Diffusion-reaction model for Drosophila embryo development

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During the early stages of gastrulation in Drosophila embryo, the epithelial cells composing the single tissue layer of the egg undergo large strains and displacements. These movements have been usually modelled by decomposing the total deformation gradient in an (imposed or strain/stress dependent) active part and a passive response. Although the influence of the chemical and genetic activity in the mechanical response of the cell has been experimentally observed, the effects of the mechanical deformation on the latter have been far less studied, and much less modelled. Here, we propose a model that couples morphogen transport and the cell mechanics during embryogenesis. A diffusion-reaction equation is introduced as an additional mechanical regulator of morphogenesis. Consequently, the active deformations are not directly imposed in the analytical formulation, but they rather depend on the morphogen concentration, which is introduced as a new variable. In this study, we show that strain patterns similar to those observed during biological experiments can be reproduced by properly combining the two phenomena. In addition, we use a novel technique to parameterise the embryo geometry by solving two Laplace problems with specific boundary conditions. We apply the method to two morphogenetic movements: ventral furrow invagination and germ band extension. The matching between our results and the observed experimental deformations confirms that diffusion-reaction of morphogens can actually be controlling large morphogenetic movements.

Keywords: morphogenesis; diffusion-reaction; cell mechanics; finite element model

1. Introduction

During the development of multi-cellular organisms, there is a combination of a biochemical pattern (Gilbert 1994) and mechanical movements that shape the embryo (Keller et al. 2000). The interdependence of these two contributions adds an enormous difficulty to the complete understanding of the essential developmental phases. The Drosophila embryo is an interesting biological model, which has been amply studied in the last decades from both the experimental and the numerical points of view. Furthermore, biologists have been able to observe the genetic control of the successive steps of embryogenesis (Farge 2003), which has been often related to the expression of specific genes for each morphogenetic movement (Leptin 1999).

In the embryo, cells do not act alone but rather collaborate with neighbouring cells and rearrange their configuration in order to maintain the highly organised structure of the system. This is a fascinating scientific aspect because it requires having a multiscale approach: at the cellular scale (microscopic) and at the tissue scale (macroscopic).

Although active cells deform locally under genetic signalling, their interaction and activity are mediated and globally transferred to their neighbouring cells. The stress state of the cell has been hypothesised as a potential source of communication (Taber 2007; Ramasubramamian and Taber 2008; Muñoz et al. 2010). However, diffusion patterns have been observed in developing systems such as the zebra fish (Kicheva et al. 2007), the fly wing (Yu et al. 2009) or in Drosophila syncytial embryo (Gregor et al. 2007). It has been noticed that the morphogen diffusion has an effect in gene expression, with subsequent implication in embryogenesis at the tissue level. In the Drosophila embryo, the dorsal morphogen has been reported to have an immediate effect on the expression of the important genes twist and snail (Steward 1989; Leptin 1999). These are responsible for the main large deformations that take place during embryo gastrulation such as ventral furrow invagination (VFI) and germ band extension (GBE) (Leptin 1999). The aim of the present study was to verify whether the coupling of the cell mechanics and the diffusion process may yield the observed deformations during the development of the Drosophila embryo.

Recently, numerical modelling in embryo biomechanics has played an important role. A major step has been the use of a deformation gradient decomposition (Smith 1993; Rodriguez et al. 1994). This method couples the large active and the passive deformations occurring in the cells. The former is usually considered a purely
local kinematical process proper to each cell and, therefore, is directly introduced into the formulation according to the experimental observations. The latter is instead a (visco)elastic response of the surrounding tissue, which then requires a mechanical analysis. Nevertheless, recent biological experiments have shown that the active deformations may depend on the passive deformations or on the stresses as a feedback mechanism (Farge 2003; Brouzès and Farge 2004), and some numerical models have introduced phenomenological laws in order to take into account this potential aspect (Taber 2007; Ramasubramaniam and Taber 2008; Muñoz et al. 2010).

1.1 Morphogens activity
Despite important progresses and results obtained in the context of the embryogenesis, there is still a relevant aspect that has not been so much explored from a numerical point of view: the process by which the active individual deformations of the cells take place. This is actually a complex process, which has been experimentally studied by analysing the presence of specific morphogens inside the cells.

Morphogens are proteins that may regulate the expression of target genes. They are believed to also provide positional information to specialised cell types within a tissue (Alberts et al. 2008). From experimental observations, it has been found that they spread from a localised source and form a concentration gradient across a developing tissue (Gregor et al. 2007; Kicheva et al. 2007; Yu et al. 2009). Morphogens are able to induce or maintain the expression of different target genes at distinct concentration thresholds. Consequently, cells close to the source of morphogens will show high gene expression depending on the concentration threshold of the target gene. Moreover, after the gene expression has taken place, the cell activity may induce active deformations that in turn may modify the concentration levels. As we show in the results section, this feedback mechanism is relevant for the control of the observed cell deformations.

The presence of diffusion phenomena in pattern formation of biological systems has already been investigated (Waddington 1940; Turing 1952) and it has led to a fruitful research line on the formation of diffusion-reaction geometrical pattern [see, for instance, a review in Maini et al. (1997) and Murray (2003)]. In these studies though, the concentration affects indeed the deformation pattern, but the influence of the latter on the concentration profile is rather low. Hence, the biological systems consisted of tissues which were mechanically sensible to the chemical concentration, but in which the diffusion-reaction equations were unaffected by the resulting deformations.

In the last decades, a series of numerical models that combine growth and pattern formation have been proposed in the literature by different authors. Among them we mention the general approaches of Crampin et al. (1999) and Neville et al. (2006), and the more applied studies of Oster et al. (1983), who used mesenchymal cells and a substrate to model their motility through strain-dependent diffusion, and Umilis et al. (2008), in which a large number of genes and product proteins were employed to simulate pattern formation during Drosophila segmentation process. Most of these studies deal with 2D or 1D pattern formation on a growing tissue. However, our main concern is to explore the influence of relatively simple chemical reaction equations on cell shape changes that occur in the 3D space as for the real embryo.

