Chapter 2: Effects of the Environment on the Dauer Decision

Introduction

Many organisms can develop into different forms, often displaying different phenotypes, known as phenotypic plasticity. Phenotypic plasticity can emerge from a genetic difference in a population or a as a response to different environmental conditions. Variance arising from genetic differences is known as polymorphism and when rare (under 5% penetrance) as a mutation. Phenotypic variance arising from environmental conditions is known as a reaction norm which is characterized by a range of phenotypes, and polyphenism when there are only 2 phenotypes (Gilbert, 2009). Animals measure their environment and make a decision of what morph they will develop into. Robust development is achieved when the genetic makeup of the organism can measure and integrate these environmental cues driving development into a morph best suited for the environmental conditions, thus increasing fecundity. The orchestration of this developmental decision is complex in multi-cellular organisms since the phenotypes of are mutually exclusive; morph A will have a distinct set of cell types and organs that morph B doesn't have, and vice versa. The understanding of genetic programs that guarantee developmental plasticity and mutual exclusivity at the same is poorly understood. For example, the desert locust *Schistocerca gregaria* can a green morph with small wings and a black-yellow morph with long wings. The former is formed under sparse population density and the latter in high population density (Tawfik et al., 1999a).

There are several recurring features in insect based polyphenisms. The inducing environment is different than the selective environment, i.e. the environment that the specific morph is selected for (Nijhout, 2003). The inducing environment in itself does

not constitute a threat, danger or unfavorable environmental condition, but it usually serves as a predictor for one. Therefore, there usually is a lag between the critical period in the inducing environment to the onset of the favored morph of the selective environment. The lag between the critical period and the morphological change is the time required to initiate the changes necessary for the favored morph, suggesting that an event or set of events during the critical period has a strong correlation with the morphology selected (Nijhout, 2003). The molecular mechanisms that correlate between the inducing period to the chosen morph in the selective period are unknown.

Another aspect, is that once committed to one morph, a change in environmental conditions will not change the fate of the morph. These mechanisms have been investigated extensively in single cell organisms such as *B. subtilis* and *Xenopus laevis. cerevisiea*. For example, commitment to sporulation in *B. subtilis* is controlled by the master regulator σ^F , a transcription factor which can transcribe multiple genes in both the forespore and mothercell (Dworkin and Losick, 2005). Amplification and positive feedback have been shown to drive commitment in the X*enopus* oocyte (Xiong and Ferrell, 2003). Molecular mechanisms that lock in the decision of a specific morph in a polyphenic switch are unknown.

The nematode *C.elegans* displays the trait of polyphenism since can develop into a sexually reproductive adult or into a larval state of suspended animation called the dauer (Cassada and Russell, 1975). *C. elegans* is an ideal organism for elucidating molecular mechanisms that shape and regulate polyphenisms since much of the molecular machinery that measures and integrates environmental conditions has been characterized. The decision is a function of environmental conditions: in a favorable

environment, comprised of sufficient food, low population density and moderate temperatures, will develop into a sexually reproductive adult. Conversely, when developing in unfavorable conditions composed of high population density, low food availability and high temperatures, the nematode will develop into the developmentally arrested dauer. The decision to develop into a dauer or adult happens in two stages during development. The first is during the first larval stage when the animal decides whether to develop into the pre-dauer L2d stage or into the L2 stage. The second decision occurs during the L2d stage when the animal decides to develop into a dauer to resume development as an L3 larva. The decision to become an L2, L3 or a dauer is irreversible; once the animals have made the decision, they commit to the specific fate and develop accordingly even if environmental conditions change.

The genetic pathways that convey measurements of food, population density and temperature have been dissected extensively. Genetic analysis of dauer formation has identified dauer constitutive (Daf-c) mutants, which form dauer larvae even in favorable conditions, and dauer defective (Daf-d) mutants, which fail to form dauer larvae even in unfavorable conditions (Albert and Riddle, 1988; Riddle, 1997). Molecular dissection of these loci reveals at least four signaling pathways. Components of neurosensory structure and guanylyl cyclase signaling are involved in sensing temperature, nutritional cues and dauer pheromone (Birnby et al., 2000), which are integrated by Insulin/Insulin-like growth factor (IIS) and TGFβ signaling. These peptide hormone pathways converge on a steroid hormone pathway, which metabolizes dietary cholesterol into several bile acid-like steroids, called the dafachronic acids (DA) (Gerisch and Antebi, 2004; Gerisch et al., 2007; Gerisch et al., 2001; Jia et al., 2002; Mak and Ruvkun, 2004; Motola et al.,

2006; Rottiers and Antebi, 2006). DAs serve as hormonal ligands for the nuclear hormone receptor transcription factor DAF-12, which regulates the life cycle fate decision (Gerisch et al., 2007; Hannich et al., 2009; Motola et al., 2006; Patel et al., 2008; Rottiers and Antebi, 2006). Notably, liganded DAF-12 promotes reproductive development (Gerisch and Antebi, 2004; Mak and Ruvkun, 2004; Motola et al., 2006), whereas unliganded DAF-12 together with the co-repressor DIN-1S direct the dauer fate (Gerisch et al., 2007; Ludewig et al., 2004; Motola et al., 2006). Thus DAF-12 serves as the switch between reproductive and dauer modes.

The dauer decision has been investigated in constant settings, i.e. food population density and temperature have been held constant. The environmental sensitive period has been characterized between the end of the first molt and the mid L2d (Golden and Riddle, 1984a). We therefore wanted to characterize the environment sensitive period in greater detail by dissecting the effects of strength, duration and fluctuation of the signal conveying population density. We also wanted understand the correlation between the environmental sensitive period (chapter 2) to the hormone sensitive period (chapter 3) and therefore developed a system to compare the two, which is described in detail in this chapter.

