Chapter 3: Effects of the Hormone Dafachronic Acid on the Dauer Decision

Introduction

Hormone networks play an important role in the regulation of development across all taxa. Since hormones diffuse easily through membrane walls they are thought to be transmitted throughout the body orchestrating many biological processes and understanding their regulation is important.

Hormones can act through the neuroendocrine system having systemic effects (Nijhout, 2003). Hormones can diffuse through membranes and act in cells by two main mechanisms; (i) binding to internal nuclear hormone receptors, or (ii) through direct binding to GPCRs in the membrane-mediated steroid signaling pathway (Denver, 2007). Nuclear hormone receptors (NHR) usually have modular domains such as a ligand binding domain which binds the hormone and a DNA binding domain which recognizes and can bind to specific sequences in the genome. NHRs are usually inactive until they bind the hormone and often homodimerize or heterodimerize when the hormone diffuses into the cell. Thus, the activity of hormones is extremely modular and can be regulated by alternative splicing, cell and tissue specific and temporal transcription of the NHRs, their combination in target tissues and the state of the genome; if the DNA binding sequence is accessible or held closed (Nijhout, 2003; Wollam and Antebi, 2010). Therefore, the hormone sensitive period is a function of the availability and activity of the receptors controlling a specific phenotype and not necessarily that of the hormone. The hormone can be synthesized, released or activated at any moment during the sensitive period, thus diffusing and binding to its receptors. Many types of hormone regulatory mechanisms that regulate different types of polyphenisms rely on

the timing and dose of hormone secretion during the sensitive period (Keshan et al., 2006).

Hormonal regulation of polyphonic traits has been studied extensively in insects. The decision between mutually exclusive fates has been characterized in a number of hormonal mechanisms in the insect class. Nijhout (2003) has characterized differences in hormone titer, threshold, timing of hormone secretion and timing of the hormone sensitive period as main regulatory mechanisms that can form switched in polyphenic decisions (Figure 1.1). The hormone sensitive period and switch mechanism are unknown in *C. elegans*.

Most traits of polyphenism are irreversible; once committed to one fate, a change in environmental conditions or exogenous addition of the hormone triggering the specific morph will not change the decision of the morph. For example, the butterfly *Araschnia levana* will develop wing pigmentation which is bright orange in the spring and black in the summer. The summer morph is regulated by photoperiod and temperature which triggers Ecdysone release for the summer morph alone. If these butterflies are treated with the summer inducing conditions in the spring, the wings will form the summer morph, and upon commitment, will remain the summer morph even if switched back to spring-morph inducing conditions or treated with Ecdysone (Gilbert, 2009).

There is still debate if the end of the hormone sensitive period is the point of commitment, the point after which a change in environmental conditions does not change the course of development. The larvae of the butterflies *Oncopeltus fasciatus*

and *Pyrhocoris apterus* treated with threshold levels of JH develop larval pigmentation on patches of their cuticle but adult surface sculpturing (Willis 1982). The metamorphosis from larvae to pupae, or Pupal commitment, in the silkworm *Bombyx mori* and the tobacco hornworm *Manduca sexta* is inhibited by JH during development ensuring that enough food has been accumulated to reach a critical mass. During the fifth instar larvae, (when nutrition is sufficient) there is an increase of the hormone 20-hydroxyecdysone, and a decrease of JH leading to pupal commitment. Addition of JH 12 or 24 hours after pupal commitment does not affect the course of metamorphosis nor does starvation (Koyama et al., 2008). The mechanism that ensures commitment to pupae is unknown.

C.elegans nematodes display polyphenism during development. In favorable environments, *C. elegans* develops rapidly through four larval stages (L1-L4) separated by molts, into a sexually reproductive adult. However, in unfavorable environments, animals can make a life cycle fate decision and develop into an alternative third larval stage, known as the dauer diapause, a developmentally arrested, long-lived form geared towards survival (Cassada and Russell, 1975; Golden and Riddle, 1984a). Accordingly, they undergo profound morphological changes including assault resistant cuticle, pharyngeal constriction and sealing of buccal cavities, which confer somatic endurance (Cassada and Russell, 1975; Golden and Riddle, 1982). Dauer larva do not feed and can endure harsh conditions, including starvation, desiccation, heat and oxidative stresses (Riddle, 1997). Whereas adult worms have a mean life span of three weeks, dauer larvae can survive for several months (Byerly et al., 1976; Cassada and Russell, 1975; Klass and Hirsh, 1976). When returned to favorable conditions, dauer

larvae molt into an L4 larvae and continue into adulthood (Cassada and Russell, 1975; Klass and Hirsh, 1976).

Molecular analysis has revealed at least four signaling pathways. Components of neurosensory structure and guanylyl cyclase signaling are involved in sensing temperature, nutrients and dauer pheromone (Birnby et al., 2000), which regulate secretion of Insulin/Insulin-like growth factor and TGFβ peptides. Working through their respective signaling pathways, IIS and TGF-beta signaling converge on a steroid hormone pathway, which metabolizes dietary cholesterol into several bile acid-like steroids, called the dafachronic acids (DAs). DAs can rescue the *daf-7* and *daf-2* Daf-c phenotypes indicating that it is downstream to the Insulin and TGFβ signaling pathways (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. Liganded DAF-12 promotes reproductive development, whereas unliganded DAF-12 directs the dauer fate together with the co-repressor DIN-1S. Thus, DAF-12 serves as a DA-responsive switch that determines whether an animal will undergo reproductive or dauer development (Gerisch and Antebi, 2004; Gerisch et al., 2007; Hannich et al., 2009; Ludewig et al., 2004; Mak and Ruvkun, 2004; Motola et al., 2006; Patel et al., 2008; Rottiers and Antebi, 2006).

