Supporting Information

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A Mathematical Model Describing Forces in Jellyfish Propulsion Captures the Recovery of Global Symmetry

How does muscle contraction drive symmetry recovery? Here, placing muscle contraction in the context of its function in propulsion and the ephyra geometry, we show that consideration of forces that normally operate in propulsion can explain the recovery of radial symmetry in injured ephyrae.

Mathematical Formulation. The components of the model are established properties of swimming pulsation (contraction and elastic response) and parameters measured directly in ephyrae (body dimensions, frequency of contraction, and percentage change of body area in response to muscle contraction). We model the angular movement of each arm with respect to the geometrical center of the body. Fig. S6 illustrates the model coordinates. $\Delta\theta_i$ is the angular span between the ith arm and the $(i+1)$th arm (Fig. S6*A*), where the corresponding body area is denoted as A_i (Fig. S6*B*).

Contraction leads to a change in the body area, ΔA (Fig. S6*C*), which in turns generates an elastic response, described using the Hooke's law:

$$F_{elastic} = k_C \frac{\Delta A}{A_i}, \qquad \textbf{[S1]}$$

where k_C is the elastic modulus of the body materials. This elastic force results into angular pivoting of the arms (Fig. S6*D*), as there is less resistance in the amputated site:

$$F_{pivot} = k_T \frac{\delta\theta}{\Delta\theta_i}, \qquad \textbf{[S2]}$$

where k_T is the tensile modulus of the body. Combining Eqs. **S1** and **S2**, we have the force balance on the ith arm:

$$k_C \frac{\Delta A}{A_i} = k_T \frac{\delta\theta_i}{\Delta\theta_i}. \qquad \textbf{[S3]}$$

The new steady-state angle $\Delta\theta_i$ after every contraction cycle can be described analytically by integrating Eq. **S**2:

$$\theta_{i,new} = \theta_{i,old}\left(e^{\frac{k_C}{k_T}\times\frac{\Delta A}{A_i}} - 1\right) + \theta_{i,old}. \qquad \textbf{[S4]}$$

Simulation. To solve the model, we estimated some parameters directly in the ephyrae: the body diameter was set at 1 mm, arm length was set at 1 mm, and the swimming contraction (coded as a sinusoidal function) was set at ∼0.5–4 pulses per s (see measurements in Fig. 5 *F* and *G*). The compressive elastic modulus of the body, k_C, was set at 20 Pa (based on measurements in refs. 61 and 36). The tensile modulus of the body, k_T, was set at 1 MPa (based on measurement in ref. 61).

Movie S3 (www.youtube.com/watch?v=VpWf74BkAbE&feature=youtu.be) shows the resulting model simulation. Every cycle of contraction and elastic recoil generates a net push into the cut site. With every cycle, the ephyra relaxes into a new stable configuration where the arms going slightly into the cut site. This continues until spacing between the arms is rebalanced. Matlab codes are available upon request.

Thus, the mathematical model consisting of known mechanical properties of swimming pulsation can recapitulate the recovery of global symmetry. Furthermore, the mathematical model also recapitulates the timescale of symmetry recovery. Eq. **S4** gives the angular pivoting per cycle as follows:

$$\frac{\theta_{i,new} - \theta_{i,old}}{\theta_{i,old}} = \left(e^{\frac{k_C}{k_T}\times\frac{\Delta A}{A_i}} - 1\right)\Delta N, \qquad \textbf{[S5]}$$

where N is the number of contraction cycles. Because k_C/k_T is small ($\sim 10^{-5}$), Eq. **S5** can be approximated as follows:

$$\frac{d\theta_i}{\theta_i} = \left(e^{\frac{k_C}{k_T}\times\frac{\Delta A}{A_i}} - 1\right)dN. \qquad \textbf{[S6]}$$

