CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2023-2024 : 18 mars, 2024

L'épigénétique à l'interface organisme-environnement

Cours III

Exemples d'impacts environnementaux sur le règne animal

E. Heard

COLLÈGE DE FRANCE 1530

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

L'épigénétique à l'interface organisme-environnement

4 mars
Cours 1: Introduction

11 mars
Cours 2: Comment l'environnement influence-t-il les phénotypes ?

18 mars
Cours 3: Exemples d'impacts environnementaux sur le règne animal

25 mars
Cours 4: Exemples d'impacts environnementaux sur le règne végétal

SUMMARY of LAST WEEK (Cours II)

How does the environment influence phenotypes?

Phenotypic plasticity within a lifetime

Environmentally programmed phenotypes

Environmentally induced cross-generational parental phenotypes

Environmentally induced trans-generational bet-hedging / phenotypic plasticity

Environmentally plastic responses that pave the way for *permanent* adaptations

Impact of rapid and dramatic changes in environment on phenotypes: stress, survival, adaptation or extinction

SUMMARY of LAST WEEK (Cours II)

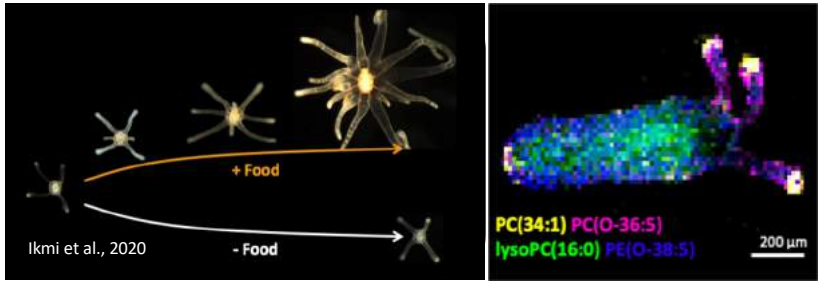
How does the environment influence phenotypes?

Phenotypic plasticity within a lifetime

Environmentally programmed phenotypes

How does the nutritional environment influence phenotypes?

Diet-induced Polyphenism of *Nematostella* sea anemone: tentacle number varies depending on nutrient supply during development (Ikmi et al, 2020)



Diet-induced Polyphenism of *Nemoria* moth caterpillars

The catkin (left) and twig (right) morphs in caterpillars of the moth Nemoria arizonaria (photo Erik Greene).

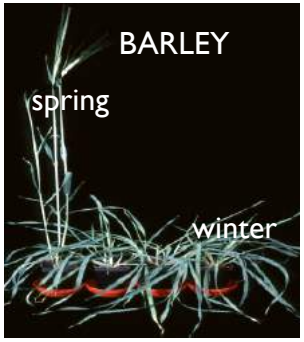
SUMMARY of LAST WEEK (Cours II)

How does the environment influence phenotypes?

Phenotypic plasticity within a lifetime

Environmentally programmed phenotypes

How does the length of cold influence flowering time?



Plants are completely dependent on subtle aspects of the weather to survive Eg Vernalization – a period of cold required for appropriate flowering timing (more next week)

Plants that need to be vernalised include important food species such as sugar beet and wheat, which feed millions and provide much-needed income globally.

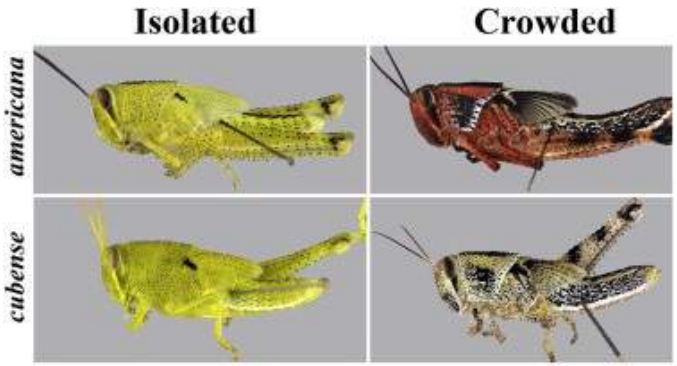
SUMMARY of LAST WEEK (Cours II)

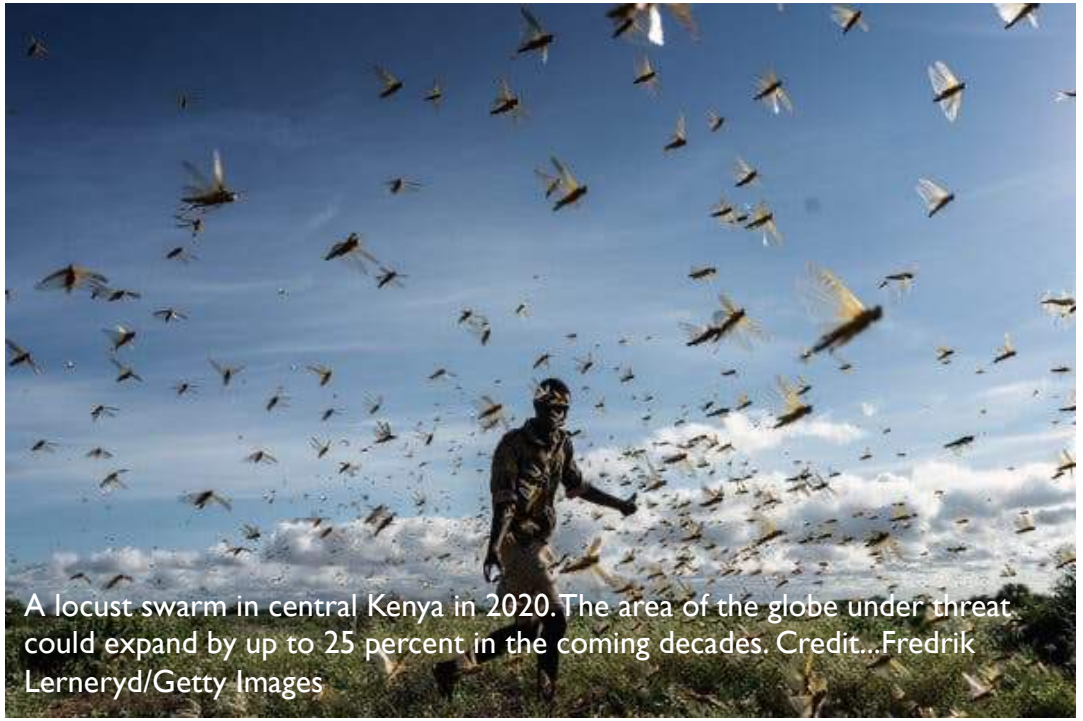
How does the environment influence phenotypes?

Phenotypic plasticity within a lifetime

Environmentally programmed phenotypes

How does dramatic phase transition in locusts occur?





A locust swarm in central Kenya in 2020. The area of the globe under threat could expand by up to 25 percent in the coming decades. Credit...Fredrik Lerneryd/Getty Images

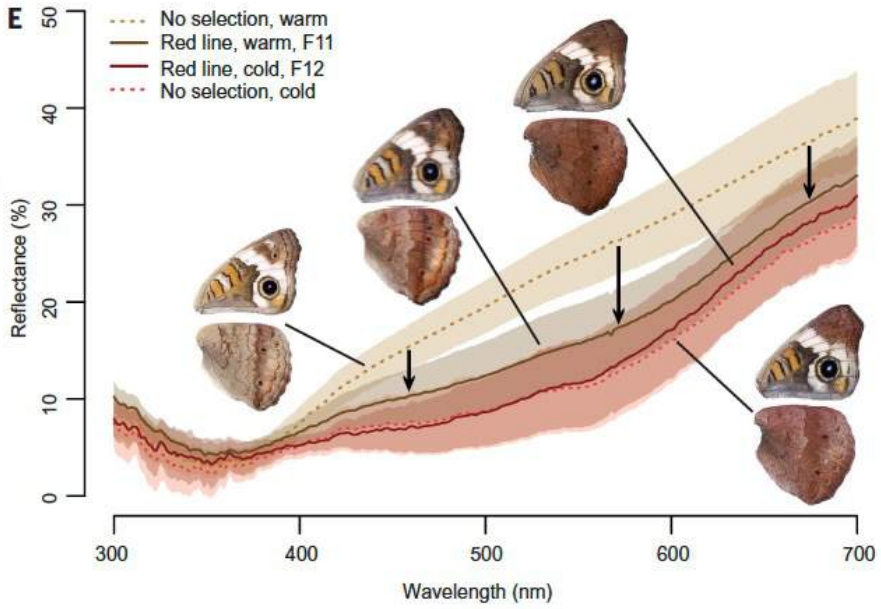
SUMMARY of LAST WEEK (Cours II)

How does the environment influence phenotypes?

Phenotypic plasticity within a lifetime

Environmentally programmed phenotypes

How do seasons affect different morphs of butterflies?



SUMMARY of LAST WEEK (Cours II)

How does the environment influence phenotypes?

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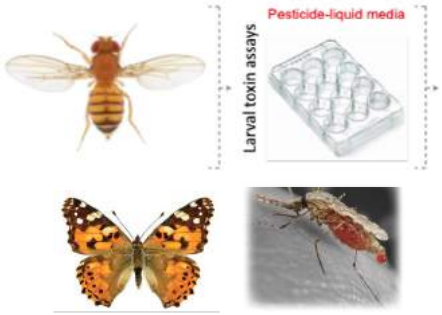
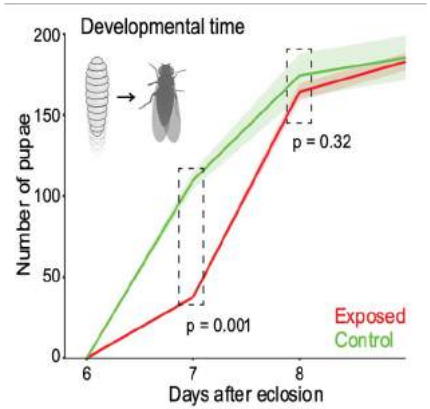
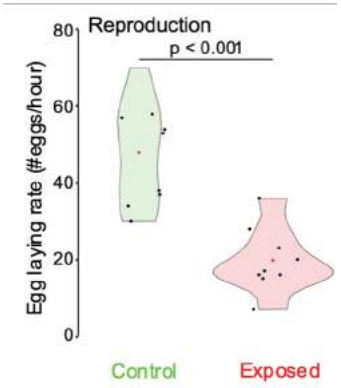
How do pesticides & increased temperature affect insect development and survival?

