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Article

Transgenerational epigenetic compensation and sexual dimorphism

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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, heritable epigenetic compensation can convert mutants into hopeful monsters, initiates speciation, and therefore determines the route of macroevolution. Transgenerational epigenetic compensation facilitates genetic assimilation of acquired characters in microevolution. The ontogenesis of females is better canalized than that one of males, and natural selection proceeds mainly through selection of males. However the presence of sexual dimorphism in transgenerational epigenetic compensation remains unclear. Here we show that the hereditary basis of transgenerational epigenetic compensation develops mainly in males. However the phenotypic results of this development are more pronounced in their female descendants, starting from F_2. This sexual dimorphism enhances the efficiency of micro- and macroevolution.

"Numerous facts go to show that changes in various sections of the body of a plant or animal organism are not fixed by the reproductive cells with the same frequency or to the same extent." (Trofim D. Lysenko, 1948; p. 535[1]). These words, entirely different from the Lamarckian ones, were written 5 years before the discovery of DNA structure. In 1953 the existence of 5-methylcytosine was considered as a problem for otherwise brilliant theory: "We have considered 5-methylcytosine to be equivalent to cytosine, since either can fit equally well into our structure." (J. Watson & F. Crick, 1953; p. 242[2]). Now, the methylation of cytosine is considered as one of the mechanisms of epigenetic inheritance, those can be used to support Lamarckian process – the inheritance of acquired characters[3]. However the phenotypic results of transgenerational epigenetic inheritance are very far from the Lamarckian expectation: "the modification in the descendants may have no visible likeness to the original one" (Henri Bergson, 1907; p. 83[4]).

Many of the changes discovered in the untreated progeny tend to be the opposite of those observed in the treated parents themselves[5-14]. This phenotypic inversion demonstrates that the main biological function of transgenerational epigenetic inheritance is a transgenerational epigenetic compensation of disturbed functionality[13]. Recently, in the course of 2-year experiment with *Per2*^{Brdm1} mutant mice under semi-natural outdoor conditions[15], it was shown that the transgenerational epigenetic compensation can dramatically increase the lifespan of homozygous mutants, not only in comparison with their initial state, but in comparison with wild-types also[13-14]. This experiment[15] may be the first study in the world in which it is shown how evolution really works, not only "natural selection", but real evolution. In all experiments with paternal or maternal drug treatment, as well as in the above-mentioned experiment with mutant mice in semi-natural conditions, the enormous difference between phenotypes of males and females was observed in the progeny. This gender-related or sex-related difference (sexual dimorphism), observed in the descendants of drug-treated parents, is greater than the difference between males

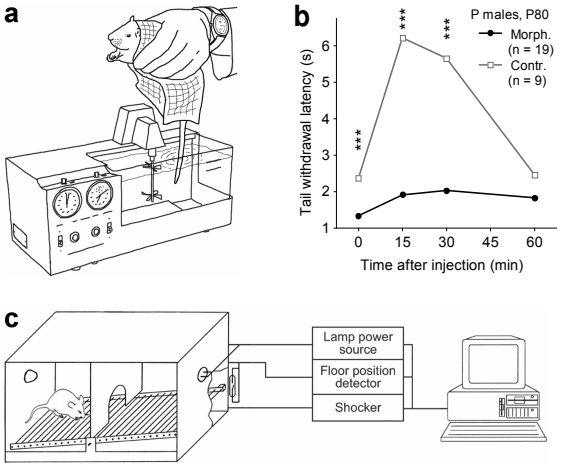

Figure 1 | Tail-withdrawal (**a**) and two-way avoidance (**c**) tests for Wistar rats and DBA/2J mice, respectively. (**b**) Male rats (P) were tested at the age of 80 days in the tail-withdrawal test (56°C), being injected with morphine 10 mg/kg, i.p., after the end of chronic (P42-P79) morphine treatment. Triple asterisk, $P < 0.001$. Mann-Whitney U test. Mean.

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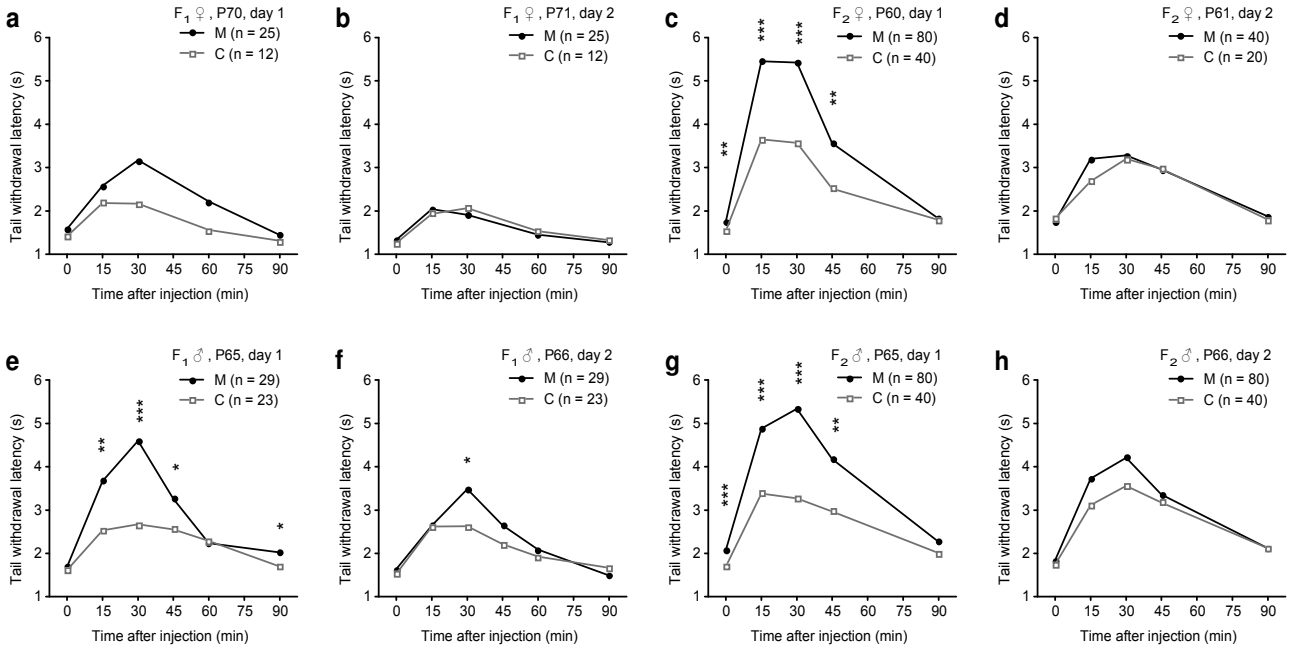

Figure 2 | Tail-withdrawal test in the F_1 & F_2 descendants of morphine-treated male Wistar rats. Each animal was tested twice (days 1 & 2) with the same dose of morphine 10 mg/kg. Morphine was administered i.p. just after the first measurement of tail-withdrawal latency (time "0"). The enhanced analgesic effect disappears at day 2 in the experimental animals, whereas control ones show stable response. In the F_2 generation the enhanced analgesic effect is present not only in males (**e**), but in both sexes (**c**,**g**). There is some difference in the basal pain sensitivity (time "0") in the F_2 generation, but only during the 1-st day (**c**,**g**). M – descendants of morphine-treated males, C – control. Hereinafter: asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$. Mann-Whitney U test. Mean (SE or SD is omitted for clarity).

and females, found in wild-type animals as a response to drug application. It means that this sexual dimorphism is a main feature of transgenerational epigenetic compensation, not just some satellite phenomenon. In 1965 it was discovered by Vigen A. Geodakian that the ontogenesis of females is better canalized than that one of males, and natural selection proceeds mainly through selection of males[16-17]. This statement is confirmed by many observations, including recent ones[18], but it is insufficient to explain the enormous sexual dimorphism in the progeny of drug-treated animals.

For our current paper we have chosen several traits (**Fig. 1**) that have demonstrated clear phenotypic inversion in the F_1-F_2 progeny. These results were obtained in the progeny of chronically (P42-P79) morphine-treated male Wistar rats and neonatally (P0-P11) tyroxine-treated male DBA/2J mice. Using these data, together with previously reported results with prenatal (E8-E14) treatments[19-24], we are going to show how the sexual dimorphism in phenotypic expression of transgenerational epigenetic compensation enhances the efficiency of micro- and macroevolution. **Supplementary Fig. 1** summarizes the microevolutionary part of our findings.

Results

I. In the F_1 generation, obtained after prenatal, neonatal or adolescent treatment of male or female parent P, the opposite phenotypic changes in many cases are equally expressed in males and females, and in many other cases they are significantly more pronounced in males.

II. In the F_2 generation, obtained by means of breeding of F_1 female with F_1 male, or breeding of F_1 female with a new naïve male, or breeding of a new naïve female with F_1 male, the above-mentioned opposite (with respect to parent P) phenotypic changes are expressed in females only, whereas males are normal.

III. In the F_3 generation, obtained by means of breeding of any F_2 animal with a new naïve animal, or breeding of F_2 animal, obtained from one new naïve parent, with any other F_2 animal, the above-mentioned opposite (with respect to parent P) phenotypic changes are expressed in males only, whereas all other animals, including males, obtained in line of incross breeding ($F_1♀ \times F_1♂$, $F_2♀ \times F_2♂$), and all females, are normal.

IV. Above-mentioned F_1-F_3 results are already sufficient for mathematical modelling of transgenerational epigenetic compensation in evolution, if we assume that the transgenerational epigenetic compensation is generated only in homozygous mutants, in males and females (or may be mainly in males), in the locus or loci, independent of the locus of mutation. Then, the transgenerational epigenetic compensation is expressed in the consecutive generations like it is described in the **I-III** and it is dominant. Being expressed, the transgenerational epigenetic compensation enhances fitness of homozygous mutants, decreases fitness of wild-types, and probably has no effect on heterozygous animals.

Tail-withdrawal (Water-immersion) test (**Fig. 1a**), in comparison with more common Hot-plate test, can be used without preliminary animal training. Synchronous Hot-plate data are available also (Figs. S54b,d[12], S55b,d[12], S57b,d[12], S58b,d[12]). Chronic morphine treatment of adolescent (P42-P79) Wistar male rats (P) has led to decreased analgesic effect of standard morphine dose 10 mg/kg (**Fig. 1b**) in these animals , but to

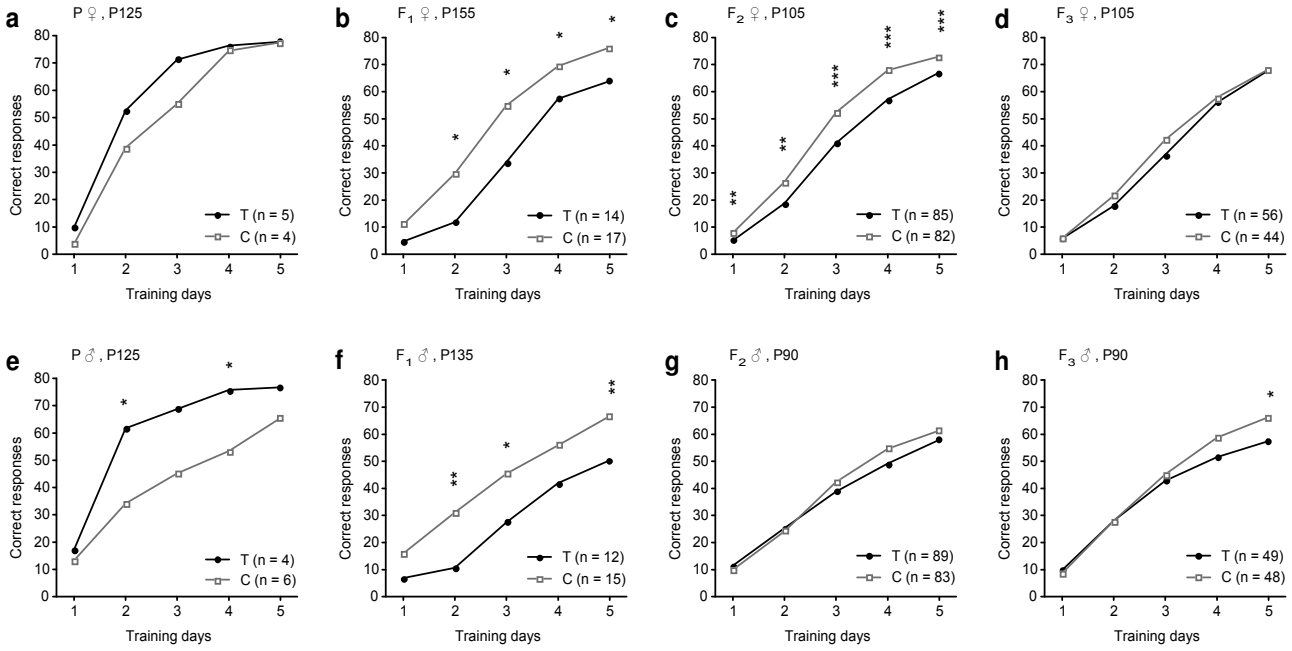

Figure 3 | Two-way avoidance in the thyroxine-treated DBA/2J mice and in the F_1-F_3 progeny of thyroxine-treated males. Note improved performance in the neonatally (P0-P11) thyroxine-treated males (**e**), but decreased performance in their descendants (**b**,**c**,**f**,**h**). Note that the performance of the F_2 males is absolutely normal (**g**), whereas the F_2 females demonstrate deviation with very high statistical significance (**c**). In the F_3 generation this significance disappears, but look at the last day in males (**h**) and see **Supplementary Fig. 3** for differences between Incross and Outcross subgroups. T – thyroxine-treated animals (generation P) or descendants of thyroxine-treated males (F_1-F_3), C – control.

