nature genetics

Supplementary information https://doi.org/10.1038/s41588-024-02059-0

Functional innovation through new genes as a general evolutionary process

Supplementary Figures and Tables

Supplementary Figure 1. The distribution of gene numbers and genome sizes across 6,794 extant species.

Supplementary Figure 2. Origination of new genes across life-origination scale evolutionary timescales. The two panels illustrate the origination of new genes on a long timescale (life-origination scale, ~4,250Mya) in humans[1] and Arabidopsis[2].

Supplementary Figure 3. New genes with documented essential phenotypes and measured selective forces. A. The proportion (α) of amino acid substitutions in a group of fixed new gene duplicates under positive selection that has evolved essential phenotypes[3] recalculated from the data[4,5] (L and S are the phenotypes of lethality and sterility detected by the knockdown experiments in the two studies). This suggests that a significant number of these substitutions are likely contributing to the development of essential functions of essential new genes. B. A genomic knockdown experiment in D. melanogaster revealed rapid evolution of functional essentiality in new genes[6]. For example, 317 genes which originated between 69 Mya and 62 Mya were tested if gene knock-down can cause lethal phenotype, eventually, 93 out of 317 genes show lethal/semi-lethal phenotype after KD. The ages of genes from GenTree[7]. "avg." means the ratio of total lethal gene number (210 genes) to total tested genes number (752 genes).

Supplementary Figure 4. The evolutionary fates of new gene via duplication and measured selective forces. The selective force of duplicate polymorphisms in various genomic regions in Drosophila: Complete gene duplicate; Intergenic region duplicate; exonic duplicate; Intronic duplicate; All duplicates; duplicates on X chromosome; Autosomal duplicate[8].

Supplementary Table 1. Fourteen molecular mechanisms of new gene structure formation.

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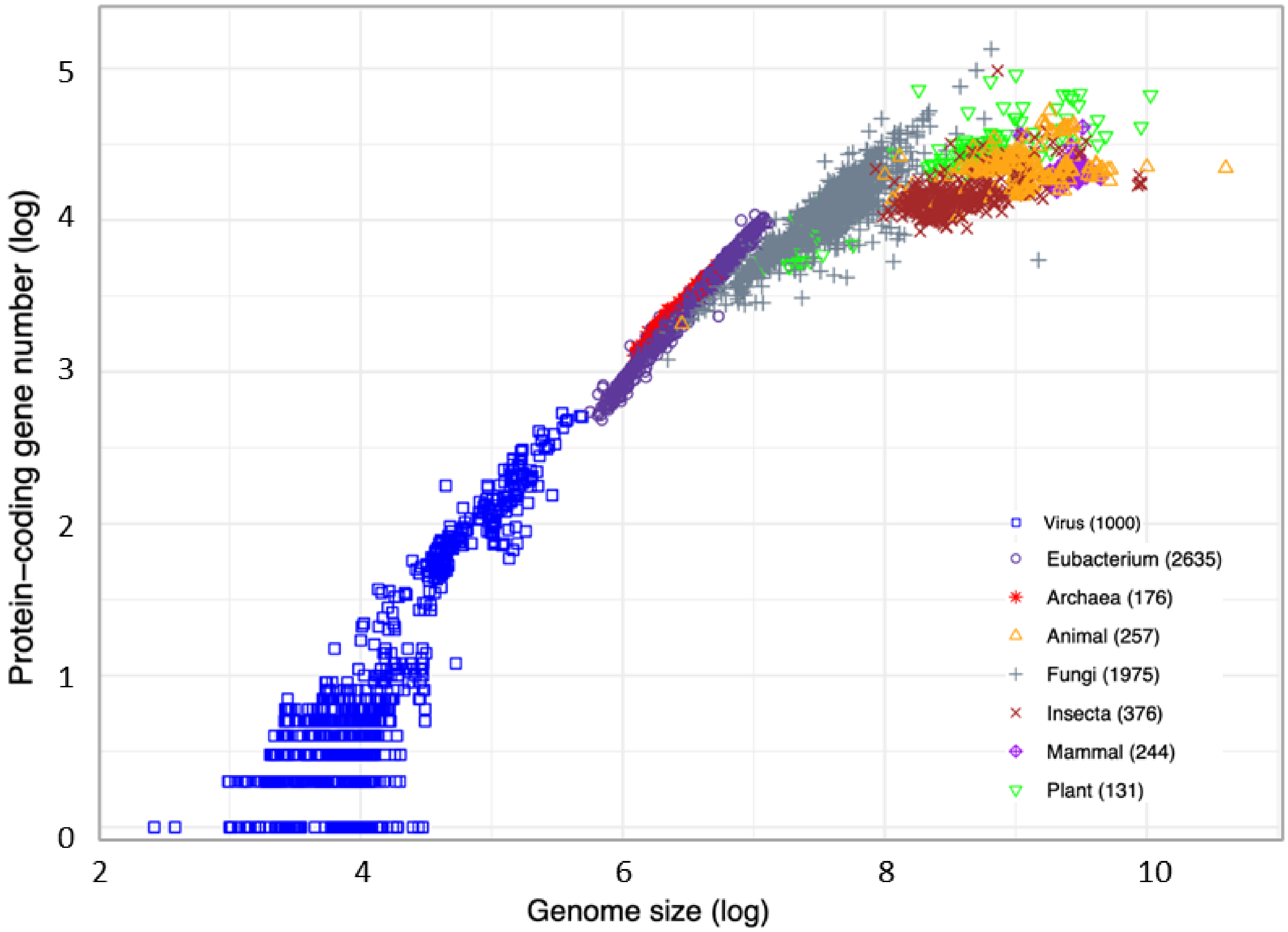
Supplementary Table 3 . References for new genes with documented phenotypes and functions.