Increasing pesticide concentrations impact behaviour and growth of caterpillar larvae
Effects are amplified when ambient temperature is increased by four degrees







Lautaro et al, biorxiv
https://doi.org/10.1101/2024.01.12.575373

Sublethal doses of agrochemicals together with temperature increases contribute to global decline in insect populations: need targeted pest control strategies

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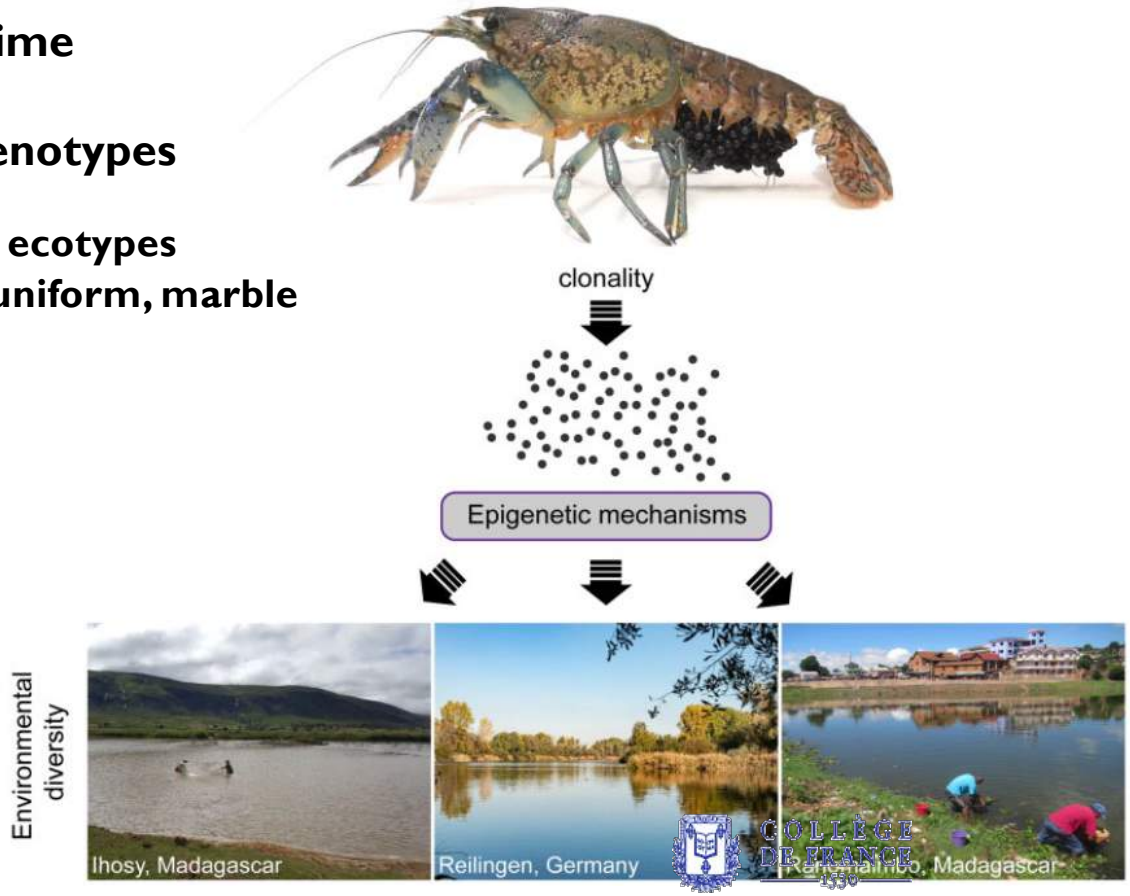
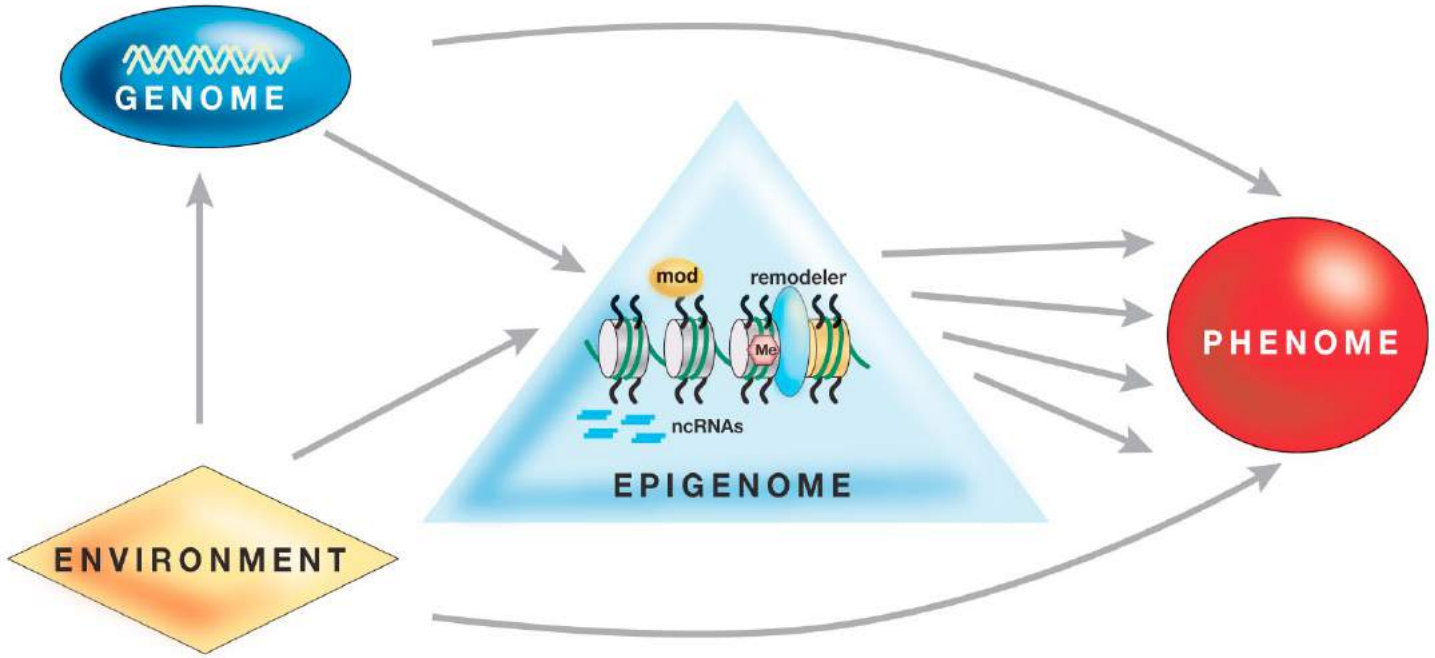
How are different epigenetic ecotypes produced by the genetically uniform, marble crayfish?



Phenotypic Plasticity and Polyphenism

Phenotypic output is defined by DNA-sequence (genetics), chromatin regulation (epigenetics and cellular memory) and environmental variables (e.g., nutritional sufficiency), and their interactions.



Epigenetic regulation underpins the stable phenotypic divergences that exemplify polyphenism. Modifications of DNA, RNA, histones, and the many proteins and metabolites that interact with transcriptional machinery, as well as the signaling circuitry that reinforces transcriptional output from one cell division to the next once an original stimulus has past.

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Phenotypic plasticity may facilitate adaptive changes and increase phenotypic diversity, thus better withstand changes in environment

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Polyphenism describes a unique sub-type of phenotypic plasticity whereby the phenotypes are not continuous, but discrete: several distinct phenotypes on the same genetic background (eg queen bees and workers)).

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Phenotypic robustness or "canalization" of phenotype describes the resistance of phenotypic development to environmental perturbations.
Plasticity and robustness are complementary rather than opposing concepts.

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Plasticity and robustness are complementary rather than opposing

Multiple physiological pathways pathways translate environmental variation into reproducible phenotypic modifications - including epigenomic changes during development that mediate and maintain the phenotypic divergences that exemplify polyphenism.

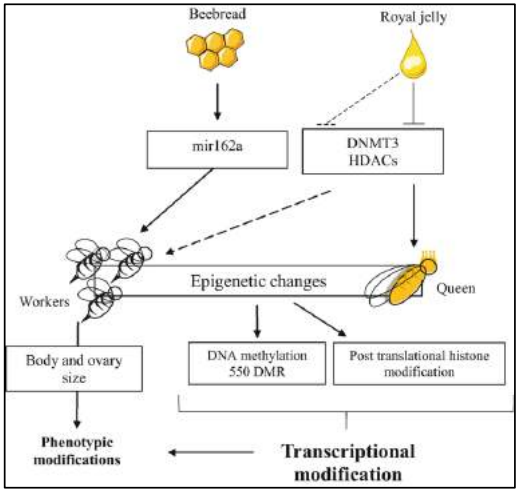
Epigenetic regulation underpins the stable phenotypic divergences that exemplify polyphenism. Modifications of DNA, RNA, histones, and the many proteins and metabolites that interact with transcriptional machinery, as well as the signaling circuitry that reinforces transcriptional output from one cell division to the next once an original stimulus has past.

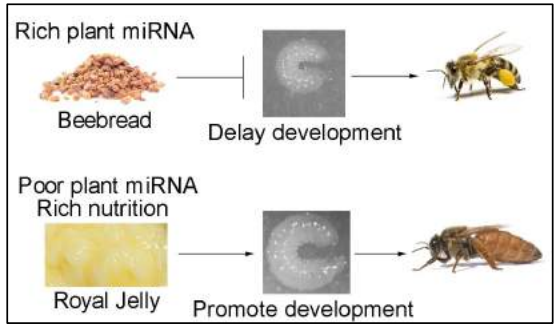
Epigenetic mechanisms underlying phenotypic plasticity and polyphenism in the Animal Kingdom

Caste Polyphenism:

Eusocial insects – queens/workers
Honey bees, ants

Eusocial mammals
Naked mole rats





See COURS 1, 2019

Mouse/Human Polyphenism in body composition/obesity?

Phenotypic variation that occurs even when both inter-individual genetic and environmental differences are controlled suggests additional dimensions must contribute to trait variation.

Eusocial Ants

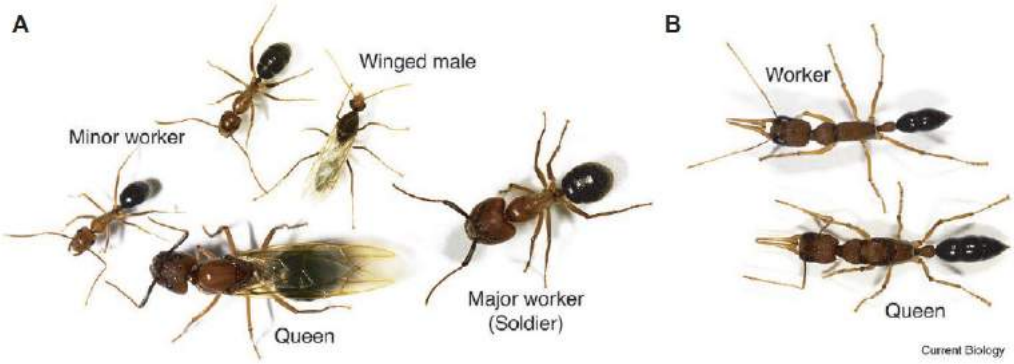
Caste Polyphenism: Camponotus floridanus
Florida carpenter ants

The ability to dynamically adapt behaviour in response to a variable environment is one of the hallmarks of complex life...