The cytochrome P450 DAF-9 is the last enzyme in the pathway of DA production and is critical to the dauer decision. *daf-9* is expressed in a bilaterally symmetric pair of neuroendocrine cells called XXXL and XXXR from hatching through adulthood; in the hypodermal syncytium from mid-L2 to L4 stages (but not during the dauer stage); and in

the adult spermatheca (Gerisch et al., 2001; Jia et al., 2002; Ohkura et al., 2003). Consistent with a hormonal mechanism, DAF-9 works cell non-autonomously to control dauer formation (Gerisch and Antebi, 2004; Mak and Ruvkun, 2004). By contrast, *daf-12* is widely expressed in most tissues and throughout development (Antebi et al., 1998; Antebi et al., 2000). With respect to dauer phenotypes, *daf-12* null mutants (Daf-d) are downstream to *daf-9* null mutants (Daf-c, Gerisch et al., 2001; Jia et al., 2002), yet hypodermal expression of *daf-9* is DAF-12 dependent, suggesting that a feedback loop regulates hypodermal *daf-9* expression (Gerisch and Antebi, 2004; Mak and Ruvkun, 2004).

The molecular and cellular basis comprising the binary nature of the life cycle fate decision remains elusive. The time of action of DA is unknown, or whether DAs are produced as a result of a decision or as a means for integrating multiple environmental stimuli and genetic inputs controlling the decision. Here we demonstrate that they act as a regulator at the point of the decision and as a messenger downstream of the decision. We show that environmental conditions affect the threshold at which levels of DA are sufficient to prevent the dauer fate and commit to adulthood, while higher amounts of DA are necessary to implement and coordinate the reproductive decision throughout the whole animal.

Results

The two forms of the dafachronic acids (DA) Δ4-DA and Δ7-DA can fully rescue the Daf-c phenotypes of the null allele *daf-9(dh6),* as well as *daf-7*/TGF-beta and *daf-2*/InsR mutants (Motola et al., 2006) . Partial reduction of *daf-9* function, results in animals that bypass the dauer stage yet exhibit incomplete gonadal morphogenesis and migration

(Mig) and occasionally aberrant cuticle shedding (Cut) defects (Gerisch et al., 2001; Jia et al., 2002) (Fig 1A). Exogenous DA can also rescue these phenotypes (Gerisch and Antebi, 2004; Gerisch et al., 2007; Mak and Ruvkun, 2004; Motola et al., 2006). We thus hypothesized that a low amount of DA is required to bypass dauer and commit to L3, whereas a high amount is required for complete development.

Dose response of daf-9 loss of function mutants to DA

To understand the physiological response to DA dose, dauer-constitutive *daf-9* loss-of-function mutants were treated with increasing amounts of Δ7-DA and measured for dauer and reproductive adult fates. Most *daf-9(dh6)* null animals developed into abnormal adults when supplemented with a minimum of 10nM DA (Figure 3.1B 74 ± 42% non-dauers), suggesting that a threshold of DA has to be crossed before committing to adult fate (dauer bypass DA threshold). Increasing the levels of DA decreased the frequency of dauers and increased the frequency of abnormal adults. 25nM DA prevented 99 ± 1% of *daf-9(dh6)* animals from developing as dauers (Figure 3.2B). Further increase of DA decreased the frequency of abnormal adults and increased the frequency of normal adults (Figure 3.2B; 50-100nM). For a distribution of Mig and Cut phenotypes, see Figure S1.1. Similar results were observed with animals homozygous for *daf-9(e1406)* or *daf-9(m540)* (Figure S1.1, worms were not synchronously hatched), both of which are strong loss-of-function alleles. By contrast, the weak loss of function allele *daf-9(rh50)* does not result in Daf-c phenotypes, but in highly penetrant Mig defects (95 ± 3%) (Gerisch et al., 2001). In these animals, only 10nM of DA was required to rescue over 90% of the Mig phenotypes (Figure S1.1), revealing a 5-fold decrease in the amount of exogenous DA required to promote

complete development compared to the stronger *daf-9* mutants (*dh6*, *e1406* and *m540)*. The *rh50* allele is an amino acid substitution D334N in a putative substrate binding domain of the Cytochrome P450/CYP27A1 (Gerisch et al., 2001). Thus, *daf-9(rh50)* animals produce sufficient amounts of DA to bypass dauer development, but require additional DA to develop into normal adults, consistent with our finding that different levels of DA are required for the two processes.

DA effects on developmental rate

To test the effects of DA on developmental rate, *daf-9(dh6)* worms were synchronously hatched in different concentrations of DA and scored for developmental stage at 48 hph (the time at which WT worms grown in favorable conditions are young adults and worms grown in unfavorable conditions are dauers) and for egg production the following day. Worms supplemented with 10nM DA arrested development at the L3 stage. At 25-50nM DA, worms developed into L4s and worms supplemented with 75-175nM DA developed into YA's. Worms that were in the L4 or YA stages at 48 hph were gravid the next day. All these trends indicate that higher DA increases growth rate (Figure 1C) and that *daf-9(dh6)* worms supplemented with DA can develop at the same rate as WT worms.

Intermediate amounts of DA increase phenotypic variation high amounts decrease it

Addition of DA at increasing concentrations bypasses the dauer but unmasks the intermediate phenotypes of mig cut and growth rate variations. Increasing the concentration even further canalizes development to adult or dauer with some variation

around growth rate. We quantified the effects of DA on the variance by calculating a Shannon-Weiner heterogeneity index (SWHI) for a diversity of phenotypes at every DA dose. The SWHI is traditionally used in quantifying the evenness of distribution of species in different niches and can be used to contrast between populations with unequal numbers of categorical data (Zar, 2009). The SWHI is defined as the ratio

$J = \frac{H`}{H_{max}} = \frac{-\sum_{i=1}^{S}(P_i lnP_i)}{lnS}$ where H` is the entropy or the amount of uncertainty or distribution in a population, H_{max} is the maximal amount of uncertainty or distribution in a population, S is the number of niches or categories that are being surveyed, and P_i is the relative abundance of species *i*. For example, a population that has no diversity will have the ratio J=0 and a population with maximal diversity where all individuals are equally spread between all categories will have the ratio of J=1. Addition of 0-5nM DA did not increase the SWHI significantly (0, 0.013, 0.02, respectively). However, at the ranges of 10-50nM DA the SWHI increased to 0.25 at 10nM peaking at 25nM with a SWHI of 0.49 due to the emergence of the abnormal mig and cut phenotypes. At 50nM the SWHI dropped to 0.24 when no more abnormal phenotypes are observed and the distribution of phenotypes is composed of arrested L3s and adults. At 75-175 another increase and decrease are observed and this is due to the distribution of growth rates (Figure 3.2). These results demonstrate that an increase of DA increases the number and distribution of different intermediate developmental states and a further increase decreases the number and distribution of it. Thus high amounts of DA can canalize the developmental states of worms bypassing the dauer decision.