Supplementary Figure 1. The distribution of gene numbers and genome sizes across 6,794 extant species



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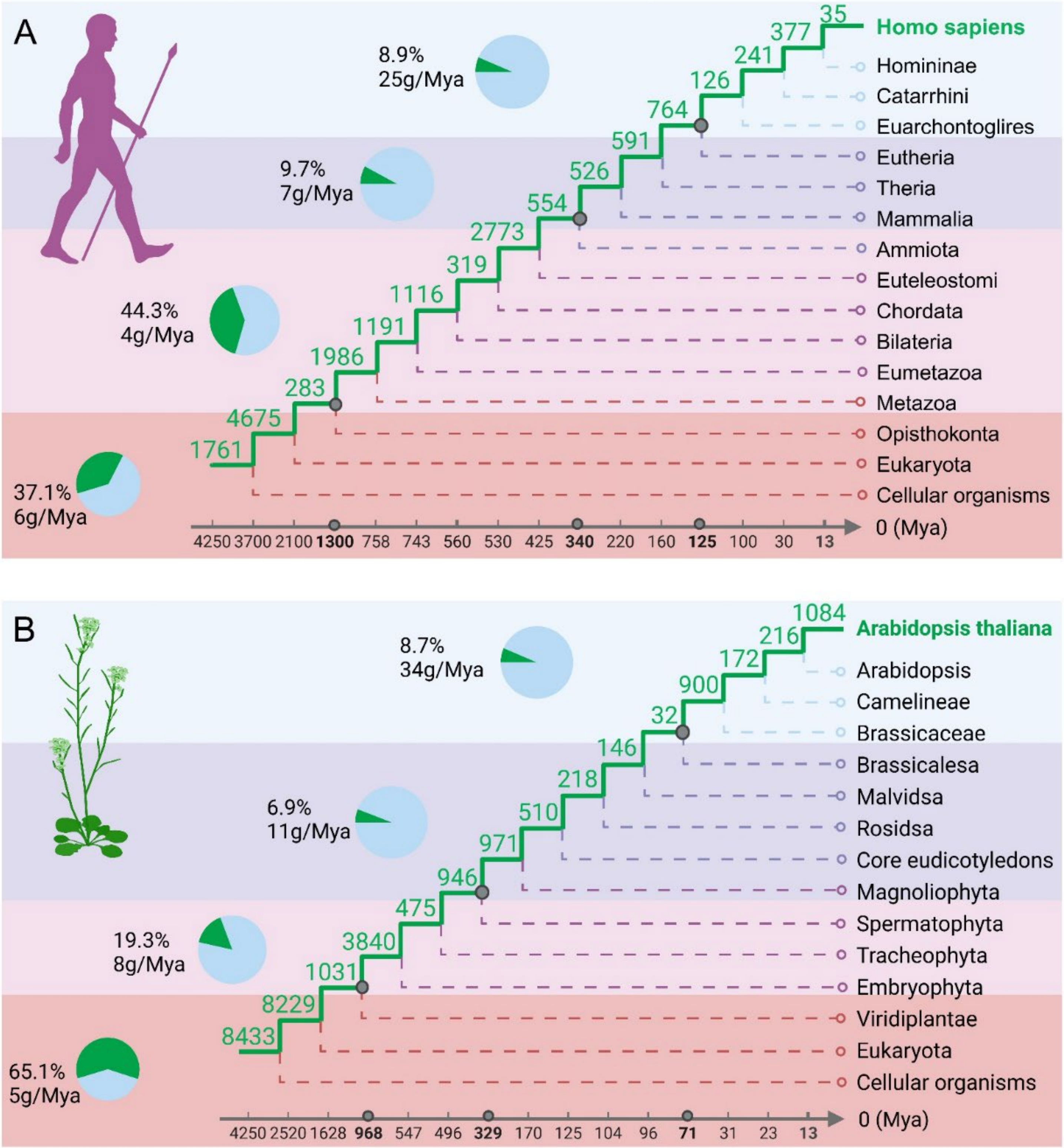