Ants acquire distinct morphological and behavioral phenotypes arising from a common genome, underscoring the importance of epigenetic regulation.

What are the molecular mechanisms governing such behavioural plasticity?

How is epigenetic regulation involved?



Chittka et al., 2012

Shelley Berger

E. Heard

Danny Reinberg

For review see: Opachaloemphan et al, 2018. "Recent Advances in Behavioral (Epi)Genetics in Eusocial Insects"

In Camponotus floridanus, "Major" workers defend the colony, but can be *epigenetically reprogrammed* to forage for food analogously to "Minor" workers.

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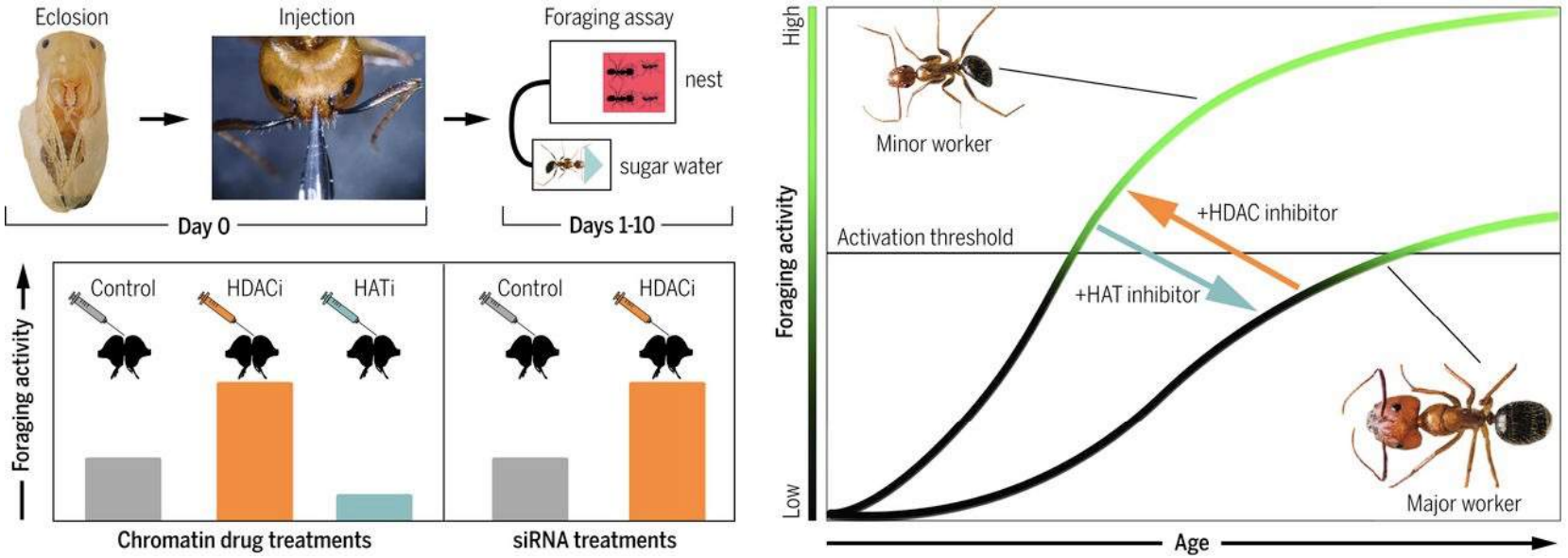
Epigenetic basis for Polyphenism in Ants

- Epigenetic (re)programming of caste-specific behavior in the ant Camponotus floridanus (Simola et al, Science 2016) (COURS 2019)

doi: 10.1126/science.aac6633



An epigenetic model for division of labour:

Left: Workers were injected at eclosion and tested for foraging activity. HDAC inhibition (HDACi) with chromatin drugs or siRNAs, enhance foraging. HATi suppress foraging. Minor and major workers express distinct behavioral ontogenies.

Right: Minors forage earlier in life and with greater intensity than majors. HDACi in majors stimulated minor-like foraging behavior, a gain of function suppressed by HATi treatment.

E. Heard, November 2018

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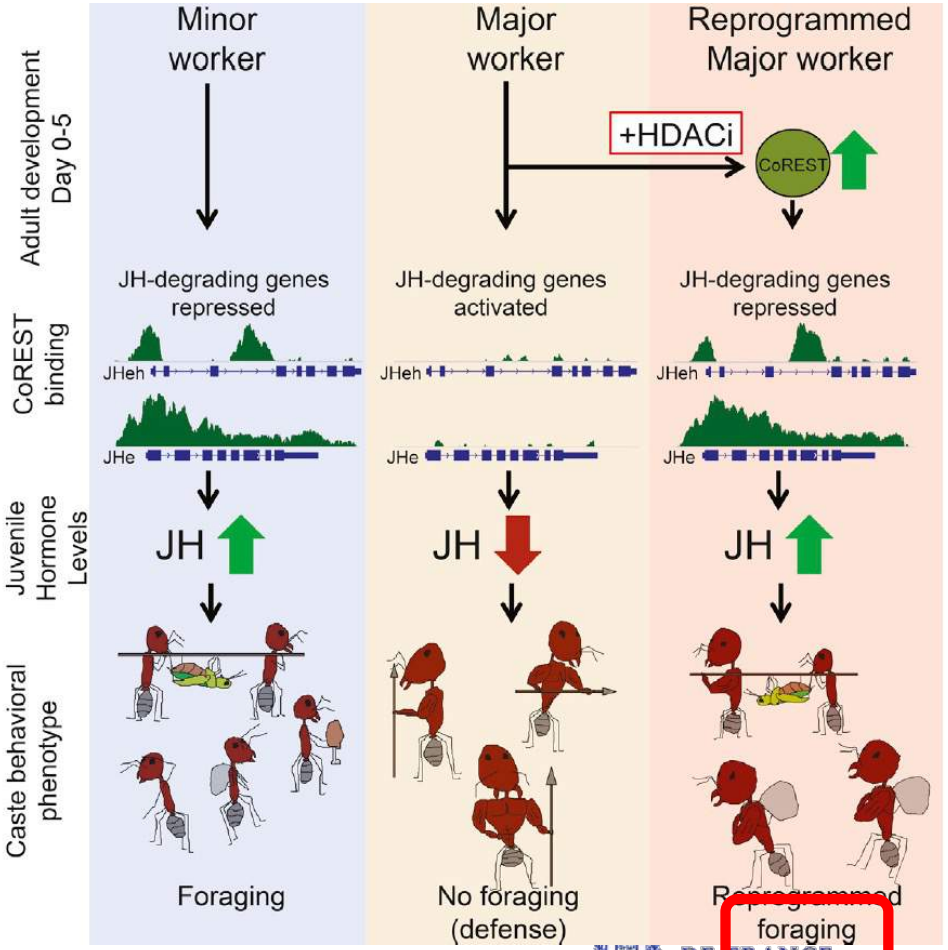
Epigenetic Control of Social Behaviour in Ants

Epigenetic Regulator CoREST Controls Social Behavior in Ants (Glastad et al, Molecular Cell, 2020)

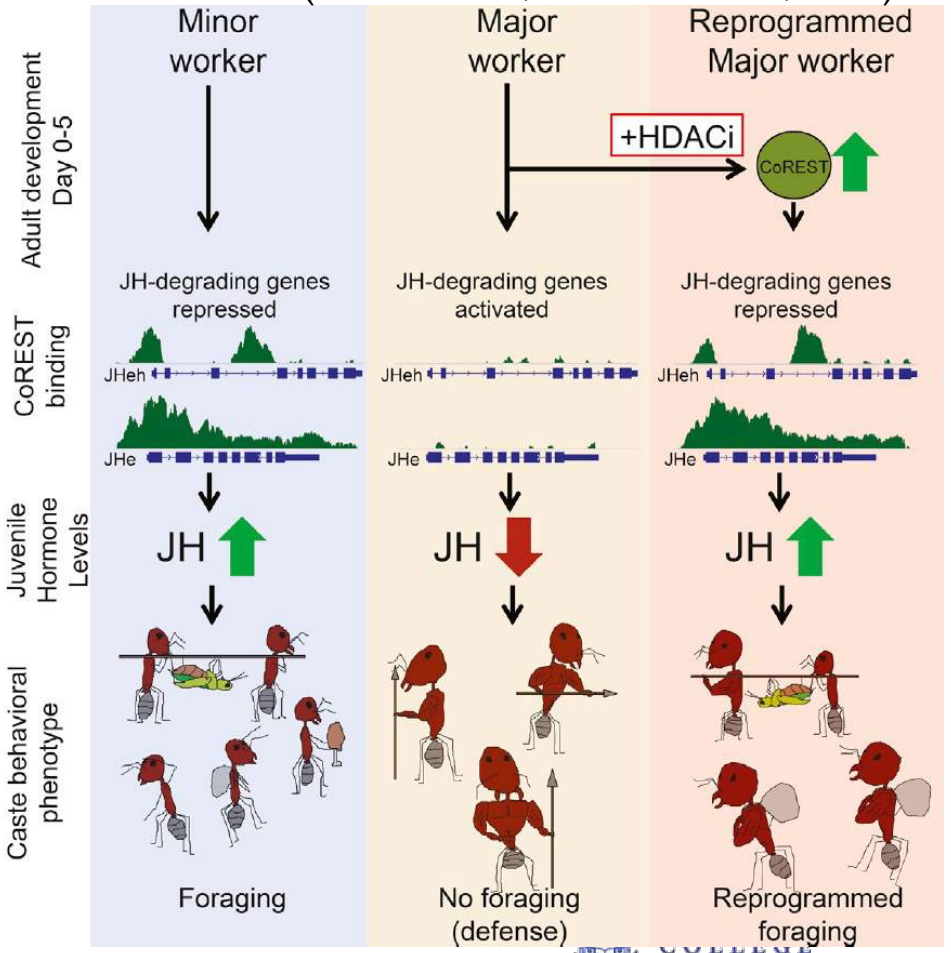
- **HDACi (Trichostatin A - TSA) reprogramming can be used to investigate natural behavioural specification (Foraging)**



Epigenetic Control of Social Behaviour in Ants

Epigenetic Regulator CoREST Controls Social Behavior in Ants (Glastad et al, Molecular Cell, 2020)

- **HDACi (TSA) reprogramming can be used to investigate natural behavioural specification:**
- During a defined window (0-5 days after eclosion) -TSA reprogramming of Majors upregulates Minor-biased genes and downregulates Major-biased genes, engaging molecular pathways involved in foraging behaviour.
- The neuronal corepressor for element-1-silencing transcription factor (CoREST) is upregulated upon reprogramming and required for the epigenetic switch to foraging.
- Genome-wide profiling of CoREST binding during reprogramming reveals that it represses expression of enzymes that degrade juvenile hormone (JH), a hormone elevated upon reprogramming.
- High CoREST, low JH-degrader expression, and high JH levels are mirrored in natural Minors, revealing parallel mechanisms of natural and reprogrammed foraging.
- **The epigenetic corepressor CoREST is a central player in experimentally-induced reprogramming of caste-specific behaviour, from soldier (Major worker) to forager (Minor worker).**

