

Supplemental Information for:

Heritability of inter-pack aggression in a wild pedigreed population of North American gray wolves

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Supplemental Note: Quantifying inter-pack aggressive behaviors of gray wolves in Yellowstone National Park

A single observer quantified inter-pack aggressive behaviors (herein, aggression) in the population of gray wolves in Yellowstone National Park (YNP) through field observations of inter-pack interactions among wolves that are individually identified during field observations. Due to the nature of these observations, not all packs were observed at equal rates. For example, northern YNP (Fig. A1) contains a higher density of and increased visibility of wolf packs due to their size and the proximity of their territories relative to road corridors and open vistas. Larger packs often have more radio-collared individuals that are tracked and therefore located more readily for observation. However, pack composition experiences substantial turnover approximately every five years as a function of dispersal, lifespan, and territory vacancies (Mech & Boitani 2003). As a result, we have averaged the scores that quantify aggression for each observed individual to reflect the dynamic nature of pack membership and frequency of observing interactions.

A team of field assistants collected behavioral data informative for aggression through direct observations, field notes, or videos. YNP staff tracks 7-14 wolf packs annually, with intense focus on 2-4 each year. A single researcher designed and scored every individual in an interaction on a scale of 1 (individual fled the interaction) to 10 (individual led an attack that resulted in a mortality) (Table A1). Intermediate scores represent increasing levels of aggression of a wolf towards another individual. These scores were averaged across all interactions per individual into a metric called the

individual aggression score, or IAS, that summarizes an individual-level representation of overall aggression (higher IAS is a consistently more aggressive individual). Further, IAS was designed to allow comparison among individuals while mitigating for differences in frequency of observations across individuals. Overall, this averaged score is expected to capture a repeatable consistent individual response in a pairwise interaction. Further, although this data is gathered at the individual level, it can be organized by pack-level.

Individuals are known to react differently to an aggressive interaction based on many variables, especially the number of pack mates present compared to the opponent, and individual factors such as age, sex, and coat color (Cassidy et al. 2017). The study by Cassidy et al. (2017) included individual wolf ID as a random variable and this study attempts to elucidate that effect on aggressive behavior. Due to the limits of studying a wild system, many variables were not measured (e.g. individual fitness at the time of the interaction, complete history of inter-pack interactions between two opponents, etc.).

A video of a representative interaction can be in the Supplemental materials. This video contains the following eight wolves and their respective aggression scores: 586M, IAS=3; UCGFpup, IAS=6; 776F, IAS=6; UCGMpup Dark, IAS=8; UCGMpup Light, IAS=8; 832F, IAS=8; 754M, IAS=9; and 755M, IAS=9.

Average IAS and minimum number of interactions

Every individual has a calculated individual aggression score based on their behavior during an aggressive interaction. These scores were averaged across the total number of observations per individual and examined in relation to the pedigree and

genotype data. By the nature of how we designed IAS, it is inherently a measure weighted by intensity. For example, if an interaction ends with a chase and no attack, the highest IAS an individual can score is 7. If an individual is attacked, the attacker in this interaction would be assigned a 9, and if the attacked individual is killed, then the attacker would score a 10 (Table A1).

Individuals with many interaction scores likely encountered a diversity of circumstances and have more moderate scores, indicative of variation in their behavioral responses to interactions. As an averaged metric, IAS based on few interactions will reflect the behavioral responses of those few interactions and may be, but not necessarily, values at the ends of the distribution. As these observational data are extremely difficult to collect, especially during such episodic events which are often chaotic, we were as inclusive as possible for our data analysis and controlled for as many known variables as possible.

Breeding status

At the time of each interaction, an individual is assigned their IAS based on the intensity (Table A1). Individuals will accumulate IAS data for as many times as they are observed in inter-pack interactions during the study period (1995-2011). The observations documented during the breeding season (December to March) were the IAS data we analyzed. We then averaged IAS data for every individual across the number of times IAS was calculated from an observation. An individual was assigned a “breeding” versus “non-breeding” status if the individual was documented at the end of the study to have reproduced at any point during the 16-year study. This variable is focused on the

overall potential difference between wolves that reproduce or not during their lifespan, which can range up to 12 years. Although this is influenced by many variables (e.g. female age at primiparity; Stahler et al., 2013; breeding status; Cassidy et al., 2017), the degree of aggression is likely influential. Further, as the IAS is an average that reflects overall consistency of an individual's response, we hypothesize that individuals with higher IAS averages are more likely to reproduce or the increased aggression is stimulated by the presence of offspring. As past analysis (e.g. Cassidy et al. 2017) did not include current breeding status, we suspect future studies will provide further insights into the role of aggression on access to reproductive.