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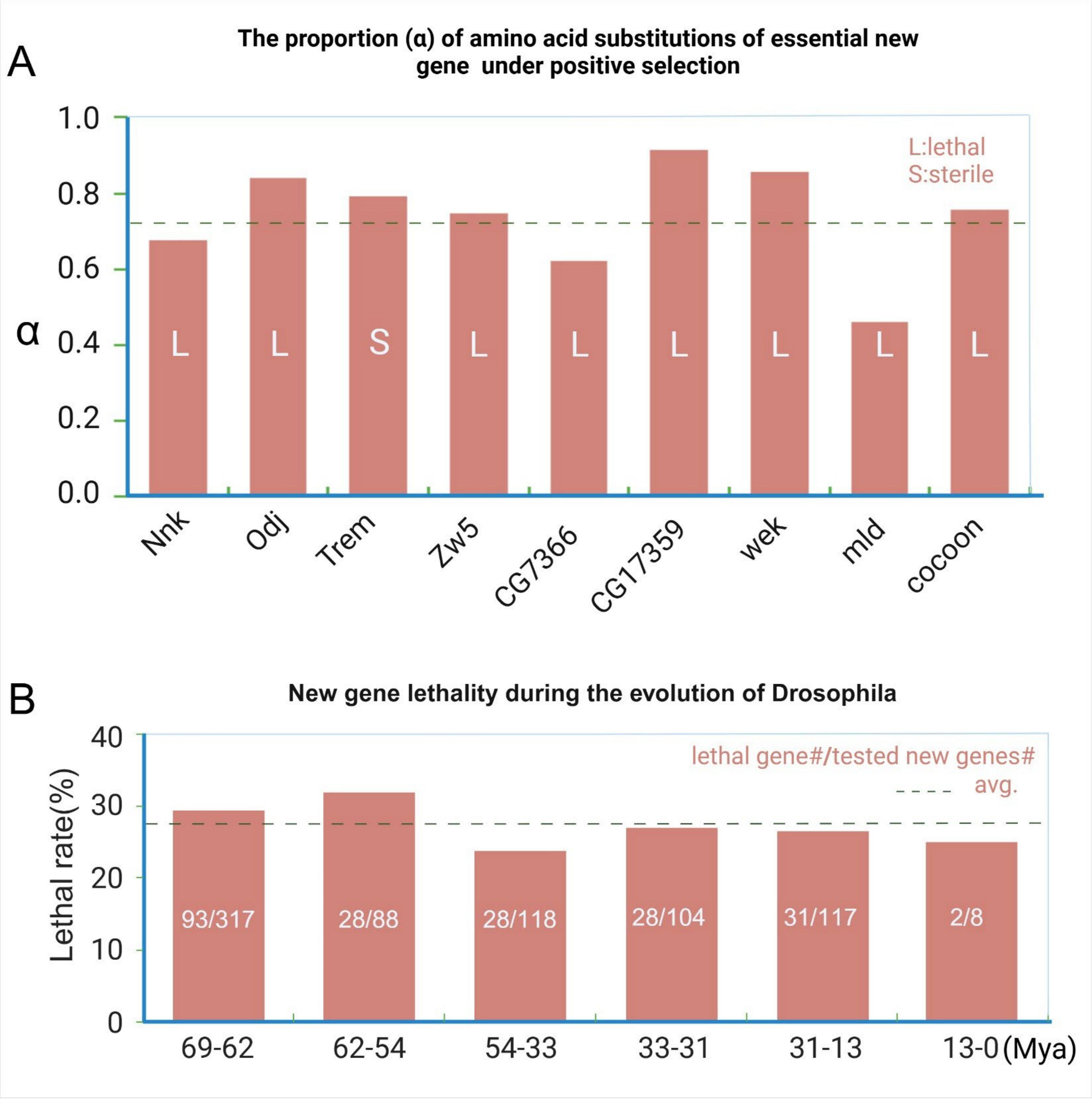