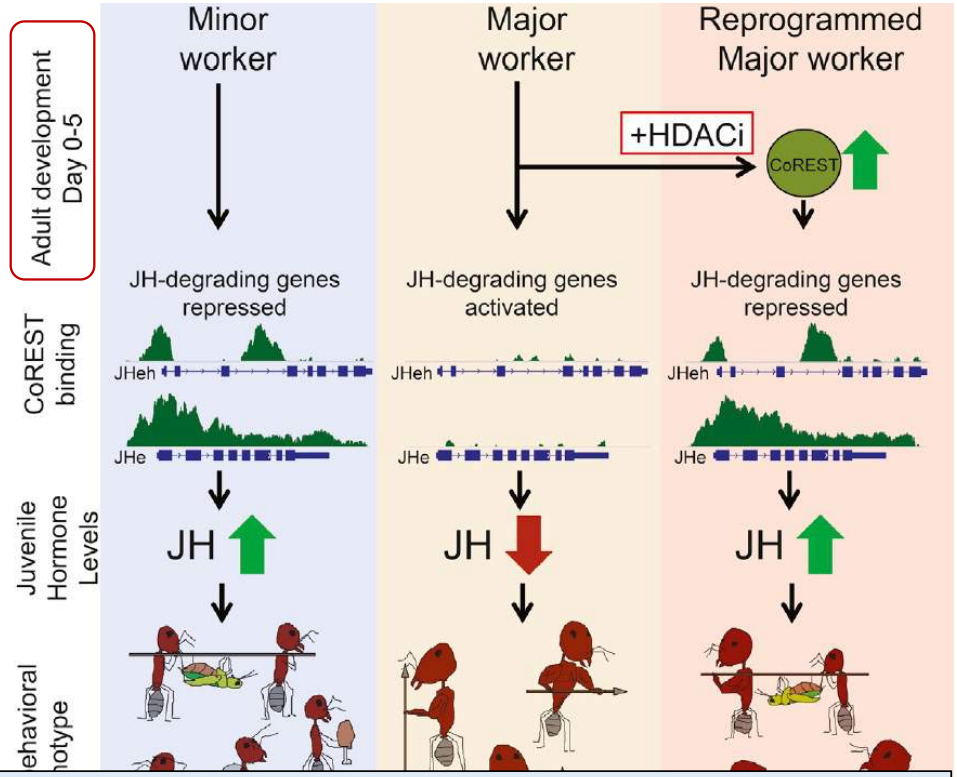


Epigenetic Control of Social Behaviour in Ants

Epigenetic Regulator CoREST Controls Social Behavior in Ants (Glastad et al, Molecular Cell, 2020)

SUMMARY: Epigenetic regulation is important for mediating programming and reprogramming of worker ant behavior. The neuronal co-repressor CoREST, together with histone deacetylation, is crucial in establishing foraging behaviour by repressing genes that degrade *juvenile hormone* JH, a behaviourally important hormone promoting foraging.

Reprogrammed worker ant behaviour mirrors natural programming via an epigenetic switch:
- Neuronal co-repressor CoREST mediates this behavioural programming and reprogramming
- CoREST controls genes that then govern levels of foraging-promoting juvenile hormone JH.

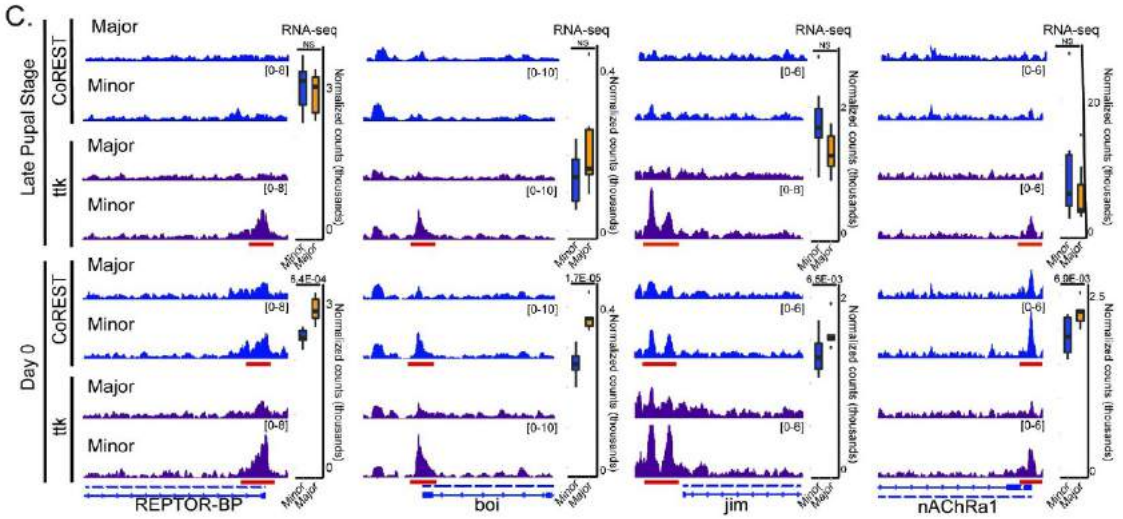


The metamorphosis-associated juvenile hormone JH, and metamorphosis-associated ecdysone (20E), are emerging mediators of caste division of labour in multiple eusocial insects (ants, bees)
These small-molecule hormones play important roles in both the developmental and behavioural division of labour.
Diverse epigenetic pathways are involved in their regulation

Epigenetic Control of Social Behaviour in Ants

Tramtrack and CoREST direct natural caste identity in ants (Glastad et al, Plos Genetics 2021)

- CoREST and the tramtrack (ttk) transcription factor, are predictive of early-life gene expression differences between Major and Minor workers.
- Tramtrack (ttk) shows a signal of poising genes in the late pupal stage for repression via CoREST upon eclosion, illustrating an order of operations i the establishment of distinct behaviors that distinguish these two worker types
- Regions bound by ttk in Minor but not Major workers also show enrichment for sequence motifs associated with hormonal regulation, implying involvement of ttk-mediated repression in attenuating hormonal signaling between castes.



Example tracks (RPM-normalized CUT&RUN signal) of genes featuring Major-biased expression at d0, pupal differential binding of tramtrack, and d0 (but not pupal) differential binding of CoREST (red bars), suggesting ttk is poising genes in Minor pupae for repression, which are then repressed by CoREST following eclosion, and thereby Major-biased in adult gene expression.

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Epigenetic Control of Social Behaviour in Ants

Tramtrack and CoREST direct natural caste identity in ants (Glastad et al, Plos Genetics 2021)

TF ttk and chromatin co-repressor CoREST together ensure caste identity by repressing Major genes in Minor workers

Many questions remain:

How does ttk get expressed in Minors?
How to ttk+CoREST actually result in caste-specification in the maturing brain?

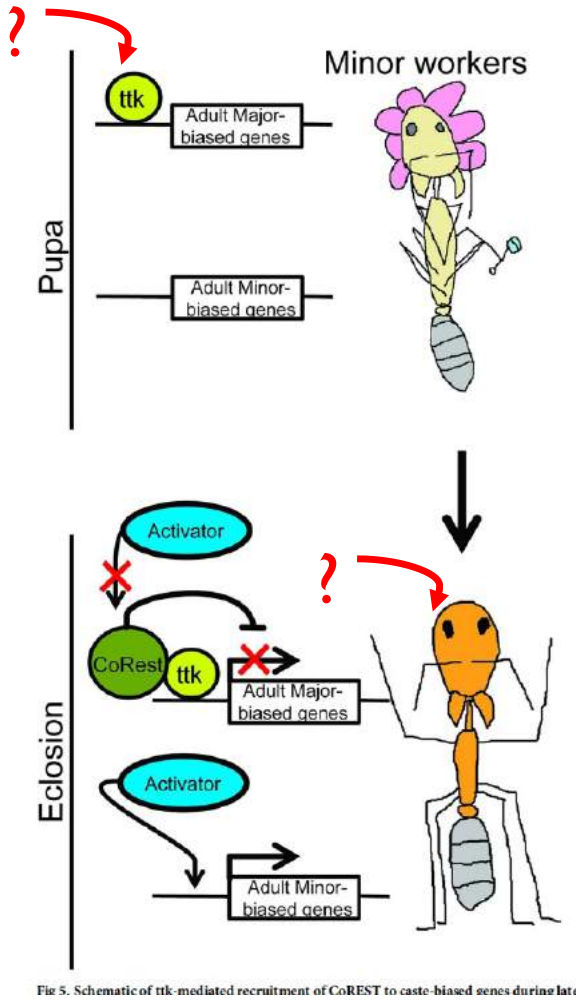

Fig 5. Schematic of ttk-mediated recruitment of CoREST to caste-biased genes during late pupal and d0

In late pupal stage, just prior to eclosion, the DNA-binding Transcription Factor, ttk, localizes to Major-biased genes in Minor Workers.
Big qu. – what are the upstream signals in larvae leading to Minor-biased ttk expression?

Upon eclosion, ttk recruits CoREST for the repression of Major-biased genes.
Big qu. how do ttk and CoREST, which maintain or repress inappropriate cell fates in differentiated cells, lead to caste-specification in the maturing ant brain?
Interplay between caste-specific differences in JH and ectodysone hormones leading to differential development of neuronal or glial populations as Majors and Minors mature?

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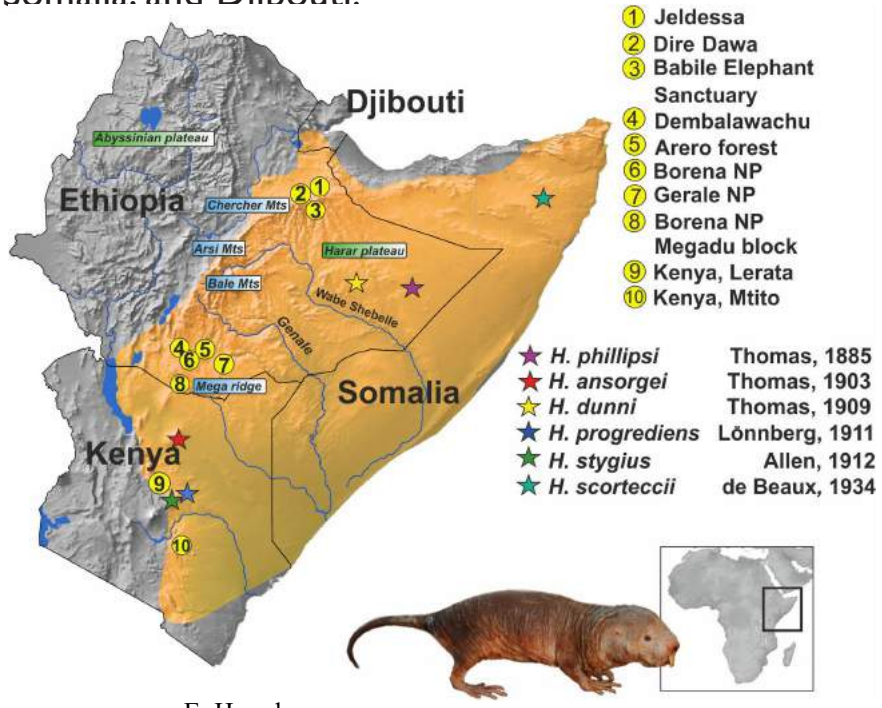
Phenotypic Plasticity and Polyphenism in Naked Mole Rats

Naked Mole Rats *(Heterocephalus glaber)*

Naked mole rats, *Heterocephalus glaber*, live underground in the wild. The pinkish, almost hairless animals have small eyes and can barely see. They have acute hearing, a well-developed sense of smell, and live in colonies underground in the grassy and semiarid regions of East Africa, including Ethiopia, Kenya, Somalia, and Djibouti.



