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Dynamical responses of oscillating yeast cells suspensions to periodic forcing

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ABSTRACT

Fermenting *S. cerevisiae* cells suspensions undergo synchronized metabolic oscillations whose amplitude and frequency depends on the population density. The asymptotic population-level dynamics is qualitatively and quantitatively accounted for by mathematical models describing the intracellular glycolytic oscillations and the coupling through the external medium. Such dynamical systems often display low-dimensional dynamics for the parameters that quantitatively fit the experimental observations. However, the high dimensionality of the chemical space and the possible existence of intermingled time scales can potentially support more complex dynamics, in particular when the system is perturbed by external forcings. Here, we explore experimentally and by means of numerical models the response of cellular populations to different kinds of periodic forcing: resonant forcing of diluted, nonoscillating populations and strongly nonresonant forcing of populations in oscillating regimes with different dynamical features. We show that, in all cases, low-dimensional semi-quantitative models reproduce the observed dynamics. Both a nonlinear analysis of the experimental time series and the models indicate that complex-looking time series correspond to quasiperiodic and not to chaotic regimes. The fact that low-dimensional dynamical systems are able to reproduce the response of biological populations in different regimes of external periodic forcing supports the use of theoretical models for inquiring the dynamical behaviour of collectively oscillating cells.

Keywords: glycolytic oscillations, forced oscillations, quasiperiodicity in biological populations, complex cellular dynamics, cell synchronization, dynamical quorum sensing.

1 INTRODUCTION

Since the first discovery, 50 years ago, of coordinated oscillations in suspensions of yeast cells [1], many other examples of oscillating populations have been evidenced. Oscillations that appear at the population level typically involve the cell metabolism [2, 3, 4]: they stem from regulatory feedbacks in the metabolic pathway and are efficiently coupled by passive diffusion of some metabolites. In some cases, genetic and metabolic changes co-occur, as in the case of ultradian oscillations in

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yeast [4]. Collective oscillations in gene expression can also be observed when cell-to-cell coupling is ensured by membrane-crossing effectors, such as quorum-sensing molecules, and has indeed been observed in bacteria endowed with engineered oscillating genetic circuits [5].

The use of bioreactors [3, 4, 6], and later of microfluidic devices [5], allowed to keep the environmental conditions so stable that a steady dynamical behaviour could be quantitatively characterized. These methodological advances have paved the way to quantitative modelling of such biological populations, and in particular for oscillations in glycolysis and in genetically engineering circuits [3, 7, 8, 5]. Quantitative approaches have complemented qualitative modelling approaches [2, 9] by elucidating how given dynamical features are implemented in a particular biological system.

It is however remarkable that, in spite of the increasing dimensionality of the state and parameter spaces, models including more and more biological details seldom display qualitatively different or more complex regimes with respect to simple qualitative models. Indeed, biological systems as complex as populations of oscillating cells seem to possess dynamical regimes that are low-dimensional, and most of the time a time-scale separation effectively decouples the asymptotic dynamics from other faster dynamical modes.

This may just be a consequence of the fact that quantitative studies are better performed once the system's attractor has been reached, and thus one tends to set up experiments so that the transient dynamic is exhausted. However, when the system is perturbed, one may expect such low-dimensional behaviour to break down, and more complex dynamical behaviour to occur, involving the excitation of fast oscillatory modes.

If the system is far enough from a bifurcation point, the simplicity of the asymptotic dynamics should be preserved under weak perturbations. Glycolytic oscillations in intact yeast cells, for instance, display a perturbation response that could be coherently explained on the basis of a two-dimensional dynamical system, by a correct projection of the space of chemical species onto the plane of oscillations [3, 7].

On the other hand, it has been shown that the glycolytic pathway can support complex dynamical regimes. This was demonstrated experimentally in continuously stirred tank reactors both under continuous [10] and varying [11, 12, 13] flow of the substrate, as well as in mathematical models [12, 14, 15, 16]. Similar results may as well hold for yeast cells suspensions. In particular, even when the asymptotic dynamics is efficiently accounted for by a simple 2-D oscillator, a sufficiently strong external forcing might drive the system towards complex, and possibly chaotic, dynamical regimes.

Here, we study the collective dynamics of *S. cerevisiae* cells suspensions under periodic perturbations of a metabolite inflow in a Continuously Stirred Tank Reactor (CSTR). This setting allows us to test perturbations of different expected impact on the population dynamics, in that we can control both the amplitude and the frequency of the forcing. The experimental observations are compared to the predictions of models of different dimensionality, reflecting different degrees of detail of the mathematical description. We show that the observed dynamics of the forced populations remains relatively simple even in the most disruptive conditions (ratio between forcing and internal frequency close to the golden ratio). The population behaviour can be well accounted for by low-dimensional semi-quantitative models already developed for this biological system, that, at least for parameter values consistent with the observed autonomous dynamics, do not display regimes more complicated than quasiperiodicity.

