MCB 5472

Types of selection

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Write script from exam

Work of Student project

Simple: dinucleotide sequence from single nucleotide sequence

Part I: Reading data

```
#!/usr/bin/perl
#hw10.pl modified from Erin Duffy
use warnings;
die "usage: hw10.pl <limit>\n" unless @ARGV == 1;
my $filename=$ARGV[0];
open(IN, "< $filename") or die "cannot open $filename:$!";</pre>
print "The file $filename is being analyzed:\n\n\n";
open(OUT, ">> table.out");
my $inseq = "";
my $ref = "";
my %nmer = ();
while(defined(my $line=<IN>)){
        chomp($line);
        if ($line=~/^>/) {
            $line =~ s/\>ref\l(\S+)\l//g;
             $ref=$1;
             print "$ref\n";}
        else {
                $inseq .= $line;
        }
}
        $inseq =~ tr/atgc/ATGC/;
        sinseq = s/s + //g;
        sinseq = \frac{s/N}{g};
       # print "$gi \n $inseq \n";
        print "$inseq \n";
```

Simple: dinucleotide sequence from single nucleotide sequence

Part 2: Analyzing data

```
#####input done, start analysis
         my @seqarray = split(//, $inseq);
                  my $count = @seqarray;
                  for (my $i = 0; $i<$count-1; $i++) {</pre>
                            my $subseq1 = $seqarray[$i].$seqarray[$i+1];
                            # my $nmer = '':
                            $nmer{$subseq1} += 1;
      my @nucs1 = ("A", "T", "G", "C");
      my @nucs2 = ("A", "T", "G", "C");
      foreach my $nuc (@nucs1) {
                foreach (@nucs2) {
                $dimer=$nuc.$_;
                print "$dimer\t$nmer{$dimer}\n";
                }}
                 # foreach (@sorted_by_value) {
                         print "dimer\t$_\toccurred$nmer{$_} times \n";
                 #
     #print "@seqarray\n";
     #print "@subseq\n";
     #my $count = @seqarray;
     #print "There were $count sequences read in.\n";
     close(IN);
```

#close(OUT);

Subroutine: dinucleotide sequence from single nucleotide sequence

```
dimfreq ($inseq); #output the last sequence into table
$inseq="";
sub dimfreq {
my $inseq = $_[0];
my %nmer = ();
        $inseq =~ tr/atgc/ATGC/;
        sinseq = s/(s+)/q;
        sinseq = \frac{s/N}{a};
        my @seqarray = split(//, $inseq);
                my $count = @segarray:
                for (my $i = 0; $i<$count-1; $i++) {</pre>
                         my $subseq1 = $seqarray[$i].$seqarray[$i+1];
                         $nmer{$subseq1} += 1;
                }
      my @nucs1 = ("A", "T", "G", "C");
      my @nucs2 = ("A", "T", "G", "C");
     foreach my $nuc (@nucs1) {
              foreach my $temp (@nucs2) {
              my $dimer=$nuc.$temp;
              if (!(exists $nmer{$dimer})){$nmer{$dimer}=0}; #defeats the purpose of using a hash, but we want a complete table
              print "\t$nmer{$dimer}";
              33
              print "\n";
               3
```

close(IN); close(OUT);

Analyze file with multiple ORFs

Part 1: Open file and print header

```
#hw10.pl modified from Erin Duffy
use warnings;
die "usage: script.pl <limit>\n" unless @ARGV == 1;
my $filename=$ARGV[0];
open(IN, "< $filename") or die "cannot open $filename:$!";</pre>
print "$filename is being analyzed:\n\n\n";
open(OUT, "> table.out");
my $inseq = "";
my $ref = "";
my \% mer = ();
my @nucs1 = ("A", "T", "G", "C");
my @nucs2 = ("A", "T", "G", "C");
##Print header##
print "REF";
                                                             Add OUT to
                             ←
 foreach my $nuc (@nucs1) {
                                                             print to file
              foreach (@nucs2) {
              $dimer=$nuc.$_;
              print "\t$dimer";
              }}
print "\n":
```

Analyze file with multiple ORFs

Part 2: Program Flow

```
my $flag="F";
while(defined(my $line=<IN>)){
        chomp($line);
         if (($line=~/^>/) && ($flag eq "T")) { #this is the second or more time a sequence is encountered
            $line =~ s/\l\:(c?\d+)//g;
                                                                                  dimfreq also
             $ref=$1;
                                                                                 prints to OUT
             dimfreq ($inseq); #calls subroutine to analyze sequence
             $inseq="";#reset sequence assembled from infile to an empty string
             print "$ref";} # print ref of next sequence to analysze
        if (($line=~/^>/) && $flag eq "F") {#this is the first sequence in the multiple fasta file
            $line =~ s/\l\:(c?\d+)//g;
            <pref=$1;</pre>
             print "$ref"; #prints seqname to table
            $flag="T"; #
             }
        else {
                $inseq .= $line;
        }
3
```

Subroutine: dinucleotide sequence from single nucleotide sequence

```
dimfreq ($inseq); #output the last sequence into table
$inseq="";
sub dimfreq {
my $inseq = $_[0];
my %nmer = ();
        $inseq =~ tr/atgc/ATGC/;
        sinseq = s/(s+)/q;
        sinseq = \frac{s/N}{a};
        my @seqarray = split(//, $inseq);
                my $count = @segarray:
                for (my $i = 0; $i<$count-1; $i++) {</pre>
                         my $subseq1 = $seqarray[$i].$seqarray[$i+1];
                         $nmer{$subseq1} += 1;
                }
      my @nucs1 = ("A", "T", "G", "C");
      my @nucs2 = ("A", "T", "G", "C");
     foreach my $nuc (@nucs1) {
              foreach my $temp (@nucs2) {
              my $dimer=$nuc.$temp;
              if (!(exists $nmer{$dimer})){$nmer{$dimer}=0}; #defeats the purpose of using a hash, but we want a complete table
              print "\t$nmer{$dimer}";
              33
              print "\n";
               3
```

close(IN); close(OUT);

Table.out in text editor

	Ľti	able.o	ut	÷													
REF AA	AT	AG	AC	TA	TT	ΤG	тс	GA	GT	GG	GC	CA	СТ	CG	CC	Sum	
55 26	14	70	45	16	25	49	77	69	43	188	101	43	85	95	181	1127	7
1235	36	26	80	68	14	37	61	70	91	47	223	116	68	73	113	148	1271
2490	27	39	78	68	8	27	68	99	102	63	227	122	73	74	142	208	1425
c4895	28	4	55	38	14	33	38	67	53	23	146	96	30	92	80	185	982
c5506	20	8	32	28	11	21	26	40	35	21	110	49	24	46	47	101	619
c7340	64	50	107	112	18	45	97	116	144	62	273	148	106	120	150	236	1848
c7993	15	4	40	25	13	28	25	40	39	32	110	54	18	42	60	101	646
c8699	39	11	69	26	4	19	27	27	72	10	92	69	28	37	57	76	663
8799	9	2	13	8	4	5	8	6	8	6	24	20	11	10	14	24	172
9022	1	2	4	3	4	10	2	9	2	2	11	10	4	11	8	11	94
12493	34	27	56	55	17	24	49	67	68	35	127	87	52	72	85	158	1013
c14311	4	12	26	33	12	33	48	75	27	26	114	84	32	97	63	144	830
14329	24	12	58	48	14	24	43	54	70	17	147	105	34	82	91	171	994
15508	17	1	46	10	4	19	13	47	35	14	99	59	19	49	49	117	598
16106	37	16	68	54	10	14	63	49	74	47	159	90	54	59	80	148	1022
17121	36	17	78	41	12	29	55	55	87	33	171	96	38	72	82	126	1028
c18677	19	6	44	29	8	23	22	41	46	7	94	59	25	58	47	106	634
c20001	24	17	74	65	30	50	67	67	87	47	246	113	38	100	107	190	1322
c20374	7	2	22	16	9	16	19	22	23	15	79	25	8	33	23	54	373
c21072	10	8	43	22	7	28	40	41	55	28	140	55	10	53	55	77	672
c21767	9	6	39	26	16	16	29	46	32	23	119	63	23	62	51	128	688
c23014	32	35	68	88	29	43	60	76	77	57		102		74	109	169	1245
23080	45	13	146	77	19	55	92	133	138	75	362	201	78	157	176	327	2094