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NMRs can handle extremely low oxygen conditions. feel no pain, do not get cancer, do not age and can live until 37yr at least, queen can reproduce until she dies. Recent study (Horvath et al 2022) revealed that based on DNA methylomes queens appear to age more slowly than nonbreeders

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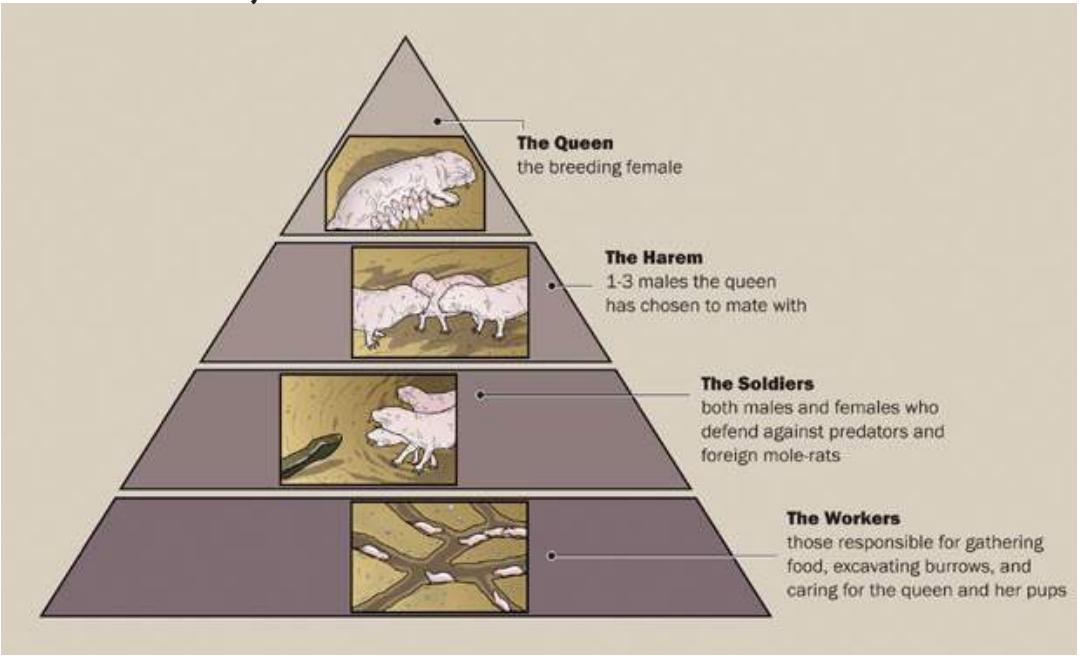

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- Eusocial colonies, with one queen, 1-3 breeding males and reproductively suppressed workers with defined tasks
- Along with having an exceptionally large reserve of healthy egg cells in their ovaries, naked mole rats generate new eggs *after* being born
- Reproductive suppression of females and males is assured by the queen
- Workers are blocked in pre-pubertal state
- Separation from colony (queen) reverses this
- Housing singly or pairing with a female increases concentrations of urinary testosterone levels and plasma luteinizing hormone (LH)
- Not known how behavioural and other sensory cues (such as signature odours and vocalisations) mediate the extreme social suppression of reproduction.
- Role of hypothalamic gonadotrophin releasing hormone (GnRH) in integrating environmental cues is well established,
- Reproductive development and puberty may be dependent on another hypothalamic peptide, kisspeptin (acting via GnRH).

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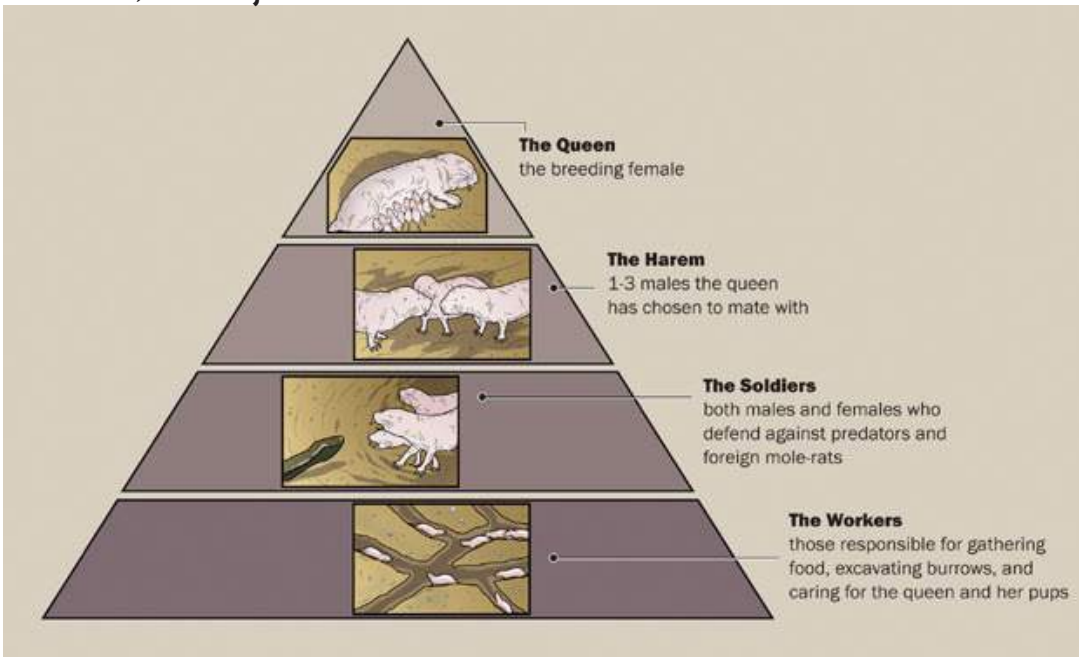

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Molecular insights into the pathways underlying naked mole-rat eusociality

Eskeatnaf Mulugeta[1,2*], Lucile Marion-Poll[1,2], David Gentien[1,2], Stefanie B. Ganswindt[3], André Ganswindt[3], Nigel C. Bennett[4], Elizabeth H. Blackburn[5], Chris G. Faulkes[6*], Edith Heard[1,2]

doi: http://dx.doi.org/10.1101/209932

Transcriptome profiling (RNA-seq) of breeding and non-breeding NMR brains and gonads (ovary and testis) – see gene expression differences that point to possible mechanisms underlying eusociality in a mammal, and extreme socially-induced reproductive suppression.

Study suggests that extreme reproductive skew, a defining features of eusociality, is associated with changes in expression of key components of dopamine pathways, which could lead to hypogonadism and a lifetime of socially-induced sterility for most NMRs

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Phenotypic Plasticity and Polyphenism in Naked Mole Rats

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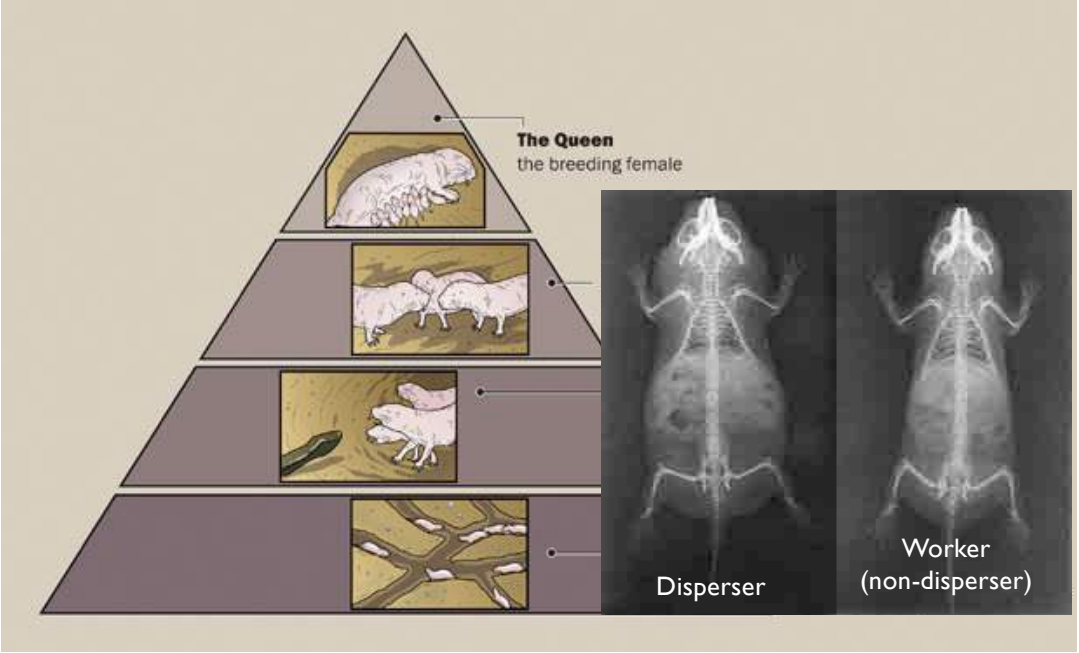


O'Riain, M.J., J.U.M. Jarvis, R. Alexander, C. Peters
Morphological castes in a vertebrate. *PNAS* 97, 24: 13194-13197

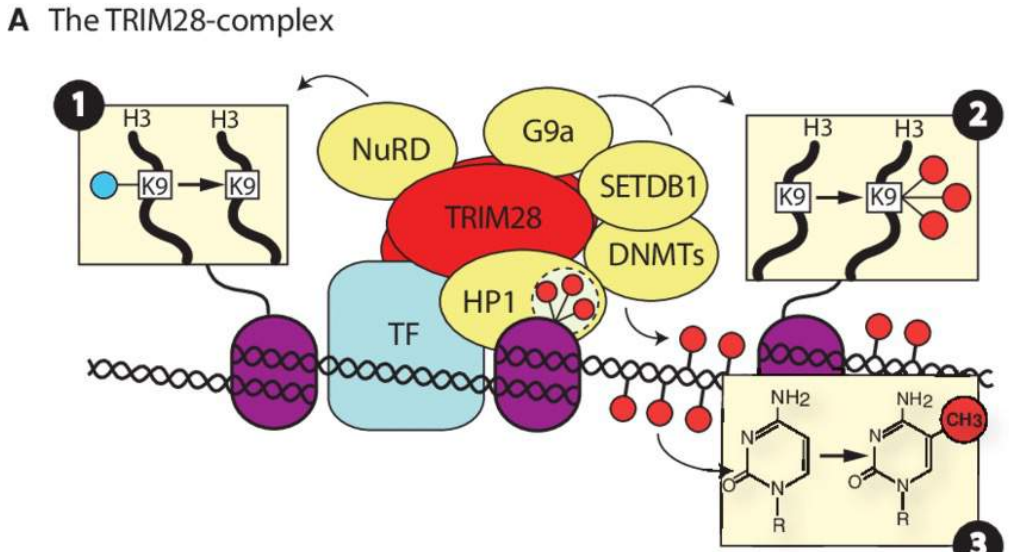
Disperser NMR: A study in 1996 discovered a new caste in some naked mole-rat colonies: the disperser. Big, fat, lazy and sexually-charged, these rare individuals seem built for dispersal.

