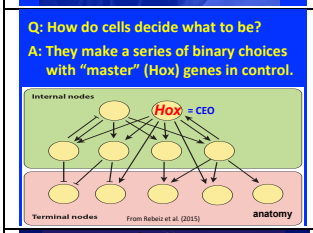
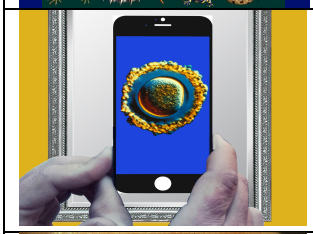
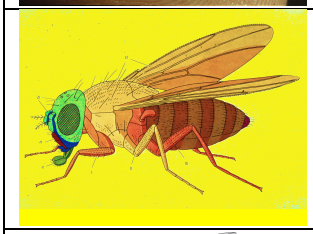
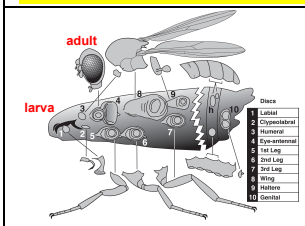
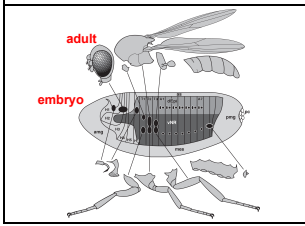
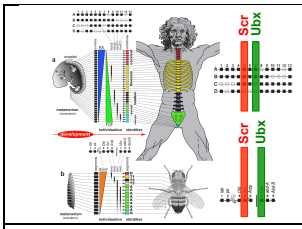
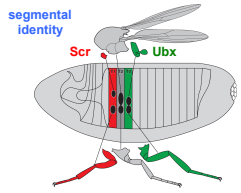
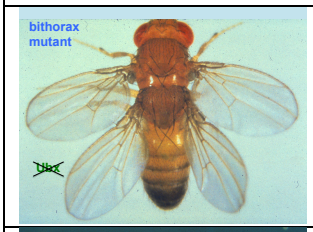
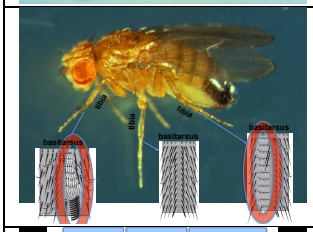
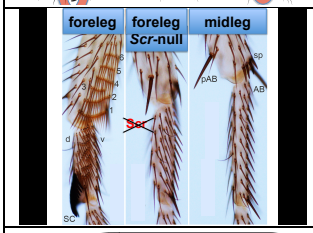
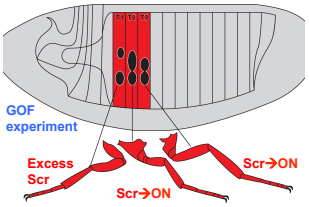
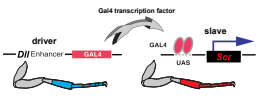
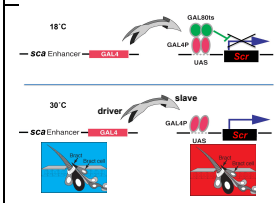
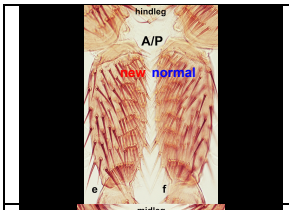
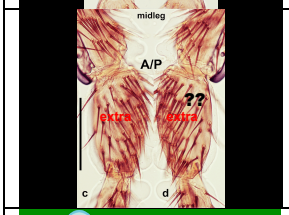