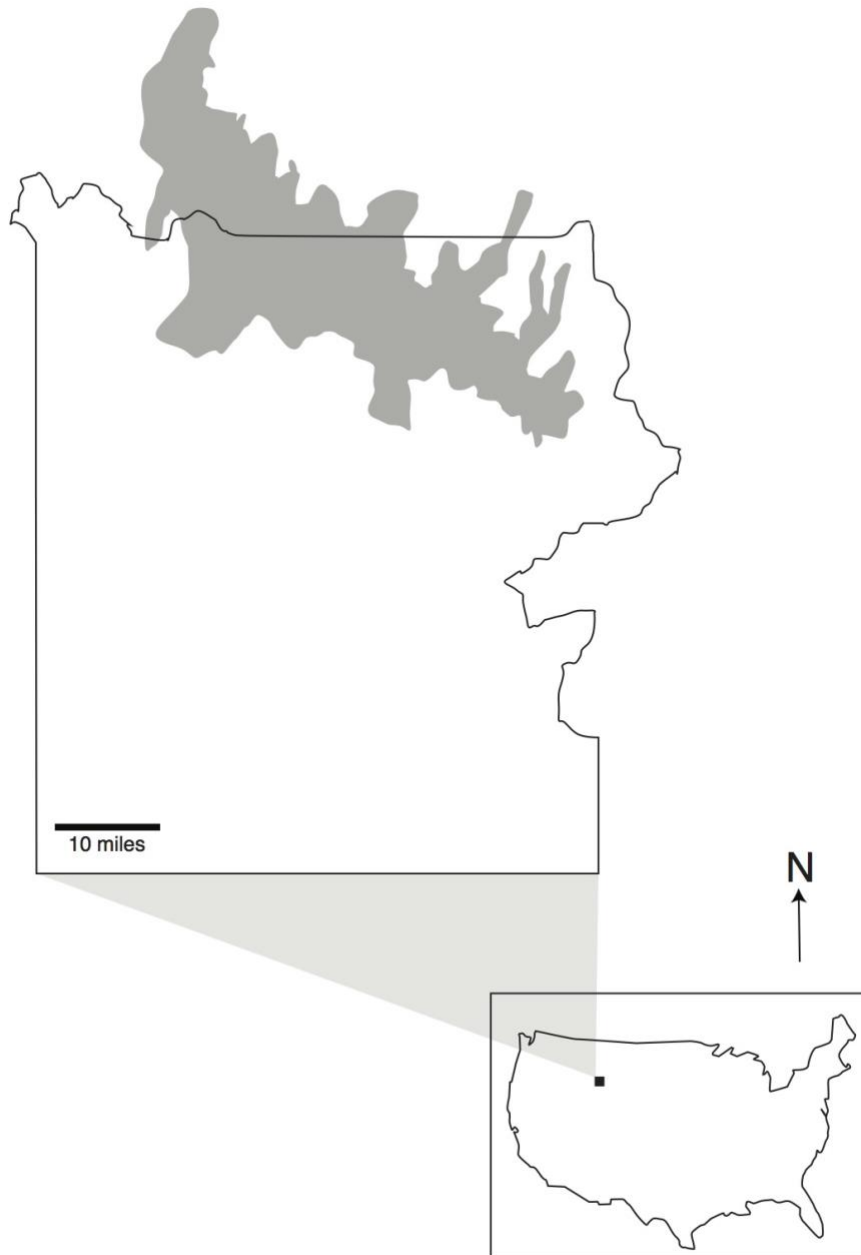
Literature Cited

- Cassidy, K.A., Mech, L.D., MacNulty, D.R., Stahler, D.R., & Smith, D.W. (2017). Sexually dimorphic aggression indicates male gray wolves specialize in pack defense against conspecific groups. *Behavioral Processes* 136, 64-72. DOI: 10.1016/j.beproc.2017.01.011
- Mech, L.D., & Boitani, L. (2003). *Wolves: Behavior, Ecology, and Conservation: Behavior, Ecology, and Conservation* University of Chicago Press.
- Stahler, D.R., MacNulty, D.R., Wayne, R.K., vonHoldt, B., & Smith, D.W. (2013). The adaptive value of morphological, behavioural and life-history traits in reproductive female wolves. *Journal of Animal Ecology* 82, 222-234. DOI: 10.1111/j.1365-2656.2012.02039.x

Table A1. Description of behaviors associated with each Individual Aggression Score (IAS) for each inter-pack interaction observed.

