A computational approach to studying ageing at the individual level

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The ageing process is actively regulated throughout an organism’s life, but studying the rate of ageing in individuals is difficult with conventional methods. Consequently, ageing studies typically make biological inference based on population mortality rates, which often do not accurately reflect the probabilities of death at the individual level. To study the relationship between individual and population mortality rates, we integrated in vivo switch experiments with in silico stochastic simulations to elucidate how carefully designed experiments allow key aspects of individual ageing to be deduced from group mortality measurements. As our case study, we used the recent report demonstrating that pheromones of the opposite sex decrease lifespan in Drosophila melanogaster by reversibly increasing population mortality rates. We showed that the population mortality reversal following pheromone removal was almost surely occurring in individuals, albeit more slowly than suggested by population measures. Furthermore, heterogeneity among individuals due to the inherent stochasticity of behavioural interactions skewed population mortality rates in middle-age away from the individual-level trajectories of which they are comprised. This article exemplifies how computational models function as important predictive tools for designing wet-laboratory experiments to use population mortality rates to understand how genetic and environmental manipulations affect ageing in the individual.

1. Introduction

Research over the last decade suggests that ageing is exceptionally malleable. Changes in mitochondrial function during the larval stages of the nematode worm Caenorhabditis elegans can regulate the health and longevity of the adult animal [1]. In the fruit fly Drosophila melanogaster, specific genetic and environmental factors, such as dietary restriction [2–4], insulin signalling [5] and mating status [6] rapidly and reversibly affect population mortality rates, often within hours or days. A thorough understanding of the dynamics of the normal ageing process, and how those dynamics respond to experimental manipulation, would provide insight into its underlying molecular mechanisms and help guide the development of anti-ageing interventions.

In addition to developing interventions, scientists and physicians interested in broadly treating age-related decline must also consider variability in the targets of their interventions. Ageing, unlike many diseases, will eventually affect all individuals, so the simplest answer is to provide everyone with such an intervention once it is developed. However, this is unlikely for many reasons, including the possibility that not everyone would benefit equally, if at all. In traditional ageing experiments, a drug that improved lifespan 15% in half the population and simultaneously reduced lifespan by 10% in the other half of the population would be difficult to distinguish from a drug that increases lifespan.
across the population by 5%. Thus, it is imperative for ageing researchers to determine whether their population measurements reflect heterogeneous or homogeneous responses to their interventions.

Unfortunately, individual patterns of ageing are difficult to measure directly. By far the most common descriptor of ageing, particularly in simple model systems, is age-specific mortality, which has been used to document rapid changes in ageing following interventions such as dietary restriction and temperature manipulation [24,7]. Because individual animals are either alive or dead, typical ageing experiments measure population mortality rates, which are estimates of the instantaneous risk of death calculated as a function of the fraction of individuals in a cohort that survive a given census interval. Individual ageing rates are often assumed to mirror the population mortality trajectory, which is only justified if all individuals in the population are identical not only genetically but in every facet, which is untenable even in the most tightly controlled ageing experiments. In most cases, however, individuals vary in their mortality characteristics. This heterogeneity, together with demographic selection (i.e. a change in cohort composition due to deaths of select individuals), make it difficult to infer individual dynamics from population measures [8].

In a broad sense, in this paper we develop computational models that explore how patterns of demographic ageing that are observed in population-level measures (as represented by age-specific mortality rates) may or may not accurately reflect the biological process of ageing at the individual level. Unfortunately, to answer this question with certainty we must understand in detail how the effects of age influence an individual’s probability of dying at each instant, which is (as described above) a near-impossible task in vivo. It is, however, straightforward to monitor in silico when the researcher defines ageing parameters. Thus, we designed a computational model that simulates the death rates of individual flies based on age and other environmental factors, including social interactions. In the narrow sense, by combining in silico and in vivo experiments, we ask whether transient increases in population mortality following pheromone exposure in Drosophila, as previously observed by Gendron et al. [9], are more likely reflective of a reversible process of ageing on the individual level or an emergent property of ageing of a highly heterogeneous population. Using a series of predictions from a stochastic, two-dimensional lattice-based simulation that models fly social interactions and the known effects of pheromones on mortality, we found that the reported effects of pheromones on ageing likely derive from both population heterogeneity and individually changing mortality rates. To the best of our knowledge, these results are the first to effectively infer individual ageing patterns primarily from population mortality rates, and they provide a template for future investigations of the dynamics of ageing.