Section 2 describes the methods of the experimental observations on yeast cell suspensions and the mathematical models of different dimensionality used in the simulations.

Section 3 presents the experimental results in the case where the forcing frequency is close to a 1:1 resonance with the free oscillators. We show there that the observations obey a scaling law that can be derived for a simple 2-D oscillator with resonant periodic forcing, and holds also for more realistic mathematical models, although with different scaling parameters.

Section 4 displays the results of forcing with a frequency far from strong resonance, resulting in complex time series, for two different densities of the cell suspension. By analysing the dynamics with different methods (attractor reconstruction, power spectrum) we found no evidence of chaos in the experimentally observed dynamics. The same conclusion is supported by the analysis of semi-quantitative models matching the observations, and confirming that extremely complex-looking time series can be quasiperiodic rather than chaotic.

We conclude in Section 5 that, contrary to the case of cellular extracts, which can be easily led to chaotic behaviour, populations of intact cells seem to be buffered against highly unpredictable dynamics, at least at the operating point where the experiments have been performed. As a consequence, low-dimensional mathematical models can efficiently describe the effective dynamics of the cellular population.

2 MATERIALS AND METHODS

2.1 Experimental materials and methods

S. cerevisiae cells suspensions have been prepared following the protocol described in [3, 8] and references therein: cells are starved in order to set a uniform initial condition throughout the population. Then, glucose is added at a concentration sufficiently high for glucose transporters to be saturated. At the same time, cyanide keeps the cells in the fermentation phase in spite of the presence of oxygen in the reactor, which is needed in order to decouple the glycolytic pathway from the respiratory metabolism. Cell suspensions are flown in a CSTR and the natural fluorescence of NADH is recorded. Similarly to what described in [8], we have added to the CSTR a periodic inflow of Acetaldehyde (Aca), whose period and amplitude can be modulated by the experimenter. In two different sets of experiments we have tested forcing amplitudes close or very different from the internal frequency of the natural glycolytic oscillations [8].

In the experiments with resonant forcing described in Section 3, a suspension at low density (OD $=$ 9.1, see the Supplementary informations of [8] for its conversion in dry weight), where the population shows damped oscillations, was subjected to a periodic change in Aca inflow, generating mixed flow oscillations of amplitude comprised between 2 and 8μM. In terms of the measure of the flux in the CSTR, these amplitudes correspond to nominal values of the forcing amplitude A, as used in the models scaled by a factor 100, i.e., between 200 and 800. The period of the forcing, 39 seconds, is close to the period of the intracellular oscillations $2\times\pi/\omega_0 = 37$ sec and to the period of the damped oscillations of the population 41 sec. We call it resonant forcing because for not too weak coupling, such a forcing is able to entrain oscillations in the 1:1 synchronization mode, that is with the same period as that of the forcing.

In the experiments with strongly nonresonant forcing described in Section 4, two different population densities have been considered. The Low Density (LD) condition corresponds to an OD of 12.2, the High Density (HD) condition to an OD of 25.2. In both cases the population oscillates, but the amplitude of the autonomous oscillations is smaller for the LD condition, and the linear stability of the limit cycle weaker, since the system is closer to the bifurcation point (OD 9.8) where the prevalence of the extracellular volume quenches the oscillations [8]. The forcing is again applied by modulating the Aca flux, with period 63 seconds and amplitude $A = 600$ in the LD condition, and $A =$ 800 in the HD condition.

2.2 The mathematical models

Models of different detail and complexity have been proposed, that account qualitatively or quantitatively for the oscillations in the glycolytic pathway in yeast cell extracts or in intact cells (see [7] and references therein).

Here, we make use of two low-dimensional models that have been quantitatively parametrized for the unperturbed *S. cerevisiae* suspension at the CSTR operating point where the experiments have been carried out [8]. To these models, we have added a term accounting for the periodic forcing.