The modelling of diffusion–mechanical coupled problems with large deformations and displacements during embryo development has been so far very little studied. We can point out some related and interesting studies in the context of plant growth (Brière and Goodwin 1988), in which diffusion equations are also coupled with a growth process, wound healing (Javierre et al. 2009), cardiac tissue with concentrations that depend on the electric intensity or the stresses (Cherubini et al. 2008 and Pustoc’h et al. 2005, respectively), or teeth growth (Salazar-Ciudad and Jernvall 2010). In all these models, diffusion-reaction equations have an effect on the rest length, active stresses or tissue growth. Our model is in fact related to the latter case, because the morphogen concentration mediates the cellular shape changes, but without actually considering any growth process. In addition, we emphasise that although we also resort to an underlying diffusion-reaction equation, the morphogen concentrations are in turn also highly affected by the model kinematics due to the presence of large deformations and displacements in the biological structure.

1.2 Interactions between morphogens activity and mechanics
To accomplish our main objective mentioned above, an extension of our previous model (Allena et al. 2010) is introduced in order to include a diffusion-reaction as an additional mechanical regulator of morphogenesis, and to consider an evolution law that relates the active deformations and the morphogen concentration. We show that strain patterns similar to those observed during biological experiments can then be reproduced by properly combining the two phenomena.

In addition, we use a technique that we have recently proposed (Allena and Aubry 2011) to parameterise the embryo geometry. This method uses the solution of two Laplace boundary problems to define the parameters that will be employed in the definition of our active deformations. Such boundary value problems can be
identified with an electrostatic problem, and although we do not directly couple such potential with our mechanical and diffusion-reaction variables, we note that the presence of the electric field may be additionally taken into account in future studies. Indeed, it has been shown that the electric field may strongly influence cell signalling (Sun et al. 2006). We therefore believe that the coupling between our electrostatic problem and the chemical and mechanical fields has a large potential in embryo modelling.

2. Harmonic parameterisation of the embryo geometry

The 3D geometry of the Drosophila embryo has been constructed from an interior and exterior ellipsoid in order to obtain a realistic shape. (Figure 1). The major axis is 500 μm long, whereas the cross axes CE and DF are, respectively, 175 and 165 μm long. The thickness is not constant, but it varies between 15 μm < h < 40 μm. Such variations have been also observed in real embryos.

It will become useful in subsequent sections to accurately parameterise this geometry. We have therefore defined a local curvilinear coordinates system described by the three parameters \( V_\theta, V_z \) and \( V_\xi \), as represented in Figure 2a. Each one of them is computed by solving Laplace’s equation on the epithelium with appropriate boundary conditions (Kellogg 1953). Similar developments have been proposed in the literature by Marchandise et al. (2011) who used the term harmonic which we now find more appropriate than electric as in our previous study (Allena and Aubry 2011). In the next sections, we briefly describe how we numerically build the normal and the tangential coordinates.

2.1 Normal and tangential variables

Let \( \partial \Omega_e, \partial \Omega_i \) and \( \Sigma_0 \) be, respectively, the outer, the inner and the middle surface of the irregular ellipsoid \( \Omega \). A harmonic variable \( V_\xi(x,y,z) \) within the thickness of the membrane is defined as follows:

\[
\begin{align*}
\Delta V_\xi &= 0 \quad \text{inside } \Omega \\
V_\xi &= +\frac{1}{2} \quad \text{on the external boundary } \partial \Omega_e \\
V_\xi &= -\frac{1}{2} \quad \text{on the internal boundary } \partial \Omega_i,
\end{align*}
\]

where \( h \) is the thickness of the ellipsoid. As shown in Figure 2b, the surface \( V_\xi = 0 \) provides a good approximation of the middle surface \( \Sigma_0 \).

From the calculated variable \( V_\xi \), the normal vector \( n_0 \) to the shell middle surface \( V_\xi = 0 \) can be estimated as follows:

\[
\mathbf{n}_0 = \frac{\nabla V_\xi}{\|\nabla V_\xi\|}.
\]

To have a complete system of curvilinear coordinates, we need now to find the two tangential parameters, \( V_\theta \) and \( V_z \). The latter parameter corresponds to the global cylindrical variable \( z \), whereas we will compute \( V_\theta \) by using boundary value problems similar to the parameter defined in Equation (1). However, since the hollow geometry of the embryo may have only two boundaries (the internal and external surfaces), special care must be taken when defining the boundary conditions for \( V_\theta \) (Brown 1983; Greenbaum et al. 1993). We will introduce a fictive slit surface \( \partial \Omega_{sl} \), defined by \( \{ z = 0, \ y < 0 \} \) so that the

Figure 2. (a) The curvilinear system of coordinates composed by \( V_\theta, V_z \) and \( V_\xi \) that allows the parameterisation of the Drosophila geometry. (b) Representation of the external (red), the middle (green) and the internal (blue) surface of the embryo (for sake of clarity, only half of the hollow geometry is reproduced).

Figure 1. The geometry of the Drosophila embryo which has been built from an anterior and an exterior ellipsoid retrieved from real embryo images. The major axis AB is 500 μm, whereas the cross axes CE and DF are, respectively, 175 μm and 165 μm. The thickness of the embryo varies between 15 μm < h < 40 μm.
coordinate which runs circumferentially around the membrane making a closed loop is assumed to be discontinuous at \( \partial \Omega_s \).

More precisely, \( V_\theta \) is chosen to be the solution of the following Laplace equation and boundary conditions with \([\bullet]\) standing for the jump of the quantities across the slit:

\[
\begin{align*}
\Delta V_\theta &= 0 \text{ inside } \Omega_0 \\
[V_\theta] &= 2\pi \text{ across the cut } \partial \Omega_s, \\
\frac{\partial V_\theta}{\partial n} &= 0 \text{ on } \partial \Omega_0 \text{ and } \partial \Omega_1, \\
\end{align*}
\]

(3)

where \( n \) is the normal vector to the inner and outer boundaries of the embryonic tissue. The boundary problems in (1) and (3) are solved using finite elements. The associated vectors \( V_\theta \) and \( V_z \) that complete the curvilinear basis are obtained, as similar as for the normal vector \( n_0 \) (Equation (2)), as follows:

\[
\begin{align*}
V_\theta &= \frac{\nabla V_\theta}{\| \nabla V_\theta \|} \\
V_z &= \frac{\nabla V_z}{\| \nabla V_z \|} \\
\end{align*}
\]