enhanced analgesic effect in their F_1 male (**Fig. 2e**), but not female (**Fig. 2a**), offspring. The enhanced analgesic effect in F_1 males in Hot-plate test after paternal morphine treatment was reported previously[9-10]. Recently, the enhanced analgesic effect in the F_1 male, but not female, offspring was observed in the Hot-plate test after adolescent (P30-P40) maternal morphine treatment (Fig. 3[25]). We did a replication of our Tail-withdrawal test with all our animals 24 hours later and have found that the previously enhanced analgesic effect was attenuated up to normal level in the experimental animals (**Fig. 2b,f**). This attenuation is equal to the enhanced rate of tolerance development. The enhanced rate of tolerance development was reported in the F_1 males, but not females, after adolescent (P30-P40) maternal morphine treatment (Fig. 4[25]). Thus, both paternal and maternal adolescent morphine treatment lead to the same phenotype in the F_1 offspring: enhanced analgesic effect of morphine in males, but not in females, and enhanced rate of tolerance development in males, but not in females.

In the F_2 generation, obtained in our experiment by incross (F_1♀ × F_1♂), the enhanced analgesic effect and the enhanced rate of tolerance development was observed in both F_2 males and females (**Fig. 2c-d,g-h**). Thus, contrary to the F_1, the F_2 females are significantly affected as well as F_2 males.

In the experiment with adolescent (P30-P40) maternal morphine treatment[26], the F_2 generation was obtained through F_1 female outcross (F_1♀ × new♂), but only males were tested[26]. The effect of repeated quinpirole (D2/D3 dopamine receptor agonist) injections on locomotor activity, namely enhanced locomotor activity, occurred to be similar for F_1 and F_2 males (Fig. 2a,b[26]). In the previous experiment with adolescent (P30-P50) maternal morphine treatment[27], the effect of morphine injection on locomotor activity after preliminary 7-day morphine treatment and 7-day abstinence was observed in the F_1 males (Fig. 3[27], *Bottom panel*, $P < 0.01$), but not females (Fig. 4[27], *Bottom panel*, N.S.); the increased locomotion was observed. In our experiment, the effect of morphine injection on locomotor activity after 5.5-day morphine treatment and naloxone-precipitated withdrawal was similar – the increased locomotion in F_1 males, whereas females were not tested (Supplementary Fig. 4a[13], $P < 0.0022$).

Thus, transgenerational epigenetic compensation can be formed by paternal or maternal adolescent morphine treatment. In the F_1 it is expressed mainly in males, even if only females in the previous generation were morphine-treated during their adolescence (P30-P40). In the F_2 the transgenerational epigenetic compensation is expressed equally in both males and females.

Transgenerational epigenetic compensation can be transmitted from F_1 to F_2 through females, by means of F_1 female outcross, as it was shown in the above-mentioned experiment of John Byrnes and co-authors (2013)[26] with adolescent (P30-P40) maternal morphine treatment, and it can be transmitted from F_1 to F_2 through males, by means of F_1 male outcross, and this result was obtained in our experiment with neonatal (P0-P11) paternal L-thyroxine treatment. Concerning morphine treatment we have to add that the basal pain sensitivity was not affected in the F_1, but it was slightly, but significantly, decreased in the F_2 in both males and females (**Fig. 2c,g**), and this effect was eliminated after the first morphine injection (**Fig. 2d,h**).

Two-way avoidance (Shuttle-box)[28] test is a fully automated operant task where an animal learns to move to the opposite (dark) compartment as a response to light stimulus presentation (**Fig. 1c**). Training consists of 80 light presentations daily, during

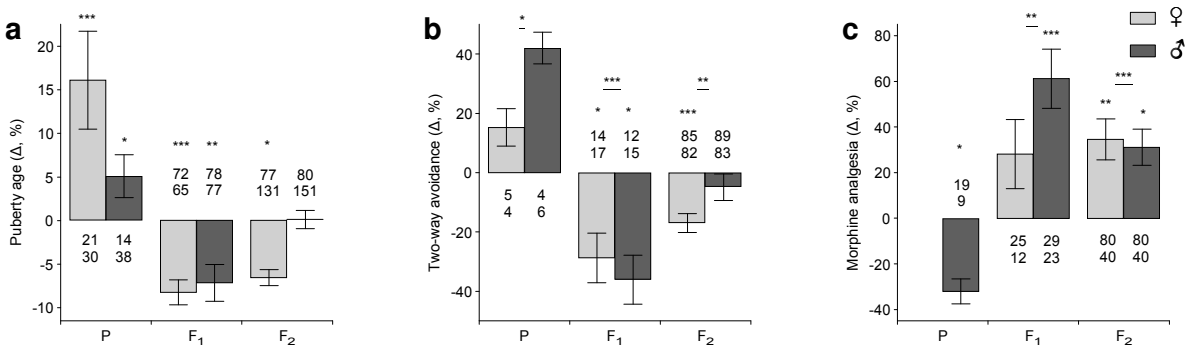

Figure 4 | Phenotypic inversion in the progeny after prenatal (E8-E14), neonatal (P0-P11) and young adult (P42-P79) parental drug treatment. (**a**) Onset of puberty in the experiment of Manikkam and co-authors (2012)[20]: Sprague-Dawley rats (P), both ♀ and ♂, were treated during E8-E14 by i.p. administration of plastic mixture to a pregnant female. (**b**) Thyroxine experiment, 2-way avoidance averaged correct responses of 5-day training, **Fig. 3**. (**c**) Morphine experiment, ratio of tail-withdrawal latency, measured 30 min after 10 mg/kg morphine injection, to baseline latency, **Figs. 1b** & **2a,c,e,g**. Each bar (Δ, %) represents the difference with respect to control (control = 100%). Underline – males and females together (for **b** & **c**). Mean ± SE.

5 consecutive training days (**Fig. 3**). Neonatal (P0-P11) L-thyroxine treatment of males and females leads to improved performance in these animals (**Fig. 3a,e**), slightly more pronounced in males (probably due to better canalization of ontogenesis in females, as usual). In the next generation (F_1), obtained from thyroxine-treated males and drug-naïve females (**Supplementary Fig. 2b**), the phenotypic inversion in the form of decreased performance is equally expressed in males and females (**Fig. 3b,f**). Thus, transgenerational epigenetic compensation can be equally expressed in the F_1 males and females. Note, however, that morphological traits, which typically do not show phenotypic inversion, but show Lamarckian inheritance, can be more deeply changed in F_1 females, than in F_1 males (Fig. 2c[13]).

In the F_2 generation, obtained by both incross (F_1♀ × F_1♂) and outcross of F_1 males (new♀ × F_1♂), the decreased performance in two-way avoidance task was observed exclusively in females (**Fig. 3c**, **Supplementary Fig. 3a-b**). Thus, in the F_2 generation, the transgenerational epigenetic compensation is expressed in females, but not in males.

In the F_3 generation all effects are absent in females, but in the F_3 males, those were obtained after outcross breeding (new♀ × F_1♂, F_2♀ × F_2♂), the transgenerational epigenetic compensation was observed (**Supplementary Fig. 3h**). Thus, transgenerational epigenetic compensation can be transmitted from F_1 to F_2 through males, and, furthermore, in the F_3 generation it is expressed only after outcross breeding. This difference between incross and outcross was observed in our experiment in many traits, not only in the Shuttle-box, namely: birth weight, hippocampal mossy fiber morphology, electrophysiological response – auditory evoked potential in the frame of mismatch negativity paradigm (Table 1[13] and Supplementary Fig. 3b[13]). The decreased Shuttle-box performance in the F_3-outcross (F_1♀ × F_1♂, new♀ × F_2♂), but not in the F_3-incross (F_1♀ × F_1♂, F_2♀ × F_2♂), was reported previously in male, but not in female, descendants of cyclophosphamide-treated male rats (Fig. 12[29]). Thus, transgenerational epigenetic compensation is expressed in the F_3 males only after outcross breeding, and it is absent in all F_3 females.

In the experiment with prenatal (E8-E14) plastic mixture treatment, conducted by Mohan Manikkam and co-authors (2012)[20], this treatment has led to delayed onset of puberty in prenatally-treated male and female rats (Fig. 4a[20]). The effect was more pronounced in females, but the sex ratio was significantly disturbed in this generation and some males probably were not born or were not born alive (Fig. S1[20]). In the next generation (F_1) the accelerated onset of puberty was observed in both males and females, but in the following generation (F_2) the accelerated onset of puberty was evident only in females (**Fig. 4a**). In the experiment of Michael Skinner and co-authors[30] with prenatal (E8-E14) vinclozolin treatment, the F_2 generation females had 1301 genes with changed expression in hippocampus (at P450) *vs.* 92 genes in males (at P360).

Fig. 4 shows that prenatal (E8-E14), neonatal (P0-P11) and adolescent (P42-P79) paternal treatments lead to the same pattern of transgenerational epigenetic inheritance: F_1 effects are equal in males and females or they are more pronounced in males, but all F_2 effects are present mainly in females.

Discussion

The F_1 and F_2 results are obtained in several independent studies with very different protocols of drug administration and animal testing and, thus, they look reliable. We can not say the same about the F_3 results, due to the lack of data. The F_4 and further results are not available at all now.

However the available data allow us to describe the following *modi operandi* of micro- and macroevolution.

The main modus of microevolution

1. In a stable random bred population, without any unusual external influence, typical quantitative trait is distributed normally among males and females, with higher variability among males (**Fig. 5a**).

2. After application of a strong environmental pressure, functionally linked with above-mentioned quantitative trait, the transgenerational epigenetic compensation will shift the mean value of female phenotype towards better adaptation (**Fig. 5b**).

3. Further increase of environmental pressure (**Fig. 5c**) will increase above-mentioned sexual dimorphism so that all females will be out of the zone of discomfort, but natural selection will be working among males; and through natural selection of males the genetic assimilation[31] of a given acquired trait will be achieved in this population.