2D model

The lowest dimensionality model that quantitatively describes the dependence of the population dynamics upon cell concentration is 2-dimensional. It approximates a higher dimensional model that explicitly accounts for the species diffusing into the suspension medium, other than for the intracellularly oscillating ones. This model has been proposed in [8] and includes a density-dependent weighting of two terms, one describing the intracellular 'decoupled' dynamics, the second accounting for the CSTR in and out flows. Adding to those equations a forcing term, the system reads:

$$\begin{aligned}\frac{dz}{dt} &= \frac{\alpha c}{\alpha c+1}\left(\lambda_0 + i\omega_0 + g\,|z|^2\right) z \\ &\quad - \frac{\tau z}{\alpha c+1} + \frac{A\beta}{\alpha c+1}F(t).\end{aligned} \tag{1}$$

We briefly review here the characteristics of the model: for the details, we refer to [8]. The parameters $\lambda_0 = 0.015$ s^{-1} and $\omega_0 = 0.17$ s^{-1} characterize the linear stability of the (unstable) equilibrium and the frequency of the oscillations. The parameter $g = -3.8$ s^{-1} weights the nonlinear term, thus determining the stiffness of the limit cycle. The turnover time of the CSTR is quantified by $\tau = 0.16$ s^{-1}. Cell density is measured by α, which is the ratio between the volume occupied by the cells and the total volume of the suspension. The rescaling parameter $c =$ 850 derives from the fact that the 2D model is constrained into a plane, even though the communication with the other cells takes place through the medium in a direction that has a component, in the space of the chemical species involved in glycolysis, orthogonal to such a plane. One way to account for the geometrical relationship between the oscillation plane and the direction of the coupling effector (in this case Aca) is to multiply the terms relative to the exchange of such molecule by the density-dependent term $1/(\alpha c+1)$. This hence also multiplies the forcing term, since a

forcing by Aca is acting in the same direction as the coupling effector. The forcing amplitude A is experimentally measured in terms of the intensity of the influx in the CSTR. The real-valued function $F(t)$ reflects the wave form of the forcing. We consider that this is well approximated by a square wave centred in zero, since in the experiments switching on and off the Aca flux results in variations around the unperturbed steady state. The constant β is measuring the effect that a perturbation in Aca will have on the intracellular oscillator. If it is too small, the forcing will not be able to entrain the internal oscillator as soon as the internal and forcing frequency are not strictly identical. However, too large couplings may also disrupt the entrainment forcing the system to a steady state. We have chosen the values for β, the only parameter of the system that is not quantitatively determined by comparison with the experiments, between 1.5×10^{-6} (non-resonant forcing) and 5×10^{-5} (resonant forcing) depending on the operating point, that is slightly different for the two sets of experiments that are presented in the following sections.

4D model

A model describing explicitly the extracellular compartment and a coupling taking place along a direction not parallel to the oscillation plane comprises two additional dimensions. Such 4D model [8] reads:

$$\frac{d\mathbf{x}}{dt} = f(\mathbf{x}) - d_{Aca}(x_3 - X) \quad (2)$$

$$\frac{dX}{dt} = \alpha\, d_{Aca}(x_3 - X) + \beta\, A\, F(t), \quad (3)$$

where $\mathbf{x} = \{x_1, x_2, x_3\}$ is the intracellular state vector (differences from the unstable steady-state concentrations of three intracellular metabolites, or linear combinations of metabolites), whose third component represents the Aca concentration. The uncoupled intracellular dynamics is defined by the function $f(\mathbf{x})$ and is chosen such that a limit cycle (Hopf normal form) exists in a plane forming an angle $\theta = 87°$ with the x_3 axis. The parameters for the intracellular oscillator are the same as for the 2D model ($\lambda_0 = 0.015$ s^{-1}, $\omega_0 = 0.17$ s^{-1}, $g = -3.8$ s^{-1}). The stability of the mode orthogonal to the oscillation plane is quantified by the parameter $\lambda_{fast} = -500$ s^{-1}. The variable X corresponds to the difference in the extracellular Aca concentration from the steady state concentration. The transport coefficient for Aca is $d_{Aca} = 300$ s^{-1}, corresponding to a fast passive diffusion that tends to equalize the intracellular and extracellular concentration of Aca. All the aforementioned parameter values correspond to those published in [8], that have been obtained by fitting experimental data. The parameter β has the same value as in the 2D model.

The forcing term is added directly to the extracellular concentration equation, reflecting the experimental setup where the forcing is imposed through a periodic modulation of the Aca flux. Such a forcing is a square wave with the same parameters as in the 2D model.

In the 4D model, the dependence of the population dynamics on the cell density is explicitly included, so that the plane of oscillations can move in response to both the coupling and the perturbations imposed to the external medium.

The collective dynamics depends in both models on cell density. This is not the same for all the experiments presented in the following, and the value of the parameter α corresponding to each experimental measure is computed by converting the Optical Density (OD) through the relation $\alpha = 0.0014$ OD (see the Supplementary Informations of [8] for the relation of those two measures of cell density with the cellular dry weight).