2. Computational procedures

(a) Experimental background

Gendron et al. [9] reported that exposure of male Drosophila to female pheromones affected population mortality rates, and several of the results of that work are relevant to our formulation. First, male population mortality rates increased exponentially with age, and mortality rates were consistently higher in cohorts of male flies when they were exposed to female pheromones. Second, removal of female pheromones was followed by a gradual reduction in population mortality such that, in roughly two to three weeks, mortality in previously exposed and unexposed cohorts was similar. Third, fly pheromones are detected by contact, through taste receptors. Fourth, genetically modified flies that lacked the required taste modality were unaffected by pheromones. Accordingly, we designed a computational model that incorporates individual behaviours such as movement through the environment and social contact with other flies, as well as dynamics of individual mortality and how they are affected by pheromone exposure.

(b) Simulation environment

We model an experiment using a 200 × 200 two-dimensional triangular lattice (each vertex has six neighbouring vertices) with cyclic boundary conditions, which maintains the fly density observed in vials used for in vivo experiments while allowing for a similar sample size. Fly movement is modelled as a random walk, under the assumption that there is no movement bias. The triangular nature of the lattice, when compared to square lattices (each vertex has only four neighbouring vertices), provides a better approximation to continuous diffusion, allowing for improved dispersal of the flies [10]. This simulation was developed using C++ and can be downloaded freely at the link provided in the Data accessibility section.

In our simulations, three distinct types of flies inhabit the lattice. Feminized males (FMs) serve as pheromone donors. These animals are genetically male but produce female pheromones. They were used by Gendron et al. to expose male flies to female pheromones without the confounding effects of mating. Wild-type males (WMs) produce normal male pheromones and have normal perceptive capabilities. Sensory mutant males (SMs) produce normal male pheromones but lack the ability to detect pheromones in their environment. In our simulations, we use SM flies as a control, as they are completely unaffected by pheromone exposure. We are interested in investigating how individual and population levels of age-specific mortality are affected in WM and SM given differential exposure to FM.

(c) Time

During each time step, our experimental flies (i.e. WM and SM) age and then have a chance to die, interact with FM, and move around the lattice. Donor flies (i.e. FM) also move around the lattice but do not age or die, which reflects the supplementation of FMs that Gendron et al. used to maintain a consistent level of exposure in the environment.

(i) Survival

At the beginning of each time step, we increase the age of each experimental fly by one, and update its probability of death (P_{death}, which is defined below). A random number (r) between zero and one is drawn for each individual, and if \( r < P_{death} \), the fly dies and is removed from the lattice.

(ii) Interactions with feminized male and movement

After experimental individuals have aged, surviving flies are chosen at random to evaluate social interactions and to move.
If an experimental male is chosen, the number of FM in the six adjacent positions is determined, and that number is added to that individual’s level of pheromone exposure ($f$), which depending on genotype may influence probability of death ($P_{\text{death}}$). For each selected fly, a destination site is randomly chosen among the six adjacent locations. If the destination is vacant, the fly will move to that site. Otherwise, it will remain in its current site. The number of randomly chosen flies at each time step is equal to the number of experimental and donor flies alive. Therefore, while an individual fly may be selected multiple times, on average each fly will move/interact once per time step.

