Modelling stripe formation in zebrafish: an agent-based approach

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1. Introduction

Zebrafish (Danio rerio), a freshwater fish with black stripes and yellow interstripes, serves as a model organism for a range of research areas, notably developmental biology [1], cancer [2] and genetic disease [3]. With a fully sequenced genome and a nearly transparent embryo, zebrafish lends itself to the study of vertebrate development [1]. Individual pigment cells are discernible on zebrafish skin, making it an excellent place to investigate how the interactions of these cells lead to the stripe pattern. Modelling the development of zebrafish skin patterns, both wild-type and mutations, will be the focus of this paper.

Three main kinds of pigment cells make up the striped pattern on zebrafish: black melanophores, yellow xanthophores and silvery iridophores [4–8]. Iridophores give the pattern its shiny appearance and appear all over the skin, though in different forms in black stripes and yellow interstripes [4,5,9]. Each of the four or five black stripes on an adult fish, shown in figure 1, consists of melanophores spread over L-iridophores and blanketed by scattered xanthophores and a thin net of S-iridophores (referred to as blue S-iridophores); the yellow interstripes, in turn, contain densely packed xanthophores over a compact layer of so-called dense S-iridophores [4,6,10,12–15]. Numerous experimental studies have been conducted to elucidate the cellular interactions that drive pattern formation. These experiments range from using laser or transgenic ablation to remove pigment cells in vivo [5,7,12,16,17] to observing pairs of cells interacting in vitro [18,19] and exploring altered cell behaviour through mutational analysis, transplantation and other methods [14,20,21]. Researchers also study time-lapse images of wild-type development [10,15,16,20,22] and analyse mutations [4,8,10,11,14–16,19–25]. While it was originally thought that melanophores and xanthophores were responsible for pattern formation, more recent research [4,6,8,10,11,15,23,26] is beginning to uncover the importance of interactions between all three types of cells.

Mathematical modelling can contribute to the study of zebrafish development in different ways than can experimental techniques. For instance, model components can be systematically removed or altered; by determining how these changes affect the resulting pattern in silico, modelling can identify possible mechanisms at work. Stripe pattern formation in zebrafish has been studied mathematically using reaction–diffusion equations [5,17,27], cellular automaton techniques [28,29], agent-based models [30,31] and integrodifferential equations [32,33]: Kondo and co-workers [5,17] both conducted and simulated ablation experiments, in addition to modelling stripe pattern formation from a larval initial condition. Moreira & Deutsch [29] simulated the development of wild-type and mutated patterns under a specific growth hypothesis, and Gaffney & Seirin Lee [27] evaluated how several reaction–diffusion models...
behave under the addition of biological feedback delays. The studies of Bloomfield et al. [32], Caicedo-Carvajal & Shinbrot [30], Woolley et al. [31] and Bullara & De Decker [28] involve reduced models in which cells interact only by differentiation/death or only by migration.

Since these mathematical studies were published, a large amount of new biological data has emerged, some of which contradicts previous modelling assumptions. Recent experiments [18] indicate that interactions between melanophores and xanthophores are not symmetric, in contrast to what was assumed in [29,30], and, furthermore, that long-range communication occurs between cells in adjacent stripes and interstripes (as is standard in empirical studies, we will refer to black stripes as stripes and yellow stripes as interstripes in the remainder of the paper) [5,7]. Informed by these advances, we present a two-population model for the formation of the striped pattern in zebrafish that describes development, ablation and mutations. To make our results amenable to direct comparisons with experimental data, we use an agent-based approach and work at the level of individual pigment cells.

We use our model to study cellular interactions (§3.1), wild-type development (§3.2), ablation experiments (§3.3), fish growth (§3.4) and mutations (§3.5). Our main findings indicate that

- fish growth widens stripes and interstripes (§3.4);
- repulsion between melanophores and xanthophores maintains stripe/interstripe separation and boundaries (§3.1); and
- the horizontal myoseptum and spreading/aggregation of iridophores (tested indirectly in our model by the sequential appearance of associated interstripe xanthophores) guide pattern directionality (§3.2).

In addition, in §3.3, we reproduce an ablation experiment [5]. We also include results on zebrafish mutations (§3.5) and investigate the importance of different aspects of pattern formation by removing cell migration or cell differentiation and death from the model (§3.6).

2. Brief model description

We begin by briefly outlining our model and refer to the electronic supplementary material for a detailed description and a discussion of the data used for parameter fitting. We reduce the complicated dynamics on the fish skin to the interplay of two types of pigment cells. These cells, which we model as independent agents, are represented by point masses marking cell centres and can interact through migration, differentiation and death on a two-dimensional domain. We will refer to the two cell groups in our model as melanophores and xanthophores, but it is important to note that the xanthophores, in particular, represent idealized particles that indirectly take into account some dense S-iridophore behaviour. This modelling simplification is based on experimental observations [13,26] that xanthophores are closely associated with dense iridophores in interstripes. We do not take into account I-iridophores, and the remaining cell types (blue S-iridophores and scattered xanthophores in black stripes), which appear at much lower density on the zebrafish skin, are not discussed in this paper.

We consider two set-ups: developing fish (growing rectangular domain) and adult fish (static rectangular domain). When included, growth takes the form of stretching the domain uniformly with rates inferred from [4,29,34] (for details, see the electronic supplementary material). For simulations of stripe/interstripe development, we follow the experimental convention of indirectly tracking time using stages associated with developmental milestones rather than days, as growth/development rates are highly variable and do not lend themselves to direct comparison with empirical data [26,34] (see §3.2). Without growth (or in the case of ablation experiments, for which the data are given in days), time is measured in days from the initialization of the simulation at t = 0. Unless otherwise noted, boundary conditions are Neumann on growing domains and periodic on static domains.

