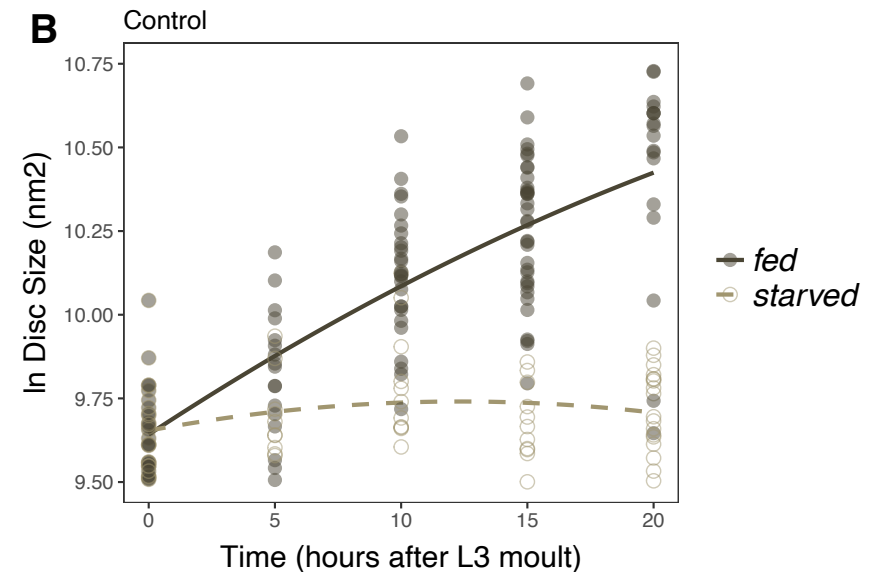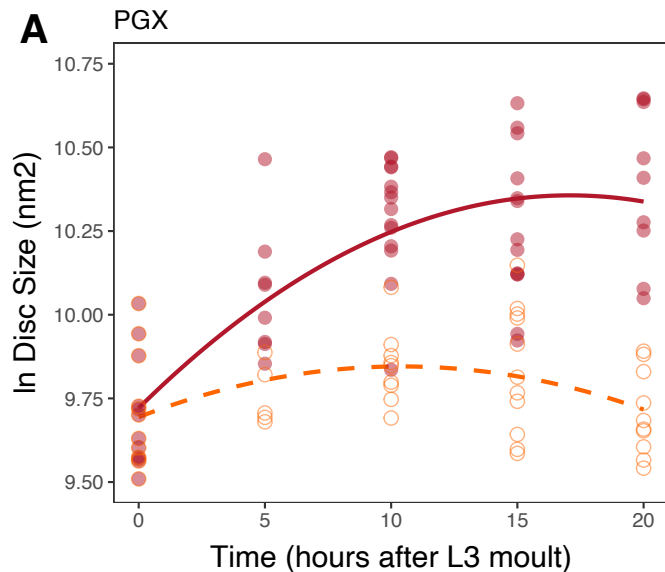