Table.out in excel

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3 55 4 1235	26 36	14 /	80 68	45 16 68 14		7 61	77 70	69 91	43 47	188 223	101 116	43 68	73 1	113	181 148	1271 0.0	0.02832415 0.0	0.02045633 0.	8 0.06294256 0	8 0.03992902 0.0 5 0.05350118 0.0	0.01101495 0.02	0.02911094 0.04	0.04799371 0.0	0.05507474 0.0	0.07159717 0.0	0.03697876	0.1754524 0	4 0.09126672 0	2 0.05350118 0	8 0.05743509 0	9 0.08890637 0	7 0.11644375	1 1.7567E-4 1 1.1294E-4
5 2490 6 c4895	27	39 -	78 68	68 8 38 14	8 27 14 33	7 68	99	102	63 23	227	122	73	74	142	208	1425 0.0	0.01894737 0.0	0.02736842 0.	0.05473684	4 0.0477193 0.0 5 0.03869654 0.0	0.00561404 0.01	0.01894737 0.0	0.0477193 0.0	0.06947368 0.0	0.07157895 0.0	0.04421053 0.	0.15929825 0	5 0.08561404 0	4 0.05122807 0	7 0.05192982 0	2 0.09964912 0	2 0.14596491	1 6.9123E-4
6 C4895 7 c5506	28			38 14 28 11			40	35	23	146	49	24		47	185					8 0.04523425 0.													1 1.56/1E-0 1 2.549E-0
1910 c1794909			81 64	64 19	19 22	76	63	83	63	227	115	71		104	150					2 0.05059289 0.0													1 7.59266-
1911 1794926 1912 1795140	12	6 1		9 7 13 4	4 5	10	12	14	10	22 27	20 20	10 15	12	17 19	40 28	208 0.0	0.03365385 0.0	0.02884615 0.	6 0.05769231		0.01923077 0.02	0.02403846 0.04	0.04326923 0.0	0.05769231 0.0	0.05769231 0.0	0.03365385 0.	0.12980769 0	9 0.09615385 0	5 0.07211538 0	8 0.05769231 0	1 0.09134615 0	5 0.13461538	1 0.00066 1 4.0863E-
1913 c1796479 1914 c1797414	31	37 7		73 17 39 11	17 25 11 33		63	85	48	164	90	81	55	90	139	1131 0.0	0.02740937 0.0	0.03271441 0.	0.06542882 0	2 0.06454465 0.0	0.01503095 0.02	0.02210433 0.05	0.05216622 0.0	0.05570292 0.0	0.07515473 0.0	0.04244032 0.	0.14500442	2 0.0795756 0	5 0.07161804 0	4 0.04862953	3 0.0795756 0	6 0.12290009	1 2.1895E-0
1915 c1798568	25		47 39 80 50	50 12	18 20	0 47	54 68	80	27 43	111 165	69 120	35 49	71 1	117	147	1119 0.0	0.02234138 0.0	0.01697945	0.0714924 0	0.04468275 0.0	0.01608579 0.0	0.0178731 0.04	0.04200179 0.0	0.06076854 0.	0.0714924 0.0	0.03842717 0.	0.14745308 0	8 0.10723861	0.0437891 0	1 0.06344951 0	0.10455764 0	4 0.13136729	1 2.4207E-0
1916 c1799172	14	9	26 3'	30 7	7 13	25	40	27	21	81	49	33	43	45	107	570 0.		0.01578947 0.	0.04561404 0	4 0.05263158 0.	0.0122807 0.02	0.02280702 0.04	0.04385965 0.0	0.07017544 0.0	0.04736842 0.0	0.03684211 0.	0.14210526 0	6 0.08596491 0	1 0.05789474	4 0.0754386 0	5 0.07894737	7 0.1877193	1 7.29E-0
1917 c1799385 1918 c1800396	0		12 2 31 37	2 1 37 J'	· · · · · · · · · · · · · · · · · · ·	14 , 45	8 50	44	28	37 152	26	3		18 90	41 169	205 886 0.0	0 01354402 1			0.0097561 0.0 0.04176072 0.0													1 0.000743 1 0.000188
1919 c1801381	31	21 6	60 57	57 20			54	72	42	169	85	46	50 1	101	105	979 0.0	0.03166496 0.0	0.02145046 0.	0.06128703 0	3 0.05822268 0.0	0.02042901 0.02	0.02757916 0.03	0.03983657 0.0	0.05515832 0.0	0.07354443 0.0	0.04290092 0.	0.17262513 0	3 0.08682329 0	9 0.04698672 0	2 0.05107252	2 0.1031665	5 0.1072523	1 1.9391E-0
1920 1801846 1921 1802567	17		34 26 91 72	26 9 72 21	9 16 21 51		35	34	18	82	64 156	25		60 124	108					9 0.04347826 0.0 3 0.04739961 0.0													1 1.3612E-0
1922 c1805269	35	18	74	2 22	14 71	1 74	117 88	76 60	54	185	156	38		124	261	1180 0.0	0.02966102 0.0	0.01355932 0.	0.06271186 0	5 0.02033898 0.0	0.01186441 0.06	0.06016949 0.06	0.06271186 0.0	0.07457627 0.0	0.05084746 0.0	0.04576271 0.	0.15677966 0	6 0.09237288 0	8 0.03220339 0	9 0.09152542 0	2 0.06440678 0	8 0.13050847	1 5.7582E-0
1923 c1805985	20	11	39 ?	34 11	.1 22	2 44	42	45	34	136	63	28	54	58	79	720 0.0	0.02777778 0.0	0.01527778 0.	8 0.05416667 0	7 0.04722222 0.0	0.01527778 0.03	0.03055556 0.06	0.06111111 0.0	0.05833333	0.0625 0.0	0.04722222 0.	0.18888889	9 0.0875 0	5 0.03888889	9 0.075 0	0.08055556 0	6 0.10972222	1 2.6664E-
1924 1806013 1925 c1806857	21		21 9 38 32	9 8 32 7	9 14	14	25	15	10	34	27	22	26 32	17 58	48 81					7 0.03146853 0.0 9 0.05673759 0.													1 5.0499E-0 1 9.9454E-0
1926 1807406		18 7	74 38	32 8 38 14	14 26		69	76	34	183	111	42	78	98	162	1114 0.0	0.03859964 0.0	0.01615799 0.	0.06642729 0	9 0.03411131 0.0	0.01256732 0.02	0.02333932 0.04	0.04308797 0.0	0.06193896 0.0	0.06822262 0.0	0.03052065 0.	0.16427289 0	9 0.09964093 0	3 0.03770197 0	7 0.07001795 0	5 0.08797127	7 0.1454219	1 0.000128
1927 1808515 1928 1809816	53		54 50	58 16 24 14	16 28		67	97	35	195	115	60	87	94	167					1 0.04654896 0.0 4 0.03003755 0.													1 0.000233
1929 c1811965	14	41 5	52 65	24 14 65 27		64	89 115	57	14	82 133	80 112	74		64 105	159	1264 0.0	0.01344937 0.0	0.03243671 0.	0.04113924 0	4 0.05142405 0.0	0.02136076 0.0	0.0403481 0.05	0.05063291 0.0	0.09098101 0.0	0.04509494 0.	0.0403481 0.	0.10522152 0	2 0.08860759	9 0.0585443 0	3 0.09018987 0	7 0.08306962	2 0.1471519	1 0.000190
1930 c1812607	15	9 4	43 27	27 9	9 18	33	35	38	32	93	61	32	36	56	97	634 0.0	0.02365931 0.0	0.01419558 0.	3 0.06782334 0	4 0.04258675 0.0	0.01419558 0.02	0.02839117 0.05	0.05205047 0.0	0.05520505 0.0	0.05993691 0.0	0.05047319 0	0.1466877 0	7 0.09621451 0	1 0.05047319 0	9 0.05678233 0	3 0.08832808 0	8 0.15299685	1 1.2975E-
1931 c1813098 1932 c1813840	4		17 22 43 38	22 y	9 24 11 10		44	19 49	17	53 99	46	24 40		41 71	78 110					0.04564315 0. 0.05198358 0.0													1 0.000359 1 8.0336E-
1933 c1814696	10	5 3	35 36	365	5 31	1 42	38 48	46	31 23	128	73 80	26	67	72	124	778 0.0	0.01285347 0.0	0.00642674 0.	0.04498715 0	5 0.04627249 0.0	0.00642674 0.03	0.03984576 0.05	0.05398458 0.0	0.06169666 0.0	0.05912596 0.0	0.02956298 0.	0.16452442 0	2 0.10282776 0	5 0.03341902 0	2 0.08611825 0	5 0.09254499 0	9 0.15938303	1 0.000207
1934 c1815741	26	10 5	51 48	48 11	11 23	3 64	54	60	50	173	100	38	69	96	172	1045 0.0	0.02488038 0.0	0.00956938 0.	8 0.04880383 0	3 0.04593301 0.0	0.01052632 0.02	0.02200957 0.06	0.06124402 0.0	0.05167464 0.0	0.05741627 0.0	0.04784689 0.	0.16555024 0	4 0.09569378 0	8 0.03636364 0	4 0.06602871 0	1 0.09186603	3 0.1645933	1 5.6692E-0
1935 c1816937 1936 1816999	30 38			45 11 58 13	11 47 13 19	9 47	74	70 67	40	172 128	125 101	47 66	82	104 86	192 170	1067 0.0	0.03561387 0.0	0.02249297 0.	0.05998126 0	3 0.03753128 0.0 5 0.05435801 0.0	0.01218369 0.01	0.01780694 0.04	0.04404873 0.0	0.07029053 0.0	0.06279288 0.0	0.02717901 0.	0.11996251 0	1 0.09465792 0	2 0.06185567 0	7 0.07685098 0	8 0.08059981 0	1 0.15932521	1 5.0201E- 1 6.9764E-
1937 c1819198	35	19 8	80 45	45 17	17 38	8 47	68	75	34	199	101	51	79 1	117	125	1164 0.0	0.03006873 0.0	0.01632302 0.	0.06872852 0	2 0.03865979 0.0	0.01460481 0.03	0.03264605 0.04	0.04037801 0.0	0.05841924 0.0	0.06443299 0.0	0.02920962	0.1709622 0	2 0.11597938 0	8 0.04381443 0	3 0.06786942 0	2 0.10051546 0	6 0.10738832	1 7.8811E-
1938 c1819771 1939 c1820835	5		26 25 82 64	25 7 64 12	7 10		22	32 100	19	93 156	44	17		44 92	62 131					5 0.0534188 0.0 8 0.06003752 0.0													1 0.000274
1940 1820901	34 14	23 2	23 28	64 12 28 6	6 27	7 55	55	100 37	30 24	137	89	58	70	68	112	795 0.0	0.01761006 0.0	0.02893082 0.	0.02893082 0	2 0.03522013 0.0	0.00754717 0.03	0.03396226 0.06	0.06918239 0.0	0.06918239 0.0	0.04654088 0.0	0.03018868 0.	0.17232704 0	4 0.10691824 0	4 0.03899371 0	1 0.08805031 0	0.08553459	9 0.1408805	1 9.3148E-0
1941 c1822297	16	3 3	37 32	32 10	10 25		45	34	24	69	45	28		42	100	584 0.0	0.02739726 0.0	0.00513699 0.	0.06335616 0	5 0.05479452 0.0	0.01712329 0.04	0.04280822 0.03	0.03938356 0.0	0.07705479 0.0	0.05821918 0.0	0.03938356 0.	0.11815068 0	8 0.07705479 0	9 0.04794521	1 0.0890411 0	0.07191781 0	1 0.17123288	1 1.8458E-0
1942 c1822704 1943 1822765	13		24 12 23 13	12 5	3	25	26	29 23	18	70	35	10	31	33 35	59					3 0.02912621 0.0 9 0.03757225 0.0													1 1.8421E- 1 0.0002465
1944 1823109	7	5 1	14 16	13 6 16 5	5 33	26	37	20	10	67	30	14	53	26	77	445 0.0	0.01573034 0.0	0.01123596 0.	5 0.03146067 0	7 0.03595506 0.0	0.01123596 0.0	0.0741573 0.05	0.05842697 0.0	0.08314607 0.0	0.04494382 0.0	0.02247191 0	0.1505618 0	8 0.08314607 0	7 0.02696629 0	9 0.11910112 0	2 0.05842697 0	7 0.17303371	1 0.0001329
1945 1823527	21	12 6	62 21	21 6	6 15		25	63	10	90	61	24	39	44	75	597 0.0	0.03517588 0	0.0201005 0	0.1038526 0	5 0.03517588 0.0	0.01005025 0.02	0.02512563 0.04	0.04857621 0.0	0.04187605 0.1	0.10552764 0.0	0.01675042 0.	0.15075377 0	7 0.10217755 0	5 0.04020101 0	1 0.06532663 0	3 0.07370184 0	4 0.12562814	1 6.2639E-
1946 1824153 1947 1825398	17	6 0		12 7 23 11	7 16 11 41	5 55	26 31	32 44	23 31	83 163	33 66	13		21 63	76 62					2 0.02564103 0.0 1 0.03328509 0.0													1 8.2145E 1 0.000163
1948 1826158	52		61 95	23 11 95 36	J6 56	72	59	71	31 61	156	105	81	74 1	105	133	1249 0.0	0.04163331 0.	0.0256205 0.	0.04883907 0	7 0.07606085 0.0	0.02882306 0.04	0.04483587 0.05	0.05764612 0.0	0.04723779 0.0	0.05684548 0.0	0.04883907 0.	0.12489992 0	2 0.08406725 0	5 0.06485188	8 0.0592474 0	4 0.08406725 0	5 0.10648519	1 0.000206
1949 c1828151 1950 c1828635	25		54 25	25 11	.4 17	41	39	53	29	152	63	21	54	51	110	752 0.0	0.03324468 0.0	0.00930851 0.	0.07180851 0	0.03324468 0.0	0.01462766 0.02	0.02260638 0.05	0.05452128 0.	0.0518617 0.0	0.07047872 0.0	0.03856383 0.	0.20212766	6 0.0837766 0	5 0.02792553 0	3 0.07180851 0	0.06781915	5 0.1462766	1 3.58E
1950 c1828635 1951 1828653	14	18	36 16 121 53	16 5 53 18	× 15 10 31	9 27 1 60	27 92	34 114	15 38	87 227	47	15 62		32 135	75 293		0.02839757 0.03205531 0.0			1 0.03245436 0.0 3 0.03331238 0.0													1 1.2909E 1 2.2982E
1952 1830250	13	10 2	24 17	17 2	2 4	4 15	92	29	38	25	23	20	13	24	38	283 0.	0.0459364 0.0	0.03533569 0.	0.08480565 0	5 0.06007067 0.0	0.00706714 0.01	0.01413428 0.05	0.05300353 0.	0.0565371 0.	0.1024735 0.0	0.03533569 0.	0.08833922 0	2 0.08127208 0	8 0.07067138	8 0.0459364 0	4 0.08480565 0	5 0.13427562	1 0.00034
1953 1830532 1954 1831050	8			22 9	9 12		29	25	9	80	50	14		51	85					2 0.04690832 0.0													1 0.00010
1955 1832902		17 5		100 28 56 21			96 48	119 51	62 23	300 126	170 84	94 54	75	138 60	280 141	919 0.0	0.04461371 0.0	0.01849837 0	0.0554951	2 0.05393743 0.0 L 0.0609358 0.0	0.02285092 0.02	0.02611534 0.05	0.05114255 0.0	0.05223069 0.	0.0554951 0.	0.0250272 0.	0.13710555	5 0.0914037 0	0.05875952 0	2 0.08161045 0	5 0.06528836 0	6 0.15342764	1 0.0003
1956 c1834280	8	4 2	23 17	17 9	9 26	5 27	17	25	13	83	54	10	36	43	74	469 0.0	0.01705757 0.0	0.00852878 0.	8 0.04904051 0	1 0.03624733 0.0	0.01918977 0.0	0.0554371 0.0	0.0575693 0.0	0.03624733 0.	0.0533049 0.0	0.02771855 0.	0.17697228 0	8 0.11513859 0	9 0.02132196 0	6 0.07675906 0	5 0.09168443 0	3 0.15778252	1 0.00010
1957 1834279 1958 1835328	32		70 43 138 96	43 13 96 30	3 37 65	7 53 5 107	65 141	68 146	33	171	98 220	54		76 195	145	1054 0.0	J.03036053 0.0	J.01897533 0	0.06641366 0	5 0.04079696 0.0 7 0.04071247 0.0	.01233397 0.04	.03510436 0.0*	.05028463 0.0	J.06166983 0.0	.06451613 0	0.0313093 0	0.16223909	0.09297913	0.0512334	0.07210626 0	0.07210626	0.13757116	1 9.60468
1959 1837695	3	3	5 0	07	1 6	4 4	2	2	0	355	9	85 5	4	3	7	64 (0.046875	0.046875	0.078125	5 0 0	0.015625 0	0.09375	0.0625	0.03125	0.03125	0	0.15625	5 0.140625	5 0.078125	5 0.0625	5 0.046875	5 0.109375	1 0.00038
1960 1837799	9		12 11	11 6	6 14	24	41	11	9	79	52	12		37	87	466 0.	0.0193133 0.0	0.01287554 0.	0.02575107 0	7 0.02360515 0.0	0.01287554 0.03	0.03004292 0.05	0.05150215 0.0	0.08798283 0.0	0.02360515 0	0.0193133 0	0.1695279 0	9 0.11158798 0	8 0.02575107 0	7 0.12017167 0	7 0.07939914 0	4 0.18669528	1 6.31728
1961 c1839208 1962 c1839999	16		56 45 48 38	+5 10	2 1b 7 24	. 51 . 44	48	65 59	39	191 130	104 75	55 32		102 66	100 117					4 0.04643963 0.0 4 0.04785894 0.0													1 0.00011
1963 c1840495	4	3	48 30 *	4 185	5 10	33	27	41	22	98	46	32	40	46	67	496 0.0	0.00806452 0.0	0.00604839 0.	0.06048387 0	7 0.03629032 0.0	0.01008065 0.02	0.02016129 0.06	0.06653226 0.0	0.05443548 0.0	0.08266129 0.0	0.04435484 0.	0.19758065 0	5 0.09274194 0	4 0.01209677 0	7 0.08064516 0	5 0.09274194 0	4 0.13508065	1 0.00036
1964 c1841086	17	13 7	36 3/	30 9	ə 9	26	34	42	25	87	35	28		40	68 138	530 0.0	0.03207547 0.	0.0245283 0.	0.06792453 0	3 0.05660377 0.0	0.01698113 0.01	0.01698113 0.0	0.0490566 0.0	0.06415094 0.0	0.07924528 0.0	0.04716981 0.	0.16415094 0	4 0.06603774 0	4 0.05283019 0	9 0.05849057	7 0.0754717 0	7 0.12830189	1 2.31758
1965 1841119 1966 1841799	21		35 26 14 6	<u>.6</u> / 5 2	2 3	9 22 3 15	40	33 15	16	90 28	60 26	25	14	53 19	43	227 0.0	0.04405286 0.0	0.02202643 0.	8 0.06167401 0	8 0.04140127 0. 1 0.02643172 0.0	0.00881057 0.01	0.01321586 0.0	0.0660793 0.0	0.04405286 0.	0.0660793 0.0	0.03524229 0.	0.12334802 0	2 0.11453744 0	4 0.03964758 0	8 0.06167401 0	1 0.08370044 0	4 0.18942731	1 3.8169 1 0.00028
1967 1842023	26	10 7	75 62	62 19	19 62	2 102	160	75	61	349	20	52	210 1	171	388	2033 0.0	0.01278898 0.0	0.00491884 0.	0.03689129	0.0304968 0.0	0.00934579 0.0	0.0304968 0.05	0.05017216 0.0	0.07870143 0.0	0.03689129 0.0	0.03000492 0.	0.17166749 0	9 0.10378751 0	1 0.02557796 0	6 0.10329562 0	2 0.08411215 0	5 0.19085096	1 0.00020
1968 c1844853 1969 c1845586	19			22 9 40 10	9 12 10 12	2 38	50 35	53 39	25	135 117	85	32		62 63	132 126					2 0.02719407 0.0 2 0.05326232 0.0													1 1.4255 1 5.3115