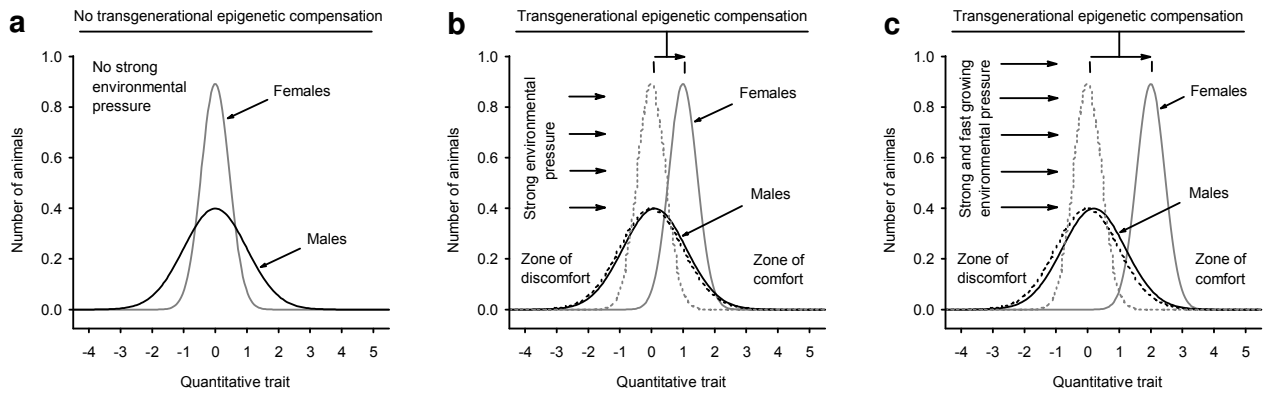

Figure 5 | Distribution of a quantitative trait among females and males in population. Original distribution in males is standard normal distribution. Chosen quantitative trait is functionally linked with given environmental influence (*e.g.*, cold-resistance – low temperatures). Without specific external pressure (**a**) the variability in males is wider than in females (the ontogenesis of females is better canalized). After application of a new environmental pressure (**b**), at least during 2-3 generations, the transgenerational epigenetic compensation will shift female distribution towards comfort zone, whereas males will remain in the zone of discomfort. The transgenerational epigenetic compensation is mainly dormant in males, it is not detectable in male phenotype, and therefore it is not helpful for their survival. If given environmental pressure will be increased further (**c**), males will proceed to develop transgenerational epigenetic compensation for females to remove them from the discomfort zone. But the number of males, suitable for breeding, will be decreased. The natural selection among males will lead to genetic assimilation of transgenerational epigenetic compensation in population.

The main modus of macroevolution

1. The appearance of a new mutation in population, its presence in some individuals (the presence of mutation is necessary for further events, even if the phenotypic results of this mutation are purely behavioural, because the biologically important behaviour is very well canalized also).

2. The application to the population of a strong environmental pressure will lead to development of transgenerational epigenetic compensation in homozygous mutants only, but not in heterozygous and wild-type animals; the loci of epigenetic compensation and mutation are usually independent.

3. In the further generations (starting from F_2), the transgenerational epigenetic compensation will be expressed mainly in females and with the following interaction with genotype: it will increase fitness of homozygous mutants; it will have no effect on fitness of heterozygous animals; it will decrease fitness of wild-types. (This was observed in the *Per2*^{Brdm1} mutant mice; Fig. 3b[14]).

4. There is a point of bifurcation here:

a) The result of transgenerational epigenetic compensation can be the accelerated replacement of wild-type allele in population by mutant one – no speciation in this case; the process starts with low selection coefficient, then the selection coefficient is increased by transgenerational epigenetic compensation, and, finally, it is low again when previous wild-type allele is completely replaced and transgenerational epigenetic compensation is significantly attenuated; this process can be helpful for genetic assimilation – for fast genetic fixation of a weak-effect mutation in population;

b) Transgenerational epigenetic compensation can lead to non-random breeding in population, namely: "wild-type ♀ × wild-type ♂" and "homozygous mutant ♀ × homozygous mutant ♂", because such breeding schema is beneficial for all animals in this population; the population will be self-separated into two independent populations: new mutant population and old wild-type population (Supplementary Fig. 1[14]). Remark: Due to the sexual dimorphism in expression of transgenerational epigenetic compensation, the beneficial phenotype will be expressed in homozygous mutant females, but not in homozygous mutant males, however, nevertheless, these females will choose homozygous mutant males (with transgenerational epigenetic compensation) as potential mates (similar result was obtained with rats and vinclozolin; Fig. 3*B*[23]).

5. After the appearance of two species (new and old), in the new species the transgenerational epigenetic compensation will be slowly replaced by weak-effect mutations through genetic assimilation; and during genetic assimilation the multiple episodes similar to the described one in the **4a** will take place.

6. After the completion of genetic assimilation there will be two species. They will avoid breeding with each other under normal conditions. However their hybrids (F_2 and further generations) will not have lack of viability, because transgenerational epigenetic compensation will be absent in both populations.

Further details can be found in the **Supplementary Information** and in our previous publication "Transgenerational epigenetic compensation in evolution"[14].

Methods

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). See **Supplementary Fig. 2a**.

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.

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. See **Supplementary Fig. 2b**.

P, F_1, F_2 and F_3 mice were tested in two-way avoidance task ("Mouse Shuttle Box", Campden Instruments Ltd., UK)[28] 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.

Mann-Whitney U test was used as a basic method for data analysis.

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Supplementary Information for

Transgenerational epigenetic compensation and sexual dimorphism

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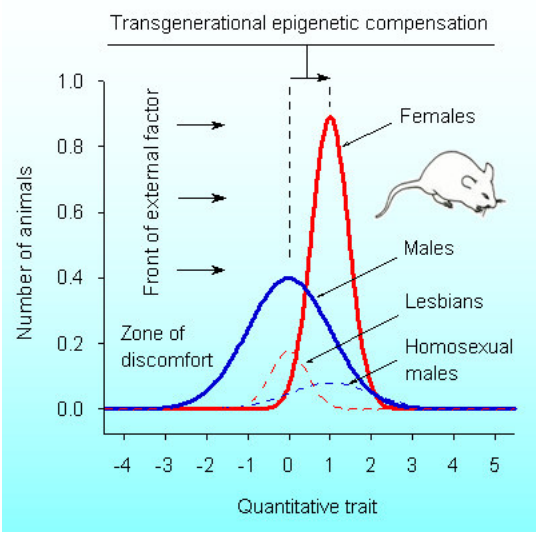
In natural population transgenerational epigenetic compensation is generated in homozygous mutants, mainly in males. It does not immediately affect the phenotype of homozygous mutants, where it was generated. Transgenerational epigenetic compensation is localized not in the same locus as original mutation and it is dominant, because it affects the phenotype even if it is received from one parent. In further generations, starting from F_2, it is more expressed in females than in males: it increases fitness of homozygous mutant females, decreases fitness of wild-type females and has weak effect on heterozygous females. If the population remains a random breeding one, the transgenerational epigenetic compensation increases the selection coefficient of mutant allele and accelerates the replacement of wild-type allele by mutant one, but the total loss of animals in population can be high. If homozygous mutant females can choose homozygous mutant males as potential mates and wild-type females can choose wild-type males as potential mates, then the population will not be panmictic anymore and it will be divided into mutant and wild-type subpopulations. The most of animals will survive and a new variety on the basis of homozygous mutants will be formed.

In 1965-1966, when the role of sexual dimorphism in evolution was first described by Vigen A. Geodakian, he considered two theoretical possibilities concerning differences between males and females: "There are two main possibilities for drawing the females' curve. The latter can either shift to the right (Fig. 9.1[17]), or it can have a smaller dispersion than the males' curve (Fig. 9.2[17])" (**Supplementary Fig. 1**). At the intuitive level Geodakian had a feeling that both possibilities could be good for evolution: "If we include all the males in the population in one team and include all the females in the other and arrange competitions between the two teams, then the champions in all personal competitions will be the males, whereas in a whole-team competition (where the results of all participants count) the females will be the winners" (p. 8[17]).

The greater variability in males could be easily achieved with a help of hormonal mechanisms, whereas the shift of the distribution of the females to the right could be explained only by unknown mechanisms, the mechanisms those were difficult to imagine, and this possibility was abandoned for over 47 years.

The higher variability in males can be a result of a not so good canalization of their ontogenesis, and the canalization of ontogenesis is modulated by sex hormones. Thus, we can imagine that the canalization of ontogenesis in males is artificially decreased in comparison with females by means of hormonal differences.

The shift of the female distribution to the right was not possible to explain from the positions of classic genetics. However this shift is a direct result of the transgenerational epigenetic compensation, more deeply pronounced in females.



Supplementary Fig. 1 | Transgenerational epigenetic compensation in microevolution. The hereditary basis of transgenerational compensation develops mainly in males. Epigenetic changes are transmitted to the next generation through both males and females. Above-mentioned epigenetic changes have more deep impact upon female phenotype.

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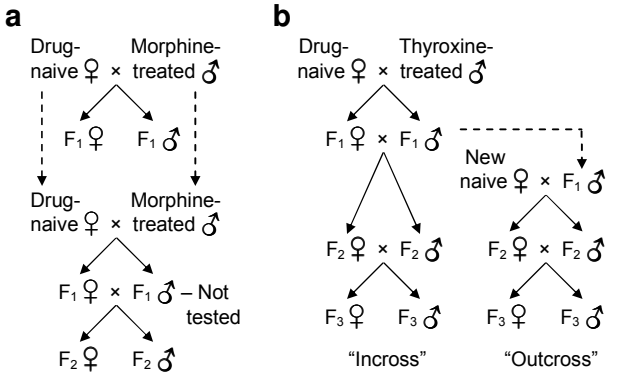


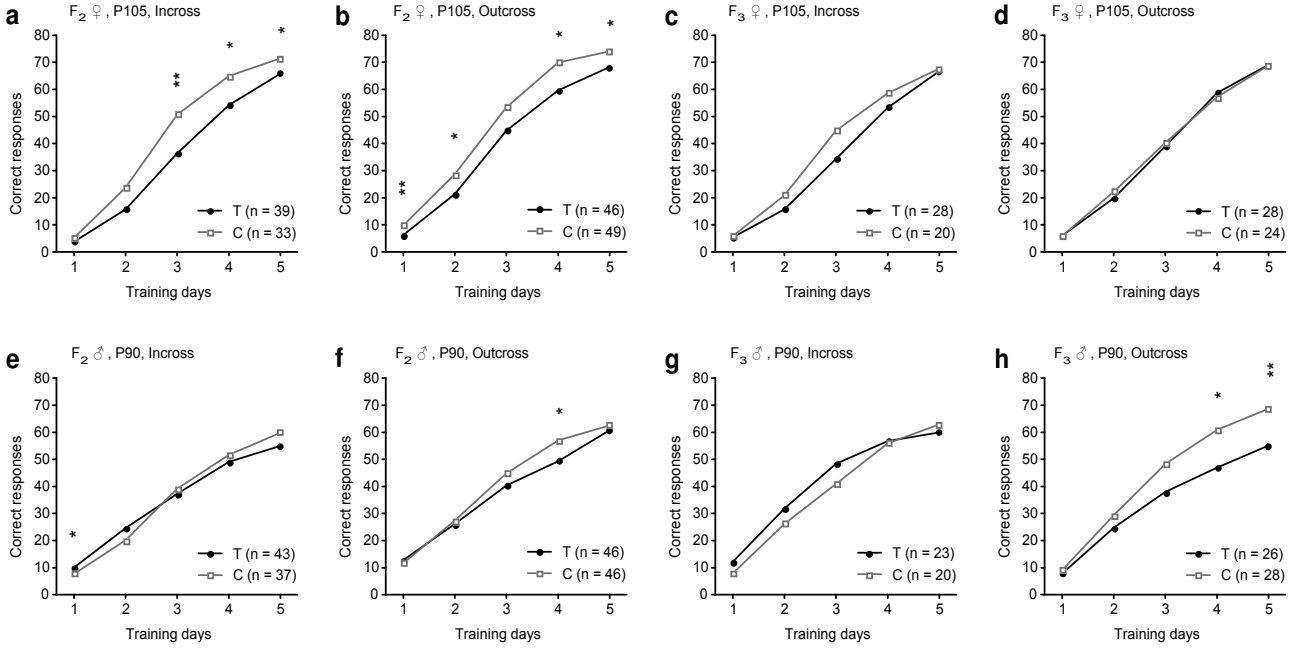
Supplementary Fig. 2 | Breeding paradigms. (**a**) Wistar rats, morphine study. (**b**) DBA/2J mice, thyroxine study. Solid arrows indicate the appearance of progeny, dashed arrows – transition of the same animals.

In some individuals, probably due to some stochastic developmental deviations, transgenerational epigenetic compensation can be unmasked in males and dormant in females. These individuals have increased probability to be homosexual males and lesbian females, respectively.