Pheromone levels regulate the adult DA threshold and reproductive development

DA and dauer pheromone have opposite effects on dauer formation, with DA preventing and dauer pheromone promoting the dauer stage. We investigated the dose-response relationship when administered together, with respect to bypass of the dauer diapause and complete reproductive development.

Synchronized populations of *daf-9(dh6)* worms were supplemented with a combination of DA and pheromone at different concentrations and scored for dauer, incomplete adult and complete adult development at 48 hph. Addition of pheromone at 1,3 or 6% (which induce 47±4, 92±2 and 95±2% dauer in WT worms, respectively, Figure 1.1A) increased the concentration of DA necessary to bypass the adult DA threshold (Figure 3.3A-D) to 30, 45, and 58nM, respectively. Moreover, 90% of the population developed into complete adults when worms were supplemented with 30nM of DA more than the amount required to bypass the adult DA threshold (Figure 3.3E,F), similar to the concentration of DA needed to bypass the adult DA threshold in *daf-9(dh6)* worms without pheromone. These experiments demonstrate that dauer pheromone increases the amount DA required to bypass the adult DA threshold and complete reproductive development.

DA time of action

To understand the time of action of DAs and their role in life cycle fate decisions we sought to identify 3 key points in the response to DA: (i) the time or stage at which *daf-9(dh6)* animals start responding to DA to bypass dauer (ii) the end of response to DA for

the dauer decision and (iii) the requirements of exposure to DA for complete development to maturity. Synchronously hatched *daf-9(dh6)* worms were shifted from media containing DA in EtOH to media containing EtOH alone (downshift) or vice-versa (upshift). Analysis of downshift experiments revealed that worms started responding to DA after 15 hph, the same time that WT worms commit to L2 mediated reproductive development (Figure 3.4A). When DA was washed away before 15 hph, worms developed into dauers, and apparently lacked a memory for previous exposures. Removal of DA at time points after 15 hph prevented dauer formation to increasing extents, which could be divided into two phases: a minimum of 3 hours on 100nM DA during the responsive period was sufficient to prevent 75% of the population from becoming dauers but these animals developed as incomplete adults (Figure 3.4A 15 to 18 hph), whereas an additional 12 hours were necessary to drive 100% of the population to complete adult development (Figure 3.4A 18 to 30 hph). To determine when *daf-9(dh6)* worms became refractory to DA, upshift experiments were performed during the L2d stage. Worms responded to DA until 33 hph, precisely at the same time that wild-type worms became refractory to pheromone (Figure 3.4B; correlation coefficient = 0.996). Next, we asked whether the total time exposed to DA or the specific time (stage) of exposure to DA were regulating the fate decision and development of normal adults. Pulse experiments revealed that worms committed to bypass dauer when exposed to DA at 15 hph for as little as 3 hours (Figure 3.4C). Also, this commitment occurs at 15-18 hph regardless of previous longer exposures to DA prior to the L2 molt (Figure 3.4D). Similar results were seen with the *daf-9(e1406)* allele (Figure S1.2). In sum, DA can affect the decision during a specific temporal window (15 to 33

hph) during the L2d stage. Worms become committed to bypass dauer with a minimal exposure of 3 hours in DA, but additional persistent exposure to DA over 12 hours is necessary for complete adult development.

Summary

Hormonal networks play a critical role in commitment to reproductive maturity throughout the animal kingdom, yet the cellular and molecular network architecture of commitment is not well understood. Here we have connected the environment sensitive period to the hormone sensitive period during the commitment to reproductive development in the nematode *C. elegans*. These studies allow us to ascribe specific time windows and pinpoint levels of hormone required to drive an endocrine network over thresholds for adult maturation.

Since DA is produced in small amounts and is detected by mass spectrometry methods which are indirect, we decided on a synthetic set of experiments to understand the molecular mechanism of DA on the dauer decision. Initially, we started with identifying the dose at which DA works as a switch of the dauer decision and at a higher dose orchestrating the decision over the whole animal. The concentration of DA sufficient to cause 100% *daf-9* null worms to develop into complete adults was used to determine the time of action which coincides with the induction and integration periods that wild type worms measure their environment suggesting that DA is the physiological mediator of the dauer decision and its outcome. We further used this baseline concentration of DA to understand the relationship with pheromone and the TGFβ pathway, and revealed that pheromone can modulate the dauer bypass DA threshold. This interaction was not revealed in the epistasis interactions.

In particular, the timing is congruent with the requirement for DA. In this view favorable conditions equate with the presence of DA, while unfavorable conditions equate with its absence. Starting from 15 hph, pulses of DA 3 hours or longer will rescue dauer formation with no memory to previous exposures to DA, eliminating it as a mediator of parental dauer history (Figure 3.4). Similarly *daf-9* mutants become refractory to DA rescue from dauer at 33 hph, mid L2d stage. Thus, these periods of DA sensitivity overlap substantially with the response to changes of population density in the environment (Figure 3.5A).

Our studies suggest that two thresholds of DA must be crossed in order to ensure proper reproductive development (Figure 3.3F). First, addition of DA to *daf-9* mutants suggest that about 10nM DA is sufficient to bypass the adult DA threshold in liquid culture and 1-5nM are sufficient on plates. Second, animals that have committed to L3 development, required an additional 30nM DA to promote normal gonadogenesis and cuticle formation. Higher levels of DA increase developmental rate. Conversely, exogenous dauer pheromone can raise the adult DA threshold and complete reproductive development, suggesting that pheromone has additional targets downstream or parallel to DA production that antagonize reproductive development (Figure 3.5B).

