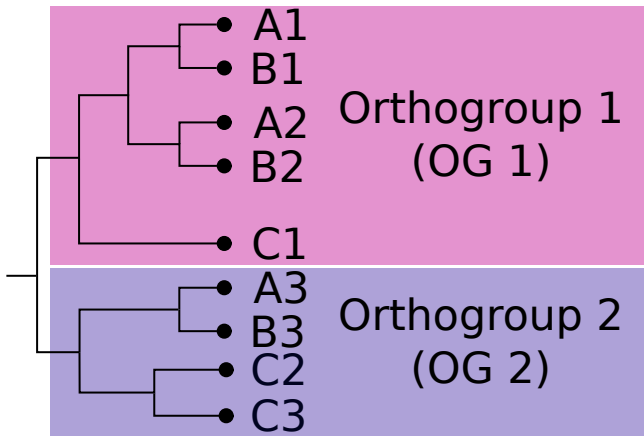


# Identification of pairwise gene relationships prior to clustering into orthologous groups

Assume for this worked example that similarity scores between genes decrease monotonically with time since divergence but that gene pairs with the same time since divergence will have different scores due to random variation. The RBBs will be,



OG1:  $\{A1, B1\}$ ,  $\{A2, B2\}$ ,  $\{A_i, C1\}$ ,  $\{B_j, C1\}$   
 where  $i, j = 1$  or  $2$  according to whether A1 or A2 has a higher similarity score with C1 and similarly for B1 and B2 with C1.

OG2:  $\{A3, B3\}$ ,  $\{A3, C_i\}$ ,  $\{B3, C_j\}$   
 where  $i, j = 1$  or  $2$  (as explained above).

Without loss of generality we can consider two cases,  
 Case 1:  $i=j$  (use  $i=j=1$ ),  
 Case 2:  $i \neq j$  (use  $i=1, j=2$ ).

## OrthoFinder

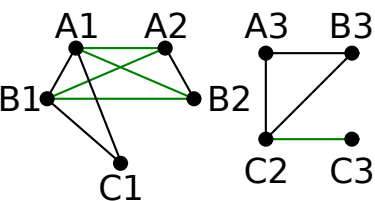
### Case 1

RBBs:

$\{A1, B1\}$      $\{A3, B3\}$   
 $\{A2, B2\}$      $\{A3, C3\}$   
 $\{A1, C1\}$      $\{B3, C3\}$   
 $\{B1, C1\}$

Better hit than RBB:

$\{A1, A2\}$      $\{C2, C3\}$   
 $\{A1, B2\}$   
 $\{B1, A1\}$   
 $\{B1, A2\}$



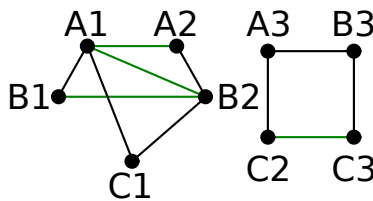
### Case 2

RBBs:

$\{A1, B1\}$      $\{A3, B3\}$   
 $\{A2, B2\}$      $\{A3, C3\}$   
 $\{A1, C1\}$      $\{B3, C3\}$   
 $\{B2, C1\}$

Better hit than RBB:

$\{A1, A2\}$      $\{C2, C3\}$   
 $\{A1, B2\}$   
 $\{B2, A1\}$



Proportions of cognate-pairs identified prior to clustering

OG 1: 8 / 10  
 OG 2: 4 / 6

OG 1: 7 / 10  
 OG 2: 4 / 6

## OrthoMCL

### Case 1

RBBs:

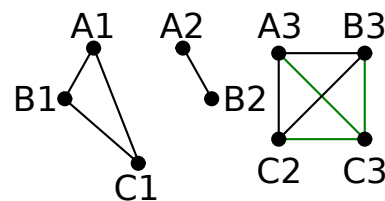
$\{A1, B1\}$      $\{A3, B3\}$   
 $\{A2, B2\}$      $\{A3, C3\}$   
 $\{A1, C1\}$      $\{B3, C3\}$   
 $\{B1, C1\}$

Paralogues:

None     $\{C2, C3\}$

Co-orthologues:

None     $\{A3, C3\}$   
 $\{B3, C3\}$



Proportions of cognate-pairs identified prior to clustering

OG 1: 4 / 10  
 OG 2: 6 / 6

### Case 2

RBBs:

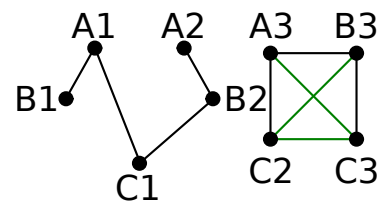
$\{A1, B1\}$      $\{A3, B3\}$   
 $\{A2, B2\}$      $\{A3, C3\}$   
 $\{A1, C1\}$      $\{B3, C3\}$   
 $\{B2, C1\}$

Paralogues:

None     $\{C2, C3\}$

Co-orthologues:

None     $\{A3, C3\}$   
 $\{B3, C2\}$



OG 1: 4 / 10  
 OG 2: 6 / 6