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# Transgenerational epigenetic compensation in evolution

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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 of disturbed functionality was discovered in the untreated progeny of drug-treated fathers as the opposite quantitative phenotypic changes (phenotypic inversion). Epigenetic changes, responsible for heritable compensation, are distributed between several independent loci and these changes disappear gradually and asynchronously during a few untreated generations. The role of hereditary epigenetic compensation in evolution remains unclear. Here we show that transgenerational epigenetic compensation of disturbed functionality converts mutants into hopeful monsters, initiates speciation and facilitates genetic assimilation of acquired characters. The increase of environmental pressure, applied to mutant and wild-type animals, induces heritable epigenetic compensation in mutants (initially less fit), whereas the development of wild-types remains canalized. In a random breeding population this heritable epigenetic compensation increases fitness and lifespan of mutants and decreases lifespan of wild-types.**

Hopeful monsters are organisms with a profound mutant phenotype that have the potential to establish a new evolutionary lineage[1,2]. The term "hopeful monster" was introduced by Richard Goldschmidt first in 1933[3] and, then, the detailed theory was provided in 1940[4]. The weakest point of this concept is a requirement that particular mutant should be initially better fit than wild-type. In our article we show that this requirement is not really necessary. Namely, the mutants, those are initially less fit than wild-types, those initially have decreased viability and decreased lifespan, can be converted into hopeful monsters by means of transgenerational epigenetic compensation in a semi-natural population. The canalization of ontogenesis, a concept proposed by Conrad Waddington[5], and the transgenerational epigenetic compensation of disturbed functionality, discovered recently[6], are necessary for understanding of speciation, but they do not provide a solution automatically. The process of genetic assimilation of acquired characters, proposed by Waddington[5], and the process of genetic assimilation of transgenerational epigenetic compensation, discussed in our paper, are important for evolution, but they are too slow to take part in the episode of speciation, which can be extremely fast (**Fig. 1**).

Transgenerational epigenetic compensation of disturbed functionality was observed in the experiments with paternal drug treatment as the opposite phenotypic changes in the untreated progeny (phenotypic inversion)[7]. Such experiments were done with rats and mice using prenatal vinclozolin treatment[8,9], neonatal thyroxine treatment[6,10-12] and young adult morphine treatment[6,12-14]. Phenotypic inversion is evident in the $F_1$ and $F_2$ after prenatal plastic mixture treatment[15] (Fig. S4[15] & Fig. 1A[15]), if prenatally-treated rats are numbered as P generation, not as $F_1$. Previously phenotypic inversion was shown in plants (*Linum usitatissimum*)[16] and insects (*Pieris brassicae*)[17].

Phenomenological properties of transgenerational epigenetic compensation were summarized the following way[6]: 1) only very small portion of all acquired compensatory (and sometimes destructive) changes becomes epigenetically heritable; 2) epigenetic inheritance promotes transgenerational compensation of disturbed functionality and entails the opposite changes in the

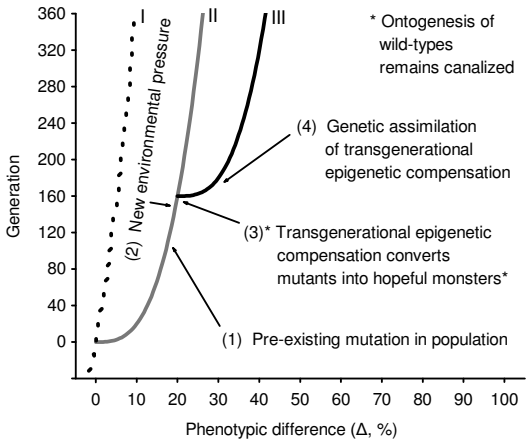


**Figure 1** | Transgenerational epigenetic compensation initiates speciation. **I**, **II** and **III** – species or races. Original mutation and its heritable epigenetic compensation are not in the same locus. Speciation demonstrated on hypothetical data.

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untreated progeny; 3) heritable epigenetic changes are distributed in several independent loci and these changes disappear gradually and independently of one another during a few untreated generations; 4) only very small portion of all changes in gene expression in the untreated progeny are primary heritable changes; others are the results of secondary adaptation and developmental compensation, initiated by heritable epigenetic changes[6]. Molecular mechanisms of epigenetic inheritance were discussed elsewhere[18-20].

## Results

The emergence of a new species (speciation) proceeds through the following 3 stages or steps.

**I**. The appearance (and further possible long-term existence) of a new mutation in population, with neutral or slightly negative effect in heterozygous organisms and weak negative effect on survival in homozygous ones.

**IIa**. The application to the population of a new unusual and rather strong environmental pressure immediately induces transgenerational epigenetic compensation in initially less fit homozygous mutants, whereas the individual development of wild-types and heterozygous organisms remains canalized.

**IIb**. The transgenerational epigenetic compensation, being found in at least one locus which is independent from the locus of mutation, in a panmictic (random breeding) population increases viability of homozygous mutants, has neutral effect on heterozygous organisms and decreases viability of wild-types.

**IIc**. Any possibility of discrimination between organisms "with" and "without" transgenerational epigenetic compensation will lead to non-random breeding inside this population: mutants will prefer to mate with mutants, wild-types – with wild-types; heterozygous organisms with strong epigenetic compensation will behave more like mutants, the ones with weak epigenetic compensation – more like wild-types.

**III**. After the formation of a new species on the basis of homozygous mutants (hopeful monsters), transgenerational epigenetic compensation will be slowly, during many generations, replaced by mutations with subtle effects on phenotype, distributed between different regulatory sites of different genes; this replacement is known as "genetic assimilation", but now the process of genetic assimilation is facilitated by transgenerational epigenetic compensation; the transgenerational epigenetic compensation is constantly updated after each episode of genetic assimilation (after each fixation of a new mutation).

**Remarks for stages II-III**. Sexual dimorphism is an important factor for facilitation of evolution. Transgenerational epigenetic compensation is building up mainly, but not exclusively, in males. It is transmitted through both males and females. Phenotypic effects of transgenerational epigenetic compensation are more pronounced in females (starting from $F_2$ generation). Genetic assimilation is working mainly through selection of males. Epigenetic compensation and genetic assimilation can start and proceed simultaneously.

The final result of genetic assimilation in morphological evolution, – many subtle-effect single-nucleotide substitutions in regulatory DNA, is described elsewhere[21].

In the **Fig. 1** the following factors are shown. (**1**) Independent appearance of mutant allele in population (some mutations are always present). (**2**) Unusual and strong environmental influence.

**P** : aaee ♀ × AAEE ♂

**$F_1$** : AaEe ♀ × AaEe ♂

**$F_2$** :

| Gamete $F_1$ ♀ \ Gamete $F_1$ ♂ | AE | Ae | aE | ae |
|---|---|---|---|---|
| AE | **AAEE** | **AAEe** | AaEE | AaEe |
| Ae | **AAEe** | AAee | AaEe | Aaee |
| aE | AaEE | AaEe | **aaEE** | **aaEe** |
| ae | AaEe | Aaee | **aaEe** | aaee |

**Figure 2** | Transgenerational epigenetic compensation promotes segregation of mutants and wild-types. **A** – mutant allele, **a** – wild-type allele; **E** – allele of transgenerational epigenetic compensation, **e** – wild-type allele. Black cells contain homozygous mutants with heritable epigenetic compensation, they have enhanced viability. White cells – wild-type animals with heritable epigenetic compensation, they have decreased viability.

(**3**) Heritable epigenetic compensation improves mutant's phenotype – converts homozygous mutants into hopeful monsters. (**4**) Genetic assimilation of heritable epigenetic compensation (facilitated by dynamic flexibility of heritable epigenetic compensation). Note that the ontogenesis of wild-types remains canalized during the whole episode. As a result of panmixia (random breeding), mutant-optimized heritable epigenetic compensation decreases fitness and lifespan of wild-types (**Fig. 2**), like paternal drug treatment decreases fitness of drug-naive descendants. After speciation there are homozygous mutants with heritable epigenetic compensation and wild-types without heritable epigenetic compensation; both avoid breeding with each other (**Supplementary Fig. 1**).

In the **Fig. 2** the transgenerational epigenetic compensation is localized in one locus, independent from the mutant one. Epigenetic compensation is useful for mutants and dangerous for wild-types. Homozygous mutants with heritable epigenetic compensation have increased fitness in comparison with all other animals. Wild-type animals with heritable epigenetic compensation have decreased fitness in comparison with both wild-type animals without epigenetic compensation and homozygous mutants with heritable epigenetic compensation. Heritable epigenetic compensation can be dominant, because a lot of abnormalities can be observed in the progeny of drug-naive females and drug-treated males.