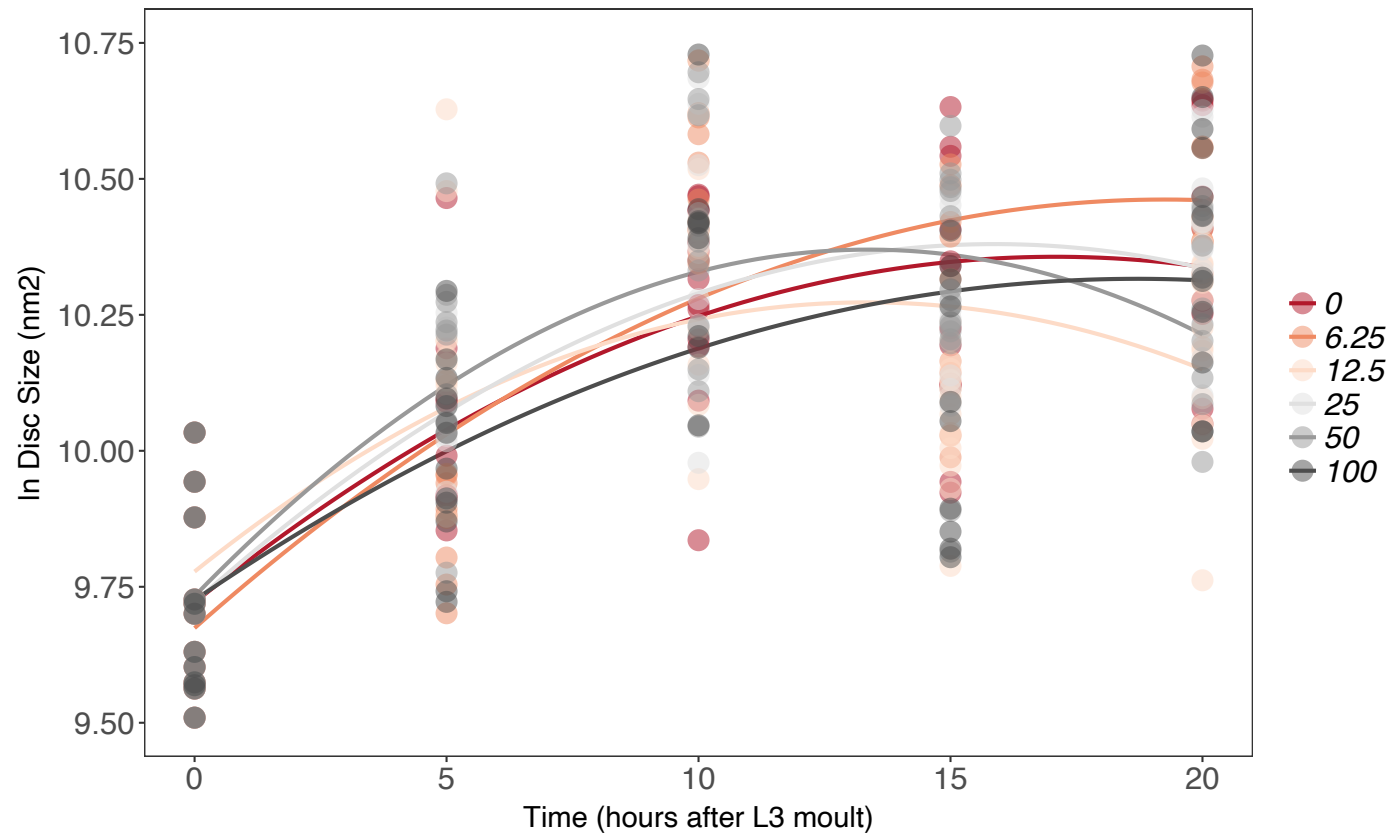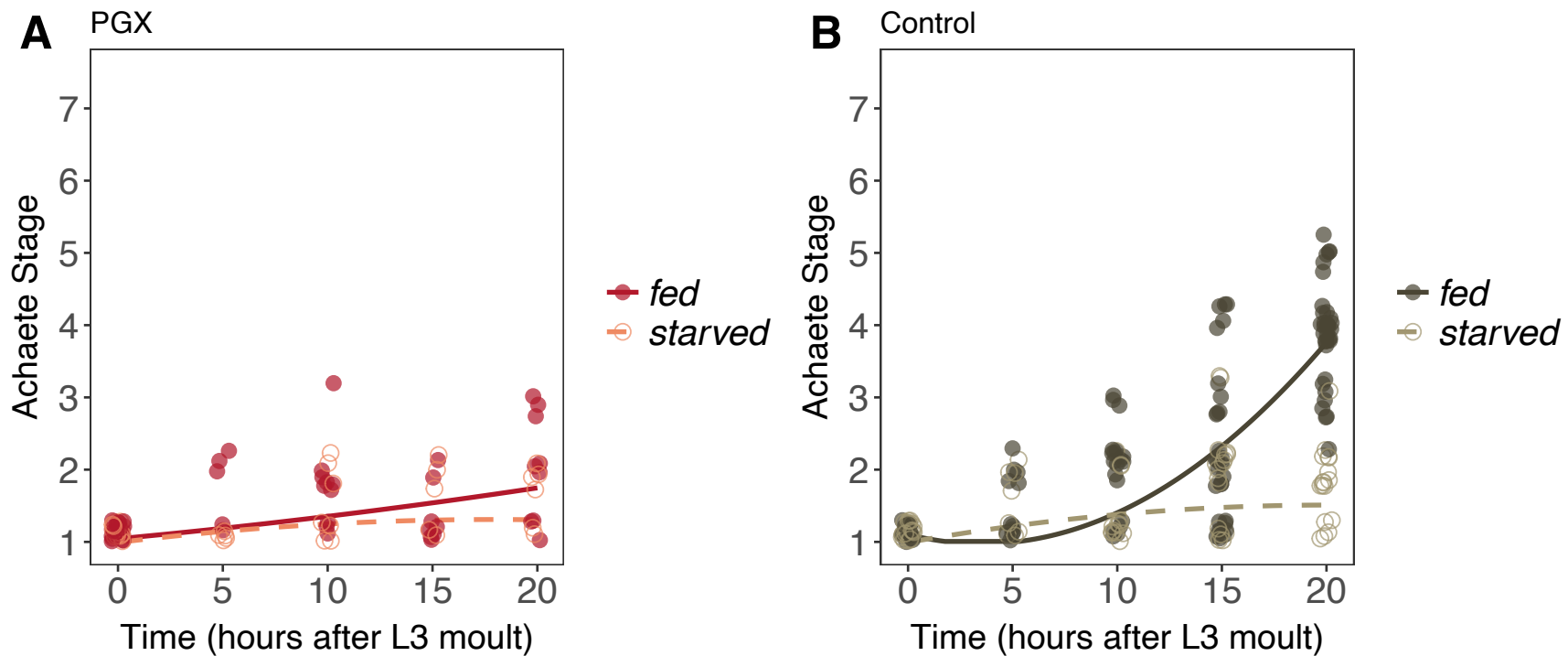# Supplementary Figures