3 RESULTS: RESONANT FORCING

Suspensions of *S. cerevisiae* cells are known to display collective oscillations due to the strong coupling among the individual cells [17]. However, when the density of the suspensions is reduced, the cells can loose the ability to oscillate due to what is known as 'dynamical quorum sensing' [8]. Both the amplitude and the frequency of the oscillations vary as a function of dilution, and are related to the corresponding features of the internal oscillator by simple density-dependent rescalings. The fact of being coupled through a medium, whose volume can be much larger than that of cells, causes oscillations to slow down as density lowers, at the same time as the amplitude decreases. If a forcing having the internal oscillator's frequency is applied to a suspension, it will be able to entrain the population-level oscillations as long as the dilution is not so high as to hamper the synchronization between the suspension and the forcing.

The first experiment we performed consisted in forcing the cell suspension with a frequency close to the internal one (that we will call hereafter 'resonant forcing', see Section 2.1). We can thus test whether the population behaves as predicted by simple models, or the nonlinearities involved in the glycolytic pathway cause a different dynamical response.

We prepared a diluted cell suspension that did not exhibit sustained oscillations due to the damping effect of the extracellular medium, and forced it with a square wave of Aca of

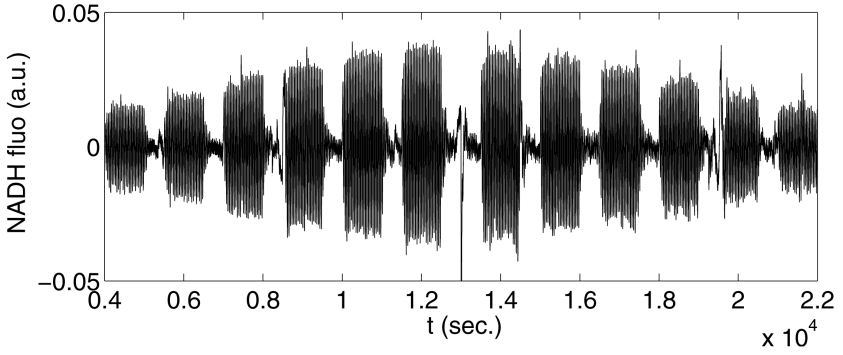

Figure 1 – Time series for NADH fluorescence of a non-oscillating yeast cell suspension subjected to a resonant forcing ($T = 39$ seconds) with different forcing amplitudes. For every forcing amplitude A, we compute the amplitude R of the oscillations induced by the forcing as the average amplitude after the transient is exhausted.

period 39 seconds and of different amplitudes (A between 200 and 800). Such a forcing entrains collective oscillations in the suspension, that after a short transient (2-3 periods) displayed regular oscillations whose amplitude depends on the intensity of the forcing (see Fig. 1).

Since the dynamics of yeast cells suspensions has been shown to be quantitatively described by a two-dimensional dynamical system that accounts for both the internal oscillatory dynamics and the presence of an external medium mediating cell-to-cell coupling, we model the autonomous dynamics as in [8].

Let us first consider the simplest possible model for a forced oscillator, and then progressively complexify it in order to reflect the experiment in greater and greater detail. We will hence start by analyzing the 2D model described in Section 2.2, but initially choose, instead of the square wave forcing, a sinusoidal forcing at the natural frequency. The forcing now takes the form of a complex exponential and acts on the complex variable that encompasses the two dimensions of the oscillator. This choice allows us to derive an analytical relationship between the amplitude of the forcing A and the amplitude $R = |z|$ of the induced oscillations.

In equations, such a simple model reads:

$$\begin{aligned}\frac{dz}{dt} &= \frac{\alpha\, c}{\alpha c + 1}\left(\lambda_0 + i\omega_0 + g\,|z|^2\right) z \\ &\quad - \frac{\tau\, z}{\alpha c + 1} + \frac{A\,\beta}{\alpha c + 1}\, e^{i\omega_0 t}\end{aligned} \tag{4}$$

Due to the simple formulation of this model, the amplitude of the oscillations entrained by the forcing can be analytically computed by writing Eq. (4) in polar coordinates $z = Re^{i\omega_0 t}$. By considering the equilibrium solution of the radial equation, we find that the amplitudes of the forcing and of the oscillations obey the following scaling:

$$\frac{A}{R} = \frac{\alpha\, c\, g}{\beta} R^2 - \frac{\alpha\, c}{\beta}\left(\lambda_0 - \frac{\tau}{\alpha c}\right), \tag{5}$$

which predicts that the ratio between forcing and oscillation amplitude linearly depends on the square of the oscillations amplitude.

Figure 2 shows that such a scaling is satisfied by the experimental data, as well as by the two different models (2D and 4D) that comprise a square wave forcing.

Although the scaling is a robust feature of the dynamics, the exact values of the slope and intercept of the linear relationship depend on the parameters and on the dimensionality of the system, and we were unable to find a simple rescaling connecting at least the 2D to the 4D models.