Results

Calibration of environmental conditions

Previous experiments determined that worms respond to food pheromone and temperature and integrate them into the dauer decision. These experiments were performed on agar plates that were prepared by mixing a crude pheromone extract with the agar, spotting bacteria as a food source on the dried plate and picking individual worms on to the plates. This technique results in a non-homogenous environment with low number of worms (Golden and Riddle, 1984a). We re-visited these experiments and modified them to liquid culture to increase scale, synchronicity of brood hatch, homogeneity and throughput and measured the frequency of dauer formation in response to dauer pheromone and food concentrations. We calibrated the synchronicity of developing broods in favorable (food with no pheromone) and unfavorable (pheromone and food) conditions as stage frequencies until they were tightly distributed and highly reproducible during early and late time periods of development (Fig – homogeneity). The synchronicity of broods during development in both condition types indicates that liquid culture conditions are homogenous.

In order to understand the points of commitment to dauer L2 and L3, I wanted to understand the values of food and pheromone that would allow growth at a constant food concentration yet amenable to changes in pheromone concentration without effecting dauer fraction and retaining a low standard deviation between technical replicates. To this end I grew worms in a series of food and pheromone concentrations

in 4 biological replicates of each pair of conditions scoring the fraction of dauers and adults in each combination (fig – heat map). I found that an optimal concentration of food is 7.5 mg/ml and pheromone at 6% (v/v) when grown at 20°C at the density of 1 worm per µl. This combination of food and pheromone allowed the entire brood to develop into adulthood when no pheromone was present, and 90% ± 2% dauer formation when 3% pheromone was present (Figure 2.1A).

Effects of pheromone on the dauer decision

Time of action

Previous experiments determined the periods in which worms respond to pheromone and commit to the pre-dauer (L2d) and to the dauer stage. Worms must be exposed to pheromone before the L1 molt in order to develop into the L2d stage and commit to the dauer fate a few hours before the mid L2d stage (Golden and Riddle, 1984a). We re-visited experiments by Golden and Riddle and measured dauer formation in response to dauer pheromone performed in liquid culture. We re-visited these experiments and modified them to liquid culture to increase scale, homogeneity and throughput and measured the frequency of dauer formation in response to dauer pheromone while grown in the presence of sufficient food for adult development. Dauer molt occurred at 48 hours post hatch (hph) and dauers were verified by measuring the percentage of survivors in 1% SDS, a standard assay for distinguishing dauers from L2 and L3 worms (Cassada and Russell, 1975) and found that all dauers in liquid culture became resistant to 1% SDS 10 hours after the molt (Figure 2.1B). Mean frequencies of life stages in favorable and unfavorable growth conditions were tightly distributed and

highly reproducible indicating the homogeneity of the liquid culture conditions (Figure 2.1C and D).

To identify the period in which worms become committed to development as L2 larvae instead of L2d larvae, consequently losing the ability to form dauers, we performed a "shift-to-unfavorable" experiment by adding a high concentration of pheromone to synchronously hatched worms. We reasoned that time points with a high standard deviation of dauer frequencies between biological replicates would indicate the transition from uncommitted to committed worms (For statistical analysis see Experimental Procedures and Table 1 in appendix to chapter). At progressive times, worms were shifted to favorable conditions by washing away pheromone. Cultures were scored for dauer formation 52 hours post hatch (hph), creating a precise time series of dauer frequencies. Worms stopped responding to pheromone at 16-18 hours post hatch (hph, 51.4% ± 37.3% dauer formation), the beginning of the L2 stage (Figure 2.2B), despite exposure to unfavorable conditions.

To precisely define the periods during which worms commit to dauer or to L3 reproductive fates we performed “shift-to-growth” experiments at two different developmental stages: L2d to dauer and L2d to L3 fates. Mean dauer to non-dauer frequencies were tightly distributed and highly reproducible during early and late time points. Analysis of four different biological samples of N2 wild type worms revealed that the point of L2d to dauer commitment occurred 33 hph (average of 29.1 ± 25.1% dauer formation) or 18 hours after the L1/L2d molt (Figure 2.1B). Shifting worms out of unfavorable conditions after this time does not affect their propensity to become dauers.

We next identified when L2d worms can commit to L3 (Figure 2.2 C). We reasoned that L2d worms exposed to longer time windows of favorable conditions have a higher propensity to develop into adults. We modified the shift-to-favorable experiment by growing synchronously hatched animals in unfavorable conditions to obtain L2d animals, followed by a shift to favorable conditions at 24 hph. Worms were then returned to unfavorable conditions after varying amounts of time (see Experimental Procedures). We find that worms shifted to favorable conditions for a three hour window (0.02% ± 0.01% dauer formation) is sufficient to commit L2d animals to reproductive development as L3 larvae despite subsequent exposure to dauer-promoting conditions, indicating commitment to L3 (Figure 2.2C). We define this consistent period in favorable conditions as the persistence window, and denote the time for 100% of the population to commit to adult the 'integration-time'. In summary, the L2d stage is divided into two distinct periods: integration (between the beginning of the L2d stage, 16 hph until the mid L2d, 33 hph) and commitment into dauer (33 hph-48 hph).

Effectors of the persistence window

We wanted to understand if the persistence window could be affected by changes of environmental conditions. Initially, we wanted to understand whether the time of the shift from unfavorable to favorable conditions could extend or shorten the integration time of pheromone. To this end we performed the modified ‘shift-to-favorable’ experiment starting at different time points in L2d and measured the fraction of dauers. We found that 99% of the population committed to adult within 3 hours of exposure to favorable conditions and that this window was uniform across different start times during the integration period of L2d (fig 3A with p=0.1, one-way ANOVA), with a

significant change at 33 hph, the time of commitment to dauer. We observed that the dauer fraction in the zero hour time point (which was actually 15 mins) decreased as worms were shifted to favorable conditions later during L2d (Figure 2.3B, P=0.0015). Interestingly, we could not detect significant differences in dauer frequencies in intermediate time points (1 and 2 hour persistence window periods, P=0.5, 0.6 respectively). These results suggest that the 'integration-time' shortens as the point of commitment to dauer draws near.