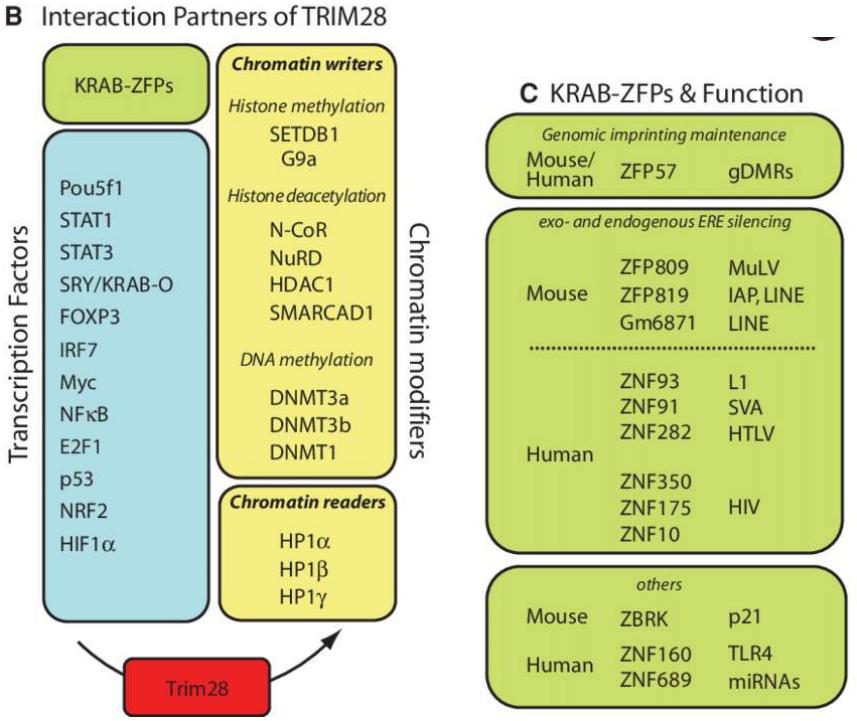
- Dispersers actively seek to leave their natal burrow whenever an opportunity for escape presents itself, and are armed with generous fat reserves to survive the journey.
- They are sexually primed, with high levels of lutenizing hormone, yet are only interested in mating with individuals from foreign colonies, not their own colony's queen. Furthermore, they are lazy, showing little interest in working cooperatively in their natal burrow.
- The disperser morphs are equipped for leaving their natal burrow, joining another colony and thus promoting exchange of individuals, and therefore genes, between otherwise isolated colonies.
- Thus gene flow via outbreeding is enabled when a disperser male leaves the colony and enters another

O'Riain, Jarvis and Faulkes, 1996

Phenotypic Plasticity in Mice and Humans?
A role for Trim28?

- TRIM28 /KAP1 is a ubiquitously expressed protein involved in critical functions including: transcriptional regulation, cellular differentiation and proliferation, DNA damage repair, viral suppression, and apoptosis.
- Sumoylated TRIM28 assembles epigenetic machinery, phosphorylated TRIM28 is involved in DNA repair.
- TRIM28 can repress transcription by binding TFs and interacts with numerous chromatin modifiers
- TRIM28 -/- knockout mice are early embryonic lethal





https://www.frontiersin.org/articles/10.3389/fcimb.2022.834636/full

https://pubmed.ncbi.nlm.nih.gov/28765213/

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Phenotypic Plasticity in Mice and Humans?
A role for Trim28?

Reduced levels of two modifiers of epigenetic gene silencing, Dnmt3a and Trim28, cause increased phenotypic noise

Nadia C Whitelaw[1,2], Suyinn Chong[1], Daniel K Morgan[1,2], Colm Nestor[3,4], Timothy J Bruxner[1], Alyson Ashe, Eleanore Lambley[1], Richard Meehan[3,4], Emma Whitelaw[1*]

Sex	Transmission	Genotype	n	Weight (g)	t-test	F-test
Combined	Paternal	Dnmt3a$^{+/+}$	63	8.4 ± 1.1	0.57	0.01*
		Dnmt3a$^{-/+}$	52	8.3 ± 1.5		
	Maternal	Dnmt3a$^{+/+}$	38	8.7 ± 1.5	0.12	0.26
		Dnmt3a$^{-/+}$	45	8.1 ± 1.8		
	Paternal & Maternal	Dnmt1$^{+/+}$	23	9.6 ± 1.8	0.95	0.71
		Dnmt1$^{Mommd2/+}$	38	9.6 ± 1.7		

Background: Inbred individuals reared in controlled environments display considerable variance in many complex traits but the underlying cause of this intangible variation has been an enigma. Here we show that two modifiers of epigenetic gene silencing play a critical role in the process.

Results: Inbred mice heterozygous for a null mutation in *DNA methyltransferase 3a* (*Dnmt3a*) or *tripartite motif protein 28* (*Trim28*) show greater coefficients of variance in body weight than their wild-type littermates. *Trim28* mutants additionally develop metabolic syndrome and abnormal behavior with incomplete penetrance. Genome-wide gene expression analyses identified 284 significantly dysregulated genes in *Trim28* heterozygote mutants compared to wild-type mice, with *Mas1*, which encodes a G-protein coupled receptor implicated in lipid metabolism, showing the greatest average change in expression (7.8-fold higher in mutants). This gene also showed highly variable expression between mutant individuals.

Conclusions: These studies provide a molecular explanation of developmental noise in whole organisms and suggest that faithful epigenetic control of transcription is central to suppressing deleterious levels of phenotypic variation. These findings have broad implications for understanding the mechanisms underlying sporadic and complex disease in humans.

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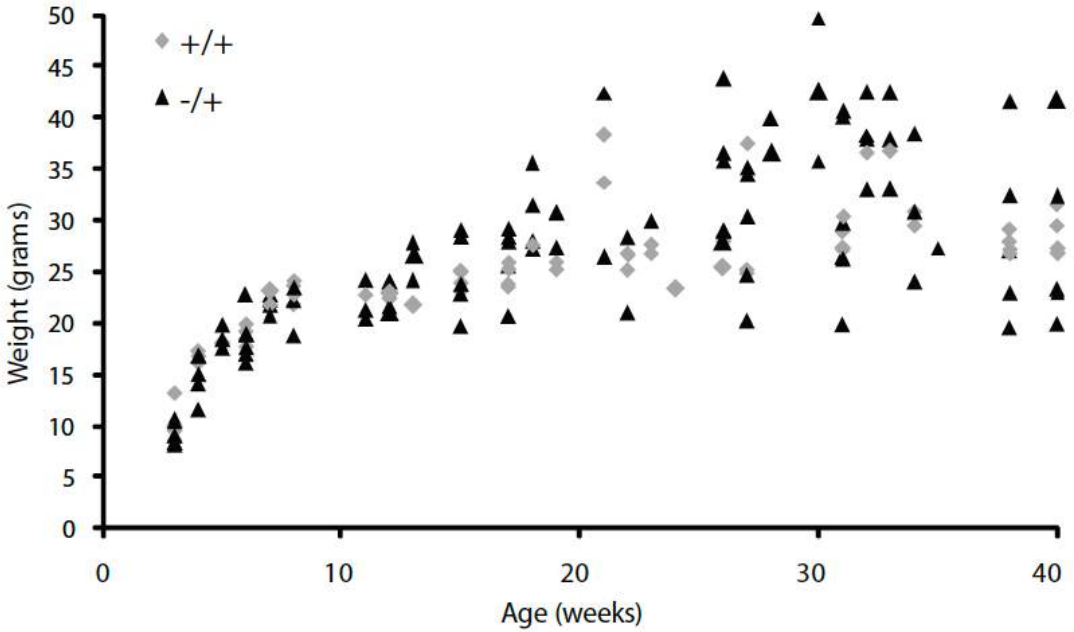
Increased variation in body weight in Trim28MommeD9/+ mice.

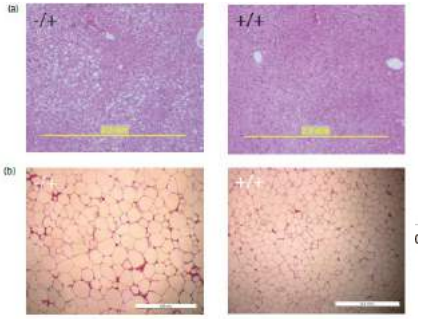
Trim28MommeD9/+ mice appear to have a greater variation in weight as they age.

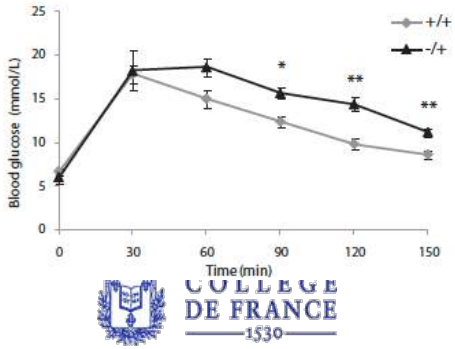
Twenty Trim28+/+ mice and 33 Trim28MommeD9/+ mice (all female) were weighed between 3 and 40 weeks of age. The data are the sum of 170 data points representing 103 Trim28MommeD9/+ and 67 Trim28+/+ body weight measurements.

Symptoms of metabolic syndrome are also seen in obese Trim28MommeD9/+ mice.