Score	Behavior
1	Flee
2	Was present during an interaction but did not participate (stood off to the side and watched)
3	Stood ground or started to chase but then fled before opponent was within 100 meters
4	Began interaction by chasing or fighting but fled after initial contact (within 100 meters)
5	Participated in a chase (last 1/3rd of the pack)
6	Participated in a chase (middle 1/3rd of the pack)
7	Led a chase (front 1/3rd of the pack)
8	Participated in a physical attack that did not result in a kill
9	Led a physical attack that did not result in a kill
10	Participated in a physical attack that resulted in a kill

Figure A1. A map of Yellowstone National Park (inset) and region referred to as northern YNP (shaded).



Supplemental Table S1. Sample information for 568 wolves, where not every individual has genotype or behavioral data quantified as the IAS (Individual Aggression Score). Negative average pack size indicates that the focal individual tended to be found in packs smaller than their adversary's pack at the time of observed aggressive interaction. A "." indicates a lack of observational data. The last four columns indicate which samples were included in specific analyses or in Figure 1 when marked with an "X" and sample size (n). The "Sample_ID" is the field that will match to RAD-seq data deposited on NCBI SRA. (Abbreviations: YOB, year of birth)

See excel file SupplementalTables_S1-S3.xlsx

Supplemental Table S2. Pedigree assignments and analytical support for SNP-based assessments of relationships. Columns headers are described as follows: Offspring_RAD, offspring identifier; Mom_Proposed, maternal parentage assignment; Mom_Support, analyses supporting maternal parentage assignment; Dad_Proposed, paternal parentage assignment; Dad_Support, analyses supporting paternal parentage assignment; LLRmom_err03, log-likelihood ratio for maternal parentage assignment made using *sequoia* with genotyping error rate set to 10^{-3} ; LLRdad_err03, log-likelihood ratio for paternal parentage assignment made using *sequoia* with genotyping error rate set to 10^{-3} ; LRRpair_err03, log-likelihood ratio for maternal and paternal pair assignment made using *sequoia* with genotyping error rate set to 10^{-3} ; LLRmom_err02, log-likelihood ratio for maternal parentage assignment made using *sequoia* with genotyping error rate set to 10^{-2} ; LLRdad_err02, log-likelihood ratio for paternal parentage assignment made using *sequoia* with genotyping error rate set to 10^{-2} ; LRRpair_err02, log-likelihood ratio for maternal and paternal pair assignment made using *sequoia* with genotyping error rate set to 10^{-2} ; Notes, special notes accompanying parentage assignments. The analytical support is represented by the following methods: *sequoia*, SNP-based parentage assignment made using the R package *sequoia*; *relatedness*, SNP-based parentage supported by relatedness estimates calculated using the R package *related* (N.B., weak support indicated with " <0.4 ") when relatedness estimate was below 0.4); *msats*, microsatellite-based parentage assignment from vonHoldt et al. (2008) (N.B., "msats only" indicates when an assignment is only based on microsatellite analyses). In the "LLR" columns, bolded values indicate parentage assignments that were based on dummy parents or putative monozygotic twins and have attendant information in the "Notes" column.

See excel file SupplementalTables_S1-S3.xlsx

Supplemental Table S3. Linear mixed model results for the association of individual aggression scores for 121 wolves genotyped for the 56K filtered SNP set (see main text for filtering parameters). (Abbreviations: N_miss, number of missing values for a given SNP; AF, allele frequency; beta, Beta estimates; SE, standard errors for beta; l_reml, restricted maximum likelihood estimate for lambda; l_mle, maximum likelihood estimate for lambda; p_lrt, *P*-values from the likelihood ratio test; VEP, variant effect predictor consequence)