1970 c1846329	15			40 10 22 9	10 12 9 22		35 51	42	27	132	78 71	40	65	60	125	749 0.0	0.02403204 0.0	0.00801068 0.	8 0.05607477	7 0.0293725 0.0	0.01201602 0.0	0.0293725 0.05	0.05073431 0.0	0.06809079 0.0	0.05607477 0.0	0.03604806 0.	0.17623498 0	8 0.09479306 0	5 0.02536716 0	6 0.08678238 0	8 0.08010681 0	1 0.16688919	1 1.0429
1971 c1848122	43	24 10	101 83	83 22	22 35	5 86	104	110	72	297	182	77	117 1	177	266	1796 0.0	0.02394209 0.0	0.01336303 0.	0.05623608 0	8 0.04621381 0.0	0.01224944 0.01	0.01948775 0.04	0.04788419 0.0	0.05790646 0.0	0.06124722 0.0	0.04008909 0.	0.16536748	8 0.1013363 0	3 0.04287305 0	5 0.06514477 0	7 0.09855234	4 0.1481069	1 1.1018
1972 1848185 1973 SUM		37 7 28073 10445		76 10 981 25371			88 111990 11	89 111268	47 61525	147 269676	119 167318 77	88 77086 13		118 49208 26	199 264213 17	1313 0. 1773900	J.0312262 U.v	J.02817974 U	J.05635948 U	8 0.05788271 0.0	.00761615 0.02	.02970297 0.0+	J4798172 U.V	.06702209 u	J.0677837 U.V	03579589 u	J.11195735	0.09063214 0	0.06702209	0.05940594 u	0.08987053	0.15156131	1 1.57
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1975																		0.00907852 0.	0.01557083 0	3 0.01197895 0.0	0.00609343 0.01	0.01391823 0.01	0.01017696 0.	0.0155053 0.0	0.01596397 0.0	0.00963836 0.	0.02568402 0	2 0.01156596 0	5 0.01371451	1 0.0187682 0	2 0.01162268 0	8 0.02710365 STDEV	
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294E-06 123E-05 671E-06 549E-05 926E-06 926E-06 926E-06 926E-06 9395E-08 83	2.14446-05 0.00013324 0.00013824 5.04E-05 3.5101E-06 0.00012822 8.4188E-06 1.3038E-09 0.00012954 0.00025045 0.000225045 0.000225045 0.000225045 0.000225045 0.000225045 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.0523E-06	1.6492E-05 1.7179E-05 8.2555E-06 5.1628E-05 0.00043116 1.4143E-06 4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	6.1619E-05 4.2752E-06 4.2752E-06 4.837E-05 1.7401E-07 2.4418E-05 3.337E-05 0.00025806 1.2896E-05 9.3826E-07 4.8723E-05 0.00015844 4.7225E-06 0.00015847 1.5137E-05 0.00015847 4.7225E-06 0.000542 2.4675E-06	1.0807E-05 7.5487E-05 2.0945E-09 1.2029E-05 5.1463E-07 0.00027792 2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 5.5918E-07 2.2801E-07	5.3303E-07 0.00011867 1.4167E-05 1.6684E-05 1.0684E-05 3.367E-05 5.9856E-05 0.00013985 0.00013925 0.00013923 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	7.4875E-07 1.2989E-06 0.00010328 4.7002E-05 0.00012589 2.1263E-05 3.1246E-05 1.0938E-05 4.5875E-06 4.5875E-06 4.575E-06 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	6.4921E-05 4.0216E-05 2.2969E-05 2.215E-06 0.00017768 0.000107 2.5591E-05 5.5192E-05 5.5192E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	7.8714E-05 7.8391E-05 7.6625E-05 3.822E-05 8.3381E-06 6.0554E-07 2.5329E-05 0.0001545 0.0001545 0.00013853 7.6866E-05 0.00023583	5.2684E-06 9.0765E-05 0.00012683 5.7423E-07 0.00022858 9.1476E-05 1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	0.00054887 5.291E-05 1.121E-05 0.00065955 0.00075198 0.00298982 0.00049358 4.9279E-05 0.00014096	9.3354E-06 7.5831E-05 1.1817E-05 0.00022989 1.1649E-05 3.3949E-05 3.3552E-06 0.00021746	0.00010091 6.041E-05 0.00016656 2.1935E-05 0.00016055 6.2746E-07 0.00082138 0.00079312	0.00034381 0.00057828 0.00031361 2.7683E-06 0.00052959 9.2266E-05 0.00033434 0.0007479	2.2977E-05 0.00024137 7.0044E-06 6.6979E-05 3.6082E-06 7.9063E-05 5.2319E-05 2.0588E-05	0.00105631 8.8791E-06 0.00155601 0.00020226 0.00092219 0.0007866 0.00020533 0.00067832	0.002343897 0.001646324 0.002609396 0.001385811 0.003184505 0.00562127 0.002551174 0.002551174	0.04841381 0.04057492 0.05108224 0.03722649 0.05643141 0.07497513 0.05050915 0.0594384
123E-05549E-05549E-05549E-05549E-05549E-05549E-05549E-05549E-05549E-05549E-05549E-056454-0554863E-055895E-08835E-055207E-05542E-06542E-075431827E-05542E-066454E-075582E-05001287568858E-050012875779755-052012855885820500127779755-052012855885820500127779755-052012855885820500127779755-052012855885820500127779755-052012855885805001287779755-052012855885805001287779755-052012855885805001287779755-052012855885805001287779755-052012855885805001287779755-052012855885805001287779755-052012855885805001287779755-052012855885805001287779755-0520128558858050012855885805001285588580500128558858050012855885805001285588580500128558858050012855885500128558850500128558850500128558850500128558855001285588505001285588505001285588505001285588555001285588505001285588550012855885050001285588505000128558850500012855885050001285588505000128558850500012855885050000000000	0.00013324 0.00013812 8.41886-06 3.51016-06 0.00016954 0.00028523 8.88676-05 1.33146-06 1.33146-06 1.33146-06 1.33146-06 1.33146-06 3.00022945 0.00022945 0.00022945 3.16396-05 5.13596-06 3.00096-07 2.84746-05 2.84746-05 2.84746-05 2.84746-05 2.84746-05 3.35238-06	1.71796-05 8.25658-06 5.16286-05 2.65238-05 0.00043116 1.41438-06 4.28678-05 2.13338-07 0.00015903 0.00015903 0.00015903 0.00015903 1.05328-06 4.10218-06 4.10218-06 4.10218-06 4.10218-06 4.10218-06 1.46718-05 2.2238-05 0.00021156	4.2762E-06 4.837E-05 1.7401E-07 2.4418E-05 3.397E-05 0.00028388 0.000358696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.000064072 2.4675E-06	7.5487E-05 2.0945E-09 1.2029E-05 5.1463E-07 0.00027792 2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 8.8818E-05 1.411E-05 3.7536E-05 5.55918E-07 2.2801E-07	0.00011867 1.4167E-05 1.6684E-05 0.000155 1.084E-05 3.367E-05 5.9856E-05 0.00013985 0.00013985 0.00013985 0.00013972 2.5103E-05 5.116E-06 9.5183E-06	1.2989E-06 0.00010328 4.7002E-05 0.00012589 2.1263E-05 3.1246E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	4.0216E-05 2.5969E-05 2.215E-06 0.00017768 0.0001007 2.9591E-05 5.5192E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	7.8391E-05 7.6625E-05 3.822E-05 8.3381E-06 6.0554E-07 2.5329E-05 0.0001545 0.0001545 7.6866E-05 0.00023583	9.0765E-05 0.00012683 5.7423E-07 0.00022858 9.1476E-05 1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	5.291E-05 1.121E-05 0.00065955 0.00075198 0.00298982 0.00049358 4.9279E-05 0.00014096	7.5831E-05 1.1817E-05 0.00022989 1.1649E-05 3.3949E-05 3.3552E-06 0.00021746	6.041E-05 0.00016656 2.1935E-05 0.00016055 6.2746E-07 0.00082138 0.00079312	0.00057828 0.00031361 2.7683E-06 0.00052959 9.2266E-05 0.00033434 0.0007479	0.00024137 7.0044E-06 6.6979E-05 3.6082E-06 7.9063E-05 5.2319E-05 2.0588E-05	8.8791E-06 0.00155601 0.00020226 0.00092219 0.0007866 0.00020533 0.00067832	0.001646324 0.002609396 0.001385811 0.003184505 0.00562127 0.002551174 0.003532924	0.04057492 0.05108224 0.03722649 0.05643141 0.07497513 0.05050915 0.0594384
671E-06 549E-05 926E-06 0006675-8863E-05 8863E-05 8855E-08 8835E-08 8835E-08 8835E-05 207E-056 0074318 0018817- 1331E-05 7.29E-06 664E-07 1459E-07 1459E-07 45	0.00013812 8.4188E-06 5.04E-05 3.5101E-06 0.00016954 0.00028523 8.8867E-05 1.3314E-06 1.3338E-09 0.00025045 0.00012794 3.1639E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.8774E-05 1.1049E-07 3.3523E-06	8.2565E-06 5.1628E-05 0.00043116 1.4143E-06 4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	4.837E-05 1.7401E-07 2.4418E-05 3.397E-05 9.00028388 0.00035696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 0.00063E-06	2.0945E-09 1.2029E-05 5.1463E-07 0.00027792 2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 3.55918E-07 2.2801E-07	1.4167E-05 1.6684E-05 1.084E-05 3.367E-05 5.9856E-05 0.00013985 0.00013985 0.00013985 0.00013972 2.5103E-05 5.116E-06 9.5183E-06	0.00010328 4.7002E-05 0.00012589 2.1263E-05 3.1246E-05 1.0938E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	2.5969E-05 2.215E-06 0.00017768 0.0001007 2.9591E-05 5.5192E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	7.6625E-05 3.822E-05 8.3381E-06 6.0554E-07 2.5329E-05 0.0001545 0.00013853 7.6866E-05 0.00023583	0.00012683 5.7423E-07 0.00022858 9.1476E-05 1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	1.121E-05 0.00065955 0.00075198 0.00298982 0.00049358 4.9279E-05 0.00014096	1.1817E-05 0.00022989 1.1649E-05 3.3949E-05 3.3552E-06 0.00021746	0.00016656 2.1935E-05 0.00016055 6.2746E-07 0.00082138 0.00079312	0.00031361 2.7683E-06 0.00052959 9.2266E-05 0.00033434 0.0007479	7.0044E-06 6.6979E-05 3.6082E-06 7.9063E-05 5.2319E-05 2.0588E-05	0.00155601 0.00020226 0.00092219 0.0007866 0.00020533 0.00067832	0.002609396 0.001385811 0.003184505 0.00562127 0.002551174 0.003532924	0.05108224 0.03722649 0.05643141 0.07497513 0.05050915 0.0594384
549E-05 926E-06 0006675 883E-05 895E-08 835E-05 207E-05 7.29E-06 0074318 00740000000000000000000000000000000000	8.4188E-06 5.04E-05 3.5101E-06 0.0016954 0.00026523 8.8867E-05 1.3314E-06 1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	5.1628E-05 2.6523E-05 0.00043116 1.4143E-06 4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	1.7401E-07 2.4418E-05 3.397E-05 0.00028388 0.00035696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	1.2029E-05 5.1463E-07 0.00027792 2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	1.6684E-05 0.000155 1.084E-05 3.367E-05 5.9856E-05 0.00013985 0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	4.7002E-05 0.00012589 2.1263E-05 3.1246E-05 1.0938E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	2.215E-06 0.00017768 0.0001007 2.9591E-05 5.5192E-05 2.55E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	3.822E-05 8.3381E-06 6.0554E-07 2.5329E-05 0.0001545 0.00013853 7.6866E-05 0.00023583	5.7423E-07 0.00022858 9.1476E-05 1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	0.00065955 0.00075198 0.00298982 0.00049358 4.9279E-05 0.00014096	0.00022989 1.1649E-05 3.3949E-05 3.3552E-06 0.00021746	2.1935E-05 0.00016055 6.2746E-07 0.00082138 0.00079312	2.7683E-06 0.00052959 9.2266E-05 0.00033434 0.0007479	6.6979E-05 3.6082E-06 7.9063E-05 5.2319E-05 2.0588E-05	0.00020226 0.00092219 0.0007866 0.00020533 0.00067832	0.002609396 0.001385811 0.003184505 0.00562127 0.002551174 0.003532924	0.05108224 0.03722649 0.05643141 0.07497513 0.05050915 0.0594384
549E-05 926E-06 0006675 883E-05 895E-08 835E-05 207E-05 7.29E-06 0074318 00740000000000000000000000000000000000	8.4188E-06 5.04E-05 3.5101E-06 0.0016954 0.00026523 8.8867E-05 1.3314E-06 1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	5.1628E-05 2.6523E-05 0.00043116 1.4143E-06 4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	1.7401E-07 2.4418E-05 3.397E-05 0.00028388 0.00035696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	1.2029E-05 5.1463E-07 0.00027792 2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	1.6684E-05 0.000155 1.084E-05 3.367E-05 5.9856E-05 0.00013985 0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	4.7002E-05 0.00012589 2.1263E-05 3.1246E-05 1.0938E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	2.215E-06 0.00017768 0.0001007 2.9591E-05 5.5192E-05 2.55E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	3.822E-05 8.3381E-06 6.0554E-07 2.5329E-05 0.0001545 0.00013853 7.6866E-05 0.00023583	5.7423E-07 0.00022858 9.1476E-05 1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	0.00065955 0.00075198 0.00298982 0.00049358 4.9279E-05 0.00014096	0.00022989 1.1649E-05 3.3949E-05 3.3552E-06 0.00021746	2.1935E-05 0.00016055 6.2746E-07 0.00082138 0.00079312	2.7683E-06 0.00052959 9.2266E-05 0.00033434 0.0007479	6.6979E-05 3.6082E-06 7.9063E-05 5.2319E-05 2.0588E-05	0.00020226 0.00092219 0.0007866 0.00020533 0.00067832	0.001385811 0.003184505 0.00562127 0.002551174 0.003532924	0.03722649 0.05643141 0.07497513 0.05050915 0.0594384
0006675 863E-05 895E-08 835E-05 207E-05 7.29E-06 0074318 0018817 391E-05 612E-06 612E-06 6172E-07 582E-06 664E-07 499E-07 454E-05 0012856 0012856 0023332 858E-05 0019077 975E-05 0035958 336E-06	3.5101E-06 0.00018954 0.00028523 8.8867E-05 1.3314E-06 1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.049E-07 3.3523E-06	0.00043116 1.4143E-06 4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7883E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	3.397E-05 0.00028388 0.00035696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	0.00027792 2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	1.084E-05 3.367E-05 5.9856E-05 0.00013985 0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	2.1263E-05 3.1246E-05 1.0938E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	0.0001007 2.9591E-05 5.5192E-05 2.55E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	6.0554E-07 2.5329E-05 0.0001545 0.00013853 7.6866E-05 0.00023583	9.1476E-05 1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	0.00298982 0.00049358 4.9279E-05 0.00014096	3.3949E-05 3.3552E-06 0.00021746	6.2746E-07 0.00082138 0.00079312	9.2266E-05 0.00033434 0.0007479	7.9063E-05 5.2319E-05 2.0588E-05	0.0007866 0.00020533 0.00067832	0.00562127 0.002551174 0.003532924	0.07497513 0.05050915 0.0594384