Supplementary Figure 1: The effect of feeding 20E to larvae reared on a regular diet (fed) or 1% agar with 20% sucrose (starved) on circulating ecdysone levels. There is a significant positive relationship between ecdysone concentration in the food and the concentration of ecdysone in the larval blood (Supplementary Table 6).

Supplementary Figure 2: Wing imaginal disc growth is suppressed in fed PGX larvae relative to controls, and in starved larvae of both genotypes. Larvae were fed on either normal food, or on 20% sucrose, 1% agar medium. Wing disc growth was modelled as a quadratic, and there was a significant interaction between genotype (PGX v. Control) and nutrition (fed v. starved) on growth (Supplementary Table 7). Solid line/closed point = fed larvae, broken line/open point = starved larvae. Control genotypes are the pooled results from both parental controls (either the *phm-GAL4; GAL80ts* or *UAS-GRIM* parental strain crossed to w[1118]).

Supplementary Figure 3: There is no effect of supplemental ecdysone dose on growth of the wing imaginal disc in fed PGX larvae. Growth was modelled as $S = E + T + T^2 + E*T + E*T^2$, where S = disc size, E = 20E concentration, and T = time. There was no significant effect of E on the linear or quadratic growth rate of the wing imaginal discs (Supplementary Table 8).

Supplementary Figure 4: Achaete patterning in wing discs from fed and starved PGX and control larvae. (A) Patterning does not progress in either fed or starved PGX larvae. (B) Patterning does not progress in starved control larvae but does in fed control larvae. There is a significant interaction between the effects of time and food on Achaete patterning in control larvae (orthogonal polynomial