If heritable epigenetic compensation is distributed between several independent loci (instead of one main locus), our conclusion remains the same: transgenerational epigenetic compensation enhances viability of homozygous mutants and suppresses viability of wild-types. This is the starting point of speciation: mutant and wild-type subpopulations would like to be separated in order to increase viability of both of them.

Currently our knowledge of molecular mechanisms of transgenerational epigenetic compensation is rather limited. However we are sure that basically the same mechanisms are involved into transgenerational epigenetic compensation of paternal drug treatment (relatively well-known at the

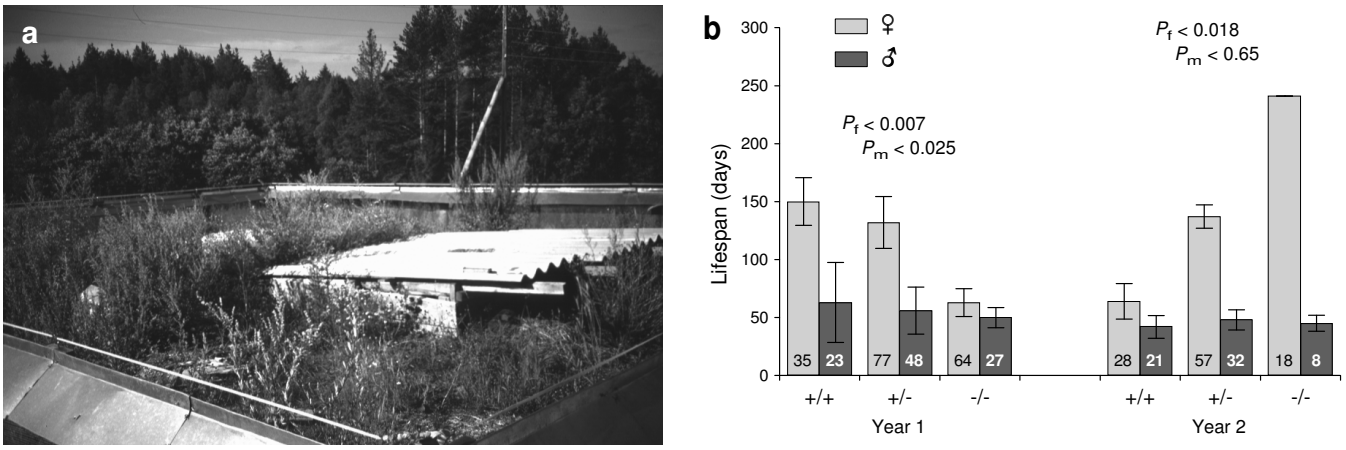


**Figure 3** | Lifespan of *Per2*$^{Brdm1}$ mice after release in semi-natural environment. (**a**) Pen 20 × 20 m with two shelters 3 × 2 × 0.7 m each. (**b**) Lifespan (days) after the first release for generations P - $F_1$ (Year 1) and $F_2$ - $F_4$ (Year 2) for all mice that were recorded at least 10 days following release. Wild-type (+/+), heterozygous (+/-) and mutant (-/-) *Per2*$^{Brdm1}$ mice. *P*-values are given for the effect of genotype (number of mutant *Per2*$^{Brdm1}$ alleles as ordinal variable) according to the Kaplan-Meijer (log rank Mantel-Cox) procedure. Median ± SE. Standard error is not shown for mutant (-/-) females during Year 2, because the most of these mice were alive at the end of experiment. Data from the experiment of Serge Daan and co-authors (2011)[22].

phenomenological level)[6,12] and transgenerational epigenetic compensation that is building up in homozygous mutants under strong environmental pressure (strong stress)[22].

Transgenerational epigenetic compensation was observed by Serge Daan and co-authors in the $F_2$-$F_3$ and further generations of transgenic *Per2*$^{Brdm1}$ mice raised under semi-natural outdoor conditions[22]. Mutant, heterozygous and wild-type male and female mice (mixed background of C57BL/6 and 129SvEvBrd), initially 250 in Mendelian ratio 1:2:1, were kept outdoors[23] as an isolated population, random breeding inside each of 4 independent pens during 2 years (each pen 20 × 20 m, **Fig. 3a**). Each mouse was individually numbered by subcutaneously injected transponder and all new mice, born in field, were genotyped and numbered twice a year. Transponders were registered by antennas, placed near feeding places. Recording equipment was working 24 hr daily, providing information about feeding activity and, finally, about lifespan of each mouse.

During Year 2 the majority of wild-type progeny had heritable epigenetic compensation in one or several loci, but it had not mutant *Per2*$^{Brdm1}$ allele *per se,* – that is why it had decreased lifespan. Simultaneously, the homozygous mutants had heritable epigenetic compensation plus mutant *Per2*$^{Brdm1}$ allele – that is why they had supernormal lifespan (**Fig. 3b**). The supernormal lifespan of 18 mutant females indicates that these homozygous *Per2*$^{Brdm1}$ females are hopeful monsters, the hopeful monsters that were proposed by Richard Goldschmidt many years ago.

The experiment of Serge Daan and co-authors illustrates steps **I**, **IIa** and **IIb** of a speciation episode. We can see that the high number of particular mutants in population (achieved in this case by artificial means, of course) makes possible the observation of initial stages of speciation despite initial low fitness of homozygous mutants. Transgenerational epigenetic compensation has converted homozygous mutants into hopeful monsters. And it was done specifically with females – with the sex that determines the quantity of descendants in the next generation. Initial stages of speciation can be investigated now experimentally. And one of the most important conditions is not only some special features of chosen mutation, but just very high percent of particular mutants in an artificially created population.

*Per2*$^{Brdm1}$ mice, used in the experiment of Serge Daan and co-authors[22], have significant deviations in opiate system, namely decreased rate of tolerance development in the experiment with morphine-induced analgesia[24]. We know that in rats the paternal morphine treatment leads to enhanced sensitivity to morphine-induced analgesia and enhanced rate of tolerance development in the $F_1$ and $F_2$[6,12]. Thus, opiate system can be a common pathway for heritable epigenetic compensation in both situations.

The next step of speciation (step **IIc**), – the discrimination of animals with and without transgenerational epigenetic compensation as potential mates by females, can be illustrated by the experiment of David Crews and co-authors[25], done with Sprague-Dawley rats and vinclozolin. Prospective parents P (both females and males) were exposed to prenatal vinclozolin treatment during E8-E14 (pregnant females received i.p. injections)[25]. We use generation numbering optimized for paternal drug treatment (prenatal, neonatal, young adult, *etc*). Prenatally treated females and males (generation P) were bred with each other to obtain $F_1$. $F_1$ females were bred with $F_1$ males to obtain $F_2$ generation. Control animals from untreated parents were bred with each other simultaneously with experimental ones. $F_2$ generation females and males were tested in mate-preference test at P90-P120 (**Supplementary Information**) and, then, $F_2$ males were tested in odour-salience test at P403 and $F_2$ females were tested in odour-salience test at P458.

In the odour-salience test males and females investigated 1-inch-round odour-carrying beads during 1 min in their individual home cages. Five beads were exposed to an animal simultaneously, each carrying one of the following odours: 1) vinclozolin subline female; 2) control female; 3) vinclozolin subline male; 4) control male; 5) self-odour.

In rodents, as well as in other mammals and many other dioecious species, including birds, the final choice of mate is produced by a female[26]. Thus, the preference, shown by a female, is the most important.

Females from vinclozolin subline at the age of 458 days have shown significant preference for odour of vinclozolin subline

males ($P < 0.01$). Males from vinclozolin subline at the age of 403 days have shown modest preference for odour of females from control subline ($P < 0.05$). Control females and males did not show significant preferences for control or vinclozolin subline in this test (Fig. 3*B*[25]). Among young animals (P90-P120) in the mate-preference test the opposite pattern was obtained: all females preferred control males ($P < 0.026$, Fig. 2*A*[25]).

In a natural or semi-natural mouse or rat population, if an animal has age of 458 days and it is still alive, this is a very strong indicator that this animal is not a bad one, indeed. Hopeful monsters in the experiment of Serge Daan and co-authors[22] at the end of experiment had age more than 241 days, calculated from the day of release. From the Daan's experiment (**Fig. 3b**) we can see that there is no such a requirement that males, homozygous mutants with heritable epigenetic compensation (*i.e.* hopeful monsters), should have an advantageous phenotype. The advantageous phenotype should exist in females, homozygous mutants with heritable epigenetic compensation, and these females should be able to identify males, homozygous mutants with heritable epigenetic compensation (but may be without advantageous phenotype), as potential mates.