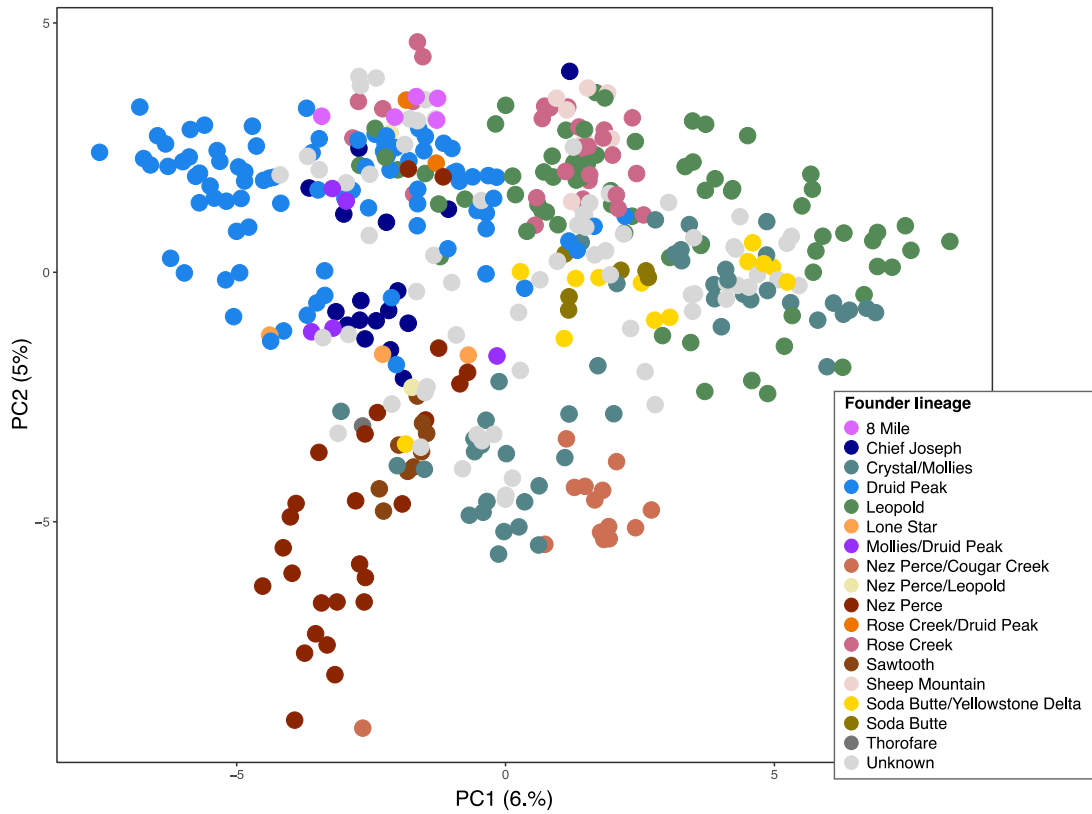
See excel file SupplementalTables_S1-S3.xlsx

Supplemental Table S4. Outlier genic SNPs associated with IAS from a likelihood ratio test of a linear mixed model, and their VEP and g:Profiler functional annotations. (Abbreviations: *P*, p-value; *N_m*, number of missing individuals per site; VEP; variant effect predictor)