0006675 863E-05 895E-08 835E-05 207E-05 7.29E-06 0074318 0018817 391E-05 612E-06 612E-06 6172E-07 582E-06 664E-07 499E-07 454E-05 0012856 0012856 0023332 858E-05 0019077 975E-05 0035958 336E-06	3.5101E-06 0.00018954 0.00028523 8.8867E-05 1.3314E-06 1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.049E-07 3.3523E-06	0.00043116 1.4143E-06 4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7883E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	3.397E-05 0.00028388 0.00035696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	0.00027792 2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	1.084E-05 3.367E-05 5.9856E-05 0.00013985 0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	2.1263E-05 3.1246E-05 1.0938E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	0.0001007 2.9591E-05 5.5192E-05 2.55E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	6.0554E-07 2.5329E-05 0.0001545 0.00013853 7.6866E-05 0.00023583	9.1476E-05 1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	0.00298982 0.00049358 4.9279E-05 0.00014096	3.3949E-05 3.3552E-06 0.00021746	6.2746E-07 0.00082138 0.00079312	9.2266E-05 0.00033434 0.0007479	7.9063E-05 5.2319E-05 2.0588E-05	0.0007866 0.00020533 0.00067832	0.00562127 0.002551174 0.003532924	0.07497513 0.05050915 0.0594384
863E-05 895E-08 895E-08 207E-05 7.29E-06 0074318 0018817 391E-05 6612E-06 664E-07 459E-07 459E-07 459E-07 459E-05 00128566 0023332 858E-05 00139505 975E-05 0035958 336E-06	0.00016954 0.00028523 8.8867E-05 1.3314E-06 1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.6974E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 2.9714E-05 3.3523E-06	1.4143E-06 4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	0.00028388 0.00035696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	3.367E-05 5.9856E-05 0.00013985 0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	3.1246E-05 1.0938E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	2.9591E-05 5.5192E-05 2.55E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	2.5329E-05 0.0001545 0.00013853 7.6866E-05 0.00023583	1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	0.00049358 4.9279E-05 0.00014096	3.3552E-06 0.00021746	0.00082138 0.00079312	0.00033434 0.0007479	5.2319E-05 2.0588E-05	0.00020533 0.00067832	0.002551174 0.003532924	0.05050915 0.0594384
863E-05 895E-08 895E-08 207E-05 7.29E-06 0074318 0018817 391E-05 6612E-06 664E-07 459E-07 459E-07 459E-07 459E-05 00128566 0023332 858E-05 00139505 975E-05 0035958 336E-06	0.00016954 0.00028523 8.8867E-05 1.3314E-06 1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.6974E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 2.9714E-05 3.3523E-06	1.4143E-06 4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	0.00028388 0.00035696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	2.4289E-05 5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	3.367E-05 5.9856E-05 0.00013985 0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	3.1246E-05 1.0938E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	2.9591E-05 5.5192E-05 2.55E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	2.5329E-05 0.0001545 0.00013853 7.6866E-05 0.00023583	1.0601E-06 6.0169E-05 3.5112E-07 1.4015E-05	0.00049358 4.9279E-05 0.00014096	3.3552E-06 0.00021746	0.00082138 0.00079312	0.00033434 0.0007479	5.2319E-05 2.0588E-05	0.00020533 0.00067832	0.002551174 0.003532924	0.05050915 0.0594384
895E-08 835E-05 207E-05 7.29E-06 0074318 0074318 0018817 391E-05 612E-06 172E-07 582E-06 664E-07 499E-07 499E-07 495E-05 0012856 0023332 858E-05 0019077 975E-05 035958 336E-06	0.00028523 8.8867E-05 1.3314E-06 1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.6974E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 2.9714E-05 3.3523E-06	4.2867E-05 2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.4671E-05 2.223E-05 0.00021156	0.00035696 1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 0.00064072 2.4675E-06	5.308E-07 1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	5.9856E-05 0.00013985 0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	1.0938E-05 4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	5.5192E-05 2.55E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	0.0001545 0.00013853 7.6866E-05 0.00023583	6.0169E-05 3.5112E-07 1.4015E-05	4.9279E-05 0.00014096	0.00021746	0.00079312	0.0007479	2.0588E-05	0.00067832	0.003532924	0.0594384
835E-05 207E-05 7.29E-06 0074318 0018817 391E-05 612E-06 612E-06 664E-07 499E-07 454E-05 0012856 0012856 0023332 858E-05 0019077 975E-05 0035958 336E-06	8.8867E-05 1.3314E-06 1.3038E-09 0.000250455 0.00012794 3.1639E-05 1.5974E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	2.1333E-07 0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	1.2896E-05 9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	1.7098E-07 3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	0.00013985 0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	4.5875E-06 4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	2.55E-05 5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	0.00013853 7.6866E-05 0.00023583	3.5112E-07 1.4015E-05	0.00014096							
207E-05 7.29E-06 0074318 0018817 391E-05 612E-06 664E-07 499E-07 454E-05 0012856 0012856 0012856 0012856 0012856 0019077 975E-05 0035958 336E-06	1.3314E-06 1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.5974E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	0.00015903 0.00017603 1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	9.3826E-07 4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	3.1805E-06 4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	0.00014323 4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	4.7022E-05 2.4994E-05 0.00037767 3.729E-06 8.1404E-05	5.5863E-06 4.9609E-05 0.00058118 4.4872E-05	7.6866E-05 0.00023583	1.4015E-05						0.00013192	0.000904197	0.03006987
.29E-06 074318 018817 391E-05 512E-06 172E-07 582E-06 564E-07 499E-07 454E-05 012856 023332 858E-05 019077 975E-05 035958 836E-06	1.3038E-09 0.00025045 0.00012794 3.1639E-05 1.6974E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	0.00017603 1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	4.8723E-05 0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	4.0872E-06 8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	4.9477E-05 0.00019772 2.5103E-05 5.116E-06 9.5183E-06	2.4994E-05 0.00037767 3.729E-06 8.1404E-05	4.9609E-05 0.00058118 4.4872E-05	0.00023583		2 0897E-05							
074318 018817 391E-05 512E-06 542E-07 582E-06 564E-07 499E-07 454E-05 012856 023332 858E-05 019077 975E-05 035958 836E-06	0.00025045 0.00012794 3.1639E-05 1.6974E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	1.1901E-07 0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	0.00128847 1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	8.8818E-05 1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	0.00019772 2.5103E-05 5.116E-06 9.5183E-06	0.00037767 3.729E-06 8.1404E-05	0.00058118 4.4872E-05		4 6597E-06							0.001547148	
018817 391E-05 512E-06 542E-07 582E-06 564E-07 499E-07 454E-05 012856 023332 858E-05 019077 975E-05 035958 836E-06	0.00012794 3.1639E-05 1.6974E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	0.00057087 5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	1.5137E-05 0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	1.411E-05 3.7536E-05 5.5918E-07 2.2801E-07	2.5103E-05 5.116E-06 9.5183E-06	3.729E-06 8.1404E-05	4.4872E-05	8.2202E-05									
891E-05 512E-06 582E-06 564E-07 499E-07 454E-05 012856 012856 019077 975E-05 035958 836E-06	3.1639E-05 1.6974E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	5.7863E-06 4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	0.00015804 4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	3.7536E-05 5.5918E-07 2.2801E-07	5.116E-06 9.5183E-06	8.1404E-05											
12E-06 72E-07 82E-06 64E-07 99E-07 154E-05 012856 012856 012332 858E-05 019077 975E-05 035958 836E-06	1.6974E-05 1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	4.1021E-06 1.0532E-06 1.4671E-05 2.223E-05 0.00021156	4.7225E-06 3.0563E-06 0.00064072 2.4675E-06	5.5918E-07 2.2801E-07	9.5183E-06			0.00017066	9.4911E-06	0.00038155	0.0001386	0.00082846	0.00017391	0.0003051	0.00174726	0.004744948	0.06888358
72E-07 82E-06 64E-07 199E-07 154E-05 012856 023332 158E-05 019077 975E-05 035958 136E-06	1.5806E-05 5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	1.0532E-06 1.4671E-05 2.223E-05 0.00021156	3.0563E-06 0.00064072 2.4675E-06	2.2801E-07		0.00014560	6.3581E-05	0.00011706	6.7527E-05	0.00042439	5.6232E-05	1.2468E-05	0.00062024	0.00036304	0.00173826	0.003801706	0.06165798
82E-06 64E-07 99E-07 54E-05 012856 023332 58E-05 019077 75E-05 035958 36E-06	5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	1.4671E-05 2.223E-05 0.00021156	0.00064072 2.4675E-06		1.394E-05	0.00014508	2.1194E-05	3.4444E-05	2.1005E-05	0.00022203	0.00016132	2.7213E-06	5.4987E-05	0.00026314	0.00100219	0.001965939	0.04433891
82E-06 64E-07 99E-07 54E-05 012856 023332 58E-05 019077 75E-05 035958 36E-06	5.1359E-06 3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	1.4671E-05 2.223E-05 0.00021156	0.00064072 2.4675E-06			0.00012847	0.000193	0.00016109	5.9167E-05	0.00150482	7.0175E-05	0.0001732	0.00018375	6.152E-06	0.00052344	0.003038199	0.05511986
64E-07 99E-07 54E-05 012856 023332 58E-05 019077 75E-05 035958 36E-06	3.0009E-07 2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	2.223E-05 0.00021156	2.4675E-06	3.34372.00													
199E-07 154E-05 012856 023332 158E-05 019077 975E-05 035958 136E-06	2.8474E-05 2.9714E-05 1.1049E-07 3.3523E-06	0.00021156		9 5139E-07													
54E-05 012856 023332 58E-05 019077 75E-05 035958 36E-06	2.9714E-05 1.1049E-07 3.3523E-06																
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58E-05)19077 75E-05)35958 36E-06																	
19077 75E-05 35958 36E-06	4 9909E-05																
75E-05 35958 36E-06						0.00019086											
35958 36E-06	0.00027593	0.00031479	3.3324E-05	4.9821E-05	0.0001104	3.1467E-06	0.00077556	0.00031082	3.2088E-05	0.00219051	3.2656E-05	0.00022767	0.000202	1.0886E-06	3.2141E-06	0.004753784	0.06894769
36E-06	2.6569E-06	7.9955E-05	9.392E-06	1.1406E-08	2.1021E-06	1.0185E-05	6.2838E-05	7.7738E-06	0.00024932	2.848E-05	3.5812E-06	4.9246E-05	0.00036844	1.7767E-05	1.642E-05	0.000921143	0.03035034
36E-06	0.00012422	0.00055752				8.7146E-07											
						2.0183E-05											
						2.6271E-05											
						0.00015339											
						1.3578E-05											
						2.3139E-05											
						7.1927E-05											
		1.1062E-05				4.4844E-05											
47E-05	5.8165E-05	0.0003255	0.00020696	9.2742E-06	0.00014442	4.4158E-05	1.6262E-05	0.00096619	4.2783E-05	3.2295E-05	0.00011734	0.00011998	0.00019778	4.8003E-06	0.00067888	0.002986253	0.05464662
18E-05	0.00017175	0.00089705	0.00010881	4.5633E-05	1.6985E-05	0.00041304	3.6606E-05	0.00026193	2.0203E-05	0.0004122	0.00015866	1.9909E-05	0.00014576	2.021E-06	6.5031E-05	0.002868731	0.05356053
58E-08	0.00011425	2.0022E-05	8.3597E-05	7.9575E-06	0.00016815	8.9784E-05	0.00019384	2.0303E-05	2.2091E-05	0.00114743	0.00029816	2.0156E-05	0.00017066	0.00014872	0.00049676	0.0030019	0.0547896
21E-05	1.3565E-06	3.9581E-07	0.00027308	4.6936E-06	4.3122E-05	0.00013973	6.3937E-10	5.8726E-05	8.1105E-05	0.00031964	8.7809E-05	0.00036802	5.395E-07	1.6127E-05	3.2957E-05	0.001445724	0.03802268
						0.00014006											
						9.1546E-05											
						7.9974E-08											
						0.00046889											
						0.00017875											
						7.7213E-05											
						3.2061E-05											
						3.4901E-05											
2E-05	2.0358E-05	0.00029485	0.00015225	8.9326E-06	0.00010726	0.00012425	2.8162E-05	7.9709E-05	0.00011662	8.7363E-05	8.8102E-05	2.0128E-05	2.4529E-05	5.466E-07	0.00124018	0.002416224	0.04915511
34876	0.00038064	0.00067206	0.00020792	5.2349E-05	0.0002467	1.7177E-05	4.3494E-05	0.00157994	4.254E-07	0.0040558	0.0001703	0.0007407	0.00090245	4.7981E-07	0.00021518	0.009634366	0.09815481
						0.00054162											0.05961939
						3.156E-05											
						5.2145E-06											
						7.5869E-05											
						2.0324E-06											
						1.2121E-05											
						0.00018608											
						6.9862E-06											
						1.4232E-05											0.08271143
2E-05	3.8875E-06	2.4707E-06	4.8733E-06	3.0099E-05	1.4874E-07	4.2989E-05	8.0567E-05	0.00013415	1.0223E-05	0.00013697	1.8584E-08	9.9439E-06	8.5812E-06	9.792E-07	2.5266E-06	0.000502662	0.02242013
36852	9.5594E-05	2.5674E-06	8.763E-05	1.7823E-05	9.3697E-05	0.00031234	7.5631E-05	0.00039745	9.3535E-05	0.00207538	2.497E-06	0.00098338	2.179E-05	7.4459E-05	0.00019221	0.004894507	0.06996076
5E-05	7.5737E-05	8.1775E-05	0.00011995	7.1757E-06	0.00016538	3.9043E-08	1.0381E-06	0.00027292	0.00015591	0.00014705	0.00080001	8.7882E-05	0.00030578	7.4672E-05	0.00042613	0.002744621	0.05238913
						0.00019119											
						0.00019119											
						1.7244E-06											
																0.005297529	
						3.5624E-06											
						0.00012235								5.0545E-08			
		7.8781E-06				3.5168E-06											
18E-05	6.0642E-06	6.9986E-06	3.1631E-07	4.2146E-06	0.00010719	9.5028E-07	2.7307E-05	2.184E-06	2.9221E-05	0.00017804	4.9199E-05	3.3944E-07	0.00011734	0.0002085	7.019E-07	0.000749581	0.02737847
72E-05	0.00015263	6.3609E-06	0.00014961	4.4706E-05	1.906E-08	7.6964E-07	1.5132E-05	2.559E-05	1.2375E-06	0.00160536	1.3616E-05	0.00055538	0.00027461	3.3149E-05	6.8467E-06	0.002900725	0.05385838