Then, for a tetramer, the total number of cycles N required to change $\Delta\theta_i$ from $\pi/4$ (the initial configuration) to $\pi/2$ (the final symmetrized configuration) is as follows:

$$N\left(e^{\frac{k_C}{k_T}\times\frac{\Delta A}{A_i}} - 1\right) = \int_{\pi/4}^{\pi/2} \frac{d\theta_i}{\theta_i} = \ln 2. \qquad \textbf{[S7]}$$

The time T to recover symmetry can therefore be analytically derived as follows:

$$T = \frac{\Delta t \ln 2}{\left(e^{\frac{k_C}{k_T}\times\frac{\Delta A}{A}} - 1\right)} \approx 47\Delta t \text{ hours}, \qquad \textbf{[S8]}$$

where Δt is the period of a contraction cycle (in seconds), or the inverse of frequency (in pulses per s). The estimated pulse frequency of an ephyra swimming in artificial seawater is 0.7–1.5 pulses/s (Fig. 5 *E*–*G*). Eq. **S8** then predicts a symmetrization time ranging from 1.3 d–7 d, which corresponds to what we typically observed in ephyra (Fig. 2*F*).

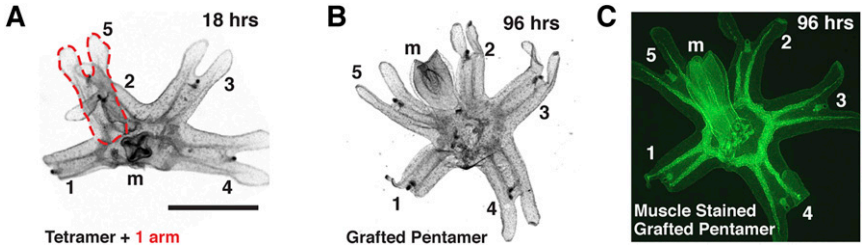


Fig. S1. Symmetrization proceeded with foreign arms. (*A*) In this experiment, the ephyra was cut in half. Subsequently, an arm from another ephyra (outlined in red) was grafted to the tetramer, between arm 1 and 2. "m" indicates the manubrium. Grafting was performed by pinning the ephyra segments next to each other on an agarose plate (1% agarose, made with artificial seawater). Pinning was done using cactus spines (ones from columnar *Espostoa* sp. worked best). The ephyrae were kept pinned overnight (~12 h), unpinned the next morning, and allowed to recover in artificial seawater. (*B*) By 4 d, the patchwork ephyra had become symmetrical. The grafted arm is outlined in red. The location of grafting looked smooth, and the ephyra had healed without obvious scarring. The extra arm was incorporated seamlessly into the host tetramer. The resulting patchwork pentamer was symmetrical and pulsed synchronously. (*C*) Phalloidin staining shows that the axisymmetrical muscle was rebuilt, and muscle from the extra arm was connected seamlessly into the host ephyra (we will discuss the muscle network in more detail in the main text and in Fig. 4).