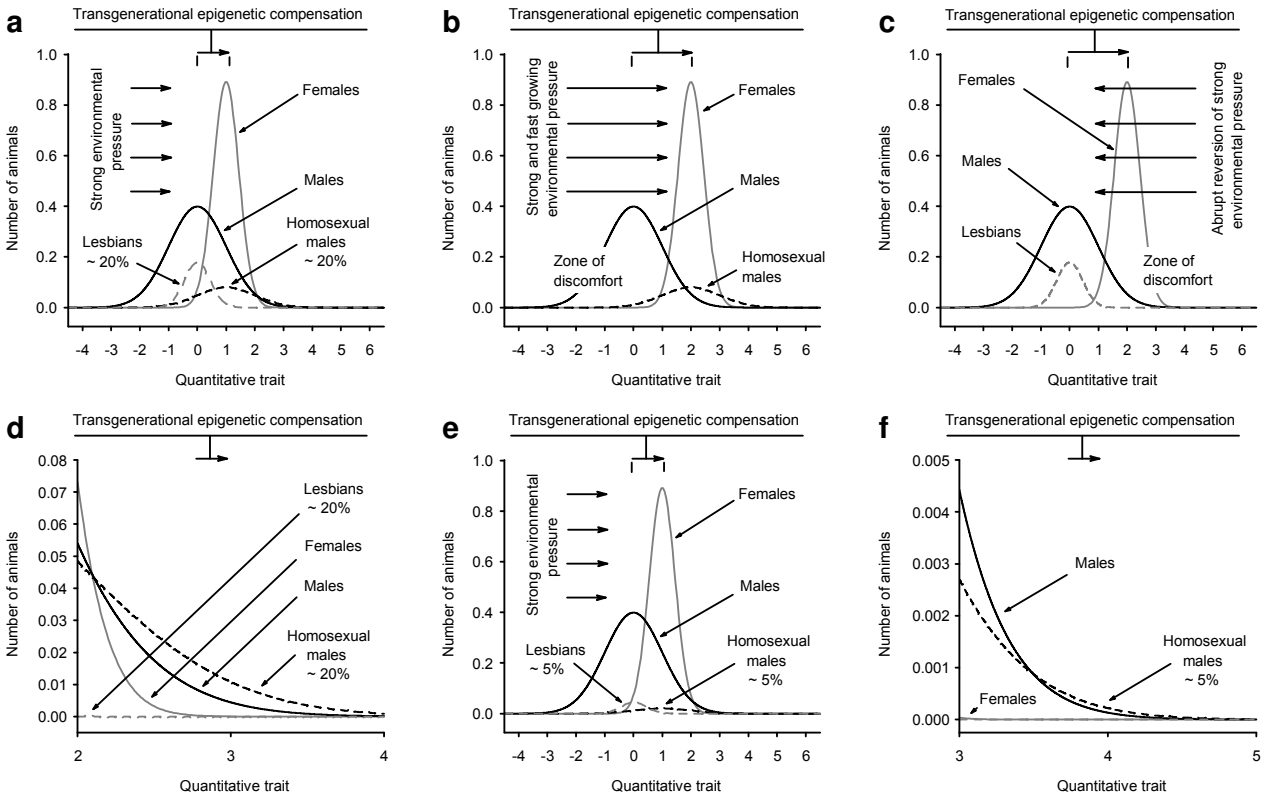
The term "canalization" was not used by Geodakian in 1966[17]; this term was introduced by Conrad H. Waddington in 1942[31] or may be even earlier. Geodakian in 1965[16] was using terminology, developed by Soviet biologist Ivan I. Schmalhausen, namely "stabilizing selection" (p. 257[32]). The terminology of Schmalhausen is unacceptable due to 2 reasons: 1) Schmalhausen was trying to be as different from Trofim D. Lysenko as possible, and it is not a good motivation is science (Lysenko was looking at biological phenomena, first, from the physiological position and, second, from the position of genetics; Schmalhausen with his "stabilizing selection" was using "correct", reversed order); 2) Waddington was using the term "canalization of ontogenesis" as a description of a final result of a given process and as a description of a set of mechanisms that leads to this final result. The "stabilizing selection" is only one part of the one of such mechanisms, because the natural selection in evolution is only one part of evolutionary process, and sometimes it is not the most important part.

The transgenerational epigenetic compensation, being more phenotypically expressed in females than in males, brilliantly explains the sexual dimorphism in a lifespan in the experiment with mutant *Per2*^{Brdm1} mice under semi-natural outdoor conditions, conducted by Serge Daan and co-authors (2011)[15].

In our experiment with thyroxine we use the term "outcross" to describe breeding with new drug-naïve animals (**Supplementary Fig. 2**). In the classic genetics the cross with an original stock, in our case with wild-type untreated animals, can be described as a "backcross". However, the backcross in a transgenerational epigenetic experiment as well can be seen as a cross of F_1 animals to their drug-treated parents (generation P or F_0). To avoid this confusion, we do not use the term "backcross", but any cross with new untreated animals is described by us as an "outcross". The term "incross" does not have similar problems – it is always a cross between animals of the same group (experimental or control).



Supplementary Fig. 3 | Two-way avoidance in the Incross and Outcross subgroups. F_1 males, obtained from thyroxine-treated males and drug-naive females, were bred with F_1 females and with new drug-naive females to produce F_2 Incross and Outcross, respectively. F_2 Incross and Outcross were bred as independent lines to produce F_3 (see **Supplementary Fig. 2b**). In the F_2 generation we can see identical changes in the Incross and Outcross, but only in females (**a-b**). In the F_3-outcross, but not in the F_3-incross, the difference has appeared in males during the last 2 training days (**h**). Similar shapes of learning curves of experimental F_3-incross and F_3-outcross, together with their relative vertical shift (**g-h**), indicate that both Incross and Outcross males have some impairment of performance during the last 2 days, but this impairment is compensated somehow in the Incross during the whole 5-day training period. T – experimental group, C – control one. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$. Mann-Whitney U test. Mean.



Supplementary Fig. 4 | Transgenerational epigenetic compensation and homosexuality. Distribution of normal males is shown as standard normal distribution. Females have always smaller variability due to better canalization of their ontogenesis. For illustrative purposes the percent of homosexual males is shown as 20% in the (**a-d**), taken the total number of males in population as 100%; the same for females. In the (**e-f**) the percent is more realistic and it is shown as 5% for both males and females.

The difference between Incross and Outcross was absent in the F_2 generation (**Supplementary Fig. 3a-b,e-f**), but the sexual dimorphism was present here. Both Incross and Outcross F_2 females have significant changes (**Supplementary Fig. 3a-b**), but both Incross and Outcross F_2 males were phenotypically normal (**Supplementary Fig. 3e-f**). The reappearance of phenotypic changes in males of the F_3-incross, obtained from the F_2-outcross, was absolutely unexpected (**Supplementary Fig. 3h**). Similar phenotypic changes were observed in several independent traits, only in males (all F_3 females were phenotypically normal). Previously, in 1990, similar differences between Incross and Outcross were observed in the F_3 male progeny, obtained from cyclophosphamide-treated male rats[29]. But they were appearing so confusing that further analysis was completely blocked due to the lack of additional data. Now we know that transgenerational epigenetic compensation can comprise complex and gender-dependent pattern of phenotypic changes, in a raw of generations. We do not know all underlying mechanisms, but we can see that such pattern is very helpful for evolution, it increases its efficiency. The phenotypic expression of transgenerational epigenetic compensation in the F_3 males after outcross breeding can increase the selection coefficient for mutant allele in a random breeding population, if this transgenerational epigenetic compensation was developed in the homozygous mutants, but afterwards was found in the wild-types as a factor, decreasing their fitness (for example, decreasing their lifespan), in a gender-dependent manner. The increase of selection coefficient of mutant allele will result in intensification of natural selection in favour of mutant allele. This will lead to faster replacement of a wild-type allele in population by mutant one.

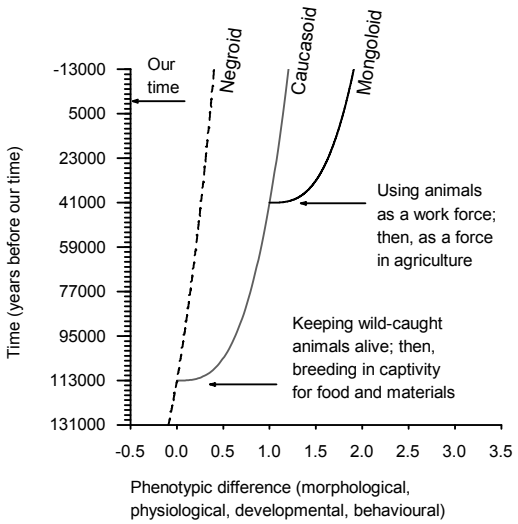
The role of transgenerational epigenetic compensation in homosexuality is shown in the **Supplementary Fig. 4**. For human population all recent changes in environmental pressure are linked with evolution of civilization. These changes are extremely fast in view of evolutionary scale (look at the previous 500 years). Transgenerational epigenetic compensation is good for females, whereas natural selection is working among males (**a**). But what we will have if environmental pressure will proceed further in the same direction, but with increased speed? Look at (**b**). Females will be saved, but the most of males will be not good for breeding. Males have higher variability and it can be a few individuals will be available, but it can be not enough for survival of population. The population will be saved, if it will be a few males with active transgenerational epigenetic compensation. Under normal conditions these males have a tendency to demonstrate homosexual behaviour. But the border between exclusive homosexuals and facultative homosexuals is not very solid even under normal conditions. Homosexual males have high variability, typical for all males, and perfect transgenerational epigenetic compensation, typical for females. This combination automatically gives the following result:

among the most talented individuals the number of homosexual males is fantastically high. The situation is not the same for lesbian females, because they are reserved in the population for other purposes (**c**). If the environmental pressure is abruptly reversed, the most of females with previously good transgenerational epigenetic compensation will not be good for breeding. And lesbian females, those have small variability, typical for all females, together with practical absence of transgenerational epigenetic compensation, typical for males, will save population from the extinction. Someone can say that these females would not like to breed. But look at the distribution in (**c**). What will be done with these females can be expressed in very simple words… *De facto*, they will not have a choice.

Supplementary Fig. 4a-d was prepared using an assumption that the number of homosexual males in population is about 20% as well as the number of lesbian females. But this percent is too high, it is far from reality. We know that the percent of homosexual males is about 5% from all males and the percent of lesbian females from all females is even less. In all cases we take distribution of normal males as standard normal distribution for particular quantitative trait. In the **Supplementary Fig. 4e-f** we show situation with 5% of homosexual males and 5% of lesbian females (very realistic situation). We see that despite total number of homosexual males in comparison with all males is only 5%, among the most talented males the probability to be a homosexual is about 50% (one half of the most famous artists can be homosexual, but it is true only for males). We assume here that the environmental pressure is linked with progressive evolution of human civilization. The most civilized humans can be homosexual males. But see again: if we take the most creative individuals as 100%, 50% of them will be normal males and 50% - homosexual ones (**Supplementary Fig. 4f**). Females are absolutely absent here (among the most creative individuals). Concerning homosexual males it is important to note that not only the right part of distribution is represented by so famous figures as Tchaikovsky, Leonardo da Vinci, Michelangelo and Plato, but the middle part of distribution is shifted to the right also: among male individuals with high education the percent of homosexuals is twice as high as percent of homosexual males among the whole male population (p. 64[18]). Here we see how homosexual males and lesbian females can play very important, but very different, roles in evolution.

The main modus of macroevolution and the origin of human races are shown in the **Supplementary Fig. 5**. A macroevolutionary event, – the appearance of a new species or race, can be dependent on many different external and internal factors. But there is a succession of events that is absolutely necessary for the final positive result – a new species. We call this succession of events "the main modus of macroevolution". The presence of profound sexual dimorphism and even the existence of two sexes are not absolutely necessary for the main modus of macroevolution, but they can help a lot, if present.

