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Article

Transgenerational epigenetic compensation and natural selection

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The term "epigenetics" defines all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself. Transgenerational epigenetic compensation was discovered in the untreated progeny of drug-treated males (rats and mice) as the opposite quantitative phenotypic changes. In natural populations, the hereditary basis of transgenerational epigenetic compensation develops mainly in homozygous mutant males, but it does not affect their phenotype. In their descendants, being in an independent locus, this heritable epigenetic compensation increases fitness and lifespan of homozygous mutant females and decreases lifespan of wild-type females, starting from F_2. Here we show that this transgenerational epigenetic compensation is a guiding agent of natural selection. Natural selection is not a directing or driving force of evolution anymore. Natural selection needs some guidance. Transgenerational epigenetic compensation can initiate speciation through segregation of mutants and wild-types and/or it can change the selection coefficient of a given mutation.

Transgenerational epigenetic compensation was discovered in the experiments with paternal drug treatment[1-4]. Prenatal, neonatal and adolescent treatment of males leads to observation of inversed phenotype in their F_1, F_2 and F_3 untreated descendants, at least in some traits[1-10].

In this article we will use these experiments with parental drug treatment in order to achieve better understanding of the results of natural selection, observed in the population of laboratory mice, consisted of wild-type, heterozygous and mutant *Per2*^{Brdm1} animals, lived under semi-natural conditions in outdoor pens (**Fig. 1**) during two years[1,11].

Four pens contained four independent populations of mice, at the beginning with 250 animals (in total), Mendelian distribution of genotypes 1:2:1 and equal numbers of females and males. Food and water were supplied by humans and both were constantly placed in two locations inside each pen. Each animal was injected with transponder (Trovan ID100). A square antenna was placed in a horizontal plane around a combination of a food pod with a water bottle, in order to register animals' visits to estimate their drinking and feeding behaviour. All mice were live trapped twice a year and all new mice (born inside pens) were genotyped and received transponders. The lifespan of each mouse was estimated using its visits of food-water places. Food and water consumption could not be analyzed separately, because each of two places contained both food and water.

Pens were protected from terrestrial predators by an electric fence on the top of slate walls. However all local aerial predators had free access to mouse populations. Aerial predators were represented by a tawny owl (*Strix aluco*) [it has been seen many times], a short-eared owl (*Asio flammeus*) [it was possible to hear it sometimes], and other aerial predators could not be excluded. Trovan transponders, injected into mice previously, were found several times in mouse residues in owl pellets, left by birds outside the pens, and this is a direct confirmation of owls' feeding behaviour. All attempts to find transponders from the missing mice inside the pens have brought negative results (practically impossible to find), but the explanation can be different, for example, a transponder can not be read, if it has gone into the wet soil.

Figure 1 | Semi-natural environment for investigation of natural selection. Wild-type, heterozygous and mutant *Per2*^{Brdm1} mice were breeding at will during two years in four pens 20 × 20 m each, each with two shelters[11]. At the beginning of experiment there were 250 mice in total with Mendelian distribution of genotypes 1:2:1 and equal presence of females and males. Tawny owls (*Strix aluco*) were hunting for mice all the year round.

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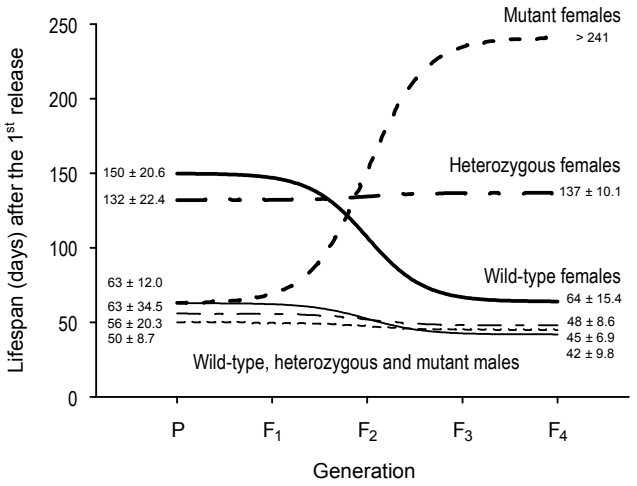

Figure 2 | Lifespan of *Per2*^{Brdm1} mice in pens after the first release[1,11]. All mice were taken out of pens (**Fig. 1**) and released back twice a year. New ones (born during previous half-year) were genotyped. Transponders, bearing individual numbers, were injected into all mice. Antennae, placed around feeding places, were used for registration of behaviour and estimation of lifespan. Lifespan, calculated from the day of 1st release, is shown here. Note an unexpected increase of lifespan in mutant females and simultaneous decrease of lifespan in wild-type females. Median ± SE.

We assume that the presence of Per2 mutant gene in a homozygous state and under harsh semi-natural conditions (*e.g.* temperature conditions) produces the same kind of transgenerational epigenetic compensation as paternal drug treatment (prenatal, neonatal or adolescent).

We know that so different parental treatments as prenatal (E8-E14) vinclozolin treatment and adolescent (P30-P50) maternal morphine treatment tend to produce common gender-specific phenotype in the F_1 and F_2 descendants, observed in the elevated plus-maze. Namely, females, but not males, of generations F_1 and F_2, show decreased time spent on open arms of elevated plus-maze (**Supplementary Fig. 2**). This is an indicator of their increased caution. Pharmacologists usually say that this is an increased "anxiety". However all observations of wild-caught voles, like bank vole (*Clethrionomys glareolus*) and root vole (*Microtus oeconomus*), in laboratory conditions, demonstrate that it is not a correct interpretation of animal behaviour. Wild-caught voles, those do not move at all in many laboratory tasks (due to so-called "freezing" behaviour), demonstrate in fact an increased "caution", but not "anxiety". It is so because the same wild-caught voles outperform any laboratory mouse strain and any laboratory F_1 hybrid, like B6D2F1, in the Morris water maze task[12]. Wild-caught voles are not more "anxious", but they are more "normal" creatures than any laboratory mouse stock.

It is possible that transgenerational epigenetic compensation, being genotype-specific, nevertheless activates some universal mechanisms, those were useful in wild nature, but useless in laboratory conditions during previous more than 100 years. The observed induction of increased caution in females (F_1 and F_2) may have the same level of generalization as general adaptation syndrome, described by Hans Selye[13].

In the **Fig. 2** we can see increased lifespan in the homozygous mutant females (starting from F_2) and decreased lifespan in the wild-type females. Thus, given semi-natural external conditions induced stress in homozygous mutants that resulted in formation of transgenerational epigenetic compensation, expressed in their descendants as increased caution in homozygous mutant females and as disrupted caution in wild-type females. Then, tawny owls have selected the least cautious mice as a source of food.

There is a belief that the main source of mouse losses in these pens is a male-male competition, during which male mice fight with each other up to death. This belief is only partially correct, because, indeed, a fighting mouse is an easy prey for an owl. Note, however, that both strong and weak fighters can be equally good food for an avian predator (an owl has very good hearing abilities and very good vision). The only way to escape from the owl is to avoid male-male fighting in general, and it seems that our laboratory male mice in these pens could not do this. That is why we have very interesting genotype-specific profile of lifespan in females and only low and genotype-non-specific lifespan in males (**Fig. 2**). Note also, that the most intense genotype-specific selection among females took place during summer, when snow was absent and owls could hunt with high efficacy (Supplementary Fig. 3[2]).

Why we are so sure that we are dealing with epigenetic inheritance[14-17] and transgenerational epigenetic compensation, but not with some other factor? Let's look now at the experiments with parental drug treatment and at very-very interesting observations on guinea pigs. We shall move through our data in the following order: 1) mice, 2) rats, 3) guinea pigs.

Neonatal (P0-P11) thyroxine treatment of inbred DBA/2J mice has led to improved two-way avoidance performance in drug-treated animals and to impaired two-way avoidance performance in the F_1 male and female descendants of thyroxine-treated males. In the F_2 animals the impaired two-way avoidance was observed only in females. In the F_3 generation the impaired two-way avoidance was observed only in males of outcross subline (**Fig. 3**).

Other significantly modified traits in all these F_1-F_3 animals, namely decreased birthweight and decreased intra- and infrapyramidal hippocampal mossy fiber projections (shortly: brain morphology), were not correlated with each other and with two-way avoidance performance (no individual correlations)! It was easy to suppose that several independent loci can be involved, but in this case it remains a mystery how all these 3 traits occurred to be recollected together in the F_3-outcross males (Table 1[1] and Supplementary Fig. 2[1]). Only guinea pigs were able to provide insight (several years later). Note that the presence of impaired phenotype in the F_1 and F_3-F_4 males, but not in the F_2 males, was described with respect to humans more than 3000 years ago (see **Supplementary Table 2**).

Adolescent (P42-P79) chronic morphine treatment of male outbred Wistar rats has led to decreased analgesic effect of standard dose of morphine (10 mg/kg) in these treated animals and to increased analgesic effect of standard dose of morphine in their F_1 male descendants. All descendants were tested twice with time interval 24 hours, in tail-withdrawal test (**Fig. 4**). In the F_1 generation, during the first day, F_1 males have shown enhanced analgesic effect, but F_1 females have shown normal phenotype. During the second day all F_1 males and F_1 females have shown normal phenotype. Very high speed, at which abnormal phenotype of F_1 males was converted into normal one, is amazing.

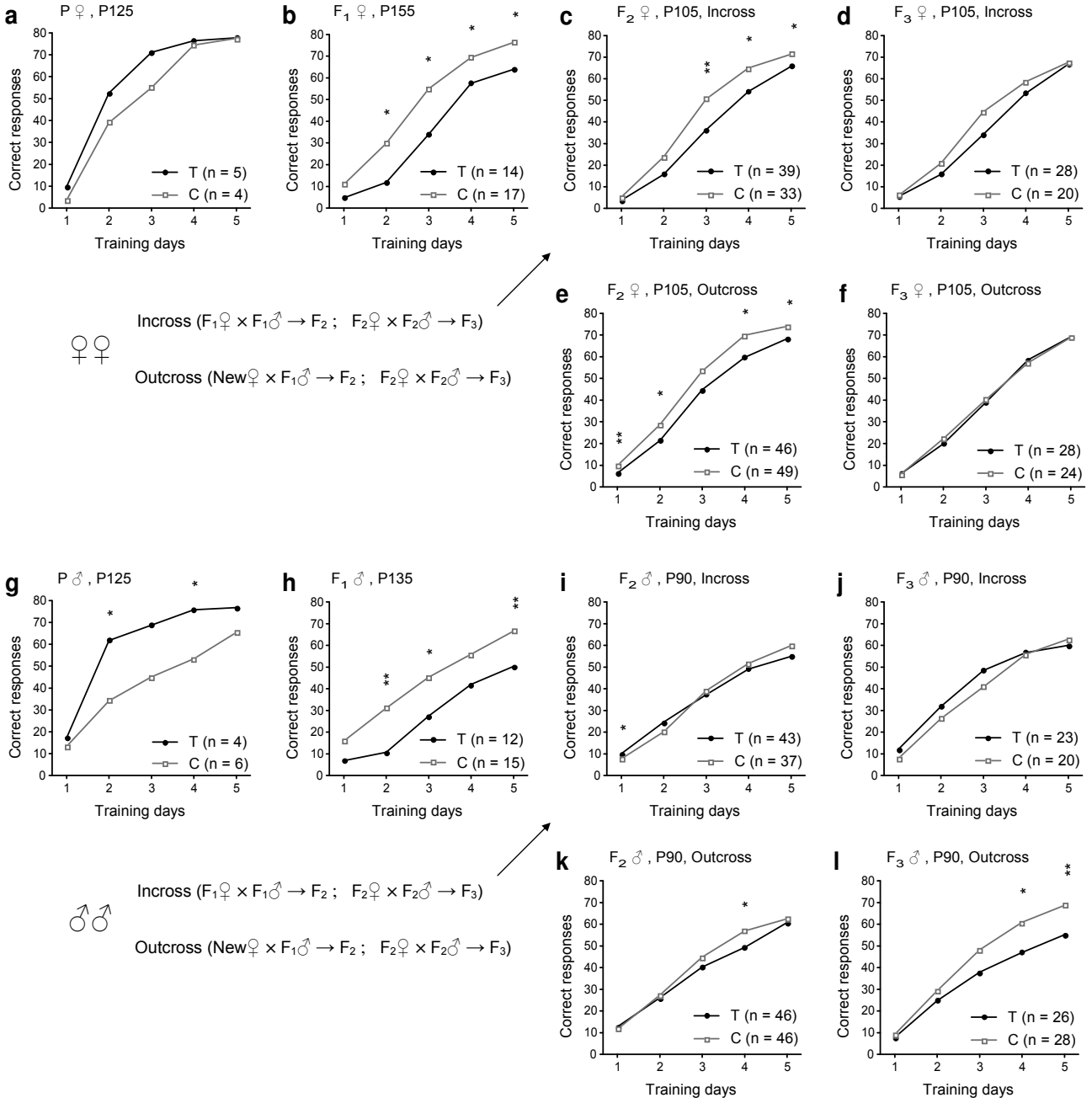

Figure 3 | Two-way avoidance in the thyroxine-treated DBA/2J mice and in the F_1-F_3 progeny of thyroxine-treated males. Note improved performance in the neonatally (P0-P11) thyroxine-treated males (**g**), but decreased performance in their descendants (**b**-**c**,**e**,**h**,**l**). Both Incross and Outcross F_2 females have decreased performance (**c**,**e**). In males the decreased performance was observed in the F_1 (**h**) and in the F_3-outcross (**l**), but not in the F_2 (**i**,**k**). Torah, the Second Commandment (Shemot 20:3-6; Devarim 5:7-10), teaches us that the misbehaviour of fathers (P) leads to problems in their sons (F_1) and problems in the third (F_3) and the fourth (F_4) generations. The second generation (F_2) is not in the original text (**Supplementary Table 2**). T – descendants of treated males, C – control. P125 – postnatal day 125. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$. Mann-Whitney U test. Mean.