(4)

\[2.2 \text{ Parameterisation of the intermediate configuration}\]

Now that the system of curvilinear coordinates has been completed, any point \( p(V_\theta, V_z, V_\xi) \) through the thickness of the embryo can be analytically located (Figure 2a). Actually, let \( p_0(V_\theta, V_z) \) be a point on the middle surface of the ellipsoid such that \( V_\xi(x, y, z) = 0 \), then we can write

\[
p(V_\theta, V_z, V_\xi) = p_0(V_\theta, V_z) + V_\xi n_0(V_\theta, V_z).
\]

(5)

As it will be explained in the next section, and as a consequence of a morphogen concentration, each point where such concentration is different from zero moves from its initial position \( p_0 \) to an intermediate position \( \tilde{x} \), with a expression similar to (5) but using a modified parameterisation \( \tilde{V}_\theta, \tilde{V}_z, \tilde{V}_\xi \):

\[
p(\tilde{V}_\theta, \tilde{V}_z, \tilde{V}_\xi) = p_0(\tilde{V}_\theta, \tilde{V}_z) + V_\xi \tilde{n}_0(\tilde{V}_\theta, \tilde{V}_z).
\]

(6)

The relationship between parameters \( (V_\theta, V_z, V_\xi) \) and \( (\tilde{V}_\theta, \tilde{V}_z, \tilde{V}_\xi) \) determine the shape of the active displacements from \( p_0 \) to \( \tilde{x} \), and will be specified in Section 4 for each morphogenetic movement. For later use in the analysis of the elastic equilibrium, it will become useful to derive the expression of the active deformation gradient \( F_a \), which is given by Smith (1993)

\[
F_a = \frac{\partial \tilde{x}}{\partial p} = \frac{\partial \tilde{x}}{\partial V_\theta} \otimes \nabla p V_\theta + \frac{\partial \tilde{x}}{\partial V_z} \otimes \nabla p V_z + \frac{\partial \tilde{x}}{\partial V_\xi} \otimes \nabla p V_\xi.
\]

(7)

The vectors \( \partial \tilde{x}/\partial V_\theta, \partial \tilde{x}/\partial V_z \) and \( \partial \tilde{x}/\partial V_\xi \) form the covariant basis at \( \tilde{x} \), and are explicit function of the covariant basis at \( p \) formed by the vectors \( \partial p/\partial V_\theta, \partial p/\partial V_z \) and \( \partial p/\partial V_\xi \). The latter are in turn computed from the contravariant basis at \( p \) according to the following relationship:

\[
\frac{\partial p}{\partial V_\theta} = \frac{\nabla p V_\xi \times \nabla p V_\xi}{g^p}
\]

with \( g^p = (\nabla p V_\xi, \nabla p V_z, \nabla p V_\xi) \) the determinant of these three vectors.

\[3. \text{ Coupled diffusive-kinematic model}\]

In this section, we introduce the governing equations and the evolution law of our chemo-mechanical system. We consider the embryonic tissue as a continuum domain under large deformations. The decomposition of the deformation gradient is used in order to take into account both the active and the passive components. The latter is assumed elastic, whereas the former, contrarily to previous models (Muñoz et al. 2007; Allena et al. 2010), depends here on the morphogen concentration. More specifically, the total observed deformation of the tissue is the result of the following steps:

- An active deformation that depends on the morphogen concentration in a simple manner. The pattern of the active deformation is kept constant, whereas the rate of its intensity depends linearly on the concentration level. Section 3.1 formalises this dependence.
- A superimposed elastic deformation that resolves the kinematic incompatibilities of the active deformation. As described in Section 3.2, the amount of the latter is found using standard equilibrium of elastic continua.

\[3.1 \text{ Chemo-mechanical framework}\]

\[3.1.1 \text{ Definition of intermediate configuration}\]

During morphogenesis, some cells at specific regions of the embryo undergo transformations in shape, which are apparently not triggered by the stress state of the tissue. These individual strains, represented by the active deformation gradient \( F_a \), change according to each morphogenetic movement, and may be kinematically incompatible (i.e. they lead to superposition of the cellular domains or discontinuities). If material compatibility is enforced at the final observed configuration, the active deformation \( F_a \) must be complemented with the so-called passive deformation \( F_m \). As a result, the total deformation gradient of tissue \( F \) is decomposed according to the following multiplicative form:

\[
F = F_m F_a.
\]

(9)
where $\hat{F}_a$ and $\alpha$ are the deformation mode and the intensity active factor, respectively. They control the shape and the magnitude of the active deformation, respectively.

The specific form of $\hat{F}_a$ determines the actual imposed deformation. In general, this form will be defined through a set of relationships between $(V_\theta, V_z, V_\xi)$ and $(V_\theta, V_z, V_\xi)$, which are now also dependent on the variable $\alpha$:

$$
\begin{align}
V_\theta &= \hat{V}_\theta(V_\theta, V_z, V_\xi, \alpha), \\
V_z &= \hat{V}_z(V_\theta, V_z, V_\xi, \alpha).
\end{align}
$$

The actual expressions of these functions will be explicitly given for each morphogenetic movement in Section 4. In all cases, the evolution of factor $\alpha$ is driven by chemical diffusion, as will be described in the next paragraphs.

We note that this formulation is in contrast with other growth processes suggested in the literature, in which the active deformations depend on the Piola–Kirchhoff (Lubarda and Hoger 2002) or the Kirchhoff stresses (Himpel et al. 2005). In our case instead, the pattern of the active deformations remains prescribed, while its intensity is modulated with the morphogen concentration. By resorting to such prescribed patterns, we are reducing the flexibility to model other potential cell shape changes. Nevertheless, we show in our results that by using suitable choices of these patterns we can reproduce the local cellular movements such as the apical constriction during the VFI or the intercalation process during the GBE, without having to explicitly model the cytoskeleton or the cell–cell contact at the membrane.