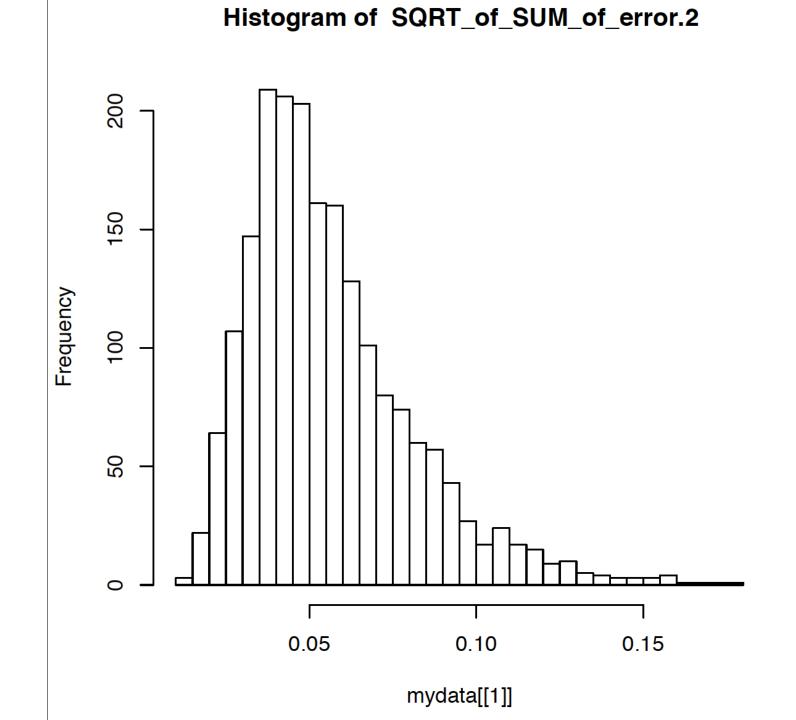


Illustration of a biased random walk

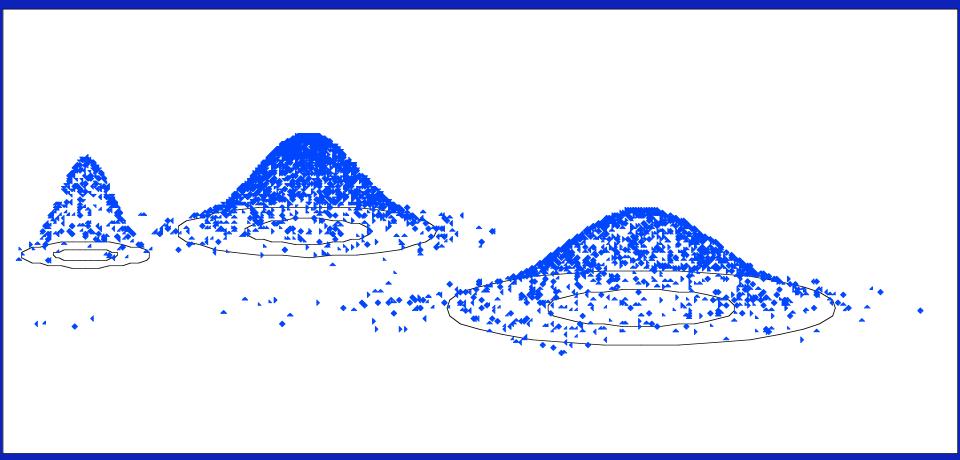


Figure generated using MCRobot program (Paul Lewis, 2001)

the gradualist point of view

Evolution occurs within populations where the fittest organisms have a selective advantage. Over time the advantages genes become fixed in a population and the population gradually changes.

This reasoning (with many more details) is known as the modern synthesis.

Note: this is not in contradiction to the the theory of neutral evolution. (which says what ?)

Processes that MIGHT go beyond inheritance with variation and selection?

•Horizontal gene transfer and recombination

- •Polyploidization (botany, vertebrate evolution) see here
- •Fusion and cooperation of organisms (Kefir, lichen, also the eukaryotic cell)
- •Targeted mutations (?), genetic memory (?) (see <u>Foster's</u> and <u>Hall's</u> reviews on directed/adaptive mutations; see <u>here</u> for a counterpoint)

•Random genetic drift

•Gratuitous complexity

- •Selfish genes (who/what is the subject of evolution??)
- •Parasitism, altruism, Morons

•Mutationism, hopeful monsters (see <u>here</u> for a critical discussion by Arlin Stolzfus)

selection versus drift

- see Kent Holsinger's java simulations at
- http://darwin.eeb.uconn.edu/simulations/simulations.html
- The law of the gutter.
- compare <u>drift</u> versus <u>select + drift</u>
- The larger the population the longer it takes for an allele to become fixed.
- Note: Even though an allele conveys a strong selective advantage of 10%, the allele has a rather large chance to go extinct.
- **Note#2: Fixation is faster under selection than under drift.**

s=0

Probability of fixation, P, is equal to frequency of allele in population. Mutation rate (per gene/per unit of time) = u ; freq. with which allele is **generated** in diploid population size N: u*2N Probability of **fixation** for each allele = 1/(2N)

Substitution rate (the rate with which mutations are fixed in a lineage) = frequency with which new alleles are generated * Probability of fixation=u*2N *1/(2N) = u

Therefore:

If f s=0, the substitution rate is independent of population size, and equal to the mutation rate !!!!

This is the reason that there is hope that the molecular clock might sometimes work.

Fixation time due to drift alone:

 $t_{av} = 4*N_e$ generations (N_e=effective population size; For n discrete generations N_e= n/(1/N₁+1/N₂+....1/N_n)

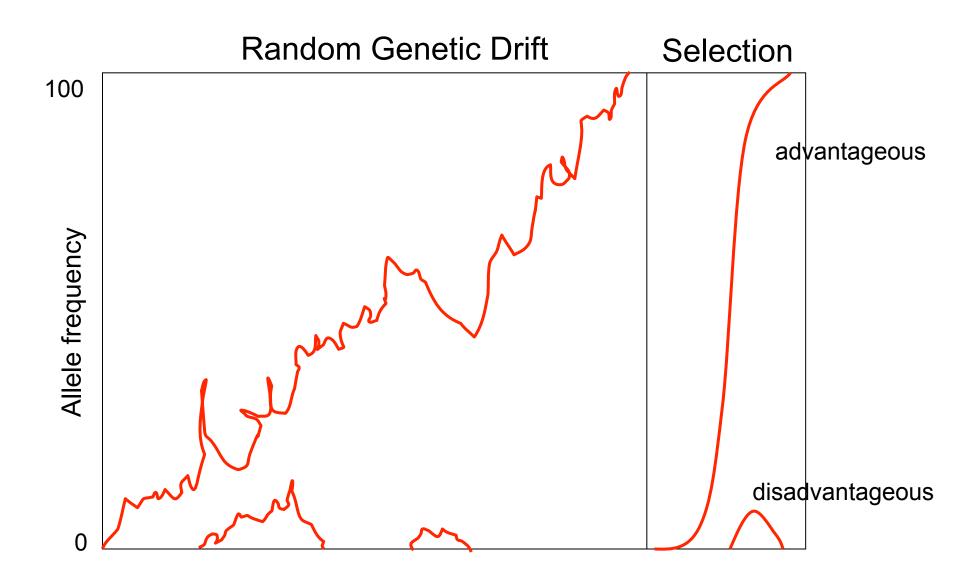
s>0

Time till fixation on average: $t_{av} = (2/s) \ln (2N)$ generations (also true for mutations with negative "s" ! discuss among yourselves)

E.g.: N=10⁶, s=0: average time to fixation: 4*10⁶ generations s=0.01: average time to fixation: 2900 generations

N=10⁴, s=0: average time to fixation: 40.000 generations s=0.01: average time to fixation: 1.900 generations

=> substitution rate of mutation under positive selection is larger than the rate wite which neutral mutations are fixed.