The experiment of David Crews and co-authors[25] provides necessary evidence for non-random breeding in population consisted of animals with and without transgenerational epigenetic modification. Adult mutant females with successful transgenerational epigenetic compensation prefer to mate with adult mutant males with transgenerational epigenetic compensation. Such animals will try to be an isolated subgroup.

Temporal geographic isolation, proposed by the theory of punctuated equilibrium of Niles Eldredge and Stephen Gould[27], will work for evolution only if the hopeful monsters will be concentrated in the isolated subpopulation, not just some randomly chosen individuals from the original population.

The next evolutionary step (step **III**) is a genetic assimilation of transgenerational epigenetic compensation (**Supplementary Fig. 2**). It is similar in principle to the genetic assimilation of an acquired character, described by Conrad Waddington[5]. The process of evolutionary development of an adaptive phenotype was represented by Waddington as several stages or steps: 1) development of quasi-proportional reaction to external influence, *i.e.* sub-optimal adaptive reaction, which is genetically fixed; 2) development of optimal reaction to external stimulus, quasi-independent from the magnitude of external influence, this canalized reaction is genetically fixed also; 3) development of replacement of external influence by internal factors or stimuli, and this replacement is also genetically fixed. Finally, previously ontogenetically acquired phenotype becomes a classic genetically fixed feature, the feature which is independent under normal conditions from the external environment, and this feature is very well canalized[5].

With respect to the genetic assimilation, the hereditary epigenetic compensation plays two roles: 1) it facilitates genetic assimilation (for example, genetic assimilation of an acquired character); 2) hereditary epigenetic compensation itself can be genetically assimilated.

Mutations in regulatory sites with subtle effect on phenotype can be easily selected (natural selection) only if the matching functional system[28], which is waiting for them, already exists. This matching functional system[28] can be developed as an acquired character during ontogenesis as a result of external environmental pressure. However in many cases, when an external pressure is applied, ontogenetic plasticity is very limited, because it happens at relatively late stage of ontogenesis. In the frame of classic genetic assimilation, without the involvement of epigenetic compensation, mutations which affect early stages of ontogenesis can exist in population, but they will not be selected, because suitable functional system, which can get benefit from them, will not exist, because it can not be developed as an acquired character under external influence.

Only heritable epigenetic compensation can develop expected functional system at earlier stages of ontogenesis in the next generations. Heritable epigenetic compensation with very high probability will disturb early ontogenetic stages in descendants. This disturbance will elicit the next wave of heritable epigenetic compensation. Finally, during several generations very efficient functional system can be developed. And each collected useful mutation will rearrange heritable epigenetic compensation further, in a way that some other, additional set of mutations will become preferable. Thus, it is some kind of a self-corrected search for mutations in a particular population.

Genetic assimilation of an acquired character, facilitated by transgenerational epigenetic compensation, can be illustrated by the experiment of Conrad Waddington (1953)[29]. In this experiment cross-veinless phenotype was induced in *Drosophila melanogaster* by heat-shock treatment. Epigenetic inheritance systems in *Drosophila melanogaster* are not the same as in mammals, especially with respect to methylation, which is practically absent in *Drosophila*[19]. However we need high numbers of animals in order to distinguish a classic genetic assimilation from its possible transgenerational epigenetic facilitation. It was found that when pupae of a wild Edinburgh strain, S/W5, were given a temperature shock (4 hours at 40 °C) starting at 21 to 23 hours after puparium formation, a fair number of crossveinless wings developed, although none appeared under normal conditions. It was decided to use this as the character to be selected. There is, of course, no reason to believe that the phenocopy would in nature have any adaptive value, but the point at issue is whether it would be eventually genetically assimilated if it were favored by selection, as it can be under experimental conditions. It was decided to concentrate on this effect, and to set up two separate selection lines. In one, only those flies which showed the crossveinless effect after treatment were bred from ("upward" selection, which should increase the frequency of response), while, in the other, the crossveinless flies were rejected, and only those still showing normal wings were used to carry on the line ("downward" selection)[29].

Observed cross-veinless phenotype, induced by heat-shock treatment, is considered by us as an indicator (direct or indirect) of some physiological adaptation to heat-shock treatment. This indicator is not adaptive *per se*, of course. Transgenerational epigenetic compensation is trying to play its role in the process of adaptation. That is why it facilitates selection in upward direction and inhibits selection in downward direction (**Fig. 4a**).

Initially this experiment has started with upward selection line only and with relatively wide window of heat-shock treatment onset (17 to 23 hours after puparium formation). Afterwards, starting from the third generation, the downward selection line was added and the time window of heat-shock treatment onset

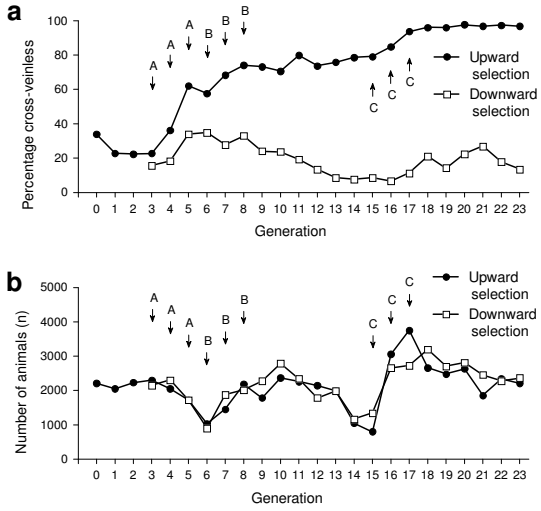


**Figure 4** | Transgenerational epigenetic compensation facilitates genetic assimilation. Assimilation of cross-veinless phenotype induced in *Drosophila melanogaster* by heat-shock treatment (40 °C) during 4 hours with onset between 21 and 23 hours after puparium formation. All shown animals (all generations) are heat-shock treated. (**a**) Percentage of animals with cross-veinless phenotypes. (**b**) Number of investigated animals. A, B and C – episodes with probable transgenerational epigenetic compensation. Other time intervals – episodes with pure classic genetic assimilation. Data from the experiment of Conrad Waddington (1953)[29].

was narrowed to 21 to 23 hours after puparium formation. We can see the impressive increase in the percentage of cross-veinless phenotype in both upward and downward selection lines (**Fig. 4a**, episode A), and this is a result of transgenerational epigenetic compensation. Note also episode C (**Fig. 4**). Before episode C we can see that the number of animals in all groups was rather low during two preceding generations (14 and 15, **Fig. 4b**) and we can suppose that a combination of this treatment with some environmental factors was rather stressful for population. This stress can be a reason of transgenerational epigenetic compensation seen in both upward and downward selection lines (**Fig. 4a**, episode C). Look next at the episode B (**Fig. 4**). Stress during episode B has induced transgenerational epigenetic compensation in upward selection line only. Between episodes B and C (generations 8 - 13) we can see the expected very regular progress in both upward and downward direction (**Fig. 4a**) and during the same period the number of animals in both lines is very stable (**Fig. 4b**). We suppose that the role of transgenerational epigenetic compensation during this time interval (generations 8 - 13) is close to zero and we can see here a classic genetic assimilation[5].

Thus, real experiment with genetic assimilation can deal with both classic genetic assimilation and transgenerational epigenetic compensation of disturbed functionality, and, furthermore, genetic assimilation can be significantly facilitated by transgenerational epigenetic compensation.

## Discussion

What can we say about macroevolution and microevolution? Microevolution, or evolution of a species without speciation, usually consists of genetic assimilation of acquired characters and genetic assimilation of heritable epigenetic compensation. Different stochastic and neutral changes of heredity belong to microevolution also. Macroevolution, or the appearance of a new species, usually consists of a systemic mutation in Goldschmidt's sense[4], which is in our terms a combination of a key mutation with its heritable epigenetic compensation.

Heritable epigenetic compensation is not only "heritable epigenetic compensation of a key mutation", but it is heritable epigenetic compensation of a complex, consisted of: (a) key mutation; (b) strong environmental influence. The origin of mutation is not specified. The requirement is that this mutation should be present in population in detectable quantity. Thus, initially it should not have too deep negative impact upon fitness and survival. Later, the enhanced fitness of homozygous mutants can be formed by transgenerational epigenetic compensation, induced by environmental pressure.