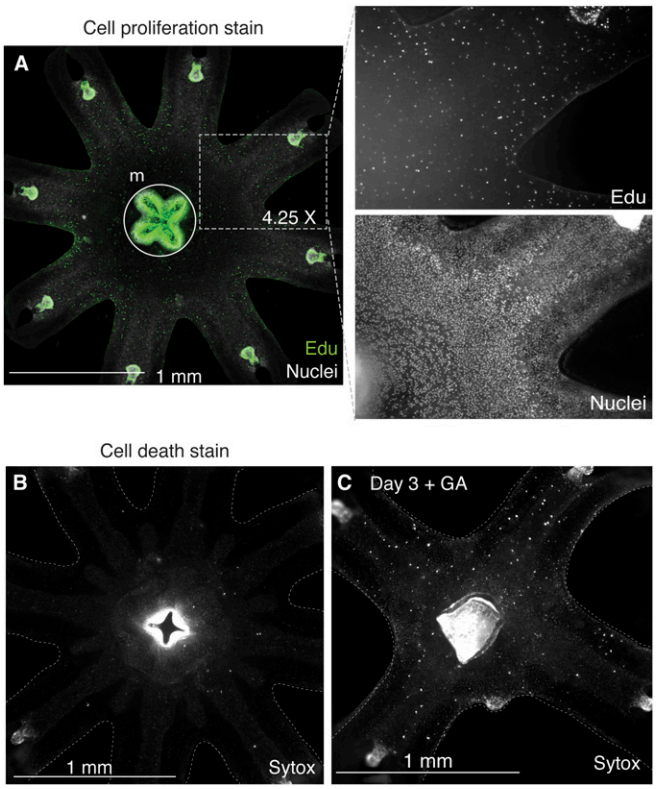


Fig. S2. Cell proliferation and cell death stains. (*A*) EdU stain (green) in an uncut ephyrae. Total nuclei were stained using Hoechst (white). The magnified regions show the EdU and nuclear stain separately. The circle indicates the manubrium. (*B*) Sparse baseline Sytox stain (white) in an uncut ephyra. (*C*) Sytox stain was increased in the presence a caspase inducer (100 nM gambogic acid; $n = 19$ of 20). In this experiment, cut ephyrae were incubated in the chemicals for 1–3 d, and then stained with Sytox (*Materials and Methods*). The 1 μM gambogic acid was lethal to ephyrae, giving us an upper limit.

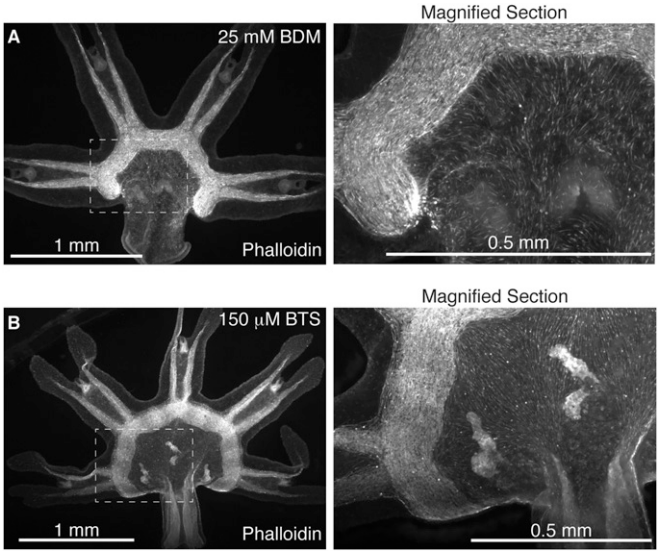


Fig. S3. Inhibitors of skeletal myosin II blocks symmetrization. In this experiment, the ephyrae were cut, and then incubated in (*A*) 2,3-butanedione monoxime (BDM) (25 mM) or (*B*) *N*-benzyl-*p*-toluene-sulfonamide (BTS) (150 mM). In both inhibitors, the ephyrae did not pulse and remained asymmetrical throughout the 4-d treatment, and the coronal muscle remained blunt ($n = 40$ of 40 for BDM; $n = 40$ of 40 for BTS). The phalloidin staining was performed on day 4 after amputation.