In the F_2 generation the vast majority of females have shown enhanced analgesic effect during the first day, but all of them have shown normal phenotype during the second day. In the F_2 generation males the situation is very complex (**Fig. 4**). First, 1/4 (20 males from 80) have shown enhanced analgesic effect during the first day. Second, 1/16 (5 males from 80) have shown enhanced analgesic effect during the second day only – it means that they had normal phenotype during day 1 and abnormal one during day 2. Third, another 1/16 (5 males from 80) have shown enhanced analgesic effect during both day 1 and day 2. Note that one or two such males were present in the F_1 generation, but the total number of experimental males in the F_1 (29 males) was not sufficient to assess whether this is a random mistake or real phenomenon. Note the absence of such strange animals in the control groups. Anyway, the change from "abnormal" to "normal" in the majority of animals and simultaneous change

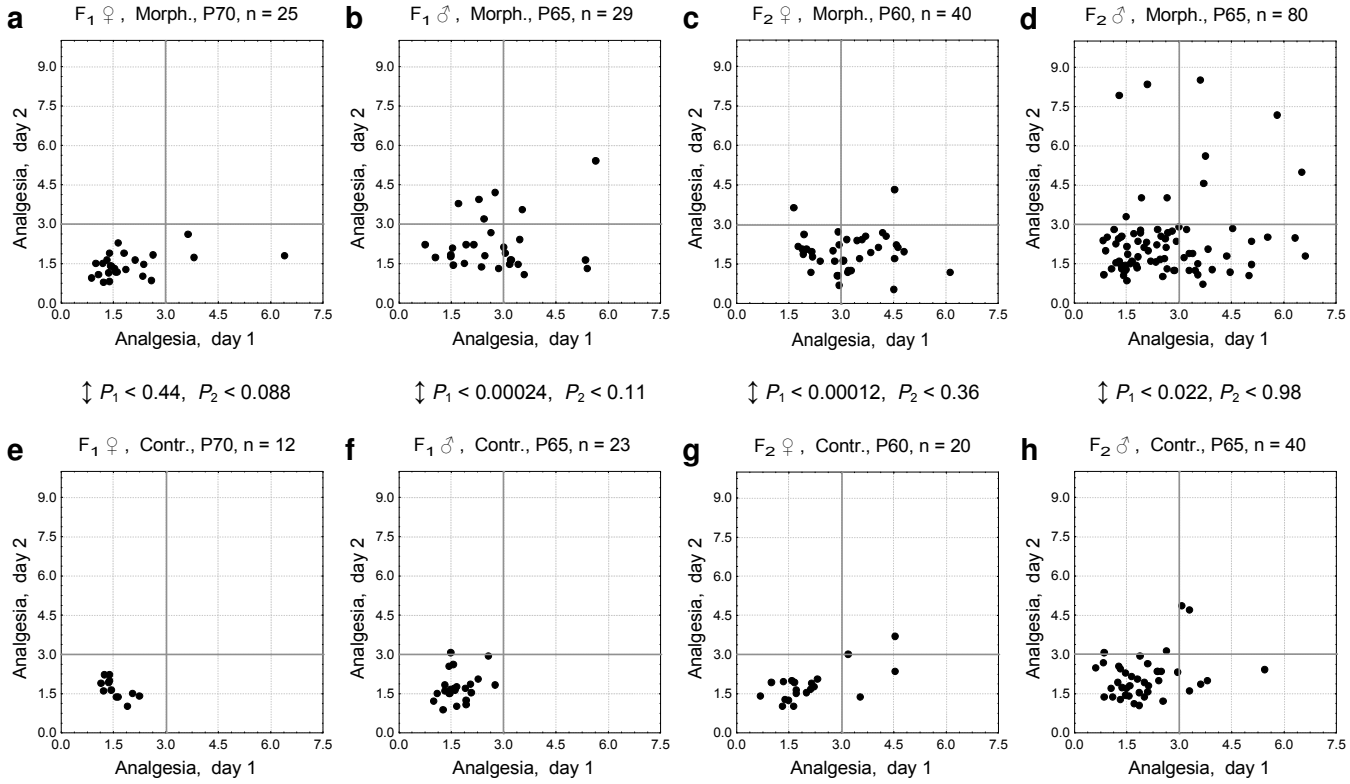

Figure 4 | Tail-withdrawal test in the F_1 & F_2 descendants of morphine-treated male Wistar rats. Each animal was tested twice (days 1 & 2) with the same dose of morphine 10 mg/kg. Morphine was administered i.p. each day after the first measurement of tail-withdrawal latency (baseline latency). Abscissa (day 1) and ordinate (day 2) of each dot (animal) show the ratio of tail-withdrawal latency, measured 30 min after 10 mg/kg morphine injection, to baseline latency. The effect is dominant in F_1 males (**b**,**f**) and F_2 females (**c**,**g**) (day 1), but recessive in F_1 females (**a**,**e**) and F_2 males (**d**,**h**; 1st day – ¼ has effect; 2nd day – 1/8, including 1/16 during both days and 1/16 during exclusively day 2). Heritable changes in two independent loci are sufficient to explain this pattern. P_1 & P_2 – statistical significance between experimental and control groups during day 1 & day 2, respectively. Mann-Whitney U test.

from "normal" to "abnormal" in few ones, during the same 24 hours and treatment procedure, does not have self-evident physiological explanation. At least, it is very unusual, when the second standard dose of morphine produces greater analgesic effect than the first one. Observations on guinea pigs have provided some clue later, more than 10 years after the end of this experiment with rats and morphine.

Once a female animal with unusual phenotype was born among our short-haired multicoloured guinea pigs (*Cavia porcellus*). This female was born in a litter of four (2 females and 2 males; all others with standard phenotype), obtained from multi-coloured female from Elm Hill Labs (Chelmsford, MA; www.elmhilllabs.com) and short-haired multicoloured male with contrasting whorl on its head (so-called "American crested"), obtained from an independent source (hybrid dysgenesis is possible). Video record, taken at postnatal day 1, is available: www.evolocus.com/Video/GuineaPigs2011-09-17.MOV . This video is not absolutely necessary for further understanding of our article, but an experienced observer can extract a lot of non-trivial information from it (all animals, including both parents, are shown). Day of birth is counted as P0 and it is 2011-09-16. At birth, at P1 and during the first several weeks this animal was not recognized as "unusual", despite post-hoc analysis of above-mentioned video record has revealed that this animal was able to demonstrate slightly increased activity already at P1, because it was called "the hard one to get". During her adolescence this female had increased locomotor activity, *e.g.* it was able to move up and down in a 3-level chinchilla's "Super Pet®" cage, using its plastic ramps and being self-motivated. This behaviour was never observed in any other laboratory guinea pig and it is more typical for animals like rats. This female was behaviourally active, but the most interesting its feature was the following: being behaviourally active, it had very low water consumption. Its water consumption, as soon as it was detected, was 3-4-fold lower than daily water consumption of any other guinea pig.

This female with low water consumption and high behavioural activity was crossed with normal male and two pups were obtained in a litter: one was found dead at P0, but another one was considered "normal" until its daily water consumption was measured. This F_1 pup was a female. Water consumption of her mother remained lower than norm during pregnancy and lactation. However water consumption of this F_1 female occurred to be 3-4-fold higher than water consumption of any control animal (**Fig. 5** and **Supplementary Fig. 3**). Increased water consumption was associated with increased urination, occurring in a different location inside the cage. This increased water consumption was stable, it was observed during several months, and it produced an impression that it will be so forever.

On the other hand, it would be interesting to see how this increased water consumption will be normalized and we were

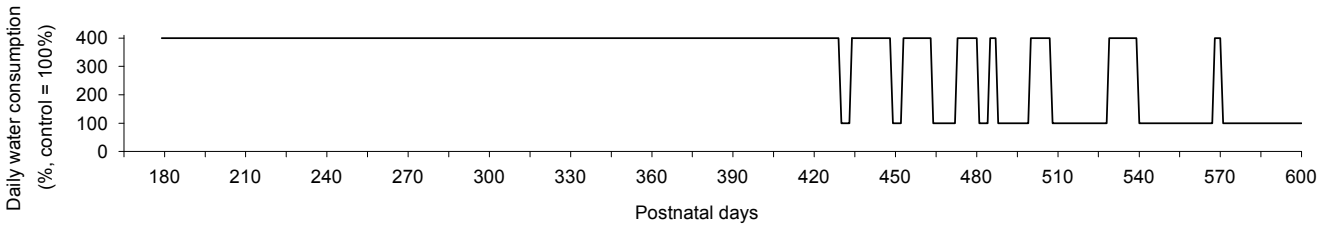

Figure 5 | Water consumption of one female guinea pig, obtained from female with unusually low water consumption (schema). In our heterogeneous outbred stock of guinea pigs (*Cavia porcellus*), one female was obtained that had unusually low (20-25%) water consumption during her adulthood. Contrary to this female, her F_1 female descendant (shown) had enormously increased (300-400%) water consumption (P180-P430). Later (P430+), some periods of normal water consumption appeared, without any intermediate state between "high" and "low" states. There is no physiological reason for the absence of gradual regulation here and, thus, "all-or-none" switch is an intrinsic feature of transgenerational epigenetic compensation.

expecting some smooth curve. We never obtained such smooth curve. At some time point water consumption was normalized abruptly – it has jumped down to the normal level in 24 hours! Water consumption was normal during few days and then it has jumped up as fast as it was jumping down previously **(Fig. 5**). There were only two stable states of this process: normal and high. Any intermediate possibility was absent.

Water consumption had a tendency to switch from "high" to "normal" each time when fresh high quality grass was becoming available on a regular basis (a guinea pig prefers the same species of grass as a white-tailed deer (*Odocoileus virginianus*) in the New York area). And water consumption had a tendency to switch from "normal" to "high" each time when grass quality was going down and, in addition, each time when bedding material in the cage was changed from old and "dirty" to new and "fresh" (we use pine bedding "PetsPick™"). May be, behavioural stress from this change together with temporal unavailability of feces, those are an important source of nutrients for a guinea pig, are the main factors for switching from normal to very high water consumption. It seems that stress of any kind can switch water consumption in this animal from normal level to very high one (**Supplementary Fig. 3**). Note that in normal animals, in both males and females, slight stress leads to slight decrease in water consumption, whereas in this female the same slight stress leads to disproportional increase.

High and abruptly switching water consumption, observed in this female, obtained from female with low water consumption and normal male, indicates that the phenotypic expression of transgenerational epigenetic compensation is not only gender-dependent (see our previous article "Transgeneraional epigenetic compensation and sexual dimorphism"[3]), but it is also stress-dependent, and it is stress-dependent in a very sharp manner in temporal dimension. For such cases Trofim D. Lysenko has introduced the term "unstable, destabilized, heredity" (p. 298[18]).

We have seen very sharp temporal response, very fast switching of transgenerational epigenetic compensation from "off" to "on" state and *vice versa*, and possibility to be "on" during different periods of ontogenesis. It means that, most likely, we do not have here something distributed among many-many independent loci, but we probably have only one change in one locus. Namely, one previously absolutely dormant gene has become transcriptionally active (that is why it is dominant), but the switching of its transcription between "off" and "on" states is heavily gender-dependent (probably, through the effects of sex hormones) and, in addition, the above-mentioned switching is heavily stress-dependent (probably, through the effects of stress hormones). Dormant genetic locus, being brought out of dormancy, becomes open for further regulation of its expression, but not for unconditional presence of its product in the organism.

The idea about dormant genes belongs to Wilhelm Jürgen Heinrich Harms, known as J.W. Harms, and it was proposed by him in 1929[19,20]. At that time it was absolutely unexpected that a re-opened dormant gene can demonstrate so sharp temporal regulation of its expression immediately, during lifespan of a single animal. Similar switching of gene activity, but between generations, was shown for genes *fused* and *star* by Dmitry K. Belyaev and co-authors in 1981[21,22]. It seems that even using 1-bit regulation of the level of expression ("on" or "off"), but having non-trivial temporal structure of this expression during ontogenesis, an organism can achieve a variety of phenotypic results, including a variety of morphological ones, uncorrelated with each other (**Supplementary Fig. 6**).