We next wanted to understand the effects of pheromone concentration on the integration time within the persistence window. We shifted worms from 3% pheromone to 0.5, 1 and 3% pheromone and measured the length of integration time. When worms were shifted from 3% pheromone to 0.5% pheromone the integration time increased from 3 hours to 6 hours. Similarly, when worms were shifted from 3% to 1% pheromone, integration-time increased from 3 hours to 8 hours (Figure 2.3C). These results indicate that the integration-time increased as the difference between the pheromone concentrations was smaller.

Epigenetic effects on the pheromone sensitivity

Since dauers can resume development and produce a brood, we asked whether this experience affected the sensitivity of F1's to form dauers. We recovered the P generation of dauers in favorable conditions and subjected the F1 F2 F3 and F4 generation to dauer pheromone. We observed a significant decrease of response to dauer pheromone as F1s exposed to 3 % pheromone resulted in 22 ± 4% dauer formation (control F1's yielded 92 ± 4% dauer, P<0.0001, one tailed t-test). F2 and F3 worms resulted in 92 ± 3% and 91 ±3% dauer fraction respectively, thus returning to

regular levels (Figure 2.4B, P_{F2}=0.52, P_{F3}=0.86, one tailed t-test). These results indicate that worms developing through the dauer molt pass this information to their offspring. The adult dauer transition memory lasts for only one generation suggesting an epigenetic component regulating the dauer decision.

Summary

The decision to become a dauer attempts to predict the future regarding the capacity of the environment to support the development of a brood (Cassada and Russell, 1975). To this end worms take into account population density (conspecific competition for resources), food availability and temperature during the environment-sensitive period. Worms then integrate these signals from the environment with their internal state composed of fat storage, previous exposures to pheromone, the history of their parents and their ability to produce hormones necessary for growth (Braendle et al., 2008). Once the decision has been made, worms develop either into a dauer with a specific morphology and behavior not displayed in the adult or in the age-matched juvenile, the L3 (Cassada and Russell, 1975). Environmental conditions often fluctuate and are seldom held constant suggesting that worms have evolved a mechanism to distinguish a true or 'stable' signal and act upon it. The mechanism that integrates the environmental cues and internal information leading to a decision committing to one of the binary outcomes remains unknown.

To dissect the process of commitment to reproduction (L3) or delayed reproduction (the dauer larvae), we modified the Golden and Riddle assay for major life cycle choices (Golden and Riddle, 1984a) by performing it in liquid culture, thus enabling a large and highly synchronized brood, amenable to facile changes in

environmental conditions. We used a high concentration of pheromone to mimic a high population density and showed that L2d worms grown in unfavorable conditions are irreversibly committed to the dauer fate by 33 hph. Conversely, L2d worms commit to reproductive fate if shifted to favorable conditions for a period of at least 3 hours before this 33 hph threshold has been crossed. Our identification of the times at which both life cycle fate decisions occur allowed us to couple changes in the environment to the known molecular and cellular components involved in this decision. We thus propose a mechanism of irreversible commitment to a life cycle fate choice.

Environmental conditions can change and be unpredictable. Worms not only integrate the ratio of food and pheromone, but also make sure that a transient signal of high or low population density is considered before committing to a fate. In engineering, this system property is called persistence detection and it is commonly used to differentiate between brief noisy signals to constant signals from the environment (Alon, 2007). We have identified that the persistence window of integrating favorable conditions during the L2d is nominally 3 hours. furthermore, worms can modulate the integration time: worms decrease the integration time as the point of commitment approaches and, conversely, increase the integration time as a function of the difference of the change in population density.

A decrease in the integration time is equated with less-strict form of integrating environmental conditions since it will allow signals with a shorter persistence to be integrated leading to development into an adult. We speculate therefore, that upon approaching the point of commitment to dauer, worms would prefer to hedge their bets against a brief signal and develop into an adult. This was observed at the zero hour

integration time length, where the decrease of integration time adjacent to the point of commitment is limited in the population since only 58 ± 8% become dauers and the rest adults.

An increase in integration time is equated with a more strict form of integrating environmental conditions since it will filter out short and intermediate length stable signals but will integrate long stable signals. Increasing the integration time slows down development and in unfavorable conditions worms that eventually decide to develop into adults will have a disadvantage. We therefore asked why worms experiencing a decreasing shift in population density increase the integration time, and have 3 hypothesis. (i) Worms experiencing a small decrease in population density measure their environment over a longer period of time thus increasing their chances for making a correct decision. (ii) The remaining amounts of pheromone in the environment may be inhibiting growth processes thus a slower growth process is extending the integration time. (iii) Fluctuating conditions may be a trigger to delay the reproductive period and to encourage migration to a new niche. Currently, we are making our conclusions on population means and not on individual worms and are unable to distinguish these hypothesis one from the other.

The dauer decision integrates the parental history since offspring (F1) to parents (P) that were dauers have an decreased sensitivity to pheromone. We speculate that the sensitivity decrease is an epigenetic trait since F2 worms show a normal response to pheromone if F1's are not stressed. A recent report has shown that worms that transiently develop through dauer have a significant portion of their genome regulated compared to age matched non-dauer adults leading to an increase of brood

size for the former (Hall et al., 2010). They identified several genes that can abolish the increase in brood size, therefore it will be interesting to test if these genes play a role in integrating pheromone in F1s.