Because stripe/interstripe width seems to vary with fish age (an observation we made from images found in [34]), we base our length scales on measurements of average distances between cell centroids, namely $\Delta_m = 50 \mu m$ (the average distance between neighbouring melanophores), $\Delta_x = 36 \mu m$ (the average distance between neighbouring xanthophores) and $\Delta_s = 82 \mu m$ (the average distance between melanophores and xanthophores at stripe/interstripe boundaries) [16], as well as on the observation [5] that stripes/interstripes are about 7–12 cells wide. Throughout this paper, we refer to interactions on three length scales that are measured between cell centroids: contact, short-range and long-range. We call contact interactions those that occur on a scale less than $\Delta_{\infty}$ short-range interactions, in turn, involve cell communication on the order of $\Delta_m$; these interactions include the contact range but also extend past it (figure 2). Contact and short-range communication may be mediated by direct contact or by short dendrites [18,19,21,35]. Lastly, long-range refers to distances of the order of $9/2\Delta_m + \Delta_s$ (approximately half a stripe width, assuming stripes of width 10 cells). These interactions may be governed by pseudopodia, which can reach a maximum of half a stripe width [7,35].
bouring melanophores and melanophores and xanthophores at stripe/interstripe boundaries, respectively, as observed experimentally in [16]. Long-range interactions may represent communication by pseudopodia [7,35]. Short-range and contact interactions may involve shorter dendrites or direct contact [18,19,21,35].

2.1. Cell migration
The movement of each cell is governed by an ordinary differential equation for its position that specifies its interactions with the other cells in the underlying two-dimensional domain. The equations are

$$\frac{dM_i}{dt} = -\sum_{j \neq i} N_m \nabla Q^{mm}(M_j - M_i) - \sum_{j \neq i} N_x \nabla Q^{xm}(X_j - M_i)$$

and

$$\frac{dX_j}{dt} = -\sum_{i \neq j} N_x \nabla Q^{xx}(X_i - X_j) - \sum_{i \neq j} N_x \nabla Q^{mx}(M_j - X_i),$$

where $M_i$ is the position of the $i$th melanophore; $X_j$ is the position of the $j$th xanthophore; $N_m$ is the number of melanophores; $N_x$ is the number of xanthophores; $\nabla = (\partial / \partial x, \partial / \partial y)$ is the gradient in two dimensions; and $Q^{ij}$ are Morse potentials of the form

$$Q^{ij}(x, y) = K^{ij} e^{-\sqrt{x^2+y^2}/\sigma^{ij}} - A^{ij} e^{-2\sqrt{x^2+y^2}/\sigma^{ij}},$$

where $K^{ij}$, $r^{ij}$, $A^{ij}$ and $\sigma^{ij}$ differ between the four types of pairwise cellular interactions and correspond to the strength and length scales of repulsion and attraction, respectively. Informed by previous studies [16,18,19,35], we model the effects of melanophores on melanophores ($Q^{mm}$), xanthophores on xanthophores ($Q^{xx}$), and xanthophores on melanophores ($Q^{mx}$) as repulsive (table 1). The forces $Q^{mm}$ on melanophores owing to melanophores are attractive at contact and repulsive at short range. This choice was based on in vitro experiments [18] that uncovered a chase–run mechanism: it was observed that melanophores extracted from fins run away from short dendrites extended towards them by xanthophores, which slowly follow in pursuit; see §3.1 for details. We note that the chase–run mechanism observed in [18] has an anticlockwise bias; as a simplifying assumption, we chose not to include this bias in our model (for a simulation of spiralling chase–run movement, see [31]).

2.2. Cell differentiation and death
Our model is informed by a set of ablation experiments performed by Nakamasu et al. [5] to deduce when cells differentiate and die (see electronic supplementary material, figure S1). Their results suggest that xanthophores in adjacent interstripes promote both the differentiation and survival of melanophores over at least half a stripe width in distance. According to [5,7], these long-range effects may be paired with short-range competition of xanthophores and melanophores (while we model short-range competition through cell death rules, it is also possible that this effect could arise owing to cell movement). It should be noted here that significantly less data were recorded on xanthophores than on melanophores in [5], so we introduce the modelling assumption that xanthophores differentiate under analogous (opposite) conditions as do melanophores. The resulting rules, outlined below, take the form of short-range activation and long-range inhibition.

We update populations for differentiation and death once per day based on the proportion of melanophores and xanthophores in two neighbourhoods. A narrow annulus $\Omega_{\text{podia}}$ models long-range communication, possibly regulated by pseudopodia [6,7], and a small disc $\Omega_{\text{xact}}$ represents short-range interactions (figure 2). At the end of each day, every pigment cell is evaluated for possible death, with rules summarized below:

— xanthophores die when the proportion of melanophores to xanthophores in their immediate vicinity ($\Omega_{\text{xact}}$) is too high; and
melanophores may also die (with small probability [5]) if the proportion of melanophores to xanthophores in $\Omega_{\text{podia}}$ is too low. This reflects the experimental evidence [5] that xanthophores promote melanophore survival in adjacent stripes.

It has been shown that melanophores and iridophores arise from a particular set of stem cells [6,36,37], and that xanthophores are derived from larval xanthophores widely scattered across the larval fish skin [12,15,38]. Based on these findings and on experimental images [17], we assume new cells appear in the fish skin domain by differentiation from precursor or stem cells, which we do not otherwise take into account. Differentiation is implemented each day by selecting a set number of random locations for possible melanophore differentiation and another equal number of random locations for possible xanthophore differentiation. At each of the selected positions, differentiation occurs according to the following rules:

- a new xanthophore appears in the selected location if it is locally surrounded by xanthophores in $\Omega_{\text{loc}}$, and the proportion of melanophores to xanthophores in $\Omega_{\text{podia}}$ is high enough;
- a melanophore differentiates under the opposite conditions: high melanophore-to-xanthophore ratio in $\Omega_{\text{loc}}$ and high xanthophore-to-melanophore ratio in $\Omega_{\text{podia}}$;
- a small amount of random differentiation is also included.

With the exception of the small amount of random birth in our model, these conditions require that new cells differentiate only in locations near populated areas, as there must be non-zero numbers of melanophores and xanthophores in $\Omega_{\text{loc}}$ and $\Omega_{\text{podia}}$ for the rules to apply. Thus, though we randomly select locations for cell birth from the entire domain, we limit differentiation to areas where cells already exist, in effect reducing the probability for differentiation away from sources spreading outwards from the centre of the fish, where the larval pattern is initialized (see §3.2). This choice allows us to preserve the sequential nature of stripe/interstripe development described in [6,10].