(d) Individual death rates

The Gompertz equation has been used for decades to study how intrinsic and extrinsic factors influence patterns of ageing. Examples include descriptions of historical trends in human population mortality rates [11] and investigations into how specific genetic manipulations impact ageing rates in nematode worms, fruit flies and mice [12]. The equation itself describes mortality rates ($\mu_t$) as exponentially increasing with age ($t$):

$$\mu_t = a e^{\beta t}, \quad (2.1)$$

where $\alpha$ represents an age-independent, or intrinsic, mortality rate and $\beta$ represents the rate at which mortality increases with age. With this formulation, in our discrete simulations the probability of death for a fly between age $t$ and $t + 1$ is $[7,13]$:

$$P_{\text{death}} = 1 - e^{-a/(\beta t+1)-\beta t}, \quad (2.2)$$

where $t$ is the number of time steps that have occurred beginning from 2 days post-eclosion (when pheromone exposure begins in our in vivo experiments). Five time steps occur in each of the simulated ‘days’ in the fly’s life, which allows for social interactions to occur on a finer timescale. The effects of pheromone exposure on mortality rates observed in Gendron et al. more closely resemble a shift in the intercept of log-mortality (determined by $\alpha$) than a change in slope (determined by $\beta$). Thus, we model the effects of pheromone exposure in terms of the age-independent component, such that

$$\alpha_t = e^{-7} (1 + ME_t) \quad (2.3)$$

and

$$ME_t = \sum_{n=1-D}^{D} (I_n \times Z_n), \quad (2.4)$$

where $I$ represents the number of encounters an experimental fly has with FM at a given time step, $D$ represents the duration of the effects of pheromone exposure and $Z_n$ represents the magnitude of effect each pheromone encounter will have on mortality. ME is therefore the mating expectation, i.e. the cumulative effect of pheromone perception on an individual fly. The parameter values $\beta = 0.04$ and $a = e^{-7}$ were estimated as those best fitting the empirically observed mortality rates in cohorts of male flies that were not exposed to female pheromones. By varying the size of the duration, $D$, pheromone effects can be made to be long-lasting or quickly lost, with subsequent effects on individual patterns of ageing that range from permanent ($D = 70$ days, the full simulation length) to rapidly reversible (e.g. $D \leq 7$ days). Differences in $Z_n$ reflect the impact of pheromone exposure; SM flies, for example, do not sense pheromones and therefore have $Z_n = 0$. For experiments where we simulate mortality rates following pheromone removal, we set $Z_n = 0$ for all ages thereafter.

(e) Initial conditions and population measures

Unless otherwise noted, we seeded simulations with 80 WM or SM flies and 400 FM flies, occupying 0.2% and 1% of the lattice points, respectively. These conditions are similar to those in pheromone exposure experiments, where the number of FM outnumbered experimental flies 5:1, and they maintain a similar density if a lattice location simulates 0.04 cm$^2$, or approximately one body length (0.2 cm) in all directions. We designed the simulations to investigate the effects of pheromone exposure over a time frame reflective of a normal fly lifespan of roughly 70 days. Five time steps per day allowed flies to explore and interact over a finer timescale, with each simulation step therefore corresponding to approximately 4.8 h. All population measures and test statistics were based on 100 replicates of each condition to obtain averages at each time step.

For each time step of the simulation, we determine the state (living or dead) for each fly, as well as the number of MEs accumulated. We calculate population mortality rates as the number of flies that died during a given time step divided by the number of flies alive at the beginning of the time step.

During any single simulation, individual flies may accumulate differences in ME based on their chance interactions with FM and the effect of each interaction, $Z$. These differences in ME will translate into heterogeneity in death rates among individuals in the population, and they will influence the extent to which population mortality rates reflect individual ageing. Because we have access to ME and $P_{\text{death}}$ for all simulated flies, we can directly calculate heterogeneity as the variance in ME across the live flies. When required, we greatly reduce heterogeneity by adding the same number of exposures (the average population value) to $I_n$ for each fly when they are selected to move.

3. Results

(a) Models of pheromone exposure demonstrate realistic increases in mortality rates

We first used our computational model to examine population mortality dynamics from cohorts of simulated male flies that were not affected by female pheromones (i.e. SMs, $Z_n = 0$). Not surprisingly given the assumptions in equations (2.1) and (2.2), mortality rates were Gompertzian, and statistical estimation of model parameters from the population-level data are generally close to the values used to determine $P_{\text{death}}$ for individuals (see the electronic supplementary material).