How is the complexity of the environment integrated eventually in to a decision with binary outcomes? In later chapters I will demonstrate that hormone sensitive period falls within the environment sensitive period. I will then demonstrate the dauer decision is mainly executed in the neuroendocrine XXX thus reducing the information complexity of the environment into those 2 cells by a yet undefined mechanism. The hierarchy of the integration process is unknown; for example, Is the persistence of pheromone exposure first measured and then integrated with food availability? Is food availability integrated with fat availability and parental history and the sun of those integrated with pheromone persistence? Is Dafachronic acid hormone availability integrated as part of the decision or the result of it? In the following chapters I will use the integration time window to correlate between changes in the environment to the effects of the hormone Dafachronic acid.

Materials and methods

All worms were handled using standard growth and cultivation techniques using the bacterial strains HB101 and OP50 as food sources (Lewis, 1995). Unless otherwise stated all liquid cultures were grown in glass flasks at ~1 worm per µl at 20°C in S complete medium supplemented with 7.5 mg/ml HB101 as described in (Lewis, 1995) in an Innova 4230 incubator at 180 RPM. The wild-type strain used was N2 (Bristol).

Synchronous hatching of large broods

Worms were hatched synchronously essentially as described by (Baugh et al., 2009); changes are described in the SOM.

Pheromone assays

Crude pheromone was prepared as described (Golden and Riddle, 1984b). Each pheromone extract was tested on N2 worms (1 worm per µl) and diluted so that 3% (v/v) would yield 90±2% dauer arrest in a culture supplemented with 7.5 mg/ml of HB101.

Shift assays

Synchronous broods were grown as described above to the L2d stage by supplementing media with 3% (v/v) pheromone, partitioned into multiple parallel cultures and grown in glass tubes. Shift to favorable: at specified times, broods were washed 3 times in S basal to remove pheromone. Cultures were re-suspended in S complete medium containing HB101 and calibrated for density. Shift to unfavorable: broods were supplemented with 3% (v/v) pheromone and grown in glass tubes. At specified time points (L2d), worms were partitioned into a control sample and experimental samples, which were washed 3 times with S basal. Worms were suspended in S complete medium and allowed to grow for specific time periods until 3% pheromone (v/v) was added.

Homogeneity of liquid culture

Stage distributions were compared between 3 biological replicates in favorable and unfavorable conditions. A Bartlett's test (Zar, 2009) was used to determine if variances were significantly different between all stages of development.

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Figures

Figure 2.1

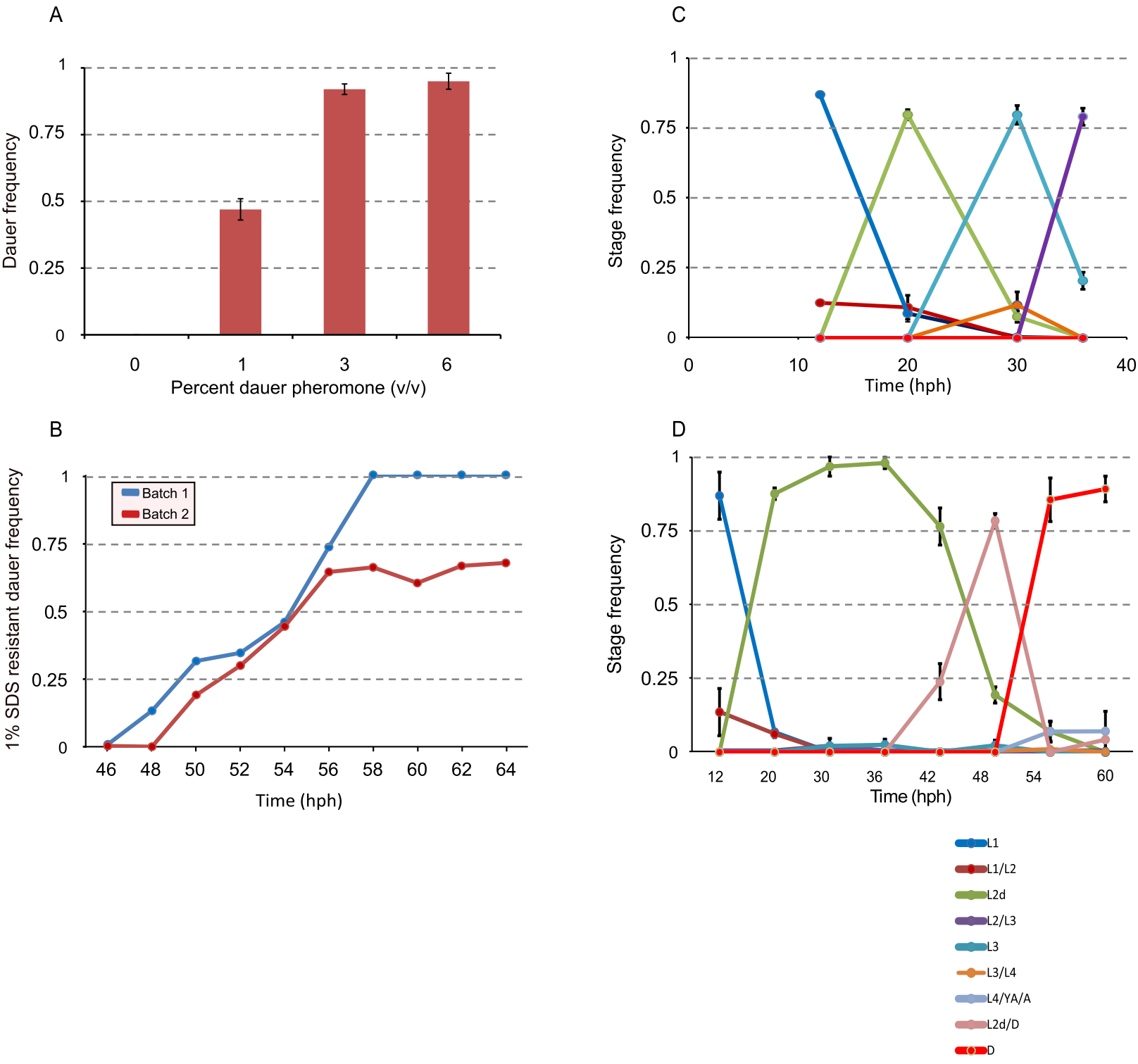
A
Dauer frequency
1
0.75
0.5
0.25
0
0
1
3
6
Percent dauer pheromone (v/v)
C
Stage frequency
1
0.75
0.5
0.25
0
0
10
20
30
40
Time (hph)
B
1% SDS resistant dauer frequency
Batch 1
Batch 2
1
0.75
0.5
0.25
0
46
48
50
52
54
56
58
60
62
64
Time (hph)
D
Stage frequency
1
0.75
0.5
0.25
0
12
20
30
36
42
48
54
60
Time (hph)
L1
L1/L2
L2d
L2/L3
L3
L3/L4
L4/YA/A
L2d/D
D