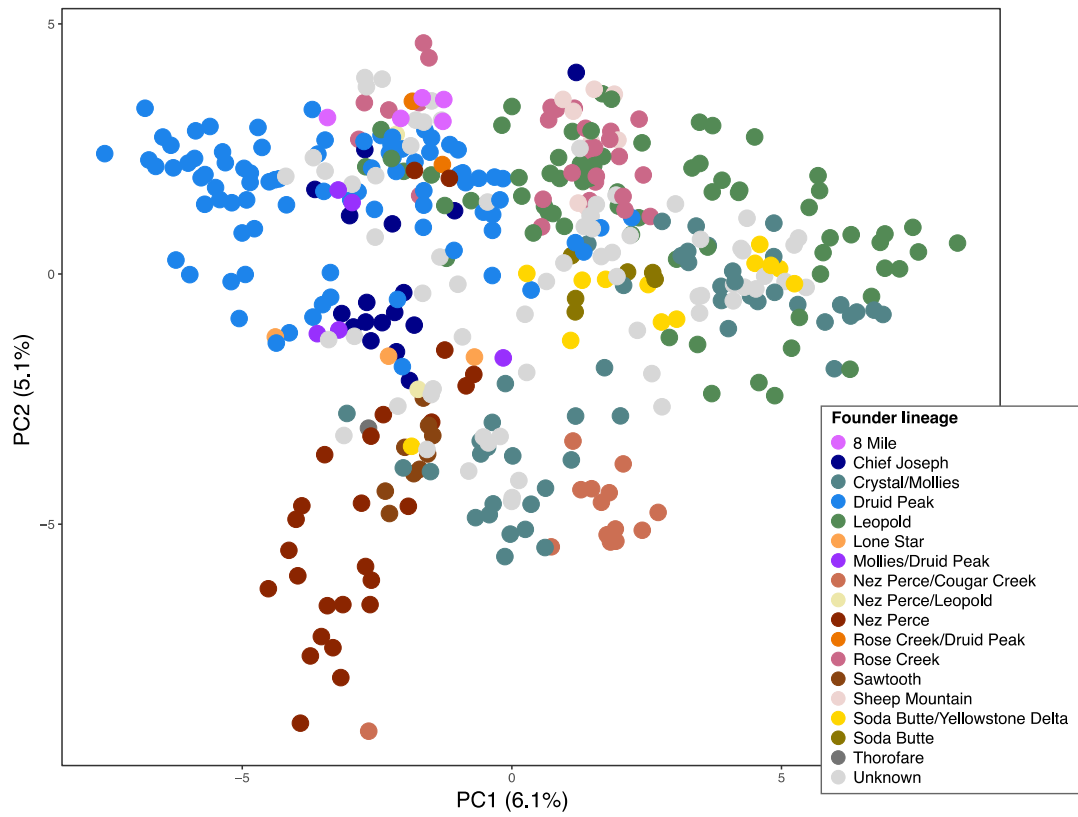
Ran k	VEP gene	Chr.Pos	<i>N_m</i>	Minor/Ma jor allele	AF	Beta (SE)	<i>P</i>	VEP consequence	VEP impact
		34.							
5	SPATA 16	3717450 5	0	G/A	0.0 91	5.35 (1.3)	3.1709E- 05	intron variant	Modifier
11	PIK3C2 B	38. 1016415	1	A/G	0.2 45	2.98 (0.7)	6.2102E- 05	upstream gene variant	Modifier
		20.							
12	MADC AM1	5798384 0	3	T/G	0.0 26	6.14 (1.5)	6.9407E- 05	upstream gene variant	Modifier
		30.							
14	MYO9A	3541263 6	4	G/A	0.6 84	1.75 (0.4)	7.7473E- 05	intron variant	Modifier
		30.							
15	CCDC3 3	3747418 7	0	T/C	0.1 41	4.04 (1.0)	9.8774E- 05	downstream gene variant	Modifier
16	SLC25A 38	23. 9027262	0	T/C	0.0 15	-6.90 (1.7)	0.0001026 9	upstream gene variant	Modifier
		23.							
17	TRAK1	1133986 5	2	A/G	0.0 1	-6.81 (1.7)	0.0001041 2	intron variant	Modifier
		7.							
21	PLXNA 2	6904064 0	0	A/G	0.0 76	3.94 (1.0)	0.0001170 8	intron variant	Modifier
		5.							
24	NTM	2833552 0	0	C/T	0.0 56	6.23 (1.7)	0.0002278 5	intron variant	Modifier
		15.							
25	SMIM3 1	6089224 3	0	A/G	0.1 41	3.08 (0.8)	0.0002579 4	intron variant	Modifier

		5.							
34	RAVER 2	4543632 2	4	T/C	0.0 68	5.94 (1.7)	0.0006421 3	intron variant	Modifier
39	NOCT	19. 3608227	1	A/G	0.0 41	3.38 (1.0)	0.0008242 9	upstream gene variant	Modifier
40	MYO15 A	5. 4140172 8	0	A/G	0.0 71	4.86 (1.5)	0.0008444 3	intron variant	Modifier
42	CCDC3 3	30. 3738967 1	4	T/C	0.0 95	3.72 (1.1)	0.0008821 6	intron variant	Modifier
43	PPFIA2	15. 2375833 9	4	G/C	0.1 89	2.33 (0.7)	0.0008901 3	intron variant	Modifier
44	EBF2	25. 3147604 3	0	A/G	0.0 15	-2.52 (0.8)	0.0009047 9	upstream gene variant	Modifier
45	EDC3	30. 3775987 1	3	A/G	0.1 25	3.62 (1.1)	0.0009071 2	intron variant	Modifier