As mentioned earlier, the rules for differentiation and death suggested by Nakamasu et al. [5] take the form of short-range activation and long-range inhibition, a patterning mechanism first proposed by Gierer & Meinhardt [39]. In [7,35], it was suggested that interactions by pseudopodia combined with repulsion of melanophores from xanthophores by dendrites could be considered a Turing-type mechanism [40] without diffusion. It is worthwhile noting that, in reaction–diffusion models, diffusion is responsible for dispersing information long range, whereas, in our model, it is the kinetics of differentiation and death that determine long-range interactions. Thus, though the abstract framework of two biological quantities interacting through short-range activation and long-range inhibition is present in both modelling approaches, the mechanisms at work are different.

3. Results

We now present a study of stripe pattern formation in zebrafish that includes development (§3.2), ablation (§3.3) and mutations (§3.5). Our main result on the effects of fish growth are described in §3.4. We also include results on cellular interactions (§3.1) and a discussion of reduced forms of our model (§3.6).

3.1. Cellular interactions

3.1.1. Short-range repulsion preserves stripe/interstripe boundaries and separation

The experimental studies [5,16] informed the repulsive form of the homogeneous interactions in our model. The asymmetric interactions between melanophores and xanthophores extracted from fins were studied in vitro by Yamanaka & Kondo [18] and Inoue et al. [25]. The results [18] suggest that heterogeneous interactions take the form of chase–run movement regulated by dendrites: melanophores move away from dendrites extended towards them by xanthophores, and xanthophores slowly follow in pursuit (note that this in vitro behaviour has not yet been observed in vivo). In contrast, other studies [4,6] indicate mutual repulsion between melanophores and xanthophores on the zebrafish body. To study these differing reports, which may represent the differences between body and fin cells, we model xanthophores as having a repulsive effect on melanophores and identify several possible mechanisms for the opposite interaction. The mechanisms of melanophore-on-xanthophore interactions we test are

(A) attraction of xanthophores by melanophores at short range;
(B) contact repulsion and short-range attraction of xanthophores by melanophores;
(C) contact attraction and weak short-range repulsion of xanthophores by melanophores; and
(D) repulsion of xanthophores by melanophores at short range.

Our results show that pure attraction, the simplest choice compatible with [18], leads to patterns with reduced separation between stripes and interstripes, as indicated in figure 3a. Woolley et al. [31] also evaluated this mechanism in a minimal agent-based model for cell movement and found that chase–run behaviour did not produce patterns. This result [31] was further supported in [33] by a stability analysis of a general non-local partial differential equation model: Painter et al. [33] found that chase–run behaviour combined with repulsive homotypic interactions could not produce unstable wavelengths. We conducted a linear stability analysis of the continuum model that we obtained as the limit of our agent-based model (see the electronic supplementary material) and, in agreement with [33], concluded that mechanism A, unlike mechanisms B–D, could not produce patterns from a uniform state.

We tested two ways of including attraction and repulsion in the forces that melanophores exert on xanthophores. Mechanism B, contact repulsion and short-range attraction, results in migration of xanthophores from interstripes into stripes (figure 3b). Our simulations show that mechanisms C and D, on the other hand, produce the correct cell-to-cell distances and support stripe/interstripe boundary separation and integrity, as shown in figure 3c. While mechanisms A and B do not seem to describe wild-type patterns, these interactions may be associated with mutations (see §3.5).

In summary, our results indicate that net repulsion between xanthophores and melanophores maintains stripe/interstripe boundaries. Because more data are available on
chase–run movement (in particular, in vitro measurements of cell speeds), we chose to use mechanism C, rather than D, in our simulations. As mentioned above, chase–run behaviour was linked in [18,35] to dendrite communication. Frohnho¨ fer et al. [4] have suggested that attractive interactions between xanthophores and the dense S-iridophores underneath them may be responsible for confining interstripes. Furthermore, Patterson et al. [23] demonstrated that overexpression of xanthophores and reduction in iridophores on the fish skin is linked to intermingling of cells at stripe/interstripe boundaries. Thus, it appears that interactions between all three types of pigment cells may be involved in preserving the sharp separation between melanophores and xanthophores (for proposed networks of such interactions, see [4,26]). The short-range repulsion in our model could be an indirect way of accounting for iridophores, which were not included in the in vitro experiments [18], or could support suggestions by Nüsslein-Volhard and co-workers [4,6,11] that cellular interactions in the fins and body are intrinsically different.

3.2. Development

Adult pigmentation evolves from a larval pattern to a set of four interstripes and four or five stripes in the metamorphic period lasting from 20 to 45 days post fertilization (dpf), as reported in [10,15] (it should be noted, however, that rates of development can vary significantly between laboratories [34]). The larval pattern consists of melanophores and iridophores in narrow stripes along the dorsal and ventral sides of the fish, as well as in a yolk–sac stripe (see, for example, [8]). Larval xanthophores are spread widely across the fish body, though they disappear as the metamorphic period continues [20,22,38]. Dense iridophores appear along the horizontal myoseptum (figure 1b) and seem to orientate the first interstripe and first two stripes [4,15]. Larval xanthophores are spread widely across the fish body, though they disappear as the metamorphic period continues [20,22,38]. Dense iridophores appear along the horizontal myoseptum (figure 1b) and seem to orientate the first interstripe and first two stripes [4,15]. Larval melanophores are also visible in a narrow strip along the horizontal myoseptum, but they withdraw from the prospective interstripe region by death or migration to make room for iridophores, which attract xanthophores [4,10,34]. This mechanism, shown in figure 4, leads to the first interstripe X0 and adjacent stripes 1D and 1V.

Nüsslein-Volhard and co-workers [6,8,10,11,15] have suggested that delayed iridophore localization (beyond the initial appearance at the horizontal myoseptum) may guide the formation of additional stripes and interstripes. By conducting iridophore ablation experiments, Parichy and his laboratory [23,26] have also shown that iridophores play an active organizational role in specifying stripe/interstripe

Figure 3. (a) Evolution of average cell-to-cell distances (measured between cell centroids) for mechanisms A (dashed), B (dotted-dashed), C (solid) and D (dotted) for representative simulations. Empirical measurements from [16] are represented as straight grey lines for comparison. (b) Pattern generated 100 days after simulation began under mechanism B; xanthophores migrate towards stripes, disrupting alignment. The initial condition used for both images is a set of adult stripes and interstripes with cells at the experimentally measured distances [16] apart (see §3.5.2). Note that the discrete cells in (b) and in the remaining images, in this paper, may appear as a continuous density in some regions owing to the scale of the figures, but this artefact disappears upon greater magnification. (Online version in colour.)