What might be the molecular and cellular correlates of these two thresholds? The cytochrome P450 DAF-9 is limiting for DA production, since its biochemical function is essential for bile acid synthesis. *daf-9* is expressed in the XXX cells from hatch and throughout development, and in the hypodermis starting from mid-L2 until L4. The timing requirements for DA described above suggest that the commitment to adult

development through the L2 stage is made early in L2 between 15-18 hph, a time that precedes visible hypodermal *daf-9* expression (Gerisch and Antebi, 2004; Mak and Ruvkun, 2004).

Increasing the amounts of DA to *daf-9* mutants, reveals several intermediate states of adulthood phenotypes and a further increase causes most animals to form normal adults. Wild type worms have only the dauer stage and the adult without any abnormal phenotypes suggesting that a canalizing mechanism is in place to avoid all of the abnormal phenotypes. We characterized the differences in these states using the Shannon- Weiner heterogeneity index and show that the addition of DA starts with a minimal entropy displaying only the dauer fate and increasing to a maximal entropy of abnormal states at 50nM. A further addition of DA decreases the entropy almost to wild type levels observed in the adult state. We speculate that the difference in adult stage entropy in the wild type state and the *daf-9(dh6)* + DA is the result of a-synchronous development. The increase and decrease of entropy suggest that the wild type worms implement a mechanism to canalize development. In the next chapter we will describe a positive feedback loop that is triggered as a result of commitment into adulthood which upregulates *daf-9* in the hypodermis giving rise to higher levels of DA in the while animal. We will argue that this upregulation is the canalizing mechanism enforcing the binary nature of the dauer decision.

Materials and methods

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.

Dafachronic acid assays

Liquid culture: Δ7-DA was solubilized in 100% EtOH to necessary concentrations. Liquid culture assays were performed by adding EtOH-solubilized Δ7-DA in S basal medium. NG agar plate assays were performed by resuspending EtOH-solubilized Δ7-DA in S basal with OP50 and spreading on plates. Worms were picked onto Petri plates not more than one day after Δ7-DA was added to those plates. For the p*daf-9*::gfp experiment Δ7-DA was added on a 3 cm NG agar plates, seeded with OP50.

Scoring incomplete development

daf-9(dh6), daf-9(e1406), daf-9(rh50) and *daf-9(m540)* worms were grown in liquid culture with different concentrations of Δ7-DA as described above. Worms were washed once with S basal medium to remove HB101 and mixed with S basal medium containing 1mM sodium azide (to limit worm movement), spotted onto a 24-well plate. Worms were scored for gonad migration and cuticle shedding. Phenotype frequencies were calculated as the means of 3 biological replicates, ± standard deviation.

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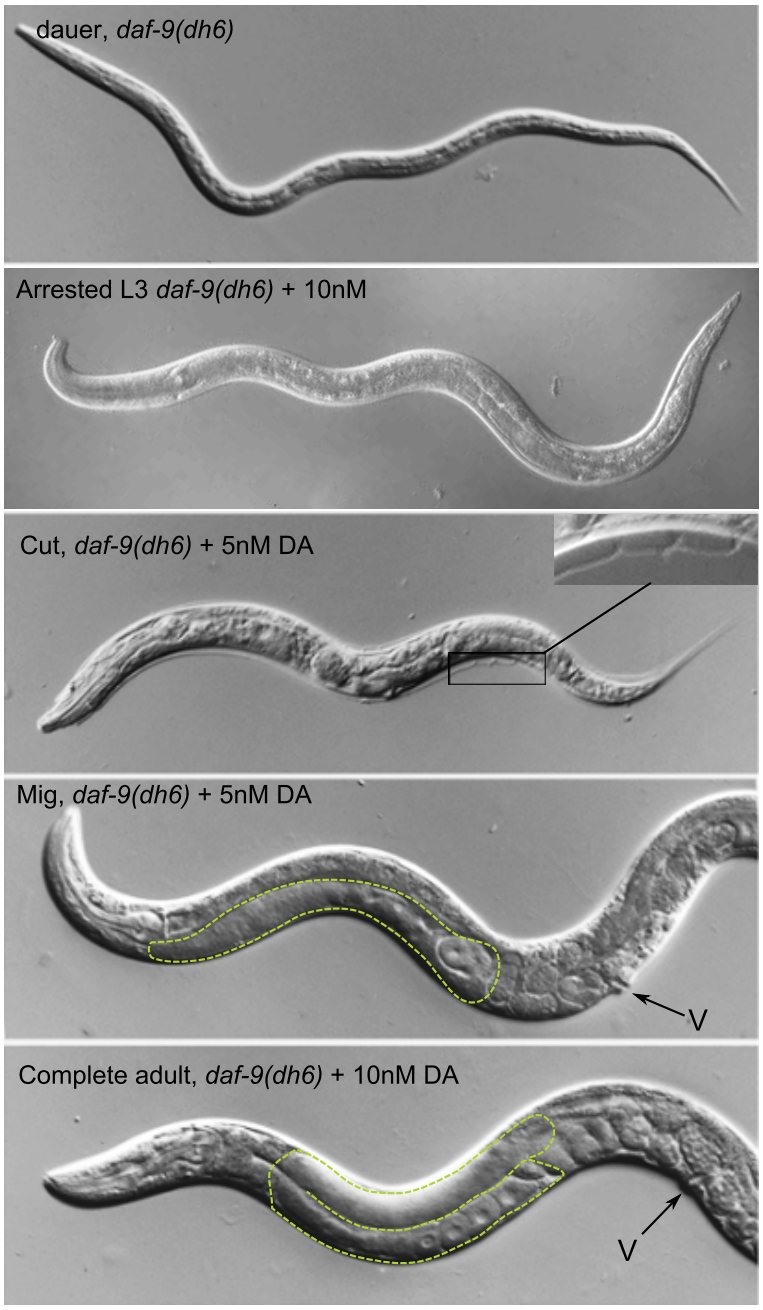
Figures