regression: $F_{food*time^2}=67.98$, P < 0.001), but not in PGX larvae (orthogonal polynomial regression: $F_{food*time^2}=1.81$, P = 0.163). See supplementary materials for details. Control genotypes are the pooled results from both parental controls (either the *phm-GAL4; GAL80ts* or *UAS-GRIM* parental strain crossed to w[1118]).
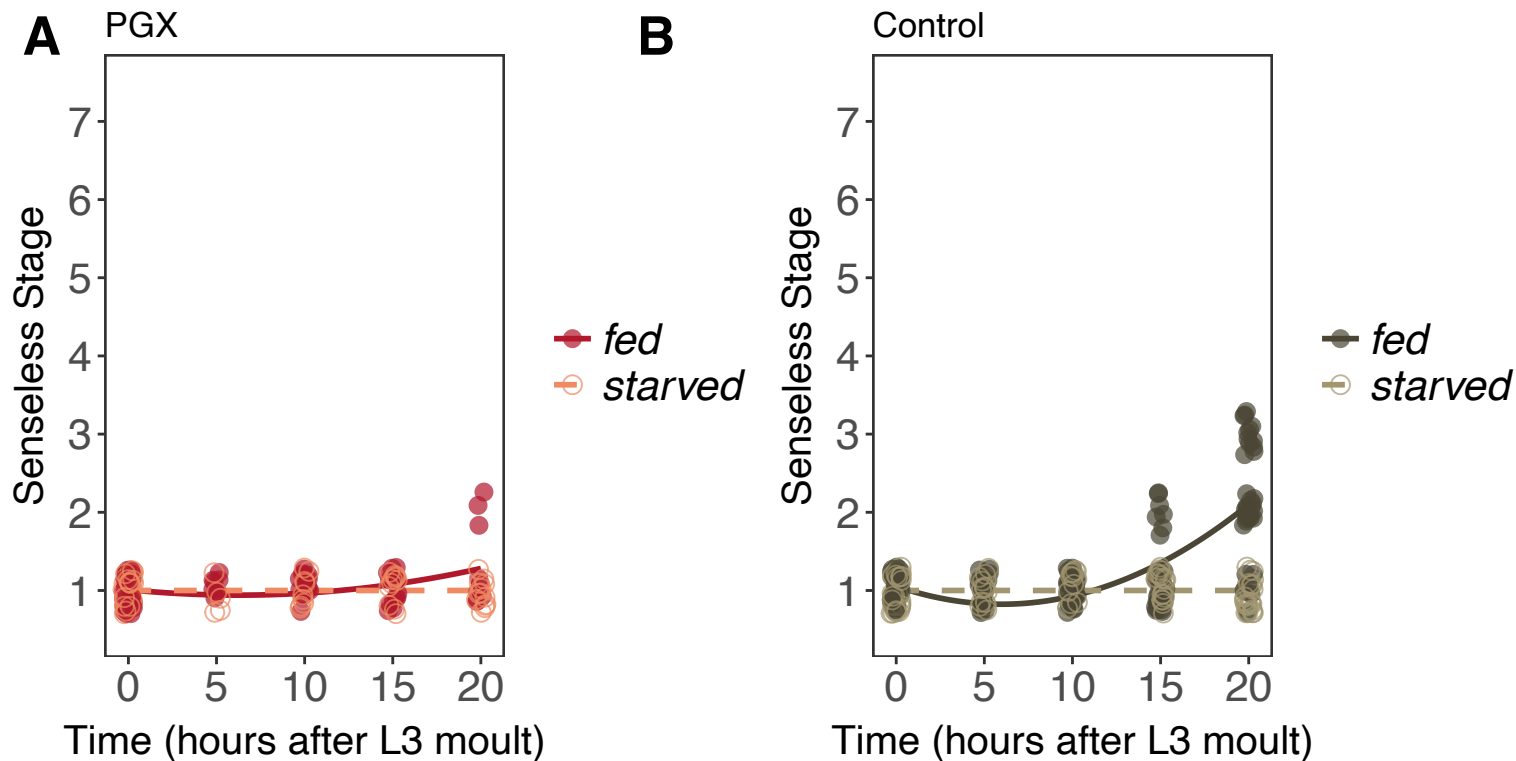
Supplementary Figure 5: Senseless patterning in wing discs from fed and starved PGX and control larvae. (A) Patterning does not progress in either fed or starved PGX larvae. (B) Patterning does not progress in starved control larvae but does in fed control larvae. There is a significant interaction between the effects of time and food on Achaete patterning in control larvae (linear regression: $F_{food*time}$=67.98, P < 0.001). In PGX larvae Senseless patterning does not progress at all in starved larvae (linear regression: F_{time}=0.057, P =0.82), but does in fed larvae (linear regression: F_{time}=9.76, P < 0.01). Control genotypes are the pooled results from both parental controls (either the *phm-GAL4; GAL80ts* or *UAS-GRIM* parental strain crossed to w[1118]).

Supplementary Tables

Supplementary Table 1: Comparing the Gompertz growth curve parameters for wing imaginal discs from PGX and control *(+>Grim* and *phm>+)* larvae.

Genotype/Condition	Asymptote (a)[A]	F[B]	Displacement along x (b)[A]	F[B]	Growth rate (c)[A]	F[B]
PGX	10.53 (10.45–10.65)	52.62***	0.11 (0.10–0.13)	68.91***	0.90 (0.85–0.94)	5.45*
Control	11.57 (11.42-11.74)		0.22 (0.21–0.24)		0.95 (0.94–0.95)	

[A] Values in parentheses are 95% confidence intervals.
[B] *F*-test of whether parameter value differs between genotype/condition. * *p* value < 0.05, *** *p* value < 0.0001.