Figure 4. Images of wild-type development. (Reproduced with permission from Frohnho¨ fer et al. [4] and licensed under CC-BY 3.0 (http://creativecommons.org/licenses/by/3.0; published by The Company of Biologists Ltd.) PB, PR, SP and J+ refer to the developmental stages shown in table 2. Scale bar, 250 μm. (Online version in colour.)
position and stripe width, and, in particular, that iridophores promote and instruct xanthophore placement. The results [4,6,10,11] indicate that iridophores spread dorsally and ventrally from the horizontal myoseptum and gather at the future locations of the next two interstripes, XIV and X1D. In this way, a repeated pattern of iridophore localization, attraction of xanthophores and adjacent gathering of melanophores could account for the full development of the adult pattern. Our goal is to study this proposed mechanism and determine how the timing of iridophore localization influences stripe/interstripe development.

As mentioned in §2, experimentalists often classify zebrafish development (figure 4) into stages using measurements of length rather than days, as size varies significantly between fish [34]. The relevant stages for our study are shown in table 2: standard length (SL) is a measurement from the snout to the point where its caudal fin begins [34]. Standardized standard length (SSL), in turn, denotes the standard length of a reference zebrafish and is used to approximate the length associated with developmental milestones despite individual variations [4,34]. We follow the convention of tracking SL measurements and label images according to the developmental stages in table 2; the electronic supplementary material contains a discussion of how our growth rates relate to these stages. Because zebrafish measure approximately 1 mm in height at three weeks when the metamorphic period begins, we begin with a 1 mm high by 2 mm long domain [10,15,29]. This field size enables us to track the full height of the zebrafish as it grows.

### 3.2.1. Sequential spreading and gathering of iridophores may promote directionality of additional stripes and interstripes

We initialize the fish skin domain with two narrow (one cell wide) strips of melanophores along its dorsal and ventral edges and two strips of melanophores surrounding a strip of xanthophores along the centre of the fish, as shown in figure 5a. Thus, we model an initial pattern at a point between the PB and PR stages; we further assume any larval melanophores left in the prospective yellow interstripe have died or migrated out and that adult melanophores are faintly visible in two stripes. We incorporate iridophores indirectly by initializing a strip of xanthophores as part of the initial condition; this narrow strip of xanthophores takes on the role of iridophores by attracting new xanthophores and promoting their differentiation. (Note that more cell types, specifically larval pigment cells and iridophores, are needed to represent the larval pattern, and, as a two-population model, our approach is somewhat limited in initial pattern choice. Our initial pattern should be thought of as capturing features of the early zebrafish that are important for instructing later stripe/interstripe development, rather than replicating the larval pattern or the PB and PR development stages.)

Our results show that, while our initial pattern (figure 5a) aligns the three base stripes and interstripes with wild-type width, it rarely leads to completion of the full pattern. Instead, later stripes/interstripes form in part and with disrupted alignment, as in figure 5b–f. In about a third of our simulations, a narrow bridge of xanthophores extends out of the central interstripe (figure 5f). Interestingly, Patterson & Parichy [26] observed that melanophores localize near iridophores and may even wrap around residual iridophores on fish skin subject to ablation of these cells; similarly, we find that melanophores wrap around partially formed yellow regions in our model as well (figure 5f). Furthermore, in the zebrafish mutations shady and rose, which feature fewer iridophores, the stripes break up into spots, with only 1D and 1V visible [4,6,8,10]. Thus, our simulations without the sequential spreading/aggregation of iridophores are qualitatively comparable to mutations and ablation experiments involving a reduction of iridophores.

While we can use our two-population model to test how the absence of iridophore spreading and aggregation negatively influences the pattern, it cannot be directly used to evaluate whether delayed localization of iridophores positively impacts directionality. Instead, we take an indirect route based on empirical observations [23,26] that iridophores promote and instruct the placement of xanthophores. In particular, we model this effect of delayed iridophore localization by introducing two narrow (one cell wide) strips of xanthophores at 20% and 80% of the current fish domain height at a delayed time. These xanthophores represent the result of iridophores spreading, accumulating there and then attracting xanthophores. Although this modelling simplification leaves out a potential cause of xanthophores appearing, namely aggregation of iridophores, it captures one possible result, and allows us to study the impact iridophores have on pattern formation without adding complexity to our model. For the remainder of this section, we will talk about the localization of iridophores (or appearance of iridophore alignment information) in interstripes XIV and X1D at different times: it is important to note, however, that though this symbolizes the dispersal and aggregation of iridophores behind the scenes, the only visible result in our model is the appearance of two narrow strips of xanthophores.

<table>
<thead>
<tr>
<th>stage</th>
<th>acronym</th>
<th>SSL from [34] (mm)</th>
<th>pattern milestone [34]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pelvic fin bud</td>
<td>PB</td>
<td>7.2</td>
<td>faint stripe of iridophores</td>
</tr>
<tr>
<td>pelvic fin ray</td>
<td>PR</td>
<td>8.6</td>
<td>1D and 1V are discernible</td>
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<tr>
<td>squamation onset posterior</td>
<td>SP</td>
<td>9.6</td>
<td>base stripes become more distinct</td>
</tr>
<tr>
<td>juvenile</td>
<td>J</td>
<td>11.0</td>
<td>base stripes are nearly complete</td>
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<tr>
<td>juvenile +</td>
<td>J+</td>
<td>13.0</td>
<td>2V forms</td>
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<tr>
<td>juvenile ++</td>
<td>J+++</td>
<td>16.0</td>
<td>2D develops</td>
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Table 2. Stages of development. Standard length (SL) is a measurement of the fish from its snout to the beginning of its caudal fin; standardized standard length (SSL) is an approximation of SL based on a representative zebrafish [34].
We consider three delayed timings for the localization of iridophores in interstripes X1V and X1D (visible in our model by the appearance of two strips of xanthophores): 4, 9 and 14 days after we begin our simulation. According to our growth rates, these time points correspond to SLs associated with the SP, J and J$^+$ developmental stages, respectively. We find that alignment improves with earlier localization of iridophores at X1V and X1D. A delay to the SP stage typically produces stripes of wild-type width and correct alignment, as in figure 5g–k, though 30% of patterns (out of 30 simulations) display bridges (figure 5m). At the J stage, quality decreases in every simulation, and 40% of the resulting patterns contain bridges (figure 5n). For iridophore localization at the J$^+$ stage, early undirected differentiation away from our initial pattern leads to patches of cells in the prospective locations of interstripes X2D, X2V and stripes 2D, 2V. The introduction of alignment information at the J$^+$ stage is not enough to smooth out these patches or reliably produce fully formed stripes of wild-type width (figure 5o).