Figure 2.1: Calibration of liquid culture for development in homogenous environmental conditions

(A) Fraction of dauers as a function of a dilution series of crude pheromone. Bars represent the mean across 3 biological experiments and error bars describe the standard deviation of those 3 biological experiments. (B) Response of dauers to 1% SDS treatment. 100 % of the population become resistant to 1% SDS, 10 hours after the dauer molt (48 hph). (C,D) Synchronized hatch and subsequent growth in liwuid culture create a homogenous environment retaining a high degree of synchronicity throughout development in (C) favorable conditions and in (D) unfavorable conditions. Points represent means of three biological experiments and error bars represent the standard deviation.

Figure 2.2

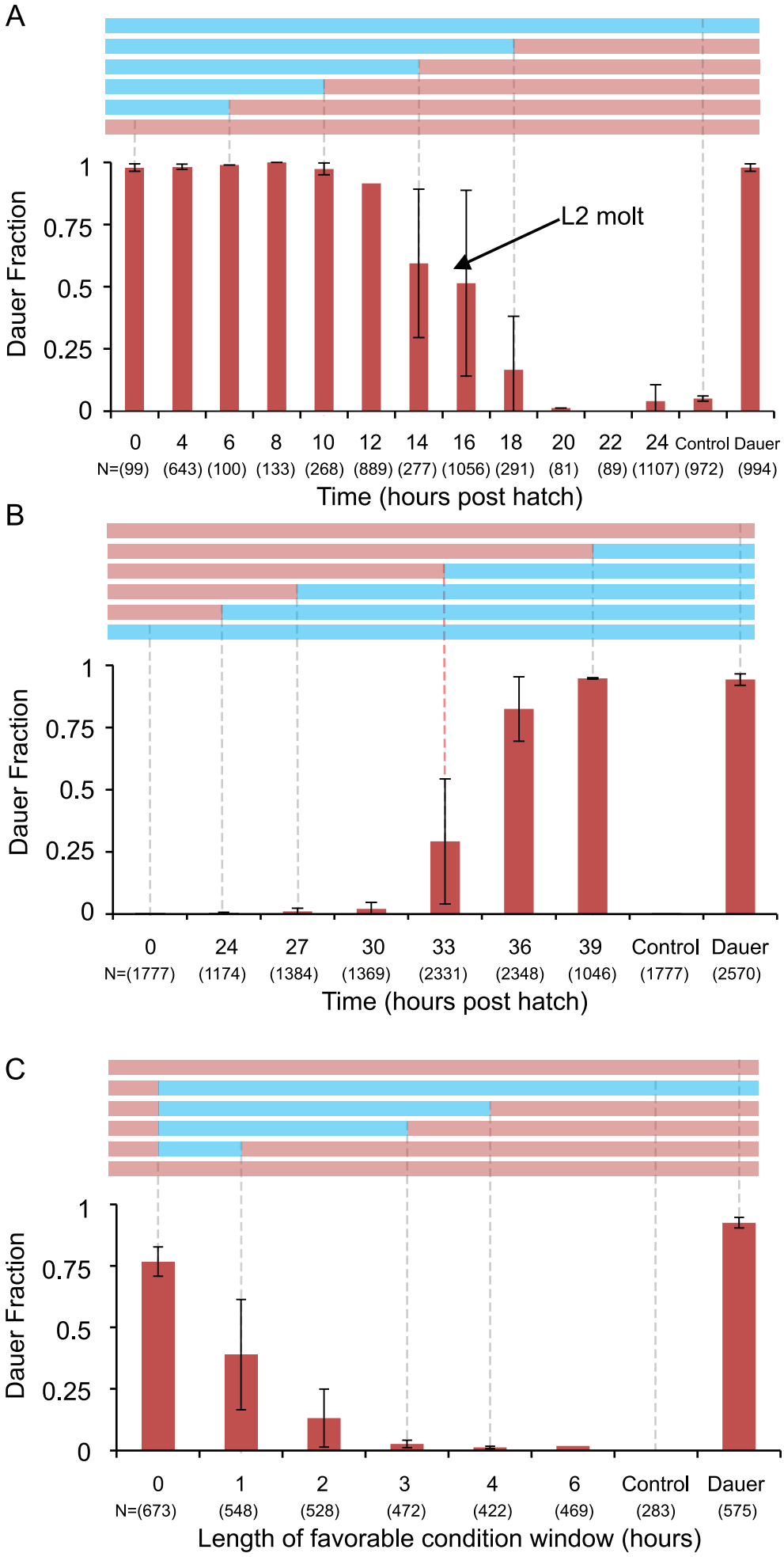
A
Dauer Fraction
1
0.75
0.5
0.25
0
L2 molt
0 4 6 8 10 12 14 16 18 20 22 24 Control Dauer
N=(99) (643) (100) (133) (268) (889) (277) (1056) (291) (81) (89) (1107) (972) (994)
Time (hours post hatch)
B
Dauer Fraction
1
0.75
0.5
0.25
0
0 24 27 30 33 36 39 Control Dauer
N=(1777) (1174) (1384) (1369) (2331) (2348) (1046) (1777) (2570)
Time (hours post hatch)
C
Dauer Fraction
1
0.75
0.5
0.25
0
0 1 2 3 4 6 Control Dauer
N=(673) (548) (528) (472) (422) (469) (283) (575)
Length of favorable condition window (hours)

