1. Motivation
The genotype-phenotype mapping is considered the major driving engine of the evolutionary developmental biology (evo-devo). Experimental data has shown that small numbers of genes form robust developmental modules, hierarchically reused throughout development. The evolutionary road that led to their existence is currently being sought by mathematical and empirical studies of fitness landscapes.

2. Objectives
We analyze the features and evolution of small modules (4 genes) towards higher diversity and robustness. Given this small size, all possible topologies and thus the entire fitness landscape can be analyzed by means of the Boolean networks approach. Inspired from the existent data on the segment polarity genetic network, we seek a description of possible underlying principles of network assemblage and evolution driven by robustness and neutrality features.

3. Results
We show that evolution leads to a class of robust (resistant to perturbations) genetic networks with an expression pattern characteristic of lateral inhibition. We identify the minimal networks responsible for robustness, with many additional interactions providing a back-up mechanism. These distinct networks of identical expression pattern denote high neutrality, with one-link mutations leading to neutral paths in the fitness landscape.

4. The model and its framework

![Image: The network model: signals are mediated by juxtacrine interactions.](image)

- (A) an 8-cells organism, with morphogenes (circles) and local genes (squares).
- (B) Network representation.

![Image: The genotype-phenotype mapping for the case (G,H) = (2, 2).](image)

\[ W_a \in W \]
\[ P_a = \Omega(W_a) \]

Why 4 genes?
We required the existence of a stripe-like expression pattern (see Fig. 2) as inspired from the segment polarity genes in insects embryos. This leads to \( N(=G+H)=4 \).

Obtaining the expression pattern?
To obtain \( P_a \) for \( W_a \) (see Solé et al. 2003, Int. J. Dev. Biol. 47, 685):

\[ g^G_{c_j}(t+1) = \Theta(G^C_{c_j}(t) + H^C_{c_j}(t)) \in \{0,1\} \]
\[ h^C_{c_j}(t+1) = \Theta(G^C_{c_j} - h^C_{c_j}(t)) \in \{0,1\} \]

where \( c=1...C, j=1...G, t = \) time, and

\[ G^C_{c_j}(t) = A_{k} g^G_{c_j} \]
\[ H^C_{c_j}(t) = B_{k} (h^{-1}_{c_j} \lor h^{C+1}_{c_j}) \]

with \( A \), the internal network, and \( B \) the external network (Fig. 2), and \( \lor \), the OR function.

5. Results
5.1 Pattern robustness
Several classes of robustness have been defined in genetics (de Visser et al. 2003, Evolution 57, 1959). Here the robustness \( R(W_a) \) is measured by obtaining \( P_a \) and flipping one by one the state of all genes in all cells \( (N \times C) \) flips, and checking the fraction of these trials \( P_a \) is recovered.

In Fig. 3, the robustness histogram of all networks with non-null stable pattern (A), and among these, of all networks producing stripes (B). There are no stripe-networks with robustness \( R=0.48 \). The majority of stripe-networks (320) are entirely robust!

All the stripe-networks are implementations of the lateral inhibition mechanism. Such pattern can be produced by at least 5 positive links, but an additional negative link (Fig. 5) is required to make the pattern robust. Among these robust stripe-networks, the ones of minimum links constitute basic modules responsible for the expression pattern and robustness (Fig. 6).

6. Overview
We have studied the entire pattern space of \( (N,H) = (4,2) \) by means of Boolean modeling. We have also identified the minimum-links robust networks producing a stripe-like expression pattern. Evolution simulations (not shown) have been conducted, showing that these networks are rapidly encountered.