We further tested how the impact of iridophores depends on timing by initializing our model with two additional strips of xanthophores as part of the initial condition rather than at a delayed time. Our results show that this disrupts the natural sequential formation of stripes and interstripes. Instead, the full set of stripes/interstripes develops at the same time and at an accelerated rate. While zebrafish

Figure 5. (a) Our initial pattern (initial condition) models features of the zebrafish pattern at a point between the PB and PR stages. (b–k) Simulated stripe pattern development from our larval pattern (figure 5a) with (b–f) no additional iridophore localization and (g–k) iridophore localization at stage SP: shown at the (b,g) PR, (c,h) SP, (d,i) J, (e,j) J$^+$ and (f,k) J$^{++}$ stages, which correspond to $t = 2, 4, 9, 13$ and 20 days after initialization in our simulation. (l–m) Bridges between stripes or interstripes occasionally emerge: pattern shown at stage J$^{++}$ for (l) no additional iridophore localization and (m) iridophore localization at stage SP. (n–p) Images at stage J$^{++}$: (n) additional iridophore information introduced at stage J, (o) at stage J$^+$ and (p) as part of the initial condition (between the PB and PR stages). PR, SP, J, J$^+$ and J$^{++}$ refer to the developmental stages [4,34] shown in table 2. (Online version in colour.)
stripes/interstripes tend to narrow as they spread outwards from the centre of the fish, the interstripes that develop in our simulations under no iridophore delay are equal in width if not wider away from the horizontal myoseptum, as shown in figure 5.

To evaluate the behaviour of our model, we calculated the average distance between neighbouring melanophores ($\Delta_{\text{mm}}$), between neighbouring xanthophores ($\Delta_{\text{xx}}$) and between stripes and interstripes ($\Delta_{\text{xm}}$). As shown in figure 6a, regardless of the timing of iridophore localization, the model outputs measurements of the average distance between neighbouring cells that match empirical results [16]. Furthermore, as indicated in figure 6b,c, we find that melanophores move $\approx$10–15 µm per day before joining complete stripes, in good agreement with the speed of 80–100 µm per week estimated by Takahashi et al. [16]. (For both the empirical estimates [16] and our measurements of melanophore migration, movement owing to growth has been accounted for separately, and the reported numbers represent change in position of melanophores due only to migration alone.)

While the average distance between xanthophores and melanophores at stripe/interstripe boundaries serves as a measure of the separation between stripes and interstripes, distributions of shortest xanthophore-to-melanophore distances across all xanthophores on the fish domain are related to interstripe width. As described in the caption of figure 7, these distributions are obtained by calculating the distance from each xanthophore to its nearest melanophore. For xanthophores at the boundary of the interstripe, the nearest melanophore will simply be a distance $\approx\Delta_{\text{xm}}$ away; for xanthophores in the
middle of an interstripe, however, the nearest melanophore will be half an interstripe width away. On average, for well-formed stripe patterns, cells are located not on the stripe boundary or in the middle of the stripe, but one-quarter of a stripe width away from the boundary. Thus, the average shortest distance from each xanthophore to a melanophore serves as a rough estimate of one-quarter of an interstripe width. Using this method to approximate the average width of interstripes across 30 simulations, we find that interstripes develop to be roughly 392 μm wide when iridophores are introduced at the SP stage (for details, see figure 7).

An alternative quantitative means for evaluating pattern quality is to track the coefficient of variation (CV) for melanophore nearest-neighbour distances [14,22]. The coefficient of variation, given by CV = 100 × standard deviation/mean, is calculated by determining the average distance from each melanophore to its nearest-neighbouring melanophore, and is a perceptive measure of pattern regularity [22]. Low CV values denote well-formed pattern elements (stripes or spots), whereas high CV values are related to irregular patterns. Parichy & Turner [22] found that the CV for wild-type zebrafish decays during development and takes on values roughly between 100 and 30 (by our approximation of the reported data [22]). It has also been shown [14] that the CV is correlated with xanthophore density, with high CV values related to decreased xanthophore numbers. As high CV values are related to irregular patterns, calculating the average shortest xanthophore-to-melanophore distance for a single simulation and subtracting Δm serves as a rough estimate of one-quarter of an interstripe width. Data points show these averages for 30 different simulations at stage J++, and the solid line denotes the mean across 30 simulations. In particular, each datum point is the result of one simulation and is related to the interstripe width generated by that simulation, whereas the solid line shows the average across 30 simulations. As shown, on average across the 30 simulations, the final width of interstripes is very roughly (180 μm − Δm) × 2 = 392 μm, where Δm = 82 μm is taken from [16]. (Note that these estimates will in general be lower estimates of interstripe width, but serve as a means of comparing simulations and extracting a measurement of pattern width.)

In conclusion, our results support the model [6,10,15] that spreading and localization of iridophores helps guide differentiation [23,26] and align stripes/interstripes sequentially, and agrees with empirical observations [23,26] that changing the timing of cell differentiation with respect to fish growth alters the resulting pattern. It has also been observed [23] that iridophores may have a role in bounding stripe width, and, in support of this empirical observation [23], we find that melanophores are more widely dispersed across the fish skin when additional iridophores are not introduced. Furthermore, our results suggest that some delay in the appearance of additional alignment information is important to preserve the sequential nature of stripe pattern formation. Nevertheless, too long a delay leads to patchy stripes with disrupted alignment. Frohnhöfer et al. [4] observed that iridophores accumulate in the future location of yellow interstripe XIV during the SP stage, as shown in figure 4. In good agreement with [4], our results indicate that introducing additional alignment information during the SP stage is significantly more successful than longer delays to the J or J+ stages.