Figure 2.2. Commitment to dauer or reproductive development.

(A-C) Time courses of commitment as a function of environmental conditions (pheromone). Top; representative colored bars indicating shifts to unfavorable conditions (red) or favorable conditions (blue). Bottom; means of dauer frequencies between biological replicates ± standard deviation. Numbers in parentheses indicate total worms per time point. Control; worms grown without pheromone. Dauer; worms grown in 3% (v/v) pheromone with no shifts.

(A) Induction period of pheromone during L1 and L2: worms respond to pheromone until 18 hph (early L2). (B) Point of commitment to dauer; worms commit to dauer 33 hph denoted by the red dashed gridline. (C) Point of commitment from L2d to L3; worms commit to L3 after a 3 hour pulse in favorable conditions when shifted at 24 hph.

Figure 2.3

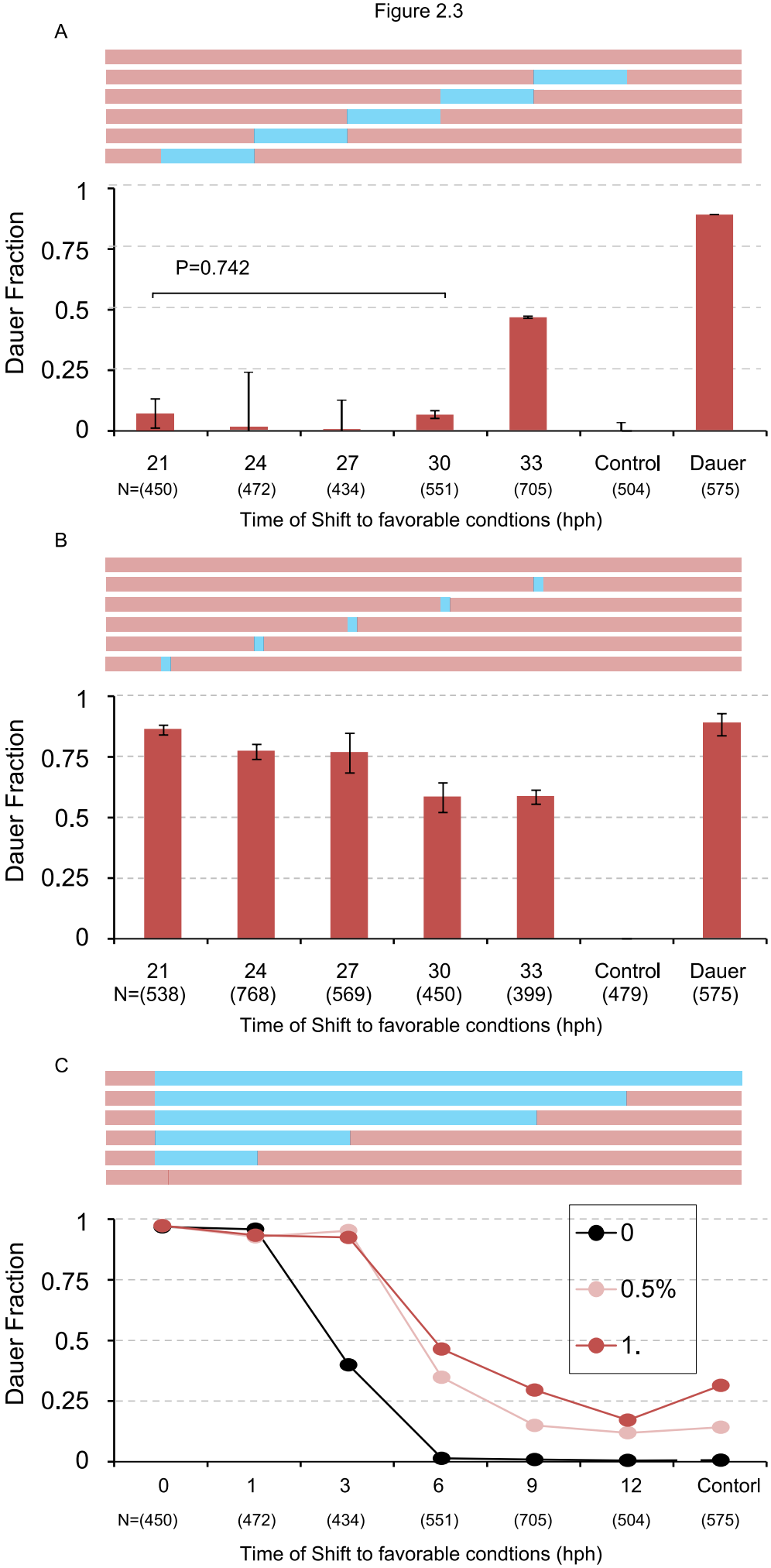
A
Dauer Fraction
1
0.75
0.5
0.25
0
P=0.742
21
N=(450)
24
(472)
27
(434)
30
(551)
33
(705)
Control
(504)
Dauer
(575)
Time of Shift to favorable condtions (hph)
B
Dauer Fraction
1
0.75
0.5
0.25
0
21
N=(538)
24
(768)
27
(569)
30
(450)
33
(399)
Control
(479)
Dauer
(575)
Time of Shift to favorable condtions (hph)
C
Dauer Fraction
1
0.75
0.5
0.25
0
0
1.
0.5%
0
1
3
6
9
12
Contorl
N=(450)
(472)
(434)
(551)
(705)
(504)
(575)
Time of Shift to favorable condtions (hph)