Figure 3.1

A



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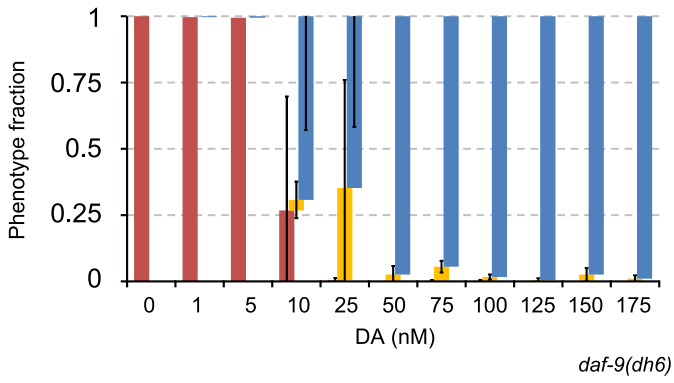

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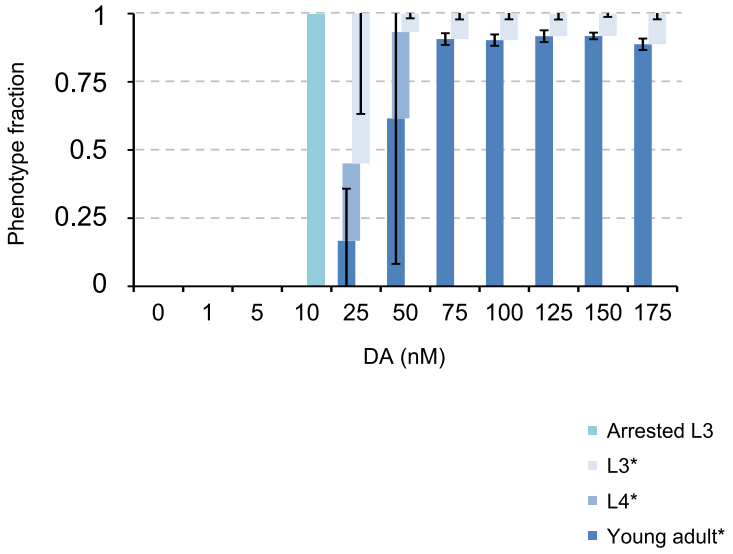

Figure 3.1. High amounts of DA are required for complete adult development

(A) Images of dauer, arrested L3, abnormal development Mig and Cut worms, and normal adults. (B) Distribution developmental stages as a function of DA, scored 48 hph. Means of dauer (red), abnormal development (arrested L3, Mig and Cut; yellow) and complete adult (L3, L4 and young adult; blue) phenotype in *daf-9(dh6)* worms. (C) Distribution of stages in the adult fraction of phenotypes. Means of population proportions of stages indicate the relative developmental rate at each concentration of DA scored at 48 hph. Wild type worms grown in these conditions are YA. Error bars represent means ± standard deviations across three biological replicates, N>500. Mig; gonad migratory defective, Cut, cuticle defective, YA; young adult. * Worms were gravid the next day.

Figure 3.2

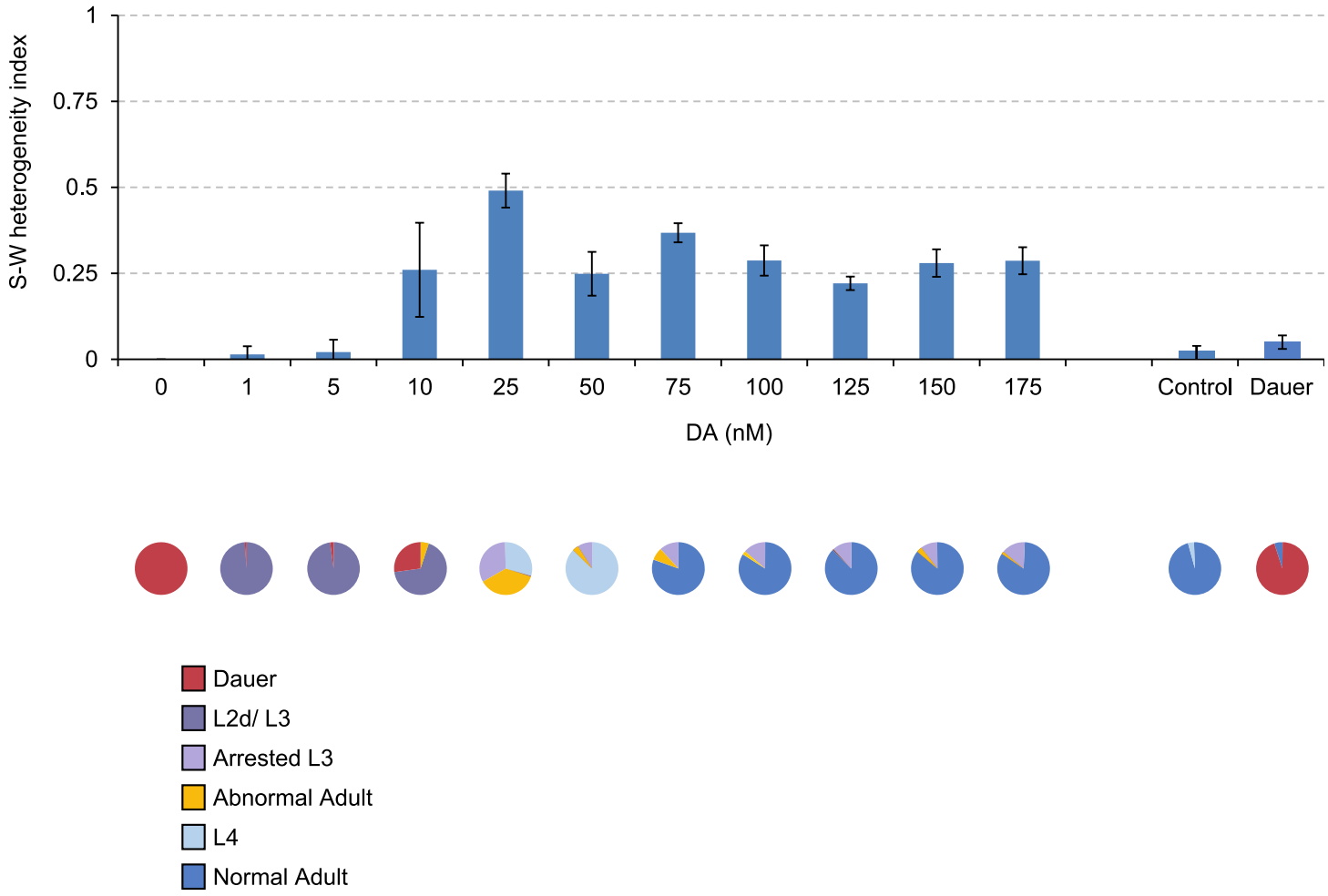
S-W heterogeneity index
1
0.75
0.5
0.25
0
0
1
5
10
25
50
75
100
125
150
175
Control
Dauer
DA (nM)
Dauer
L2d/ L3
Arrested L3
Abnormal Adult
L4
Normal Adult

Figure 3.2: An increase in DA leads to canalization of developmental fates.