### 3.1.2 Evolution law and diffusion-reaction equations

The chemical and the mechanical fields are coupled with a simple linear relationship between the rate of the intensity active factor $\alpha$ and the actual concentration per unit of deformed volume $c$ as follows:

$$
\frac{d\alpha}{dt} = \beta c,
$$

where $\beta$ is a positive constant. This equation is motivated by the following three facts: (i) at points with larger morphogen concentration, the genes will be more likely to trigger the driving active forces, (ii) we aim to achieve a homeostatic state, with $c = 0$, in which no further active deformations are introduced and (iii) at the end of the morphogenetic movement, when $c = 0$, the value of $\alpha$ must remain unchanged, i.e. the process will not be reversed. The latter fact has prevented us from setting a linear relationship between $\alpha$ and $c$. The parameter $\beta$ controls the influence of the concentration onto the active deformations, which is a consequence of multiple factors. In the two morphogenetic movements analysed here, we have assigned a single value to $\beta$ such that the deformed configuration resembles the observed configuration. This is of course a preliminary simple choice which can be modified in the future.

We note that, since $c \geq 0$, $\alpha$ will always increase. However, its rate may increase or decrease according to the concentration rate itself. We recognise that as far as $c \neq 0$, $\alpha$ will keep increasing, which seems a priori unrealistic. In fact, Equation (12) is a linear approximation of multiple concurrent complex phenomena that occur during a larger time-span than the duration of our analysis. Our model does not aim to fully reproduce all this myriad of processes with such a linear relationship, and consequently will become more approximated during the end-time instants. We will limit our attention and conclusion to a subinterval of this whole period, in which Equation (12) seems better justified and reproduces better the experimental observations.

We define an active region as the domain in which $\alpha \neq 0$, and therefore a region in which some active deformations are present. We will assume an initial condition in which the morphogen concentration is uniform on a restricted region. Due to Equation (12), such a region will be subjected to active deformations, but due to diffusion-reaction of this morphogen, the active region will vary its extension as a function of time.

Let us consider the motion $\Omega_T \rightarrow \Omega_T$, from the reference to the actual configuration and assume that the concentration $c$ follows a diffusion-reaction equation at the instant $t$. Thus, if $\text{div}_c$ and $\nabla_c$ are, respectively, the divergence and the gradient with respect to the actual position $\mathbf{x}$, the balance of morphogen onto a control volume yields the following equation:

$$
\frac{dc}{dt} + \text{div}_c \mathbf{v} = \text{div}_c (k_D \nabla_c c) + k_R c,
$$

where $\mathbf{v}$ is the velocity of the material, $k_D$ the diffusivity scalar constant and $k_R$ the chemical reaction coefficient.

For large deformation problems, and with the aim of spatially discretising the model with finite elements, we will express the above differential equation with respect to the reference configuration. Recalling that $(dJ/dt) = J \text{div}_c \mathbf{v}$ (where $J = |\det F|$), and $\nabla_c c = F^{-T} \nabla_p c$, and according to Piola’s identity $\text{div}_c \mathbf{a} = (1/J) \text{div}_p (J F^{-T} \mathbf{a})$ (Marsden and Hughes 1983), where $\mathbf{a}$ is any vector, we have that Equation (13) turns into

$$
\frac{dJc}{dt} = \text{div}_p (J k_D C^{-1} (\nabla_p c)) + J k_R c,
$$

where $\mathbf{F}$ is the deformation gradient and $\mathbf{C}$ is the right Cauchy-Green tensor. This equation is solved in Section 4.
where \( C = F^T F \) is the Cauchy–Green deformation tensor. We emphasise that we are solving the diffusion-reaction equation on a deformable domain, and therefore some additional terms have arisen with respect to diffusion-reaction equations on a fixed domain. We note that the effects of the medium deformation on the concentration evolution are twofold. On one hand, the initial scalar diffusion term \( k_D \) is now replaced by an anisotropic diffusion tensor \( J k_D C^{-1} \). Thus, for highly deformed tissues, the diffusion term decreases along the principal direction of deformation. On the other hand, we can identify a reaction term equal to \( c( J k_R - (dJ/dt) ) \). Consequently, if the material is compressed \( (dJ/dt) < 0 \), we will observe that the morphogen concentration augments due to the increase of \( (J k_R - (dJ/dt)) \).

Due to the diffusion-reaction process given in Equation (14) and to the evolution law given in Equation (12), we will observe a gradient of the active deformation. This is in contrast to our previous studies (Muñoz et al. 2007; Allena et al. 2010) in which the intensity factor \( \alpha \) was assumed uniform throughout the active region.

The corresponding weak form of Equation (14) is obtained in the usual manner: by multiplying this equation by the test concentration \( \tilde{c} \) so that, assuming vanishing concentration flux outside the embryo, we have

\[
\int_{\Omega_r} \tilde{c} \frac{d(Jc)}{dt} \, dV = - \int_{\Omega_r} J k_D \left( C^{-1} \nabla_p \tilde{c}, \nabla_p c \right) \, dV + \int_{\Omega_r} J k_R \tilde{c} c \, dV, \quad (15)
\]

where \( (a, b) \) is the dot product of two vectors \( a \) and \( b \). Equation (15) will be discretised by a standard finite element method on the initial configuration.

### 3.1.3 Qualitative analysis of the model

Before detailing the numerical simulation on the full embryo, let us consider from a qualitative point of view the influence of the diffusion coefficient \( k_D \) and the reaction coefficient \( k_R \). In order to illustrate and evaluate this specific aspect, we consider a 1D domain \( \infty < x < \infty \) with an initial active region at the centre \(( -1 \leq x \leq 1 )\) that has a morphogen concentration \( c = 1 \) at \( t = 0 \) s. The differential equation (15) and the evolution law given in (12) with \( \beta = 0.1 \) have been symbolically solved with Mathematica®. Figure 3(a) and (b) show the evolution of \( c \) and \( \alpha \), respectively, as a function of time \( t \) and space \( x \), in the case without reaction \( (k_R = 0) \). As expected, it can be observed that morphogen concentration grows faster initially, when \( c \) is higher. The region in which the intensity active factor \( \alpha \neq 0 \) spreads outside the initial active region and increases monotonously as mentioned above.