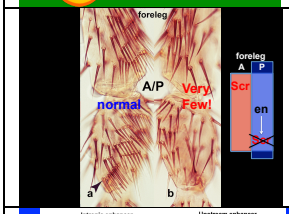
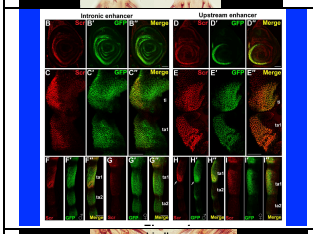
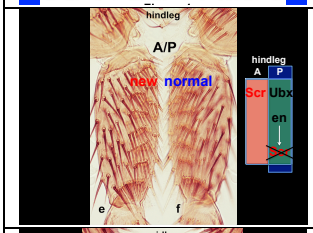
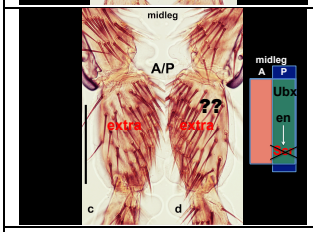
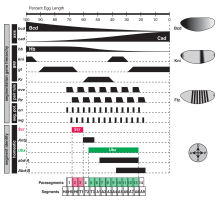
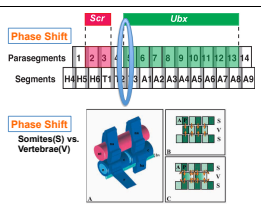
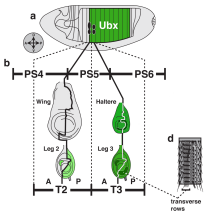
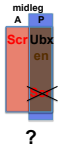
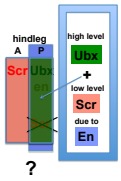
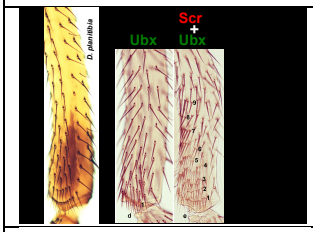
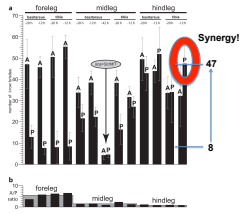
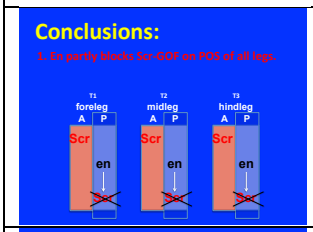
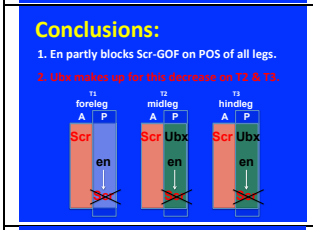
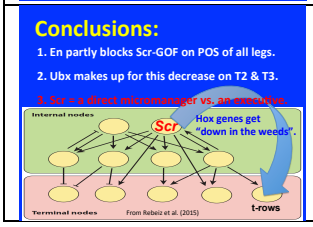
Hamlet on the Fly

