

Diagram illustrating the ASR (Ancestral State Reconstruction) workflow:

- extract orthologues:** A sequence alignment of orthologous genes across six species, showing the ancestral sequence: AAAAADESEAEAAA.
- build phylogeny:** A phylogenetic tree is constructed based on the orthologous sequences.
- ASR (Ancestral State Reconstruction):** The tree is analyzed to infer the ancestral state (A) at the root node.
- simulated evolution:** The tree is simulated to show the evolution of the sequence from the ancestral state (A) to the tips, resulting in the reconstructed sequences: AAAAAEESEAEAAA, AAAAADASEAEAAA, AAAAAATEAEAAA, AAAAADESEAEAAA, and AAAAADETEAEAAA.

Stacked bar chart showing the relative frequency of three age classes (young, intermediate, old) across different substrate quality categories. The y-axis represents relative frequency from 0.00 to 1.00. The x-axis shows substrate quality bins: 0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, and 0.7-1. Sample sizes (n) are indicated above each bar: 10, 23, 28, 54, 58, 60, 47, and 24. The legend indicates: young (light blue), intermediate (medium blue), and old (dark blue).

| Substrate quality | n | old | intermediate | young |
|-------------------|----|------|--------------|-------|
| 0-0.1 | 10 | 0.20 | 0.50 | 0.30 |
| 0.1-0.2 | 23 | 0.43 | 0.30 | 0.27 |
| 0.2-0.3 | 28 | 0.29 | 0.54 | 0.17 |
| 0.3-0.4 | 54 | 0.37 | 0.41 | 0.22 |
| 0.4-0.5 | 58 | 0.45 | 0.25 | 0.30 |
| 0.5-0.6 | 60 | 0.50 | 0.33 | 0.17 |
| 0.6-0.7 | 47 | 0.53 | 0.35 | 0.12 |
| 0.7-1 | 24 | 0.50 | 0.25 | 0.25 |

Evolutionary rate (z-score)

Substrate quality

$r_s = -0.205$
 $p = 1.1 \times 10^{-4}$

real

simulated

Figure 1 consists of two panels. The left panel is a scatter plot titled "G3BP1 S149" showing the relationship between SQ mean (x-axis, ranging from 0.4 to 0.7) and SQ variance (y-axis, ranging from 0.00 to 0.04). The data points are grey circles, and a red circle highlights the point at approximately (0.75, 0.00). The right panel is a bar chart titled "All sites (n=299)" showing the relative frequency (y-axis, ranging from 0.0 to 0.6) for four categories of SQ mean and variance: "high M low V", "low V", "high M", and "no difference". The bars are red, and the "no difference" category has the highest relative frequency, exceeding 0.6.