Dormant genes, being brought out of dormancy by transgenerational epigenetic compensation, are changing the evolutionary landscape faster than natural selection does.

Methods

***Per2*^{Brdm1} mouse experiment.** Mutant *Per2*^{Brdm1} allele is known to compromise circadian organization and entrainment and to cause multiple physiological disturbances[23]. Male and female animals (1/4 homozygous mutants, 2/4 heterozygous and 1/4 wild-types; 250 mice in total; mixed background of C57BL/6 and 129SvEvBrd) were individually numbered by means of injected transponders, which can be read by an external antenna, and were placed in 4 independent (20 × 20 m each) open outdoor pens, isolated from each other and terrestrial predators by slate walls (1 m high and sunk 50 cm into the soil, covered by zinc-plated iron on the top)[11]. Each pen had 2 wooden roofed shelters (3 × 2 m each, 70 cm depth, filled with hay, straw and branches). Inside each pen, but outside of both shelters, there were two feeding places (food + water), each equipped with antenna, which allowed monitoring of animal visits during 2 years in a non-stop manner. The end of feeder visits provided precise information about lifespan of each animal. All animals were live trapped and new (born in field) animals were genotyped and injected with transponders twice a year.

Animals were released into the shelters at the field station Chisti Les (Clear Forest), Bubonizi (Pozhnia, Tvier Region, Western Russia, 56°44'7.99"N; 31°31'34.44"E) on May 21, 2005, at the age of 76 ± 5.4 days (mean ± SD).

Thyroxine experiment. DBA/2J mice (P) were treated as neonates during the first 12 days (P0-P11) by subcutaneous injection of a daily dose of 2 μg L-thyroxine dissolved in 0.05 ml 0.9% NaCl made alkaline (pH 9.0) by adding a few drops of NaOH. Solution was prepared once 24 hr before the first administration (kept at +4°C). All pups in a given litter received the same treatment (between 17:00 and 18:00) and were kept in an original litter under their native DBA/2J mother (110-day-old at breeding). Control animals were left undisturbed. Reversed day-light cycle was used (8:00-20:00 – dark, 20:00-8:00 – light). Adult mice were housed individually.

To have F_1, each DBA/2J male (P) at the age of 60 days was housed with 2 or 3 nulliparous 90-day-old naive DBA/2J females during 7 days. At birth pups were numbered and placed under primiparous NMRI foster-mothers to have 4 experimental and 4 control pups in each foster litter. To have F_2-incross, F_1 males at the age of 200 days were housed with F_1 females (2 females × 1 male, incross, but without inbreeding). To have F_2-outcross, F_1 males at the age of 230 days were housed with naive DBA/2J nulliparous 110-day-old females (2 females × 1 male). To have F_3, F_2-incross males at the age of 180 days were housed with F_2-incross females and F_2-outcross males at the age of 150 days were housed with F_2-outcross females (1 female × 1 male), simultaneously. NMRI foster-mothers were used in F_1, F_2 and F_3.

P, F_1, F_2 and F_3 mice were tested in two-way avoidance task ("Mouse Shuttle Box", Campden Instruments Ltd., UK)[24] at the age 90-155 days. Training: 5 days, 80 trials daily. The condition stimulus was light (5 sec), the negative reinforcement was foot-shock 0.15 mA (10 sec), which was supplied together with additional 10 sec of light, but both could be terminated by escaping to another compartment. This termination had a 0.8 sec delay – in order to have optimal DBA/2J training. Inter-trial interval: 5-15 sec.

Morphine experiment. Male Wistar rats, 42-day-old initially (P42; body weight 197 ± 20 g, mean ± SD), housed in groups 5-10 under normal day-light cycle, were injected intraperitoneally (i.p.) with morphine during 38 days. The first 7 days – twice daily (morning-evening, 8 hr between, mg/kg): 5-10, 15-15, 20-20, 25-30, 35-40, 45-50, 55-60 (10 mg/ml in 0.9% NaCl). Next day – 60 mg/kg in the morning and 6 hr later – injected i.p. with 2 mg/kg of naloxone (2 mg/ml) to induce early in life naloxone-precipitated morphine withdrawal. Next day – injected with morphine 60 mg/kg. The rest 29 days – injected with morphine 60 mg/kg twice daily Monday-Friday, and 60 mg/kg daily Saturday-Sunday. Control males were left undisturbed.

During the last 5 days of morphine treatment P males were housed individually with drug-naive 75-day-old nulliparous Wistar females. To have F_1-2 (F_1, second brood), P males at the age of 175 days (*i.e.* 95 days of withdrawal) were housed individually with familiar females. To have F_2, F_1-2 males at the age of 85 days were bred individually with F_1-2 females (incross, but without inbreeding).

P, F_1, F_2 animals were tested in tail-withdrawal test at the age of 60-95 days. The distal part of the tail of a lightly restrained animal was dipped into circulating water thermostatically controlled at 56 ± 0.2°C. Latency to respond to the heat stimulus, by a vigorous flexion of the tail, was measured to the nearest 0.1 sec, cutoff latency – 15 sec. The test was done once before i.p. 10 mg/kg morphine injection (baseline latency) and 15, 30, 45, 60 and 90 min after. This testing was repeated 24 hours later to assess acute tolerance.

Guinea pig experiment. Outbred short-haired multicoloured guinea pigs (*Cavia porcellus*) were used. Multicoloured female was obtained from Elm Hill Labs (7 Kidder Rd., Chelmsford, MA 01824; www.elmhilllabs.com) and it was bred with short-haired multicoloured male with contrasting whorl on its head (so-called "American crested"), obtained from Petland Discounts #17 (439 Tarrytown Rd., White Plains, NY 10607). Two females and two males were born 2011-09-16. One female from this litter demonstrated low water consumption being an adult.

We had cages "RB100" (100 × 54 × 44.5 cm) and Super Pet "My First Home Chinchilla Cage Kit" (76 × 45.5 × 76.5 cm; a 2-shelf cage, each shelf 44 × 25 cm, placed at 26 cm and 44 cm from the floor in the opposite parts and connected consequently by two ramps 42.5 × 12 cm each). Bottles 500 ml from LM Animal Farms were refilled daily and their weight was measured at 11:00 PM using electronic scale KS/B-2000 (Max: 2000 g, d = 0.1 g). Pine bedding "PetsPick" and bowls with standard guinea pig food were always in cages. Fresh grass was supplied daily, when available. During snow periods animals received "Kaytee Timothy Hay Ultra" and apples. We kept 1-2 adult animals per cage under normal day-light cycle. Each adult animal had its own plastic house "Super Pet Big Igloo" (D = 24.5 cm (lower), d = 19 cm (upper), H = 16 cm (ext.), h = 13.5 cm (int.); entrance tunnel: L = 6 cm, H = 11.5 cm, W = 10 cm).

Above-mentioned female with low adult water consumption was crossed with normal male (her littermate), and from this cross a female with high adult water consumption was obtained, born 2012-03-09.

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Additional information

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Supplementary Information for

Transgenerational epigenetic compensation and natural selection

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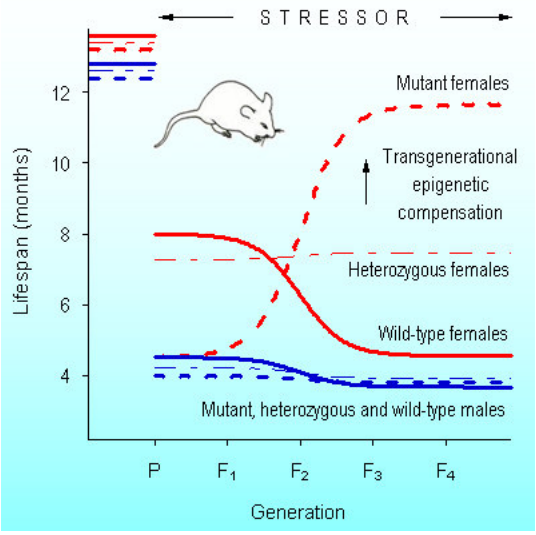
Transgenerational epigenetic compensation is induced in animals by significant disturbance of parental early ontogenesis. Transgenerational epigenetic compensation consists of unblocking of one (and usually only one) previously absolutely dormant gene. This gene is selected from the whole population of dormant genes inside this organism and we can call this process "*Orthoselektion*" (after J.W. Harms, 1934, S. 201). Being unblocked, this gene becomes dominant immediately (can affect phenotypes of all animals where it is present in either heterozygous or homozygous state). The expression of dormant gene has two "locks": the first "lock" is removed by strong disturbance of parental early ontogenesis, the second "lock" is always present and its current state ("locked" or "unlocked") is controlled by sex-hormones and stress-hormones inside particular descendant. The expression of this gene is strictly binary (all-or-nothing) and the shifting of expression episodes in time during ontogenesis can produce enormous variety of phenotypes.

Transgenerational epigenetic compensation can be induced in animals by significant disturbance of parental early ontogenesis. This disturbance can be genetic (*e.g.* presence of mutant gene in homozygous state), environmental (prenatal, neonatal or adolescent drug treatment), or their combination (exposure of a homozygous mutant to environmental stressor during early ontogenesis). The exposure of an adult animal to unusual conditions usually has either no transgenerational effect (weak stressor) or "*Tier tot*" (strong stressor).

The expression of previously dormant gene in descendants can be described by binary function (on-off; this is an easy part), but episodes of its expression can be distributed in time during ontogenesis in a very complex fashion (this is a difficult part of this process). The changes in time of expression of one previously dormant gene can produce a multitude of phenotypes.

The expression of previously dormant gene in a given descendant can be "zero" under normal conditions and "maximum" under stressor application (example: water consumption in our guinea pig). In an independent experiment, with different treatment and different previously dormant gene, its expression can be "maximum" under normal conditions and "zero" after stressor application (example: morphine analgesia during the second day of testing in the progeny of morphine-treated males). It can be "zero" in males and "maximum" in females (example: F_2 generation, thyroxine experiment), or *vice versa* (example: F_1 generation, morphine experiment).

All changes, distributed among multiple independent loci, known as commonly discussed epigenetic changes, like changes in DNA methylation, or histone modifications, are all secondary changes, they all are not primary heritable changes, but consequences of activation of one previously dormant gene.



Supplementary Fig. 1 | Gender-specific transgenerational epigenetic compensation in mutant mice. Heritable epigenetic compensation, developed by mutants for mutants under stressor, was introduced into all genotypes, due to breeding "at will". It has changed behaviour of mutant and wild-type females only. Natural selection was conducted by avian predators, tawny owls (*Strix aluco*), by elimination of incautious animals.

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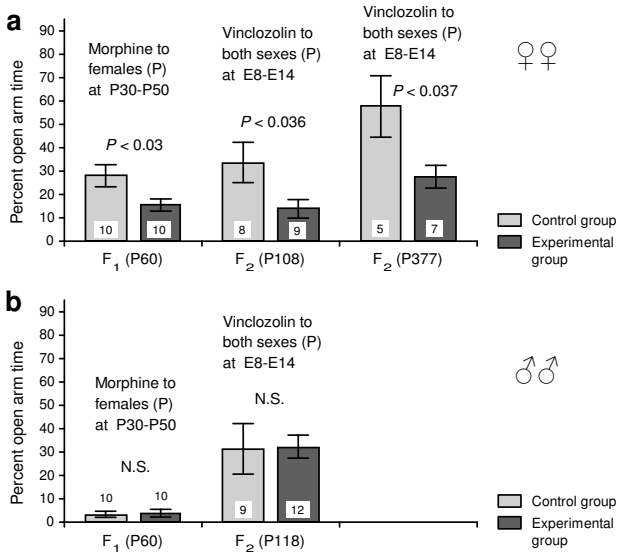


Supplementary Fig. 2 | F_1 and F_2 descendants of drug-treated parents in the elevated plus-maze. F_1 – descendants of morphine-treated (P30-P50) females, Sprague-Dawley rats[25]. F_2 – descendants of prenatally (E8-E14) vinclozolin-treated males and females, Sprague-Dawley rats[26]. (**a**) Females. (**b**) Males. Both treatments entailed very similar gender-specific results in the F_1 and F_2 – increased caution (decreased time spent on the open arms), observed exclusively in females. Student's t-test. Mean ± SE.

The fate of mutant animals with given mutation in a natural population is determined by the efficacy of transgenerational epigenetic compensation (orthoselection and deblocking of one previously dormant gene – in homozygous mutant animals and for homozygous mutant animals) and the efficacy of natural selection (elimination of mutant and other animals from population). If orthoselection goes faster than natural selection – mutants will survive and even can replace normal animals, otherwise they will be eliminated from population completely and irreversibly.