Supplementary Table 2: Comparing the Gompertz curve parameters of Achaete patterning against time for wing imaginal discs from PGX and control *(+>Grim* and *phm>+)* larvae.

Genotype/Condition	Asymptote (a)[A]	F[B]	Displacement along x (b)[A]	F[B]	Growth rate (c)[A]	F[B]
PGX	3.98 (3.56–4.41)	12.62***	1.03(0.84–1.21)	12.05***	0.045 (0.029-0.063)	0.56
Control	6.34 (6.20–7.08)		1.64 (1.42–1.87)		0.074 (0.059-0.090)	

[A] Values in parentheses are 95% confidence intervals.
[B] *F*-test of whether parameter value differs between genotype/condition. * *p* value < 0.05, *** *p* value < 0.0001.

Supplementary Table 3: Comparing the linear Senseless patterning parameters for wing imaginal discs from PGX and control *(+>Grim* and *phm>+)* larvae.

Genotype/Condition	Intercept (a)[A]	F[B]	Slope (b)[A]	F[B]
PGX	1.25 (0.94–1.55)	25.89***	0.01(0.00–0.02)	583.26***
Control	0.46 (0.21–0.72)		0.13 (10.12–0.14)	

[A] Values in parentheses are 95% confidence intervals.
[B] *F*-test of whether parameter value differs between genotype/condition. * *p* value < 0.05, *** *p* value < 0.0001.

Supplementary Table 4: Comparing the parameters of the linear relationship for Achaete pattern against (log) disc size, between Samarkand larvae reared at 18°C, 25°C, and 29°C, between phm > InR and their parental control line (+ > InR), and between P0206 > PTEN and their parental control line (+ > PTEN).

Genotype/ Condition	Intercept (A) [A]	F[B]	Slope (B) [A]	F[B]
phm>InR	-14.99 (-17.32–-12.65)		1.93 (1.70-2.15)	
P0206>PTEN	- 12.23(-13.72–-10.75)	22.42***	1.61 (1.44–1.78)	660.67**
Control	-16.40 (-18.45–-14.23)		1.97 (1.83-2.10)	

[A] Parameter values are for a linear model $y = B + Ax$, where x is disc size and y is Achaete pattern. If a parameter value was not significantly different between genotypes/conditions, the model was re-fit with a single value for that parameter across genotype/conditions, and the remaining parameter values were recalculated.
[B] *F*-test for when the parameter value differs between genotype/condition. [NS] *p* value > 0.05, **p* value < 0.05, **p* value < 0.01, *** *p* value < 0.001.

Supplementary Table 5: Comparing the parameters of the logistic relationship for Senseless pattern against (log) disc size, between Samarkand larvae reared at 18°C, 25°C, and 29°C, between phm > InR and their parental control line (+ > InR), and between P0206 > PTEN and their parental control line (+ > PTEN).

Genotype/ Condition	Minimum (A)[A]	F[B]	Maximum (B)[A]	F[B]	Point of inflection (C)[A]	F[B]	Logistic Growth rate (D)[A]	F[B]
Phm>InR	0.92 (0.32 – 1.52)		7.13 (5.72–8.54)		10.42 (10.14–10.69)		2.13 (1.40 – 4.45)	
P0206>PTEN	1.00 (0.73-1.27)	0.12[NS]	7.15 (6.31–8.00)	3.61*	10.97 (10.84–11..10)	6.94**	4.65 (3.31–7.84)	5.89**
Control	1.04 (0.80-1.29)		8.91 (7.08–10.73)		11.22 (10.99–11.45)		2.39 (1.86–3.35)	

[A] Parameter values for the four-parameter logistic model $y = A + (B - A)/(1 + e^{D(C-x)})$, where x is disc size and y is Senseless pattern. Values in parentheses are Bonferroni-corrected 95% confidence intervals. If a parameter value was not significantly different between genotypes/conditions, the model was re-fit with a single value for that parameter across genotype/conditions, and the remaining parameter values were re-calculated.
[B] F-test for when the parameter value differs between genotype/condition. [NS] p value > 0.05, *p value < 0.05, *p value < 0.01, *** p value < 0.001.