It has been verified that when the chemical reaction is taken into account, \( k_R \neq 0 \), there is a morphogen production (consumption) when \( k_R \) is positive (negative), and consequently we have that \( \alpha \) grows at a faster (slower) rate. Figure 3(c) and (d) shows the evolution of \( c \) and \( \alpha \) when \( k_R < 0 \). In this case, \( c \) decreases much faster and the evolution of \( \alpha \) is much slower.

These preliminary results confirm that when the activation factor \( \alpha \) is driven by a diffusion-reaction equation, the activity that was initially concentrated in
specific areas is transferred to neighbouring zones. This concentration will affect the mechanical response of the system, and as it will be shown in the next section, the converse will also be true: the mechanical deformation will affect the morphogen concentration, similar to the mechanotransduction phenomenon observed within the tissue.

3.2 Mechanical equilibrium and behaviour

Let \( \mathbf{x} \) stand for the actual position of a material particle and \( \mathbf{y}_m \) stand for a Cartesian frame. Then the total deformation may be described by the deformation gradient

\[
\mathbf{F} = \sum_{m=1,2} \frac{\partial \mathbf{x}}{\partial \mathbf{y}_m} \otimes \mathbf{y}_m.
\]

We remind that this total deformation gradient may be decomposed in its active and passive part according to Equation (9). It is illustrative to contemplate two extreme situations: (a) when the cell is completely free, without any boundary condition imposed, \( \mathbf{F}_m = \mathbf{I} \) and \( \mathbf{F} = \mathbf{F}_a \), i.e. the final deformation coincides with the active deformation itself and (b) when the cell is fully constrained by other cells and by the boundary conditions, as in the case of the embryo, \( \mathbf{F}_m \) is the actual response to the active deformation \( \mathbf{F}_a \). In general, the two situations are mixed to provide the suitable final consistent deformation imposed by the continuity of the material.

The weak form of the mechanical equilibrium condition in the initial configuration \( \Omega_p \) is expressed through the first Piola–Kirchhoff stress \( \mathbf{\pi} \) as follows:

\[
\int_{\Omega_p} \text{Tr}(\mathbf{\piD_p w^T}) \, dV = \int_{\partial\Omega_p} (\mathbf{wF_a}^{-1}\mathbf{F}^{-1}(\mathbf{n})) \, dS,
\]

where \( \mathbf{w} \) indicates the pressures exerted on the inner and the outer surfaces of the embryo and \( \mathbf{w} \) is a kinematically admissible displacement test function.

The first Piola–Kirchhoff tensors are computed as \( \mathbf{\pi} = \mathbf{J}_u \mathbf{F}_a^{-1}\mathbf{S}_m \), where \( \mathbf{J}_u = \text{Det}[\mathbf{F}_u] \) \( \mathbf{S}_m \) is the second Piola–Kirchhoff tensor with respect to the intermediate configuration, and it is defined as

\[
\mathbf{S}_m = \lambda \text{Tr}[\mathbf{E}_m]\mathbf{I} + 2\mu \mathbf{E}_m,
\]

where \( \lambda = E/(1+\nu)(1-2\nu) \) and \( \nu = E/(2(1+\nu)) \) are the Lamé material parameters, with \( E \) and \( \nu \) being Young’s modulus and Poisson’s ratio, respectively. The Green–Lagrange strain tensor \( \mathbf{E}_m = (1/2)(\mathbf{C}_m - \mathbf{I}) \), where \( \mathbf{C}_m = \mathbf{F}_m^T\mathbf{F}_m = \mathbf{F}_a^{-1}\mathbf{F}^T\mathbf{FF}_a \), measures the elastic passive deformation.

By using a purely elastic model, we are disregarding any viscous dissipative phenomenon and the viscous forces. It must be noted though that some elastic contribution does exist, as the experiments in Supatto et al. (2005) and in Farge (2003) show, in which the original shape of the embryo is recovered when an imposed deformation is removed. Indeed, the viscoelastic nature of embryo epithelia is still a controversial debated issue, and in fact, although some researchers have modelled the Drosophila embryo resorting to solely fluid equations (Pouille and Farge 2007), others have used a purely elastic tissue (Turing 1952).

In our case, by considering a purely elastic material, the passive deformations that accommodate the kinematic incompatibilities of the active deformations will induce some elastic stresses. We are aware that these may be actually different from a representative stress state of the cell. We show in our numerical results that the actual value of the stresses is proportional to the material stiffness, which can be just estimated for embryo tissue (Wiebe and Brodland 2005). Nonetheless, in Section 4.1 we have also compared our stress values with those reported in other models.

More importantly, the active deformations of our model are independent of the material properties, and are those that largely contribute to the total deformation. The aim of this study was to reproduce the active deformations and to analyse the plausibility of a diffusion-driven mechanism. We do not intend to match the in-vivo stresses, which, to the authors’ knowledge, have not been reported so far.

The equilibrium Equation (17) must be complemented with the particular boundary conditions. In the Drosophila embryo, they correspond to the vitelline membrane contact conditions and the internal yolk volume preservation. Due to the irregular shape of the ellipsoid, these conditions prevent any rigid body motion. They are modelled by including additional terms in Equation (17) [see Allena et al. (2010) for further details].

In summary, mechanical and chemical phenomena are coupled according to the following dependences:

- The elastic deformation on the morphogen concentration \( c \), \( \mathbf{\pi} \) depends on \( \mathbf{F}_a \), which is mediated by the active intensity factor \( \alpha \) that depends on the morphogen concentration \( c \).
- The morphogen concentration \( c \) on the total deformation: the diffusion equation depends on \( \mathbf{C} \) and \( \mathbf{J} \), reflecting the fact that compressed areas will increase their concentration \( c \).

The two Equations (15) and (17) are simultaneously solved through a multistep time integration and a Newton scheme for the displacement \( \mathbf{u} \) and the morphogen concentration \( c \) at each time step.

4. Morphogenetic movements

The coupled mechanical-diffusion model and the parameterisation of the active deformations developed in the
previous sections will be applied here to two morphogenetic movements: the VFI and the GBE.