3.3. Ablation

Yamaguchi et al. [17] used laser irradiation to ablate a large central region of pigment cells on young (≈ three weeks) zebrafish and then observed the regeneration process. Their
experiment led to stripes/interstripes with arbitrary direction but fairly normal width, as shown in figure 9a. They further studied this phenomenon by simulating a reaction–diffusion model [17] on a static domain and obtained similar results. We simulated ablation [17] under two conditions: developing fish (growing domain) and adult fish (no growth). This choice enables us to compare results with both the in vivo work of Yamaguchi et al. [17] and their corresponding reaction–diffusion simulations on a static domain. Our results have interesting repercussions for the effects of growth on stripe/interstripe width and on the necessary scale for long-range interactions, as discussed in §3.4.

3.3.1. Ablation destroys directionality on developing fish

We model ablation [17] under the condition of growth by initializing the domain at roughly 20 dpf (between PB and PR developmental stages) with our standard initial pattern with one difference: a large central 1 mm² square region is removed, as indicated in figure 9b. In good agreement with [17], we find that artificially disturbing the initial pattern results in regenerated stripes/interstripes with lost directionality but similar width (figure 9).

3.3.2. Ablation on adult fish selects narrower stripes and interstripes

We also studied ablation on adult fish skin domains under the setting of no growth. The initial condition we used is a set of six perfectly formed 10-cell-wide stripes and interstripes with a large central region removed, as in figure 10a. Our simulations show random differentiation followed by segregation of cells into stripes and interstripes. This trajectory is similar to those observed in our simulations with fish.

**Figure 8.** (a) Evolution of average coefficient of variation (CV) for melanophore nearest-neighbour distances; average is calculated across 30 simulations, and standard deviation is shown. CV decays in time, representing the development of better formed patterns, comparable with Parichy & Turner [22] (empirical measurements of the CV take on values roughly between 100 and 30, by our approximation of the reported data [22]). (b) Scatter plot shows melanophore nearest-neighbour CV values at stage \( J^+ + \) (for 30 simulations) as related to xanthophore density; in agreement with Parichy & Turner [14], we find that a lower CV is associated with higher xanthophore density. (Online version in colour.)
growth and in the in vivo experiments [17]. The most interesting area of comparison is stripe/interstripe width: as shown in figure 10b, four interstripes appear in the domain in place of the three interstripes initialized in figure 10a. To see this, it may be helpful to imagine drawing a line vertically across the domain in figure 10b; in the process, the line will intersect four yellow regions, though the simulation began with three interstripes. Thus, the width of the regenerated stripes/interstripes is roughly 75% of the width of the initial stripes/interstripes. This result was unexpected, and it suggests that the relationship between fish growth and stripe/interstripe width should be further explored. We turn to this exploration in §3.4.

3.4. Effects of domain growth
3.4.1. Fish growth plays a role in selecting stripe/interstripe width

As discussed in §3.3, while stripes and interstripes of natural width form when growth is included, the stripes/interstripes that form on static domains after ablation are approximately 25% narrower. To test the idea that growth increases stripe/interstripe width, we simulated stripe pattern formation from an empty initial condition (no cells, no initial pattern) on both static and growing domains. As shown in figure 11a,b, the stripes and interstripes selected under conditions of growth are significantly wider. From a biological perspective, this suggests that fish growth is an integral part of the pattern formation dynamic: in particular, if a zebrafish were to grow to adult size and only then develop stripes and interstripes, our model indicates that these stripes/interstripes would be narrower than those that appear on fish for which pattern formation and growth occur concurrently. While our model shows that growth widens stripes and interstripes, it does not explain how growth does so. Interestingly, Parichy et al. [34] found that slowing body growth (and altering temperature) in wild-type fish led to a decoupling of melanophore pattern state from developmental stage. Taken together, these results suggest that the interplay of growth and pattern development should be further explored to elucidate how growth impacts patterns.

3.4.2. Growing domains support shorter long-range interactions

Because long-range interactions seem to be largely responsible for selecting stripe/interstripe width and maintaining the width of stripes [6,7], we hypothesized that simulations on a static domain with longer-range interactions could lead to the same widths as simulations on a growing domain with the original length scales. As expected, we find that the stripe and interstripe widths selected with no growth increase as the scale of long-range interactions increases. Lengthening these interactions by 100 μm (an increase of roughly 30%) widened stripes and interstripes, though not enough to match the widths found on growing domains (figure 11c). An increase of 200 μm, however, generated interstripes on static domains of similar width to those that form with growth (figure 11d). As an alternative means of presenting these results, we chart average distributions of shortest xanthophore–melanophore distances in figure 11c (as discussed in the caption of figure 7, average distributions are related to interstripe width); the distribution obtained on a static domain with long-range length scales increased by 200 μm is close to that obtained with growth, whereas the distributions from static domains with shorter long-range length scales drop off more quickly, signifying narrower interstripes.

Intrigued by what this relationship between growth and stripe/interstripe width might mean for our long-range interaction scale, which was set using simulations on a static domain, we returned to studying development from our initial pattern, now with a reduced length scale. We find that decreasing the scale for long-range interactions by 50 μm (roughly 15%) produces slightly narrower stripes. In contrast, a decrease of 100 μm disrupts the pattern and enables long-range death signals to break up stripes. These long-range death signals, which may be regulated by pseudopodia [7], strongly control stripe width and enforce an interaction scale long enough for mid-stripe melanophores to be able to communicate with xanthophores.

From a mathematical perspective, we conclude that domain growth supports wider stripes/interstripes and, hence, shorter long-range interactions. Because our results indicate that a much larger interaction scale is needed on a static domain to obtain patterns similar to those on a growing domain, studying pattern formation on constant grids for problems involving growing organisms may not lead to

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**Figure 10.** Simulated ablation on adult fish with no growth. (a) Initial condition: square region of stripes and interstripes is ablated. (b) Resulting stripes and interstripes (70 days later) are narrower than those initialized. (Note that groups of xanthophores in the initial condition may appear together as large squares: this is due to their initial ordered arrangement and is an artefact of the image size that disappears upon further magnification.) (Online version in colour.)
accurate approximations of interaction distances, especially for problems that do not involve long-range death signals. Accounting for growth may bring the length scales that arise from mathematical studies closer to their biological counterparts.

3.5. Mutations

Zebrafish mutations are classified into two types: those that affect the pattern at the early development stage and those that lead to fish with disrupted adult stripes/interstripes despite initially normal pigment cell development [24]. We discuss both...