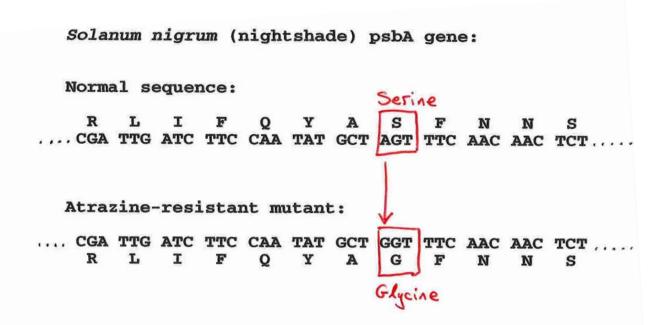
Positive selection

- A new allele (mutant) confers some <u>increase</u> in the **fitness** of the organism
- Selection acts to favour this allele
- Also called adaptive selection or Darwinian selection.

NOTE: **Fitness** = ability to survive and <u>reproduce</u>

Advantageous allele

Herbicide resistance gene in nightshade plant



Negative selection

- A new allele (mutant) confers some <u>decrease</u> in the fitness of the organism
- Selection acts to remove this allele

• Also called purifying selection

Deleterious allele

Human breast cancer gene, BRCA2

5% of breast cancer cases are familial Mutations in BRCA2 account for 20% of familial cases

Normal (wild type) allele

2780 2790 4800 2810 2820 2830 2846 2850 2860 2870 2880 2890 2900 ICCA TO GTTTTATATOGAGACACAGOTGATAAACAAGCAACCCAAGTGTCAA TTAAAAAAAGATTTGGTTTATGTTCTTCCAGAGGAGAACAAAAATAGTGTAAAGCAGCATATAAAAATGACIC ICCATGGTTTTTATATGGAGACACAGGTGAT---thrMetValLeuTyrGlyAspThrGlyAsp LysGlnProLysCysGlnLeuLysLysIleTrpPheMetPheLeuGlnArgArgThrLysIleVal Mutant allele (Montreal 440 Stop codon Family) 4 base pair deletion Causes frameshift Modified from from www.tcd.ie/Genetics/staff/Aoife/GE3026/GE3026 1+2.ppt

Neutral mutations

- Neither advantageous nor disadvantageous
- Invisible to selection (no selection)
- Frequency subject to 'drift' in the population
- **Random drift** random changes in small populations

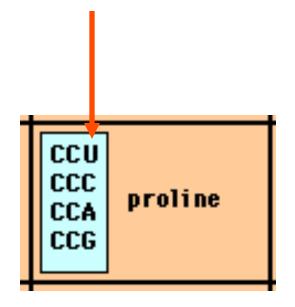
Types of Mutation-Substitution

- Replacement of one nucleotide by another
- Synonymous (Doesn't change amino acid)
 - Rate sometimes indicated by Ks
 - Rate sometimes indicated by d_s
- Non-Synonymous (Changes Amino Acid)
 - Rate sometimes indicated by Ka
 - Rate sometimes indicated by d_n

(this and the following 4 slides are from mentor.lscf.ucsb.edu/course/ spring/eemb102/lecture/Lecture7.ppt)

Genetie	c Code –]	Note dege	eneracy
of 1 st	vs 2 nd vs 3	rd positio	n sites
UUU phenyl UUC alanine	UCU UCC Net serine	UAU UAC tyrosine	UGU UGC cysteine
UUA UUG leucine	UCA UCG	UAA UAG stop	<mark>UGA</mark> stop UGG tryptophan
CUU CUC CUA CUA CUG	CCU CCC CCA CCG	CAU CAC histidine CAA glutamine	CGU CGC CGA CGG
AUU AUC AUA AUA MUG methionine	ACU ACC ACA ACG	AAU AAC AAA AAA AAG 1ysine	AGU AGC AGA AGA AGG arginine
GUU GUC GUA GUG	GCU GCC GCA GCG	GAU aspartic GAC acid GAA glutamic GAG acid	GGU GGC GGA GGG

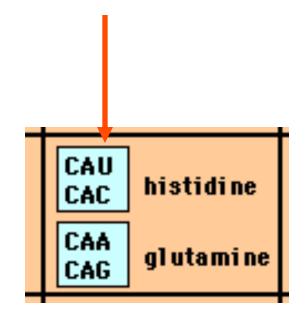
Genetic Code



Four-fold degenerate site – Any substitution is synonymous

From: mentor.lscf.ucsb.edu/course/spring/eemb102/lecture/Lecture7.ppt

Genetic Code



Two-fold degenerate site – Some substitutions synonymous, some non-synonymous

From: mentor.lscf.ucsb.edu/course/spring/eemb102/lecture/Lecture7.ppt

Measuring Selection on Genes

- Null hypothesis = neutral evolution
- Under neutral evolution, synonymous changes should accumulate at a rate equal to mutation rate
- Under neutral evolution, amino acid substitutions should also accumulate at a rate equal to the mutation rate

From: mentor.lscf.ucsb.edu/course/spring/eemb102/lecture/Lecture7.ppt

Counting #s/#a

SerSerSerSerSerSpecies1TGATGCTGTTGTTGTSerSerSerSerSerSerAlaSpecies2TGTTGTTGTTGTGGT

#s = 2 sites	To assess selection pressures one needs to
#a = 1 site	calculate the rates (Ka, Ks), i.e. the
	occurring substitutions as a fraction of the
#a/#s=0.5	possible syn. and nonsyn. substitutions.

Things get more complicated, if one wants to take transition transversion ratios and codon bias into account. See chapter 4 in Nei and Kumar, Molecular Evolution and Phylogenetics.

Modified from: mentor.lscf.ucsb.edu/course/spring/eemb102/lecture/Lecture7.ppt

dambe

Two programs worked well for me to align nucleotide sequences based on the amino acid alignment,

One is <u>DAMBE</u> (only for windows). This is a handy program for a lot of things, including reading a lot of different formats, calculating phylogenies, it even runs codeml (from PAML) for you.

The procedure is not straight forward, but is well described on the help pages. After installing DAMBE go to HELP -> general HELP -> sequences -> align nucleotide sequences based on ...->

If you follow the instructions to the letter, it works fine.

DAMBE also calculates Ka and Ks distances from codon based aligned sequences.

dambe (cont)

MBE Data	a Analysis in Molecu	lar Biology and Evolution
File E	De Alleumente Conserva	a Can Analysia Osenhina Dhylananatian Cantah Tasla Hala La 🗖
	OAMBE Help	_ 🗌 🗙
		Options Help
	Help Topics Back Print	
	Dontents Search	Align nuc. seq. against aligned aa. seq.
If you	Overview Main Menu	Why: One frustrating experience I have often had with aligning protein-coding nucleotide sequences is the introduction of many frameshift indels in the aligned sequences, even if the protein genes are known to be all
will b	File	functional and do not have these frameshifting indels. In other words, the introduced frameshifting indels in the aligned sequences are alignment artefacts, and the correctly aligned sequences should have complete codons, not
is incl	e w Frite	one or two nucleotides, inserted or deleted.
the eld	e - [©] Sequences	One way to avoid the above alignment problem is to align the protein-coding nucleotide sequences against amino acid sequences. This obviously requires amino acid sequences which can be obtained in two ways. First, if you have
and de	Align seque	nucleotide sequences of good quality, then you can translate the sequences into amino acids. Second, if you are working on nucleotide sequences deposited in GenBank, then typically you will find the corresponding translated
wish t	Align nuc.	amino acid sequences. DAMBE can read both the nucleotide sequence and the corresponding amino acid sequence in a GenBank sequence.
If you	Sequences	How: Here I illustrate the use of this special feature by assuming that you already have a file containing unaligned
inform	🗌 🖳 🖹 View Seque	protein-coding nucleotide sequences, say unaligned.fas , in your hard disk.
also a assist	🗌 🕒 🗎 Get Rid of 🛀 📗	Open the unaligned.fas file. When asked whether to align the sequences, click No . The unaligned sequences will then be read into DAMBE's buffer. Now click Sequences Work on Amino Acid Sequences to translate the
assist	🛛 🖳 🖻 Delete seq 👘	protein-coding nucleotide sequences into amino acid sequences. If the translation results in a number of termination codons embedded in the sequences (represented by "*"), then either your nucleotide sequences are of poor quality
Citatic	🛛 🕒 🖻 Delete dup	or they might be from pseudogenes. In either case you should give up aligning your nucleotide sequences against
Xia, Y	Work on Co	these junky amino acid sequences.
e	Work on A	If the translation looks good, then click Sequence Align sequences with Clustal to align the translated amino acid sequences. Once this is done, you have a set of aligned amino acid sequences in the DAMBE buffer for you to align
Xia, Y	🛛 🖳 🖹 Work on cc	your nucleotide sequences against.
h	🛛 🕒 🖹 Work on cc	Click Sequence/Align nuc. seq. against aligned aa seq. A standard file Open/Save dialog box will appear. Choose the unaligned.fas file again, which contains the unaligned nucleotide sequences. DAMBE will align the
	🛛 🖳 🖹 Work on cc	nucleotide sequences against the aligned amino acid sequences in the buffer. This procedure ensures that no frameshifting indels are introduced as an alignment artefact.
	🛛 🖳 🖹 Work on cc	If your sequences were retrieved from GenBank, then most protein-coding genes will already have translated amino
	📄 🖹 Restore sei	acid sequences included in the FEATURES table of GenBank files. You can use DAMBE to first read in all amino acid sequences, align these amino acid sequences, and then ask DAMBE to splice out the corresponding CDS, and
Triley Mr. Ct.	🗌 🖳 🖹 Change sec	align the CDS sequences against aligned amino acid sequences in DAMBE to splice out the corresponding CDS, and align the CDS sequences against aligned amino acid sequences in DAMBE buffer.
File: No file	📄 🕒 🖻 Get Comple	
S. 6 9. 5	🛛 🖶 🗢 Seq. Analysis 📃	

aa based nucleotide alignments (cont)

An alternative is the tranalign program that is part of the emboss package. On bbcxsrv1 you can invoke the program by typing tranalign.

Instructions and program description are <u>here</u>.

If you want to use your own dataset in the lab on Monday, generate a codon based alignment with either *dambe* or *tranalign* and save it as a nexus file **and** as a phylip formated multiple sequence file (using either clustalw, PAUP (export or tonexus), dambe, or <u>readseq</u> on the web)

PAML (codeml) the basic model

$$q_{ij} = \begin{cases} 0, & \text{if the two codons differ at more than one position,} \\ \pi_j, & \text{for synonymous transversion,} \\ \kappa \pi_j, & \text{for synonymous transition,} \\ \omega \pi_j, & \text{for nonsynonymous transversion,} \\ \omega \kappa \pi_i, & \text{for nonsynonymous transition,} \end{cases}$$

The equilibrium frequency of $\operatorname{codon} j(\pi_j)$ can be considered a free parameter, but can also be calculated from the nucleotide frequencies at the three codon positions (control variable CodonFreq). Under this model, the relationship holds that $\omega = d_N/d_S$, the ratio of nonsynonymous/synonymous substitution rates. This basic model is fitted by specifying model = 0 NSsites = 0, in the control file codeml.ctl. It forms the basis for more sophisticated models implemented in codeml.

sites versus branches

You can determine omega for the whole dataset; however, usually not all sites in a sequence are under selection all the time.

PAML (and other programs) allow to either determine omega for each site over the whole tree, *Branch Models*, or determine omega for each branch for the whole sequence, *Site Models*.

It would be great to do both, i.e., conclude codon 176 in the vacuolar ATPases was under positive selection during the evolution of modern humans – alas, a single site does not provide any statistics

Sites model(s)

work great have been shown to work great in few instances. The most celebrated case is the influenza virus HA gene.

A talk by Walter Fitch (slides and sound) on the evolution of this molecule is <u>here</u>.

This article by Yang et al, 2000 gives more background on ml aproaches to measure omega. The dataset used by Yang et al is here: <u>flu data.paup</u>.

sites model in MrBayes

The MrBayes block in a nexus file might look something like this:

```
begin mrbayes;
set autoclose=yes;
lset nst=2 rates=gamma nucmodel=codon omegavar=Ny98;
mcmcp samplefreq=500 printfreq=500;
mcmc ngen=500000;
sump burnin=50;
sumt burnin=50;
end;
```

Vincent Daubin and Howard Ochman: Bacterial Genomes as New Gene Homes: The Genealogy of ORFans in *E. coli. Genome Research* 14:1036-1042, 2004

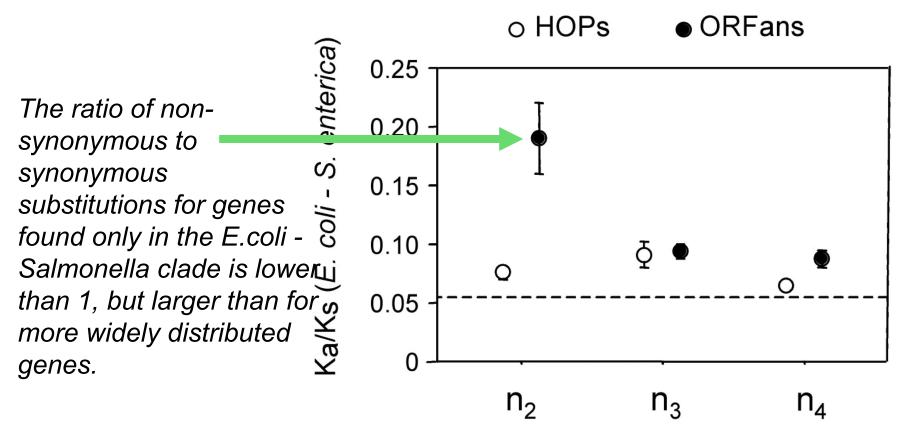


Fig. 3 from Vincent Daubin and Howard Ochman, Genome Research 14:1036-1042, 2004

Trunk-of-my-car analogy: Hardly anything in there is the is the result of providing a selective advantage. Some items are removed quickly (purifying selection), some are useful under some conditions, but most things do not alter the fitness.