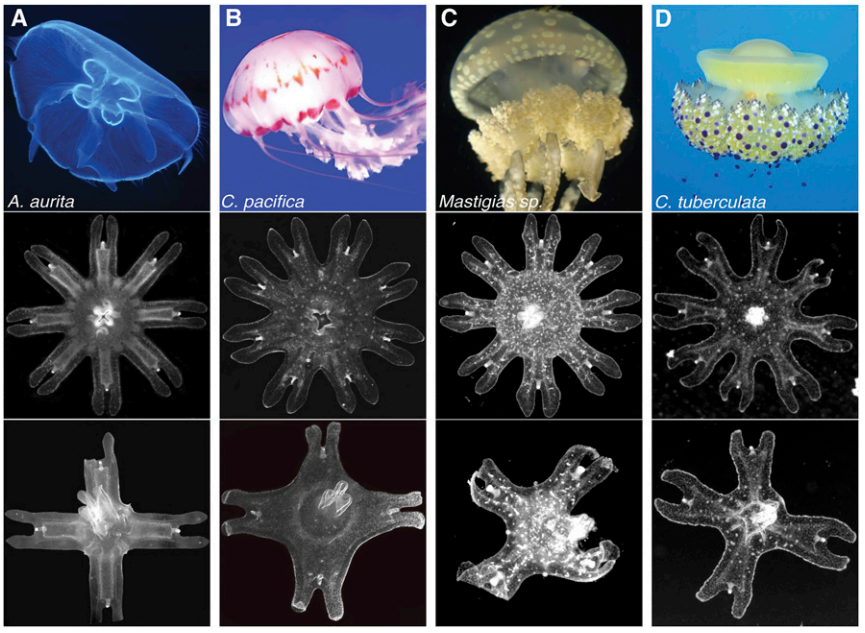


Fig. S4. Symmetrization was observed across four species of Scyphozoan jellyfish. (*A*) The moon jellyfish *Aurelia aurita*. Image courtesy of Wikimedia Commons/Hans Hillewaert. Image © Hans Hillewaert. (*B*) The sea nettle *Chrysaora pacifica*. Image courtesy of Sofi Quinodoz. (*C*) The lagoon jellyfish *Mastigias* sp. Image courtesy of Wikimedia Commons/Captmondo. (*D*) The Mediterranean jellyfish *Cotylorhiza tuberculata*. Image courtesy of Wikimedia Commons/Antonio Sontuoso. For each column, row 1 shows the adult medusa, row 2 shows the uncut ephyra, and row 3 shows the symmetrized tetramer from amputation. Freshly strobilated ephyrae were cut in half and allowed to recover in seawater. Symmetrized tetramers were observed within 4 d in all species.

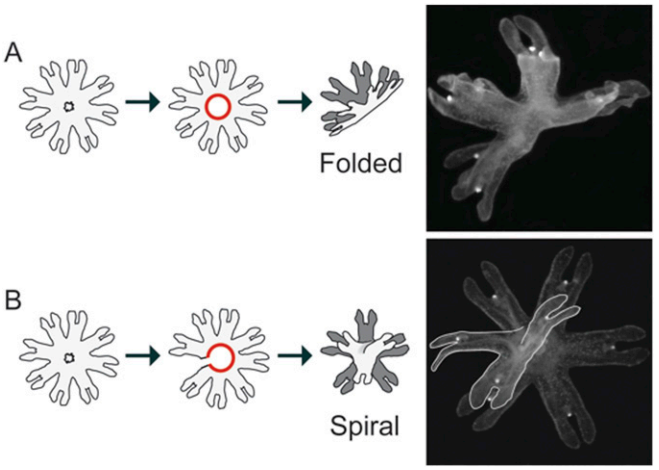


Fig. S5. Removing the manubrium broke planarity. (*A*) Punching out the manubrium led the ephyrae to adopt a fan shape. We used a P200 pipette tip to make a clean hole and removed the entire manubrium. (*B*) Punching out the manubrium and linearizing the ribbon led the ephyrae to adopt a spiral shape, with threefold symmetry stacked on fivefold symmetry. The new symmetry was observed within 1–4 d after amputation. The ephyrae pulsed synchronously and remained alive for over 3 wk until the experiment was ended.