Figure 11. Pattern formation from an empty initial condition on (a) a growing domain; (b) a static domain; (c,d) a static domain with long-range interactions increased by 100 or 200 \( \mu \text{m} \), respectively. Growing domains produce wider stripes and interstripes. Images are from 25 days into simulation; Neumann boundary conditions are used. (e) Bar graph shows the average distribution of shortest xanthophore-to-melanophore distances across 30 simulations; to assemble these distributions, we calculated the distance from each xanthophore to its closest neighbouring melanophore, binned these distances in 30 \( \mu \text{m} \) wide boxes, and divided by the number of xanthophores for each simulation (at 35 days); we then averaged the results over 30 simulations. (Note that for simulations involving domain growth, growth was stopped at 25 days and distributions were calculated at 35 days, allowing 10 days for stripes/interstripes to relax and removing any artefact owing to stretching of the cell positions.) For further details on this method of evaluating interstripe width, see figure 7. As shown, bars drop off sharply without growth, but increasing long-range interactions by 200 \( \mu \text{m} \) on static domains widens the support of the distribution, bringing it closer to the results obtained on growing domains. (Online version in colour.)
these broad categories of zebrafish mutations, which we will refer to as early-stage and late-stage mutations, below.

3.5.1. Early-stage mutations: directionality is lost without a horizontal myoseptum

The choker mutation lacks a horizontal myoseptum, resulting in adult stripe patterns with normal width but arbitrary direction, so that locally parallel stripes/interstripes curve and branch off on the fish skin [4]. To model choker development, we omit the initial pattern along the horizontal myoseptum and instead initialize the fish skin domain with only two narrow (one cell wide) strips of melanophores at its dorsal and ventral edges. The resulting simulations agree with the patterns observed on choker fish, as shown in figure 12. We conclude that horizontal stripes and interstripes do not form in the absence of a horizontal myoseptum. This finding is in agreement with experimental observations [4] suggesting that the timely presence of iridophores along the horizontal myoseptum may orientate stripes and interstripes.

3.5.2. Late-stage mutations: disrupting heterotypic interactions alters stripe/interstripe width

Experimentalists have shown that many late stage mutations affect the interactions between melanophores and xanthophores [18,19]. In jaguar/obelix zebrafish, a mutation characterized by wider stripe patterns with ambiguous boundaries, fin melanophores seem to move around, instead of away from, fin xanthophores; fin xanthophores, in turn, exhibit reduced attraction towards fin melanophores in vitro [18]. As reported in [24], homozygous obelix zebrafish feature broad interstripes, and, as noted in [21], the heterozygous variation of this mutation displays fewer and wider stripes that lack strict separation from interstripe regions. The dali phenotype, which is similar in appearance to jaguar/obelix, is another example of a mutation displaying altered cell migration [25].

To better understand how changes in heterogeneous cell interactions are related to mutations, we simulated the evolution of fully formed adult stripes/interstripes initialized with natural width and correct cell-to-cell distances without growth; we then altered different aspects of migration and compared the results with simulations done using the original wild-type parameters (figure 13b). Setting $R_{mx} = 0$ (see equation (2.1)) corresponds to removing the run part of chase–run movement; this alteration led to patterns featuring slightly widened stripes with less regular boundaries, as in figure 13c. Disrupting the full chase–run movement by setting $A_{mx} = R_{mx} = 0$ gave similar results (figure 13d). In contrast, removing all heterogeneous migration forces resulted in wide interstripes with reduced xanthophore–melanophore distances like those in homozygous obelix zebrafish (figure 13e). Setting $R_{mx} = 0$ alone was also enough to generate wider interstripes, shown in figure 13f. As discussed in the caption of figure 7, distributions of shortest xanthophore–melanophore distances are a quantitative means of describing interstripe width; as an alternative measure of how altered migration parameters impact stripe/interstripe width, we chart these distributions and their averages in figure 13g,h.

Through several transplantation experiments, Maderspacher & Nüsslein-Volhard [21] found that wild-type melanophores can form wild-type stripe patterns with obelix xanthophores, but obelix melanophores and wild-type xanthophores interact to form obelix-like patterns. These data [21] indicate that it is the genotype of melanophores that drives the obelix pattern. Our model generates wide interstripes under two different parameter tests and wide stripes (associated with narrower interstripes) under two parameter alterations as well. In particular, setting $R_{mx} = 0$ could be thought of as a weaker mutation than removing all heterogeneous interactions, though both parameter changes reduced repulsion between melanophores and xanthophores and widened interstripes. Likewise, disrupting only the run movement of melanophores had a similar impact on stripe/interstripe width as did the stronger alteration that removed the full melanophore-run and xanthophore-chase movement, lending support to the empirical observation in [21] that a mutation in melanophores alone is enough to generate an obelix-like pattern.

In conclusion, our results support experimental observations that changes in stripe pattern width are directly related to changes in heterogeneous migration interactions. We propose that the widening of interstripes and narrowing of stripes in the homozygous obelix mutation may be a product of the reduction in distance between melanophores and xanthophores: when boundaries are ambiguous and cells no longer repel (or repel less strongly in the case when $R_{mx}$ alone is zero), short-range competition takes over, leading to the death of melanophores owing to invading xanthophores.
3.6. Reduced model

3.6.1. Different roles: cell differentiation selects pattern width and migration maintains stripe/interstripe boundaries

One advantage of having a well-fitted model is that we can systematically test which mechanisms of cell interaction are most important at different times during stripe pattern development. It has been suggested by Mahalwar et al. [15] that differentiation, rather than migration, drives stripe pattern formation. According to this viewpoint, cells appear in the appropriate locations and reorganize only locally to smooth out stripe/interstripe boundaries. In addition, Singh et al. [10] put forth that net repulsion of melanophores and xanthophores may maintain stripe/interstripe boundaries. We test these hypotheses by simulating our model under conditions of no migration or migration alone.