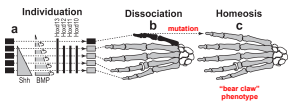
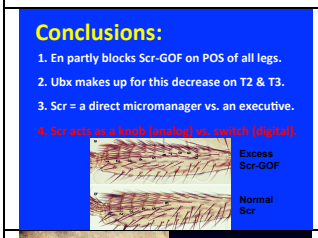
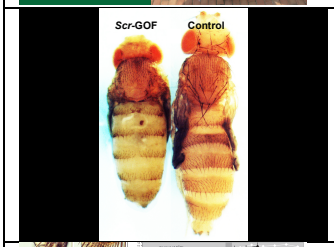
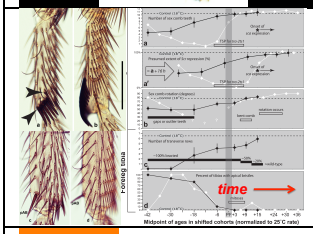
7 February 2018

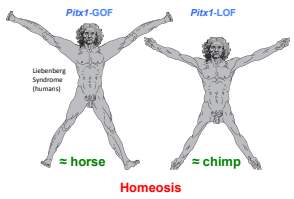
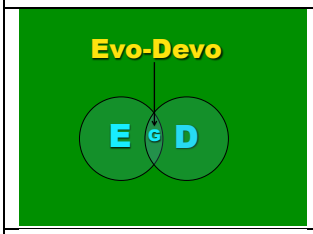
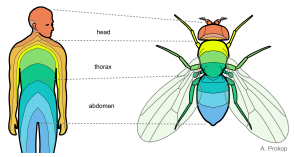
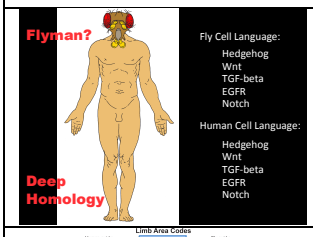
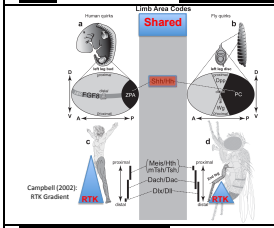
	One reason I chose the Hamlet theme: There will be lots of plot twists in the story.
	Humans have ~200 diff. cell types: skin, nerve, muscle, etc. Q: How do cells adopt correct identities during dev't? A: ~BINARY (be/not be) decisions. Genes = ON/OFF. By the end of talk: show that this view is partly incorrect.
	Glorious diversity of animals on Earth. We normally think about animals as ADULTS. But every animal starts as a fertilized EGG.
	That includes you. = Your earliest selfie! Fertilized egg of a human is so tiny ... ... that it can fit on the head of a pin.
	Can't do expts on humans, so use other animals instead. Flies = useful. Fly dev't is interesting b/c the baby looks nothing like adult. Larva = maggot. Analogous to tadpole. Metamorphosis.
	Adult (imago) body is assembled like a quilt (jigsaw puzzle) from individual pieces.
	Pieces = imaginal discs (≈ modules). They grow inside larva (≈ balloons) & evert. 10 different identities.
	Discs (black ovals) come from diff. segments in the embryo. 6 head segments, 3 thoracic segments, & 10 abd. segments.

	Segmental identity is handled by Hox genes (clusters). Transcription factors w/ DNA-binding domain (homeobox). Same area codes in humans & flies (common ancestor). Humans have genes that are homologs of Scr & Ubx.
	Today: Focus on the 3 thoracic segments. Identities of T1 and T3 controlled by Hox genes Scr & Ubx.
	Many of you know the story of the "bi-thorax" mutation. Disable Ubx: T3 → T2. Haltere → wing.
	Hindleg also transforms → midleg. Foreleg & hindleg differ from midleg. Transverse rows = brushes to clean the eyes & wings. Also foreleg has sex comb = grasps female during mating.
	If you disable Scr, T1 leg → T2 identity. T-rows & sex comb disappear. Defaults to T2 state just like Ubx-LOF.
	What happens if you force Scr to be expressed in all legs? Interesting b/c it confuses the cells → Reveal secrets? But backfired: confused me! GOF (Gain of Function). T1: no change expected. Q: T2 & T3 → T1 identity?
	Method: Gal4 & UAS transgenes. Distal-less driver (master) with Scr "slave". UAS = Upstream Activating Sequence.
	Additional details of the approach we used. Gal80ts control timing via 18° vs. 29° C. Scabrous driver forces Scr to be expressed in bristle cells (& surrounding cells) only.