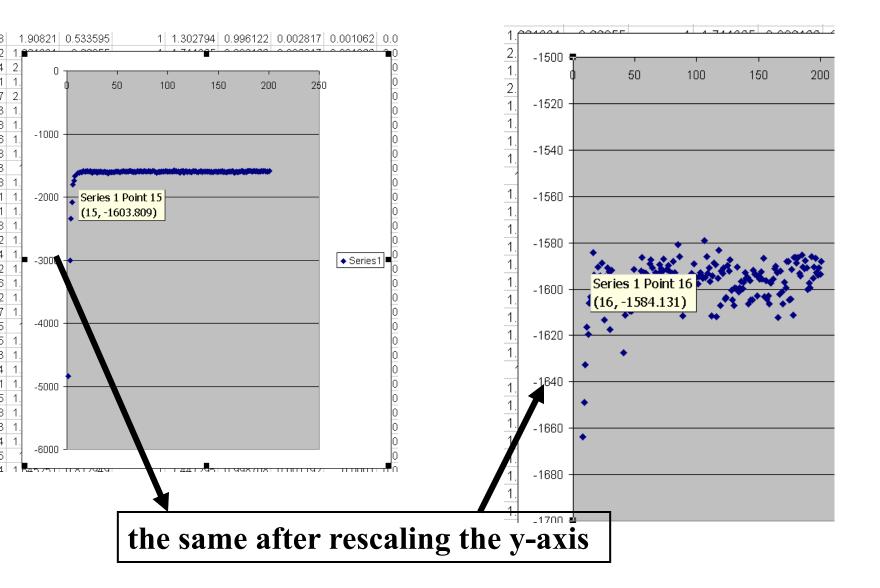


Could some of the inferred purifying selection be due to the acquisition of novel detrimental characteristics (e.g., protein toxicity)?

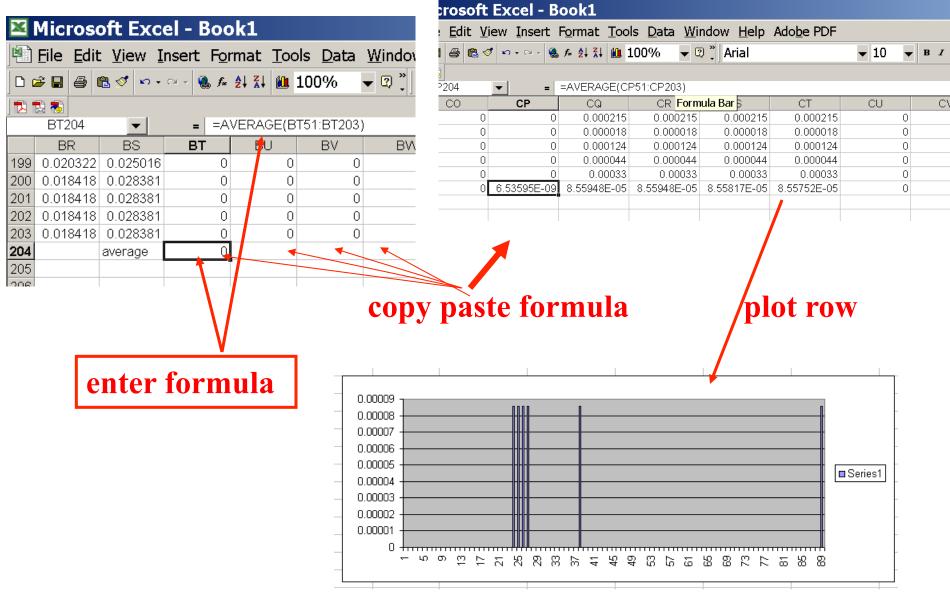
MrBayes analyzing the *.nex.p file

- 1. The easiest is to load the file into excel (if your alignment is too long, you need to load the data into separate spreadsheets see <u>here</u> execise 2 item 2 for more info)
- 2. plot LogL to determine which samples to ignore
- 3. for each codon calculate the the average probability (from the samples you do not ignore) that the codon belongs to the group of codons with omega>1.
- 4. plot this quantity using a bar graph.

plot LogL to determine which samples to ignore



for each codon calculate the the average probability



MrBayes on bbcxrv1

- If you do this for your own data,
- •run the procedure first for only 50000 generations (takes about 30 minutes) to check that everthing works as expected,
- •then run the program overnight for at least 500 000 generations.
- •Especially, if you have a large dataset, do the latter twice and compare the results for consistency. (I prefer two runs over 500000 generations each over one run over a million generations.)

The preferred wa to run mrbayes is to use the command line: >mb

Do example on threonlyRS

PAML – codeml – sites model

the paml package contains several distinct programs for nucleotides (baseml) protein coding sequences and amino acid sequences (codeml)

and to simulate sequences evolution.

The input file needs to be in phylip format.

By default it assumes a sequential format (e.g. here).

If the sequences are interleaved, you need to add an "I" to the first line, as in these example headers:

6 467 Т gi|1613157 ----- MSDNDTIVAQ ATPPGRGGVG ILRISGFKAR EVAETVLGKL gi|2212798 ------ MSTTDTIVAQ ATPPGRGGVG ILRVSGRAAS EVAHAVLGKL qi|1564003 MALIQSCSGN TMTTDTIVAQ ATAPGRGGVG IIRVSGPLAA HVAQTVTGRT qi|1560076 -----M QAATETIVAI ATAQGRGGVG IVRVSGPLAG QMAVAVSGRQ gi|2123365 ----MN--- -ALPSTIVAI ATAAGTGGIG IVRLSGPQSV QIAAALGIAG qi|1583936 ----MSQRS TKMGDTIAAI ATASGAAGIG IIRLSGSLIK TIATGLGMTT PKPRYADYLP FKDADGSVLD QGIALWFPGP NSFTGEDVLE LQGHGGPVIL 5 855 т PKPRYADYLP FKDVDGSTLD QGIALYFPGP NSFTGEDVLE LQGHGGPVIL LRPRYAEYLP FTDEDGOOLD OGIALFFPNP HSFTGEDVLE LOGHGGPVVM human LKARHAHYGP FLDAGGQVID EGLSLYFPGP NSFTGEDVLE LQGHGGPVVL goat-cow LQSRHARYAR FRDAQGEVID DGIAVWFPAP HSFTGEEVVE LQGHGSPVLL rabbit rat LRPRYAHYTR FLDVQDEVID DGLALWFPAP HSFTGEDVLE LQGHGSPLLL marsupial GTG CTG TCT CCT GCC GAC AAG ACC AAC GTC AAG GCC GCC TGG GGC AAG GTT GGC GCG CAC C ...T A.. ... A.TAA ... A.C ... AGCC ... G.A .ATA ... A.. ... AA. TG.G ... A.. ..T .GC ..T ..C ..G GA. ..TT C.. ..G ..A ... AT.TG ..A .GC ... 61 GCT GGC GAG TAT GGT GCG GAG GCC CTG GAG AGG ATG TTC CTG TCC TTC CCC ACC AAG .G.C ..C ... G. ... G. ... T. GG. ... T. G.

PAML – codeml – sites model (cont.)

the program is invoked by typing codeml followed by the name of a control file that tells the program what to do.

paml can be used to find the maximum likelihood tree, however, the program is rather slow. Phyml is a better choice to find the tree, which then can be used as a user tree.

An example for a codeml.ctl file is <u>codeml.hv1.sites.ctl</u> This file directs codeml to run three different models: one with an omega fixed at 1, a second where each site can be either have an omega between 0 and 1, or an omega of 1, and third a model that uses three omegas as described before for MrBayes. The output is written into a file called <u>Hv1.sites.codeml_out</u> (as directed by the control file).

Point out log likelihoods and estimated parameter line (kappa and omegas)

Additional useful information is in the <u>rst</u> file generated by the codeml

Discuss overall result.

PAML – codeml – branch model

For the same dataset to estimate the dN/dS ratios for individual branches, you could use this file <u>codeml.hv1.branches.ctl</u> as control file.

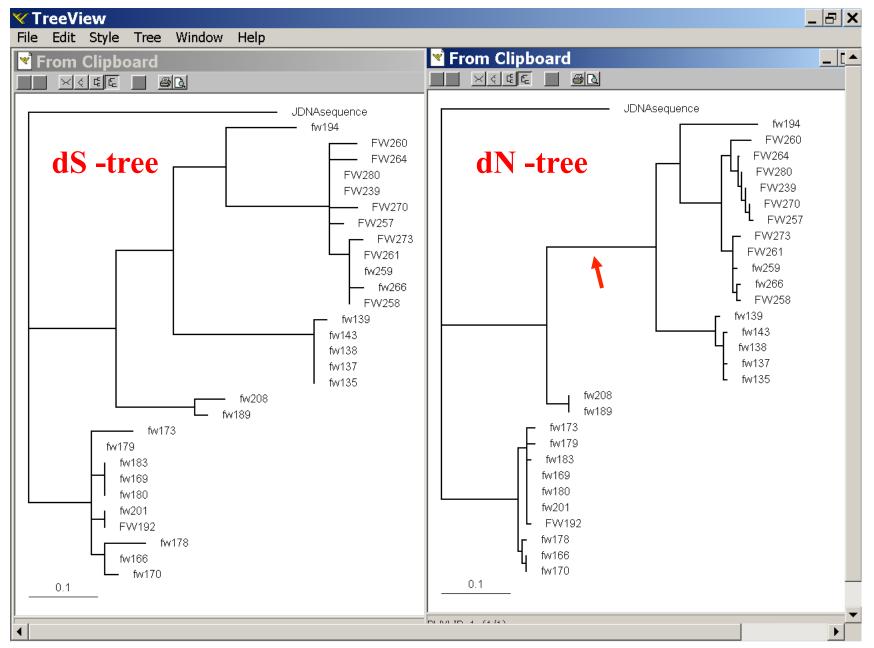
The output is written, as directed by the control file, into a file called <u>Hv1.branch.codeml_out</u>

A good way to check for episodes with plenty of non-synonymous substitutions is to compare the dn and ds trees.

Also, it might be a good idea to repeat the analyses on parts of the sequence (using the same tree). In this case the sequences encode a family of spider toxins that include the mature toxin, a propeptide and a signal sequence (see <u>here</u> for more information).

Bottom line: one needs plenty of sequences to detect positive selection.

PAML – codeml – branch model



where to get help

read the manuals and help files check out the discussion boards at <u>http://www.rannala.org/phpBB2/</u>

else

there is a new program on the block called <u>hy-phy</u> (=hypothesis testing using phylogenetics).

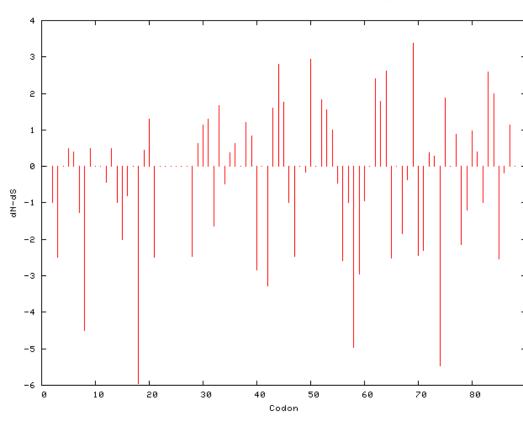
The easiest is probably to run the analyses on the authors datamonkey.