We first study how stripe pattern formation is affected when cell migration is removed. For simulations of our reduced model with no movement, we consider the evolution of an initially empty domain with fish growth. Because no starting pattern

![Figure 13](http://rsif.royalsocietypublishing.org/)

**Figure 13.** (a) Initial condition used for (b–f) is a set of fully formed adult stripes and interstripes of width 10 cells with the experimentally observed cell-to-cell distances [16]. Images are shown at 100 days after the start of the simulation. (b) Wild-type. (c) Run component of chase–run movement removed: \( R^\text{run} = 0 \). (d) Full chase–run movement removed: \( R^\text{run} = A^\text{run} = 0 \). (e) All heterogeneous migration interactions disrupted: \( R^\text{het} = A^\text{het} = R^\text{run} = 0 \). (f) Only repulsion of xanthophores from melanophores disrupted: \( R^\text{xan} = 0 \). (g) Average distribution of shortest xanthophore-to-melanophore distances across 20 simulations; as shown, altering heterogeneous migration interactions impacts both interstripe width and stripe/interstripe separation. Distributions were assembled by calculating the distance from each xanthophore to its closest melanophore (at 100 days), binning these distances in 30 μm wide boxes, and dividing by the number of xanthophores for each simulation; we then averaged these results over 20 simulations. (h) Scatter plot shows the average xanthophore-to-melanophore distances for each of 20 different simulations and the solid line charts the mean across all simulations. For well-formed stripe patterns, calculating the average xanthophore-to-melanophore distance and subtracting the distance between stripes and interstripes serves as a rough estimate of one-quarter of an interstripe width. For further details on this method of evaluating interstripe width, see figure 7. (Online version in colour.)
is initialized, the resulting stripes and interstripes form with arbitrary directionality. Interestingly, we find that similar stripe pattern widths are selected under the reduced and full model, as indicated in figure 14a, but cell-to-cell distances vary dramatically. This result is in agreement with the minimal model [28]: Bullara & De Decker [28] found that birth and death rules leading to an overall short-range activation and long-range inhibition dynamic could produce stripes and interstripes of wild-type width, a mechanism they call differential growth. We further investigate the reduced model by simulating a random initial distribution of cells interacting on a static domain through movement alone. Shown in figure 14b, the resulting patterns consist of small aggregates of xanthophores and melanophores. The pattern width selected is narrow, as the cells only migrate enough to gather into homogeneous conglomerates, but not far enough to reach natural stripe and interstripe widths. The average distances between pigment cells, however, are upheld.

We conclude, in agreement with Bullara & De Decker [28], that differentiation and death may be responsible for selecting stripe pattern width; in addition, our results indicate that migration supports cell-to-cell distances and ensures that short-range competition of melanophores and xanthophores does not degrade stripe/interstripe boundaries. As discussed in §3.5, we found that altering the net repulsion between melanophores and xanthophores disrupts stripe pattern width. Thus, while differentiation specifies stripe pattern width, migration may maintain it. These observations are instructive when compared with the results of reaction–diffusion models. As mentioned by Mahalwar et al. [15], and shown for an ablation simulation by Yamaguchi et al. [17], Turing-type models can achieve stripe pattern formation from a random initial distribution of melanophores and xanthophores that separate into stripes and interstripes by lengthy movements. In contrast, simulations (figure 14b) of our model indicate that movement alone cannot transform a random initial condition into stripes and interstripes of natural width, at least not on a biological time scale.

4. Discussion

We have presented a two-population model for the development of stripe patterns in zebrafish. Melanophores and xanthophores are modelled as independent agents interacting according to differential equations for migration and discrete-time rules for differentiation and death. We account for both short-range cell communication, potentially regulated by dendrites, and long-range interactions, which may be governed by pseudopodia extensions [7].

We argue that fish growth and long-range interactions help select the width of zebrafish stripes and interstripes. The horizontal myoseptum and delayed localization of iridophores promotes stripe pattern alignment. At the same time, growth and development create a set-up in which stripes and interstripes can gradually appear, directed and informed by the existing pattern. Long-range interactions, the larval pattern and iridophores work together to guide cell differentiation. Growth widens stripes/interstripes and thus supports a shorter, more biological scale for long-range interactions. Lastly, net repulsion of melanophores and xanthophores ensures that clear boundaries are maintained between stripes and interstripes. These clear boundaries prevent short-range competition from upsetting stripe pattern width.

As more biological results emerge about zebrafish, our approach towards xanthophores is one of the main places our model could be improved. Because less is known about these cells, which are hard to observe on zebrafish skin [34], we model their differentiation as similar to melanophore differentiation. The ablation experiments of Nakamasu et al. [5] have demonstrated, however, that melanophores and xanthophores react very differently to long-range signals, both for differentiation and for survival. In addition, our initial pattern could be altered to better match the biology and adjusted to simulate stripe pattern development from an earlier age than the PB stage.

Regarding future work, one avenue a modelling perspective naturally lends itself to studying is the impact of domain growth on pattern formation, and many interesting questions can be raised in this area. In particular, Parichy & Turner [22] have shown that slowing body growth in the puma mutation can enable fish to recover wild-type stripes and interstripes. Furthermore, it is known that zebrafish stripes/interstripes do not form perfectly parallel to the horizontal myoseptum, but are actually tilted a few degrees from centre [34], though the reason remains hidden. In the future, we plan to explore these questions and better understand the interplay of growth and stripe pattern development on the zebrafish skin.
In this study, we focused on late-stage mutations such as jaguar/obelix and dali that alter stripe pattern width, and we did not discuss the spotted leopard phenotype. Promisingly for future work, however, we found that one set of altered migration parameters (see §3.1) disrupted the stability of wild-type stripes and interstripes in such a way as to lead to an undulating stripe pattern with clear boundaries. This pattern, shown in figure 3b, is akin to the undulating stripes/interstripes found in weak forms of the leopard or luchs mutations [11,21], and we are especially interested in further exploring this possible connection and the full development of spotted phenotypes in future work.

With only two cell populations and no direct precursor cells, our model successfully reproduces a wide range of experimental data. Nevertheless, many other types of pigment cells appear on zebrafish skin: sparse xanthophores in stripes, L-iridophores, blue S-iridophores and dense S-iridophores [4,6,11,15]. Because these cells are segregated into either stripes or interstripes, we were able to combine their known effects into two populations with good results. In the future, we plan to introduce iridophores into our model directly and investigate how their effectively directional diffusion influences stripe pattern alignment. With a three-population model, we will be able to more closely investigate mutations [4,6,11] in which one or two types of pigment cells misdevelop. Lastly, it would be interesting to see whether the presence of sparse xanthophores at low density in stripes can reduce the scale of long-range interactions further.

Authors’ contributions. A.V. and B.S. constructed the model, designed simulations and analysed results. A.V. carried out simulations and drafted the manuscript. Both authors gave final approval for publication.

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