The placement of laboratory mice into stressful semi-natural environment leads to the enhancement of viability of homozygous mutant females and, simultaneously, to the suppression of viability of wild-type females (**Supplementary Fig. 1**). The suppression was presumably achieved due to penetration of transgenerational epigenetic compensation, developed for homozygous mutants, into the animals without given mutation. This suppression is not a result of some kind of competition between animals, because heterozygous animals do not demonstrate any change in their survival during this time period. During 2 or 3 consecutive generations, the applied stressor has changed behaviour of mutant and wild-type females in the opposite direction. Further natural selection was conducted by avian predators, mainly tawny owls (*Strix aluco*).

In the **Supplementary Fig. 1** we can see population of mutant, heterozygous and wild-type mice and the role of transgenerational epigenetic compensation. This schematic picture is based on real experiment with *Per2*^{Brdm1} mutant mice (**Fig. 2**). Transgenerational epigenetic compensation is induced in homozygous mutant animals by harsh external conditions, presumably low temperatures and high amplitude temperature fluctuations. Some previously dormant gene has become unmasked and opened for further regulation of its expression. Its expression was "maximum" in females and "zero" in males. This previously dormant gene remains unknown for us in this experiment.

We know that in some experiments with parental drug treatment we can see gender-specific effects in the progeny, namely increased caution in the F_1 and F_2 females in the elevated plus-maze (decreased time spent on open arms). No such effect was observed in males (**Supplementary Fig. 2**).

It seems that the increased caution in females can be a general consequence of orthoselection and activation of any one gene from many-many very different previously dormant genes. However here we can see that this increased caution is genotype-specific and is obvious in homozygous mutant females only, whereas the same activation of previously dormant gene leads to decreased caution in homozygous wild-type females (**Supplementary Fig. 1**).

Natural selection is conducted by tawny owls (*Strix aluco*). They are the most common aerial mouse predators in this area. Other aerial predators, like a short-eared owl (*Asio flammeus*), can take part in this process also. Aerial predators eliminate the most incautious animals from mouse population. These are the same mice that would show the longest time spent on the open arms of elevated plus-maze. And we know that so different drug treatments as adolescent (P30-P50) maternal morphine treatment and prenatal (E8-E14) vinclozolin treatment (of both parents) lead to increased caution in the F_1 and F_2 generation females (and only in females), observed in the elevated plus-maze as decreased open arm time (**Supplementary Fig. 2**).

Concerning experiment with vinclozolin we know that the increased caution in females is a result of increased gene expression. If we look at all genes with changed expression in different organs (**Supplementary Table 1**), we see that the number of genes with increased expression in hippocampus (in comparison with control) in increased only in females. We assume that the activation of one dormant gene leads to activation of a cascade of other genes. And most of them are usual, well-known genes, expressed in normal animals at some detectable level.

Supplementary Table 1 | Number (*n*) of genes with changed expression

F_2	♀♀		♂♂	
	↑	↓	↑	↓
Hippocampus	1086	215	29	63
Amygdala	49	123	97	173
Hippocampus + Amygdala	1135	338	126	236
Heart	336	70	57	115
Kidney	83	67	385	340
Liver	36	63	166	100
Heart + Kidney + Liver	455	200	608	555
Ovary	211	94		
Uterus	138	141		
Prostate			276	836
Seminal vesicles			105	169
Testis			203	349
Gender-specific organs	349	235	584	1354

F_2 rats, descendants of prenatally (E8-E14) vinclozolin-treated males & females (P). ↑ - increased transcription. ↓ - decreased transcription. Gene overlap is ignored. Brains were taken at P450 from females and at P360 from males (Skinner *et al.*, 2008)[26]. Other organs were taken at P120 (Skinner *et al.*, 2012)[27].

Supplementary Table 2 | The Second Commandment

	Translation	Original
Shemot - Exodus - Chapter 20 (3-6)	**3**. You shall not have the gods of others in My presence. **4**. You shall not make for yourself a graven image or any likeness which is in the heavens above, which is on the earth below, or which is in the water beneath the earth. **5**. You shall neither prostrate yourself before them nor worship them, for I, the Lord, your God, am a zealous God, Who visits the iniquity of the fathers upon the sons, upon the third and the fourth generation of those who hate Me, **6**. and [I] perform loving kindness to thousands [of generations], to those who love Me and to those who keep My commandments.	**ג.** ל֣א יִהְיֶ֥ה לְךָ֛ אֱל֥הִים אֲחֵרִ֖ים עַ֣ל פָּנָ֑י: **ד.** ל֣א תַעֲשֶׂ֣ה לְךָ֔ פֶּ֖סֶל | וְכָל תְּמוּנָ֡ה אֲשֶׁ֣ר בַּשָּׁמַ֣יִם | מִמַּ֡עַל וַאֲשֶׁ֣ר בָּאָ֣רֶץ מִתָּ֑חַת וַאֲשֶׁ֥ר בַּמַּ֖יִם | מִתָּ֥חַת לָאָֽרֶץ: **ה.** ל֣א תִשְׁתַּחֲוֶ֣ה לָהֶ֔ם וְל֥א תָעָבְדֵ֖ם כִּ֣י אָנֹכִ֣י יְהוָ֣ה אֱלֹהֶ֑יךָ אֵ֣ל קַנָּ֗א פֹּקֵ֤ד עֲוֹן֙ אָב֣וֹת עַל בָּנִ֔ים עַ֖ל שִׁלֵּשִׁ֣ים וְעַל רִבֵּעִ֑ים לְשֹׂנְאָֽי: **ו.** וְעֹשֶׂ֥ה חֶ֖סֶד לַאֲלָפִ֑ים לְאֹהֲבַ֖י וּלְשֹׁמְרֵ֥י מִצְוֹתָֽי:
Devarim - Deuteronomy - Chapter 5 (7-10)	**7**. You shall not have the gods of others in My presence. **8**. You shall not make for yourself a graven image, or any likeness which is in the heavens above, which is on the earth below, or which is in the water beneath the earth. **9**. You shall not prostrate yourself before them, nor worship them, for I, the Lord your God, am a zealous God, visiting the iniquity of the fathers upon the sons, upon the third and the fourth generations of those who hate Me. **10**. And [I] perform loving kindness to thousands [of generations] of those who love Me and to those who keep My commandments.	**ז.** ל֣א יִהְיֶ֥ה לְךָ֛ אֱל֥הִים אֲחֵרִ֖ים עַ֣ל פָּנָ֑י: **ח.** ל֣א תַעֲשֶׂ֣ה לְךָ֔ פֶּ֖סֶל כָּל תְּמוּנָ֡ה אֲשֶׁ֣ר בַּשָּׁמַ֣יִם מִמַּ֡עַל וַאֲשֶׁ֣ר בָּאָ֣רֶץ מִתָּ֑חַת וַאֲשֶׁ֥ר בַּמַּ֖יִם מִתַּ֥חַת לָאָֽרֶץ: **ט.** ל֣א תִשְׁתַּחֲוֶ֣ה לָהֶ֔ם וְל֥א תָעָבְדֵ֖ם כִּ֣י אָנֹכִ֣י יְהוָ֣ה אֱלֹהֶ֑יךָ אֵ֣ל קַנָּ֗א פֹּקֵ֤ד עֲוֹן֙ אָב֣וֹת עַל בָּנִ֔ים וְעַ֖ל שִׁלֵּשִׁ֣ים וְעַל רִבֵּעִ֑ים לְשֹׂנְאָֽי: **י.** וְעֹשֶׂ֥ה חֶ֖סֶד לַאֲלָפִ֑ים לְאֹהֲבַ֖י וּלְשֹׁמְרֵ֥י מִצְוֹתָֽי:

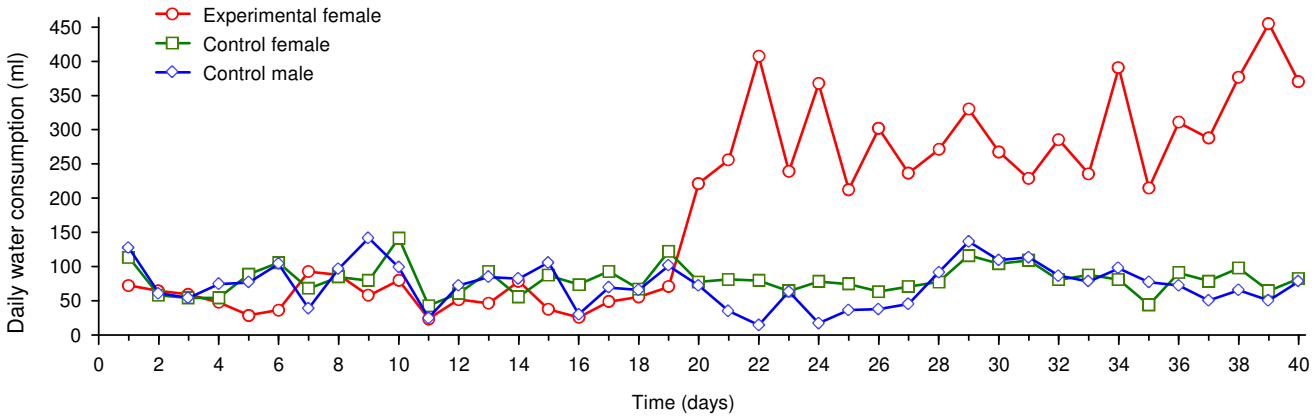
The source of translation: http://www.chabad.org/library/bible_cdo/aid/9881 ; http://www.chabad.org/library/bible_cdo/aid/9969 . In colloquial Modern Hebrew, the plural יְלָדִים is generally used to refer to children (of mixed or unknown sex) while בָּנִים is generally used to refer to boys. In Christian translations, the following two simplifications are typically introduced: 1) "the sons" are replaced with "the children" (without any material basis) and 2) the continuity of generations is artificially added (without any material basis also): "visiting the iniquity of the fathers on the children to the third and fourth generation of those who hate me" (Christian Bible, English Standard Version (ESV), 2011). We do not use Christian Bible, of course. The reference to Christian Bible is provided here only due to known popularity of this deviated (inaccurate) text in the internet.

The Christian interpretation, being expressed in modern biological terms, implies that the following generations are involved: P ("parental" generation), F_1, F_2, F_3 and F_4 (four "filial" generations, *i.e.* generations of descendants). This interpretation was obtained by means of blind simplification and it is obviously wrong. We can consider at least two alternative attempts of understanding. In the first alternative attempt we can assume that this sentence can be logically divided into two semi-independent parts: 1) "visiting the iniquity of the fathers upon the sons"; 2) "visiting the iniquity [of the fathers] upon the third and the fourth generation(s) of those who hate Me". If we assume that the phrase "those who hate Me" is logically linked mainly with "generation(s)", but not with "the fathers", we can think that the following generations are involved: P, F_1, F_2 and F_3 (expressed in modern terms). Thus, the fathers are "the first generation" in the text, and this generation numbering should be shifted by 1 to be compatible with modern biological terms. However there is one feature of this understanding, which makes it questionable. If we apply "those who hate Me" to "generation(s)", we can expect the appearance of an infinite loop. Thus, in the second alternative attempt of understanding we assume that the phrase "those who hate Me" is applicable exclusively to "the fathers", but not to "generations". In this case all "generations" occur to be "filial" generations (generations of descendants), and in accordance with modern biological classification the following generations are present here: P, F_1, F_3 and F_4. And we remember that all above-mentioned subjects are males, *i.e.* this text describes males only.

Note that due to the increase in expression, this heritable change becomes "dominant" (detectable in phenotype of both homo- and heterozygote). Note also that the increase in expression of previously dormant gene leads to the increase of expression of many other genes, but not to their suppression. Thus, the sign of change in gene expression is not changing at all in these cascades (the increase in expression of one gene leads to the increase in expression of other genes, but not to the suppression of expression of other genes). Therefore the situation in real life is asymmetrical, in spite of the fact that from pure mechanistic viewpoint we can expect equal probability of increased and decreased expression of other genes. Note that this general increase in expression is specific for females, where we can see detectable phenotype. In males, where specific phenotype is absent, the number of genes with decreased expression is higher than the number of genes with increased expression (**Supplementary Table 1**). This change in gene expression in males can lead to recessive phenotype only. Remember that all above-mentioned changes in gene expression are probably secondary changes, *i.e.* they are consequences of the activation of one dormant gene.

It is interesting to note that when methylation patterns were measured by Michael K. Skinner and co-authors (2013)[28] at two different, but closely related, developmental stages, namely at E13 and E16, in the F_2 progeny of prenatally (E8-E14) vinclozolin-treated male and female rats, no common methylation changes were found in the primordial germ cells, with an exception of one gene, identified as *Pigb*, they all were different. It means that the demethylation of 5-methylcytosine is not a primary mechanism of dormant gene deblocking in transgenerational epigenetic compensation. The main mechanism remains unknown for us. But some of its features are already known and they are very interesting, and most of them were absolutely unexpected previously.