The Shannon-Weiner heterogeneity index was calculated for the developmental phenotypes of each dose of DA. The index spans from 0 indicating that onlu one phenotype is displayed in a population and therefore it is homogenous, to 1 where an equal representation of all other phenotypes are observed equally indicating a heterogeneous population. Dauer: wild type worms grown in unfavorable conditions. Control; wild type worms grown in similar conditions without DA. The high amount of arrested L3 worms in the *daf-9(dh6)* population treated with DA was conserved over all concentrations above 50nM and if it is disregarded, the heterogeneity index approaches that of the control.

Figure 3.3

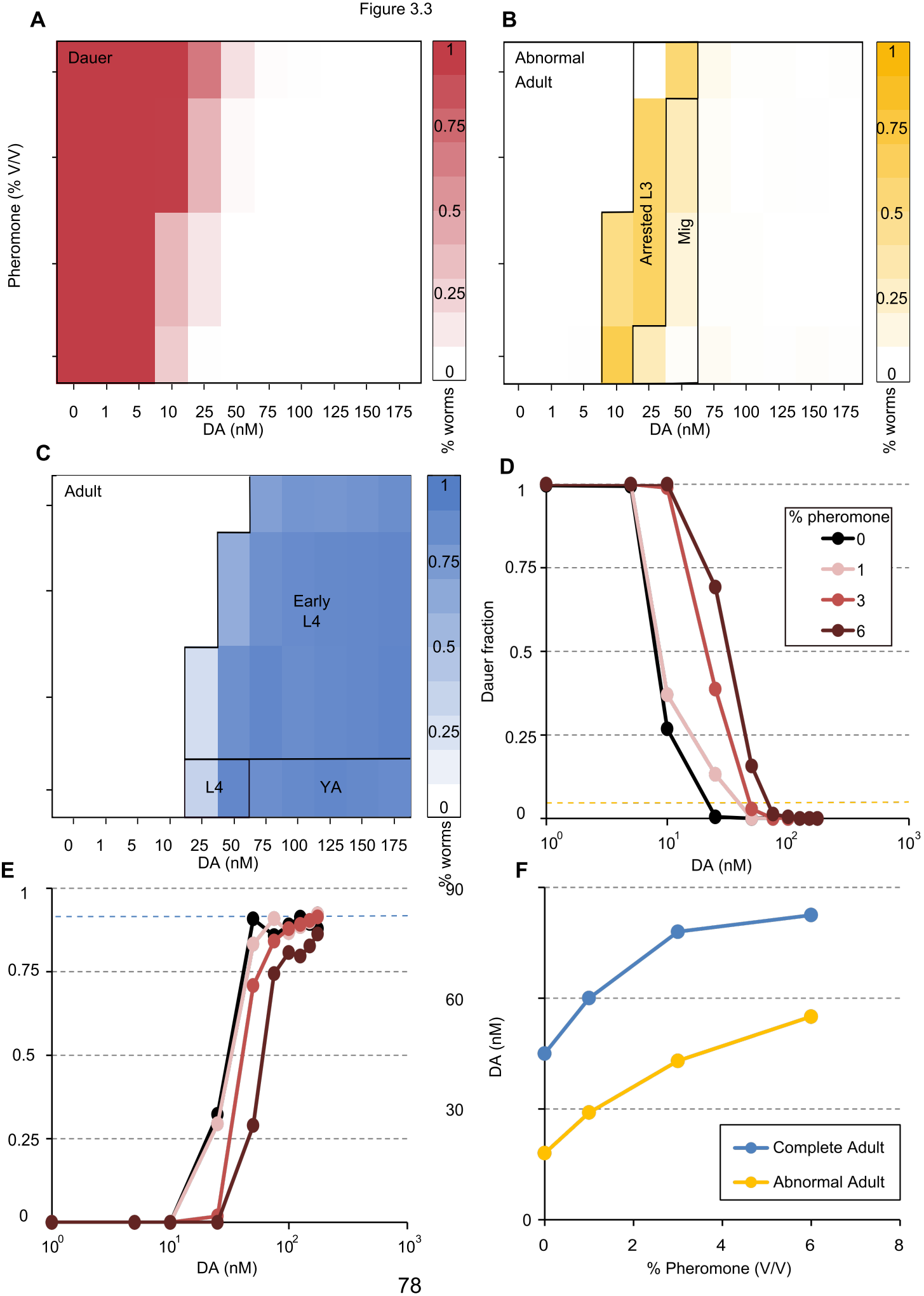
A
Dauer
Pheromone (% V/V)
1
0.75
0.5
0.25
0
0 1 5 10 25 50 75 100 125 150 175
DA (nM)
% worms
B
Abnormal
Adult
Arrested L3
Mig
1
0.75
0.5
0.25
0
0 1 5 10 25 50 75 100 125 150 175
DA (nM)
% worms
C
Adult
Early
L4
L4
YA
1
0.75
0.5
0.25
0
0 1 5 10 25 50 75 100 125 150 175
DA (nM)
% worms
D
% pheromone
0
1
3
6
Dauer fraction
1
0.75
0.5
0.25
0
10^0 10^1 10^2 10^3
DA (nM)
E
Complete Adult fraction
1
0.75
0.5
0.25
0
10^0 10^1 10^2 10^3
DA (nM)
90
60
30
F
DA (nM)
Complete Adult
Abnormal Adult
0
2
4
6
8
% Pheromone (V/V)

Figure 3.3: Dauer pheromone regulates the threshold for reproductive development.