Supplementary Table 6: Effect of ecdysone supplementation on titres of ecdysone in the larval blood of control (*phm > +* and *+ > grim*) and PGX larvae under starved and fed conditions.

Factor[A]	df	Chi Square	P
Ecdysone	1	59.61	<0.001
Food	2	179.13	<0.001
Genotype	2	2.71	0.32
Ecdysone: Food	1	8.02	0.005
Ecdysone: Genotype	2	13.16	0.001
Food: Genotype	2	2.46	0.29
Ecdysone: Food: Genotype	2	11.93	0.003

Supplementary Table 7: Effect of diet type on wing disc growth in PGX and control (*phm > +* and *+ > grim*) larvae under starved and fed conditions.

Factor	df	*F value*	*P*
Genotype	1	1.4	0.24
Food	1	278.5	<0.001
Time^2	2	202.5	<0.001
Genotype: Food	1	8.7	0.003
Genotype: Time^2	2	13.2	<0.001
Food: Time^2	2	98.7	<0.001
Genotype: Food: Time^2	2	5.31	0.005

Supplementary Table 8: Effect of ecdysone concentration on growth of the wing imaginal disc in starved and fed PGX larvae.

Factor[A]	Starved				Fed			
	SS	df	*F*	*P*	SS	df	*F*	*P*
Ecdysone	1.5	1	27.3	<0.001	0	1	1.35E-01	0.7137
Disc Age^2	7.2	2	64.6	<0.001	36.6	2	29.8	<0.001
Ecdysone: Disc Age^2	1	2	8.88	<0.001	0	2	4.01E-01	0.6697
Residuals	22.3	403			27.8	453		

[A] Factors where fit using an orthogonal polynomial regression.

Supplementary Table 9: Parameters describing the effect of ecdysone on growth of the wing imaginal disc in starved PGX larvae.

Parameter[A]	Parameter Value[B]	t-value[C]	P
Intercept	9.48	604.39	<0.001
Ecdysone	0.0002	5.22	<0.001
Disc Age	0.059	10.06	<0.001
Disc Age^2	-0.0021	-5.20	<0.001
Ecdysone * Disc Age	0.0004	3.80	<0.001
Ecdysone * Disc Age^2	<0.0001	-1.81	0.0707

[A] Disc size through time was modelled as an orthogonal polynomial regression $S = E + T + T^2 + E*T + E*T^2$, where S = disc size, E = ecdysone concentration, and T = disc age.

[B] Parameter values are for non-orthogonal polynomial regression to facilitate interpretation.

[C] t-value is for orthogonal parameters

Supplementary Table 10: Comparing the linear/Gompertz curve parameters of Achaete patterning against time for wing imaginal discs from fed PGX larvae supplemented with different quantities of ecdysone

Ecdysone (ng/ml of food)	Intercept [C]	F [D]	Slope [C]	F [D]	Asymptote (a) [C]	F [D]	Displacement along x (b) [C]	F [D]	Growth rate (c) [C]	F [D]
0 [A]	1.03 (0.82-1.24)	0.10	0.03 (0.01-0.05)	2.41						
6.25[A]	1.08 (0.79-1.38)		0.05 (0.03-0.08)							
6.25 [B]					2.08 (1.39-2.77)	7.89***	0.77 (0.28-1.25)	6.49***	0.19 (-0.10-0.58)	1.20
12.5 [B]					3.5 (3.06-3.95)		1.3 (0.96-1.65)		0.22 (0.10-0.34)	
25 [B]					4.7 (3.76-5.65)		1.55 (1.24-1.85)		0.14 (0.08-0.22)	
50 [B]					4.54 (3.74-5.33)		1.55 (1.24-1.85)		0.16 (0.09-0.23)	
100 [B]					5.27 (4.21-6.33)		1.72 (1.40-2.04)		0.14 (0.08-0.20)	

[A] Patterning through time was modelled as a linear function $S = E + T + E*T$, where S is patterning stage, E is supplemental ecdysone level (categorical) and T is time (continuous).

[B] Patterning through time was modelled as Gompertz function fit to each supplemental ecdysone level.

[C] Values in parentheses are Bonferroni-corrected 95% confidence intervals for all pairwise comparisons.

[D] F-test of whether parameter value differs between ecdysone levels. * p value < 0.05, *** p value < 0.0001. See supplementary methods for details.