Observations on guinea pigs have changed interpretation of all previous experiments dramatically. In all previous experiments with transgenerational epigenetic compensation we never had non-stop indicator of any important physiological trait, available through the whole life of an experimental subject, with an exception of body weight, but it is a very generalized characteristic of an animal. We had only measurements, taken at a given time point. Only in 2012 we obtained a female guinea pig, born from mother with low water consumption and normal father, which demonstrated very high water consumption as soon as it became detectable by a direct observation. We describe this "metamorphosis" of phenotype (in this case between the mother



Supplementary Fig. 3 | Guinea pig water consumption, an episode with an abrupt increase. Experimental female was obtained from one female with naturally very low water consumption and normal male. Obtained F_1 female had enormously increased water consumption, observed during her adolescence and later (up to P430, **Fig. 5**). Afterwards, during spring and summer, when high quality grass was available, some periods of absolutely normal water consumption have appeared stochastically. Each such period appeared and disappeared abruptly. After relatively long period of normal water consumption, during the next winter, the water consumption has jumped up again. 40-day period (≈ P615-P655) is shown here, from 2013-11-13 to 2013-12-22. All animals received the same standard food, including fresh grass that was available practically the whole year round (Tarrytown, NY).

and her daughter) as "phenotypic inversion". This animal (daughter) never showed a smooth curve of normalization of her water consumption. Instead, her water consumption was always binary, it could be either "normal" or "maximum", and the switching from one to another was obviously stress-dependent (**Fig. 5** and **Supplementary Fig. 3**).

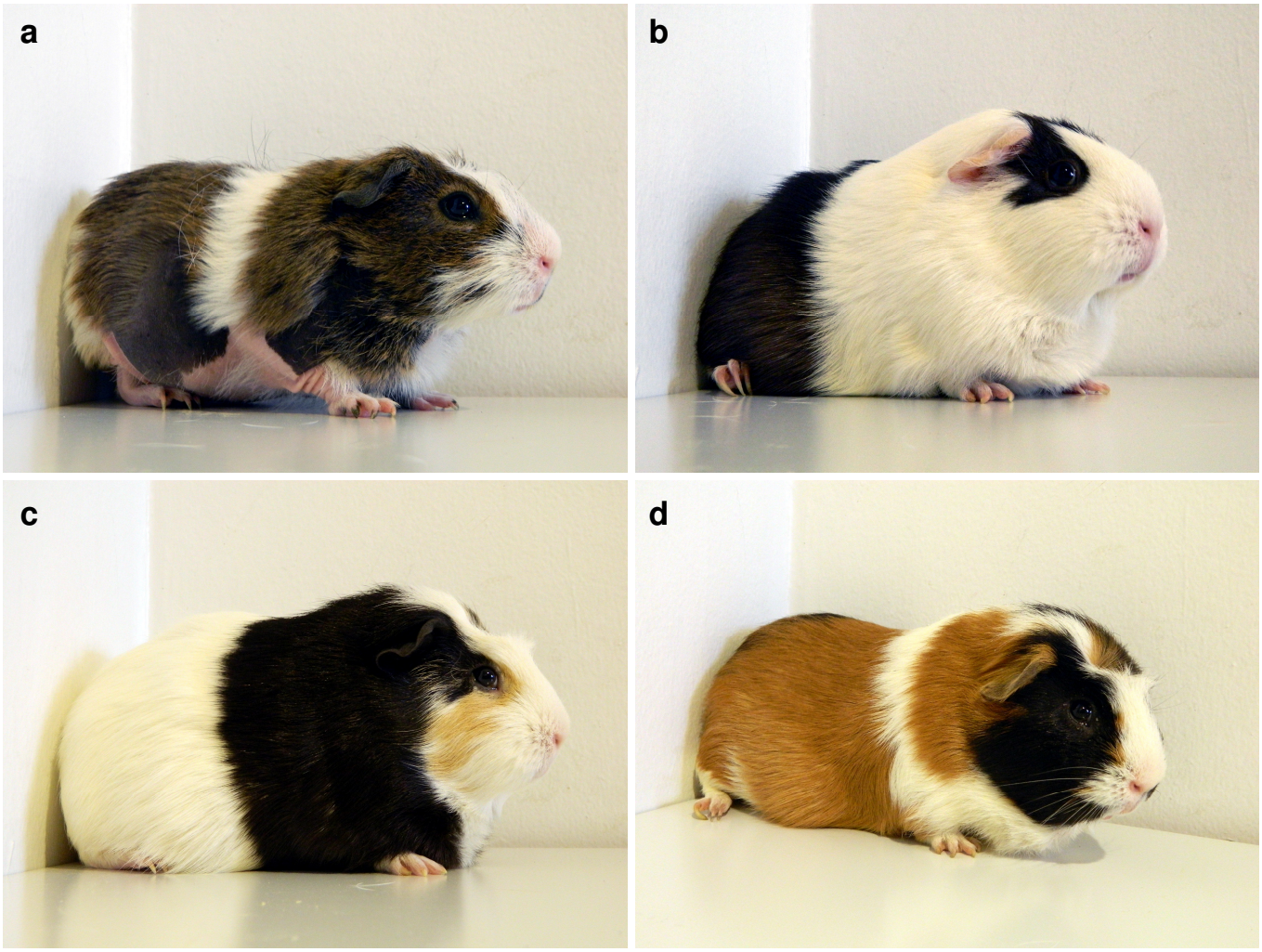
This is an example of very sharp regulation in time. It is compatible only with a single-gene effect that can not be promoted by many independent heritable changes in many-many independent loci. The locus is only one, but its switching from "on" to "off" state in time (and *vice versa*, from "off" to "on"; very abrupt, very sharp switching) can provide unbelievable variety of phenotypes (**Supplementary Fig. 6**).

In the experiment with neonatal thyroxine treatment: previously dormant gene was activated in the F_1 and F_2 females and in the F_1 males. However in the F_3-outcross males it was initially inactivated, but due to the stressful nature of two-way avoidance task it has become activated in the middle of 5-day training period (**Fig. 3l**). Obviously, if animal behaviour in two-way avoidance task can be strongly modulated by dynamic activation-deactivation of a single gene, any individual correlation between this behaviour and brain morphology should be absent. And, in fact, no individual correlation between hippocampal mossy fiber morphology and two-way avoidance behaviour was found in the F_1-F_3 generations, descendants of thyroxine-treated males, despite both traits, taken separately, had statistically significant changes (Figs. S3-S4[4], S17-S19[4]).

In the experiment with adolescent morphine treatment: previously dormant gene was initially activated in the F_1 males, but the first 10 mg/kg morphine injection plus tail-withdrawal and hot-plate testing have led to its inactivation. Therefore the analgesic effect during the second testing was normal (**Fig. 4b**). In the F_1 females this previously dormant gene was inactivated (**Fig. 4a**). In the F_2 females this gene was initially activated, but the first testing has led to its inactivation (**Fig. 4c**). In the F_2 males all theoretically possible combinations of activation-deactivation were observed (**Fig. 4d**).

Note that these observations support the existence of two semi-independent locks of a dormant gene: the first lock is very difficult to remove and it can be removed only by very specific conditions during parental early ontogenesis; the second lock is relatively easy to remove and place back, this lock is strongly gender-dependent and stress-dependent, and the stress can be rather weak in this case. Removal and placement back of this second lock entail binary expression pattern of this previously absolutely dormant gene in time.

We suppose that rather wide variety of treatments can lead to deblocking of some previously dormant gene, whose expression was permanently switched off in a given laboratory line of animals previously, as it was supposed by J.W. Harms many years ago (1929, 1934)[19,20]. The choice of this gene is genotype-specific, and it is useful only for given genetic constitution (*e.g.*: good for given homozygous mutants, bad for wild-types). However the process of its deblocking starts as a non-specific response to stressor, as it was proposed by Hans Selye (1952)[13], despite he did not discuss transgenerational effects of stress, to the best of my knowledge. And it is very important that among the results of transgenerational epigenetic compensation, in the progeny, there is a batch of common non-specific changes, typical for unblocking of different dormant genes (example: increased caution in F_1 and F_2 females). These non-specific transgenerational changes are physiologically linked with expression of specific dormant gene. May be due to this reason, may be due to some other reasons, but their appearance in phenotype, despite they are common (non-specific), is strictly genotype-specific. *I.e.* above-mentioned non-specific changes can be observed only with given genotype, not with any possible genotype, where unblocked dormant gene can be introduced (introduced, for example, by means of regular breeding, like in the experiment of Serge Daan and co-authors[11]). Important, that

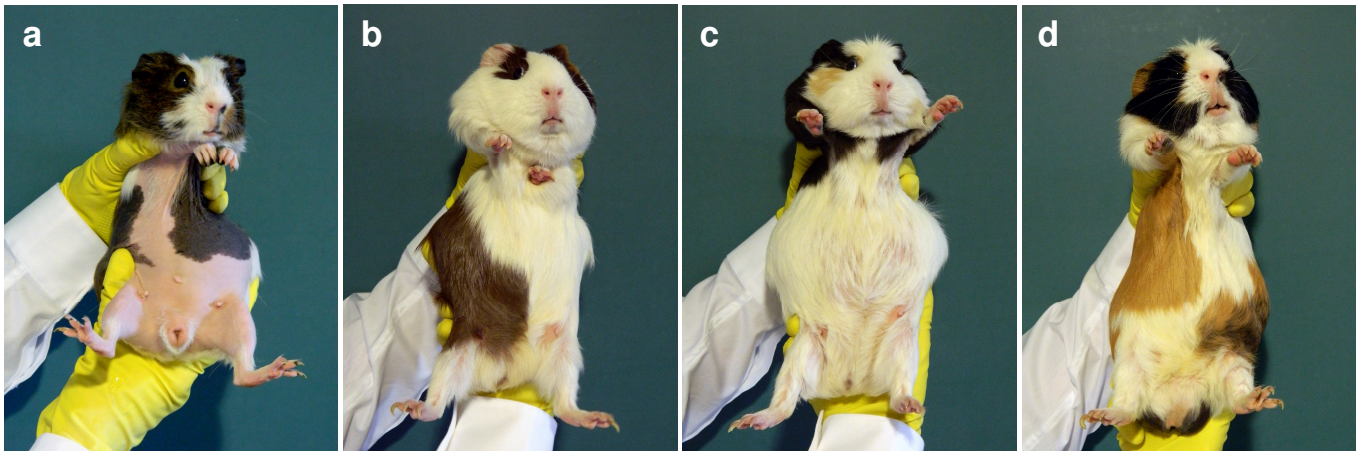


Supplementary Fig. 4 | Experimental female guinea pig (**a**), control females (**b**,**c**) and control male (**d**). Note rather relaxed posture in the control animals (**b**-**d**), and "freezing" posture in the experimental one (**a**), known from fear-conditioning task in rats and mice. Note also partial hair loss (**a**). This animal (**a**) does not like handling and it is relatively "wild" (it is trying to escape from human hands). Body weight: 994.4 g (**a**); 1370.1 g (**b**); 1474.6 g (**c**); 1533.0 g (**d**). Body weight is given for day 20 shown in the **Supplementary Fig. 3** (2013-12-02). Photographs were taken on day 3 (2013-11-15).

non-specific phenotypic changes can be formed during different stages of ontogenesis than changes, associated with transgenerational epigenetic compensation. Common changes can be build up during early ontogenesis, when expression of dormant gene can be "On", whereas phenotypic inversion could be formed during later ontogenesis, when dormant gene can be "Off" in some animals and, thus, in these animal phenotypic inversion will not be observed at all – they will be phenotypically normal with respect to phenotypic inversion, but abnormal with respect to common (non-specific) changes – they will have these non-specific changes. Honestly, the experimental data, available today with respect to the fate of non-specific adaptive changes, are even more limited than our knowledge concerning the fate of transgenerational epigenetic compensation, typically observed in the form of phenotypic inversion. However the potential importance of non-specific adaptive changes, induced by deblocking of one previously dormant gene, in evolution is obvious. They can be used as markers by females during formation of breeding pairs, and through this mechanism the non-random breeding can proceed in real population, where breeding "at will" is possible. Non-random breeding can lead, in principle, to formation of a new species. We do not have any experimental data concerning the role of transgenerational epigenetic compensation in real speciation, but, hopefully, we can use other means in order to solve this problem.