In the last decades, these two movements have been amply studied from both the experimental and the numerical points of view. In fact, some interesting computer models have been proposed in the literature (Alberch et al. 1981; Jacobson et al. 1986; Weliky and Oster 1990; Clausi and Brodland 1994; Davidson et al. 1995; Taber 1995; Conte et al. 2007; Muñoz et al. 2007; Pouille and Farge 2007; Taber 2007; Ramasubramanian and Taber 2008). However, in all these references, the cell activity is either imposed or is a function of the stress state of the cell. In this study instead, motivated by the presence of diffusion profiles of dorsal morphogen which control the expression of the genes twist and snail during the VFI and the GBE (Steward 1989; Leptin 1999) or the concentration profiles of Fgf8 and Dpp in other developmental processes (Kicheva et al. 2007; Yu et al. 2009), we will reproduce the VFI and the GBE according to the evolution law in (12), the diffusion-reaction equation in (15) and the mechanical equilibrium in (17).

In all of the following analyses, we have assumed the constant representative values: $E = 100\, \text{Pa}$ (Wiebe and Brodland 2005), $\nu = 0.45$, $k_0 = 10^{-9}\, \text{m}^2\, \text{s}^{-1}$ and $\beta = 10^{-1}\, \text{m}^3\, \text{s}^{-1}\, \text{mol}^{-1}$. However, due to a lack of experimental information, we have set $k_0 = 0$, except for the simulation of the VFI in which a parametric analysis has been done.

### 4.1 Ventral furrow invagination

The VFI is an orthogonal invagination that takes place along the ventral embryonic midline. It extends between 6% and 85% egg length and it involves about 800 cells that will become internalised. The movement is highly controlled by two developmental genes: twist and snail. In addition, the former induces the ventral expression of Fog and T48 (Kolsh et al. 2007), two proteins that recruit RhoGEF2 to constrict a contractile actin–myosin II network that leads to the deformation of the active cells (Nikolaidou and Barret 2004). The VFI is triggered by a series of synchronised cell shape changes that provide the final form of this furrow (Alberch et al. 1981; Leptin and Grunewald 1990; Sweeton et al. 1991; Costa et al. 1993; Leptin 1999). Similar to our previous study (Allena et al. 2010), the apical constriction along the transversal section of the embryo is only responsible of VFI. Apical constriction occurs primarily through the contraction of cytoskeletal elements and, specifically for the *Drosophila* embryo, the actin–myosin filaments. It consists in the contraction of the apical basis of the cells, which is in contact with the vitelline membrane, and it causes the cells to take a wedge shape.

Such deformation can be reproduced by applying the intermediate position in Equation (6) and particularising Equation (11) to the following expressions:

\[
\begin{align*}
\dot{\mathbf{V}}_\theta &= V_\theta + \alpha \frac{2V_c}{h} m(V_\theta), \quad (19a) \\
\dot{\mathbf{V}}_z &= V_z, \quad (19b)
\end{align*}
\]

where $m(V_\theta)$ is a periodic function that mimics the cell boundaries by appropriately modulating the intensity of the active deformation. Its explicit expression is

\[
m(V_\theta) = 2 \left[ \frac{V_\theta}{V_{\theta,\text{cell}}} - \frac{1}{2} \right] \cdot \text{round} \left( \frac{V_\theta}{V_{\theta,\text{cell}}} - \frac{1}{2} \right), \quad (20)
\]

where $V_{\theta,\text{cell}}$ corresponds to the angular extension of a material cell and round represents the classical step function which gives the integer number of $(V_\theta/V_{\theta,\text{cell}}) - (1/2)$.

This function subdivides the embryonic tissue into several sub-domains corresponding to the real cells. By using this periodic function, we can evaluate the individual cellular deformations. It depends on the hypothesised dimensions of a real cell of the embryo which have been set equal to $15\, \mu\text{m}$ along $V_z$ and $10\, \mu\text{m}$ along $V_\theta$ and $V_z$. From Figure 4(a), it is possible to observe the physical distribution of the pseudo-cells along the transversal section of the embryo. Each white domain represents a cell, whereas the black domains are triggered by the smoothing effect of the Heaviside function by which the material cells are obtained (Allena et al. 2010).

The values of the intensity factor $\alpha$ are determined by the evolution law in Equation (12). By inserting Equation (19) into Equation (6), the active deformation gradient $\mathbf{F}_a$ can be then computed according to Equation (7).

Initially, a uniform non-zero concentration is introduced in a restrained region of the embryo, as it is

---

Figure 4. Representation of the material cells by which the embryonic tissue is subdivided (Equation (20)) during the VFI simulation. Each white domain represents a cell, while the black domains are triggered by the smoothing effect of the Heaviside function by which the material cells are obtained. (a) Initial configuration ($t = 0$): the cells show a columnar shape, (b) final configuration ($t = 1\, \text{s}$): the apical constriction has occurred and it is maximal at the apex of the furrow.
observed in reality. Figure 5 shows this region with the initial conditions for concentration \( c \). Such an area is obtained using a Heaviside function which is equal to 1 in the region with non-zero concentration values and equal to 0 everywhere else. The discontinuities of this function have been smoothed to ease the computations (Allena et al. 2010).

The successive steps of the VFI are shown in Figure 6(a), (b) and (c). The embryonic tissue is more constricted in the area corresponding to the active region, due to the higher initial morphogen concentration. As the diffusion phenomenon takes place, the initial concentration decreases. Thus, at \( t = 0 \) s we have a concentration \( c = 1 \) mol m\(^{-3}\) in the centre of the active region (Figure 6(a)), whereas at the end of the simulation (\( t = 1 \) s) we find \( c = 0.36 \) mol m\(^{-3}\) (Figure 6(c)). The experimental values of dorsal morphogen concentration in the embryo during the VFI are not detailed, and just the general profiles can be found in the literature (Steward 1989). Furthermore, the value of parameter \( \beta \), which controls the influence of the concentrations on the active deformations, is hard to measure and has just been estimated in our simulations.