We therefore asked how exposure to female pheromones influenced population-level mortality rates by allowing flies to be affected by pheromone exposure ($Z_n > 0$). We found that when male flies were exposed to female pheromones throughout life and the effects of exposure were long-lasting ($D = 35$ days), population mortality rates were significantly increased compared to those unaffected by pheromones, with increasing values of $Z_n$ resulting in progressively greater increases in population mortality (figure 1a).
When \( Z_n > 0 \), population mortality rates deviate significantly from Gompertz dynamics, with mortality deceleration becoming apparent at advanced ages. This pattern of deceleration is a population-level phenomenon because the expected value of individual mortality rates increases exponentially throughout life. Population-level mortality estimates are expected to level off when individuals vary significantly in their mortality characteristics [8]. In our simulations, the source of this heterogeneity is variability in ME. Each time step, experimental flies on the lattice experience \( n \) encounters with FM. Each interaction augments ME, which leads to a distribution of mortality rates among same-age individuals in the population (electronic supplementary material, figure S1). To determine whether variability in ME is driving mortality deceleration, we repeated the simulations but removed this source of heterogeneity. Reducing heterogeneity in this manner resulted in a return to strict Gompertzian dynamics, with mortality rates continuing to increase at an exponential rate even at older ages (figure 1b).

(b) Heterogeneity in individual mortality characteristics produce population-level reversals

We next investigated the conditions for which, in our simulations, removal of the pheromone stimulus at a given age resulted in a reversal of mortality increases, as was observed in the published work [9]. We began by limiting pheromone exposure to the first 7 days of life (\( Z_n = 0 \) for \( n > 7 \)) while maintaining their long-lasting effects (\( D = 35 \) days). We found that population mortality rates in exposed cohorts remained high until age 30, after which they rapidly declined such that at age 42 they were indistinguishable from cohorts comprised of flies that had never been exposed to pheromones (figure 1c). This is not surprising considering that the effects of exposure at the individual level were reversible. They could not, for example, persist after 42 days of age (as the latest possible age of exposure is day 7 and MEs effects are maintained their long-lasting effects (\( D = 35 \) days). We therefore repeated the simulations (removing pheromone perception at day 7 and constraining \( D = 35 \) days) with heterogeneity removed. We found that heterogeneity contributed to an earlier and more abrupt mortality reversal (figure 1c). These results prompted us to ask whether heterogeneity alone is sufficient to cause population mortality rates to exhibit a mortality reversal after removal of the pheromone stimulus. For these simulations, we eliminated individual reversibility by making pheromone effects permanent (\( D = 70 \) days, which is the length of the simulation) and stopped pheromone exposure at day 7. We found that overall population mortality reverted to levels observed for unexposed cohorts (figure 1d).

Figure 1. Mortality dynamics can be explained by individual reversibility and/or population heterogeneity. (a) Increases in mortality rate from continuous pheromone exposure are proportional to the magnitude of pheromone effect, \( Z_n \). Exposure is throughout life, with long-lasting pheromone effects (\( D = 35 \) days, equation (2.4)). (b) Late-life mortality deceleration is driven by heterogeneity in ME. Removal of heterogeneity (light blue line) restores Gompertzian dynamics reflective of ageing at the individual level. Exposure is permanent with \( D = 35 \) days and \( Z_n = 10^{0.7} \). (c) Removal of pheromone exposure at day 7 leads to reversal of mortality rates to never-exposed control rates in both heterogeneous and homogeneous populations when the duration of pheromone effects is long (\( D = 35 \) days), using \( Z_n = 10^{2} \). (d) Removal of pheromone exposure at day 7 when pheromone effects are permanent (\( D = 70 \) days) leads to reversal of mortality rates in heterogeneous, but not homogeneous, populations, using \( Z_n = 10^{2} \). Shading in panels represent when pheromones were present.
despite individual death probabilities that do not decrease (electronic supplementary material, figure S2a). Without heterogeneity the reversal did not occur (figure 1d; electronic supplementary material, figure S2b).

