The rate of synonymous substitution (D_s or K_s) The rate of nonsynonymous substitution (D_n or K_a)

D_s (Ks) = the number of synonymous substitution/the average number of synonymous sites for two seq

D_n (Ka) = the number of nonsynonymous substitution/the average number of nonsynonymous sites for two seq

Approximately:

61 codons, ~19 synonymous codons and ~42 nonsysnonymous codons

The ratio of the synonymous site and nonosynonymous site ≈ 1:2~3

The stringency of synonymous substitutions in the codon position: 3^{rd} codon position > 1^{st} codon position > 2^{nd} codon position

Case study to calculate the substitution sites

```
Seq (SPECIES) 1 TCA ACT GAG ATG TGT TTA

Seq (SPECIES) 2 TCG ACA GAG ATA TGT CTA
Ser Thr Glu Ile Cys Leu

The total number of synonymous sites for seq 1 =1+1+1/3+0+1/3+5/3= 4.333
The total number of nonsynonymous sites for seq 1 =2+2+8/3+3+8/3+4/3=13.677

The total number of synonymous sites for seq 2 =1+1+1/3+2/3+1/3+5/3 =5
The total number of nonsynonymous sites for seq 2 =2+2+8/3+7/3+8/3+4/3 =13
```

The average of synonymous sites as $N_s = (4.333+5)/2 = 4.667$

The average of nonsynonymous sites as $N_A = (13.677+13)/2=13.333$

Case study to calculate Ka, Ks, and Ka/Ks

```
Glu
                Ser
                     Thr
                              Met
                                    Cys
                                         Leu
Seq (SPECIES) 1
                TCA
                     ACT
                          GAG
                              ATG
                                    TGT
                                         TTA
Seq (SPECIES) 2
                TCG ACA
                          GAG
                               ATA
                                    TGT
                                         CTA
                     {	t Thr}
                          Glu
                              Ile Cys Leu
                Ser
```

The average of synonymous sites as $N_s = (4.333+5)/2 = 4.667$ The average of nonsynonymous sites as $N_A = (13.677+13)/2 = 13.333$

- 3 synonymous substitution 1 nonsynonymous substitution
- Ka=1/13.333=0.0750Ks=3/4.667=0.643

$$Ka/Ks = 0.075/0.643 = 0.1166$$