If mutation is not present in population in detectable quantity, the population will respond to a new strong environmental pressure without speciation. Initial reaction of population to external influence will be quasi-Lamarckian: transgenerational epigenetic compensation will be formed during a few generations. Afterwards, if above-mentioned environmental pressure will be still present, the epigenetic hereditary changes will be replaced by genetic changes (mutations) during relatively slow process of genetic assimilation.

Natural selection remains a part of evolutionary theory, just because it is a part of evolutionary process. Genetic assimilation proceeds through natural selection, especially through natural selection of males. However natural selection is not a "driving force" or "directing force" of evolution, because the efficacy of transgenerational epigenetic compensation determines the direction of natural selection during each evolutionary episode (during any episode with or without speciation).

Sexual dimorphism was found to be important for evolution in the frame of classic genetics by Vigen Geodakian[26,30]: females have better canalization of their ontogenesis, smaller variability in natural populations, and mutations and harmful external influences have lesser impact on their phenotype and survival; whereas the ontogenesis of males is less canalized, mutations have more direct projections to their phenotype, males have higher variability in natural populations; and, as a consequence, natural selection is working mainly in males, whereas females promote sufficient quantity of descendants in each generation.

Transgenerational epigenetic compensation was shown to be highly significant in the progeny after paternal drug treatment – after treatment of males. And it is extremely interesting to see that in their progeny the results of this treatment are more pronounced in females than in males. It is not so evident in the first generation ($F_1$): there are experiments with equal changes in $F_1$ males and females (Fig. S4[15], Fig. 2b[6]) and there are experiments with even more pronounced changes in $F_1$ males (Fig. 4b[6]). However in the second generation ($F_2$) all changes are more pronounced in females: here we have experiments with prenatal treatment with plastic mixture (Fig. 1A-B[15]), neonatal treatment with L-thyroxine (Fig. 2b[6]) and young adult treatment with morphine (Fig. 4b[6]). The enhanced transgenerational epigenetic compensation in females can be observed despite better canalization of their ontogenesis, typical for all females.

In the experiment of Serge Daan and co-authors[22], with mutant mice in semi-natural environment, all hopeful monsters were

exclusively females. Transgenerational epigenetic compensation is in the process of its development mainly in the organisms of males, but the phenotypic results of this process are more beneficial for their female offspring. This distribution of evolutionary functions between males and females allows to have practically adapted females (as a result of transgenerational epigenetic compensation) and males, those are still working for further improvement of transgenerational epigenetic compensation and/or working for its genetic assimilation (which will be a result of natural selection, active among males only). In a natural population the transgenerational epigenetic compensation, more beneficial for females, and the canalization of ontogenesis, more pronounced in females, are working for the same final goal: to have maximum quantity of females, suitable for breeding. These females will be bred with a few the most advanced males, those are the best in production of transgenerational epigenetic compensation and are the best with respect to mutations, useful for genetic assimilation of the above-mentioned transgenerational epigenetic compensation.

## Methods

Methods for *Per2*$^{Brdm1}$ mice experiment are given in the refs.[6,22]. Methods for mate preference experiment are provided in the ref.[25]. Methods for genetic assimilation experiment can be extracted from the ref.[29], but it should be noted that the description given in the ref.[29] can produce false impression that the narrowing of the time interval of the onset of heat-shock treatment from 17-23 hr to 21-23 hr after puparium formation was introduced at Generation 5. Indeed, Generation 5 was chosen as the first generation for demonstration in the Fig. 2[29]. However the data from the Table 1[29], namely identical changes during Generations 3-5 in the "upward" and "downward" lines, shown in our **Fig. 4**, indicate that the above-mentioned narrowing of the time interval was introduced synchronously with the introduction of "downward" selection line at Generation 3. There is no legal contradiction between this statement and the description, provided by Waddington, because 21-23 hr time interval is completely included into the officially declared for these Generations 3-4 time interval 17-23 hr.

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## Acknowledgments

I would like to thank Alexei L. Vyssotski and Oxana O. Litvine for their persistent help.

## Additional information

**Supplementary Information** accompanies this paper at http://www.evolocus.com/evolocus/v1/evolocus-01-007-s.pdf

**Competing financial interests:** The author declares no competing financial interests.

**How to cite this article:** Vyssotski, D.L. Transgenerational epigenetic compensation in evolution. *Evolocus* **1**, 7-12 (2012). www.evolocus.com/evolocus/v1/evolocus-01-007.pdf

Received: 25 May 2012
Accepted: 28 May 2012
Published online: 17 June 2012

*Evolocus*

***Supplementary Information for***

# Transgenerational epigenetic compensation in evolution

Dmitri L. Vyssotski

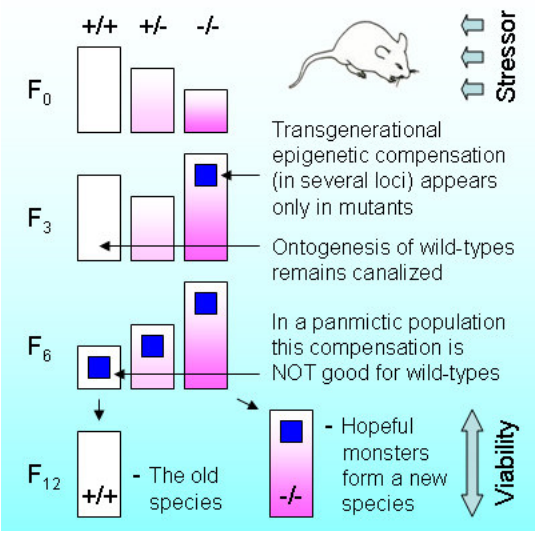
Published 17 June 2012, *Evolocus* **1**, 7-12 (2012): http://www.evolocus.com/evolocus/v1/evolocus-01-007.pdf

**Previously, many-many years ago, hopeful monsters of Richard Goldschmidt[4], nomogenesis of Leo Berg[31] and canalization of ontogenesis with genetic assimilation of Conrad Waddington[5] were at the periphery of evolutionary knowledge. Lamarckism and psycho-Lamarckism were treated as anti-scientific theories[32]. Sexual dimorphism of Vigen Geodakian[26,30] was practically unknown. Natural selection of Wallace[33] and Darwin[34] and its modern variant, known as neo-Darwinism or synthetic theory[35-37], were considered as a synonym for evolution. Most of these theories were discussed as alternatives, incompatible with each other. Here we show how the transgenerational epigenetic compensation[6] links all above-mentioned concepts in a highly cooperative and very efficient evolutionary process (Supplementary Fig. 1). Natural selection takes its rather modest place mainly in the frame of genetic assimilation and mostly among males, if we consider dioecious species (Supplementary Fig. 2).**

In the experiment of Serge Daan and co-authors[22], the new environmental pressure, applied to the heterogeneous population, was consisted of new climatic conditions. These conditions have induced transgenerational epigenetic compensation in homozygous mutants.

In the modern natural populations, the new environmental pressure may be more often a new virus infection. We did not discuss this opportunity in our article due to the absence of related experimental data. Hope, these data will be accumulated soon and we will be able to see how new virus infection can induce transgenerational epigenetic compensation. Up to now it is absolutely unknown field and we even do not know which level of novelty the virus should have in order to be considered new enough to induce transgenerational epigenetic compensation. We assume that the rate of transgenerational epigenetic compensation can be faster than the rate of mutation of typical viruses in natural conditions. May be the transgenerational epigenetic compensation is not so helpful for elimination of a virus, but it can be very helpful for co-adaptation of a species to particular virus, if the virus itself is unavoidable.

In the experiment of Serge Daan and co-authors[22] the best fitness and lifespan occur in homozygous mutants with heritable epigenetic compensation, the second – in wild-types without heritable epigenetic compensation, the third – in heterozygous animals, and the less fit are wild-types with heritable epigenetic compensation, because heritable epigenetic compensation is optimized for mutants, but not for wild-types (**Supplementary Fig. 1**).




**Supplementary Fig. 1** | Transgenerational epigenetic compensation converts mutants into hopeful monsters. Transgenerational epigenetic compensation enhances viability of mutants and, simultaneously, in a random breeding (panmictic) population it suppresses viability of wild-types. Segregation of mutants and wild-types becomes beneficial for both of them.