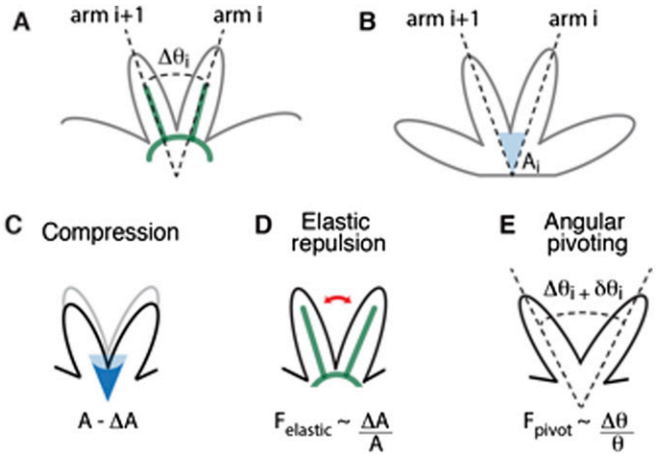
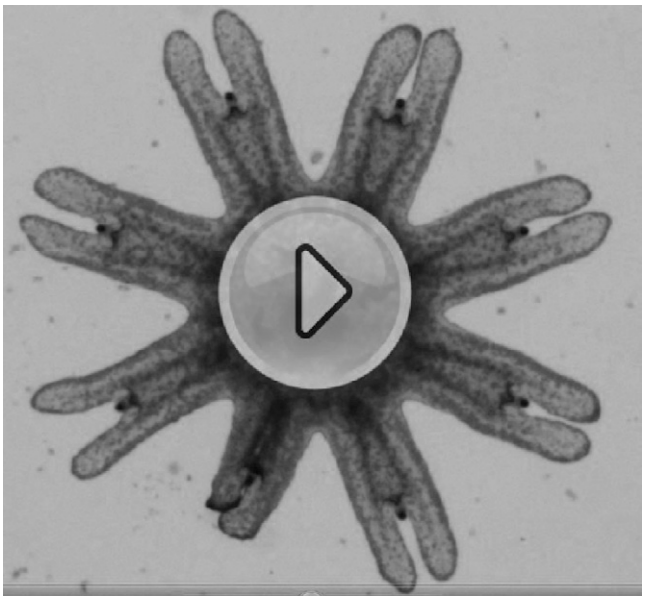
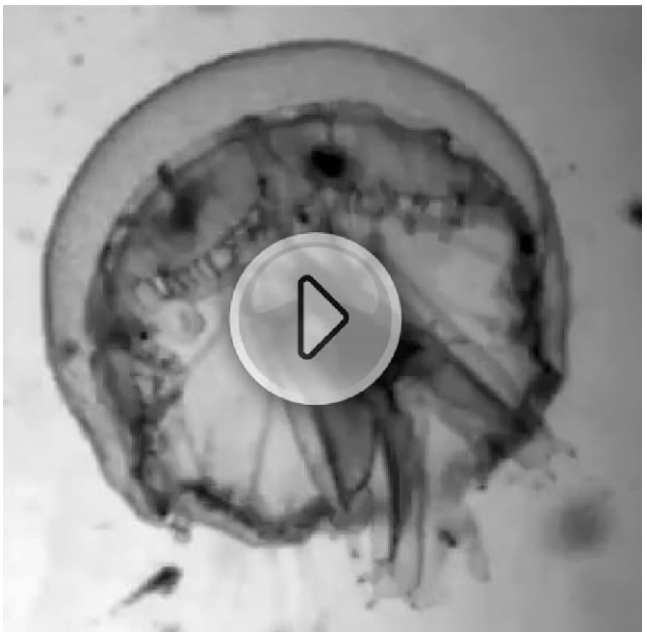


Fig. S6. Model geometry and coordinate. (*A*) We consider a $\Delta\theta_i$, the angular span between arm i and arm $i+1$. (*B*) The corresponding body area is denoted as A_i. Muscle contraction compresses the body by ΔA (*C*) and generates an elastic response (*D*), which in turn leads to angular pivoting of the arms (*E*).