The fact that the dynamical properties of the response to resonant periodic perturbations of the system are the same as in the low-dimensional models implies that such approximations should capture the essential properties of the biological system even in less idealized environmental conditions than those that can be sustained in a laboratory setup. In particular, even perturbations that substantially displace the system from its equilibrium condition do not excite dynamical modes that, hidden when the asymptotic regime is attained, could generate chaotic dynamics.

In this section, we have studied the dynamics of a yeast cell suspension under a periodic resonant forcing. Deviations from the predictions of low-dimensional models could be expected for large values of the forcing amplitude. Nevertheless, the amplitude of the resulting oscillations depends on the forcing intensity as predicted by a simple model of a forced limit cycle, and the whole

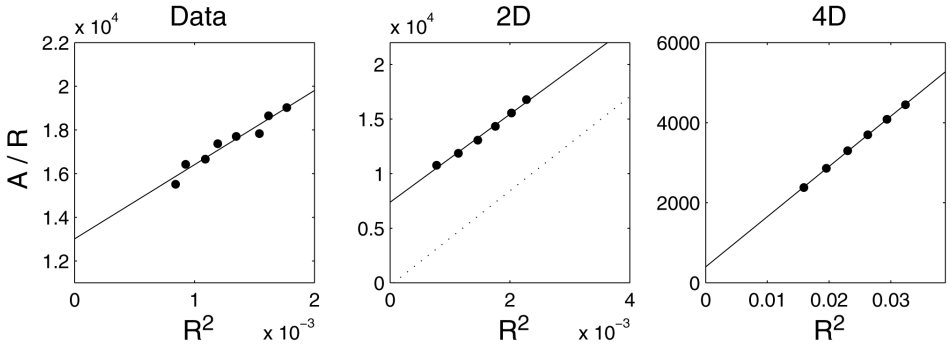

Figure 2 – The linear scaling of the ratio A/R with respect to the R^2, predicted in the text by analyzing a simple forced oscillator. a) Experimental observations, obtained by analyzing the time series in Figure 1 with the same method as in [8]; b) 2D model, the dotted line indicating the relationship identified by Eq. (5) for the model parameters values; c) 4D model. The models parameter values can be found in Section 2.

population behaves as one single macroscopic oscillator. In the next section, we will test the impact on the system of forcings of different frequencies.

4 RESULTS: STRONGLY NON-RESONANT FORCING

In general, the entrainment to a forcing with frequency close to the natural one (1:1 resonance) is very robust, since the synchronization will be maintained for a large set of parameters (the so-called Arnold's tongue) where the frequency difference can become relatively large as the intensity of the forcing increases [18]. The possibility of biological oscillators to exhibit resonances has been recently demonstrated in bacterial populations endowed with a synthetic genetic clock [19].

On the other hand, when the difference between forcing and natural frequency become too large, the 1:1 synchrony is lost, and the system can attain another entrained state whereby the ratio between those two frequencies is a rational number. This will occur for forcing frequencies close to the resonant one (in this general case, such that a multiple of the natural frequency exactly equals another multiple of the forcing frequency). However, the interval of frequencies whereby the entrainment can be maintained shrinks as the order of the resonance increases.

We wanted to test the response of the yeast cells oscillations to a strong perturbation, and therefore we chose a forcing frequency that was far from the strong resonances. The weakest resonance thus occurs for frequency ratios that, even if rational, are close to the golden mean $(1+\sqrt{5})/2$. We have thus chosen a forcing frequency of 63 seconds, such that the ratio to the natural frequency of the intracellular oscillations (about 39 seconds) was approximately the golden mean. The fact that frequencies in such a relation are the most difficult to synchronize has been recently suggested to be relevant for synchronization processes taking place in the brain [20].

We have performed the experiments for two different values of the population density such that the population displayed self-sustained oscillations, that is for sufficiently dense suspensions. The rationale behind this choice is that an oscillatory system should be differentially affected by perturbations depending on its transversal stability, that is if it is close or far away from a bifurcation point. We have therefore chosen two populations densities, one (OD 25.2) deep in the region where self-sustained oscillations occur, and the other (OD 12.2) close to the critical density where oscillations disappear through a Hopf bifurcation [8]. In the following, we will refer to the first case as High Density (HD) and to the second case as Low Density (LD).

Figure 3, upper plots, displays the two time series at LD and HD (left and right, respectively). In both cases, the dynamic is complex and may look chaotic.

If we simulate the 4D system with the appropriate forcing frequency and corresponding densities, we obtain a behaviour that displays no qualitative difference from the observed one (Fig. 3, lower plots). Instead, the 2D system with a square-wave forcing, in spite of maintaining some qualitative agreement with the experimental time series, displays much more regular oscillations (data not shown). In the following, therefore, we will only consider the 4D model.