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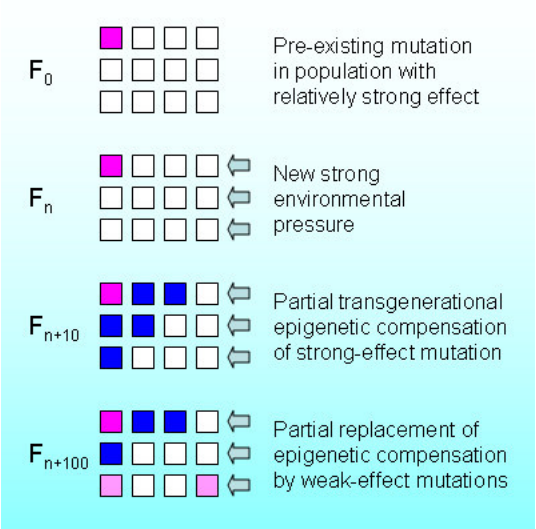
In order to increase average fitness of mutants with transgenerational epigenetic compensation and wild-types without transgenerational epigenetic compensation, it is favourable for mutants to be extracted from original population into a new subpopulation, which can be achieved in mammals initially by means of breeding preferences: mutant (-/-) ♀ × mutant (-/-) ♂; wild-type (+/+) ♀ × wild-type (+/+) ♂. In a geographic dimension it is favourable for mutants to migrate into the periphery of the old population. Geographic isolation is not a driving force of speciation, but a helpful mechanism, which helps mutants to avoid breeding with wild-types. Natural selection is not a driving force of evolution, but a part of evolutionary process, whereas an outcome of any episode of speciation depends on efficacy of epigenetic compensation, its heritability, proportion and spatial distribution of mutant animals in population, acuity of environmental change and level of environmental pressure, its temporal dynamics, and other factors. If mutants are absent in population, an extremely high environmental influence can induce heritable epigenetic compensation in wild-types, but in this case population will evolve as a whole, without speciation.

If some mutants are present in sufficient quantity, heritable epigenetic compensation will convert mutants into hopeful monsters and with a help of breeding preferences new subpopulation will be formed. These two subpopulations and, then, two species are not equal: one of them is old and another one is new, formed by hopeful monsters. Originally hopeful monsters were formed from mutants by heritable epigenetic compensation of disturbed functionality. In further generations heritable epigenetic changes will be replaced by genetic changes through genetic assimilation of acquired characters. Heritable epigenetic changes in gene expression will be exchanged for genetic changes in regulatory sites, probably for many changes, each with relatively small (subtle) effect. Heritable epigenetic compensation facilitates genetic assimilation of primary heritable epigenetic compensation in mutants the same way as it does with respect to any acquired character.

Heritable epigenetic compensation of disturbed functionality increases fitness and lifespan of homozygous mutants up to absolutely new, previously impossible, level – it converts homozygous mutants into hopeful monsters. In a random breeding population the same epigenetic compensation, being optimized for mutants, disrupts fitness and lifespan of wild-types. Non-random mating, namely (-/-) ♀ × (-/-) ♂ and (+/+) ♀ × (+/+) ♂, becomes preferable. The discrimination of mutant and wild-type prospective mates by females, the discrimination of males with and without heritable epigenetic compensation by females, the self-recognition of females as mutant and wild-type (or with and without heritable epigenetic compensation), the migration of mutants to the periphery of the original population and the partial geographic isolation are beneficial for both wild-types and mutants. Note that the first generation hybrids are healthy and fertile, and only slightly less fit, but problems appear in the later generations ($F_2$ generation hybrids can be weak and sometimes cannot produce viable offspring due to the segregation of mutation and heritable epigenetic compensation). Mammalian brain, olfactory system and other sensory systems should be able to handle this relatively complex situation, because they should be helpful in choosing an appropriate and probably the best possible mate.



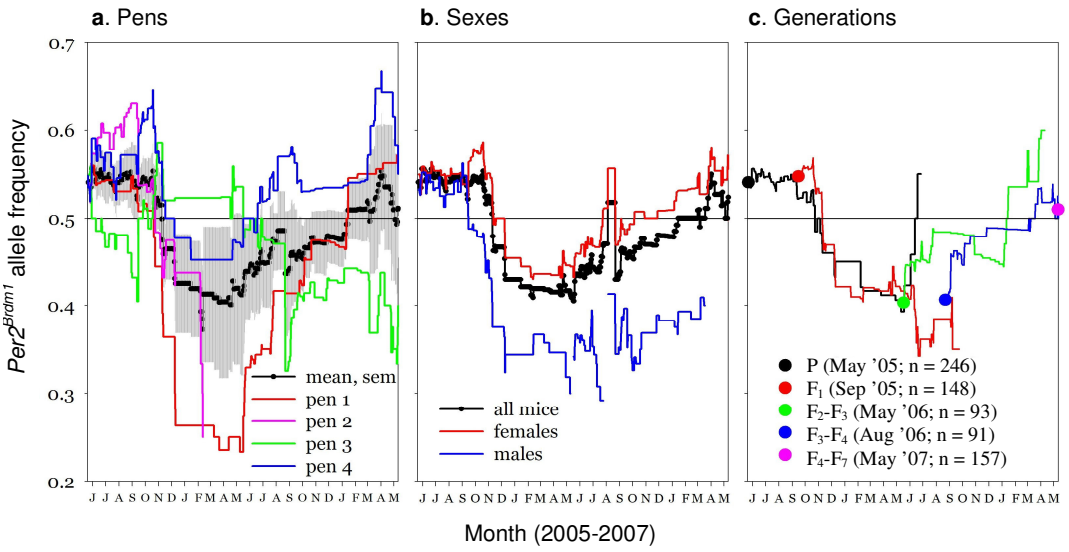

**Supplementary Fig. 2** | Transgenerational epigenetic compensation and its genetic assimilation. Each small square represents schematically one hereditary unit with its genetic or epigenetic modification (colour). Wild-type units are white. Each cluster of 12 small squares represents the hereditary basis of chosen functional system. Phenotypic traits are NOT shown in this figure. They are different in females and males.

All above-mentioned factors facilitate speciation. Reproductive isolation appears mainly not due to some external factors, like geographic isolation, but because it is helpful for successful survival of both new and old species. Heterozygous animals with and without heritable epigenetic compensation have typically intermediate lifespan between mutants and wild-types (mentioned above mutants and wild-types can be with or without heritable epigenetic compensation themselves). Sometimes low fitness of hybrids was discussed as a necessary prerequisite of speciation without geographic isolation. As we can see here, supposed "low fitness" of hybrids is not required for speciation in accordance with above-mentioned mechanism. In addition, it is known that first generation hybrids have very often the increased fitness and better learning abilities than both parental stocks – so called hybrid vigour. Behavioural hybrid vigour can be dramatically enhanced by early in life enrichment of living conditions – this feature is a result of active development; the initial combination of genes is important, but the active development is important also (see pp. 65-71[12]).

Temporal geographic isolation, proposed by the theory of punctuated equilibrium[27,38-40] and discussed earlier by Ernst Mayr[41], will work for evolution only if the hopeful monsters will be concentrated in the isolated subpopulation, not just some randomly chosen individuals from the original population.

Heritable epigenetic compensation not only supports mutants (*e.g.* increases their lifespan), but it suppresses wild-types (*e.g.* decreases their lifespan) in a random breeding population. That is why the appearance of a new species is a so rapid process.

It becomes clear why a new species originates not from main the most advanced, well adapted group, but from some small satellite group of animals (probably mutant animals). It occurs because epigenetic compensation begins earlier in mutant animals. Epigenetic compensation is actively working in mutant




**Supplementary Fig. 3** | Day-to-day changes in the frequency of the *Per2*$^{Brdm1}$ allele in mice under semi-natural outdoor conditions. Four independent random breeding (panmictic) populations were kept in 4 pens during two years. Gene frequencies were only calculated when 10 or more individuals were present. (**a**) Four pens separately and the mean of the four pens (black dots) +/- standard error (grey area). (**b**) Two sexes separately and combined (black dots). (**c**) Five cohorts separately: P, $F_1$, $F_2$-$F_3$, $F_3$-$F_4$, $F_4$-$F_7$. Large symbols indicate the initial frequency in each cohort. Starting from the $F_2$-$F_3$ generation, the generation numbers should be considered as an estimate. Data from the experiment of Serge Daan and co-authors (2011)[22].

subpopulation, whereas main population is still "sleeping", it is doing nothing in terms of epigenetic compensation. Several generations are necessary for development of epigenetic compensation. Not many, 3-7 generations can be enough. This is a very short period in view of evolutionary process. However it means extremely fast appearance, extremely fast formation of a new variety. And at the behavioural level, mutants will prefer to breed with mutants and they will avoid breeding with wild-types, whereas wild-types will prefer to breed with wild-types and they will avoid breeding with mutants, because after the appearance of epigenetic compensation the most viable animals will be either wild-types without heritable epigenetic compensation (old species) or mutants with heritable epigenetic compensation (prospective new species). Any mechanism that prevents breeding between old and prospective new species will be beneficial for both of them, at least during this evolutionary period, during the establishment of a new species.