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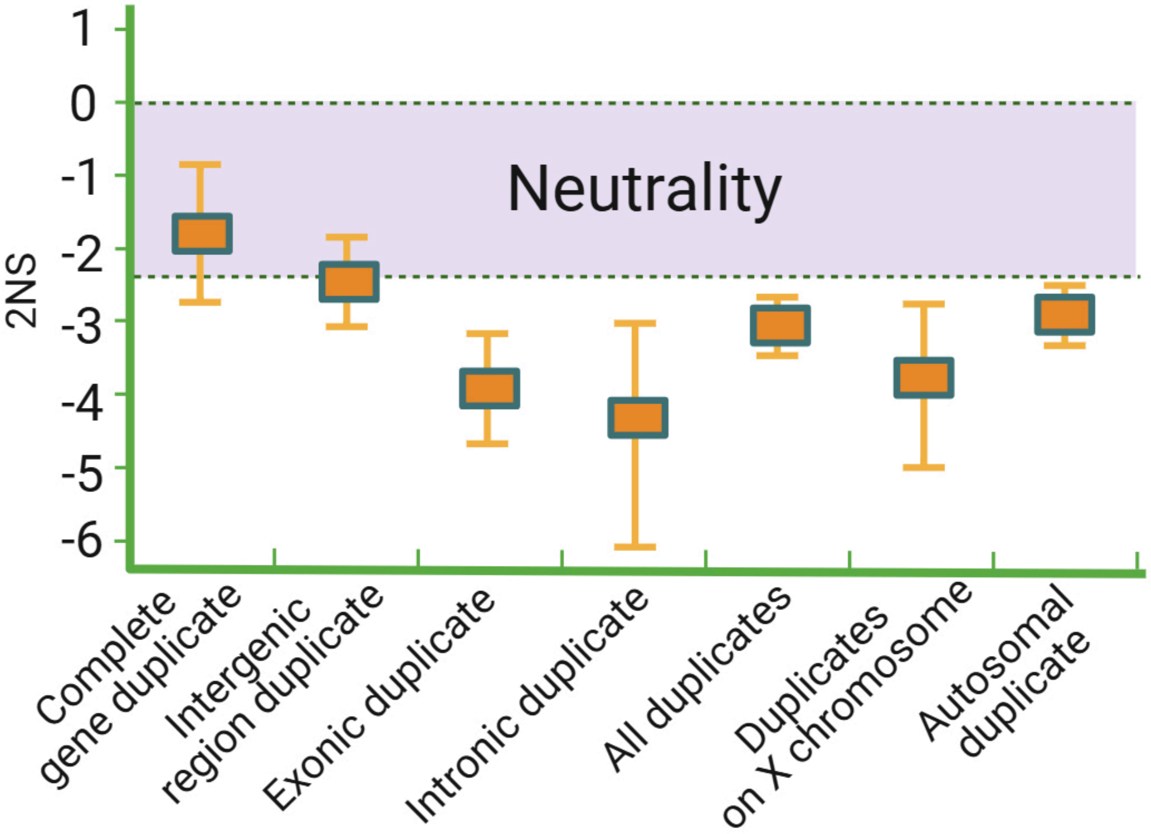