1. For the beginning of a macroevolutionary episode the population should have a subgroup of animals with some mutation in heterozygous and homozygous state. This mutation should not be beneficial in any dimension, but it should be compatible with survival of homozygous mutants. Then, some environmental pressure, may be new, may be unusual, should be applied to this population, to the population consisted of wild-types, heterozygous mutants and homozygous mutants. Sometimes people assume that this environmental pressure should be "good for mutants" in some respect. It is not a realistic presupposition and it is wrong. Environmental pressure will be bad for all animals of this population and it will be especially bad for homozygous mutants. Under this environmental pressure the average lifespan of homozygous mutants can be several times shorter than the lifespan of wild-types.



Supplementary Fig. 5 | The main modus of macroevolution and the origin of human races. Shown temporal scale is based on DNA analysis and it is taken from the publication of Masatoshi Nei (1985)[43]. The accuracy is 113000 ± 34000 and 41000 ± 15000 years for the moments of appearance of Caucasoid and Mongoloid races, respectively. The appearance of each race was a very fast process, as it is shown in this picture.

2. However there is an interesting moment here. The ontogenesis of wild-types is usually very well canalized. The ontogenesis of heterozygous animals is not deeply disturbed also. And only ontogenesis of homozygous mutants, especially under this environmental pressure, is significantly destabilized. The transgenerational epigenetic compensation begins to work in homozygous mutants to improve their phenotype and it takes into account two factors: particular environmental pressure and special features of particular mutation.

3. Transgenerational epigenetic compensation is distributed in several independent loci and these loci are typically very far from the mutant locus. Now the situation depends on how the population or this local subgroup of animals will be "infested" by this transgenerational epigenetic compensation. This transgenerational epigenetic compensation will increase lifespan of homozygous mutants and it will decrease lifespan of wild-types, because it is optimized for mutants, but not for wild-types. If local concentration of mutants, including both heterozygous and homozygous, is relatively high or at least equal to concentration of wild-types, the process of selective breeding can be started.

4. The process of selective breeding can rely on any mechanism, which can be specific for particular species level of organization, but selective breeding is always beneficial for all participants of this situation. Wild-types will prefer to breed with

wild-types only, to avoid infestation by transgenerational epigenetic compensation, very destructive for wild-types. And homozygous mutants would like to be bred with homozygous mutants in order to acquire and keep transgenerational epigenetic compensation, very useful for them, very positive for their lifespan and survival. Homozygous mutants and wild-types would like to be separated as separate species in order to increase survival of both of them. These 4 modules constitute the main modus of macroevolution.

Afterwards transgenerational epigenetic compensation will be slowly replaced by mutations (mainly weak-effect mutations) though genetic assimilation. Mutations, useful for genetic assimilation, can be distributed between very different genes and they can be found not only in the regulatory sites, but in the coding sites also. It means that, despite transgenerational epigenetic compensation is purely "regulatory" mechanism, its genetic assimilation can be not so exclusively "regulatory". After many-many generations the morphological phenotype of a new species can be supported by newly acquired mutations in both regulatory and coding sites. From this point of view, the final genetic result of morphological evolution should be even more "classic" (more close to the original statements of classic genetics) than the one described by Rudolf Raff & Thomas Kaufman in the book "Embryos, Genes, and Evolution" (1983)[33] (morphological evolution through modification of regulatory sites and regulatory subsystems). Our viewpoint is also supported by the article of David Stern and Virginie Orgogozo "The loci of evolution…" (2008)[34].

After genetic assimilation of transgenerational epigenetic compensation previously detected sexual dimorphism in phenotypic expression of transgenerational epigenetic compensation will not be observed anymore. Instead, the phenotypic result of genetic assimilation will be equally expressed in males and females.

Transagenerational epigenetic compensation can intensify natural selection. It happens during the whole period of genetic assimilation and during each step of genetic assimilation, *i.e.* during each fixation of a new weak-effect mutation in population. Above-mentioned mutation is typically thought to be a single-nucleotide substitution in a *cis*-regulatory site, like discussed in the article of Nicolas Frankel and co-authors "Morphological evolution caused by many subtle-effect substitutions in regulatory DNA" (2011)[35]. The intensification of natural selection by transgenerational epigenetic compensation is an important feature of genetic assimilation and it was not discussed in our previous articles "Transgenerational epigenetic compensation" and "Transgenerational epigenetic compensation in evolution", and it is not a self-evident consequence of general mechanisms of transgenerational epigenetic compensation.

How weak-effect mutations, if their final effects are really so weak, can be efficiently selected in evolution? David Stern & Virginie Orgogozo (2008)[34] have mentioned that "There is a fundamental disconnect between population genomics approaches to studying adaptations and genetic studies of "obviously" adaptive traits, especially of polymorphisms maintained in populations. Population genomics approaches generate estimates of very small selection coefficients, on the order of 10^{-5} for most adaptive fixations in Drosophila (Andolfatto, 2007)[36]. In contrast, when measured, selection in the wild is often about four orders of magnitude greater (Hoekstra *et al*., 2001[37]; Kingsolver *et al*., 2001[38])" (p. 2176[34]). Let's imagine that some mutation with slight positive effect has appeared in a reasonably sized population. Let's suppose that it has at least slight physiological effect in homozygous mutants. In these homozygous mutants this mutation inevitably elicits transgenerational epigenetic compensation – heritable epigenetic changes, distributed between several independent loci. These loci are independent of each other and of the locus of mutation. In the next several generations (minimum 2-3 generations are necessary) we will have at least 2 distributions, those will not be absolutely independent, but, on the other hand, will be clearly distinctive: 1) distribution of mutation; 2) distribution of transgenerational epigenetic compensation, developed for this mutation. Above-mentioned transgenerational epigenetic compensation will increase positive effects of mutation in homozygous mutants and it will induce negative effects in homozygous wild-types.

The development of a transgenerational epigenetic compensation is an extremely fast process and it will lead to the following: the selection coefficient for a given mutation will not be a constant anymore (in any sense and in any evolutionary reasonable timescale). The selection coefficient, initially low, will jump up 10-fold or more due to transgenerational epigenetic compensation. Afterwards it will be low again, when all transgenerational epigenetic compensation will disappear. In the experiment of Serge Daan and co-authors (2011)[15] the selection coefficient between Year 1 and Year 2 has undergone a 9-fold change. We can speculate, without any material basis up to now, that the described above difference "about four orders of magnitude" can be completely covered by transgenerational epigenetic compensation. When a mutation just has appeared in the population, its effect is rather weak, selection is weak, and its selection coefficient is low. As soon as its transgenerational epigenetic compensation is developed and somehow distributed in the population, the fitness of homozygous mutants goes up, the positive effect of mutation is increased, but the fitness of homozygous wild-types goes down, their fitness is impaired. Therefore, the natural selection with respect to this mutation becomes strong, and the selection coefficient of a given mutation becomes really high. When this mutation will completely replace wild-type allele in population, and probably many years will pass away, the transgenerational epigenetic compensation will not be present anymore, the selection will be weak again, and the selection coefficient of a given mutation will be again low.

The described above mechanism provides unbelievable possibilities for fast selection of mutations with weak positive effects, selection either in the frame of genetic assimilation or independently. The selection coefficient goes "low – high – low" and it is high only during the episode of fixation of particular mutation in a given population. If someone would like to invent an efficient mechanism for similar purpose, exactly this mechanism will be deployed. This mechanism does not make the life of a species "easy" during particular episode, but it makes the replacement of a wild-type allele by a mutant allele fast. And particular mutant allele has positive effect on fitness and survival in a long term run.

Any mutation, even with a weak effect, including discussed above, usually have several pleiotropic effects, some of them are good, the other ones – bad, and the description "weak positive effect" means in reality that positive effects are in average

stronger than negative ones. Negative effects as a rule can be eliminated or diminished by transgenerational epigenetic compensation. We remember that the epigenetic compensation is transgenerational, and the population will be contaminated by these epigenetic changes, because they will not be physically linked with a given mutation, they will be distributed in independent loci, as a rule. Heritable epigenetic changes will be good for homozygous mutants and bad for homozygous wild-types.

Most of our knowledge about transgenerational epigenetic compensation came from the experiments with parental drug treatment (paternal, maternal, or both; morphine, thyroxine, vinclozolin). There is only one experiment with real mutation ($Per2^{Brdm1}$) in semi-natural environment and for interpretation of this experiment we use our data, obtained with paternal drug treatments. In the experiment with semi-natural environment all transgenerational epigenetic effects were more pronounced in females (both increase of lifespan in mutants and decrease of lifespan in wild-types were registered starting from F_2-F_3 generation). In the experiments with parental drug treatment (both paternal and, independently, maternal) in the F_1 generation the transgenerational effects can be equal in males and females, or more pronounced in males, or more pronounced in females (this is not only treatment-dependent, but trait-dependent also). For example, paternal morphine treatment during P42-P79 and maternal morphine treatment during P30-P40 or P30-P50 produce very similar and very impressive phenotype in F_1 males, but not in F_1 females. However in the F_2 untreated generation in the vast majority of studies all effects are more pronounced in females. It sounds fantastic, but it looks like transgenerational effects should be briefly checked in males in F_1, and only afterwards they are detectable in females in F_2. Even more fantastic is the fact that in the F_3 generation the effect appears once again only in males, but only after outcross breeding ("outcross" means in this case breeding with animals without transgenerational epigenetic compensation), this outcross breeding can be realized at the F_2 or F_3 level. If there is no outcross breeding, the effects are absent in both F_3 males and females.

It is obvious that for long-term perspective the transgenerational epigenetic compensation should be optimized for females, because the number of females, suitable for breeding, determines the quantity of descendants. However for genetic assimilation of any adaptation it would be better for population to work with males, just because several males can mate with a lot of females. For the existence of above-mentioned intensification of natural selection by transgenerational epigenetic compensation, this transgenertational epigenetic compensation should be expressed in male phenotype. And it is expressed, but only in F_1 males AND in F_3 males after outcross breeding. In the F_2 the epigenetic compensation appears in females, but not in males. F_1 and F_2 generations are officially present in the experiments with paternal drug treatment, whereas in the experiment with mutation there are direct descendants of homozygous mutants with different previous breeding history, descendants of heterozygous mutants with different previous breeding history and descendants of wild-types with also different previous breeding history. Previous breeding history is important for expression of transgenerational epigenetic compensation. Homozygous animal, obtained from mutant male and heterozygous female, will not be the same as homozygous animal, obtained from heterozygous parents; and parental history is important also.

If we suppose that the transgenerational epigenetic compensation develops in homozygous mutants, it can be found in their homozygous mutant descendants in F_1, F_2 and F_3 and in their wild-type descendants in F_2 and F_3-outcross only (we suppose that this mutant animal was bred with heterozygous and wild-type animals). Thus, F_1 homozygous males can have improved phenotype, whereas F_1 homozygous females have original phenotype; afterwards, F_2 homozygous males can have original phenotype, if they were obtained from heterozygous animals, and they will have improved phenotype, if one of the parents was homozygous mutant, F_2 homozygous females will have improved phenotype in any above-mentioned case and F_2 wild-type females will have impaired phenotype in any above-mentioned case, whereas F_2 wild-type males will have normal phenotype. F_3 wild-type males, descendants of homozygous male, will always have impaired phenotype, because in order to be a wild-type, they should be obtain through outcross – from heterozygous parents or from one heterozygous and one wild-type parent. It is difficult to believe that the main purpose of F_3-outcross effects is to suppress fitness of wild-type males in order to increase the relative survival of male mutants, to facilitate natural selection in the direction of faster replacement of wild-type allele with mutant one. However, if someone would like to "intelligently design" the process, the final process would be as described.

We will not consider phenotype of heterozygous animals, because for quantitative traits it will be intermediate (independently of epigenetic compensation) with reasonably high probability.

If there is an opportunity for homozygous mutants to move somewhere in a geographic dimension, it can be helpful for separation of two species. It is a helpful condition, but it is not the main driving force of macroevolution. The more helpful factor can be a sexual dimorphism in phenotypic expression of transgenerational epigenetic compensation. If transgenerational epigenetic compensation will be more phenotypically expressed in females than in males, it will help to keep higher quantity of females (homozygous), suitable for breeding, whereas homozygous males will be working for further improvement of transgenerational epigenetic compensation. This distribution of functions will accelerate the separation of two species and it will result in faster improvement of the phenotype of homozygous mutants.