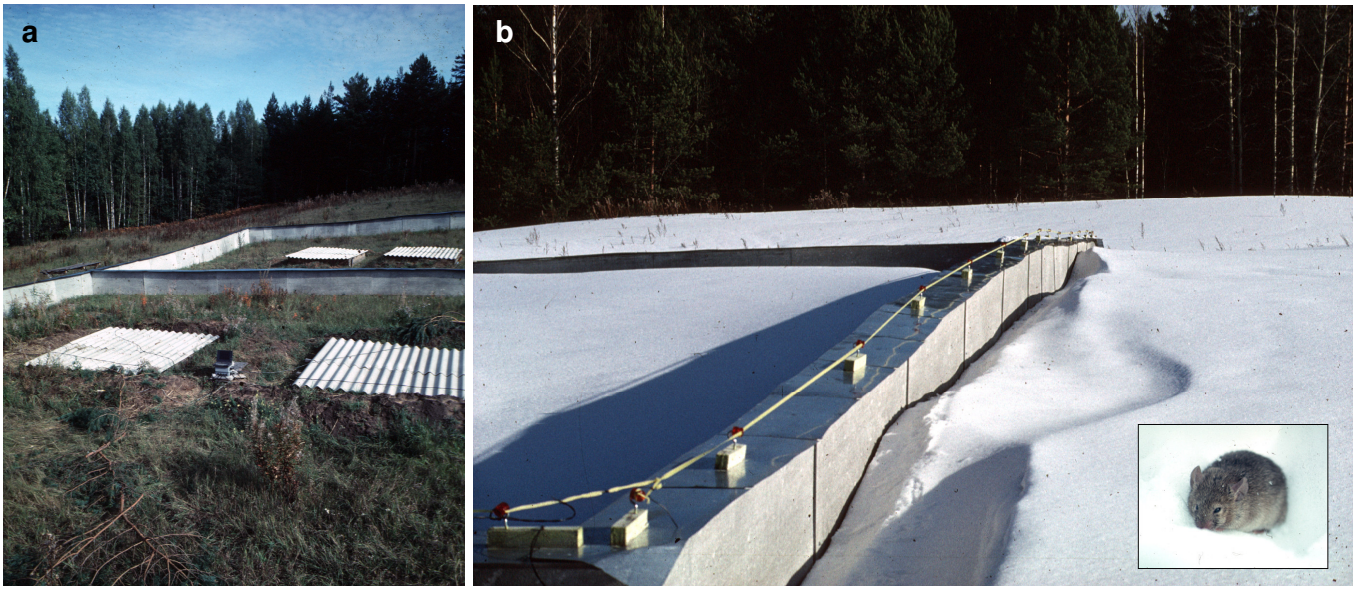
**Supplementary Fig. 2** illustrates the replacement of transgenerational epigenetic compensation by mutations through genetic assimilation. For simplicity the gender-related differences are not expressed in this figure. However it is extremely important that the phenotypic results of the transgenerational epigenetic compensation are not equal in males and females. Transgenerational epigenetic compensation is more beneficial for females. Namely, epigenetic compensation can be practically complete in females and, thus, they can be very well adapted, but males will be still under strong environmental pressure. Natural selection will be active in males and genetic assimilation of epigenetic compensation will proceed through selection of males. Thus, one population can have: a) perfectly adapted females; b) males under strong environmental pressure. This environmental pressure, acting on males, will lead to further improvement of transgenerational epigenetic compensation (developing in males, but useful for females) and, simultaneously, will lead to genetic assimilation in this population, working through natural selection of males, but useful for both sexes.

It seems that the transgenerational epigenetic compensation can never produce a perfect male phenotype. Males are permanently busy with genetic assimilation. Natural selection of males improves the genetic basis of the whole population, without diminishing the number of descendants in this population. The above-mentioned distribution of evolutionary functions between males and females entails very high efficiency of evolutionary change. By the way, the results of Serge Daan and co-authors[22], shown in our **Supplementary Fig. 3b**, are nicely compatible with the described above roles of males and females in evolution.

## Experiment of Serge Daan and co-authors (2011)

The process of formation of transgenerational epigenetic compensation is not a straightforward one. It is a complex process which depends on many factors, sometimes seemingly subtle. In the experiment of Serge Daan and co-authors[22] there were 4 independent pens (4 independent populations). All of them should be technically identical (**Supplementary Fig. 4**). And we can estimate the reproducibility of the results looking at the differences between these 4 pens (**Supplementary Fig. 3**).

Population of mice in the Pen 2 was eliminated by some ground predator due to high snow (**Supplementary Fig 3a**, see the first "F" – February 2006). In the other three pens the *Per2*$^{Brdm1}$ allele frequency recovery was observed only in the Pen 1 and Pen 4 (**Supplementary Fig. 3a**), whereas in the Pen 3 no initial drop in allele frequency was observed during the first year and afterwards we can see steady decline during the second year. Probably, epigenetic compensation was not formed in the Pen 3




**Supplementary Fig. 4** | Semi-natural environment for mice. (**a**) Summer, the installation of experimental setup several years before the beginning of the experiment of Serge Daan and co-authors[22], in 1998. This experimental setup had long evolution *per se*, it was established and persistently improved by Hans-Peter Lipp. (**b**) Winter, before the Daan's experiment. There are four pens 20 × 20 m each. Slate walls (with electric fence on top) are 1 m over and 0.5 m under the ground. Each pen has two shelters 3 × 2 × 0.7 m each. Water and standard food for mice were provided by humans[22].

(despite rather similar external conditions in all three pens). Thus, the observed reproducibility of these results (two of three) is not very high and it is rather close to the reproducibility of the transgenerational epigenetic experiments with paternal drug treatment. In the Pen 2, where the population was eliminated by some predator, we can see the same initial pattern as in the Pen 1 and Pen 4, and someone can expect that in the absence of predator the Pen 2 might have been similar to the above mentioned Pen 1 and Pen 4. There is some possibility that in the severely deviated Pen 3 some other epigenetic compensation was formed, because something has helped to keep relatively high frequency of mutant allele during the first winter, even after initial summer mutant allele frequency drop. But during the next summer the mutant allele frequency drop was unavoidable (**Supplementary Fig 3a**). Someone can speculate that in the Pen 3 epigenetic compensation was optimized "for winter", whereas in all other three pens it was optimized "for summer", but we have not enough solid data for this conclusion yet.

Number of mice (*n*) for all 4 pens combined is shown in the **Supplementary Table**.

**Supplementary Table** | Number (*n*) of *Per2*$^{Brdm1}$ mice in the field

| | Initial release 2005--05-21 | Recapture 1 after 116 days 2005-09-14 -2005-09-22 | | Recapture 2 after 236 days 2006-05-08 -2006-05-13 | | Recapture 3 after 86 days 2006-08-02 -2006-08-20 | | Recapture 4 after 282 days 2007-05-11 -2007-05-16 | |
|---|---|---|---|---|---|---|---|---|---|
| | P | P | **$F_1$** | P-$F_1$ | **$F_2$-$F_3$** | P-$F_3$ | **$F_3$-$F_4$** | P-$F_4$ | **$F_4$-$F_7$** |
| ♀ (+/+) | 28 | 11 | **19** | 12 | **20** | 8 | **21** | 5 | **24** |
| ♂ (+/+) | 26 | 3 | **12** | 4 | **14** | 3 | **23** | 1 | **22** |
| ♀ (+/-) | 53 | 25 | **45** | 19 | **38** | 24 | **34** | 15 | **55** |
| ♂ (+/-) | 67 | 7 | **38** | 8 | **20** | 2 | **27** | 3 | **31** |
| ♀ (-/-) | 40 | 13 | **35** | 5 | **9** | 8 | **12** | 6 | **22** |
| ♂ (-/-) | 36 | 6 | **13** | 1 | **6** | 2 | **8** | 1 | **15** |
| Total | 250 | 65 | **162** | 49 | **107** | 47 | **125** | 31 | **169** |
| % *Per2*$^{Brdm1}$ | 54.4 | 53.8 | **55.2** | 39.8 | **41.1** | 48.9 | **40.4** | 51.6 | **47.3** |
| *P* | | 0.79 | | 0.82 | | 0.15 | | 0.54 | |
| Total | 250 | 227 | | 157 | | 172 | | 200 | |
| % *Per2*$^{Brdm1}$ | 54.4 | 54.85 | | 40.71 | | 42.73 | | 48.00 | |

Initial release at the age of 76 days. New individuals born in the field are indicated in bold type by generations **$F_1$**, **$F_2$-$F_3$**, **$F_3$-$F_4$**, **$F_4$-$F_7$**; previously marked individuals – P, P-$F_1$, P-$F_3$, P-$F_4$. *P*-values (*Chi*-square, df = 1) show the difference in the number of mutant alleles (-) in the old cohort survivors against new cohorts caught in the same trapping session. Data from the experiment of Serge Daan and co-authors (2011)[22].