Obese Trim28MommeD9/+ mice and WT littermates were fasted for 15 hours and a blood glucose measurement was taken at t = 0. Mice were injected with 2 g/kg of a 20% glucose solution and blood glucose measurements were taken every 30 minutes for 150 minutes







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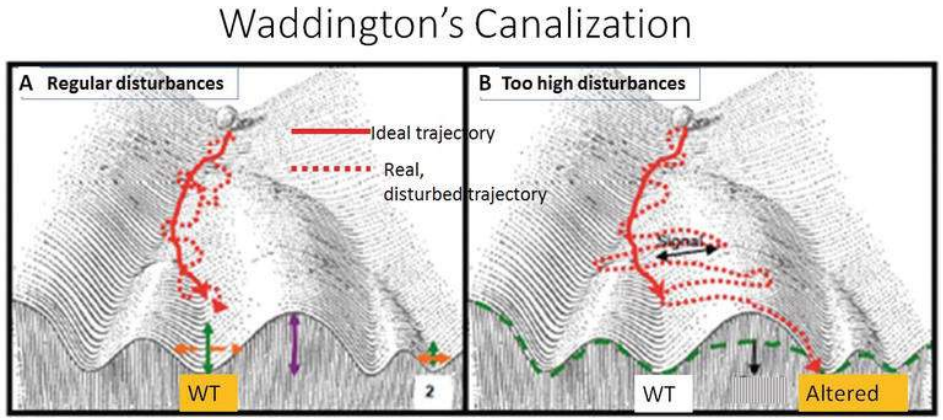
Trim28 – an epigenetic factor that buffers against phenotypic variation
A canalization factor...

Canalization: the capacity of an organism to ensure the production of a standard phenotype in spite of environmental disturbances.



This study shows that modifiers of epigenetic gene silencing are fundamental to this process and suggest that their levels have been fine-tuned by evolutionary pressures to allow cells to acquire different patterns of gene expression during differentiation, but at the same time to lock-in the transcriptional profile of differentiated cell types.

Numerous studies on vertebrates and invertebrates using isogenic individuals raised in controlled environments show considerable variance for many phenotypic traits, for example, body weight, bristle number etc.

This is the first report of any mechanism that can change the degree of variance at the level of the whole organism in mammals.

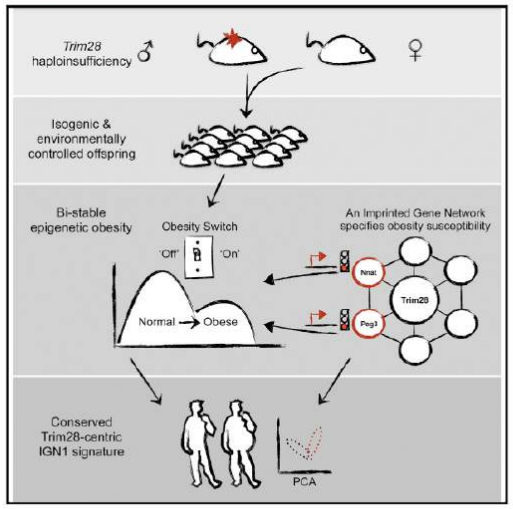
Implications for mechanisms underlying phenotype and disease in all multicellular organisms.

Obesity Polyphenism in Mice and Humans revealed by TRIM28/KAP1 Haploinsufficiency

Cell

Trim28 Haploinsufficiency Triggers Bi-stable Epigenetic Obesity

Graphical Abstract



Authors

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Correspondence

pospisilik@ie-freiburg.mpg.de

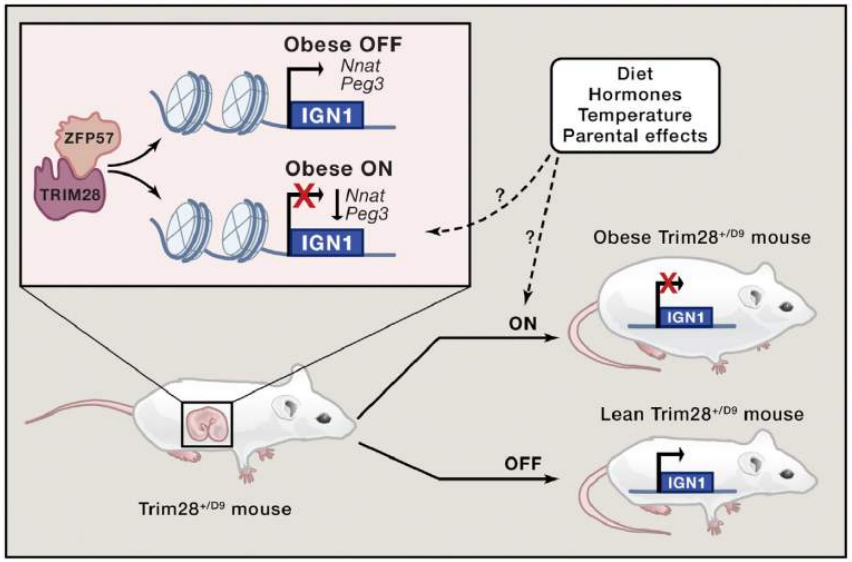
In Brief

TRIM28 insufficiency in both mouse and human leads to polyphenism, wherein lean and obese phenotypes can arise from the identical genotypes through dysregulation of an imprinted gene network.

Highlights

- *Trim28* haploinsufficiency triggers stochastic bi-stable obesity or polyphenism
- Non-classical imprinted gene dysregulation specifies "on" versus "off" obese states
- *Peg3* and *Nnat* perturbation trigger stochastic bi-stable obesity
- Human BMI distributions and transcriptomes suggest Trim28-associated subpopulations

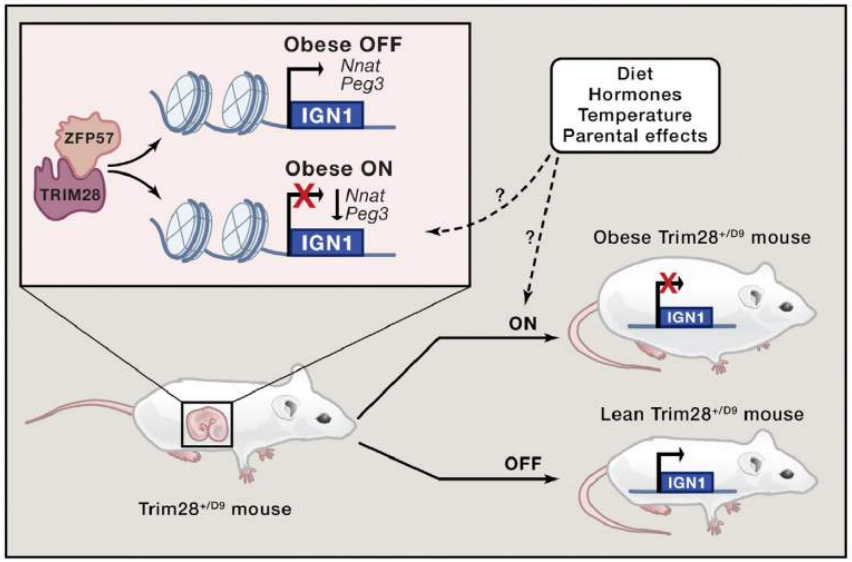
Obesity Polyphenism in Mice and Humans revealed by TRIM28/KAP1 Haploinsufficiency



Mutations in the chromatin-associated co-repressor Trim28/KAP1 (Trim28$^{+/D9}$) affect the formation of functional TRIM28-ZFP57 complex, which in turn affects chromatin states and modifications durin embryonic development. Such (not yet identified) epigenetic alterations might be linked to a dowr regulated expression of an imprinted cluster of genes named IGN1. Reduced levels of components of th IGN1 network, including Nnat and Peg3, could lead to an obese-ON program. This program of obesit susceptibility is linked to a phenotypic switch in the body weight in adult genetically identical Trim28$^{+/}$ mice, rendering them either normal or obese but without an intermediate phenotype. Such polyphenis might be influenced by environmental signals including dietary conditions, hormonal signaling, an changes in the environmental temperature, occurring in early life stages, during development, or poter tially in the prior generation.

- TRIM28 is important for transcriptional programming during development
- Haploinsufficiency affects formation of TRIM28-ZFP57 complex, which leads to down-regulation of imprinted cluster of genes IGN1
- Reduced levels of genes such as *Nnat* could result in "obese-ON" program...
- This program of obesity susceptibility is linked to polyphenism : Obese/normal in genetically identical Trim28 +/- adults (can be as much as 100% difference in body weight)
- Obese or not – no intermediate phenotypes
- Polyphenism could be influenced by diet, hormones or changes in temperature in early life stages (development) or even inprior generation

Obesity Polyphenism in Mice and Humans revealed by TRIM28/KAP1 Haploinsufficiency



Is this adaptive? BMI/adiposity polyphenism in the mouse might confer multiple evolutionary advantages: difficulties associated with requiring marginally larger burrows might be offset by resistance to famine/food deprivation, reduced necessity to forage under higher-risk conditions, advantage during mate competition, improved lactation and maternal care, improved resistance to cold, physical injury, and drowning.

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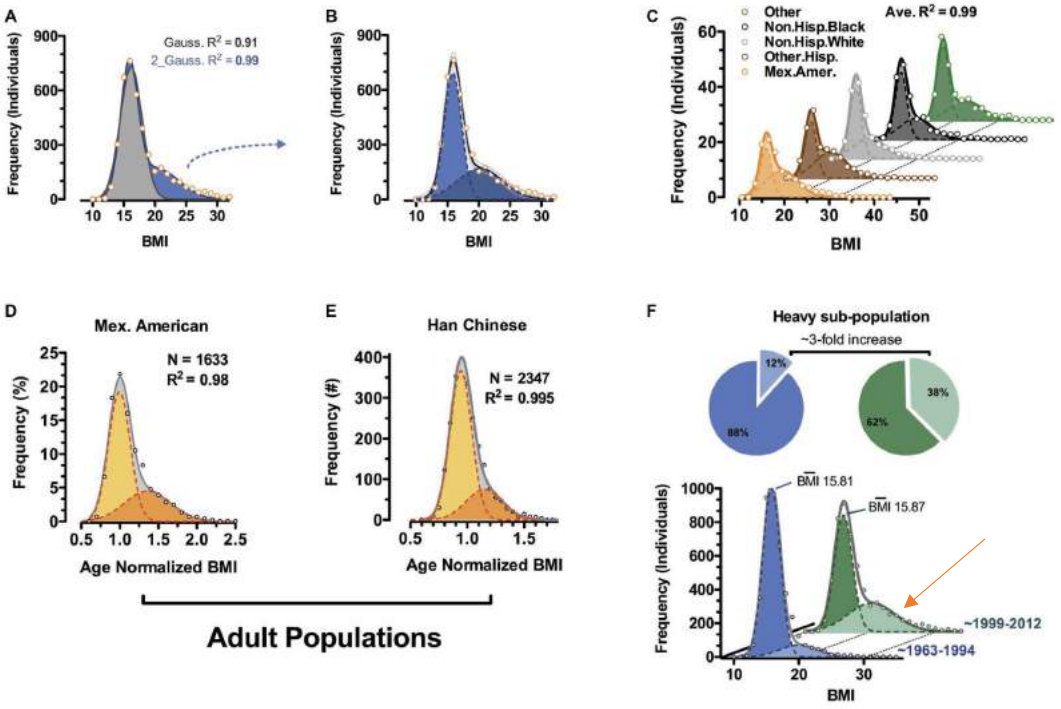
Human Polyphenism (Homo sapiens)
Body Composition Polyphenism?