Thus, sexual dimorphism in phenotypic expression of transgenerational epigenetic compensation is very important for the main modus of macroevolution, but it is not its necessary prerequisite. In the same fashion the same sexual dimorphism in phenotypic expression of transgenerational epigenetic compensation is very helpful for the main modus of microevolution (for progressive evolution of one given species). The same mechanism serves as universal tool for both micro- and macroevolution.

Concerning the origin of human races, first of all we have to start from terminology. There are several classifications of human races and not all of them are scientific. The most famous (or infamous?) is Apartheid classification, where races are represented as White, Black and Coloured. The last one is a

mixed category, which includes Mongoloids and people from India and Pakistan and different hybrids, obtained from White and Black. In order to discriminate Coloured from Black so-called "pencil test" was used (if a pencil can be held by the hair – the person is Black, otherwise – Coloured; this test can not be used to distinguish Whites from Coloured, obviously). Of course, Apartheid classification is not a scientific one, but, unfortunately, many other known social classifications are really not very far from this infamous Apartheid classification. "White", "Black" and "Other" or "White", "Afro-American" and "Asian" do not provide more precise description.

To avoid any mixture with any social or sociological classification, we will use exclusively biological classification of human races, where the most famous 3 human races are called "Negroid", "Caucasoid" and "Mongoloid". Simultaneously, we do not care whether it can cover all real human populations, whether it is useful for their "classification", whether it can or cannot "adequately describe human variation", because the presence of hybrids with unknown or poorly determined history is not a question of our interest; only the first generation hybrids (F_1) can be used for biological analysis; other hybrids occur to be useless, they are good for nothing in the frame of biological research (however we remember that they can be important for sociologists, but sociologists prefer their own classification of human races anyway).

For biological classification the skin colour had always very limited importance. Historically, in biological science races were discriminated using morphological traits (and this approach is obviously correct). We are going to describe humans as biological objects and, of course, categories like "ethics" are not applicable here. We can leave these categories for social demagogues, outside of biological science. There is famous story that races are "equal" and have originated "simultaneously". This story belongs to the field of social propaganda. All biological data accumulated during previous century (and even earlier) demonstrate that these statements are absolutely wrong.

There is also a statement that human race "is not a biological reality"[39]. This statement comes from biologically illiterate people. In accordance with our current view the origin of Caucasoid and Mongoloid races (at the level of biological mechanism) is very close to the origin of a new species and both proceed through the appearance of hopeful monsters, but we can leave this new view aside, for some time, at least. Classic biological concept about racial chains (clines) and "rassenkreis" was developed before WW2 using non-human species, like *Lymantria*[40]. Our knowledge concerning human races, accumulated up to now, precisely coincides with our knowledge of races in other species.

An example, frequently quoted in European literature (p. 120[40]), is the case of *Parus major* (a titmouse). This little bird has formed an Eurasian rassenkreis, *major* (green-necked), *bokharensis* (gray), and *minor* (yellow-necked), as indicated in the map (Fig. 22[40]). *Major* spreads as a northern form from Europe across Siberia into northern East Asia. In the south *major* gradates into Persian *bokharensis*, and this, in turn, in India and southern China, into *minor*. The latter reaches north through China and Amur. Here it meets an eastern branch of the Siberian *major* and two end-points of the series are shown converging. These two subspecies live side by side in this region without hybridizing. We do not know what keeps them apart; but it might be a very small physiological or biological difference of the same order as individual micromutational differences, whether geographical or local. But this is not what real species differences consist of (pp. 120-121[40]).

People, rejecting human races, usually do not have any personal experience with any other species and typically are not informed about concepts of "race" and "racial chains" in biology, developed before WW2 by Richard Goldschmidt and others. Or may be someone would like to say that, for example, above-mentioned groups *major*, *bokharensis* and *minor* are also "not a biological reality"? Individuals, claiming that human races are not real, are either social demagogues or biologically illiterate people.

We do not claim that all races of animals and all distinctive human groups have originated through the main modus of macroevolution, through hopeful monsters. Most of them can be a result of microevolution, of adaptation to local conditions. We do not have sufficient information concerning the origin of Negroid race, although the hypothesis of C. Owen Lovejoy (1981)[41] looks attractive in several dimensions (and it is a macroevolutionary mechanism; the explanation is given below). However, concerning specifically the origin of Caucasoid race and the origin of Mongoloid race, we assume that both these events have happened through the main modus of macroevolution (not microevolution), each time through subgroup of mutant humans with a strong deviation in their phenotype, through so-called "hopeful monsters".

The main modus of macroevolution, described in our paper, insists that if one species splits into two new species, this split is never symmetrical: one of them is always "old species" and another one – really "new species", based on mutant individuals, those can be described as "hopeful monsters".

The old formula "two species have originated from their common ancestor" in reality is less precise than the phrase like "one species has originated from another species". For example, the phrase that "White bear has originated from Brown bear" provides more detailed and more correct description of reality than formula like "White and Brown bears have originated from their common ancestor". Of course, the Brown bear has evolved somehow after the appearance of a White bear, but the White bear has changed during the same time period significantly more. That is why in view of the White bear, the Brown bear has evolved just slightly.

We will use the term "ape" to describe very ancient human ancestors (it is not precise, of course, but slightly better than folkloristic "monkey"). Thus, Negroid race has originated from apes through several rather complex intermediate stages[41]. Molecular data show that man has diverged from chimpanzee about 6.5M years ago[43]. For simplicity we can say that Negroid race has originated from apes less than 6.5M years ago. Caucasoid race has originated from Negroid one approximately 113000 years ago[43]. Then, Mongoloid race has originated from Caucasoid one about 41000 years ago[43]. We can say that Negroids are intermediate stage in development between apes and Caucasoids. And we can say that Caucasoids are intermediate stage in development between Negroids and Mongoloids. Each time a new race has appeared through the main modus of macroevolution, through subpopulation of mutant individuals – hopeful monsters. At the moment of appearance of a new species, the new species was obviously more biologically

advanced than the old one. This is an absolutely necessary condition for the appearance of a new species. And we suppose that this rule is true for the appearance of Caucasoid and Mongoloid races.

The formation of a new species or race starts with a new mutation. Even if this process will proceed through new behaviour, in real life new mutation is necessary.

First of all, biologically important behaviour is very well canalized also. Small disturbance typically will not change biologically important behaviour, even if this change will be finally beneficial. We can see deep canalization of biologically important behaviour when, for example, someone suggests to volunteers to take part in the experiment, in which some unusual forms of sexual behaviour are really involved (homosexual behaviour, zoophilic behaviour, *etc*). The vast majority of volunteers will go out from the experiment as soon as this fact will be discovered. Of course, there are good reasons for this, despite these forms of behaviour are technically easy.

Second, we can imagine, at least in theory, that some new form of behaviour can be induced by very strong new environmental pressure. Biologically important forms of behaviour can be easily modulated by a mutation, because effects of mutation will exist during early ontogenesis and many of them will be corrected by means of very high early developmental plasticity. On the other hand, similar effects, if they will be induced by environmental pressure, will exist at relatively late stages of ontogenesis, for example, during adolescence, and plasticity during this period is incomparable with very high plasticity of prenatal and neonatal development. As a result, if the external pressure will be mediocre, a new behaviour will not be formed, but if the external pressure will be high, the species will be wiped out, and new behaviour still will not be formed. However if there is a mutation, it can change behaviour (and also morphology and physiology) significantly, and the animal will be still alive. It will be alive, because it will be saved by early ontogenetic events, early ontogenetic plasticity and early ontogenetic adaptation. Thus, in real life, new behaviour will be facilitated by new mutation.

We remember so-called "Baldwin effect": a new evolutionary episode starts from new behaviour. But new behaviour will be induced by a new mutation. It will not be induced by new environmental pressure.

Thus, for the emergence of a new species or race several factors are necessary: 1) the presence of mutation in some members of original population; 2) this mutation should facilitate some new behaviour; 3) this new behaviour should be beneficial, at least for a group of homozygous mutants, under given external conditions.

The origin of Negroids from apes, or "the origin of man" was brilliantly explained by the hypothesis of C. Owen Lovejoy (1981)[41]. This hypothesis put together bipedalism, sexual attractiveness of human females without sharp seasonal maximums and minimums and so-called "nuclear family" (female + male) in order to receive more efficient system, than it was present previously in apes. Briefly and a little bit simplistic: the main negative effect (cost factor) of previous system was high mortality rate of the infants, when a female was fed together with her infant in natural environment (young animal can fall down from its mother and can be injured, mother and infant can be attacked by a predator, *etc*). To avoid this, the following system was developed in humans: the food is collected by a male and it is brought to his female and infant (here bipedalism is important), the female is sexually attractive during whole year (to exchange "sex for food"). This system works only in the frame of "nuclear family", between given male and given female (the paternity is clear for everybody). Big brain is also helpful here: it can be useful for social behaviour and to invent tools which can help to bring food to a female by the most efficient way.

It is known that the size of human brain was increasing fast during some period of evolution and, then, this increase was completely stopped. There is a hypothesis that "biological evolution was replaced by cultural evolution". I assume that this hypothesis is wrong, because it uses atypical solution. Typically, one biological process or object is replaced by more efficient biological process or object. Big brain is useful, if it works in accordance with hypothesis of Gerald Edelman (1987)[44], through neuronal group selection from pre-existing repertoire (1993)[45]. Big brain is important to have ready pre-existing repertoire. But for adult animal, if new forms of behaviour should be formed, even more important to have some fresh part of pre-existing repertoire, newly available neuronal groups, available for further specialization. If the number of neuronal groups can not be increased anymore with the size of the brain, there is another possibility: in adult animal some neuronal groups should be destroyed and replaced by new neuronal groups through adult neurogenesis. The process should proceed through the following waves: first, during several days, some neuronal groups or even small brain regions should be destroyed by the immune system; then, during the next several days, new neuronal groups should be build up by the adult neurogenesis. Above-mentioned process can bring better result than "bigger brain". Obviously, for a long-living organism, the "fresh brain" (at least, in part) can be more beneficial than a "big brain".

The origin of Caucasoids from Negroids will be explained here. The key or clue can be a difference in behaviour of modern Negroids and Caucasoids with respect to animals. The difference in handling of domestic and laboratory animals by Negroids and Caucasoids is very demonstrative and it is easy to assess. The only negative side of these observations is that they can provide scientific support for racism. Racism itself is not a scientific theory, it is a social program (in accordance with it some race(s) should be prosecuted). We know that the results of classic genetics were widely and successfully used by German Nazi, but it does not mean that these results were false. The same situation is here. Common laboratory animals like mice, rats and Guinea pigs are good for this purpose. Adult Guinea pigs and 10-day-old ones (P10) are absolutely the best. Sometimes wild-caught insects of significant size (American cockroach and mantis) can be used also.

From the human side, young adults about 20-30 years old and kids in range 4-6 years old are the best groups for such observations. The origin of humans is important. Negroid group, if possible, should be consisted of people, who came directly from Africa (in our example, from Ghana). Caucasoid group can be easily formed from Jews (in Tarrytown and Upstate New York in general, at least). An independent Caucasoid group can be formed from Hispanic people (in our example, from Ecuador). For adult groups, the level of education is not important for real results, but in view of further discussion, the top-level education

of participants helps to avoid some standard speculations (in our example, all with high education, US).