(A-C) Population distribution of dauers (A), abnormal adults (B) and adults (C), as a function of DA and pheromone. Each pixel on the heat map is the mean fraction of population (see bar on the right for quantification) developing in the specific category, N>300 per pixel. Partition of abnormal adults and adults into sub-categories is detailed in Fig S3. (D) Concentrations of DA required to pass the dauer bypass DA threshold as a function of pheromone. Yellow dashed line indicates 90% non-dauers in the population. (E) Concentrations of DA required for normal adult development without any arrested L3, Mig or Cut phenotypes. Blue dashed line indicates 90% complete adults in the population. (F) Normal adult development requires an additional 30 nM DA above the amount for dauer bypass. Yellow plot denotes the concentrations of DA required for 90% of the population to develop into non-dauer fates and blue plot denotes the concentration required for 90% of the population to become normal adults. * denotes animals that developed into gravid adults the next day. Mig; gonad migratory defective, YA; young adult.

Figure 3.4

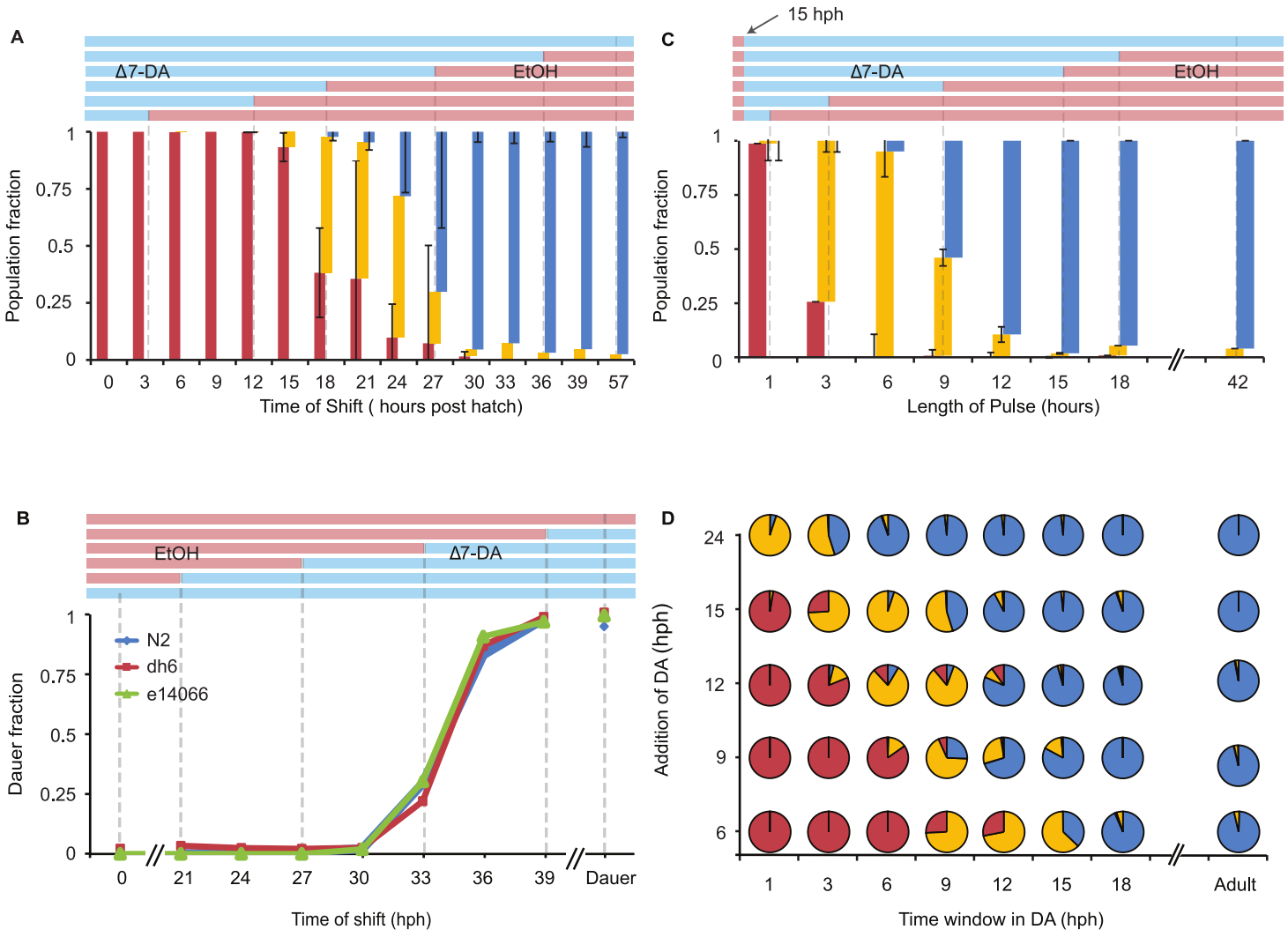
A
Δ7-DA
EtOH
Population fraction
1
0.75
0.5
0.25
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 57
Time of Shift (hours post hatch)
C
15 hph
Δ7-DA
EtOH
Population fraction
1 3 6 9 12 15 18 42
Length of Pulse (hours)
B
EtOH
Δ7-DA
N2
dh6
e14066
Dauer fraction
0 21 24 27 30 33 36 39 Dauer
Time of shift (hph)
D
Addition of DA (hph)
24
15
12
9
6
1 3 6 9 12 15 18 Adult
Time window in DA (hph)

Figure 3.4. Temporal activity of Δ7-DA

Dauer, abnormal development and normal adult fates as a function of exposure times to Δ7-DA. (A) *daf-9(dh6)* worms start responding to ∆7-DA at 15 hph and require an additional 12 hours of ∆7-DA for complete adult development. Top; representative colored bars indicating the shift experiment: red bars indicate EtOH carrier and blue bars indicate Δ7-DA. Bottom; Normal adult (blue), abnormal adult (yellow) and dauer (red) bars indicate the population fraction per time point. (B) Worms become refractory to Δ7-DA at 33 hph, the same time that they commit to dauer. N2 indicates worms shifted from unfavorable to favorable conditions as indicated in Fig 1C, and points indicate dauer proportions (abnormal development is considered non-dauer in this panel). (C) Pulses of Δ7-DA indicate the minimal time necessary for normal development when added at 15 hph. Top; length of pulses. Bottom: Normal adult (blue), abnormal development (yellow) and dauer (red) bars indicate the population fraction per time point. (D) Worms have no memory of previous exposure to Δ7-DA before the L1/L2 molt. Pie charts indicate proportions of dauers (red), abnormal development (yellow) and normal adults (blue) as a function of total amount of time exposed to Δ7-DA (x-axis) when exposed to Δ7-DA at different hours post hatch (y-axis). N > 100 for all time points in all panels, see SOM for error bars.