Supplementary Table 11: Comparing the linear/Gompertz curve parameters of Achaete patterning against time for wing imaginal discs from starved PGX larvae supplemented with different quantities of ecdysone

Ecdysone (ng/ml of food)	Intercept [C]	F^D	Slope [C]	F^D	Asymptote (a) [C]	F^D	Displacement along x (b) [C]	F^D	Growth rate (c) [C]	F^D
0 [A]	1.01 (0.77-1.26)		0.02 (0.00-0.04)							
6.25 [A]	0.96 (0.62-1.30)	0.07	0.03 (0.00-0.06)	7.65***						
12.5 [B]	1.00 (0.66-1.34)		0.07 (0.04-0.10)							
12.5 [B]					4.24 (-12.90 - 21.37)		1.44 (-2.47-5.36)		0.04 (-0.13-0.26)	
25 [B]					9.00 (1.37- 16.63)	1.05	2.28 (1.54-3.04)	0.81	0.06 (0.02-0.11)	1.51
50 [B]					5.54 (3.94-7.15)		1.84 (1.44-2.25)		0.12 (0.06-0.20)	
100 [B]					6.41 (4.17-8.65)		2.00 (1.59-2.41)		0.11 (0.05-0.17)	

[A] Patterning through time was modelled as a linear function $S = E + T + E*T$, where S is patterning stage, E is supplemental ecdysone level (categorical) and T is time (continuous).

[B] Patterning through time was modelled as Gompertz function fit to each supplemental ecdysone level.

[C] Values in parentheses are Bonferroni-corrected 95% confidence intervals for all pairwise comparisons.

[D] F-test of whether parameter value differs between ecdysone levels. * p value < 0.05, *** p value < 0.0001. See supplementary methods for details.

Supplementary Table 12: Comparing the linear model parameters of Senseless patterning against time for wing imaginal discs from fed PGX larvae supplemented with different quantities of ecdysone

Ecdysone[A] (ng/ml of food)	Intercept[B]	F^C	Slope[B]	F^C	Tukey HSD[D]
0	0.97 (0.77–1.17)		0.01 (-0.01–0.03)		A
6.25	0.99 (0.51–1.46)		0.02 (-0.03–0.06)		A
12.5	1.03 (0.55–1.5)	0.2627	0.03 (-0.02–0.07)	45.1206***	A
25	0.95 (0.47–1.42)		0.08 (0.04–0.13)		B
50	0.97 (0.5–0.26)		0.09 (0.05–0.14)		B
100	0.93 (0.46–1.4)		0.1 (0.05–0.14)		B

[A] Patterning through time was modelled as a linear function $S = E + T + E*T$, where S is patterning stage, E is supplemental ecdysone level (categorical) and T is time (continuous).

[B] Values in parentheses are Bonferroni-corrected 95% confidence intervals for all pairwise comparisons.

[C] F-test of whether parameter value differs between ecdysone levels. * p value < 0.05, *** p value < 0.0001. See supplementary methods for details.

[D] Tukey HSD comparison of slopes among ecdysone levels. Levels with different letters are significantly different.

Supplementary Table 13: Comparing the linear parameters of Senseless patterning against time for wing imaginal discs from starved PGX larvae supplemented with different quantities of ecdysone

Ecdysone[A] (ng/ml of food)	Intercept [B]	F[C]	Slope [B]	F[C]	Tukey HSD[D]
0	1 (0.74–1.26)		0 (-0.02–0.02)		A
6.25	0.98 (0.35–1.6)		0.01 (-0.05–0.06)		A
12.5	0.97 (0.34–1.59)	0.1958	0.02 (-0.03–0.08)	53.9490***	A
25	0.9 (0.28–1.53)		0.09 (0.03–0.14)		B
50	0.95 (0.33–0.27)		0.1 (0.05–0.16)		B
100	0.91 (0.29–1.53)		0.14 (0.08–0.19)		C

[A] Patterning through time was modelled as a linear function $S = E + T + E*T$, where S is patterning stage, E is supplemental ecdysone level (categorical) and T is time (continuous).

[B] Values in parentheses are Bonferroni-corrected 95% confidence intervals for all pairwise comparisons.

[C] F-test of whether parameter value differs between ecdysone levels. * p value < 0.05, *** p value < 0.0001. See supplementary methods for details.

[D] Tukey HSD comparison of slopes among ecdysone levels. Levels with different letters are significantly different.