(c) Switch experiments distinguish population from individual mortality dynamics

Our results highlight the potential discordance between patterns of ageing that are occurring in the individual and those observed at the population level. We showed that the observed reversal of pheromone effects on mortality rates can result either when effects are permanent but the population is highly heterogeneous, when effects are temporary and individual death probabilities are reversible, or some combination of both. To identify experimental designs that would allow us to distinguish these competing effects, we used the simulation structure to explore predicted mortality dynamics when the age of pheromone removal was manipulated.

Our simulations revealed that patterns of individual ageing may be inferred from population mortality when a set of wet-laboratory experiments are executed, each of which involves removing pheromone exposure at a different age. When pheromones were removed at day 7, for example, we found that individual reversibility alone (i.e. no heterogeneity) could be clearly differentiated from a pure heterogeneity model (i.e. \( D = 70 \) days) if the duration of pheromone effects was sufficiently short. If pheromone effects lasted 5 days (\( D = 5 \) days), then pheromone exposure early in life had no effect on population mortality rates. If, however, pheromone effects lasted longer (e.g. 35 days), then population mortality trajectories were similar to those observed from the pure heterogeneity model (i.e. effects are permanent; mortality rates were significantly increased for much of early life before reverting to unexposed control levels (figure 2a). On the other hand, when pheromones were removed later in life (at day 30), then the slower individual reversibility condition could be differentiated from a pure heterogeneity model (i.e. no reversal, \( D = 70 \) days). Whether pheromone effects were quickly reversed without heterogeneity (\( D = 5 \) days) or permanent with heterogeneity (\( D = 70 \) days), population mortality dropped soon after pheromone removal. However, if \( D = 35 \) days there was no significant reversibility in population mortality rates in the absence of heterogeneity (figure 2b). Together, these results indicate that an experiment in which pheromones are removed early in life (i.e. before flies begin to die) should differentiate rapid reversibility at the individual level from heterogeneity as the primary cause of population mortality reversal. Removal in middle-age (i.e. in the midst of the exponential rise in mortality rates) should distinguish slower individual reversibility from a pure heterogeneity model.

(d) In vivo experiments rule out rapid individual reversal of pheromone effects

We next applied the predictions from our model to a set of in vivo experiments, with removal of pheromones occurring at days 7, 28 and 35. Cohorts that were exposed to pheromones throughout life and those never-exposed served as positive and negative controls, respectively. We observed that male flies exposed to female pheromones only during the first week of life exhibited significantly higher post-exposure mortality rates than flies that were never-exposed to female pheromones (figure 3a). These results are not consistent with the predictions generated when individual effects are rapidly reversed. When pheromone exposure was terminated during middle-age (28 or 35 days post-eclosion) mortality rates began to separate from the lifelong exposed cohort roughly one week after pheromone removal; they trended downward towards (though did not join) the mortality curve associated with non-feminized exposure for the remainder of the animals’ lifespans (figure 3b,c). These observations are consistent with both slow reversibility at the individual level (on the order of weeks but not days) and a pure heterogeneity model.

One necessary condition of our simulations is that pheromone exposure will lead to increased heterogeneity in the probability of death of individuals. As this prediction cannot be directly tested in vivo, we instead examined a measure of health correlated to ageing: the variance of climbing ability (see the electronic supplementary material). We found that 28 days of pheromone exposure led to a large decrease in average climbing ability when compared with both never-exposed and 7-day-exposed cohorts (electronic supplementary material, figure S3a,b). Twenty-eight days of exposure was also sufficient to increase the variance proportional to the mean when compared with both never-exposed and 7-day-exposed cohorts (\( p < 0.0001 \) and \( p = 0.008 \), respectively, comparing % deviation by F-test). Exposure for 7 days (followed by 21 days of recovery) led to a slight, but not statistically significant, increase in variance compared with the never-exposed control (\( p = 0.16 \),
comparing % deviation by F-test). Thus, the condition demanded by the simulation appears to be fulfilled in vivo, and these experiments allowed for an additional, somewhat unexpected observation. It is notable that both exposed populations, (but not the never-exposed population) display significant bimodal tendencies as determined by Hartigan’s dip test ($p = 0.05$ for 28 day exposure, $p = 0.01$ for 7 day exposure, $p = 0.71$ for never-exposed). This suggests the development of distinct populations of flies that are affected versus unaffected by pheromone exposure. This is consistent with the distributions observed in our heterogeneous simulations (see the electronic supplementary material, figures S1 and S2).