The general trend of the strains due to the diffusion phenomenon agrees with the experimental observations. Indeed, while the morphogen concentration reduces during the simulation, the active intensity factor increases and consequently the final strain occurring to the cells (Figure 7(a)). At the initial configuration (Figure 4(a)), the cells show a columnar shape, whereas at \( t = 1 \) s, once the furrow has formed (Figure 4(b)), the apical constriction is clearly evident, especially for the cells in the active region. Figure 8 shows the value of the total stretching along \( V_\theta \), which is computed as the projection of the Cauchy–Green deformation tensor \( C \) along \( V_\theta \) as follows:

\[
(C(V_\theta), V_\theta).
\]

We find that the final maximal stretching for the mechano-diffusion model at the apex of the invagination is equal to 0.74 (Figure 8(b)), which is almost double compared with that found in our previous simulation of the VFI with the imposed active deformations (0.32) (Figure 8(a)) (Allena and Aubry 2011). According to the chosen dimensions of a real cell, we can estimate that the maximal apical constriction at the apex of the furrow is equal to 7.5 \( \mu m \). In general, the final strain simultaneously depends on the initial position of the cell as well as on the diffusion phenomenon implemented here.

Although no in-vivo stresses have been reported in the literature, we have computed the stresses along the direction \( V_\theta \) that is the value of \((S_m(V_\theta), V_\theta)\) at point \( E \) in Figure 1(b), and compared the values with those reported in Brodland et al. (2010). From the expression of \( S_m \) in Equation (18), we deduce that \((S_m(V_\theta), V_\theta) = \lambda \text{Tr}[(E_m)] + 2\mu((E_m(V_\theta), V_\theta) - \mu))^2\), which is directly proportional to the Young modulus \( E \). In Figure 7(a), we have plotted the evolution of \((S_m(V_\theta), V_\theta)\) for the value employed in our model, \( E = 100 \) Pa, the stress value approaches 400 Pa (Figure 7(c),(d)). In order to compare this value with the tractions reported in Brodland et al. (2010) (Figure 4(a)), we converted our stress value into a traction per unit of \( \mu m \) depth and applied it onto a cell of width 15 \( \mu m \), which yields a force of 6 nN. This value is between one and two orders of magnitude higher than that in Brodland et al. (2010), which as the authors in the reference recognise is proportional to the viscosity, which in turn may vary in different orders of magnitude. It is also worth noting that our stresses and forces monotonically increase, whereas those in Brodland et al. (2010) have a parabolic trend. This is in agreement with the different material model considered, as discussed in Section 3.2: while the forces in Brodland et al. (2010) are proportional to the strain rate, ours are proportional to the accumulated elastic strain.

We also remark that, as pointed out by Butler et al. (2009), the elongation of the invaginating tissue on both sides of the furrow towards the midline could be the direct cause of the antero-posterior tensile force deforming the germ band in the early fast phase of its extension. Therefore, the VFI, as well as other three morphogenetic movements (cephalic furrow formation, posterior midgut invagination and amnioserosa cell elongation), could be a good candidate to contribute to the cell shape changes leading to the GBE, which are then considered as a passive response to the mechanical forces occurring during the VFI.
To conclude, we remark that the elastic deformations at any point in the initially non-active region of the tissue are originated by two contributions. For the regions in which \( a = 0 \), the deformations in active areas with \( a < 0 \) will induce global elastic deformations. On the other hand, due to the diffusion phenomenon, \( a \) may eventually be positive and consequently superimpose an additional active deformation. Therefore, the total deformation at each point is due to the activity at neighbouring cells, plus the active deformations that this point may have. This is in contrast to our previous simulation in which the active deformations were exclusively localised in the initial active zone (Allena et al. 2010).

As a final and qualitative test, we have introduced the reaction term into Equation (14) with \( k_R = 0.1 \text{ s}^{-1} \). The main objective of this specific simulation is to show that, as similarly as in Section 3.1.3, the reaction term may influence the global behaviour of the biological system. Let us consider the point \( E \) of the ventral furrow as represented in Figure 1. As expected, since \( k_R > 0 \), we have a morphogen production which leads to an increase in the morphogen concentration \( c \), as observed in Figure 7(b).

Figure 7. The trends of the morphogen concentration \( c \) (blue line), of the active intensity factor \( a \) (red line) and of the total apical constriction defined as \( (C(V_y), V_y) \) (green line) over time \( t \) during the VFI simulation (the curves are traced for the point \( E \) of Figure (1)). In (a) \( k_R = 0 \) and the concentration \( c \) progressively decreases. In (b), \( k_R = 0.1 \text{ s}^{-1} \) which leads to an increase in the concentration \( c \) at the end of the simulation. In both cases, the initial condition at point \( E \) is \( c = 1 \) and \( a = 0 \). As expected \( a \) always increases. (c) and (d): computation of the stress \( S_{\text{mx}} \) along the direction \( V_y \) (Section 4.1) for the case in which \( k_R = 0 \) (c) and \( k_R = 0.1 \text{ s}^{-1} \) (d).

Figure 8. Final deformation of the tissue during the VFI represented by the projection of the Cauchy–Green tensor \( C \) along the tangential vector \( V_y \) (Equation (21)). (a) Results when simulating VFI with imposed active deformations, as modelled in Allena and Aubry (2011), yielding a maximal apical constriction equal to 0.32. (b) Present mechano-diffusion simulation of the VFI with a maximal final stretch equal to 0.74.
This is in contrast to the previous case (Figure 7(a)) with \( k_R = 0 \) and in which \( c \) globally decreases due to diffusion. For what concerns the evaluation of the active apical constriction, we remark that the final invagination is smaller than the model with \( k_R = 0 \). Although further studies may be necessary, we believe that the proposed analysis can be considered as a first step to investigate the mechanotransduction phenomenon. So far we have introduced a linear reaction term \( (k_R c) \), even though we are aware that for chemical analyses a nonlinear term is usually involved \( (k_R c^n) \).

### 4.2 Germ band extension

The germ band is located at the ventral region of the embryo and it starts to extend at the end of gastrulation (Keller et al. 2000). This event leads to an elongation of the germ band to about 2.5 times its initial length. It is due to a convergent–extension movement of a population of cells at the central-lateral region of the embryo. This process is triggered by an intercalation of the cells that interpose themselves between their dorsal or ventral neighbours, resulting in a decrease in the number of cells along the dorsal-ventral axis and in an increase in the number of cells along the anterior-posterior axis (Keller et al. 2000). Recently, it has also been shown that during the fast early phase, the GBE depends on cell shape changes in addition to intercalation, and that these changes in shape are a passive response to the mechanical forces caused by the invagination of the ventral tissue (Rauzi et al. 2010).