Let us now make the comparison more precise by considering different representations of the orbit. If we project the attractor onto a plane by performing a Hilbert transform of the experimental time series (a method detailed in [8]), as shown in Figures 4a and b, we can see that the trajectory looks less regular in the LD than in the HD case, in spite of the fact that the amplitude

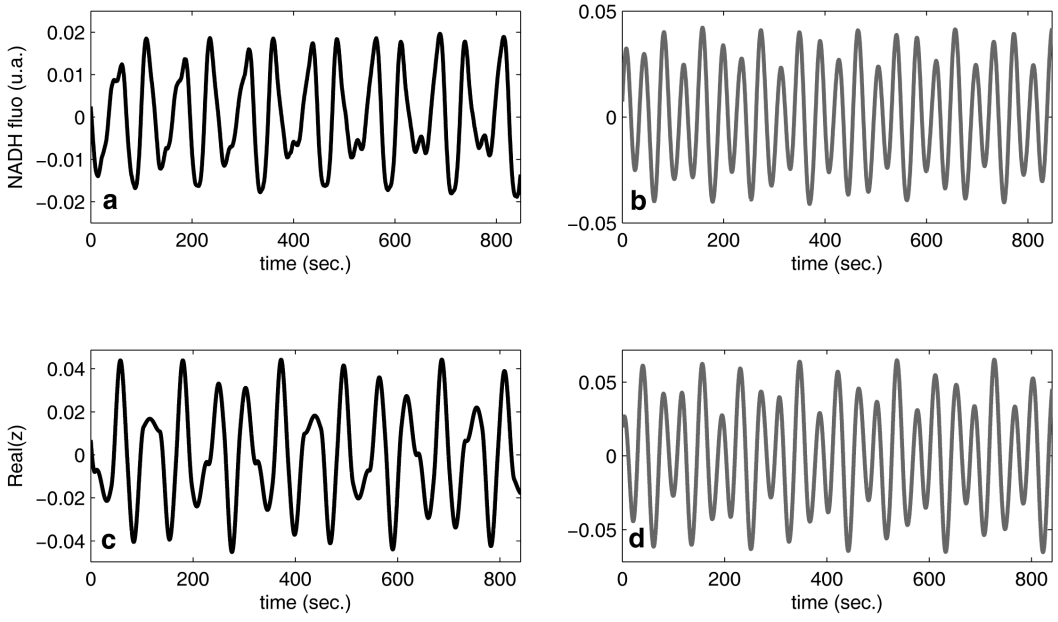

Figure 3 – Comparison of experimental and simulated time series for Low Density (black) and High Density (gray) suspensions and strongly nonresonant coupling. a) and b) experimental measures of NADH fluorescence. c) and d) one variable (the real part of z) in the 4D model, obtained by numerical simulations with parameters specified in Section 2 and $A = 600$ in LD, $A = 800$ in HD.

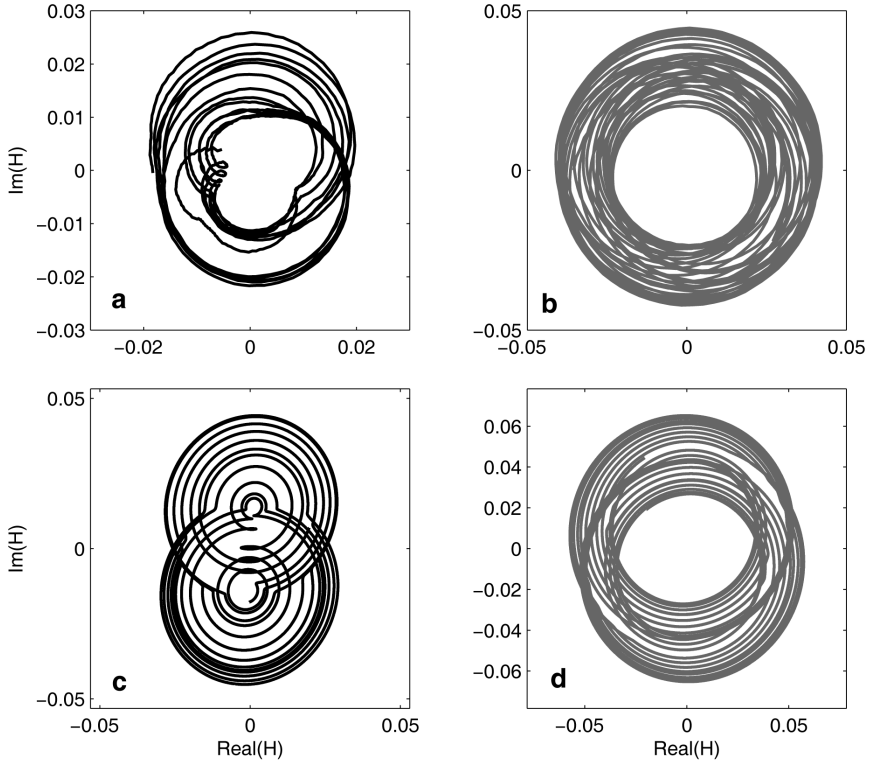

Figure 4 – Attractors reconstructed by Hilbert transform of the time series in Figure 3 (black, LD condition; grey, HD condition): a,b) experimental observations; c,d) numerical simulations of the 4D model. The reconstructed attractors look alike in the observations and in the model simulation. Moreover, in both cases they appear more complex at low cell densities than at high cell densities.