(e) Pheromone effects on mortality reverse over the course of weeks

To better examine the extent to which heterogeneity and individual reversibility contribute to the observed changes in population mortality, we undertook a more detailed analysis of our new experimental data using simulations designed to reflect the observed dynamics. We first examined the values of duration ($D$) and magnitude ($Z_a$) of pheromone effects that best fit the in vivo mortality rates from males exposed to female pheromones throughout life. Simulations were executed using parameter values chosen from a grid comprised of five values of $D$ (ranging from 5 days to permanent) and 25 values of $Z_a (Z_a = 10^x \text{ where } x = (0, 0.1, 0.2, \ldots, 2.4))$. For each set of parameter values, we executed 100 simulations (with pheromone exposure throughout life) and obtained the average mortality rate at each age. We then calculated the McFadden pseudo $R^2$, also known as $\rho^2$ (figure 4a) and the mean average deviation (electronic supplementary material, figure S4a) between the simulated and the observed in vivo mortality rates for each set of parameters. We found that the best fits were obtained along a ridge (red/orange coloured in the plot) where a larger magnitude of pheromone effects was offset by a shorter duration (figure 4a).

We next chose the combination of parameter values along this ridge of best fit and executed simulations where the effects of pheromones were removed at day 7, 28 or 35. Results of these simulations were then compared with the observed data from the pheromone removal experiment, again using mean average deviation (electronic supplementary material, table S1) and $\rho^2$ (electronic supplementary material, table S2). We found that simulations in which $D = 35$ days and $Z_a \approx 5$ most accurately predicted the effects of pheromone removal at all three time points (figure 4d–f) as determined by both mean average deviation and $\rho^2$. These results suggest that pheromone effects on individual mortality patterns dissipate, albeit slowly.

To determine the extent to which heterogeneity is also influencing population mortality rates in these experiments, we repeated these simulations without heterogeneity. In the absence of heterogeneity, removal of pheromone exposure early in life led to population mortality rates that accurately reflect reversibility seen on an individual level (figure 5a). However, when pheromone exposure is terminated later in life, the absence of heterogeneity leads to divergence from the experimentally observed mortality dynamics (figure 5b,c). Indeed, removing heterogeneity significantly worsens the fit of the middle-age removal curves, but not the early removal curves (electronic supplementary material, table S3). Thus, we have demonstrated through a combination of in vivo and in silico experiments that pheromone exposure leads to highly heterogeneous, reversible changes in individual mortality rates, with individual reversibility driving changes in population-level mortality early in life while heterogeneity dominates population-level dynamics once flies reach middle-age.

4. Discussion

Understanding how genes and environment influence the dynamics of ageing in individuals is essential to many areas of ageing biology. Reliable biological indices of the rate of ageing, often called biomarkers of ageing in the scientific literature, have proved elusive, and population measures of age-specific mortality have served as the primary method to infer patterns of ageing at the individual level. Indeed, roughly 17 years ago it was observed that mortality rates decelerated in the oldest-old of several species, suggesting that the pace of ageing slows in individuals of advanced age [15]. However, it was recognized at the time that
Figure 4. *In silico* analysis of *in vivo* data reveals individual mortality rates reverse slowly. (a) McFadden’s pseudo $R^2$ statistic, also known as $\rho^2$, [14] colourmap as a function of the duration of pheromone effects $D$ (x-axis, in days) and magnitude of pheromone effects $Z_n$ (y-axis). Outcomes represent the difference between experimental fly mortality rates data with permanent pheromone exposure and simulation outcomes for combinations of $D$ and $Z_n$. Further simulations (see the electronic supplementary material, tables S1 and S2) examining best fit to the *in vivo* effects of pheromone removal determined that the optimal $D$ and $Z_n$ correspond to 35 days and $10^{3.7}$, respectively (shown in panels b–f). *In vivo* mortality data closely match simulated data with a pheromone effect duration of 35 days for no exposure (b), constant pheromone exposure, (c), 7 day removal (d), 28 day removal (e) and 35 day removal (f). See the electronic supplementary material, tables S1 and S2 for fitting statistics. Shading in panels represent when pheromones were present.