Body mass index (BMI) bimodality in humans

- Unbiased examination of publically available epidemiology datasets reveals bimodality at least in human body weight distributions and specifically body mass index *(BMI)*.
- Bimodality can be observed in children of all recorded ethnic classes of the National Health and Nutrition Examination Survey (NHANES) 1968–2012 survey and in select adult cohorts (Mexican American and Han Chinese).
- Comparing BMI distributions from NHANES data gathered between 1963 and 1994 (CDC and NIHS, 1994) with the more recent 1999–2012 (continuous NHANES) data (CDC and NCHS, 2012), **the frequency of individuals within the heavy sub-population of BMI triples** while that of the lighter (less heavy) sub-population decreases (Dalgaard et al., 2016; 1999–2012 NHANES vs. 1963–1994 NHANES).
- Almost exclusively a change in <u>frequencies</u>, as the magnitude of BMI for each of the sub-populations remains essentially fixed over the same timescale.
- These data are in agreement with the notion of polyphenism.
- These data suggest increased incidence of a distinct category of "triggered" individuals, consistent with the rodent obese and dispersal morphs.
- Argues against the general statement that the whole population is becoming much more obese.



These data suggest increased incidence of a distinct category of "triggered" individuals consistent with the rodent Trim28 +/- obese and dispersal morphs - the notion of body mass polyphenism in humans

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A Case for Human Polyphenism (Homo sapiens)
TRIM28/IGN1-Associated Obesity in Select Human Cohorts?

- Authors also observe that humans, like mice, appear to stratify into subpopulations defined by adipose Trim28 expression.
- Subjects displaying low Trim28 levels exhibited increased obesity incidence and a distinct transcriptome characterized again by IGN1 dysregulation.

- Authors then analyzed adipose tissue samples from **monozygotic twins displaying differences in their body weight**.
- Both reduced Trim28 levels and reduced IGN1 gene expression found in obese relative to lean isogenic co-twins.

- Altogether, these data support a possible role of the Trim28/IGN1 axis in increasing the susceptibility to obesity in humans.

- => a switch in the regulation of body weight can occur in genetically identical individuals as a consequence of impairment of the chromatin-sensitive Trim28/IGN1 molecular axis.

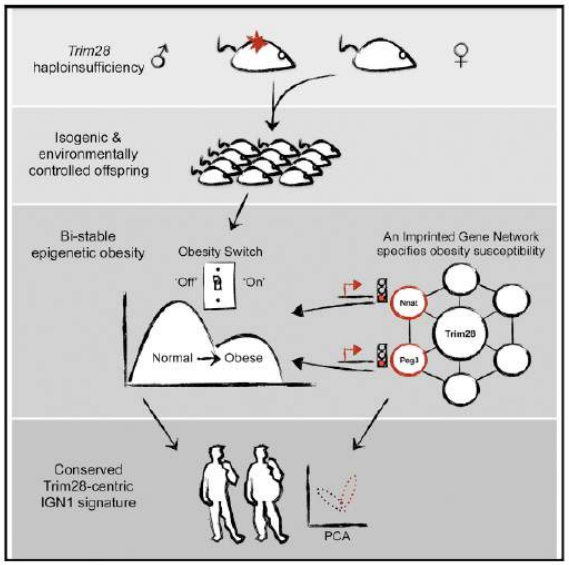
- Can environmental factors contribute to this epigenetic-based mechanism of body weight regulation?

- Humans are continuously exposed to environmental factors, such as hormones, metabolic factors, or dietary conditions, that might induce epigenetic remodeling.

- Whether environmentally regulated epigenomic ON/OFF switches for obesity via the Trim28/IGN1 axis operate in humans remains to be found.

Trim28 Haploinsufficiency Triggers Bi-stable Epigenetic Obesity

Graphical Abstract



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Nnat insufficiency also triggers an overgrowth polyphenism (increased fat and lean mass) but this is distinct and independent of Trim28!

- Trim28 loss has been linked to the dysregulation of imprinted genes during development (Messerschmidt et al., 2012).
- IGN1 genes have been implicated in placentation (Sekita et al., 2008),, development, and growth. Insulin and IGF1 signaling are potential regulatory candidates for generating bi-stability during embryonic development.
- NNAT (in IGN1 network), has been correlated with human obesity (Vrang et al., 2010; Gburcik et al., 2013) and very recently with regulation of hormone maturation in endocrine cells (Millership et al., 2018).
- **Paternal *Nnat* deletion (+/-) triggers bistable epigenetic overgrowth in mice** (Yang et al, 2021) – similarly to Trim28?

Even though *Nnat* expression is downregulated in Trim28 haploinsufficient mice, compound mutants demonstrate three distinct phenotypes, showing that the mechanisms by which each gene buffers against UPV are different!

This shows the potential for a given mammalian genome to deliver three discrete and reproducible phenotypic outcomes that are probabilistically determined.

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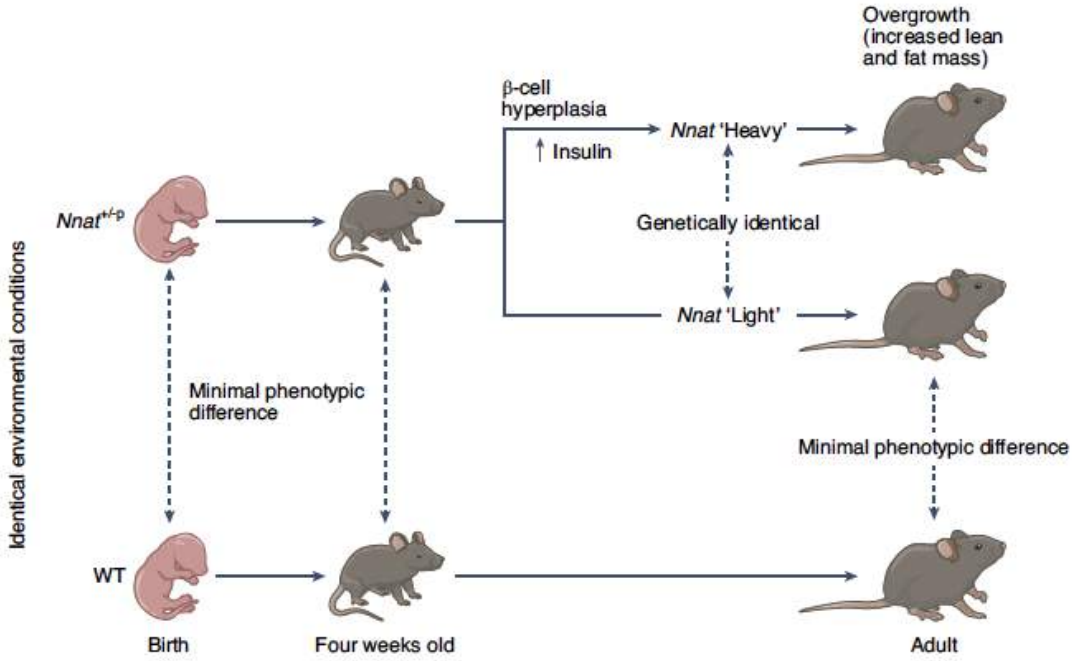
Unexplained phenotypic variation (a future course!)

- UPV = phenotypic variation that occurs even when both inter-individual genetic and environmental differences are controlled- additional dimensions contribute to trait variation?
- The basis of this 'unexplained phenotypic variation' (UPV) I are probably multifactorial (often dismissed as random biological noise).
- Such variation can originate even from single loci being regulated in a probabilistic manner to establish an 'on' or 'off ' gene expression state.
- The implication of such bimodal regulation is that it can give rise to predictable outcomes, which may be important in the context of human disease.
- In Nnat +/- mice: the bimodal overgrowth phenotype in genetically and environmentally identical mice was found to be driven by hyper-insulinaemia, due to increased proliferation of pancreatic β-cells (bot due to alterations in growth hormone/insulin-like growth factor signalling).
- **=> Nnat functions to buffer against activation of HDAC-driven β-cell hyperplasia in early life**



"The big question we are still asking is the origin of the UPV, as well as how and when this specific neuronatin-buffered process is most relevant," says co-first author Chih-Hsiang Yang. "Our data suggest UPV might be traced back to the embryo stage or even triggered in the previous parental generations." Nature Revlews Endocrinology 2022.

Unexplained phenotypic variation

HUMAN MONOZYGOTIC TWINS

- identified two recurrent discordant morphological patterns
- in pairs of monozygotic twins:
type A UPV (characterised by a relative adiposity)
type B UPV (characterised by a relative overgrowth)
- Monozygotic twins with type B UPV showed reduced NNAT expression, increased histone deacetylase-responsive gene signatures and increased clinical outcomes linked to insulinaemia.
- Furthermore, type B UPV signature stratified cohorts of both children and adults into four distinct metabolic states, including two phenotypically and molecularly distinct sub-types of obesity.

=> this study identifies new subtypes of obesity.

Mouse/Human Polyphenism in body composition/obesity?


ARTICLES nature metabolism

OPEN

Independent phenotypic plasticity axes define distinct obesity sub-types

Chih-Hsiang Yang, Luca Fagnocchi, Stefanos Apostle, Vanessa Wegert, Salvador Casaní-Galdón, Kathrin Landgraf, Ilaria Panzeri, Eraz Dror, Steffen Heyne, Till Wörpel, Darrell P. Chandler, Di Lu, Tao Yang, Elizabeth Gibbons, Rita Guerreiro, Jose Bras, Martin Thomasen, Louise G. Grunnet, Allan A. Vaag, Linn Gillberg, Elin Grundberg, Ana Conesa, Antje Körner, PERMUTE and J. Andrew Pospisilik

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SUMMARY SO FAR
Phenotypic Plasticity and Polyphenism in the Animal Kingdom

- Polyphenism is found in many species from insects to mammals
- In some cases, eg naked mole rat eusocial phenotypic plasticity, phenotypes are still malleable after adulthood
- This is similar to physical attributes associated with alphas in pack animals, a social and physiological divergence that is widespread across the mammalian kingdom.
- The blurriness between concepts of polyphenism and hierarchy-associated phenotype reflects the limited understanding of interplay between environment (social or physical) and molecular regulation of developmental switches.
- How "stable" are the developmental switches in other forms of polyphenism?
- What are the similarities or differences in molecular mechanisms that lead to adult phenotypic plasticities?
- Will molecular principles between socially reinforced phenotypic differences (alphas and wrasse), robust nutritionally conditional systems (royal jelly of the queen bee), and classical developmental switch polyphenisms be similar?
- In addition to genetic and environmental factors, phenotypic outcomes in mammals are defined by **probabilistic factors** with the potential to canalize multiple distinct, stable and reproducible outcomes. A substantial fraction of human metabolic disease variation (and potentially associated processes such as cancer and inflammation) are defined by such processes (Yang et al, 2021 Nature Metabolism - *https://doi.org/10.1038/s42255-022-00629-2*)