprint	B Evene Image: D Image: D Image: D Evene Image: D Image: D <
1 2 3 4 8 6 7 6 7 6 7 6 7 6 7	1 2 3 4 5 6 7 6 6 7 6 15 15 15 16 17 18 19 100 10 103 171 100 100 144 137 186 155 155 155 15 16 17 18 19 40 100 100 100 164 15 16 17 18 19 40 100 </th
BXD2 repertor 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	BU3 reprint 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Burgering 1 <td< th=""><th><i>BUS2 negative</i></th></td<>	<i>BUS2 negative</i>
Substrain Substrain <t< th=""><th>BXD79 repertoire 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 155 151 131 130 129 122 130 16 15 11 12 11 100 160 160 16 15 13 14 15 16 17 16 16 16 15 13 12 110 160 160 16 16 15 13 14 15 16 17 16</th></t<>	BXD79 repertoire 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 155 151 131 130 129 122 130 16 15 11 12 11 100 160 160 16 15 13 14 15 16 17 16 16 16 15 13 12 110 160 160 16 16 15 13 14 15 16 17 16

Figure S5. Syllable repertoires for each strain.

(A-L) Syllable repertoires of size 100 repertoire units generated for each strain.

Figure S6, Related to Figure 6





Figure S6. Cross Repertoire Similarity Boxplots for each strain.

(A-L) Cross Repertoire Similarity Boxplots using each individual strain as the reference strain (indicated by the title) with the 11 comparison strains shown on the X-axis.

Figure S7, Related to Figure 6



Figure S7. Cross Repertoire Similarity Matrix for the C57BL/6 and DBA/2 reference syllable repertoires in comparison to B6D2F1 as well as the most and least similar strains.

(A-C) Cross Repertoire Similarity Matrix used to assess the similarity of the spectral shapes of pairs of repertoire units (RUs) learned from the C57BL/6 reference syllable repertoire and the (A) BXD77, (B) B6D2F1, and (C) BXD48 comparison syllable repertoires. The matrix diagonal gives the Pearson correlation for sequential pairs of C57BL/6 and other-strain RUs ranked from most to least similar (e.g., Unit 1 in both repertories are highly similar). The Cross Repertoire Similarity Boxplot metric (shown in **Figure 6B**) identified BXD77 and BXD48 as, respectively, the most and least similar offspring syllable repertoires compared to C57BL/6. The F1 cross shows moderate similarity to the C57BL/6 syllable repertoire.

(D-F) Cross Repertoire Similarity Matrix used to assess the similarity of the spectral shapes of pairs of RUs learned from the DBA/2 reference syllable repertoire and the (A) BXD43, (B) B6D2F1, and (C) BXD48 comparison syllable repertoires. The matrix diagonal gives the Pearson correlation for sequential pairs of DBA/2 and other-strain RUs ranked from most to least similar. The Cross Repertoire Similarity Boxplot metric (shown in Figure 6C) identified BXD43 and BXD48 as, respectively, the most and least similar offspring syllable repertoires compared to DBA/2. The F1 cross shows higher similarity to the DBA/2 syllable repertoire compared to C57BL/6 syllable repertoire.

Figure S8, Related to Figure 8



Figure S8. Pearson correlations and strain of origin analyses for different master repertoire build sizes.

(A-C) Pearson correlations for different master repertoire sizes (i.e., number of repertoire unit (RU)-clusters) used to model the 12 strain syllable repertoires. Pearson correlations are shown as a percentage of the total number of **(A)** RU-clusters (5-100), **(B)** RUs (1200), and **(C)** syllables (138606) meeting different threshold correlation values. Boxes highlight master repertoire sizes that maximize the Pearson correlations while minimizing the proportion of RU-clusters, and proportion of RUs and syllables that are present within RU-clusters, that contain a small number of RUs (e.g., 1 or \leq 5).

(D-F) Strain of origin analysis for master repertories of different sizes. Sizes were selected based on the optimal modeling determined in A-C (see boxes). For each RU-cluster the strain of origin was determined based on whether the RU-cluster contains RUs (and consequently syllables) learned from both parental strains, a single parental strain, the F1 cross but neither parental strain, multiple BXD strains (but not the parental strains or F1 cross), or a single BXD strain. Data are presented as the percentage of (D) RU-clusters, (E) RUs, and (F) syllables that originate from each of the possible strains of origin.

(G) Images of master repertoire sizes analyzed in D-F.

Figure S9, Related to Table 1

Α																							
	Female Urine (UR) repertoire																						
1	2	3	4	5	6	7	8	9	10	11	12	13	14 J	15	16	17	18	19	20	21	22	23	24
389	360	357	339	330	318	306	303	297	295	289	279	277	263	261	252	251	250	244	240	240	237	235	232
25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
230	222	219	219	217	217	213	212	211	211	210	210	210	197	196	196	195	194	194	192	192	188	188	187
49	50	51	52	53	54	55	56	57	58	⁵⁹	60	61	62	63	64	65	⁶⁶	67	68	69	70	71	72
185	184	183	183	182	179	178	177	176	175	175	174	173	172	172	171	170	169	167	166	166	165	165	163
73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
161	159	159	158	157	150	149	147	147	146	145	145	145	143	143	142	140	139	134	134	134	132	129	125
97	98	99	100	101	102 🗯	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
124	120	119	115	111	110	109	108	106	105	104	104	103	100	99	94	85	79	78	78	77	71	66	38





Figure S9. Syllable repertoires built from vocalization data in Chabout et al., 2015.

(A-C) Syllable repertoires generated from recordings of sexually-experienced B6D2F1 males vocalizing in response to same-strain: (A) female urine, (B) anesthetized female or (C) awake female.

Figure S10, Related to Table 1





1.

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Figure S10. Cross Repertoire Similarity Matrix and Boxplot measures for vocalization data in Chabout et al., 2015.

(A-C) Similarity matrices for each pair-wise syllable repertoire comparison. (D) Boxplot similarity metrics with each condition serving as the reference syllable repertoire.

Figure S11, Related to Table 1





С



Anesthetized Female (AF)



Awake Female (FE)



Shared (UR, AF, FE) UR Only AF Only UR & AF



Figure S11. Master repertoire and shared and unique RU-clusters across vocalization datasets in Chabout et al., 2015.

(A) Master repertoire of 35 RU-clusters generated from a cluster analysis of the female urine (UR), anesthetized female (AF), and awake female (FE) syllable repertoires. The total number of RUs and syllables in each cluster are shown in blue and red, respectively. (B) Each RU-cluster was categorized as based on whether it contains RUs from each social condition ("shared") or only from a subset of conditions ("unique"). (C) For each social condition, the proportion of syllables that are present within RUs, which are present within shared or unique RU-cluster categories (see B and legend), is shown on the left. Within each shared or unique category, the most-to-least prevalent RU-cluster types are indicated by boxes, with prevalence indicated by a gradation of dark-to-light colors. The percentage of syllables within each RU-cluster is noted within each box and the representative image for each cluster is shown on the right. *Designates the proportion of syllables that are present in RU-clusters that are present in RU-clusters that are present in RU-clusters are summed and no image is given. RU-cluster images can be seen in Panel B.