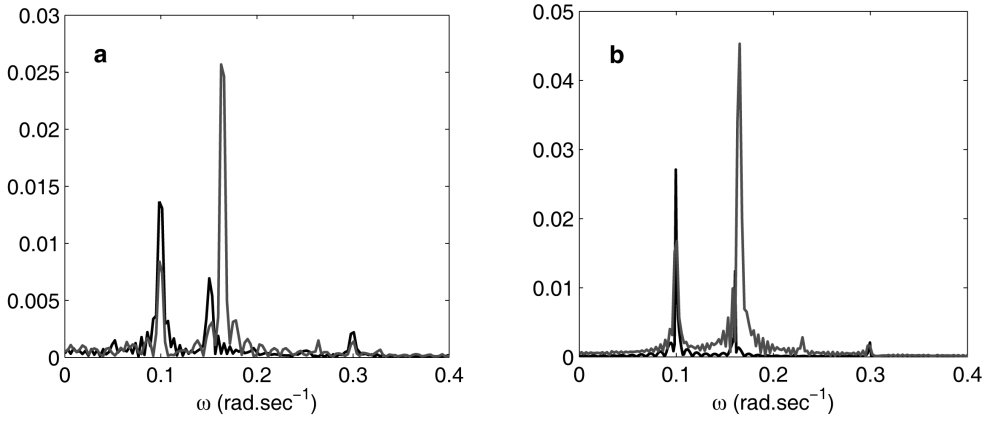

Figure 5 – Fourier spectra for the time series in Figure 3: experimental observations (a) and numerical simulations of the 4D model (b). The spectra relative to the low density (LD) condition are in black, those relative to the high density (HD) condition are in grey. Both the observations and the model show a displacement of the weight in the power spectrum towards the forcing frequency as the cell density is lowered and the linear stability of the limit cycle for the collective oscillations moves close to the neutral bifurcation condition. At the meantime, a peak appears at the third subharmonic of the forcing frequency. The fact that the peak corresponding to the internal frequency also displaces is a consequence of the 'dynamical quorum sensing' effect on the collective oscillations, which implies a slowing down of the oscillations as dilution increases.

of the forcing is larger in the latter. This is consistent with the fact that the LD attractor should be more fragile to external perturbations because it is, unperturbed, closer to a bifurcation point.

The numerical simulations (Figs. 4c and d) provide a similar result, although the attractor for the LD condition looks considerably more regular for the simulations than for the experimental data.

The difference between low and high density conditions is also confirmed by examining the Fourier spectra of the time series, that differ in the weight of the two components relative to the natural and the forcing frequencies. Whereas in the HD condition the principal oscillatory component is given by the intracellular oscillator, in the LD condition this is less stiff and the spectrum is concentrated onto the forcing frequency, as illustrated in Figures 5a and b.

Exactly the same variation in the relative weight of the two peaks is observed in the numerical simulations (Figs. 5c and d). The appearance, in the LD condition, of a third peak at the $\omega = 0.3 \approx 3\ \omega_F$ subharmonic of the forcing frequency $\omega_F = 0.1$ is also reproduced. This correspondence supports the use of the 4D system as a semi-quantitative model for the yeast population, where the effect of the perturbation onto the intracellular oscillator is mediated by a proportionality constant (β) that is chosen sufficiently weak not to quench the oscillations.

It is noticeable that both the experimental and the model time series, in spite of their complex outlook, are quasiperiodic, with a coexistence of natural and forcing frequency.

If we accept that the model represents the biological reality of the forcing experiment, we can use it to make the analysis of the dynamics more precise, which is possible with longer time series than those available in the experimental recordings. We computed the Lyapunov exponents for the asymptotic attractor of the forced 4D model for different values of the cell density ($\alpha \in [16 \times 10^{-3}, 20 \times 10^{-3}]$, corresponding to $OD \in [11.4, 14.3]$) and of the coupling intensity ($\beta \in [2 \times 10^{-6}, 12 \times 10^{-6}]$), and found no instance of the dominant exponent becoming positive.