Concerning both adults and kids there is expected sexual dimorphism. Males and females of both ages should be discussed separately. Briefly, adult Negroid males, contrary to Caucasoid ones, do not like to handle Guinea pigs and for them this idea sounds like an inappropriate proposition. Adult Negroid females, contrary to Caucasoid females, not only avoid Guinea pigs, but can sometimes demonstrate very specific behaviour, which will be described below. In laboratory practice this type of behaviour is often observed in rodents (in rats and mice) in behavioural tasks with strong negative reinforcement (*e.g.*, electric foot-shock, supplied through grid floor), especially in fear-conditioning paradigm. Fear-conditioning paradigm is a one-trial learning task, in which during training day an animal is placed in a new chamber for relatively short period about 2-3 min and during the last minute there is typically 20-30 sec presentation of a new rather load sound, and during the last 2-3 seconds of sound presentation the animal receives electric foot-shock through grid floor. It is usually removed from the chamber in 10-30 sec after foot-shock. The next day an animal can be placed into the same chamber for 5-6 min. Or, alternatively, in a new chamber it can be exposed to the previously applied loud sound during 3 min. In both cases during significant percent of time an animal shows reaction called "freezing". During "freezing" an animal does not produce any movements, except movements, necessary for breezing. It is an indication of fear and memory: the animal remembers that this environment and/or sound were dangerous[43]. Freezing reaction can be demonstrated by an adult Negroid female (20-30 years old) in the vicinity of a Guinea pig, handled by another person. Note that Guinea pigs are grass-eating laboratory animals. They are good swimmers (can swim very fast for their size and can be successfully trained in the Morris water maze task). Simultaneously, they are absolutely harmless. Guinea pigs never bite (ok, that's not absolutely true, once some Guinea pig has bitten one Chinese student (male), but it was during practice course in Zurich University, when this student was trying to do s.c. saline injection to this relatively rare, in comparison with mice and rats, laboratory animal).

This is not the first attempt to understand human behaviour using animal models. Fear conditioning in rats is an example of very well developed animal model. However what is different if we compare not human behaviour in general, but specifically behaviour of a Negroid with behaviour of a rat? This approach is not new also. With respect to morphology it was successfully used even in 18th century. Richard Goldschmidt has published the following example in 1940: "The mechanism of walking in man consists, among other things, of the level system of the foot with the heel-bone as the shorter arm. To this is attached the tendon of the gastrocnemius muscle which moves the level. Marey (1887)[47] has shown that in the white races the short arm of the level is relatively short. In connection with this the muscle is compact and powerful, showing a characteristic featherlike arrangement of the fibers, and the tendon is long. In black races, however, the short level arm of the heel-bone is much longer, and correspondingly the muscle has a completely different structure (absence of a pronounced calf), and the tendon is short. This is, of course, a hereditary difference. The leg of the cat is build like that of the Negro. If the cat's heel-bone is shortened by operation, regulation sets in after some time and the muscle of the calf assumes the type of structure found in the white man" (pp. 292-293[40]).

We would not like to represent behaviour of Caucasoids in idealized manner. For adult Caucasoid females the same Guinea pigs can be not very attractive also: "What is so interesting in these animals? They are looking like big rats without tails". On the other hand, Caucasoid adult males typically can willingly play with animals like Guinea pigs.

There are also other observations, those usually can not be discussed, because they are touching professional performance directly (thus, they are forced to be "racist"). Originally we were trying to describe the biological situation with human races in an objective way. The result occurred to be very sharp and it has produced even more deep negative impression than known racists' statements. It has happened so due to so-called "objectivity". The behaviour-related argument attracts disproportional attention. It could be purposively removed from the text of article and SI, however… Is it really necessary to wait the next 200 years in order to start the discussion of race-related behavioural differences? It is a difficult question without clear answer up to now.

I have seen Negroid animal-care-takers (from Ghana also) in mouse facilities. And I have seen animal-care-takers from the Arab countries (Caucasoid control). We can think about social factors here: may be Negroids have too many animals or too low salary, may be it is difficult for them to find any other job in Switzerland. It can be true, but the direct observation is that it will be better for everybody, including mice, if these people will do something else instead. They do not like animals, they hate them. They would not like to develop any intuition concerning mice and they even do not understand the case, because for them each mouse is "just an animal". Yes, a mouse is an animal. That is why it has long-term memory and remembers such kind of animal-care-takers very well.

Well, laboratory animals can be used for different purposes. May be for some situations the handling is not important at all (we can imagine this, at least). Animals, mice as well, have long-term memory and usually remember previous history. In a multi-generational experiment we have animals born in the frame of experiment and raised in the frame of experiment. They were handled starting from day of birth (P0) in a systematic manner and practically exclusively by a researcher. As a result, they behave more like pets (domestic animals) than like laboratory animals, obtained from an external source. It is good, because such animals are nice to work with, and it is bad, because they behave differently in the experimental tasks and sometimes require different protocols. For example, in order to see the same level of "freezing" in a fear-conditioning task, the foot-shock (electric current) for Wistar rats, born and raised in the frame of experiment, should be practically doubled (*e.g.*, increased from 0.28 mA to 0.48 mA)[12]. Otherwise "domestic rats" will not demonstrate freezing at all!

Concerning kids 4-6 years old the situation is even more demonstrative than concerning adults. Kids older than 6 years old are not very good for such observations, because they are trying to behave more like adults and they may have some idea about electronic means of observation and recording. It is very important for the results of this observation to keep kids together with Guinea pigs "unattended". 10-day-old Guinea pigs with their mother are the best for this task. Of course, nobody should

be informed about any "experiment". Caucasoid girls of above-mentioned age, both Jewish and Hispanic, are happy to play with Guinea pigs, taking them in hands like dolls, *etc*. (this observation remains true for other small creatures, like frogs, toads and even caterpillars). Negroid girls would like to avoid Guinea pigs and would not like to hold them in hands (this is also true for more exotic creatures). Caucasoid boys would like to play with Guinea pigs and sometimes can handle them rather professionally (some Hispanic kids are amazing here). Negroid boys 4-6 years old, being left "unattended" with Guinea pigs, demonstrate the most interesting behaviour. They are not afraid of Guinea pigs, they are brave enough (real *Homo sapiens*). But the first hypothesis, which they would like to test with respect to a 10-day-old Guinea pig, is how this animal can be killed, how it can be killed the most efficient way. First, the youngest kids from this group start with taking and throwing Guinea pig onto the ground like a stone. If there is grass around, this method does not bring positive result and 10-day-old Guinea pig remains alive. Then, the next method is applied: the body of a Guinea pig is squeezing in a human hand. This method works good and 10-day-old Guinea pig is typically dead as a result of its application. Interesting, that after killing one animal, the efficacy of this method should be confirmed by killing the next 10-day-old Guinea pig (4 animals were available at the beginning of experimentation, not counting mother). Note, that it is a natural behaviour. It was probably very helpful many-many years ago.

Sexual dimorphism, observed between Negroid girls and Negroid boys, looks compatible with hypothesis of C. Owen Lovejoy (1981)[41] about the origin of man (about the origin of Negroids from apes in our terms). Concerning Mongoloids, we do not have relevant information about their behaviour in similar situations, but on the basis of other observations it is possible to suppose that behaviour of Mongoloids with laboratory animals will be similar to behaviour of Caucasoids (or even better, because Mongoloids have a tendency to keep more exotic pets than Caucasoids, as it was mentioned by Spurgeon M. Keeny, *Director for Asia 1950-63*, UNICEF)[48]. Concerning F_1 hybrids, obtained from Caucasoid female and Negroid male, and F_1 hybrids, obtained from Negroid female and Caucasoid male, we currently have very limited information (not enough samples – only 1 sample of each above-mentioned hybrid, all males in range 4-6 years old; no information about F_1 hybrid females). Two available hybrids have produced the following impression: F_1 male hybrid from Negroid female and Caucasoid male behaves with respect to Guinea pigs like typical Negroid male of particular age, whereas F_1 male hybrid from Caucasoid female and Negroid male behaves more like typical Caucasoid male. However both observations should be considered as very pilot ones, they are not reliable in any sense.

Some people believe that the analysis of hybrids is "offensive". It is not. Exactly the same type of analysis was applied by us to the behaviour of mice, namely F_1 hybrids, obtained from inbred mouse strains C57BL/6J and DBA/2J (pp. 65-71[12]). One of the features of F_1 hybrids is so-called hybrid vigour (heterosis). Among other things, the development of hybrid vigour depends on the enrichment of living conditions of F_1 animals. The enrichment of living conditions (P21-P63) enhances behavioural hybrid vigour in mice, observed during the rest of their life (after P63)[12]. What about Caucasoid kids? Are they ideal with respect to Guinea pigs? Obviously not. Caucasoid kids of above-mentioned age 4-6 years old can kill Guinea pig also, but by different way. Both Jewish and Hispanic kids, both girls and boys, do not have appropriate intuition that temperature conditions can be dangerous for a Guinea pig, too could or too hot; they typically do not have an internal feeling that, for example, the weather can be too cold for a Guinea pig. The final result will be the same: dead animal.

Phenotypic inversion can be observed in humans and Guinea pigs. Human-related observation concerns the first generation, obtained from parents, whose childhood was during WW2 in Europe. Being adults, they had height, significantly higher than the height of their parents and sometimes higher than typical height before WW2. The usual explanation is that they had basically the same heredity as their parents, but living conditions during WW2 were very difficult in comparison with post-war period. Thus, their genotype has received an opportunity to develop high-height phenotype only during relatively nice post-war period. But this explanation is wrong.

First of all, some samples look like they have significant "overshoot" – they are too high in comparison with all known previous generations. This observation looks suspicious itself, but someone can say that living conditions during all known previous generations were not very good. This explanation can be ruled out in Guinea pigs.

Once I had a female Guinea pig with rather unusual phenotype. These Guinea pigs are outbred stock with relatively high variability, including variability in fur colour. Littermates of this female were all phenotypically normal. This Guinea pig, being young, was jumpy and it was moving a lot, more like a rat, but without signs of pathology. Being adult and having normal weight, it consumed 3-4-fold less water daily than usual female Guinea pig of similar age. This female was bred with phenotypically normal male. It consumed 3-4-fold less water than norm, being pregnant and during lactation period, which should be considered as pathology and it could be dangerous for its progeny. Among 2 born pups one was dead, but another one was a female, which was developing normally, except one feature. This female, being adult, consumed 2-3-fold more water on a daily basis than any other female or male Guinea pig in population! Here we may have an example of transgenerational epigenetic compensation (with an overshoot), similar to the situation with human height during and after WW2 in Europe.

Caucasoids have originated from Nergoids the following way. Some group of mutant Negroids occurred to be formed in Africa. This mutation had an impact on their behaviour and it allowed them to keep some wild-caught animals alive for some short period, instead of killing them immediately. However there were no much benefits to keep domestic animals in Africa at that time due to 2 reasons: 1) concentration of wild animals was relatively high and it was economically easy just to catch the next wild animal; 2) concentration of dangerous predators was also relatively high and any domestic animal could be eaten by a predator instead of human. But when these mutant humans (hopeful monsters) have moved to the North out of Africa (really to the North-East, due to geographic restrictions, to the territory of modern Pakistan and India)[42], the situation occurred to be different: 1) lower concentration of wild animals – it is more difficult to get them; 2) lower concentration of dangerous predators – it is relatively easy to protect domestic animals from predators.

Supplementary Fig. 6 | Taiwan, near Taitung, 1965. Girl of sixteen ploughing a hemp field with a simple wooden plough and a water buffalo. In the tropics there are no seasons in the Western sense; thus there are ripe crops next door to freshly tilled fields. Photograph # 53 from the book: Breitenbach, J. *Women of Asia* (The John Day Company, New York, 1968)[48].

In accordance with molecular data Caucasoid race has originated from Negroid one about 113000 years ago[43]. So-called "farming" is assumed to be invented only about 10000 years ago (*i.e.* relatively recently). We guess that it is incorrect and this statement is based on a complete lack of information. Of course, farming was present 10000 years ago. But before the exploitation of animals in agriculture, some preliminary steps should be done, and they were done many-many years before so-called "farming". First of all, some animals should be kept alive in captivity during some time. And it is not so easy task *per se*. Then, may be many years later, animals should be bred in captivity. Both tasks were successfully solved by mutant Negroids outside of Africa and these mutant Negroids are known as Caucasoids now. Thus, in accordance with our hypothesis, the main factor in formation of Caucasoids was the acquisition of the first domestic animals and their further breeding in captivity. These animals were used as a source of food and materials during many years, but they were not used as a work force and, of course, they were not used as a work force in agriculture.

Agriculture was virtually non-existent during many-many years, but the first domestic animals were already there. We know that this hypothesis is in contradiction with widely accepted views about "hunter-gatherers". But these views are based on nothing (complete lack of information). Nobody can distinguish the bones of wild-caught animals from the bones of domesticated ones. Some other approach should be used here. Today people believe that farming came from Turkey about 8000 years ago (p. xxi[42]). We assume that domestication of animals has started long before this time, but also outside of Africa. It is known that many African species of animals are very difficult for domestication. The classic example is a comparison between Indian and African elephants. Yes, there are successfully domesticated African animals, like ostrich[49], but in general the difference in domestication between African and out-of-Africa species does exist, and it concerns not only elephants. Why African species are so "wild"? The real answer is unknown, as usual, but we can suppose that the domestication of some animals outside of Africa has started not about 10000 years ago, but about 113000 years ago.