Supplementary Table 1. Fourteen molecular mechanisms of new gene structure formation.

	Mechanisms	Comments	Examples
Protein to protein	**1. Exon shuffling** Ectopic recombination of exons and domains from different genes or through retroposition[9].	A common process to create chimeric genes in Eukaryotes[10,11]	*jingwei*[12], *AK111451_3437611*[13], *CG18217*[14], *chirons*[15,] Chimeric TE-gene transcripts[10], *Sphinx*[16], Chimeric genes by truncated duplications[11], *wom*[17].
	2. Retroposition An RNA-based duplication creating new gene by reverse transcription[18].	A widely existing process in metazoans[19-21].	*PMCHL2*[22], *Sphinx*[23], *Pgk2*[24], *Zeus*[25], *HAPSTR2*[26].
	3. Gene duplication-divergence Chromosomal duplication, ectopic recombination, and genome duplication can duplicate genes[27,28].	A main process of new gene evolution, duplicates often evolved new and important functions[29,30].	*Apollo*[31], *Artemis*[31], *CGβ*[32], *Cid*[33], *Odysseus*[34], *COX4-like*[35].
	4.Horizontal gene transfer A gene is transferred between organisms[36-38].	Reported to frequently occur from bacteria to both eukaryotes and prokaryotes and play important role in genome innovation and evolution[39-45].	Vip3A[46], FHY3/FAR1 genes[47], tet(X) variants[48], Pismi_683611/Pismi_689601[49], LOC105383139[50]
	5.Gene fusion Two adjacent genes fuse into a single gene[51].	Involved formation of ~0.5% of prokaryotic genes[52].	fatty-acid synthesis enzymes[53,54], *Kua-UEV*[55], *Sdic*[56], *rpoB* and *rpoC*[57].
	6.Gene fission One single gene split into two genes[58]	Involved formation of ~0.5% of prokaryotic genes[59].	*kms1+*[60], *RPW8-NBS*[61].
	7.Isoform divergence The production of multiple, non-overlapping isoforms can drive distinct gene identities[62].	*NURF-1.D* and *NURF-1.B* are split isoforms from *NURF-1* in C. elegans with distinct roles in male and female gametogenesis, respectively[62]	NURF-1.D and NURF-1.B[62]
	8.Reading-frame shifting In a protein-coding gene, can generate novel proteins[63,64].	Frame shifts have been detected in a large number in human genomes and mosses[65,]	*YAN/AltYAN*[66], *CYP3A5*7 allele*[67], *MT-ATP6 frameshift mutation*[68], *SRY* gene[69], tRNA modification[70], Leucine-Rich Repeat Protein[71] ribosomal frameshift[72]. Cdkn2a and Rbm4[73].
Noncoding to protein	**9.De novo origination** A novel protein-coding gene generated from a noncoding ancestral sequence[74-79].	Detected in flowering plants, mammals, Drosophila, polar fish and yeast. Orphan genes may also be de novo gene candidates[80-87].	*C20orf203*[88],XLOC_175402[89], OsDR10[90], TWISTED1[91].
	10.Short repeat expansion Novel proteins of simple structures were created by repeating short repeats[92-94].	Antifreeze proteins essential for the viability of some polar fish were detected to be recently created by the expansion of short peptides[95].	*AFGP*[79,95-99], *Cb10*[100], *Bs_AFGP8*[101].
	11.TE domestication A transposable element (TE) sequence can be directly recruited by host genes to form a novel gene[102].	Generated 4% of new exons in human protein-coding genes and large proportion of promoters[103-106].	*HLA-DRβ1*[107], *hRED1*[108], *human DAF*[109], lungerkine mRNA[106], *mNSC1*[106], *PGBD1*[110].
	12.Bi-directional promoters It can drive expression both upstream and downstream, allowing for the creation of novel genes[111].	A large number of genes in S. cerevisiae are expressed under bi-directional promoters and inferred to be able to create new genes in upstream regions[112].	Pigt[113], peroxygenase[114], RPBSA promoter[115], designed bi-directional duplex promoters[116], catalase promoter system[117], TH2A and TH2B[118], PACRG[119].
Protein to noncoding	**13.Pseudogene regulators** Gene duplicates through recent pseudogenization regulate parental genes[120].	Mammalian pseudogenes were found to be transcribed and play a role as 'sponges' for microRNAs. It has been identified involving cancer in human[121-125] and rice[126,127].	*SUMO1P3*[128], *HBBP1*[129], *hYRNAs*[130].