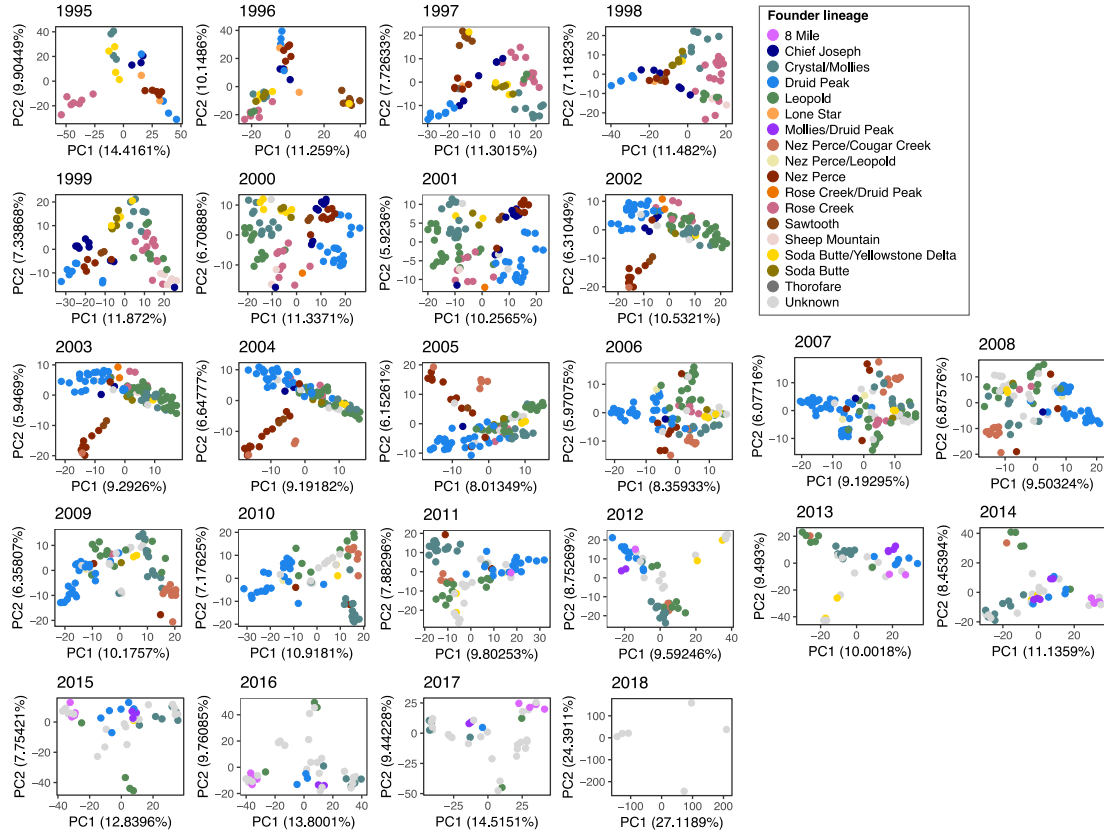
Supplemental Figure S1. Principal component analysis of 413 wolves from 598 SNPs genotypes identified for parentage and pedigree reconstruction.



Supplemental Figure S2. Principal component analysis of 384 wolves from 598 SNPs genotypes identified for parentage and pedigree reconstruction.



















Supplemental Figure S3. Annual principal component analysis between 1995-2018 of wolves from 598 SNPs genotypes identified for parentage and pedigree reconstruction.



MOLECULAR ECOLOGY

The colors for different evidence codes in the table:

Gene Ontology	
	Inferred from experiment [IDA, IPI, IMP, IGI, IEP]
	Direct assay [IDA], Mutant phenotype [IMP]
	Genetic interaction [IGI], Physical interaction [IPI]
	Inferred from High Throughput Experiment [HDA, HMP, HGI, HEP]
	High Throughput Direct Assay [HDA], High Throughput Mutant Phenotype [HMP]
	High Throughput Genetic interaction [HGI], High Throughput Expression pattern [HEP]
	Traceable author [TAS], Non-traceable author [NAS], Inferred by curator [IC]
	Expression pattern [IEP], Sequence or structural similarity [ISS], Genomic context [IGC]
	Sequence Model [ISM], Sequence Alignment [ISA], Sequence Orthology [ISO]
	Biological aspect of ancestor [IBA], Rapid divergence [IRD]
	Reviewed computational analysis [RCA], Electronic annotation [IEA]
	No biological data [ND], Not annotated or not in background [NA]
Biological pathways	
	KEGG, Reactome
Regulatory motifs in DNA	
	TRANSFAC TFBS, miRTarBase
Protein databases	
	Human Protein Atlas, CORUM protein complexes
Human Phenotype Ontology	
	Human Phenotype Ontology (sequence homologs in other species)

The colors for log scale:

