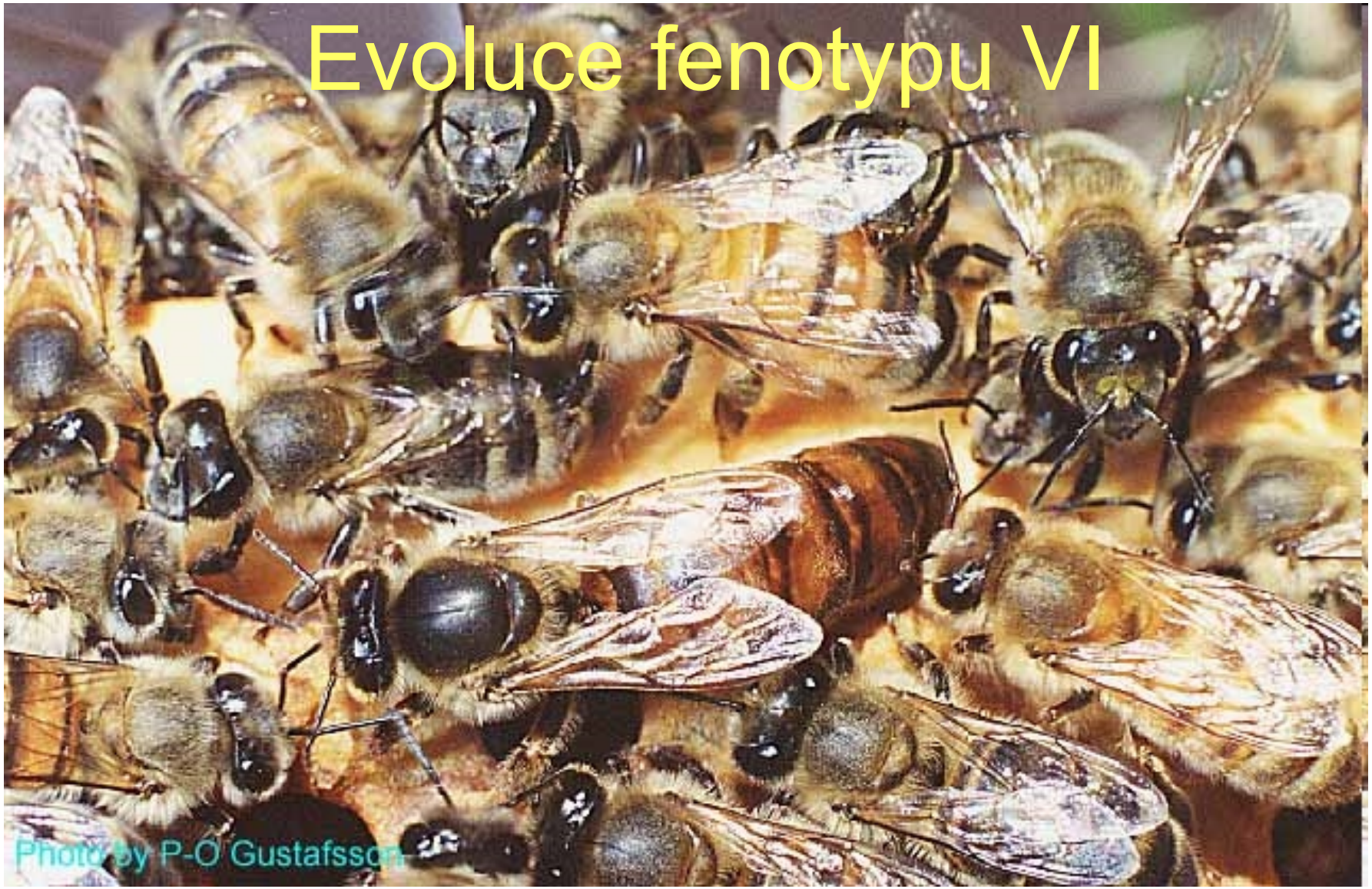
Evoluce fenotypu VI



Fenotypová plasticita jako důsledek externích podmínek

Zebrafish Behavioral Profiling Links Drugs to Biological Targets and Rest/Wake Regulation

Jason Rihel,[1]*† David A. Prober,[1]*‡ Anthony Arvanites,[2] Kelvin Lam,[2] Steven Zimmerman,[1] Sumin Jang,[1] Stephen J. Haggarty,[3,4,5] David Kokel,[6] Lee L. Rubin,[2] Randall T. Peterson,[3,6,7] Alexander F. Schier[1,2,3,8,9]†

15 JANUARY 2010 VOL 327 SCIENCE

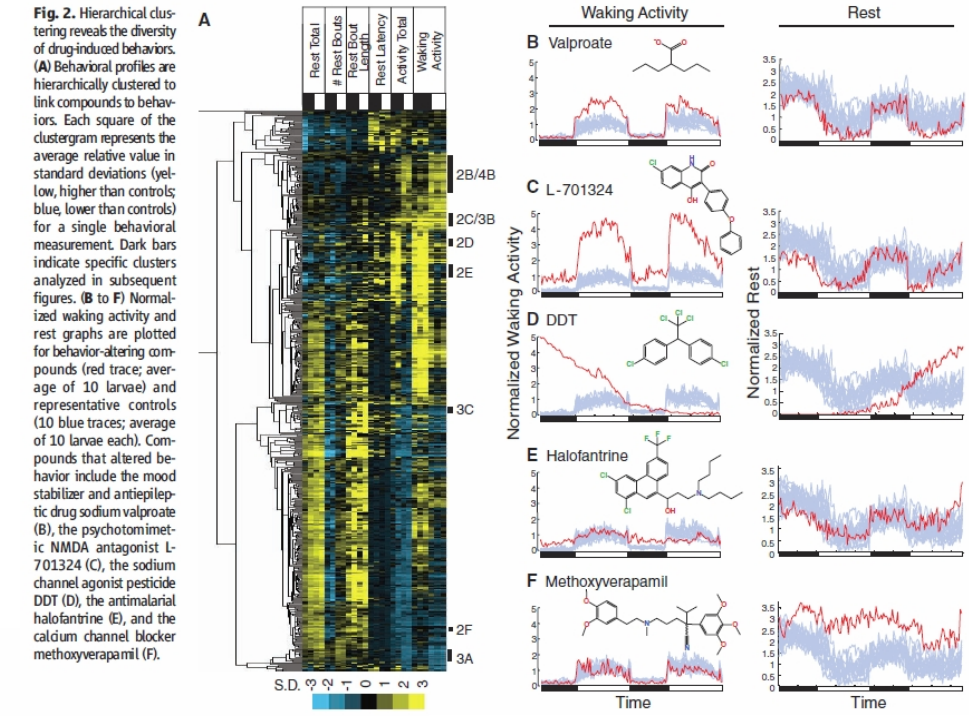


Fig. 2. Hierarchical clustering reveals the diversity of drug-induced behaviors. (**A**) Behavioral profiles are hierarchically clustered to link compounds to behaviors. Each square of the clustergram represents the average relative value in standard deviations (yellow, higher than controls; blue, lower than controls) for a single behavioral measurement. Dark bars indicate specific clusters analyzed in subsequent figures. (**B** to **F**) Normalized waking activity and rest graphs are plotted for behavior-altering compounds (red trace; average of 10 larvae) and representative controls (10 blue traces; average of 10 larvae each). Compounds that altered behavior include the mood stabilizer and antiepileptic drug sodium valproate (B), the psychotomimetic NMDA antagonist L-701324 (C), the sodium channel agonist pesticide DDT (D), the antimalarial halofantrine (E), and the calcium channel blocker methoxyverapamil (F).

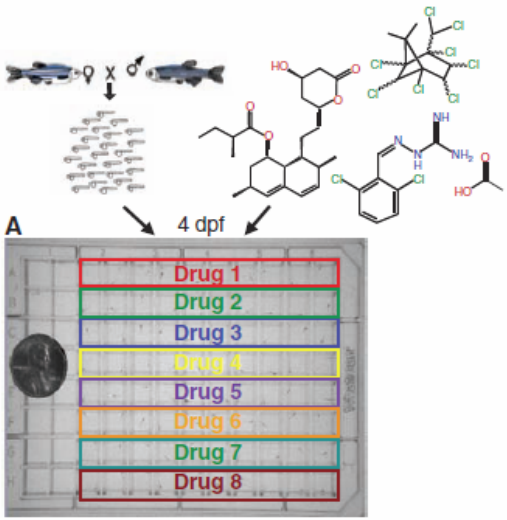
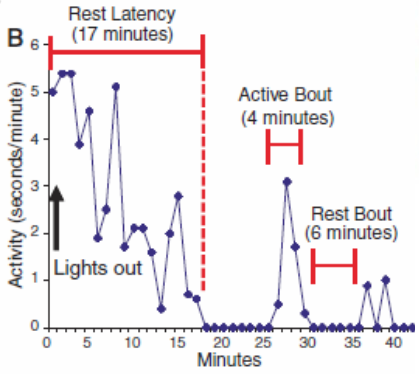
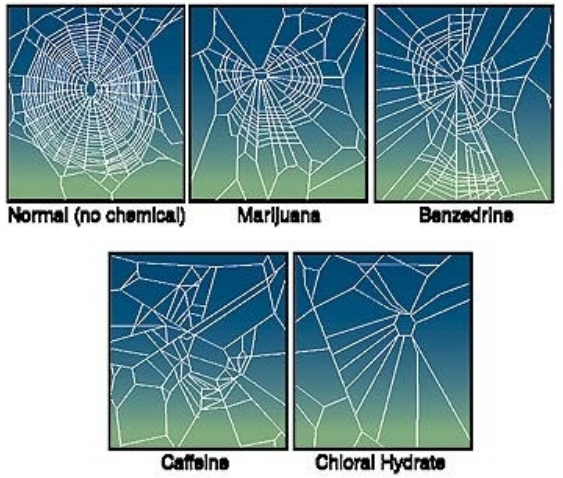
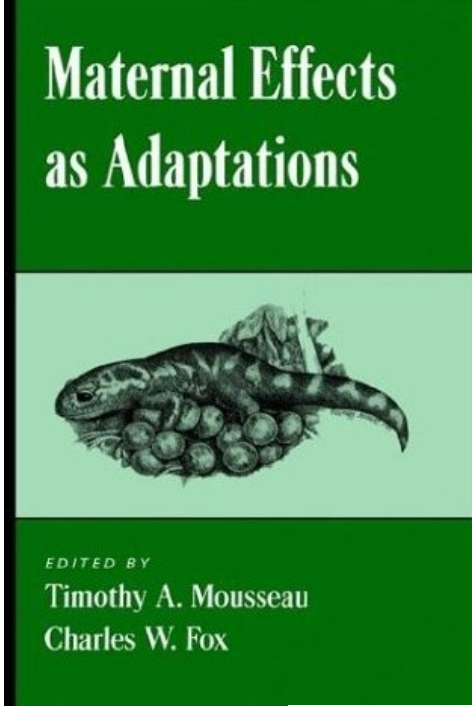


Fig. 1. Larval zebrafish locomotor activity assay. (**A**) At 4 days post-fertilization (dpf), an individual zebrafish larva is pipetted into each well of a 96-well plate with small molecules. Automated analysis software tracks the movement of each larva for 3 days. Each compound is tested on 10 larvae. (**B**) Locomotor activity of a representative larva. The rest and wake dynamics were recorded, including the number and duration of rest bouts [i.e., a continuous minute of inactivity (*8*)], the timing of the first rest bout after a light transition (rest latency), the average waking activity (average activity excluding rest bouts), and the average total activity. Together, these measurements generate a behavioral fingerprint for each compound.





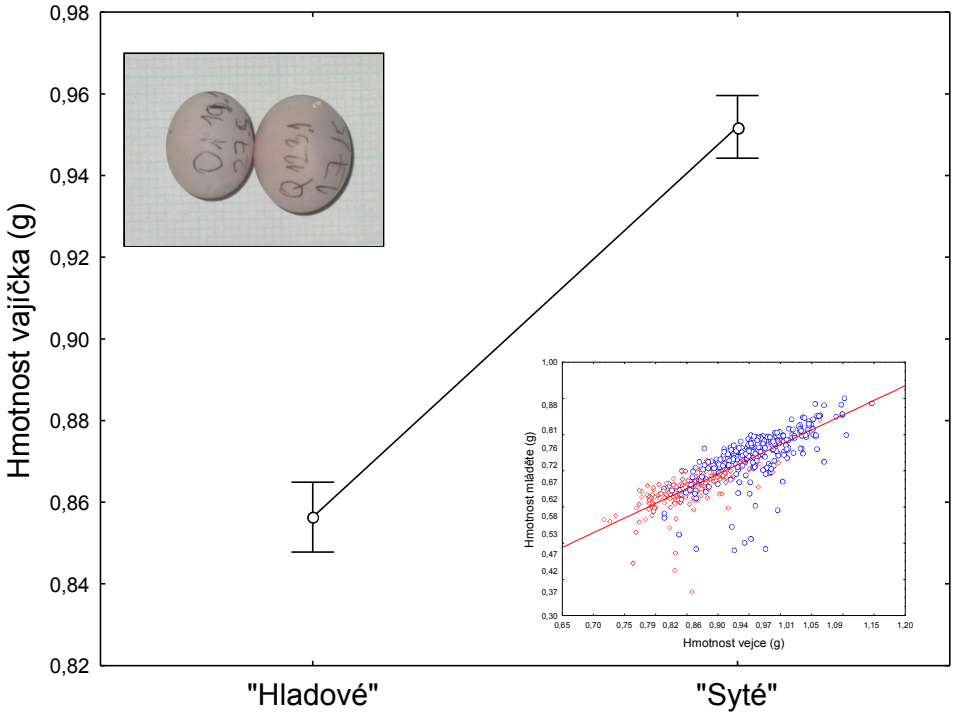


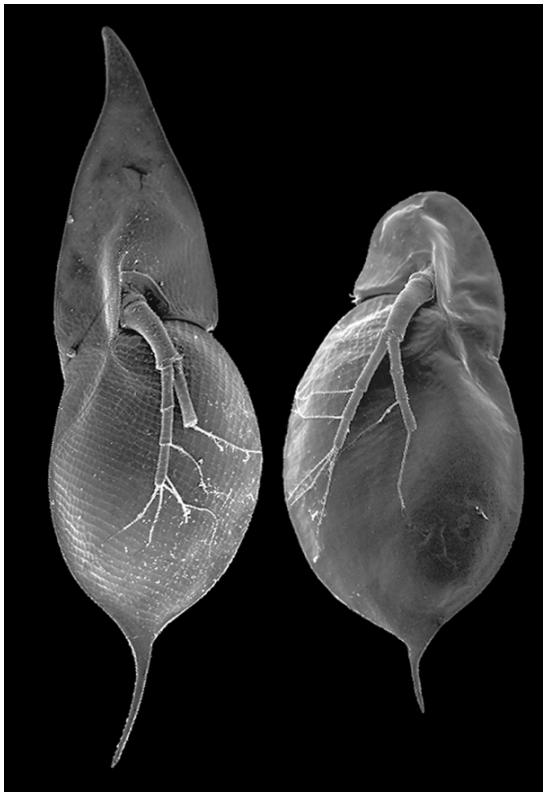
Je fenotypová plasticita adaptivní?

Maternální efekt:

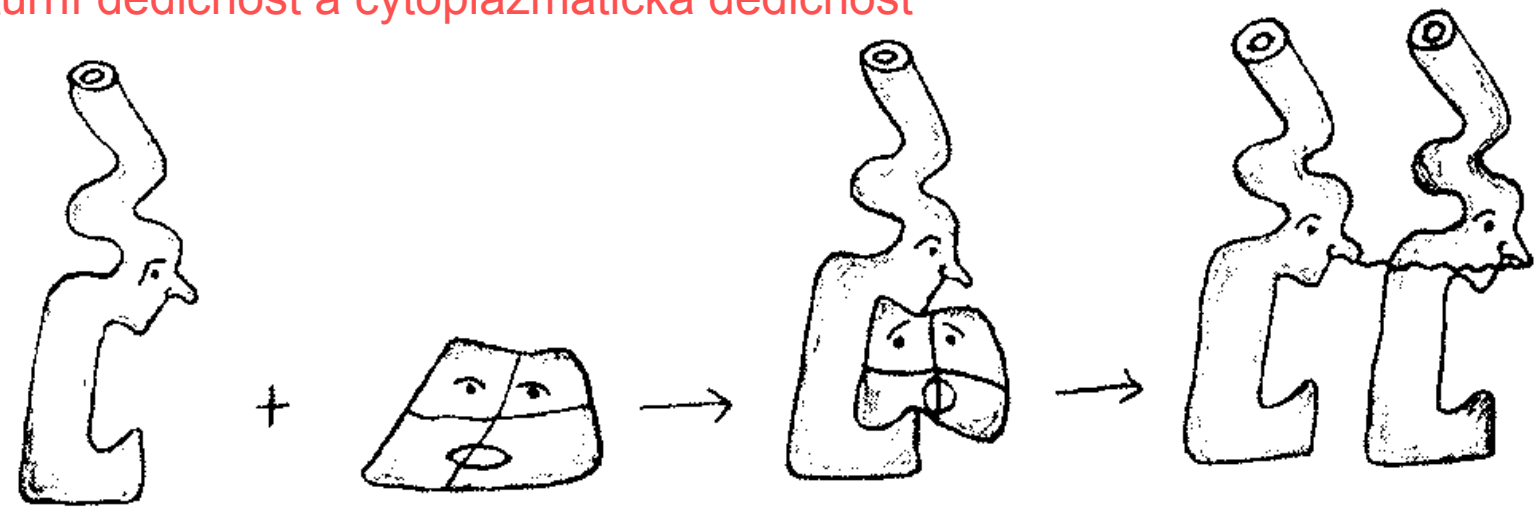
- rodiče optimalizují fenotyp potomků na prostředí, ve kterém sami žili

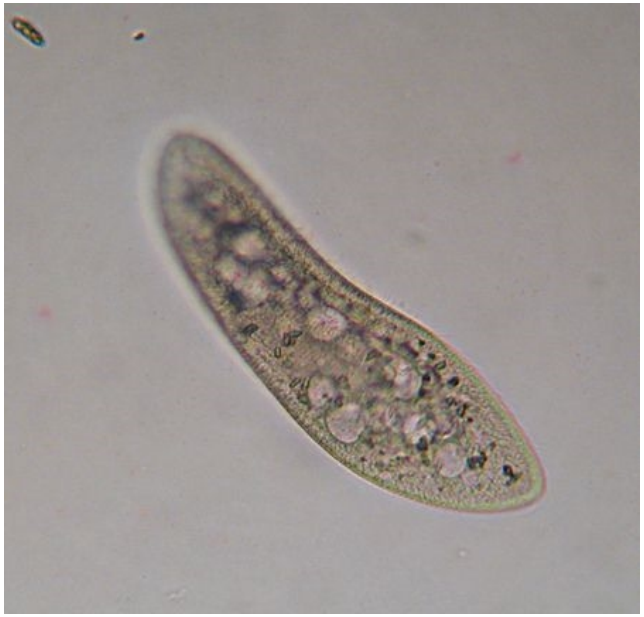
- jedinci v dobré kondici získané díky prostředí bohatému na zdroje přenášejí dobrou kondici na potomky (ale ta se hodí i ve špatném prostředí) - ostatní dělají „the best of bad jobs“





Strukturní dědičnost a cytoplazmatická dědičnost





Fenotypová plasticita (a maternální efekty) jsou často spojeny s metabolismem steroidních hormonů

Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 11446–11450, December 1993
Neurobiology

Yolk is a source of maternal testosterone for developing birds

(egg/steroid hormone/embryo/sexual differentiation/aggression)

HUBERT SCHWABL

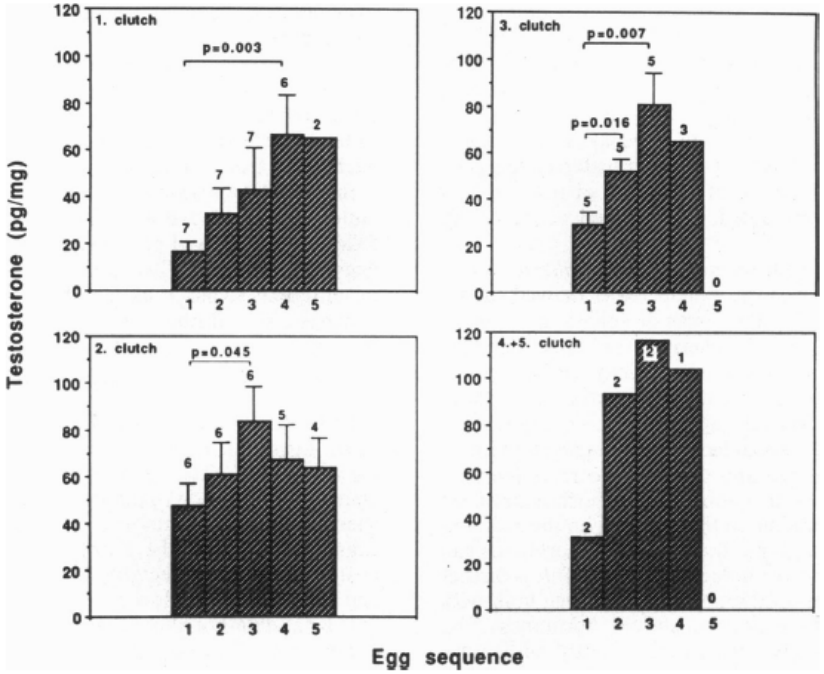
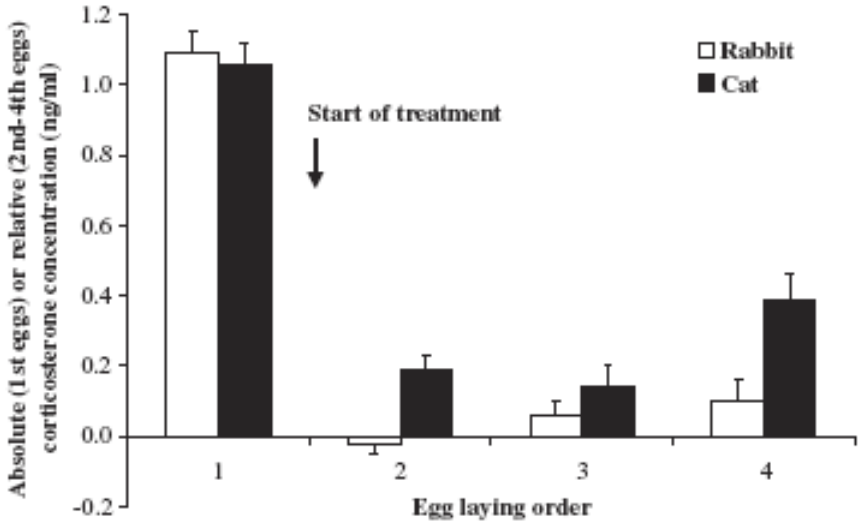


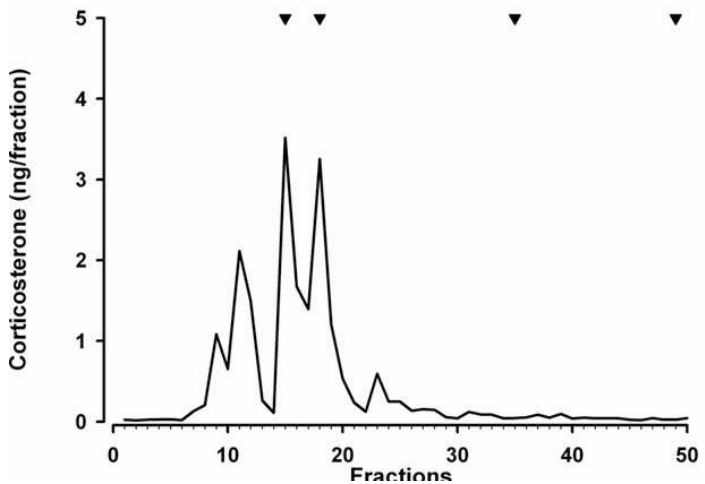
FIG. 2. T concentration (in picograms per mg of yolk; means ± SEM) of eggs from successive clutches laid by seven pairs of canaries, showing the order of laying in a clutch. Numbers on top of columns indicate numbers of eggs. T levels varied with order of laying (clutch 1: $F = 4.92$, $P = 0.019$; clutch 2: $F = 6.71$, $P = 0.011$; clutch 3: $F = 0.69$, $P = 0.54$). Statistical analysis was not done on clutches 4 and 5, here shown together, because of the small number of eggs laid. Significant differences in T contents between eggs are indicated by brackets.

…ale pozor na artefakty způsobené metodou měření hormonů

Saino et al. *J. Exp. Zool.* 2005



„Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring“



Gestagens and glucocorticoids in chicken eggs

S. Rettenbacher [a,*], E. Möstl [a], T.G.G. Groothuis [b]

General and Comparative Endocrinology

Maternální efekt může být způsoben formou rodičovské péče

1956 – Seymour Levine – handling potkaňat vede k zlepšené toleranci vůči stresu v dospělosti

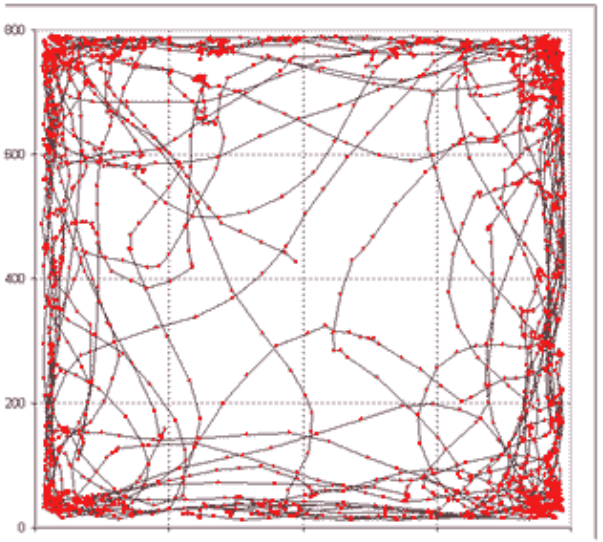
později: - i větší tendenci k exploračnímu chování, větší rodičovské péči

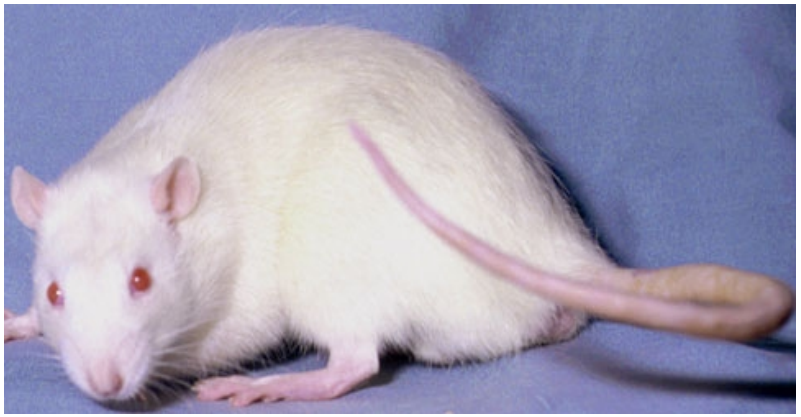
- předává se transgeneračně negeneticky (cross-fostering)

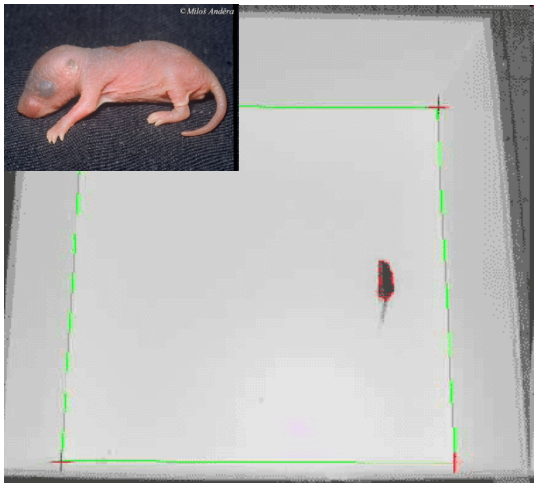
Nongenomic Transmission Across Generations of Maternal Behavior and Stress Responses in the Rat

Darlene Francis, Josie Diorio, Dong Liu, Michael J. Meaney*

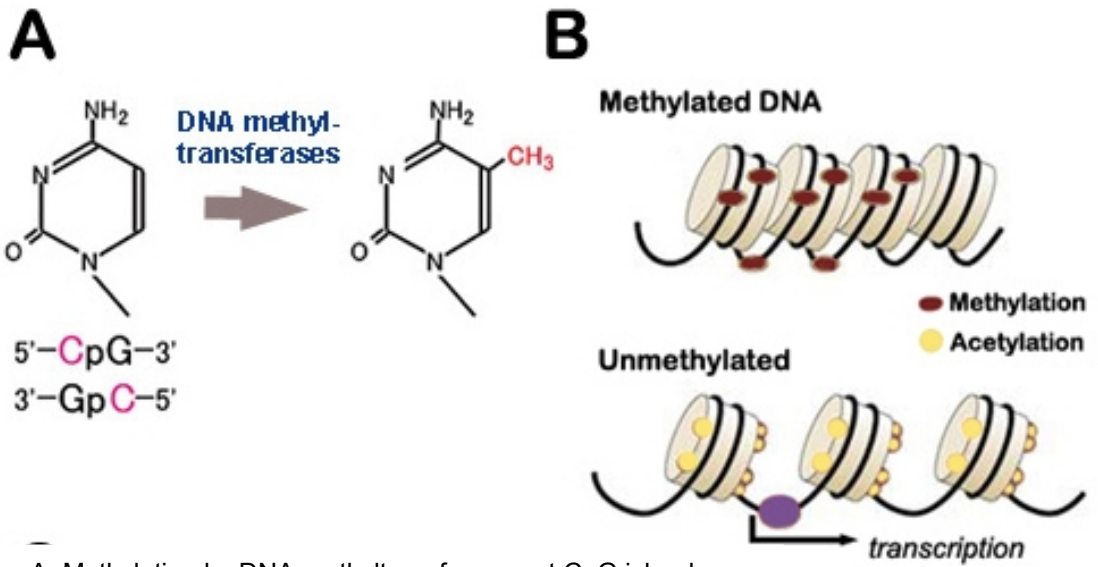
Science 5 November 1999



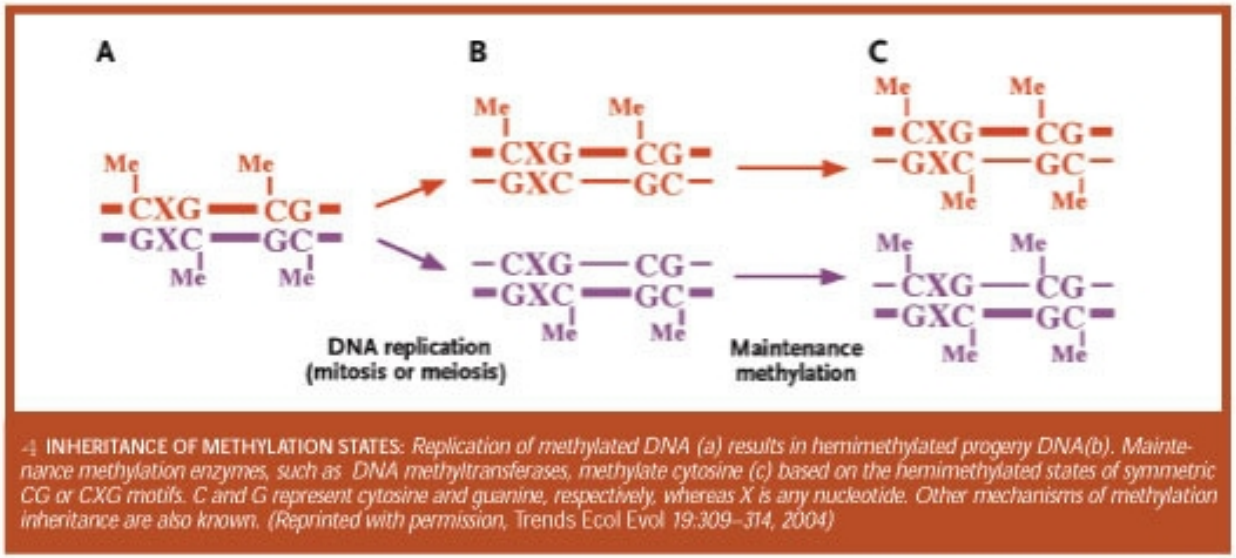




Fenotypová plasticita může souviset s epigenetickými změnami chromatinu



A. Methylation by DNA methyltransferases at CpG islands.
B. DNA demethylation relaxes chromatin structure allowing histone acetylation and the binding of transcriptional complexes.



4 INHERITANCE OF METHYLATION STATES: *Replication of methylated DNA (a) results in hemimethylated progeny DNA(b). Maintenance methylation enzymes, such as DNA methyltransferases, methylate cytosine (c) based on the hemimethylated states of symmetric CG or CXG motifs. C and G represent cytosine and guanine, respectively, whereas X is any nucleotide. Other mechanisms of methylation inheritance are also known. (Reprinted with permission,* Trends Ecol Evol *19:309–314, 2004)*

Fenotypová plasticita může souviset s epigenetickým reprogramováním chromatinu

Epigenetic programming by maternal behavior

Ian C G Weaver[1,2], Nadia Cervoni[3], Frances A Champagne[1,2], Ana C D'Alessio[3], Shakti Sharma[1], Jonathan R Seckl[4], Sergiy Dymov[3], Moshe Szyf[2,3] & Michael J Meaney[1,2]

NATURE NEUROSCIENCE VOLUME 7 | NUMBER 8 | AUGUST 2004

- **mateřská péče (doloženo cross-fosteringem) vede ke změně metylace cytosinu v promotoru genu pro receptor glukokortikoidů (GR) v hipokampu (mění vazbu NGFI-A na promotor) během prvního týdne po narození, což vede ke změně exprese GR**

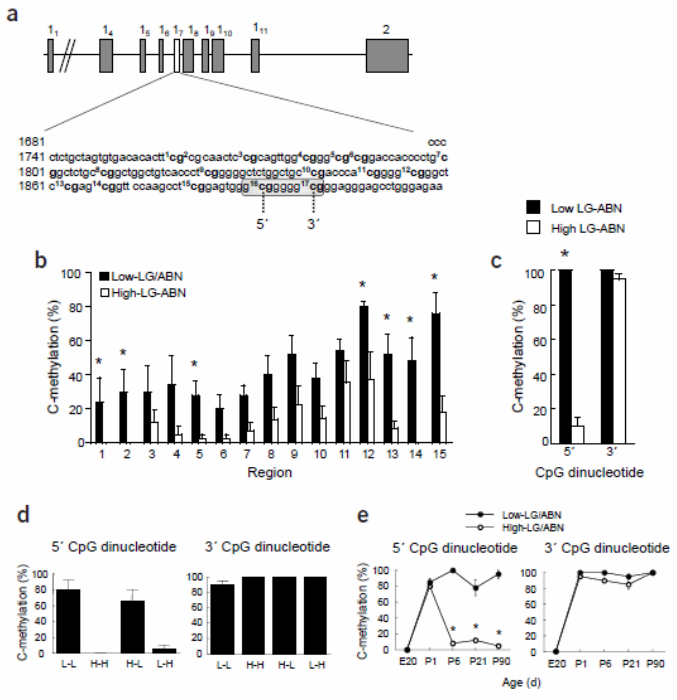
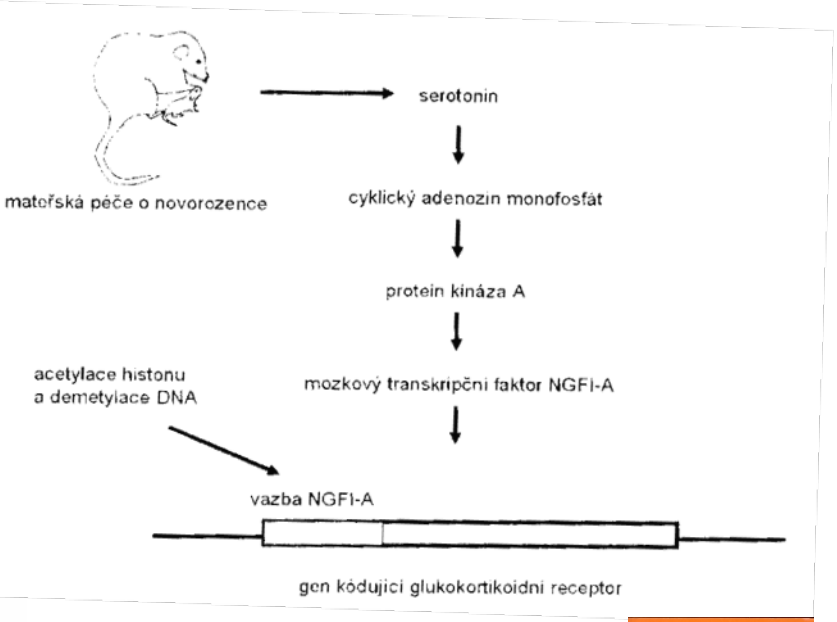
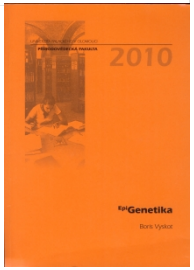


Figure 1 Maternal care alters cytosine methylation of GR promoter. (**a**) Sequence map of the exon 1_7 GR promoter including the 17 CpG dinucleotides (bold) and the NGFI-A binding region[16] (encircled). (**b**,**c**) Methylation analysis of the 17 CpG dinucleotides of the exon 1_7 GR promoter region from adult high- and low-LG-ABN offspring (6–10 clones sequenced/animal; $n = 4$ animals/group; $^*P < 0.01$). (**b**) Percentage of cytosine residues that were methylated (mean ± s.e.m.) for the first 15 CpG dinucleotides ($^*P < 0.05$). (**c**) Percentage of methylated cytosines (mean ± s.e.m.) for the 5′ (site 16) and 3′ (site 17) CpG dinucleotides within the NGFI-A binding sequence ($^*P < 0.0001$). (**d**) The effect of cross-fostering the offspring of high- and low-LG-ABN mothers on cytosine methylation of the 5′ and 3′ CpG dinucleotides within the NGFI-A binding sequence of the exon 1_7 GR promoter gene in adult hippocampi ($n = 5$ animals/group) L-L: animals born to and reared by low-LG-ABN mothers; H-H: animals born to and reared by high-LG-ABN mothers; H-L: animals born to high-LG-ABN mothers and reared by low-LG-AB mothers; L-H: animals born to low-LG-ABN mothers and reared by high-LG-ABN mothers. (**e**) Percentage of cytosine methylation (mean ± s.e.m.) of the 5′ and 3′ CpG dinucleotides with the NGFI-A binding region of the exon 1_7 GR promoter gene in the offspring of high- or low-LG-ABN mothers ($n = 5$ animals/group; $P < 0.001$) as a function of age. There were no differences at any postnatal age in level of cytosine methylation of the 3′ CpG (site 17).





- **změna metylace, acetylace histonů a vazby NGFI-A**

Fenotypová plasticita a exprese genů

NATURE REVIEWS | GENETICS VOLUME 9 | AUGUST 2008 | 575

The environmental contribution to gene expression profiles

Greg Gibson

- *cis*-regulace
- *trans*-regulace nebo společná regulační síť

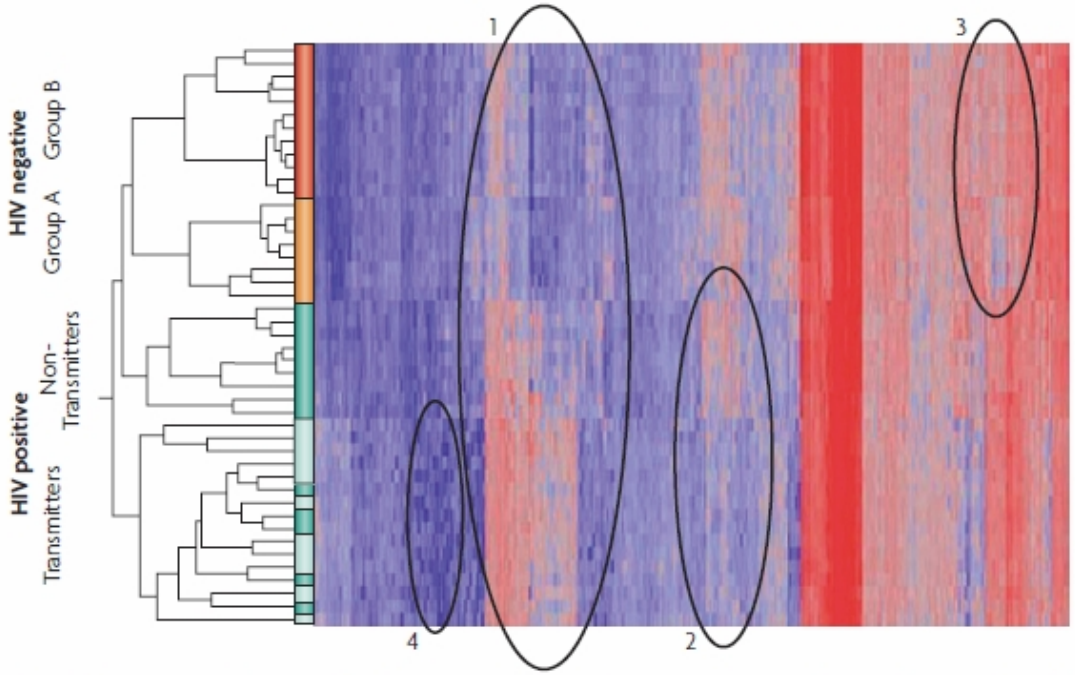


Figure 1 | **Sources of expression heterogeneity.** This heat map shows the expression profiles of several thousand genes that are differentially expressed in a sample of 45 Botswanan women[4]. Each row is the profile of an individual woman, each column represents the abundance of one transcript from very low (dark blue) to low (light blue) to very high (dark red) expression. The clustering suggests four groups, but the analytical challenge is to identify statistically significant groupings of subsets of genes, and to explain the sources of variability. Visually, the genes within ellipse 1 demarcate HIV-positive from HIV-negative women. Those in ellipse 2 identify a subset of non-transmitting mothers (top dark green bar), whose profile is distinct from a group consisting of both mothers who did transmit the virus (top light green bar) and of some of the transmitters (other dark green bars). Ellipse 3 highlights a group of genes that differ in abundance between two subgroups of the HIV-negative women, and ellipse 4 highlights a group of genes that are especially heterogeneous within the transmitting mothers, as implied by the variety in the depth of blue shading. A simple prediction would be that a common *trans*-acting genetic factor produces group 3, because there is clear co-regulation of the transcripts, whereas *cis*-acting genetic factors might independently regulate the transcripts in group 4. Feasibly, joint genotyping and expression profiling can be used to test the hypothesis. Alternatively, numerous loci spread throughout the genome could lead to clustering of relatives, in which case pedigree or relatedness analysis should suggest that individuals with similar expression profiles are more closely related by descent.

Fenotypová plasticita a exprese genů

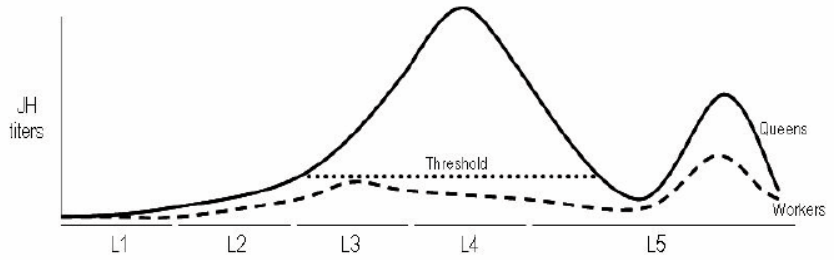


Figure 1
Reaching of the juvenile hormone (JH) threshold in developing females is proposed not only to allow for the general body growth and ovary development, but also to act by negatively regulating the development of some organismal systems that are characteristics of adult workers and are also present in the original developmental pattern. JH titres during larval development (L1–L5) data are modified from Hartfelder and Engels [5]

SHORT COMMUNICATION

P. WIRTZ * & J. BEETSMA *: *Induction of caste differentiation in the honeybee* (Apis mellifera) *by juvenile hormone.*

Ent. exp. & appl. **15** (1972) 517—520. *N. Holl. Uitg. Mij Amsterdam*

Molecular determinants of caste differentiation in the highly eusocial honeybee *Apis mellifera*

Angel R Barchuk*[1,4], Alexandre S Cristino[2], Robert Kucharski[4], Luciano F Costa[3], Zilá LP Simões[1] and Ryszard Maleszka[4]

BMC Developmental Biology 2007, **7**:70 doi:10.1186/1471-213X-7-70

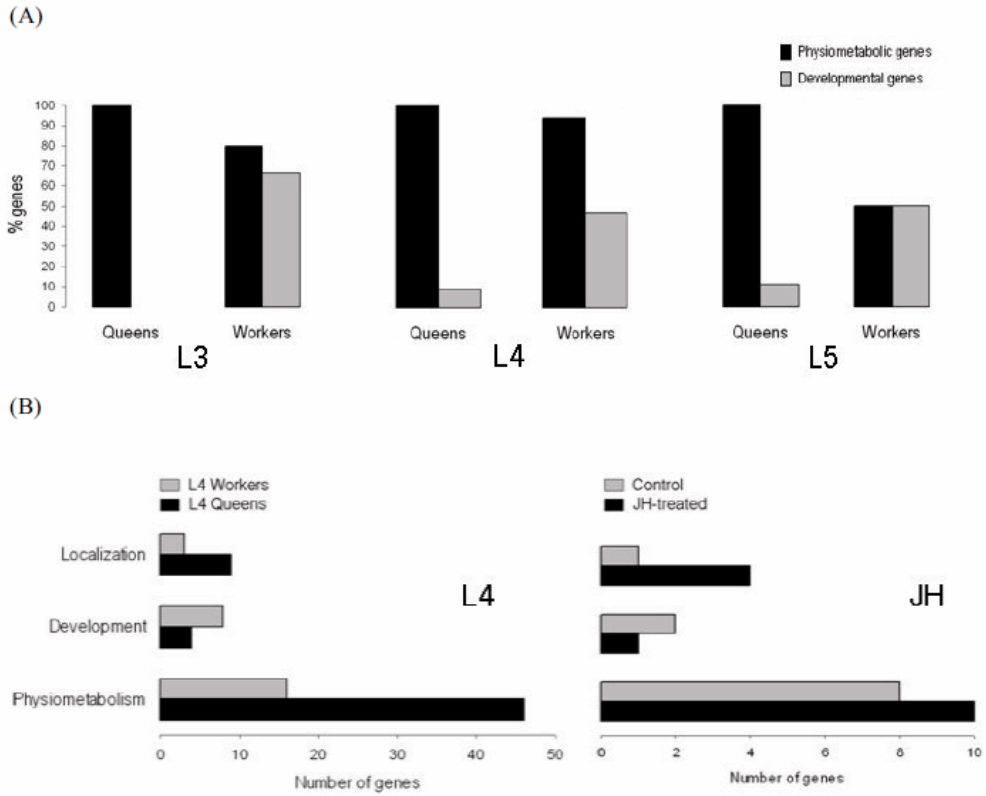


Figure 2
Functional trends of DEGs classified according to the Biological Process terms defined by GO consortium. (A) Developing workers up-regulate more developmental genes than queens in all studied larval instars. Physiometabolic genes are always more up-regulated than developmental genes (B) Juvenile hormone (JH) treatment induces a queen-like gene expression profile. Left panel: up-regulated genes in L4 queens/workers. Right panel: up-regulated genes in L4 Control/JH-treated workers. The proportion of Physiometabolic and Localization genes is higher in normal queens and JH-treated workers, whereas more Developmental genes are up-regulated in normal and in Control workers.

Fenotypová plasticita a exprese genů

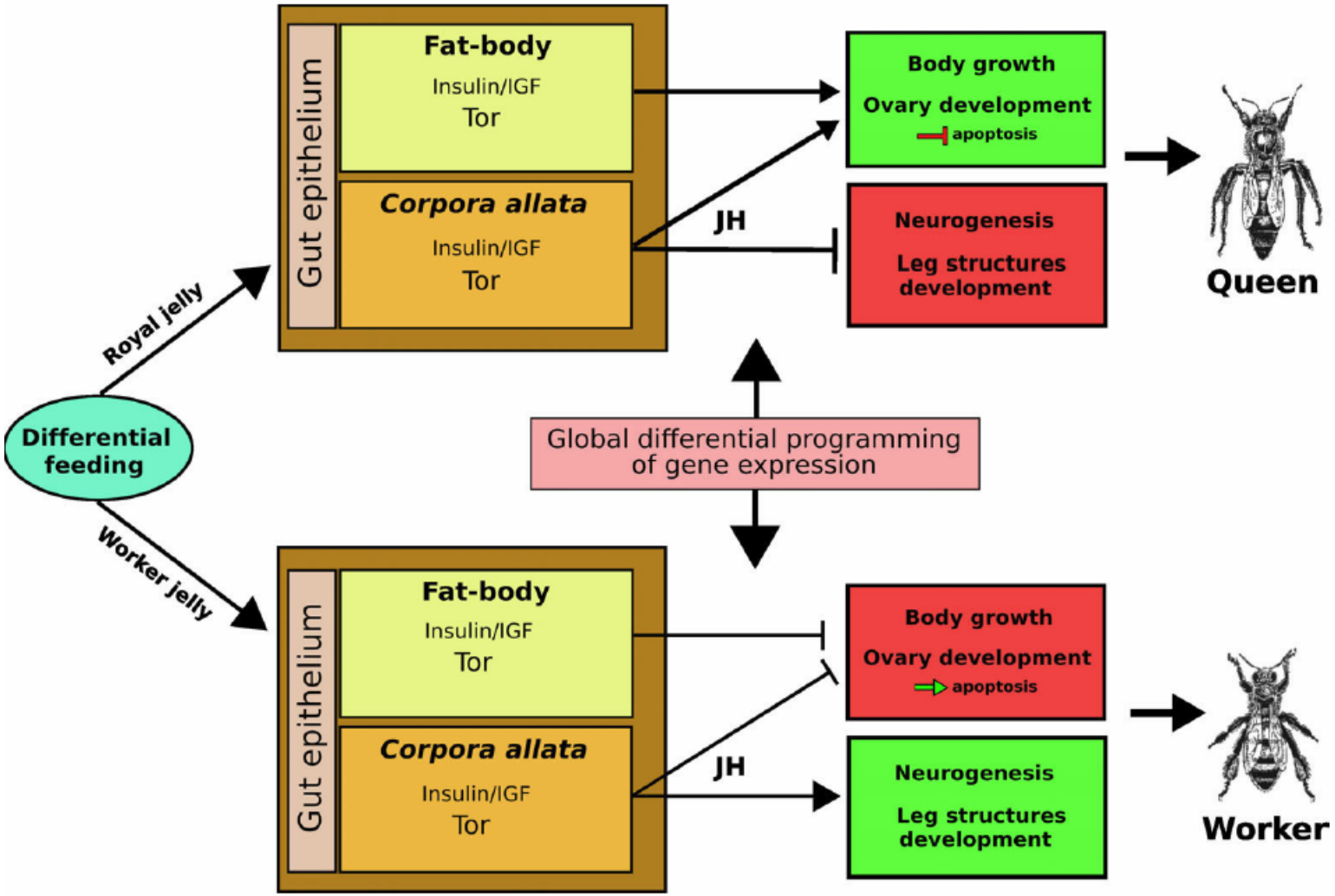


Figure 4
Proposed general model of caste differentiation in *Apis mellifera*. Arrows thickness indicates the relative action levels of the considered factors. Recent studies by our group suggest that the global differential programming of gene expression in the honeybee is controlled by DNA methylation mechanism in a manner similar to epigenetic transcriptional changes inducible by environmental factors in vertebrates (Maleszka et al., in preparation). For details see Section "Towards a unified model of caste differentiation in the honeybee".

Fenotypová plasticita a exprese genů

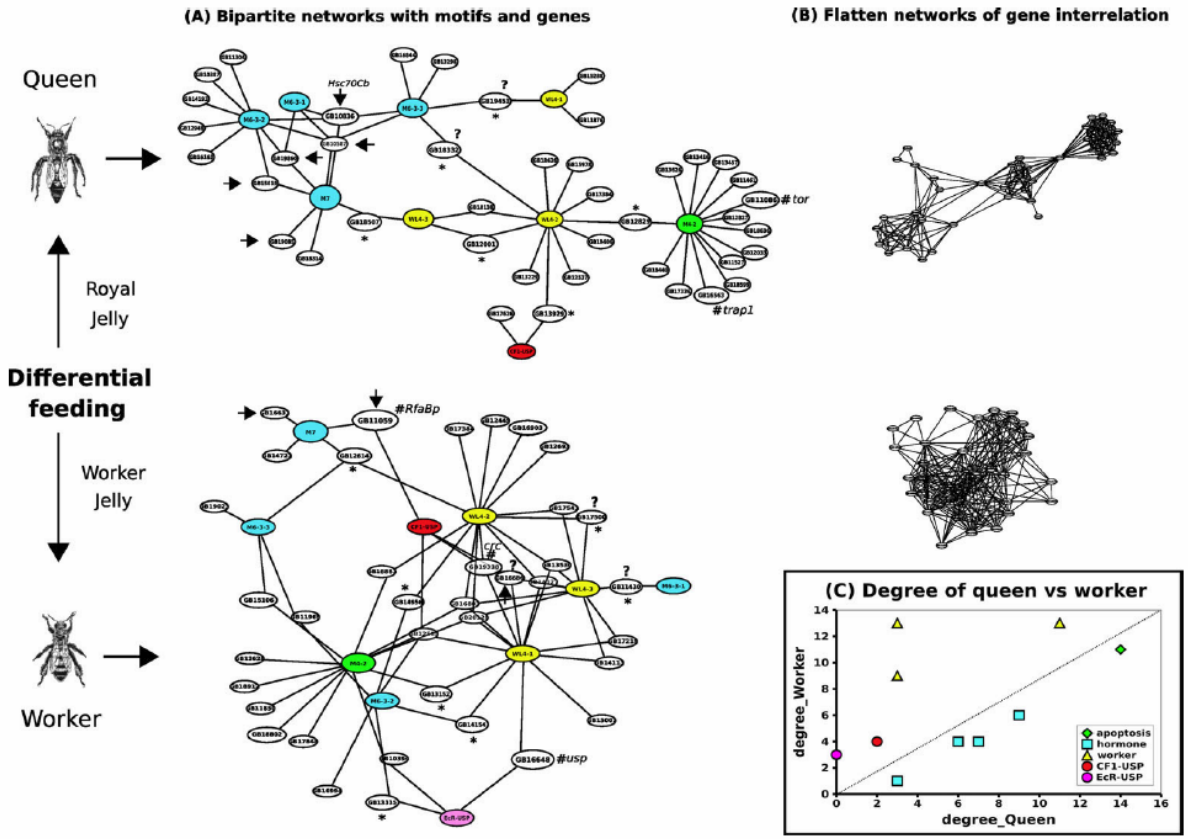


Figure 3
Networks depicting putative gene interactions based on the occurrence of overrepresented motifs in the UCR of DEG between *A. mellifera* castes. (A) Bipartite graph representing the occurrence of motifs (colorized circles) in the UCR of DEG in queen and worker castes. Motifs represented in blue were found in the functional group "JH responsive" (M6-3-1, M6-3-2, M6-3-3) and "hormone+caste" (M7), those in green were found in the functional group "apoptosis/other proteins" (M4-2), in yellow in top10-WL4 genes (WL4-1, WL4-2, WL4-3) and in magenta are motifs found experimentally in other insects (CF1-USP and EcR-USP). The black arrows point to genes coherently up-regulated in caste stages and JH assay. Genes with unknown function are marked by a question mark (?). Genes marked by an asterisk (*) were not in the training dataset for motif discovery. The worker DEG marked by a hash (#) are *usp, crc* and *RfaBp*, repressed by hormones. The queen DEGs marked by a hash (#) are *tor* and *trap1*, negative regulators of cell death in response to nutritional availability. (B) One layer graph (subsumed) designed to obtain measures of complex networks. Clustering coefficient (cc) and degree (d) show that worker's network (d = 62.21 ± 28; cc = 0.37 ± 0.23) is more interconnected than queen's network (d = 31.23 ± 15.67; cc = 0.36 ± 0.25). This suggests the worker DEGs share much more conserved *cis*-elements when compared to queen DEGs. (C) A plot obtained by representing each motif by a point with abscissa equal to its degree in the queen network and the ordinate equal to its degree in the case of the worker network. The fact that most nodes resulted above the main diagonal line (represented by the dashed line) objectively indicates that most promoters, except for "hormone" and "apoptosis" motifs, regulate more genes in the latter case (workers).

RNA interference

Mechanisms of gene silencing by double-stranded RNA

Gunter Meister & Thomas Tuschl

NATURE | VOL 431 | 16 SEPTEMBER 2004

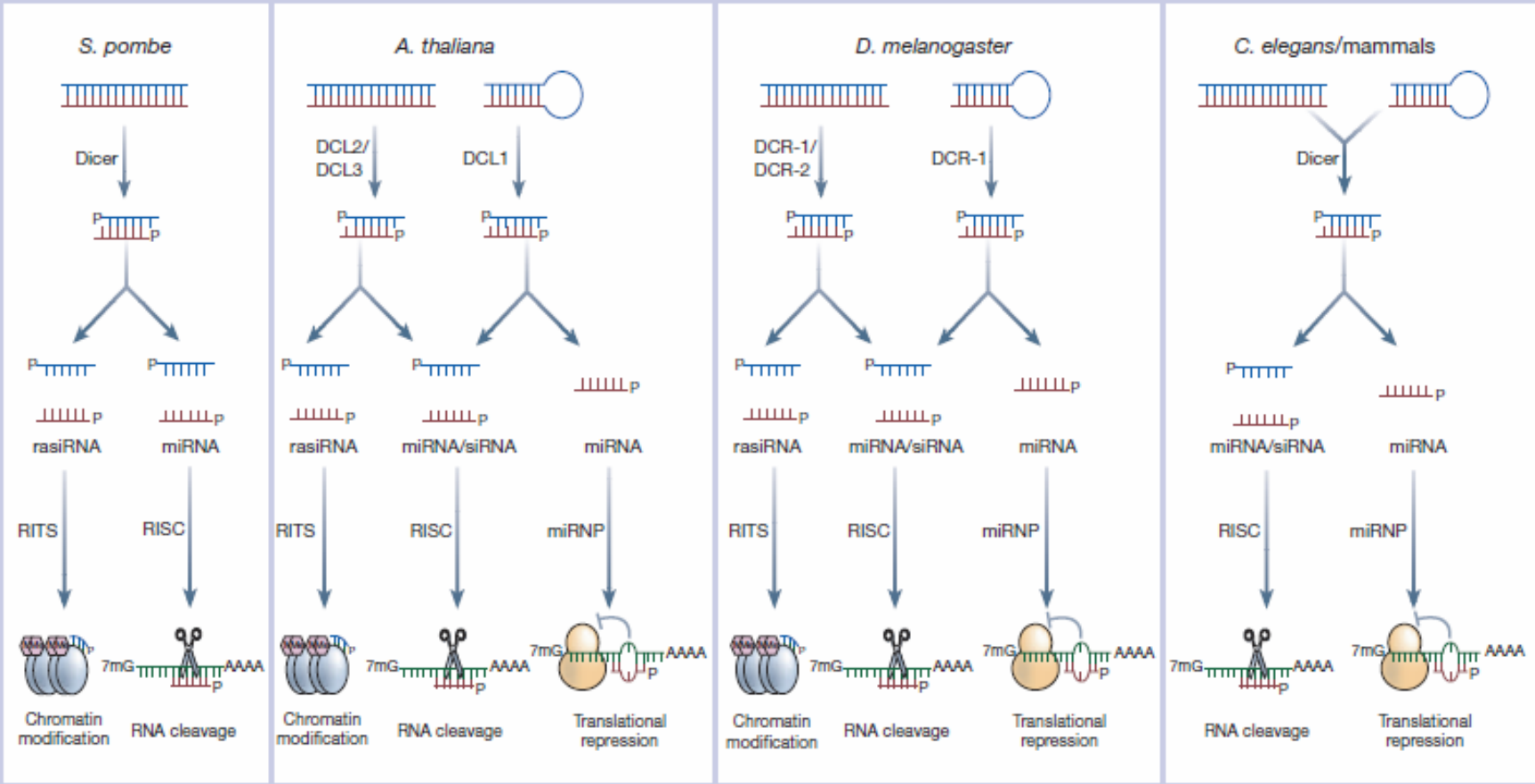


Figure 1 RNA silencing pathways in different organisms. Long dsRNA and miRNA precursors are processed to siRNA/miRNA duplexes by the RNase-III-like enzyme Dicer. The short dsRNAs are subsequently unwound and assembled into effector complexes: RISC, RITS (RNA-induced transcriptional silencing) or miRNP. RISC mediates mRNA-target degradation, miRNPs guide translational repression of target mRNAs, and the RITS complex guides the condensation of heterochromatin. In animals, siRNAs guide cleavage of complementary target RNAs, whereas miRNAs mediate translational repression of mRNA targets. rasiRNAs guide chromatin modification. *S. pombe*, *C. elegans* and mammals carry only one Dicer gene. In *D. melanogaster* and *A. thaliana*, specialized Dicer or DLC proteins preferentially process long dsRNA or miRNA precursors. 7mG, 7-methyl guanine; AAAA, poly-adenosine tail; Me, methyl group; P, 5′ phosphate.

Exprese genů souvisí s epigenetickou modifikací DNA

- manipulace DNA cytosine-5-methyltransferase 3 (Dnmt3) pomocí RNA interference (RNAi) u včel

Nutritional Control of Reproductive Status in Honeybees via DNA Methylation

R. Kucharski,* J. Maleszka,* S. Foret, R. Maleszka†

SCIENCE VOL 319 28 MARCH 2008

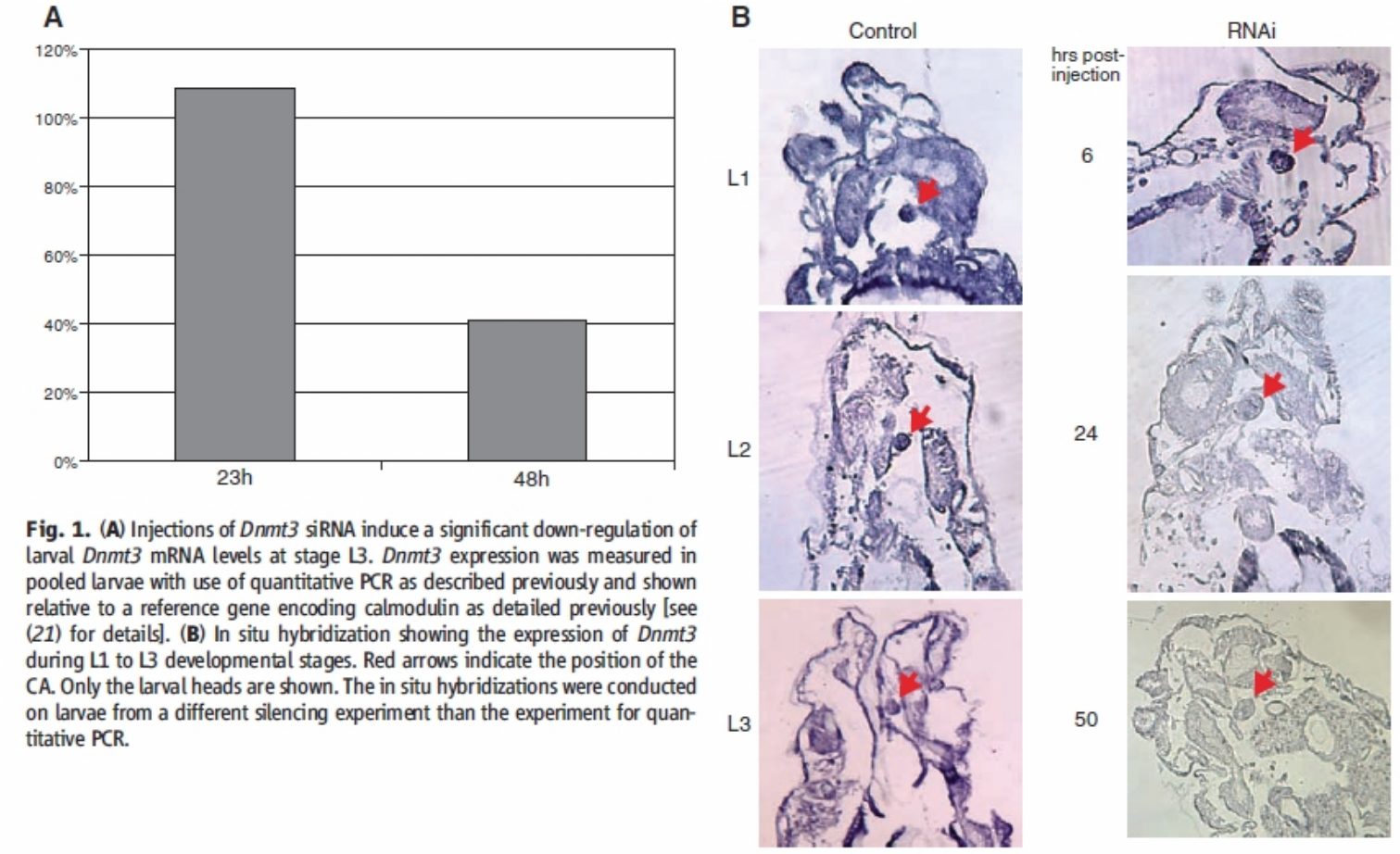


Fig. 1. (**A**) Injections of *Dnmt3* siRNA induce a significant down-regulation of larval *Dnmt3* mRNA levels at stage L3. *Dnmt3* expression was measured in pooled larvae with use of quantitative PCR as described previously and shown relative to a reference gene encoding calmodulin as detailed previously [see (*21*) for details]. (**B**) In situ hybridization showing the expression of *Dnmt3* during L1 to L3 developmental stages. Red arrows indicate the position of the CA. Only the larval heads are shown. The in situ hybridizations were conducted on larvae from a different silencing experiment than the experiment for quantitative PCR.

Exprese genů souvisí s epigenetickou modifikací DNA

- manipulace DNA cytosine-5-methyltransferase 3 (Dnmt3) pomocí RNA interference (RNAi) u včel

Nutritional Control of Reproductive Status in Honeybees via DNA Methylation

R. Kucharski,* J. Maleszka,* S. Foret, R. Maleszka†

SCIENCE VOL 319 28 MARCH 2008

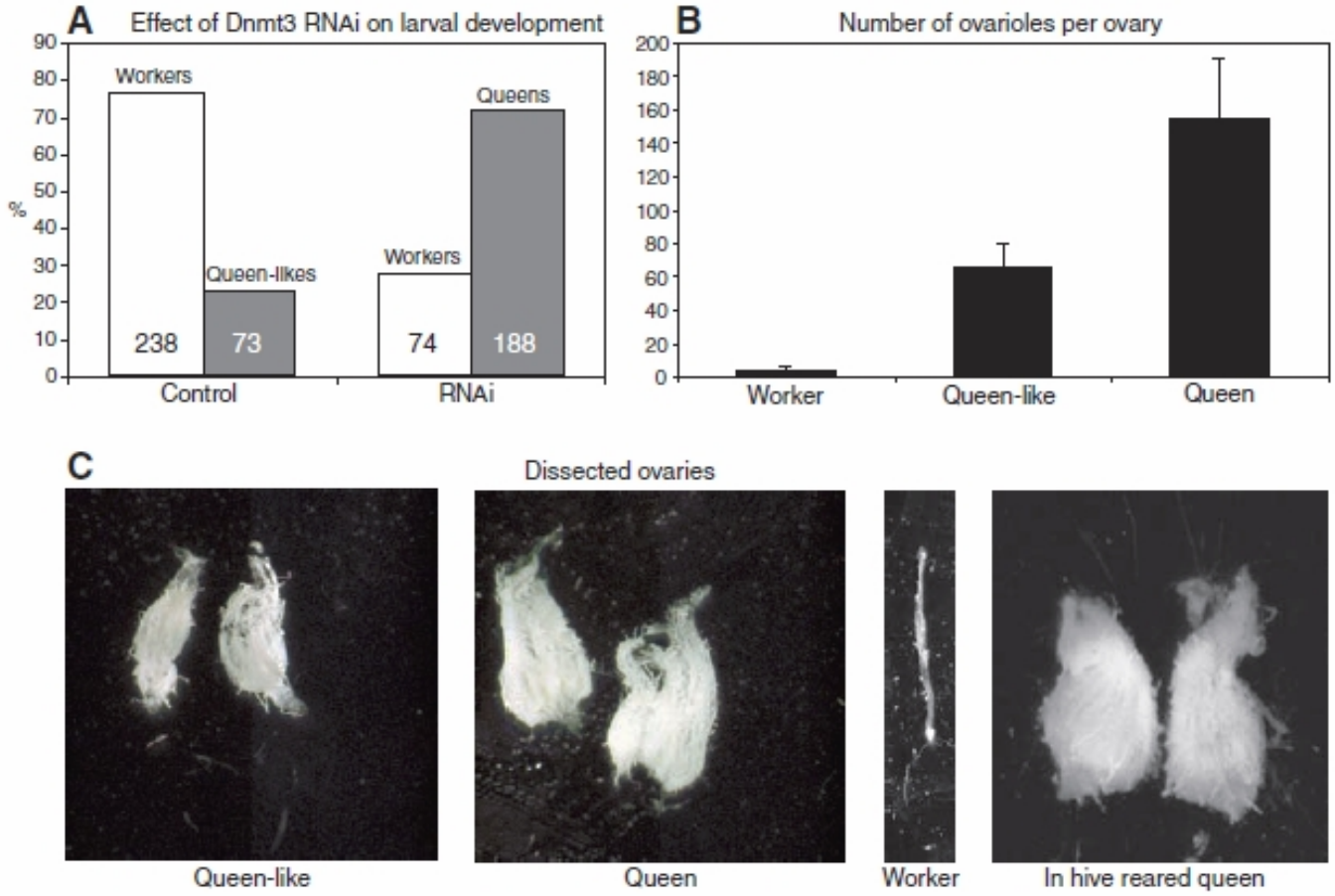


Fig. 2. Effect of Dnmt3 silencing on caste development in honeybees. Newly emerged larvae were injected either with a nonlarval control gene, *uth*, siRNA or with *Dnmt3* siRNA and allowed to develop until adulthood in a climate-controlled incubator. In both groups, the larvae developed normally, but the emerging adults displayed contrasting phenotypes. (**A**) The number of adults in each phenotypic category (workers, queens, and queenlikes). (**B**) The number of ovarioles per single ovary in each phenotypic class. Range error bars encompass the lowest and highest values. (**C**) Examples of ovaries dissected from each category and, for comparison, from a virgin queen reared in the hive on royal jelly. Queenlikes have queen morphological features but fewer ovarioles per ovary than queens [see (B)]. Workers have only rudimentary ovaries with two to six ovarioles. The figure is a compilation of four independent experiments. See (*21*), table S2, and fig. S1 for more details and results from individual experiments.

Exprese genů souvisí s epigenetickou modifikací DNA

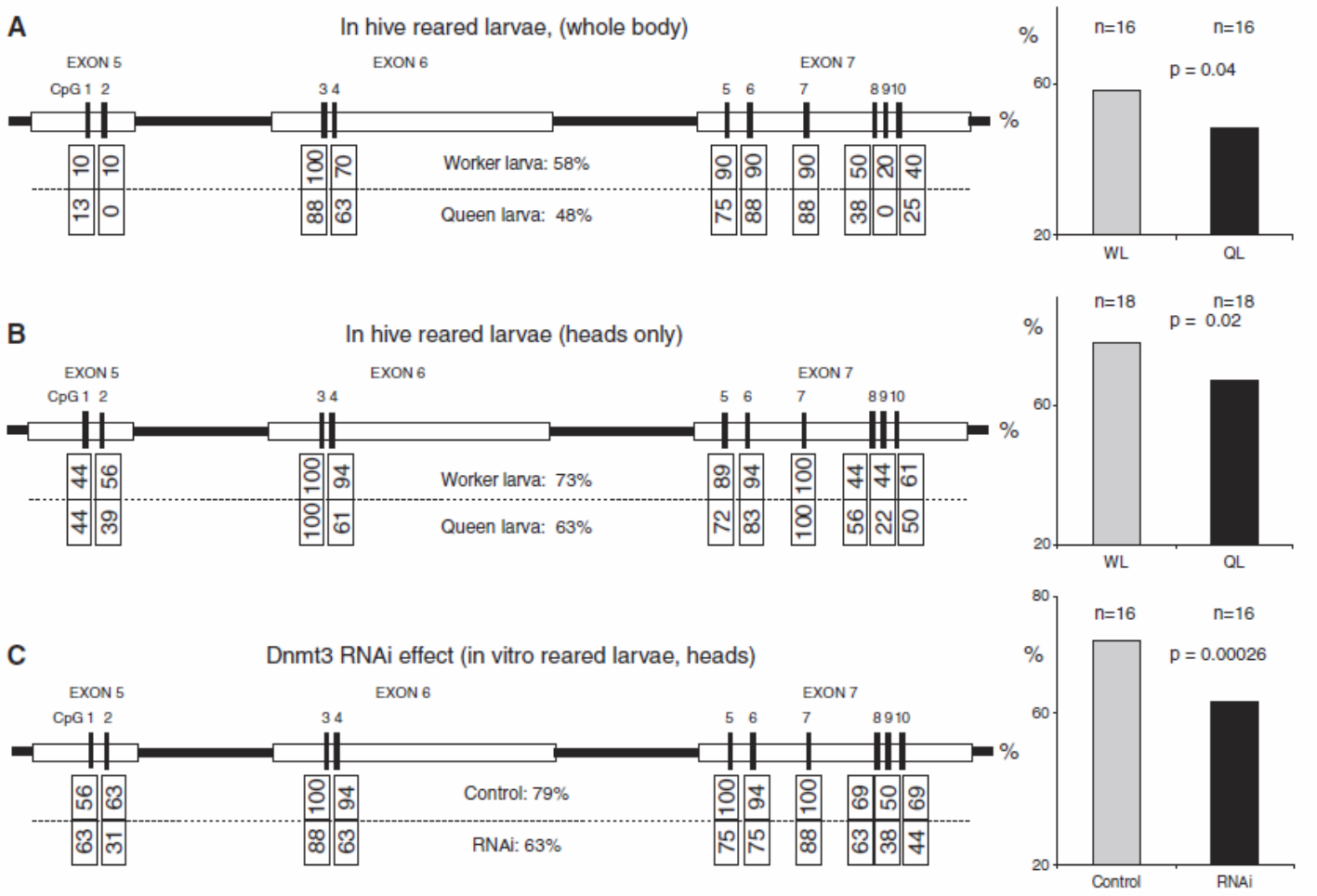
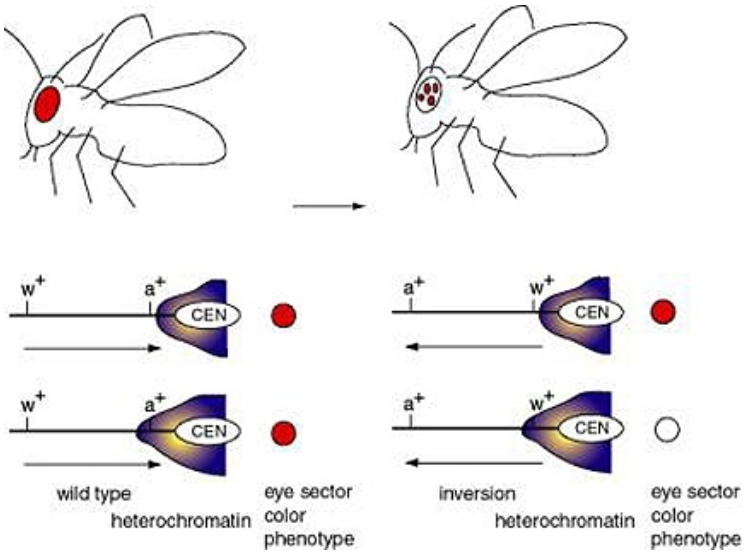
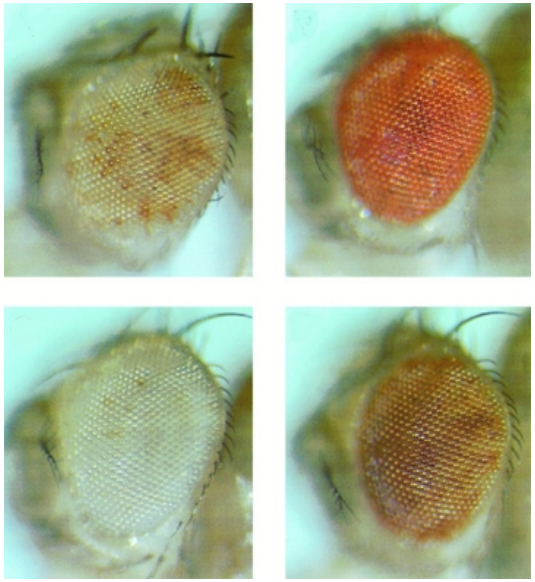
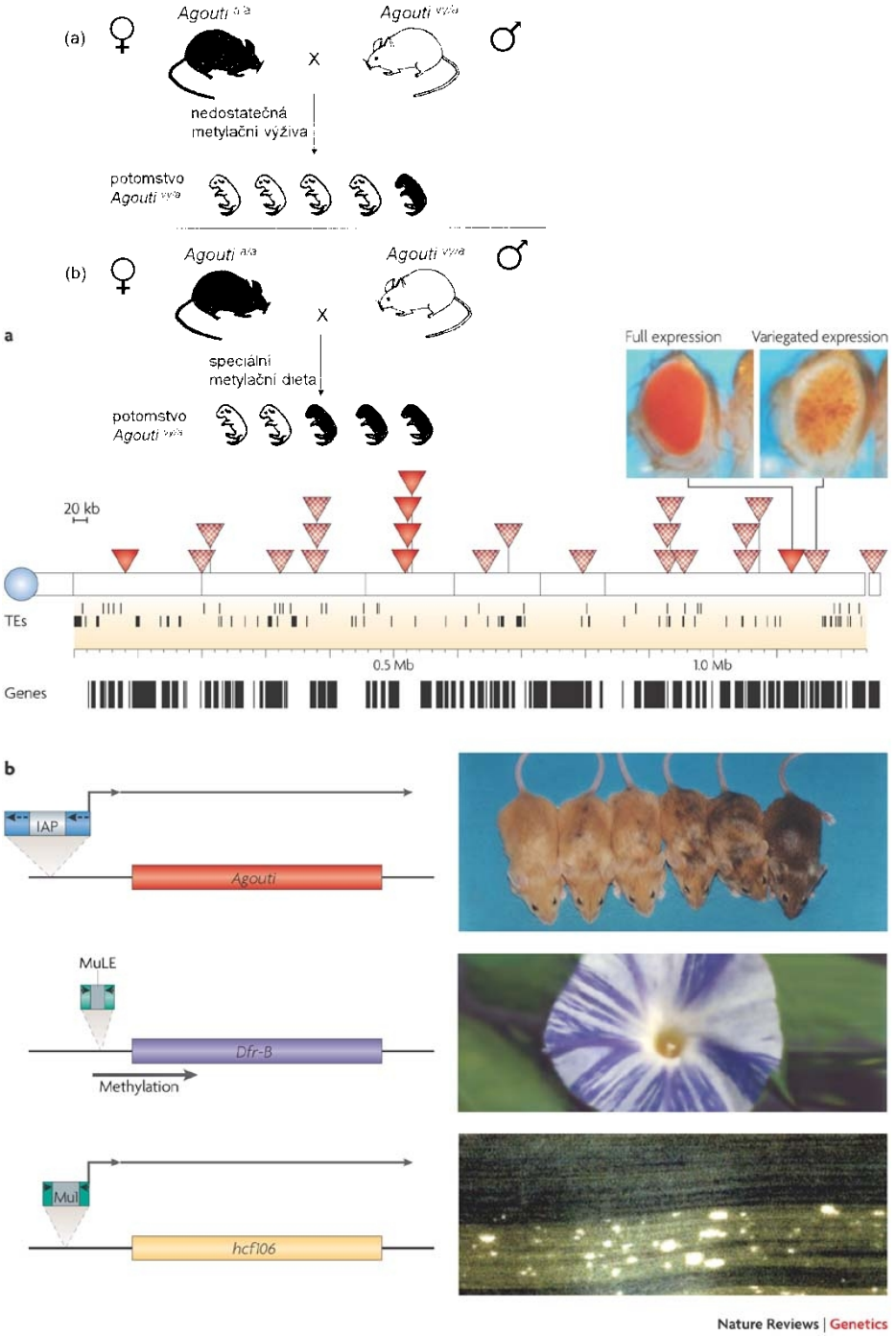


Fig. 3. Methylation status of cytosines in CpG dinucleotides of *dynactin p62*. The percentage of methylation for individual CpGs is shown in boxes, and the overall methylation in the right-hand graphs. DNA was isolated by using larvae collected (**A** and **B**) from the hive [for (A), pooled whole late-L3 larvae, $n = 7$; for (B), heads only, $n = 20$ for workers and $n = 14$ for queen larvae] and (**C**) from pooled heads of late-L3 in vitro reared larvae ($n = 7$). The number of clones sequenced for each category is shown above the bars in the right-hand graphs. Methylation quantities along this gene were analyzed with a general linear model of the binomial family (*31*) by using treatment (diet or RNAi) and position as factors to model the state of each CpG. The differences between queen larvae (QL) and worker larvae (WL) as well as the effect of RNAi are statistically significant.

Poziční efekt a umlčování transpozonů







Alternativní „splicing“

Heredity (2008) 100, 111–120

www.nature.com/hdy

SHORT REVIEW

Quantitative and evolutionary biology of alternative splicing: how changing the mix of alternative transcripts affects phenotypic plasticity and reaction norms

JH Marden

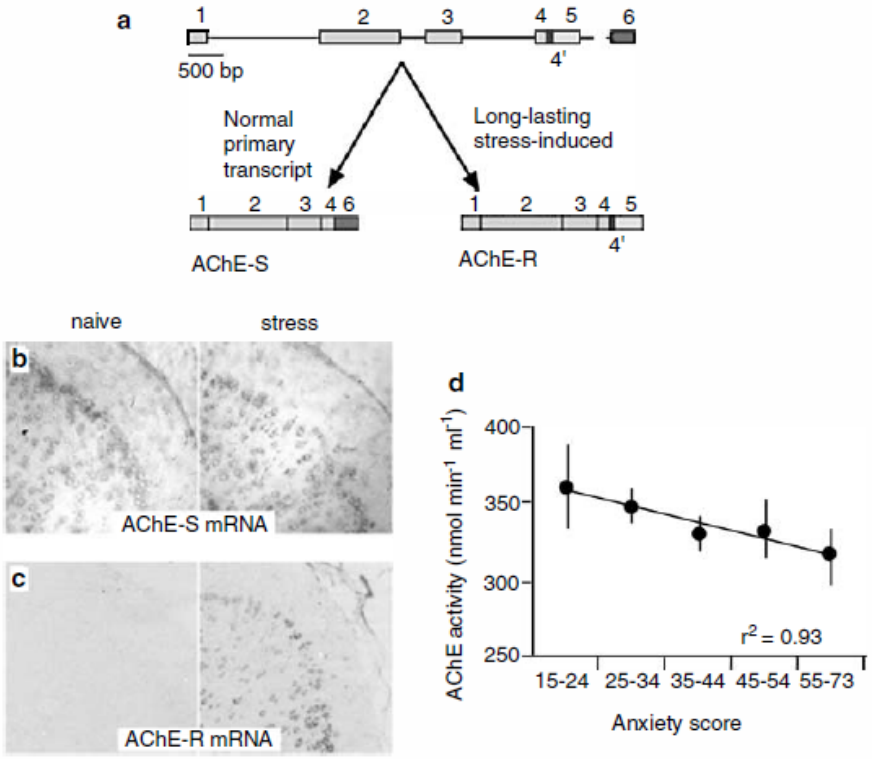
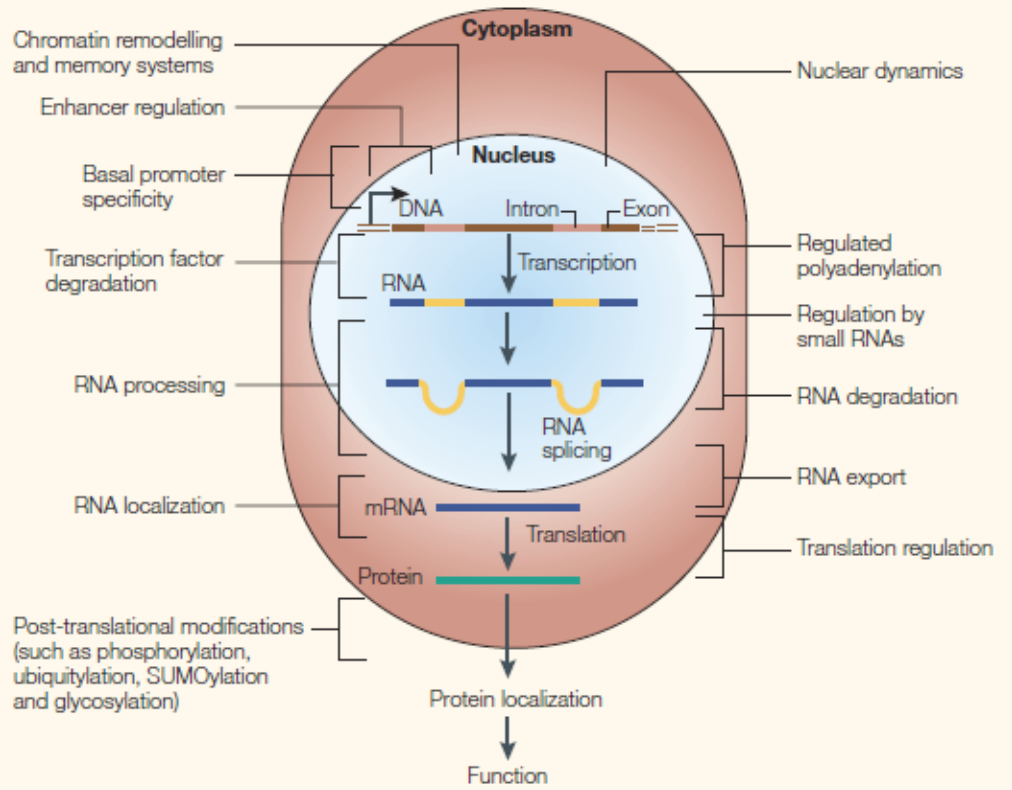
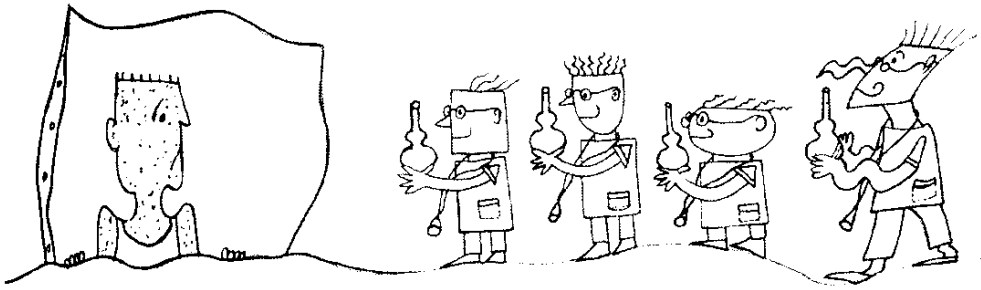


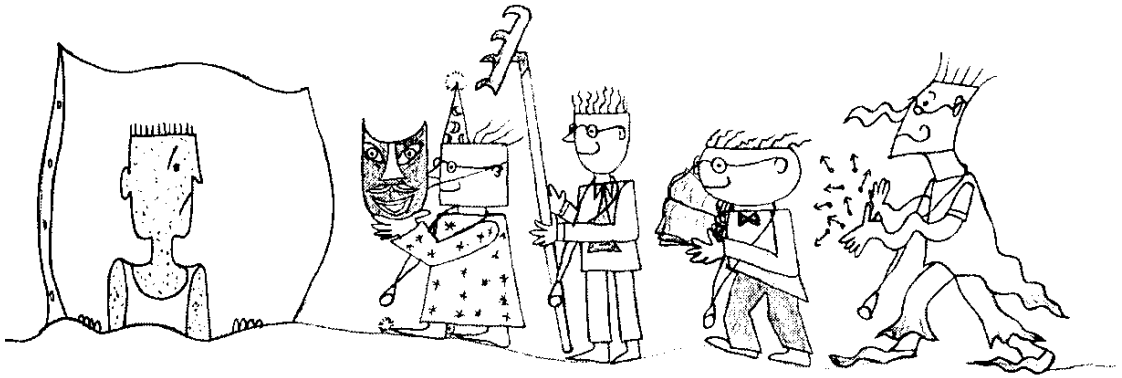
Figure 1 (**a**) Intron–exon structure and alternative exon inclusion at the 3′-end of transcripts of the mammalian AChE gene. (**b** and **c**) Fluorescent *in situ* hybridization with mRNA of AChE-S (**b**) and AChE-R (**c**) in the prefrontal cortex of mice before and 2 weeks after stress. (**d**) Relationship between human self-reported anxiety scores and the level of AChE enzyme activity in their blood serum; this reflects abundance of the freely circulating AChE-R isoform. (**a**–**c**) are adapted from Meshorer *et al.* (2005); (**d**) is adapted from Sklan *et al.* (2004).

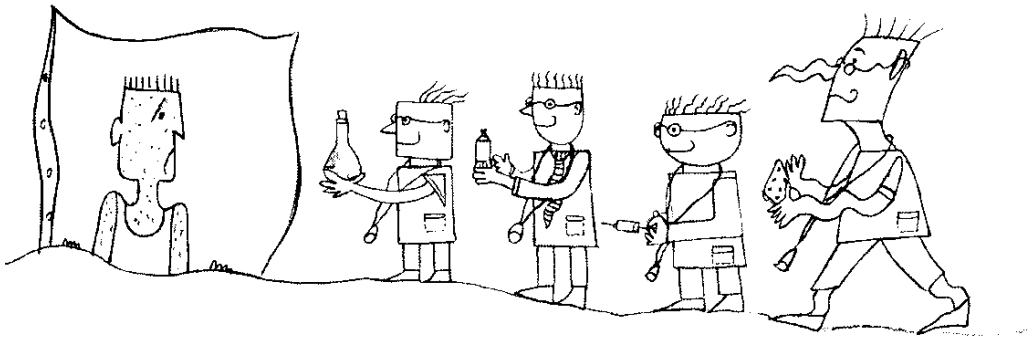
Další proximátní mechanismy fenotypové plasticity – jak jsou jednotlivé procesy ovlivněné prostředím?

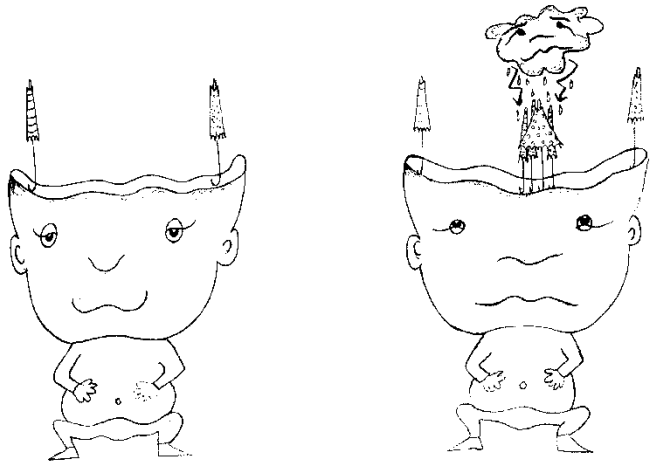


Indukované lokální mutace?







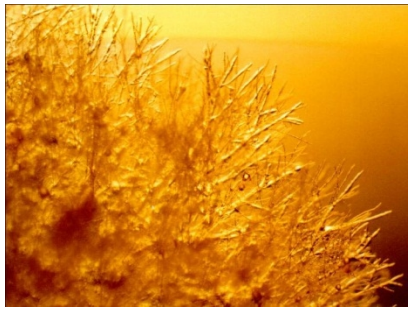


Mol. Cell. Biol., 1995, 5586-5597, Vol 15, No. 10

DNA methylation associated with repeat-induced point mutation in *Neurospora crassa*

MJ Singer, BA Marcotte and EU Selker

- změna G/C – A/T



Shrnutí

- Fenotypová plasticita je často spojena se změnami metabolismu hormonů a alternací v expresi genů
- Změny exprese genů jsou často spojeny se změnami struktury chromatinu
- Fenotypová plasticita však může být spojená i s postranslačními procesy