Figure 3.5

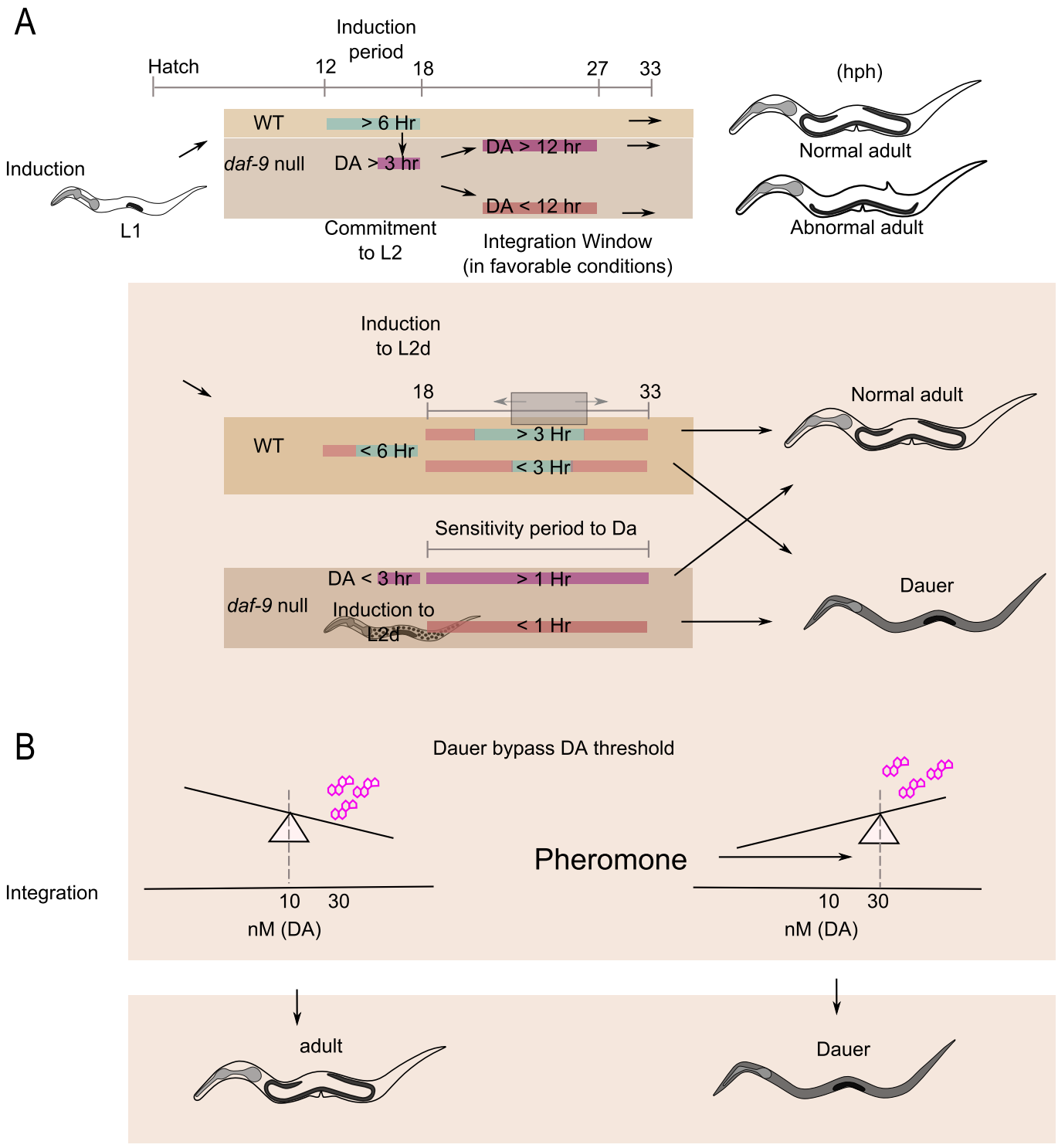
A
Induction period
Hatch
12
18
27
33
(hph)
WT
> 6 Hr
DA > 12 hr
Normal adult
Induction
daf-9 null
DA > 3 hr
DA < 12 hr
Abnormal adult
L1
Commitment to L2
Integration Window (in favorable conditions)
Induction to L2d
18
33
Normal adult
WT
< 6 Hr
> 3 Hr
< 3 Hr
Sensitivity period to Da
DA < 3 hr
> 1 Hr
Dauer
daf-9 null
Induction to L2d
< 1 Hr
B
Dauer bypass DA threshold
Integration
Pheromone
10
30
nM (DA)
10
30
nM (DA)
adult
Dauer

Figure 3.5: Effects of dose and temporal exposure of DA on *daf-9* worms

(A)Top; A tight correlation between the induction period of wild type worms exposed to pheromone and the sensitivity period of *daf-9* nulls to DA. Bottom; The integration window of wild type worms overlaps with the sensitivity period to DA. When wild type worms commit to dauer at 33 hph, *daf-9(dh6)* worms become refractory to DA leading to dauer development. (B) Integration of pheromone and DA into the dauer decision. *daf-9* worms display 2 thresholds for DA; the dauer bypass DA threshold at around 10nM is sufficient to bypass dauer, whereas an additional 30nM are required for complete adulthood. I modeled the mechanism as a balance when tipped to the right, causes commitment to adult development, whereas if maintained on the left leads to duaer commitment. Nascent DA levels can flip the balance to the right leading to adult commitment. Pheromone influences the position of the fulcrum; As the fulcrum is shifted to the right, more DA is required to flip the balance.