Noncoding to noncoding	**14.Noncoding RNA genes** Noncoding RNAs can be generated from noncoding sequences[131].	These noncoding genes that do not encode proteins but can have various important functions[132,133] were detected in a large number in multiple species[134-140]. there are several mechanisms can promote noncoding RNA genes formation[141].	*Sphinx*[23,142,] HAR1[143-145], miR-941[146-148], Xist[149], roX[150], HOTAIR[151].

Supplementary Table 2. Lineage-specific origination rates of new genes across species.

Species/ lineage	Mechanism	Gene #/ Time (mya)/ origination rate
Vertebrate		
Homo sapiens (Homo)	Duplication & putative de novo genes	846/70/12.1[7]
	De novo	60/6.4/9.4[153]
	Retrogenes	76/63/1.2[155]
	Retrocopy with exon fusion	36/63/0.6[157]
Platichthys stellatus (Platichthys)	DNA duplication	1541/73.7/20.9[159]
	RNA duplication	96/73.7/1.3[159]
	De novo	128/73.7/1.7[159]
Mus musculus (Mus)	DNA/RNA duplication and de novo	2316/70/33.1[7]
D. rerio (Danio)	Orphan genes	66/320/0.2[164]
	Duplication	135/320/0.4[164]
Insecta		
Anopheles gambiae (malaria mosquitoes)	RNA-based duplication	28/100/0.3[167]
Bombyx mori (Bombyx)	Orphan genes	738/125/6[168]
Drosophila melangster (Sophophora)	Duplication & putative de novo genes	1848/62/29.8[134]
	Retrogenes	94/63/1.5[169]
	Exon shuffling	14/55/0.3[14]

Species/ Lineage	Mechanism	Gene #/ Time (mya)/ origination rate
Amoebozoa		
Dictyostelium discoideum (Dictyostelium)	DNA/RNA duplication and orphan	3304/520/6.4[152]
Fungi		
Saccharomyces cerevisiae (Saccharomyces)	De novo genes	415/82/5.1[154]
Lachancea fantastica (Lachancea)	De novo genes	288/82/3.5[154]
Escherichia coli	proto genes	9/0.05/1.4[156]
Nematoda		
Caenorhabditis elegans(Caenorhabditis)	Orphan genes and de novo genes	893/19/47[158]
	Orphan genes	5645/193/29[160]
Angiosperms		
Arabidopsis thaliana (Arabidopsis)	DNA/RNA duplication and de novo	2353/96/25[161]
Phyllostachys edulis (Phyllostachys)	Orphan genes	1622/46/35[162]
Citrus sinensis (Citrus)	Orphan genes	1039/8/129[163]
B. rapa (Brassica)	Orphan genes	529/26/20[165]
Triticum aestivum (Triticum)	Orphan genes	3812/19/200[166]
Oryza Sativa (Oryza)	RNA/DNA Duplication and de novo	7845/36/218[161]
	De novo genes	175/3.4/51[81]
	Gene fusion	310/4.9/63[119]
	Pack-Mule-mediated transduplication	76/63/1.2[170]
	Retropseudogene	337/53/6.4[13]

Supplementary Table 3 . References for new genes with documented phenotypes and functions.