	Mug shots of hindleg basitarsus. T-rows on ANT = expected. But surprise: t-rows remain on POS! Confusing: Scr doesn't overrule Ubx?
	Midleg basitarsus: t-rows on both faces. Confusing: Scr normally only affects ANT. But evidently it can work on both POS & ANT.
	We had assumed that Scr → foreleg identity. **RETHINK:** Maybe its role is to induce t-rows? And it just so happens to only be expressed in ANT.
	No! Scr-GOF hardly affects POS of foreleg at all! Why not? Maybe engrailed is suppressing Scr? (Is that why Scr is normally confined to the ANT?)
	Although Scr is strongly expressed in t-rows & sex comb. I had assumed Scr is activated only on the ANT side. [C = Scr expression (dots = nuclei); C' = GFP (all cells).]
	Mug shots of hindleg basitarsus. T-rows on both faces. Easy to explain if Ubx → t-rows (like Scr → t-rows). On a roll. But then … hit brick wall.
	Why isn't engrailed inhibiting Scr on the midleg? We argue that it is doing so, but Ubx makes up for it. Ubx is expressed in T2??? Yes! At low levels.
	The reason Ubx is expressed in the POS half of T2? Initially, Scr, Ubx, etc. = expressed in "parasegments." Segmentation gene hierarchy.
	Parasegments = offset from segments by engrailed stripe. Enigma. But humans also have phase shift: somites vs. vertebrae.

	Parasegment 5 includes POS side of T2. Ubx fades from wing disc. (High level in t-rows on hindleg.) But retained at low level (light green) in POS of midleg.
… so when we force *Scr* to be expressed on both sides, *en* partly suppresses *Scr*, but there might be enough Scr left to interact with Ubx so as to make t-rows on P side. 	RECAP: Scr = partly suppressed by en, but enough remains for it to cooperate with low-level Ubx to elicit t-rows. Low Scr + Low Ubx → Exceed threshhold. If so, then Scr should interact synergistically with Ubx … WHEREVER they overlap. So re-examine the hindleg.
Test: See whether there are excess t-rows on the P side on the hindleg. 	On the hindleg, Scr overlaps with HIGH-level Ubx.
	Yes! Synergy! Normally POS = only 1 row of 8 bristles. This is an evol. conserved hard-wired trait (50 MY old). But notice that Scr-GOF elicits as many as 9 t-rows total!
	Summary of quantitative data: Number of t-row bristles increases from 8 to 47 (mean). Review: Effects of Scr-GOF on T1, T2, T3. A/P ratios!
	1. Scr gets confined to ANT b/c En inhibits it. **Test:** en-LOF clones on P side of leg . *Done by Teresa.*
	2. Scr and Ubx interact synergistically. **Test:** Express both together.
	3. Hox genes (at late stages) ≈ paintbrushes for touch-up? **Test:** Disable Scr at late stages (sca-GAL4). DIRECTLY affecting downstream decisions. Leaving penthouse suite @ Trump Tower → assembly line.

	Similar thing in vertebrates. Late role ≠ early role. Re-purposing of Hox genes for non-segment roles. Ex.: Recruiting of Hox genes for finger identity
	Mutate Hox13 (d cluster) → "bear claw" phenotype. Thumb transforms into an ordinary finger.
	4. Scr acts as a knob (analog) vs. switch (digital). Ubx also. Best example of dosage effect = tibia in 1st leg of Scr-GOF. Excess Scr causes additional t-rows (10 vs. 6) on foreleg.
	Given these dosage effects, title → Measure for Measure?
	Or … given that we've demoted these exalted Hox genes to the level of functioning of ordinary transcription factors, Maybe the title of talk → Much Ado About Nothing
	Acknowledgements: 3 undergrad apprentices: Andrew Davis, Rachel Aybar, and Zack Fitzgerald. Teresa Orenic did the staining of discs for Scr expression.
	Enigmatic result. Scr-GOF. Deleted macro- but not microchaetes.
	Summary of LOF effects. Time course: disable Scr.

	
	Pitx1-LOF ≈ Liebenberg Syndrome.
	Qs → Evo-devo (E & D). G = Genetics. Playground.
	
	Same signaling pathways! DEEP homology. Conserved TOOLKIT.
	Fly legs vs. human legs. Look diff., but same recipe! [Gerard Campbell]