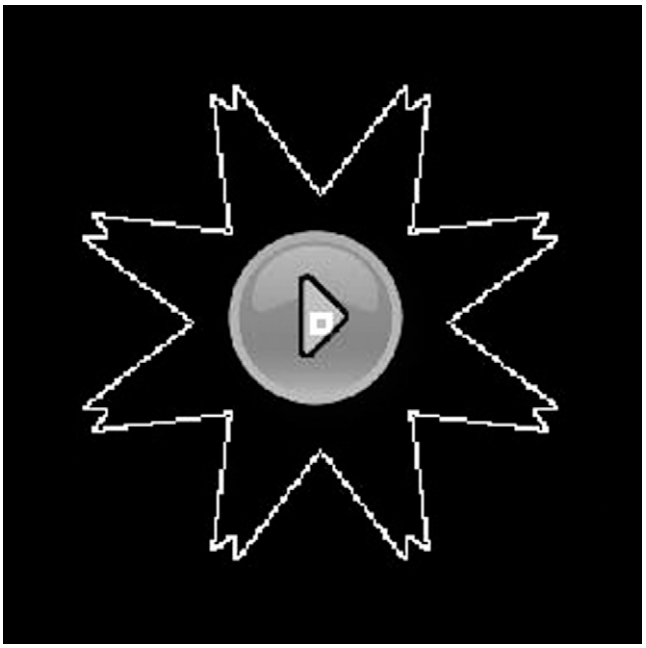
Movie S1. An *Aurelia* ephyra swimming in seawater. A 1-d-old ephyra. The movie is in real time. The movie can also be viewed through the following YouTube link: www.youtube.com/watch?v=fdFkjwWrI-U&feature=youtu.be.

Movie S1



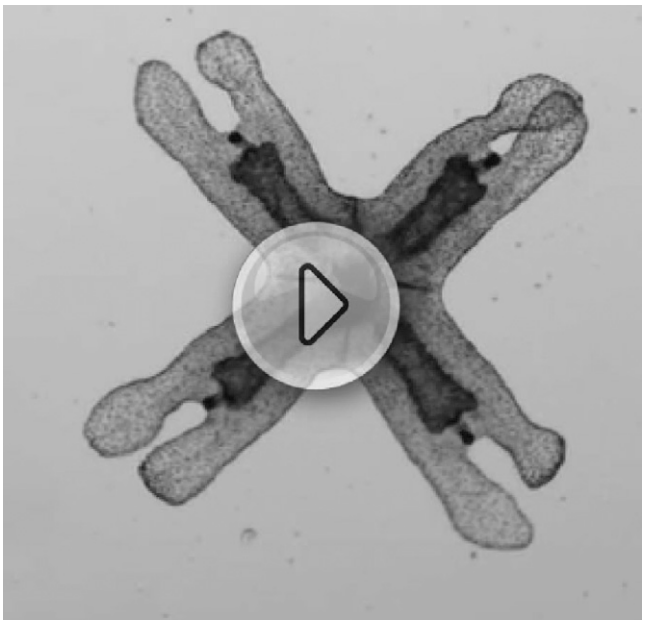
Movie S2. Symmetrization facilitates maturation into adult medusae. The medusae were filmed 4 wk after amputation. The movies are in real time. Part 1: Medusa growing from symmetrized pentamer. Part 2: An abnormal medusa from ephyrae that do not symmetrize. The movie can also be viewed through the following YouTube link: www.youtube.com/watch?v=lQdx-rwvPFM&feature=youtu.be.

Movie S2



Movie S3. Simulation of a tetramer symmetrizing using our mathematical model. Every cycle of contraction and elastic recoil generates a net push into the cut site. With every cycle, the ephyra relaxes into a new stable configuration where the arms go slightly into the cut site. This continues until spacing between the arms is rebalanced. Matlab codes are available upon request. The movie can also be viewed through the following YouTube link: www.youtube.com/watch?v=VpWf74BkAbE&feature=youtu.be.

Movie S3



Movie S4. Frequency of muscle contraction dictates the speed of symmetrization. The movies are in real time. Part 1: Tetramer swimming in seawater. Part 2: Tetramer swimming in seawater with reduced $MgCl_2$ (50% of the concentration in normal seawater). Part 3: Tetramer swimming in seawater with 80 μM menthol. The movie can also be viewed through the following YouTube link: www.youtube.com/watch?v=DxrNW0qcWy4&feature=youtu.be.

Movie S4