Discussion: Other ways to detect positive selection? Selective sweep -> fewer alleles present in population Repeated episodes of positive selection -> high dN

If time discuss http://online.itp.ucsb.edu/online/infobio01/fitch1/

hy-phy

Results of an anaylsis using the SLAC approach



	F	DUND	4	POSITI	VELY	SELECTED	SITES	(0.2	significance	level)
$\left \right $										
		Codo	n	dN-dS	Norma	dized dN-dS	p-val	ue		
$\left \right $		43	5 2	.80905		1.57283	0.17414	48		
		51	12	.94548		1.64923	0.10914	44		
$\left \right $		63	5 2	.62064		1.46734	0.19757	79		
		70	3	.37001		1.88693	0.12480	68		

FOUND 13 NEGATIVELY SELECTED SITES (0.2 significance level)

Codon	dN-dS	Normalized dN-dS	p-value
4	-2.5	-1.39979	0.111111
9	-4.5	-2.51963	0.0178326
19	-5.94245	-3.32728	0.0243467
22	-2.5	-1.39979	0.111111
41	-2.84041	-1.59039	0.193214
48	-2.45744	-1.37597	0.0793724
59	-4.96667	-2.78093	0.0236379
60	-2.96058	-1.65768	0.108898
66	-2.51831	-1.41004	0.15211
71	-2.45417	-1.37413	0.129462
72	-2.31427	-1.2958	0.162177
75	-5.47043	-3.06299	0.0388673
86	-2.54472	-1.42483	0.151309

more output might still be <u>here</u>

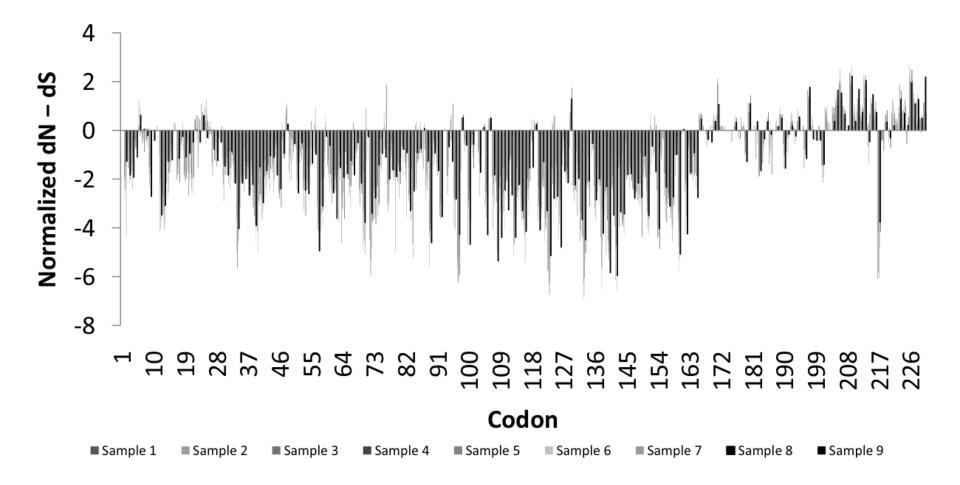


Fig 1. Patterns of substitutions: Bars represent dN > dS (positive) or dN < dS (negative) in random samples of 148 - 150 sequences (A) and the whole dataset of 1312 viruses (B). Included in B are regions of mapped activity and 3D structures of the RNA-binding domain (RBD, panel I) [21] and Effector domain (ED, rotated to expose the 7 β -sheets (panel II) and 2 α -helices (panel II)) [7] with residues under negative (yellow/brown), neutral (gray) or positive (red) selection highlighted. Residues 208-230 not included in the 3D structure of the ED are disordered (compare with figure 5). Note sites with dN > dS map on the helix motifs of the ED or the linkers flanking them or the disordered region.

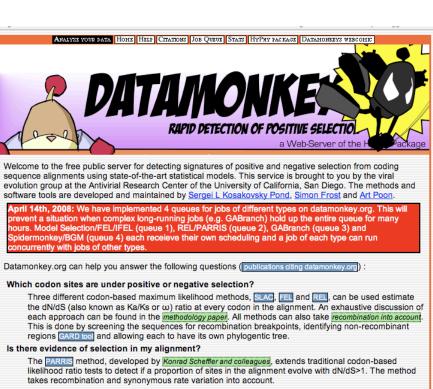
Hy-Phy ·

Hypothesis Testing using Phylogenies.

Using Batchfiles or GUI

Information at http://www.hyphy.org/

Selected analyses also can be performed online at http://www.datamonkey.org/



Which codon sites are under positive or negative selection at the population level?

The codon-based maximum likelihood real method can investigate whether sequences sampled from a population (e.g. viral sequences from different hosts) have been subject to selective pressure at the



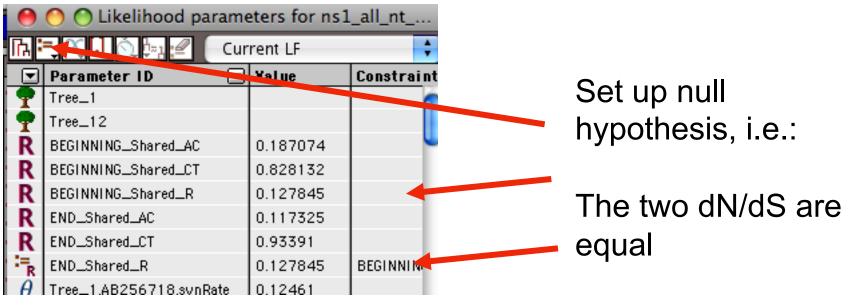
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5738		GAAGACG GT	GCTATAGT	GGCTGAAATA	TCTCCTATTC	COTCOATGOO	AGGACATTCI	T ACAGAGGATG	TCAA <mark>A</mark> AATGCA	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
6718		GAAGATG GT	GCTATTGT	GGCTGAAATT	TOTOCOATOC	CCTCTATGCC	AGGACATTCI	FACAGAGGATG	TCAA AATGC A	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
7385		GAAGATG GT	GCTATTGT	GGCTGAAATT	TCTCCCATTC	CCTCCATGCC	AGGACATTCI	FACAGAGGATG	TCAA <mark>A</mark> AATGCA	ATTGGAAT	гс <mark>стсетсеете</mark>	GACTTGAATGG	AATGAT
3503		GAGGATG GT	GCTATTGT	GGCTGAAATT	TCTCCCATTC	CCTCCATGCC	AGGACATTCI	FACAGAGGATG	TCAA <mark>A</mark> AATGC <mark>A</mark>	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
5579		GAAGATG GT	GCTATTGT	GGCTGAAATT	TCTCCCATTC	CCTCCATGCC	AGGACATTCI	FACAGAGGATG	TCAA <mark>A</mark> AATGC <mark>A</mark>	ATTGGAA1	IC CTCATCGGTG	GACTTGAATGG	AATGAT
5593		GAAGATG GT	GCTATTGT	GGCTGAAATT	TCTCCCATTC	CCTTCATGCC	AGGACATTCI	FACAGAGGATG	TCAA <mark>A</mark> AATGCA	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
4259		GAAGATG GT	GCTATTGT	GGCTGAAATA	TCTCCCATTC	CCTCCATGCC	AGGACATTCI				IC CTCATCGGCG		
4307	IC ACAI	GAAAGTG GT	GCTATTGT	GGCTGAAATA	TTTCCCATTC	COTCOGTACO	AGGACATTTI	T ACAGAGGATG	TCAA AATGC A	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
8445	IC ACAI	GAAAGTG GT	GCTATTGT	GGCTGAAATA	TCTCCCATTC	CCTCCGTACC	AGGACATTCI	T ACAGAGGATG	TCAA <mark>A</mark> AATGCA	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
5773	IC ACA	GAAGATG GT	GCTATTGT	GGCTGAAATA	TOTOCOATTO	COTCOATGOO	AGGACATTCI	T ACAGAGGATG	TCAA <mark>A</mark> AATGCA	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
1454	IC ACG	GAAGATG GT	GCTATTGT	GGCTGAAATA	TCTCCCATTC	CCTCCATGCC	AGGACATTCI	T ACAGAGGATG	TCAA <mark>A</mark> AATGCA	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
1119	IC ACA	GAAGATG GT	GCTATTGT	GGCTGAAATA	TCTCCCATTC	CCTCCATGCC	AGGACATTCI	FACAGAGGATG	TCAAAAATGC A	ATTGGAAT	IC CTCATCGGTG	GACTTGAATGG	AATGAT
3644		GAGGATGGT	GCCATTGT	АССТБАААТА	TCTCCCATTC	CTTCCATGCC	AGGACATTCI	TACAGAGGATG	TCAA AATGC A	ATTGGAAT	сстовтовате	GACTTGAATGG	AATGAT
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		GACGATGGT		AGCTGAAATT		CTTCTATGCC					CCTCATCGGTG		
7171		SAAGATGGC		AGCTGAAATT							CCTCATCGGTG		
9957		GACAATGGC		AGCTGAAATA							CCTCATCGGCG		
4270		GACGATGGT		GGCTGAAATA		CTTCTATGCC					CCTCATCGGTG		
4370		GATGATGGT		AGCTGAAATA							CCTCATCGGTG		
3280		GACGATGGA		AGCTGAAATA		CTTCTATGCC					CCTCATCGGTG		
1137		GACGATGGC		AGCTGAAATA							CCTCATCGGTG		
6399		GACGATGGC		AGCTGAAATA							CCTCATCGGTG		
1201		GACGATGGC		AGCTGAAATA							CCTCATCGGTG		
1633		GACGATGGC		AGCTGAAATA							CCTCATCGGTG		
4691													
		GATGATG GC		AGCTGAAATA							CCTCATCGGTG		
05		GATGATGGC		AGCTGAAATA							CCTCATCGGTG		
4773		GATGATGGC		AGCTGAAATA							CCTCATCGGTG		
4972		GATGATGGC	GCCATTGT	AGCIGAAAIA	TOTOCCATTO	CUTUTATOCC	AGGACATICI	TACAGAGGATG	TCAABAATGCA	ATTGGAAT	CCTCATCGGTG	GACTIGAATGG	AAIGAI
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Set up two partitions, define model for each, optimize likelihood

\varTheta 🕙 🔿 Likelihood parameters for ns1_all_nt											
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	Parameter ID 💌	Yalue	Constraint								
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7	Tree_12										
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R	BEGINNING_Shared_CT	0.828132									
R	BEGINNING_Shared_R	0.127845									
R	END_Shared_AC	0.117325									
R	END_Shared_CT	0.93391									
R	END_Shared_R	0.946316									
θ	Tree_1.AB256718.synRate	0.12461									
θ	Tree_1.AF001672.synRate	0.016737									
θ	Tree_1.AF009898.synRate	0									
θ	Tree_1.AF055424.synRate	0.017357									
θ	Tree_1.AF074267.synRate	0									
θ	Tree_1.AF074279.synRate	0.0527182									
θ	Tree_1.AF084286.synRate	0.0176037									
θ	Tree_1.AF144307.synRate	0.0528252									
θ	Tree_1.AF256183.synRate	0									
θ	Tree_1.AF256188.synRate	0.0174124									
θ	Tree_1.AF523503.synRate	0.0527042									
θ	Tree_1.AJ344036.synRate	0									
θ	Tree_1.AJ410594.synRate	0.0350104									
θ	Tree_1.AJ410598.synRate	0.0174538									
θ	Tree_1.AM502792.synRate	0.0174516									
0	THE LAMEODOOT HERE										

Save Likelihood Function then select as alternative

The dN/dS ratios for the two partitions are different.



(to do, select both rows and then click the define as equal button on top)

Example testing for dN/dS in two partitions of the data --John's dataset DataSet ns1_all_nt_8

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	385 T		Tree_12					AGGACATTCTAC
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	579 TI 593 TI		BEGINNING_Shared_CT		0.891995			AGGACATTCTAC
	259		BEGINNING_Shared_R		0.19809			AGGACATTCTA(AGGACATTCTA(
	307 T		END_Shared_AC		0.126137			AGGACATTTTA
	445 T	R	END_Shared_CT		0.770683		TACC	AGGACATTCTAC
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	644 T		Tree_1.AF001672.synR	ate	0.0364501			AGGACATTCTAC
	8 <mark>T</mark> I		Tree_1.AF009898.synR	ate	0			AGGACATTCTAC
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	370 T	C A	Tree_1.AF084286.synR	ate	0.0419524			AGGACATTCTA
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	399 T		Tree_1.AF256183.synR		0			AGGACATTCTA
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Example testing for dN/dS in two partitions of the data --

John's dataset

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	9	тс	θ	Tree_1.AF055424.synRate	0.0414451		TGCC AGGACATTCT AC	
	171 957	ТС, ТС,	θ	Tree_1.AF074267.synRate	0		TGCC AGGACATTCT AC	
	270	тс	θ	Tree_1.AF074279.synRate	0.131262		TGCCAGGACATTCTAC	
	370	тс	θ	Tree_1.AF084286.synRate	0.0419524		TGCC AGGACATTCT AC	
	280 137	ТС. ТС	θ	Tree_1.AF144307.synRate	0.129191		TGCC AGGACATTCT AC	
		тс	$\tilde{\theta}$	Tree_1.AF256183.synRate	0		TGCCAGGACATTCTAC	
	201	тс	$\tilde{\theta}$	Tree_1.AF256188.svnRate	0.0415364		TGCCAGGACATTCTAC	
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	972	TC	θ	Tree_1.AJ410 Input			🚓 🖓	
		3	θ	Tree_1.AM502			11	
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_				DataSet ns1_									
0	O O Likelihood parameters for ns1_all_nt												
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	Parameter ID		Yalue	Constraint 500									
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¦:= _R	END_Shared_R	Likelihood Ratio Test											
θ	Tree_1.AB256718.synR		2*LR = 225.88	31									
θ	Tree_1.AF001672.synR		DF = 1 P-Value = 0										

After selecting LRT (= Likelihood Ratio test), the console displays the result, i.e., the beginning and end of the sequence alignment have significantly different dN/dS ratios.

Alternatively, especially if the the two models are not nested, one can set up two different windows with the same dataset:

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[
	530		540	550	560	570	580	590		500 6:	10	620		
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AF523503														
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O Nucleot	ide Dati	a. 690 sites	; (403	l B distinct patterns),	150 species. Cu	urrent Select	tion:1-690							

Simulation under model 1, evalutation under model 2, calculate LR Compare real LR to distribution from simulated LR values. The result might look something like this or this