## Experiment of David Crews and co-authors (2007)

In the experiment of David Crews and co-authors[25] $F_2$ generation descendants of prenatally vinclozolin-treated males and females were tested in mate-preference test at the age of 3-4 months.

In mate-preference test an animal was investigating two other animals of opposite sex during 10 min through a wire mesh (one animal from control subline and another one from vinclozolin-treated subline). The preference in investigation was classified as a mate preference.

During mate-preference test, at the age of 3-4 months, males from vinclozolin and control sublines did not show any preference for vinclozolin or control subline females. However females from both vinclozolin and control sublines have investigated more males from control subline ($P < 0.026$, total time spent in behaviours directed toward the stimulus males [wire mesh, facial investigation, and Plexiglas]; Fig. 2*A*[25]).

Prenatal vinclozolin treatment, contrary to neonatal thyroxine treatment or young adult morphine treatment, is a very early influence; it takes place when particular functional system[28] may not have enough developed feedback loops for correction of phenotypic effects. This leads to maladaptive disease phenotype, which does not vanish during several untreated generations[8].

However during odour-salience test, applied at the age of 15 months, all females can analyze the odour only and they can not

see reduced testis size and tumors of vinclozolin subline males. That is why the decision of experimental females to prefer experimental males as potential mates looks reasonable. At the given age of 15 months both females and males with transgenerational epigenetic compensation can be typical hopeful monsters. Young animals (age 3-4 months) behave differently and it is reasonable also.

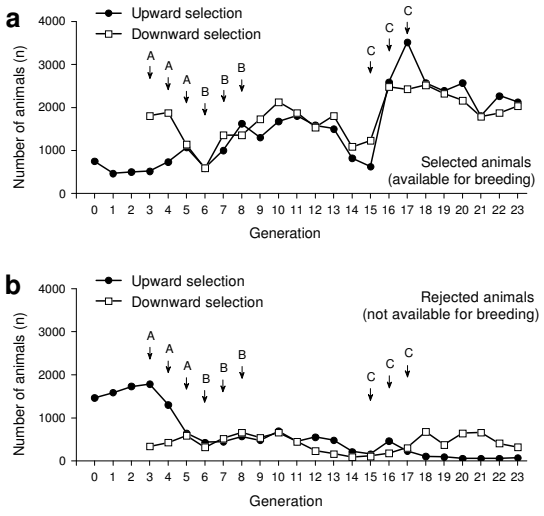
## Conrad Waddington and genetic assimilation

Genetic assimilation was first introduced by Conrad Waddington as genetic assimilation of an acquired character[29]. We will discuss genetic assimilation of heritable epigenetic compensation. But first of all we will describe classic genetic assimilation of an acquired character and we will show how heritable epigenetic compensation can facilitate genetic assimilation of an acquired character.

During each step of genetic assimilation the genetic fixation of a particular feature occurs by means of collection of pre-existing mutations in the population into one organism, but if some mutation occurs in the course of experiment and it is useful for particular stage, it will be collected also. Typically, however, the result of genetic assimilation is several mutations in several regulatory sites of different genes, each with subtle effect on phenotype, collected into one genotype.

What is the main feature of genetic assimilation, proposed by Conrad Waddington, as an evolutionary mechanism? The subdivision of a process into several local stages makes possible the process of collecting of those mutations, which will never be collected without genetic assimilation. At the beginning of each stage a specific functional system[28] is created or modified, and collectable mutations are useful only in the frame of this functional system.

If we compare the stochastic appearance of some useful mutation with the process of genetic assimilation, proposed by Waddington, we will see that a stochastic useful mutation, useful in as-is state, will be a very rare event, because it should be highly specific and it should combine in itself very specific features, whereas in the case of genetic assimilation at any its stage roughly 50% of process-related mutations are useful and pull the process in correct direction and the rest 50% mutations are not good, because they are pushing the process in the opposite direction. Requirements for collectable mutations in the process of genetic assimilation are relatively low. That is why this process can go relatively fast despite its several stages. The existence of several stages not attenuates, but accelerates evolutionary process. And it makes possible the appearance of functional results those can not be achieved at all without genetic assimilation.




**Supplementary Fig. 5** | Number of selected and rejected animals in upward and downward selection lines. Note that fluctuations in the number of selected animals were not a result of stronger or weaker artificial selection, but they were a result of different numbers of animals, available for selection. Data from the experiment of Conrad Waddington (1953)[29].

It is very important that the process of genetic assimilation of hereditary epigenetic compensation can be facilitated by local hereditary epigenetic compensation at each specific step. So, with respect to more global heritable epigenetic compensation, which is supported by more or less permanent external pressure during significant period of time, local heritable epigenetic compensation acts like with respect to an acquired character.

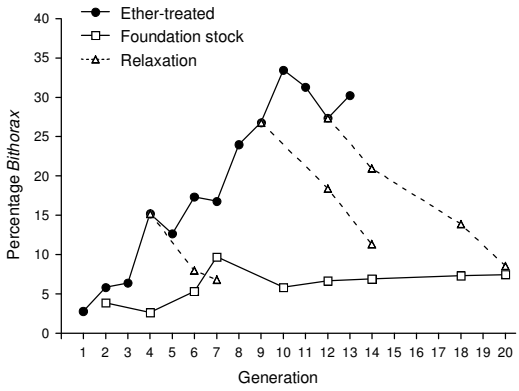
Thus, heritable epigenetic compensation facilitates genetic assimilation of an acquired character. "Facilitates" means not only "accelerates", but it makes possible the appearance and further existence of results, those can not be achieved without such facilitation.

**Supplementary Fig. 5** shows the number of selected and rejected animals in upward and downward selection lines. And we can see that the fluctuations in the number of selected animals were not a result of stronger or weaker artificial selection, but they were a result of different numbers of animals, available for selection. Thus, above-mention fluctuations in the number of animals, available for selection, were the results of application (probably – unintentional application) of some stressor to all populations under discussion (in addition to heat-shock treatment, of course). The stress, induced by this unidentified stressor, has led to activation of the process of transgenerational epigenetic compensation, observed during episodes B and C in the **Fig. 4**.

Thus, in the experiment of Conrad Waddington (**Fig. 4**) we can see a combination of classic genetic assimilation with transgenerational epigenetic compensation.

Thirty years later, in 1983, in a different experimental setup, but also in *Drosophila*, Mae-Wan Ho and co-authors[42] have shown in the frame of experiment with multi-generational treatment, but without any artificial selection, that it is possible to observe pure transgenerational epigenetic compensation, practically without any classic genetic assimilation. It was shown in the experiment with so-called "relaxation" of treatment, in the experiment with induction of *Bithorax* phenocopy in *Drosophila melanogaster* by ether treatment (**Supplementary Fig. 6**).

The relatively fast dissipation of the acquired phenotype during the relaxation stage of this experiment (**Supplementary Fig. 6**) reminds the dissipation of transgenerational epigenetic compensation in the successive untreated generations in the experiments with neonatal paternal L-thyroxine treatment and young adult paternal morphine treatment[6]. Mae-Wan Ho and co-authors (1983)[42] have mentioned that these results are reminiscent of *Dauermodifikationen* of Victor Jollos (1921)[43]. *Dauermodifikationen* (long-term modifications) are a class of




**Supplementary Fig. 6** | Induction of *Bithorax* phenocopy in *Drosophila melanogaster* by ether treatment without artificial selection (solid circles) and relaxation of ether treatment (unfilled triangles). All shown dots are formed by ether-treated animals. Data from the experiment of Mae-Wan Ho and co-authors (1983)[42].

environmentally induced modifications which linger on, disappearing gradually over a number of generations after the inducing environmental regime is discontinued.