Figure 2.3: Effectors of the persistence window

Persistence window is modulated by environmental conditions. Top; representative colored bars indicating shifts to unfavorable conditions (red) or favorable conditions (blue). Bottom; means of dauer frequencies between biological replicates ± standard deviation. Numbers in parentheses indicate total worms per time point. Control; worms grown without pheromone. Dauer; worms grown in 3% (v/v) pheromone with no shifts. (A) Constant persistence window: start time of pulse shifts to favorable conditions during L2d. Pheromone was added to worms 3 hours post shift to favorable conditions. Cultures shifted to favorable conditions at 33 hph show a higher ratio of dauers since worms commit to dauer at 33 hph. (B) Worms shifted to the 0 time length window at times approaching the point of commitment decrease the length of the integration time. (C) Down shifting worms to 0% (black) 0.5% (pink) and 1% (red), and then returning them to 3% pheromone increases the integration time of the persistence window (N> 200 per pheromone concentration per time point).

Figure 2.4

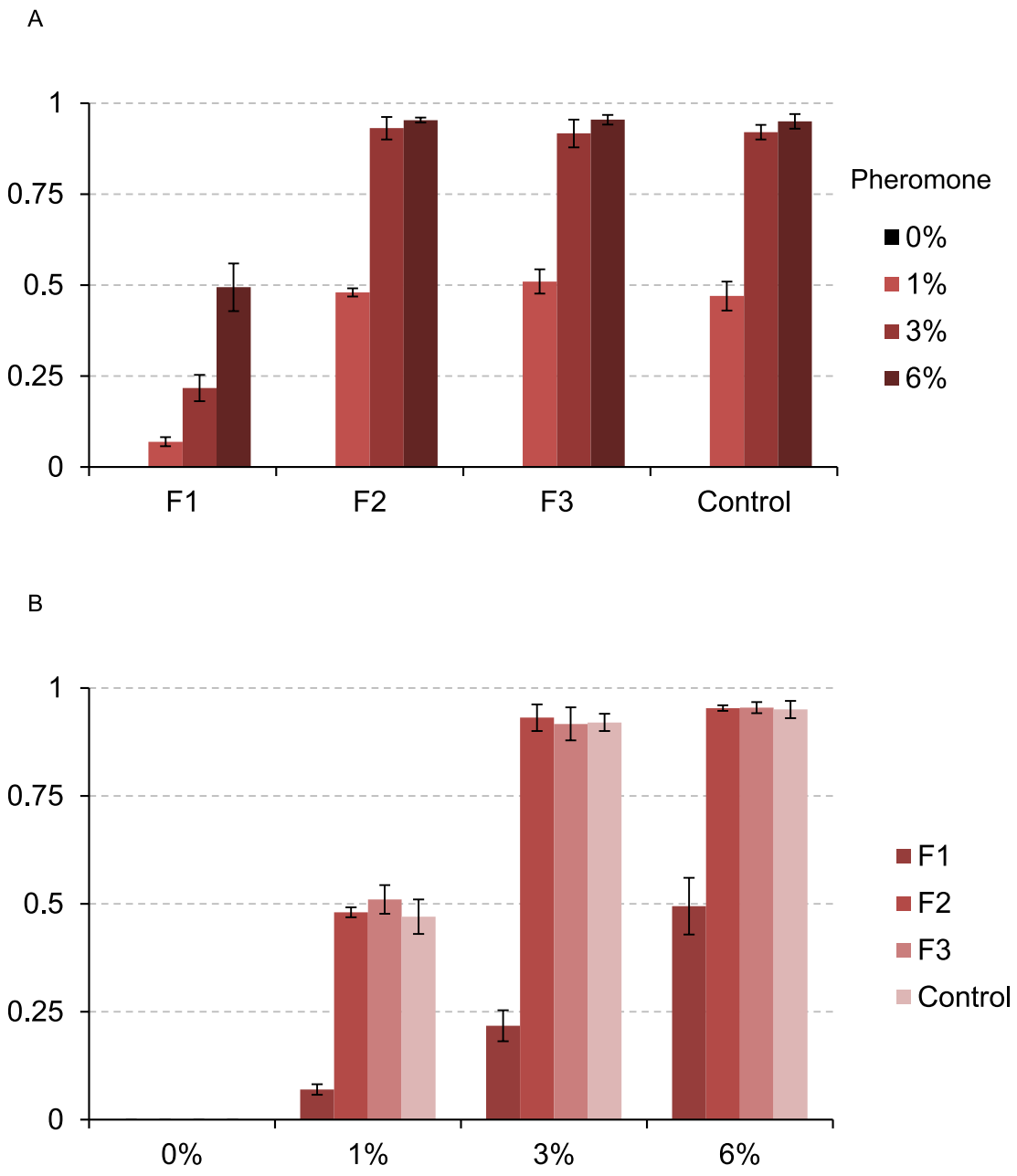
A
1
0.75
0.5
0.25
0
F1
F2
F3
Control
Pheromone
0%
1%
3%
6%
B
1
0.75
0.5
0.25
0
0%
1%
3%
6%
F1
F2
F3
Control

Figure 2.4: Dauer history is reflected in offspring

Parents that transitioned through the dauer stage pass the experience to offspring. (A) F1 generation offspring are significantly less sensitive to pheromone than control animals (at least 5 generations of favorable growth). See text for analysis of significance. Bars represent means of 3 biological replicates and error bars represent the standard deviation across the 3 biological replicates. F1 F2 and F3 represent the first second and third generation to parents that transition through dauer, respectively.