Here, since the cells are not individually modelled, we do not precisely simulate the intercalation process. Instead, we propose a continuous movement of compression–extension tangential to the middle surface of the blastoderm. As in the VFI, the active deformations are introduced on a limited region of the embryo (Figure 9(a)): the germ band at the ventral region in which the initial morphogen concentration is not 0. The intermediate position in Equation (6) uses the following particular expressions for \( \tilde{V}_\theta \) and \( \tilde{V}_z \):

\[
\tilde{V}_\theta = (1 + \alpha)V_\theta, \quad (22a)
\]

\[
\tilde{V}_z = (1 + \alpha)V_z. \quad (22b)
\]

The active deformation gradient \( \mathbf{F}_a \) may be then computed by inserting these expressions into \( \dot{\mathbf{X}} \) in Equation (6) and evaluating Equation (7).

The results for this simulation are plotted in Figure 9(b). As we can observe, the convergence–extension movement is evident even though the amplitude of the uniform compression from the dorsal to the ventral region is not so pronounced as in our previous study (Allena et al. 2010). We can still notice the vortex movements forming towards the anterior pole and the posterior pole. In addition, we have been able to evaluate the maximal value of the two active strains by projection of the Cauchy–Green deformation tensor along \( V_\theta \) (shortening, \( (\mathbf{C}(\dot{V}_\theta), V_\theta) \)); and along \( V_z \) (extension, \( (\mathbf{C}(\dot{V}_z), V_z) \)). We find, respectively, 0.06 for the convergence and 0.21 for the extension. Thus, if we consider the dimensions given in Section 4.1. for a single cell, we may observe an extension of each cell along \( V_z \) of about 2.1 \( \mu \text{m} \) and a shortening along \( V_\theta \) equal to 0.6 \( \mu \text{m} \).

Like in the VFI simulation, the morphogen concentration decreases during the diffusion phenomenon. Consequently, at \( t = 0 \) we find an initial maximal value
of $c = 1 \text{mol m}^{-3}$ in the active region (Figure 10(a)), whereas at the end of the simulation ($t = 1 \text{s}$), we have $c = 0.08 \text{mol m}^{-3}$ (Figure 10(c)).

In Figure 11, the trends of the intensity active factors, the two active strains and the morphogen concentration at point $E$ of Figure 1 are reported as a function of $t$. As we can observe, the concentration $c$ decreases while $\alpha$ progressively increases, which affects the tendency of the active deformations. The absolute value of stretching in the directions of $V_\alpha$ and $V_z$ increases, but of course in the former case it is negative (shortening) whereas in the latter it is positive (lengthening).

5. Conclusions
We have coupled the diffusion-reaction equations on a deforming domain with the equilibrium equation of an elastic domain subjected to active deformations, which are in turn dependent on morphogen concentration. In addition, a novel technique to parameterise the embryo geometry and obtain a set of covariant basis has been employed, which has allowed us to deduce the expressions of the concentration-dependent active deformations. We have applied the model to simulate VFI and GBE in the *Drosophila* embryo.

Despite an increasing number of experiments analysing the diffusion of morphogens, their effect on the biological developing tissue has not been taken into account. Here, we couple these diffusion profiles with the mentioned two morphogenetic movements. The promising results show that the employed set of equations reproduces the trend of the active deformations observed during morphogenesis. Clearly, in order to obtain more realistic strains, the reaction term and the initial morphogen concentration should be regulated, especially for a consistent comparison with the experimental observations and values. Also, additional nonlinear source terms are probably needed.

We recognise that by using a purely elastic material, some errors in the resulting stresses and the actual timing of the cellular response may have been introduced. We have though compared our stress values with those in Brodl et al. (2010), and remarked their good agreement. The lack of *in-vivo* measurements, similar to those described for other morphogenetic movements in Gregor et al. (2007), Kicheva et al. (2007), Yu et al. (2009), does not allow us to give further rigorous quantitative analyses. Qualitatively, it is worth pointing out that the usual smooth profiles encountered in diffusion process may have sudden mechanical effects, as in the case of the ventral furrow invagination modelled here. These severe folds in the epithelium may in turn drastically change the concentration profile, triggering non-smooth evolutions of the concentrations and strains, as shown in Figure 6 and 7.

We note that due to the presence of the vitelline membrane in the *Drosophila* embryo it is not possible to measure stresses at the embryo epithelium. However, the development described here is easily extensible to other embryos such as the *Zebrafish*, in which these data can be measured. We intend in future studies to test our model in these embryos.

We note that in contrast to the stress field, which is transferred nearly instantaneously, the diffusion process allows to match the timing of the morphogenetic movement, without artificially imposing a set of incremental active deformations. This is one of the features that has motivated the present study: the coupling of the active deformation with a physical quantity that enables to successfully control the rate and magnitude of the cell shape changes. In this paper, we have verified that the diffusion process is a plausible mechanism, able to govern the driving forces of the invagination process.

In our simulations, we have imposed a set of initial conditions for the concentrations, displacements, stresses and active deformations. We recognise that such conditions are certainly not always fully determined, and in fact correspond to the final conditions of other processes which we have not modelled. For this reason, our comparisons have more of a qualitative than a quantitative character. Nonetheless, the agreement between the observed deformations and our computations is certainly satisfactory. Furthermore, we point out that in our case, the active deformations are not directly imposed, but are a
consequence of the morphogen diffusion and the evolution law proposed for the intensity factor $\alpha$.

The simulations show that the dynamics of the diffusion process is compatible with an active genetic movement triggered by morphogens. The coupling between chemicals and mechanics through a diffusion-reaction in embryo development has not been attempted so far. The simulations presented here constitute then an original contribution for a better understanding of the whole 3D problem. We are aware that the complex signalling process between morphogen concentrations and gene activity may further regulate the relationship between embryo phenotype and its genotype. We have shown that without explicitly modelling the mechanotransduction path within the cells, morphogen activity may be included with simple rules that successfully match the complex synchronisation between chemicals and mechanics during the embryo development.

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