Canalization of ontogenesis and the absence of so-called "stabilizing selection" in nature

The term "canalization of ontogenesis" and the sentence "Ontogenesis in females is better canalized than ontogenesis in males" describe situation when any disturbance of ontogenesis is handled by an animal in a way that it remains alive and able to produce offspring. This statement has nothing common with the claim that morphology or some quantitative traits remain "unchanged" or "fixed". An animal remains alive usually due to the efficacy of existing feedbacks inside the most important functional systems. These systems control the achievement of

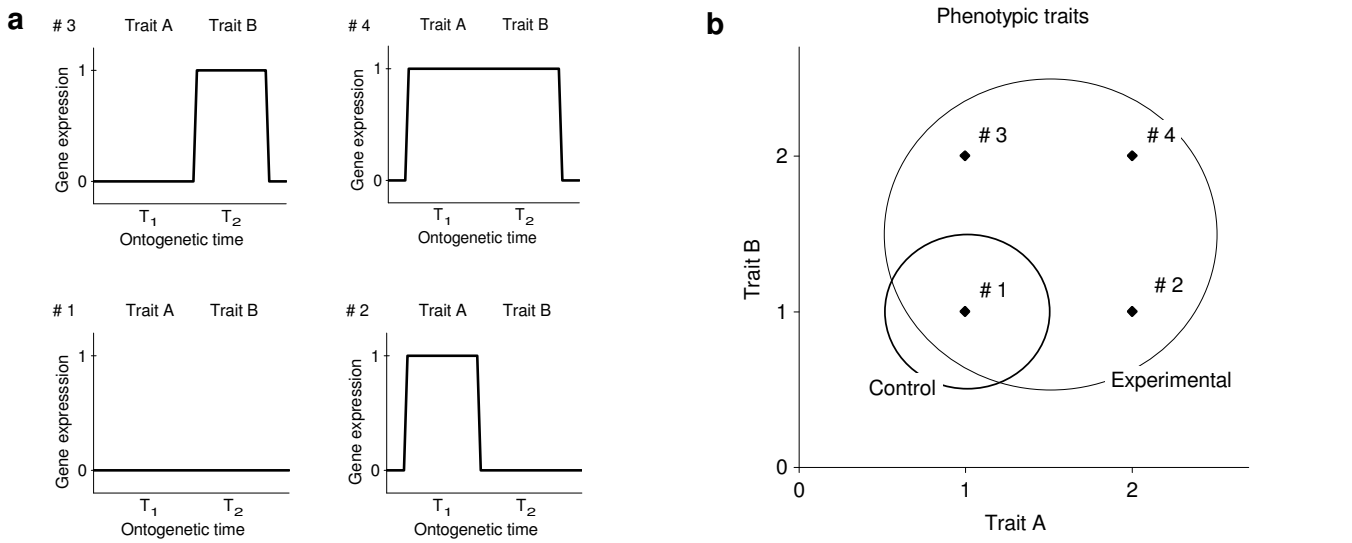


Supplementary Fig. 5 | Experimental female guinea pig (a) and control animals (b-d). The observed hair loss and visible morphological changes can be a result of excessive urination. These morphological changes can be a result of secondary adaptation, developed to suppress urination, but this statement can be wrong and it remains unconfirmed. Photographs made by Alexei L. Vyssotski. Green background is a chalkboard.

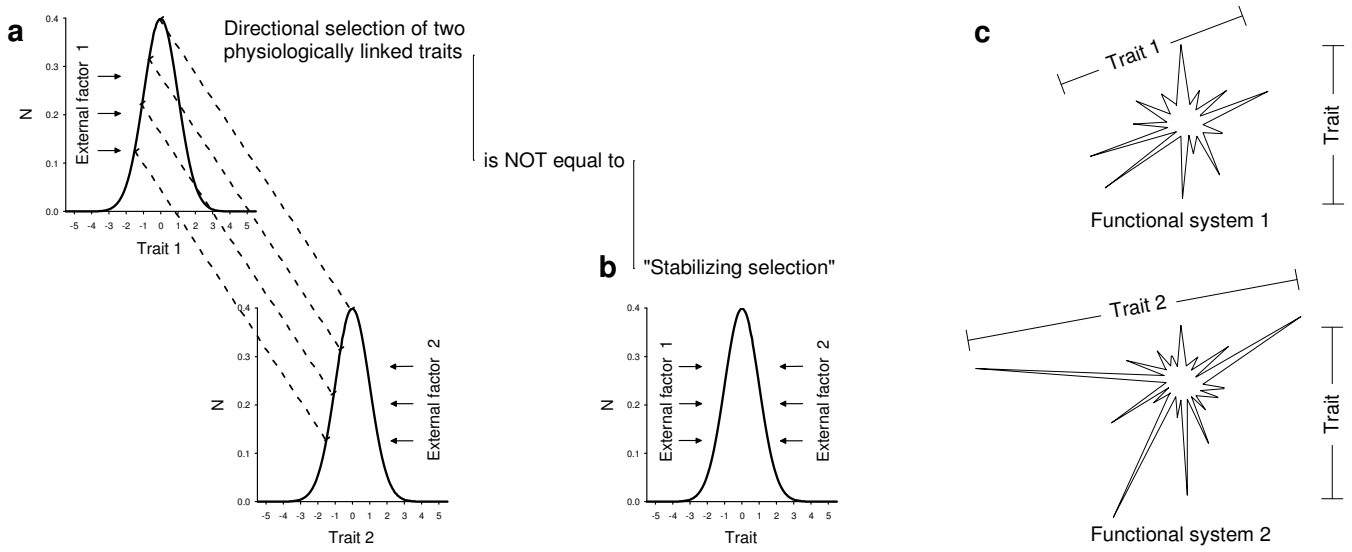
vital physiological results. Above-mentioned functionality has nothing common with so-called "stabilizing selection" – with elimination of extremes from the both sides of distribution curve of chosen quantitative trait (**Supplementary Fig. 7b**; see, for example: Solbrig & Solbrig, 1979, p. 146)[29].

First of all, stabilizing selection, being a simplistic model, does match to nothing in nature, and, second, being modelled in laboratory, it does not lead to canalization of phenotype, so common for wild species. Even more, it was shown in 1959 by Thoday (p. 197)[30] in *Drosophila melanogaster* that stabilizing selection results in weak phenotype (in general weak phenotype – animals with decreased viability), which is directly the opposite feature with respect to traits, observed in the wild nature.

"Stabilizing selection", being mathematically and logically correct, force us to assume that under stable conditions there is some "optimal" phenotype, which can be changed only due to some change in external conditions. That's wrong. First of all, as a rule, chosen indicator, convenient for measurement or observation, is linked with the most important characteristic of a given functional system in a rather correlative manner (**Supplementary Fig. 7c**). And despite this indicator is chosen



Supplementary Fig. 6 | Environmentally-sensitive timing of expression of one gene imitates multiple loci. Transgenerational epigenetic compensation makes at least one previously dormant gene available for expression. However this expression is not well optimized and it is not continuously/gradually regulated, it works as an "all or nothing gate" with two positions: 1) zero expression and 2) some technically-limited maximum. The current position of this "on-off" switch is not only gender-specific, but it is stress-sensitive, and it may be sensitive to external factors in general. Despite above-mentioned sensitivity, the answer of the gene is always 1-bit: zero expression (0) or maximum (1). The different timing of expression of one gene in four different animals (**a**) can completely imitate the presence of several independently modified loci, both during investigation of individual correlations (**b**) and during investigation of the disappearance of phenotypic traits in a raw of generations (not shown). T_1 & T_2 – ontogenetic periods of formation of traits A and B.



Supplementary Fig. 7 | Contradictory directional selection of two physiologically linked traits. Contradictory or conflicting directional selection of two physiologically linked traits (**a**) appears when one characteristic of an animal, let say animal size, is important for two different processes, for example for male-male competition (bigger is better) and for hiding from avian predators (the small size is easy to hide). Commonly, this situation was described as "stabilizing selection" (**b**), and it was supposed that it can be modelled by elimination of animals with extreme phenotypes from both sides of distribution. It was assumed that stabilizing selection is a common type of selection in nature and that it produces well canalized phenotype. Both ideas are wrong.

by a researcher as identical for two different functional systems, it is very rarely the most important dimension for both of them (**Supplementary Fig. 7c**). Second, the existing physiological link between different traits can be modified in evolution, it is a structure-dependent feature, and this structure can evolve. Animals are always under the pressure of mixed contradicting forces, and solution of some contradiction can be the appearance of a new species. It is not an often event, but it is always theoretically possible. All evolutionary limits, derived from the model of stabilizing selection, are false. The term "canalizing selection", as a synonym for "stabilizing selection", is also wrong, because canalization of ontogenesis is a result of directional selection, and very often a result of cyclic selection. The feature that is canalized is a set of positive results, physiological results or states, provided or achieved by phylogenetically old functional systems. Organisms that have functional systems those can achieve phylogenetically old results, despite unusual external influences, unexpected mutations and significant developmental noise, are the only ones good for further evolution. An organism can acquire in evolution something really new, only if it is able to support minimum functionality of its own old functional systems. This minimum functionality is the purpose of canalization. Morphology *per se* is not the object of canalization. Behaviour *per se* is not the object of canalization either.

Disruptive selection, as it can be organized in laboratory, is more close to real nature, than stabilizing selection, because selection of animals with opposite phenotype and their interbreeding provide load to stabilizing abilities of an organism and, as a result, the line will have improved (better stabilized) phenotype in a row of generations. This was also shown in 1959 by Thoday (pp. 196-197)[30]. See also the next article of Thoday & Boam (1959)[31].

Concerning natural populations, Kingsolver and co-authors have shown in 2001[32], using a bunch of published articles, that both stabilizing and disruptive selection are typically quite weak. In addition, they have shown that there is no evidence that stabilizing selection is stronger or more common than disruptive selection in nature. Taken these conclusions seriously, we can say that neither disruptive, nor stabilizing selection does exist in nature as a class. All known phenotypic results can be achieved by means of directional selection, including contradictory directional selection of two physiologically linked traits.

As we have seen previously, any randomly chosen quantitative trait, suitable for easy observation and recording, is not the thing that should be canalized. But the achievement of all positive physiological results, necessary for survival and reproduction, must be canalized.

Canalization is comprised by a set of active feedback loops. In the mechanical automates, developed by humans during previous century (XX), we typically can see one feedback loop (or, at least, one major feedback loop). In the leaving creatures, each function is supported by a set of semi-parallel feedback loops, and the inactivation one of them has relatively small negative impact on the functionality of this functional system (the impact that is usually undetectable under normal conditions). For example, the ontogenesis of females is slightly better canalized than that one of males, as it was shown by Vigen A. Geodakian in 1965[33-35]. It can be that some percent of semi-parallel feedback loops is disabled in males (*i.e.*, the expression of some of their components is dependent on sex-hormones, and this expression is possible only when female sex-hormones are present and male sex-hormones are absent). We suppose, as usual, that the expression of the above-mentioned components is binary ("all-or-none"): some semi-parallel feedback loops are completely disabled in males, but active in females.

We know that the transgenerational epigenetic compensation, visible at the level of phenotype as phenotypic inversion, is often present in the F_2 generation females, but not in the F_2 generation males (Fig. 4^3). It can be that this sexual dimorphism in the expression of phenotypic inversion, namely expression of some previously dormant gene in F_2 females, but not F_2 males, is the result of the same mechanism that provides better canalization of ontogenesis in females. And this mechanism is dependent on sex-hormones. For example, both previously dormant gene and some genes, involved into some semi-parallel feedback loops, can have the same regulatory sites, accessible directly or indirectly by sex-hormones. And the presence of female sex-hormones allows the expression of both groups of above-mentioned genes (previously dormant ones and the ones involved into feedback loops). Less simplistic view can be that both female and male sex-hormones are involved somehow. The question is, how.

We do not know the answer yet, but as a result of such functionality, females have better canalization of their ontogenesis and higher probability of expression of at least one deblocked previously dormant gene. In real life the expression of this previously dormant gene will lead to activation and expression of a lot of physiologically linked genes. We can see this, for instance, in the **Supplementary Table 1**. Note a lot of genes with increased expression in the F_2 females, and only a few genes with increased expression in the F_2 males; both are descendants of prenatally vinclozolin-treated males and females.

By the way, there is no contradiction or competition between "canalization" and "plasticity", because "plasticity" is not an independent entity, but it is a part of canalization process (canalization is a process, and not only a result of above-mentioned process). Of course, the result of any mechanical or chemical damage *per se* is not an example of "plasticity". But the following compensation of disrupted functionality is the one.

Relative fitness and selection coefficient

Relative fitness is determined usually with respect to the most fitted phenotype, which is usually a phenotype of wild-type animal, and it is usually taken as "1" (Solbrig & Solbrig, 1979, pp. 141-142)[29]. Transgenerational epigenetic compensation can increase fitness of homozygous mutant phenotype so dramatically and, simultaneously, it can decrease fitness of wild-types by a so impressive way, that selection coefficient occurs to be completely reversed (**Supplementary Fig. 1**). Here we can suppose that fitness is proportional to lifespan duration. Obviously, before the appearance of transgenerational epigenetic compensation, the best animals, *i.e.* animals with the longest lifespan, were wild-type females. After the appearance of transgenerational epigenetic compensation and after its distribution in population, the longest lifespan belongs to homozygous mutant females. And their lifespan is superior with respect to any lifespan seen before (in this experiment, of course). Thus, selection coefficient should be calculated with respect to homozygous mutant animals, starting from some time point (such time point is around generation F_2 in the **Supplementary Fig. 1**). After overwhelming distribution of transgenerational epigenetic compensation in population, the selection coefficient by chance could be even numerically the same as previously (before the appearance of epigenetic compensation), but its biological meaning will be completely inversed. This is a result of transgenerational epigenetic compensation in evolution: it can convert homozygous mutants with previously impaired phenotype into hopeful monsters – into the animals with the best phenotype ever seen.