This indicates that the biological system is, at least close to the operating point where the experiments are performed, sufficiently robust to perturbations to avoid becoming chaotic even under an extreme mismatch with the forcing frequency.

5 DISCUSSION

Populations of cellular oscillators in interaction are known to display collective synchronized dynamics, that is often reproducible in a qualitative or even quantitative way by models possessing low-dimensional attractors. The correspondence between models having simple asymptotic dynamics and experimental observations has been demonstrated for fermenting *S. cerevisiae* cell suspensions both in closed [21, 22] and in open [7, 23, 8] reactors.

In principle, the accuracy of such relatively simple models in describing the biological oscillations may rely strongly on the fact that one usually observes the asymptotic dynamics (in open reactors) or a quasi-steady state (in closed reactors). In these cases,

the fast oscillatory modes that may perturb the population behaviour have decayed prior to the time of observation, and only the dynamics onto the oscillations manifold is evident [24].

There are however a number of assumptions underlying the models formulation that are likely not to hold strictly in real cellular populations, most importantly the time scale separation between slow and fast oscillatory modes and the absence of cell-to-cell heterogeneity. Relaxing one or both of these hypotheses may lead to complex behaviour and may be worthwhile of study in further analyses.

The existence of complex, quasiperiodic and chaotic, dynamics has been demonstrated theoretically both at the level of the intracellular oscillations [15, 16] and at the population level, where it is due to differences in the natural frequency of globally coupled oscillators [25]. The idea that the intracellular dynamics might be inherently chaotic is also underlying the use of potentially chaotic dynamical systems as qualitative models for the intracellular oscillatory behaviour, as for instance in [26].

Complex dynamical behaviour is even more likely if the system undergoes external perturbations. Even though yeast cells suspensions have been shown to have a linear response to the addition of pulses of acetaldehyde [7, 24], compatible with a model with a clear-cut time scale separation between oscillatory modes, one might expect that deviations from the low-dimensional behaviour would appear when the perturbation is stronger or has a frequency that excites the glycolytic pathway with a frequency different from the natural one. The fact that a nonresonant periodic forcing by glucose was able to excite complex and seemingly chaotic behaviour in yeast extracts has been indeed demonstrated in [11, 12, 13].

In this paper, we have studied the response of yeast cells suspensions to periodic modulations of the extracellular concentration of acetaldehyde, the metabolite primarily responsible for cell-to-cell coupling [27, 23]. The population has been forced in two ways.

First, a forcing of different amplitude has been applied to a diluted suspension, such that the autonomous dynamics displayed no self-sustained oscillations. The forcing, at a frequency close to that of the intracellular oscillations, entrained rapidly the population into macroscopic oscillations, whose amplitude increases with the intensity of the forcing. We have shown that the amplitude of the entrained oscillations obeys a scaling law with respect to the amplitude of the forcing oscillations. Such a scaling has been analytically obtained for the simple case of a Hopf oscillator with complex resonant forcing, but goes on holding when increasingly realistic models are considered: the 2D model, comprising a planar oscillator with square wave forcing, and the 4D model, comprising a three dimensional intracellular chemical space and explicitly accounting for the extracellular medium.

Second, we have forced an oscillating suspension with a signal of frequency very different from the natural one. We have repeated the experiment at two different cellular densities, such that the population was more (Low Density) or less (High Density) close to the density where oscillations disappear through a Hopf bifurcation. In both cases, we observe complex behaviour, whereby the population is not entrained by the forcing, but displays instead quasiperiodic behaviour. The features of the forced population dynamics (time series, attractor shape, power spectrum) are well reproduced by the 4D model, while the 2D model has a less good qualitative resemblance to the experimental data.

This results confirm on the one side that models supporting low-dimensional dynamical regimes seem to provide an accurate description of the biological population's behaviour, at least at the operating points that we have considered. This is probably due to the fact that the time scale separation between the 'slow' modes involved in the collective oscillations and other fast modes, related for instance to other intracellular nonlinearities or to cell-to-cell heterogeneity, is not impaired by the forcing we applied. One reason for this might be that the presence of an external medium that buffers the intracellular dynamics reduces the range of possible dynamical regimes that the biological system can exhibit, and notably dampen expansive, chaotic, regimes.

On the other side, such semi-quantitative or qualitative models do not support regimes more complex than quasiperiodicity in the region of parameters where they have been shown to reproduce the unperturbed dynamics [8].

The question whether chaotic dynamics may be functional in certain biological populations, or rather cells may have been selected in order to keep oscillatory regimes as simple as possible, is still entirely open. Our results suggest that obtaining chaos in a population of biological oscillators is not straightforward. Further investigations aimed at evidencing complex dynamical features in autonomous or forced yeast cells suspensions may exploit semi-quantitative mathematical models to predict the conditions where such regimes are expected to occur.

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