Mongoloid race has originated from Caucasoid one relatively recently. Molecular data give time point about 41000 years ago[43]. It is well known that Mongoloids differ from Caucasoids by their paedomorphic appearance[42,50,51]. Paedomorphosis – the appearance of ancestral juvenile traits in adult descendants (p. 2[50]). See **Supplementary Fig. 7**.

As it was mentioned by Stephen Oppenheimer, "An interesting hypothesis put forward by palaeontologist Stephen Jay Gould many years ago was that the package of the Mongoloid anatomical changes could be explained by the phenomenon of neoteny, whereby an infantile or childlike body form is preserved in adult life. Neoteny in hominids is still one of the simplest explanations of how we developed a disproportionately large brain so rapidly over the past few million years. The relatively large brain and the forward rotation of the skull on the spinal column, and body hair loss, both characteristic of humans, are found in foetal chimps. Gould suggested a mild intensification of neoteny in Mongoloids, in whom it has been given the name "paedomorphy"[50]. Such a mechanism is likely to involve only a few controller genes and could therefore happen over a relatively short evolutionary period" (p. 217[42]).

There was a mutation or a set of mutations in the direction of neoteny. But what immediate benefit under what conditions can be extracted from paedomorphism? Mongoloids are the most paedomorphic humans. We suppose that paedomorphic appearance is linked with gaming behaviour. But with what objects the earliest Mongoloids should play? With each other? With stones, sticks or bones? Probably, not. We suppose that early Mongoloids were playing with animals. Mainly with domestic animals. As a result of these games the first domestic animals, suitable for riding, were obtained. Then, may be many-many years later, the first domestic animals, those can be used as a force in agriculture, were obtained. Of course, working animals can increase the efficiency of agriculture dramatically. See **Supplementary Fig. 6** for primary impression.

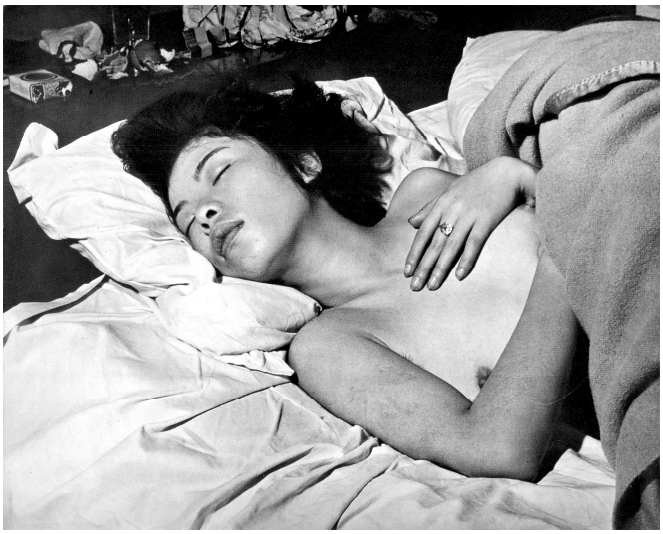
Spurgeon M. Keeny (*Director for Asia 1950-63*, UNICEF), an amazing observer, has provided the following description in 1968.

"No sketch of an Asian household is complete without a mention of its pets. I should place first the water buffalo except that that ungainly animal is more a member of the family than a pet. Every country child, boy or girl, looks forward to the day when he or she can take the family buffalo out for a graze and a wallow.

Next come dogs, whose ancestry usually won't bear examination. By day they play with the children; by night they bark challenges to neighbour dogs or howl at the moon just as they do in the West…

Cats seem to me less plentiful than on American farms, but no country is without them. In Thailand the favourite is not the Siamese with the scrannel voice so prized in the upper levels of Western society, but the bigger one from up-country in Korat.

As for the rest, the range is enormous – from mongoose to talking birds. In one house in Bangkok, in addition to dogs and cats, we have pigeons, a duck, and a gibbon – known to the



Supplementary Fig. 7 | Sleeping Japanese girl. This Mongoloid creature, despite its paedomorphic appearance, is an absolutely adult female (look at her nipples). Mongoloid anatomical changes could be explained by the phenomenon of neoteny, whereby an infantile or childlike body form is preserved in adult life[50]. Photograph made by Josef Breitenbach before 1968, not dated, # 78[48].

servants as "the thief" because of her skill in stealing anything eatable. She has recently adopted two half-grown kittens, which she picks up by the neck with either hand or foot. Then she takes one for a wild run over the rooftops while the other patiently waits its turn" (p. 13[48]).

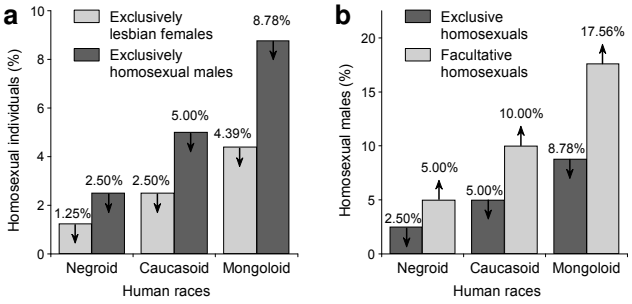
We know that many Mongoloids like exotic pets and pets in general. This is an attractive part of paedomorphism. But not so attractive part of paedomorphism is an enormous cruelty. Adolescent sadism is a very well known phenomenon. Persistent cruelty to other people is also a direct consequence of paedomorphism. There are known records about WW2, previous history, the behaviour of Tatar-Mongols on the territory of modern Russia many-many years ago. However the main factor in the appearance of Mongoloids was a gaming behaviour that has led to deep domestication of animals, useful as a work force. Afterwards, may be many years later, this tradition (animals as a work force) has spread to Caucasoids by means of cultural diffusion. Northern Caucasoids (*e.g.*, Germans), in addition, are not pure *Homo sapiens*, they are hybrids with Neanderthals, but it is another story[52,53].

Now we know the temporal order of the origin of human races (**Supplementary Fig. 5**). Does this order automatically mean that the most ancient race is the most "biologically primitive" and the newest race – the most "biologically advanced"? We do not have specific information concerning human races, but some general knowledge about the origin of new species is available. First of all, at the moment of appearance of a new variety (species or race), the new variety is definitely more biologically advanced than the old one. Otherwise new variety will not be formed – new mutants (the core of a new variety) will not be extracted from the old species into a distinctive subgroup, and this mutation will just silently disappear from the population. Whether the same order exists now? Honestly, it is difficult to say – a lot of things have changed. But we know that during the period of canalization of phenotype of a new species a lot of independent traits occur to be improved. Any new species is improved in many dimensions, not just in a leading dimension, for example in handling of domestic animals. Many independent traits are improved during genetic fixation, genetic assimilation of a new phenotype.

What can we say about the rate of evolution of modern human races today? What can we say about the rate of microevolution of each particular race? We can not assess the rate of microevolution directly, but indirectly we can obtain some knowledge through transgenerational epigenetic compensation. It is clear that human environment has changed dramatically during the last 500 years. It means the existence of a new environmental pressure and acceleration of evolution in accordance with the most known evolutionary theories (Darwinism, Lamarckism, our current views, *etc*). We know that the accelerated evolution is linked with the increased transgenerational epigenetic compensation in population. The increased transgenerational epigenetic compensation is linked with the increased percent of individuals those can demonstrate homosexual behaviour under conditions of normal society (especially, male individuals). It is well known that the least homosexual race is Negroid one, the most homosexual race – Mongoloids (Caucasoids are in between, as usual). Thus, in accordance with this logic, Mongoloid race is not only the youngest race, but the race with currently the highest rate of evolution (of microevolution).

We know that China officially reports low percent of homosexual individuals, atypical for Mongoloid race (reported values are much closer to Caucasoid race). We guess that here we have an example of significant difference between the real biological situation and the results, obtained by means of sociological studies. In the countries with relatively strong social propaganda in the sex-related areas, the results of sociological studies indicate not biological situation, but mainly the public opinion about particular biological situation (please, understand: sociological data are not "falsified", but people, providing raw information, *de facto* provide information about their expectations, but not about real biological situation).

The solution is a direct observation in the countries, like Thailand. For a biological observation it is better to choose countries, where more or less clear information can be collected (Thailand, Holland, Switzerland), than to collect lots of contaminated data in large countries like USA, China or Russia.



Supplementary Fig. 8 | Lesbians and homosexuals among the three major races of man, Negroids, Caucasoids, and Mongoloids. This estimation is constructed on the basis of sociological studies and direct observations in the countries with relatively weak social propaganda in the sex-related areas (Thailand, Holland, Switzerland). Values for Negroid race were estimated using US data.

Thus, it is better to assume that Thailand data are valid for China, than to use Chinese sociological reports. Reconstructed data will be more close to reality. It can be some real difference between populations of China and Thailand in percent of homosexuals, but real values should be close to each other and typical for Mongoloid race in general. The estimation, shown in the **Supplementary Fig. 8**, should be considered only as a preliminary hypothesis, showing further direction of research, but not as a confirmed truth. Arrows indicate that the real values are probably lower than shown (for exclusive homosexuals) and they are higher than shown (for facultative homosexuals).

P.S.

For our article we have selected traits that demonstrate clear phenotypic inversion (between P and F_1). These traits include: 1) time point of the appearance of particular developmental stage (acceleration-retardation of eye opening[7,8], retardation-acceleration of puberty onset[20]); 2) performance in behavioural operant tasks (improved-impaired two-way avoidance performance[12,13,*]); 3) sensitivity to morphine-induced analgesia (decreased-increased sensitivity)[9,10,12,13,25,*] and initial tempo of tolerance development (retardation-acceleration of acute tolerance formation)[12,13,25,*]. Simultaneously in the same animals or elsewhere the other traits show Lamarckian inheritance. These traits include: 1) morphological changes in adult animals (brain morphology – hippocampal mossy fibers[12,13] and synaptophysin contents in hippocampus[12], testis morphology[19], ovary morphology[54]); 2) opiate dependence after application of standard protocol of chronic drug treatment (morphine treatment)[12,13,27]. In all examples we have phenotypic inversion in time points of ontogenesis and operant behaviour and, in the same animals, Lamarckian inheritance in morphology and final stages of development (*e.g.*, of opiate dependence development).

* – present article.

P.P.S.

Above-mentioned results can be obtained using inbred mouse strains[12,13,*] and outbred rat[12,13,19,23] and mouse[55] stocks, as well as mixed background, obtained from crossing of two inbred mouse strains[15], namely C57BL/6 and 129SvEvBrd. However, not all inbred mouse strains are equally good for observation of transgenerational epigenetic effects[56,57]. DBA/2J is obviously good[12,13,*]. C57BL/6J is not recommended (its ontogenesis is very well canalized, pp. 70-71[12]). Strain 129 may be not so good[55], but mixed background C57BL/6 × 129SvEvBrd was perfect[15]. DBA/2J strain, being very good for observation of transgenerational epigenetic effects, should be used with foster-mothers, for example with primiparous NMRI (outbred stock)[12,13,*], in order to have low postnatal mortality. DBA/2J strain, in comparison with C57BL/6J, has high susceptibility to audiogenic seizures (P21-P30) and can show significant effect of the adolescent enrichment of housing conditions (P21-P62) on the adult behaviour (P63+)[12]. "A transgene, pHRD, is highly methylated in 12 independent mouse lines when in a C57BL/6 strain background, but becomes progressively less methylated when bred into a DBA/2 background. Transgenes inherited from the mother are generally more methylated; however, this parental effect disappears following continued breeding into the nonmethylating strain" (p. 939[57]). Thus, the better canalization of ontogenesis of C57BL/6J strain in comparison with DBA/2J, as well as the better canalization of ontogenesis of females in comparison with males, can be explained as a direct correlate of the increased basal methylation, despite this idea sounds too simplistic to be true.

Supplementary References (references 1-31 are given in our main paper)

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