Gene name	Function categories	phenotypical and functional studies references
Cocoon	*Development and morphogenesis*	[4]
Desr	*Development and morphogenesis*	[171]
ms(3)K81	*Development and morphogenesis*	[172]
Umbrea	*Development and morphogenesis*	[173,174]
bicoid[175]	*Development and morphogenesis*	[175]
ZAD-ZNF gene	*Development and morphogenesis*	[5]
EXOV	*Development and morphogenesis*	[176]
sORF2146	*Development and morphogenesis*	[177]
panish	*Development and morphogenesis*	[178]
chirons	*Development and morphogenesis*	[15]
XIST	*Development and morphogenesis*	[179-182]
Syncytin 1(ERVW1)	*Development and morphogenesis*	[183-185]
INSL4	*Development and morphogenesis*	[186]
FGF4	*Development and morphogenesis*	[187,188]
Prod1	*Development and morphogenesis*	[189]
JAUP1	*Development and morphogenesis*	[190]
jingwei	*Adaptation*	[191,192]
Adh-Finnega	*Adaptation*	[193]
CYP98A8/CYP98A9	*Adaptation*	[194,195]
CYP84A4	*Adaptation*	[196]
QQS	*Adaptation*	[197-204]
AtEWR	*Adaptation*	[2,205]
BrOG1	*Adaptation*	[206]
GapC	*Adaptation*	[207]
TCAF1/TCAF2	*Adaptation*	[208]
TRIMCyp	*Adaptation*	[209]
bcs-6	*Adaptation*	[210]
AFGP	*Adaptation*	[79]
GIG2	*Adaptation*	[211]
Xcbp1	*Brain and nervous system*	[171]
SRGAP2B/2C/2D	*Brain and nervous system*	[212-215]
ARHGAP11B	*Brain and nervous system*	[216-218]
NOTCH2NLA,B,C	*Brain and nervous system*	[219-221]
ENSG00000205704	*Brain and nervous system*	[222]
Olfactory receptor genes	*Brain and nervous system*	[223]
KRAB–ZNF family	*Brain and nervous system*	[224-226]
Primate opsin genes	*Brain and nervous system*	[227]
Odysseus (OdsH)	*Speciation*	[228-232]
JYAlpha	*Speciation*	[233]
iORF3 and iORF4	*Speciation*	[234,235]
HWS1 and HWS2	*Speciation*	[236]
YELLOW UPPER(YUP)	*Speciation*	[237]
Xmrk-2	*Speciation*	[238-242]
Apl	*Reproduction and Sexuality*	[31]
nsr	*Reproduction and Sexuality*	[243]
Sflc	*Reproduction and Sexuality*	[244]
Sdic family	*Reproduction and Sexuality*	[245,246]
p24-2	*Reproduction and Sexuality*	[173,247]
Zeus	*Reproduction and Sexuality*	[25,169,248,249]
Pros28.1A	*Reproduction and Sexuality*	[173,250]
Poseidon	*Reproduction and Sexuality*	[25]
sphinx	*Reproduction and Sexuality*	[16,251,252]
Tssor-3 and Tssor-4	*Reproduction and Sexuality*	[253]
POLDI/ Pldi	*Reproduction and Sexuality*	[254,255]
Bnams4b	*Reproduction and Sexuality*	[256-258]
INSL3	*Reproduction and Sexuality*	[259]
Goddard, Saturn, Atlas	*Reproduction and Sexuality*	[260-261]

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