Horizontal gene transfer

Transgenerational epigenetic compensation, processing through unblocking of previously dormant genes, changes our views on horizontal gene transfer in evolution of eukaryotes. Very important role of horizontal gene transfer in evolution of prokaryotes was shown by Boris Mirkin and Eugene Koonin in 2003[36,37]. Transgenerational epigenetic compensation, as we see it now, does not change probability of horizontal gene transfer in higher eukaryotes directly, but it makes horizontal gene transfer less dangerous for ontogenesis and survival. If any (or practically any) transferred gene, new for a given organism, can be effectively blocked, the positive effects of horizontal gene transfer can be much higher than its negative effects.

Dormant genes and genetic assimilation

The deblocking of a dormant gene, being the main route of transgenerational epigenetic compensation, changes our expectations concerning the main biological function of genetic assimilation. Previously it was assumed that the genetic assimilation could be a replacement of epigenetic hereditary changes by genetic ones, and, speaking honestly, the purpose of this supposed replacement was a mystery, because it was seen as a rather complex process without clear benefits. Now we can see that the result of unblocking of one previously dormant gene is the functional appearance in the genotype of an organism of one gene with poorly determined and often unacceptable temporal pattern of expression (expression during "wrong" stages of ontogenesis and the absence of expression during "correct" developmental stages) and with poorly determined and often unacceptable dependence on sex hormones and stress hormones (expression in males instead of females, or *vice versa*, expression during the absence of stress instead of expression during stress, or *vice versa*).

The main purpose of the following genetic events, which coincide in time with the period of "genetic assimilation", is optimization of the temporal pattern of expression and optimization of sensitivity (in a very wide sense) to sex hormones and stress hormones. If the average level of expression should be changed (usually it should be decreased), it is achieved first of all by means of sinusoid-style level of expression, by means of regulation in time. And only afterwards, we can suppose, the average level of expression can be decreased in accordance with the existing requirements (but we do not have relevant experimental data now).

The question about spatial differences in expression (tissue-specific expression) seems very important, because specific expression in some structures can be extremely beneficial. Probably, the specification of the location of expression can be determined at the last steps of the discussed process, the process that can be entitled (in accordance with tradition) "genetic assimilation". Thus, the main purpose of genetic assimilation is not a replacement of transgenerational epigenetic compensation, but optimization of its results those are unexpected in many dimensions at the beginning of this process.

It can be, by a lucky chance coincidence, that previously dormant gene will be absolutely ready for normal usage immediately, without any change in the functionally-linked transcription factors and other neighbouring elements. However more realistic expectation is that at least some changes will be highly desirable.

The priority of "dormant genes"

We assume that the priority of dormant genes belongs to J.W. Harms, despite he did not use the term "dormant gene", but, instead of this, he was speaking about "active" and "passive" genes. However he discussed "active" and "passive" genes at the levels of both genotype and phenotype, he discussed an activation of a "passive" gene by external environmental factors, and above-mentioned activation was useful for a given organism. It is important that above-mentioned activation was supposed to be heritable. He discussed an activation of a "main", the most critical, gene among a set of passive genes, and he has introduced the term "orthoselection" to describe activation of one gene among lots of irrelevant passive genes. He supposed that activated gene can control a group of other genes with their associated phenotypic traits. And he has assumed that above-mentioned currently passive gene was used during previous evolutionary history of this species, together with above-mentioned cascade of genes, functionally linked with each other, that is why "orthoselection" [in German: "*Orthoselektion*"].

There are at least two other opinions about priority of "dormant genes". Eva Jablonka and Marion J. Lamb (2014)[17] assume (p. 14[17]) that the basis of the idea of dormant genes was expressed by Darwin in his theory of "pangenesis", included in his book "*The Variation of Animals and Plants under Domestication*" (1868)[38], where he described hypothetic hereditary particles – "gemmules" and stated that some gemmules can be dormant, and nutrition or climate could reawaken dormant gemmules. If we assume that a "gemmule" is a sufficient prototype of "gene", we can agree with above-mentioned claim. However it seems that the gap between "gene" and "gemmule" in the ideation space is too wide for such generalization.

Dmitry K. Belyaev and co-authors (1981)[21,22] have provided references with respect to dormant genes to publications of Muller (1932)[39] and Zuckerkandl & Pauling (1962)[40]. I have read the article of Muller (1932) from the beginning to the end and I have found nothing that can be associated with "dormant genes". There is a statement that there are mutations which lead to activation of some genes, *i.e.* lead to the appearance of some previously absent functionality, but that is all (see section "*Neomorphic mutations*", pp. 245-249[39]). Article of Zuckerkandl & Pauling (1962)[40] was published too late, really too late in view of publications of J.W. Harms (1929, 1934)[19,20], and just due to this reason they can not pretend to any priority.

Despite the article[19] and the book[20] of J.W. Harms were unknown to D.K. Belyaev, the role of Dmitry Belyaev *per se* in the story of dormant genes should not be neglected. It was Dmitry K. Belyaev who has written the following.

1. "Once entered into an active state, the gene subsequently behaves like a discrete Mendelian unit. The idea of functional activation-inactivation of genes has permeated through the literature. It has been shared by Muller[39] and explicitly stated as the dormant gene hypothesis by Zuckerkandl and Pauling[40]. There was no experimental substantiation of this idea.

As suggested by *star* studies, once brought out of dormancy, a gene would become functionally active and relatively stably inherited in the new state; sometimes it would become inactive, thereby distorting classical segregation patterns. The phenomenon of gene activation-inactivation cannot be explained only by the influence of the genotypic environment for the reason that within the same genotypic environment (the same organism) one of the homologous genes can be active and the other inactive" (p. 273[22]).

2. "The explanation offered relies on the supposition that inactive ("dormant") genes may be activated and reverted toward an inactive state. A similar reliance on gene activation-inactivation has been implied by many investigators, including Muller[36]. Since then, Zuckerkandl and Pauling[40] formulated it in their "dormant gene" hypothesis, and Belyaev[41] extended it to the process of domestication.

It is worth emphasizing that "dormant" genes are activated by evolutionary stimuli eventually resulting in profound metabolic changes in the cell. With regard to mice, a strong stimulus has been selection in the course of domestication" (p. 112[21]).

3. "One can imagine that under the influence of an altered hormonal equilibrium a number of "dormant" genes could be expressed, resulting in a high frequency of appearance of a whole complex of morphological characters (position of the tail, ears, etc.) mentioned above" (p. 306[41]).

Emile Zuckerkandl and Linus Pauling have described dormant genes in 1962[40] in the article "Molecular disease, evolution, and genic heterogeneity", seemingly without prior knowledge about publications of J.W. Harms (1929[19], 1934[20]). Their description is provided in the section "D" of above-mentioned article. The section "D" is entitled "The destiny of duplicate genes and the function of genic multiplicity" (pp. 206-213[40]). Pages 206-210[40] are reprinted below as they were published in 1962 (only the reference numbers are added by us in order to be compatible with our reference style).

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"D. The Destiny of Duplicate Genes and the Function of Genic Multiplicity

If gene duplication is one of the means of increasing the output of a given protein, one may distinguish two phases in this respect. Up to an optimum number of duplications, the duplicate gene will be selectively retained with a structure identical to and a position rather near to that of the mother gene. Beyond this point, duplicate genes will be progressively more strongly selected against. During this latter phase they will be in part eliminated with their carriers, and in part subjected to progressive change. When they are preserved and changed, their destiny may be of three types. They might evolve new useful functional properties. In this case they will be retained as active genes, and to the extent to which polypeptide output depends on gene duplication their own duplicates will be kept unchanged by natural selection. Secondly, functionless or unfavourable duplicates will not maintain duplicates to their own likeness and may be themselves be translocated to other chromosome parts and be reduced to minor-component genes by a position effect. Some such minor-component genes, more or less profoundly

changed in the meantime, may be selected for later in evolution and be changed"

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"into major-component genes. Thirdly, the activity of the duplicate may be reduced to zero.

This elimination of gene activity may again take three forms. The changed structural gene may be bodily eliminated through the loss of the part of the chromosome that carries it; or it may be modified to such an extent that its products, although significant in amount, are no longer recognizable in terms of the original protein; or it may be preserved in a modified state, but totally or subtotally deprived of the power of expression.

The existence of such "dormant genes", although difficult to verify, is a plausible inference from two types of observations: firstly, that within a given tissue, say the hematopoietic tissue, major and minor structurally distinct components of a given type of polypeptide chain are found. Since at a given time the relative quantities of hemoglobin chains vary between 100 and 1 %, other genetically distinct minor components may be present in such small amounts that they are practically undetectable. Secondly, there are numerous examples of proteins that are produced exclusively in one type of tissue, and of which no trace is found with presently available analytical means in other tissues. The nonproduction of hemoglobin in muscle sells and of myoglobin in reticulocytes is one example. This example shows that some among even relatively closely related structural genes may, within a given tissue, be the ones strongly expressed, the others unexpressed. We must assume that all the structural genes have during embryological development been communicated to all cell lineages. It is therefore quite likely that there exist in every organism numerous structural genes that do not find in any of the existing tissues conditions favorable to their expression and thus remain permanently dormant.

Furthermore the relative structural similarity of minor hemoglobin components to one of the major components affords yet another argument in favor of the existence of dormant genes. Indeed, as we have seen, the human δ- and β-chains are quite similar. Likewise structurally distinct components of orangutan hemoglobin have been found to be quite similar, and the same holds for pig hemoglobin components (E. Zuckerkandl, R. T. Jones, Y. Nishiwaki and L. Pauling, 1959-1961, unpublished). If duplicate genes remained usually expressed, one would expect to find a series of minor-component chains differing from all the other chains as much as the human β-chain. This is, however, not"

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"the case. One is led to think that, in the long run, duplicate minor-component genes most often cease to be expressed. There is no apparent reason why one should assume that they have most times been bodily eliminated. Other possibilities are that they have been implied in a further translocation, that a mutation or transposition of a controlling element (repressor) has occurred, or that the structurally modified mutated gene possesses specificity characteristics that fail to comply with the specificity requirements for polypeptide production under the conditions prevailing in the cell.

Dormant genes of course are conceived as dormant only as far as their expression, and not as far as their mutability goes. Mutations in dormant genes and in minor-component genes will never be lethal, unless the latter have some distinct specific function, which would then lead us to consider them as "major components" of another protein type. Minor-component genes and, mainly, dormant genes may thus furnish an important and perhaps the principal part of the genic raw material for macro-evolutionary experiments of nature. A new translocation, or the transposition of a controlling element such as those described by McClintock (1956)[42], or some other genetic modification may reactivate the dormant gene after a very long period of time during which mutations have changed it enough so that it now controls the production of a new kind of protein. In this fashion new enzymes, new functions can arise without the corresponding loss of old enzymes, old functions. We have recalled earlier the importance to evolution of the loss of functions through the mutations of active genes. But it is evident that evolution, while it makes the best of such losses and of molecular disease, could not be based on them along. There are a great many more different functions to be carried out by a great many more different types of enzymes than we allowed to suppose can have existed in early evolutionary times. Primordial living matter must have been limited to a few simple functions. Therefore the notion of evolution by gain is a necessary complement to the notion of evolution by loss.

Horowitz (1945)[43] made a lasting contribution to our thinking about evolutionary gain at the enzymatic level. He described how new reaction chains might arise in certain circumstances through the chance combination of the necessary genes and furthermore proposed a general mechanism for the stepwise building up of complex enzyme-systems, presenting us with a plausible scheme of macro-evolution at the molecular level. Obviously, as enzyme systems"

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"become more complex, more different enzyme molecules are needed. There are reasons to think that the same molecules cannot usually be expected to carry out several different enzymatic functions. Therefore, new genes are needed for the building up of new functions. This is where the concept of the mutational reactivation of dormant genes complements Horowitz's picture. Minor-component genes are not to be excluded from a similar role, but may usually not yet be different enough from their parental genes to be fit for carrying out novel functions. Most minor-component genes, as stated above, are liable to be eventually turned into dormant genes because natural selection will not prevent their transfer to synthetically inactive chromosome regions, nor their coming otherwise under the influence of a repressor gene, nor structural changes that might place the genes outside the range of specificity requirements of the available macromolecules that collaborate with the structural gene in protein synthesis. As dormant genes become reactivated after periods of cryptic existence corresponding perhaps to geological ages, they may produce potential enzymes that do not disrupt existing chains of reactions, but are able to add new processes to the old ones, perhaps in the ways described by Horowitz. One of the possible mechanisms of the reactivation of dormant genes is the reactivation of the chromosomal region where it is located through a change in intracellular environment. The hope of demonstrating the existence of dormant genes rests on this possibility. Such a change in intracellular environment could result from the adaptation of the organism to changes in external milieu. In adaptational changes, during an initial phase, gain and loss mutations may be balanced, or loss mutations only

may occur, so that the total complexity of the organism either remains constant or tends to decrease. In the process, however, the environment of the chromosomes may be altered in some tissue and, on account of this alteration, genes be activated in some regions of the chromosomes, inactivated in others. Inactivation of this kind will be mostly lethal and genomes with corresponding inactivation-resistant chromosome regions will be selected for. The newly activated genes on the other hand will now be available to respond to further adaptational needs and will furnish a series of gain mutations without corresponding losses. Parts of this concept are supported by observation. Changes in conformation of the genic DNA molecules are presumably related to changes in the activity of the genes, and Schmitt (1956)[44] has shown that the state of chromosomal DNA"

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"depends on the chemical environment of the chromosomes. Furthermore, genes in mice that display the same activity characteristics during the individual's life time have been found to be closely linked on the same chromosome (Paigen and Noell, 1961)[45].