population heterogeneity could produce the observed deceleration even when individual mortality rates continued to rapidly increase. Subsequent efforts to disentangle these influences have been largely unsuccessful.

The complexity of experiments that investigate the interactions among ageing, behaviour and the environment will continue to expand. Exploration of how complex social hierarchies and dominance among individuals affect lifespan, for example, would significantly increase the potential number of factors that influence an individual’s ageing rate, which would make it difficult for experimentalists to generate specific individual-level hypotheses and corresponding predictions on population mortality. Here we demonstrate the value of a computational framework capable of generating testable predictions while incorporating the intrinsic heterogeneity resulting from varying behaviours and life-history experiences.

When we re-examined the conclusions from Gendron et al. [9], who used demographic analysis to assert that pheromone perception had short-term effects on individual ageing, we found that transient changes in population-level mortality rates can be caused by a combination of heterogeneity (i.e. variability in mortality parameters among individuals) and demographic selection (i.e. those individuals with the highest mortality tend to die first) even when effects of pheromone exposure on individual ageing were permanent. Our *in silico* results suggested that carefully timed removal of pheromone exposure in flies of multiple, specific ages would provide better insight into individual-level effects. Subsequent *in vivo* experiments revealed that exposure to female pheromones almost surely does transiently affect individual mortality rates in male *Drosophila*, but the effects are likely longer lasting than previously suggested. This distinction is important. Pheromone exposure alters other physiological phenotypes, such as fat stores and stress resistance, on a timescale of approximately 2 days. Thus, the longer lasting effects of pheromones on mortality suggest that distinct mechanisms drive these different phenotypic outcomes. These results reinforce the benefits of ‘switch experiments’, in which genetic and/or environmental manipulations known to affect lifespan are applied only at specific ages and for defined durations (e.g. [1,4,16]), while demonstrating how computational modelling provides the specification of design parameters that maximize their inferential power.

Beyond the laboratory setting, heterogeneity must be considered when analysing human interventions or treatments in middle-aged populations, as such interventions are, by their very nature, a type of switch experiment. With the recent increased focus on both personalized medicine and manipulations that can alter the ageing process during middle-age, it is becoming more important than ever to distinguish individual and population effects. Identification of individuals that are (or conversely, are not) affected by various interventions will not only improve our understanding of mechanisms of action, but also enhance the efficacy of treatment guidelines.
Figure 5. Heterogeneity drives late reversibility but not early reversibility. (a) When heterogeneity is removed from the simulation, the in vivo effects of early (day 7) pheromone removal are still replicated. However, when pheromone removal occurs on day 28 (b) or day 35 (c), removal of heterogeneity leads to deviation from the observed in vivo mortality rates (see the electronic supplementary material, table S3). Removal of heterogeneity does not prevent replication of never-exposed (d) or constantly exposed (e) mortality rates. Shading in panels represent when pheromones were present.

For example, studies have found differential effects of cardiovascular medications between various ethnicities [17]. It is reasonable to expect such heterogeneous effects may also occur between groups that are not so obviously differentiated, such as variation between patients' gut microbiomes, which may alter the effectiveness of dietary regimens in treating type-2 diabetes [18]. It is our hope that this article will become a roadmap for improving the design, methodology and analysis of experiments examining the effects of various treatments on an individual level.


Competing interests. We declare we have no competing interests.

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