Figure 2.5

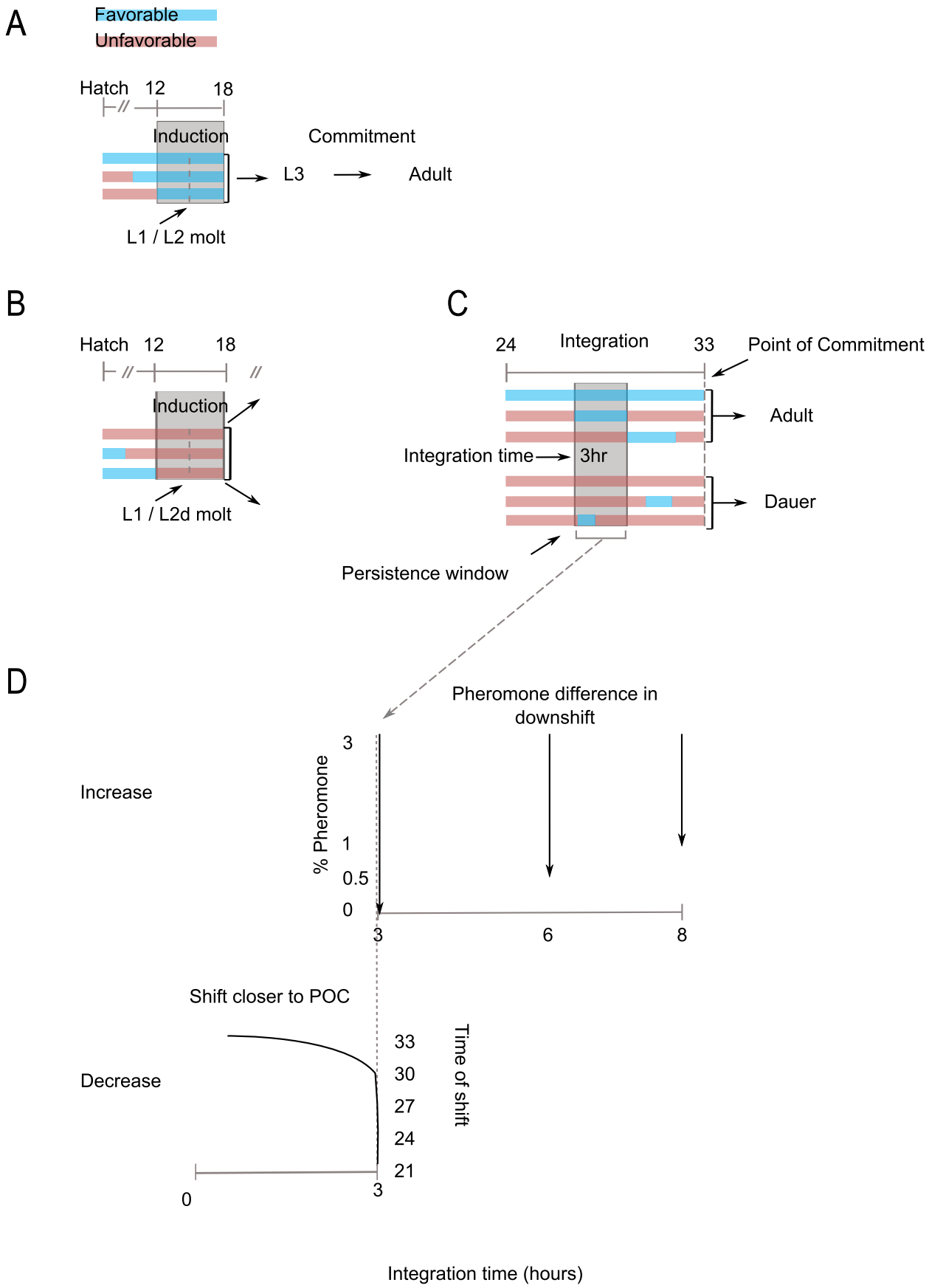
A
Favorable
Unfavorable
Hatch
12
18
Induction
Commitment
L3
Adult
L1 / L2 molt
B
Hatch
12
18
Induction
L1 / L2d molt
C
24
Integration
33
Point of Commitment
Adult
Integration time
3hr
Dauer
Persistence window
D
Pheromone difference in downshift
Increase
% Pheromone
3
1
0.5
0
3
6
8
Shift closer to POC
Decrease
Time of shift
33
30
27
24
21
0
3
Integration time (hours)

Figure 2.5: Effects of environmental conditions on the dauer decision

(A) Induction period is defined by the response to pheromone during 12-18 hph. Top; If animals aren't exposed to pheromone at that time worms develop into adults through the L2 stage. (B) If worms are exposed to pheromone during the induction period they develop into the L2d stage where they integrate the environmental conditions. (C) The L2d stage is divided into integration (18-33 hph) and commitment (33-48 hph). If worms are exposed to favorable environmental conditions during the integration period for an integration time that is equal to or greater than the persistence window (grey box) then they develop into L3's and into adulthood, otherwise they develop into adults. (D) The integration time is regulated by environmental conditions. Top; the integration time can increase if the difference in the pheromone concentration during the downshift is small. Bottom; the integration time can increase if the downshift is performed near the Point of commitment (POC) to dauer.