Through the reactivation of dormant genes by a modification of the intracellular environment an initial adaptational stress of great magnitude would appear instrumental in producing a rise in complexity of the organism. Thus would be solved the old paradox expressed in this question: why should organisms get to be more complex, since simpler organisms are evidently adapted as well to the environment? Once more Biology will show that it can do without any "élan vital" or "entelechy".

The present concept leads one to predict that the fastest evolution toward more highly organized forms would take place after the occurrence of major environmental stress. Evolutionary history bears out this expectation. For a long time paleontologists have noted that the initial phases in the development of new types of forms has an "explosive" character (consult, for instance, Rensch, 1954)[46]. According to the present theory, we assume that at the onset of such an explosive evolutionary phase a change in intracellular environment has brought about the reactivation of a number of previously dormant genes. Rensch believes that the phenomenon of explosive evolutionary phases is adequately accounted for by increased natural selection accompanying the conquest of new biotopes. He thus considers as instrumental a change in *external* environment only. This however does not explain the trend toward increased complexity of the forms.

In the sense that has been laid out here a marked change in environmental conditions may lead to what a layman would call a shake-up of the genome, and this may be the part of reality behind the poorly documented and falsely interpreted observations of Soviet anti-geneticist A. Lysenko.

To sum up, mutations of active genes controlling "major" protein components suffice to explain how an organism can adapt to changes in its environment. Mutations of minor-component genes and dormant genes, however, seem to be able to furnish the organism with the genic raw materials that eventually allow it not only to adapt to a new environment but also, in the process, to become more highly organized. Minor-component genes and dormant genes may thus prepare the major steps in evolution."

The concept of dormant genes, provided by Emile Zuckerkandl and Linus Pauling, is expressed in more modern terms than the article and book, written in 1929 and 1934, respectively, by Wilhelm Jürgen Heinrich Harms. However some major and minor remarks, given form the view from the 21st century, might be useful. "Dormant gene" is a catchy term. However the term "genetic locus", proposed instead of the term "gene" by James A. Shapiro in 2011 (p. 30[47]), provides more precise description of real situation in the field of transgenerational effects. The activation of a dormant genetic locus does not lead to the expression of this gene at some constant level. Instead, it will be expressed only if necessary combination of stress-hormones and sex-hormones will be present in a given descendant at a given time. During some generation (*e.g.*, F_2) and in some gender (*e.g.*, males), the gene, being brought out of absolute dormancy, and being potentially dominant, will be completely unexpressed during the whole life cycle, due to the lack of combination of stress-hormones and sex-hormones, necessary for its expression. Thus, dormant genetic locus, being brought out of dormancy, becomes open for further regulation of its expression, but not for unconditional presence of its product in the phenotype. As we have seen in the experiments with morphine and thyroxine, together with observations on guinea pigs, the expression of previously dormant genetic locus is obviously gender-dependent, significantly stress-dependent, and is sharply regulated in time during lifespan of a single organism through the effects of short periods of stress.

"Dormant genetic locus" can be not only the whole dormant gene together with all its *cis* and *trans* regulatory sites, but it can be only a part of its regulatory sites. Thus, dormant genetic locus can contain a part of regulatory sites of a given gene. Even in this case the effect of this genetic locus is strictly binary: it can be dormant and coexisting with normal level of expression of a given gene, or, alternatively, it can be brought out of dormancy and (an additional condition) in the presence of necessary stress-hormones and sex-hormones it can entail very high, enormously high, level of expression of this gene.

The case, when previously dormant genetic locus will entail decreased expression, is not interesting for us and for evolution, because it will be a recessive trait, undetectable in the heterozygous organism. The asymmetry between activation and repression is very important here. If we will speak only about "regulation of expression" in general terms, we will miss the most important features of the process.

The role of "junk DNA", including the role of potentially regulatory sites in it, was discussed in the article of Emile Zuckerkandl and Giacomo Cavalli "Combinatorial epigenetics, "junk DNA", and the evolution of complex organisms" (2007)[48] from similar standpoint. Of course, we can discuss "the transcriptional rate of individual genes" in general. However we should remember that the transcriptional rate has minor importance, unless it is increased by a factor of four, at least. And then, when it is increased, it can be regulated only in "all-or-none" fashion and its ontogenetic windows of "on" *vs*. "off" states is the most important. Then, logically, tissue-specific expression must be optimized. We suppose that time-specific expression and stress-dependent expression will be optimized first of all.

We know that the primary removal of dormancy is a site-specific effect, the effect which is associated with a given locus of a given chromosome. Experiments of Dmitry K. Belyaev have

shown that the same gene can be dormant in one chromosome and, simultaneously, it can be expressed in homologous chromosome of the same organism. Thus, extra-chromosomal factors and intracellular conditions can not be solely responsible for above-mentioned effect.

We have not material basis to claim that the heritable unblocking of dormant genetic locus is solely epigenetic effect in modern sense, *i.e.* that it does not formed by a change of DNA sequence in a given locus. Alternatively, taking into account known variety of natural genetic engineering systems, summarized by James A. Shapiro[47], it can be equally supposed that site-specific reversible genetic modification of DNA can take place with a help of specific proteins. The involvement of proteins in general in this process was shown by the experiments with Hsp90 (for a short summary, see pp. 262-263[17]).

Interesting that even if it will be shown that site-specific DNA sequence modification is involved as a primary factor, we still will be able to use the term "epigenetic", but in this case only in the Waddington's wide sense (not in the modern sense, given, for example, in the abstract of our current article).

Previously, the hypothesis of demethylation of 5'-methylcytosine in specific DNA regions was considered as a parsimonious explanation of transgenerational effects in mammals. However it was rejected by recent experiments with vinclozolin, conducted by Michael K. Skinner and co-authors in 2013[28]. These experiments have shown that methylation patterns in the germ cells of F_2 generation are very different at E13 and E16 (they are so different that the demethylation process can not be considered as a primary activator of dormant genetic loci in evolution anymore).

Zuckerkandl and Pauling discussed "mutational reactivation of dormant genes" (p. 209[40]) and "reactivation of dormant genes by a modification of the intracellular environment" (p. 210[40]). J.W. Harms described reactivation of passive genes as an adaptive response to a new environment. This transgenerational adaptive response consists of orthoselection among many-many passive genes and further hereditary transmission of the activated state of a chosen gene to the next generation (p. 201[20]). Harms did not discuss the situation when the dormancy of a passive gene can be completely removed, but the gene still will not be expressed in the progeny due to the lack of some gender-related or stress-related activation factors. Zuckerkandl and Pauling did not discuss this possibility as well.

We assume that the priority of "dormant genes" is currently held by J.W. Harms, but we can change our opinion, if some other publication with early priority date will be found. It is interesting to note that Richard Goldschmidt (1940)[49] described theoretical views of J.W. Harms the following way: "Harms explained the whole set of adaptive evolutionary changes on a purely Lamarckian basis; we refrain from discussing his arguments, which may be used as a fine example of the fallacies of that doctrine" (p. 276[49]).

Trofim D. Lysenko, mistakenly called by Zuckerkandl and Pauling as "A. Lysenko", discussed "unstable heredity" [in Russian: "расшатанная наследственность"] in his own publications a lot (see the term "destabilized heredity" in the Subject Index of his book "Agrobiology", published in English in 1954[18]). Concerning the "poorly documented observations", one can mention that they were rather "poorly accepted by Soviet Morganists" than "poorly documented". "Soviet Morganists", of course, had no relation to Thomas Hunt Morgan, the author of many articles, including popular "The theory of the gene" (1917)[50] and "Chromosomes and heredity" (1910)[51]. Whether "Soviet Morganists", existed in 1962, had read Morgan's articles? It is a difficult question. We know how they have read Darwin's books: they widely exploited the term "Darwinism" as the opposite to "Lamarckism", without any knowledge that Darwin himself supported the idea of "the inheritance of acquired characters"[38]. Contrary to many "Soviet Morganists", Trofim D. Lysenko discussed Darwinism not only as "natural selection", but as real teaching of Charles Darwin[38]. For an objective and compact description of Darwinism, see section "Darwin's Darwinism" (pp. 10-16[17]) in the book of Eva Jablonka and Marion J. Lamb "*Evolution in Four Dimensions: Genetic, Epigenetic, Behavioral, and Symbolic Variation in the History of Life*", revised edition (2014)[17]. Views of T.D. Lysenko can be found in a compact form in his publication "The Situation in Biological Science" (1948), see pp. 515-554[18], one can look at p. 537[18] specifically for "destabilized heredity".

Concerning "entelechy" the situation should not be simplified too much. Just because if we have two species of animals and one of them believes into "entelechy", and another one does not believe in such non-material entities, the first one will evolve faster and in more efficient way. Especially if species are faced with the task that can not be solved during lifespan of one generation and the efforts of several consecutive generations are required. Without believe into "entelechy" an animal will not have any motivation even to try to find a solution of the task that is obviously too difficult for her. However, if she believes that her attempts, described in details, will be used as a basis for further attempts of further generations, even if all her above-mentioned attempts will never be included into the "gold fund", and will be just all wrong, all in wrong direction, all completely rejected, but showing that particular field of possibilities is carefully and honestly investigated with final negative result, she may start more enthusiastically, just because our knowledge of honestly excluded possibilities may help us to find a positive solution. By the way, such animal as "rat" (as well as "mouse" and "guinea pig") is always "she" in Russian, even if we are talking about male rat (or male mouse, or male guinea pig).

Further details can be found in the books of Henri Bergson (1907)[52], Genrich Altshuller (1973, *e.g.* pp. 25, 48, 52)[53] and Ludwik Fleck (1935)[54].

Sometimes we can speak about the necessity of a "new synthesis"[16]. But may be "natural selection" and Darwin's Darwinism are already upside down? Transgenerational epigenetic compensation and dormant genetic loci have placed "natural selection" exactly into the above-mentioned position. If some population has a mutation, slightly deleterious in homozygous subjects and practically neutral in heterozygous ones, then if this population will be placed in a hard conditions, the conditions, difficult for survival, then, in accordance with the laws of natural selection, this mutation will be wiped away from the population together with the bearing it animals.

However, in real life, the hard environmental conditions will bring out of dormancy at least one genetic locus, only in the above-mentioned homozygous mutants, and we will have in population not only one previously known recessive mutation, but, in addition, one unexpected dominant mutation. Original mutant gene is typically recessive, whereas the deblocked

genetic locus always bears dominant trait. Of course, original mutant gene and deblocked genetic locus are not necessarily homologous (as it was discussed by Zuckerkandl and Pauling), but they are participants of the same functional system. We use the term "functional system" exactly as it was proposed by Peter K. Anokhin (1974)[55], but not in the sense of "systems biology".

The old mutant gene (*e.g.*, Per2 in the following example) and the unblocked previously unknown dormant gene are not necessarily homologous. However if they are homologous due to some unexpected lucky coincidence, it will simplify further experimental investigation enormously.

The deblocked genetic locus may change the direction of natural selection towards homozygous mutants, bearing original mutation, exactly as it is shown in the **Fig. 2** and **Supplementary Fig. 1**. These figures show the experimental data with $Per2^{Brdm1}$ mutant mice under semi-natural outdoor conditions, obtained in the experiment of Serge Daan and co-authors, first described in 2011[11]. In the most interesting case this situation can develop into a speciation (formation of a new species on the basis of homozygous mutants having independent deblocked genetic locus). Such homozygous mutants can be called "hopeful monsters", as it was proposed by Richard Goldschmidt many years ago[49,56]. Above-mentioned new species will be reproductively separated form the old one. The old species consists of wild-type animals without any change in the above-mentioned dormant genetic locus. This possibility was described in the section "The main modus of macroevolution" in our article "Transgenerational epigenetic compensation and sexual dimorphism"[3].

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