## Neo-Darwinism

What can we say about the residues of neo-Darwinism[35-37] in view of new evolutionary theory? Not much, really. Natural selection remains a part of evolutionary process. However natural selection is not a whole real process. Obviously, it is not a "driving force" or "directing force" of evolution, but it is a solid process inside general process of evolution (this statement is very close to the original view of Alfred Wallace[33], not Charles Darwin[34]). Nobody will reject natural selection. But we can not say the same about other quasi-philosophical and pseudo-scientific parts of neo-Darwinism, which forbid the existence of any other evolutionary process in view of natural selection. If someone still would like to speak in terms of driving forces of evolution: living beings would like to be alive, not dead, – that's the "driving force". Heritable epigenetic compensation is more important for this task, it is more important for speciation and it is more important for progressive evolution than natural selection. In other words: local processes, surrounding epigenetic compensation, and epigenetic compensation *per se* have more impact upon final result of any evolutionary episode than natural selection during the same time interval. The results of transgenerational epigenetic compensation determine further route of natural selection, but not *vice versa*. Why should we always say that natural selection is a "directing force" of evolution? Natural selection is a part of evolutionary process – nothing more, nothing else.

## Classic genetics

What can we say about classic genetics in view of evolution and new evolutionary theory? The role of classic genetics remains the same. Previous theory described evolution as a transition from one genetic constitution to the next genetic constitution. New evolutionary theory also says that initial genetic constitution of a species will be finally replaced by the next genetic constitution (without involvement of any epigenetic heredity into initial and final states). All heritable epigenetic changes will be finally replaced by genetic changes through genetic assimilation. Someone can say: if the initial states and the final results are anyway purely genetic, who cares what was in between? The answer is: heritable epigenetic compensation and genetic assimilation not only accelerate evolutionary process in several orders of magnitude, but they make possible a lot of evolutionary results, those were previously physically impossible to achieve (by means of natural selection or by means of its combination with different forms of Lamarckism). As we can see, the proposed evolutionary theory is not a combination of Darwinism with any known subtype of Lamarckism – it is an entirely new thing. However, some Lamarckian processes are allowed inside new theory through epigenetic inheritance and genetic assimilation, but it is not its new part; these possibilities were discussed previously by other authors[18].

In a random breeding population heritable epigenetic compensation of a mutant allele increases lifespan of homozygous mutants and decreases lifespan of wild-types, because typically epigenetic compensation is localized in the loci, which are independent, *i.e.* not in the same locus as mutant allele. Transgenerational epigenetic compensation works in all living beings those have epigenetic inheritance.

## Natural selection

After discovery of heritable epigenetic compensation, natural selection should be classified as a part of evolutionary mechanism, not as a main mechanism and not as a main driving force of evolution. The outcome of an evolutionary episode is dependent mainly on the local features of epigenetic compensation. Different stochastic events during this evolutionary episode can change the outcome dramatically. It is not correct to say that the outcome is dependent only on particular mutation and external conditions. Heritable epigenetic compensation which can convert some mutants into hopeful monsters is a main factor of evolution. Natural selection is just a part of evolutionary process.

Sometimes we can meet the argument that heritable epigenetic compensation and epigenetic inheritance in general are the results of natural selection. This statement is partially correct. Heritable epigenetic compensation is a result of previous evolutionary history. And this evolutionary history includes both natural selection and previous heritable epigenetic compensations (a list of previous heritable epigenetic compensations).

The role of previous history is more important for new evolutionary theory than for neo-Darwinism, because in the neo-Darwinism the outcome of an evolutionary episode was dependent on current situation (genetic contents of population plus current environmental pressure) and on appearance of new mutations, whereas in the new evolutionary theory the outcome of evolutionary episode is dependent mainly on the efficacy of heritable epigenetic compensation (the efficacy which was formed by the previous evolutionary history). Further natural selection, further course of natural selection and the result of natural selection are completely determined by the efficacy of heritable epigenetic compensation. The direct involvement of heritable epigenetic compensation into evolutionary response to the environmental pressure makes possible the observation of many nomogenetic regularities in evolution (nomogenesis – evolution determined by law)[31]. Nomogenetic regularities were

previously incompatible with neo-Darwinism, but they are compatible with new evolutionary theory.

### Genetic assimilation and "organic selection"

And, of course, the genetic assimilation of heritable epigenetic compensation is very important also. Genetic assimilation of heritable epigenetic compensation is not only a classic genetic assimilation, but it is genetic assimilation, facilitated by additional processes of heritable epigenetic compensation.

Genetic assimilation and canalization of development are processes moved by the same unitary force. All known attempts to explain assimilation by relatively simple threshold-based model are interesting only as intellectual exercises, because in the real nature the assimilation is linked with canalization. What is the fun to declare that the threshold model is "sufficient" for explanation, if in the real nature the less simple, but more efficient, scenario is realized?

The same we can say about "organic selection", proposed independently by James Mark Baldwin, Lloyd Morgan and Henry Fairfield Osborn at the end of 19th century. The statement that "organic selection" of Baldwin and "genetic assimilation" of Waddington are "practically the same" is not correct and it sounds for us like an unacceptable simplification. The process proposed by Waddington is not only much more detailed, but it is much more efficient than previously described ones. We will use exclusively Waddington's interpretation of genetic assimilation for further discussion.

### Epigenetic compensation *vs.* epigenetic inheritance

We prefer the term "heritable epigenetic compensation of disturbed functionality" instead of just "epigenetic inheritance", because "epigenetic inheritance", being more general term, has led to serious misunderstanding. It forces us to assume that epigenetic inheritance and genetic inheritance, despite their known different molecular mechanisms, are functionally equivalent or similar: some features are heritable through genetic mechanisms, some others – through epigenetic ones. However the main role of epigenetic inheritance in evolution (and ontogenesis) is hereditary compensation of disturbed functionality. It is a temporal compensation of disturbed functionality, during a few, sometimes very few consecutive generations. That is why we have introduced a new distinction – the distinction between "epigenetic inheritance systems" and "hereditary epigenetic compensation of disturbed functionality". Of course, the last one is a part of the first one, but it is the most important part. We can imagine that under some experimental conditions epigenetic inheritance can play the role of genetic inheritance (during some relatively short time period), but we know that under such conditions as soon as possible epigenetic inheritance will be replaced by genetic inheritance. It will be done through genetic assimilation – through genetic assimilation of heritable epigenetic compensation of disturbed functionality.

### Richard Goldschmidt and systemic mutations

Richard Goldschmidt has written in 1940 that hopeful monsters should appear through so-called "systemic mutations"[4] – general rearrangement of chromosomes which changes expression of many genes simultaneously ("simultaneously" means "during one or several generations"). Now we see that he was absolutely right at the functional level. In fact, a combination of important mutation with heritable epigenetic compensation, distributed between many genes of genome, forms a complete functional equivalent of a "systemic mutation".

Another way of appearance of hopeful monsters, also proposed by Richard Goldschmidt, – mutations in the important genes, playing key role during development[4], in reality also belongs to the same category of "systemic mutations", because these mutations will also entail heritable epigenetic compensation of disturbed functionality: heritable epigenetic changes in many other genes. During further course of evolution heritable epigenetic changes will be step by step replaced by regular genetic changes – replaced by mutations in regulatory sites of functionally linked genes, mutations with typically subtle effect. Mutations in the same gene, which has heritable epigenetic compensation, are possible in principle, but the probability of their appearance in the regulatory sites of exactly the same gene is not very high (really relatively low).

### Lamarckism and psycho-Lamarckism

We show here that the new evolutionary theory is compatible not only with Lamarckian processes (in a modified, *i.e.* not original, interpretation)[6,12], but with psycho-Lamarckian processes also. Suppose that we have a case with strong psychological influence that induces psycho-somatic disease (measurable physiological changes in the organism). If this disease is rather severe, it can induce transgenerational epigenetic compensation (partial, as usual). In a raw of generations hereditary epigenetic compensation can be converted by means of genetic assimilation into genetic changes.

In the experiments of Nikolai P. Studentsov[44], reported by Ivan P. Pavlov[45], the extremely loud sound, applied to the young mice (the loudness up to the level of audiogenic seizure induction) has produced hereditary change in a raw of 7 treated generations, probably of epigenetic nature (*i.e.* relatively fast; see discussion in the Supplementary Information of the ref.[6]). The exposure of mice of particular age (P21-P30) to loud sound is known to be able to induce a pathological reaction – audiogenic seizures. On the other hand, it can be considered as an example of strong psychological influence.

### Cost factors of epigenetic compensation

If heritable epigenetic compensation is a so strong tool, why is it replaced by normal mutations in a raw of consecutive generations? Real reasons are not known yet, but we can guess that some cost factors of heritable epigenetic compensation are relatively high for an organism and population. One of such cost factors can be relatively high dominance of heritable epigenetic compensation. If there is a mutation in a regulatory site with subtle, but negative, effect, and particular animal is heterozygous, the effect of this mutation on phenotype will be negligible. But if there is a heritable epigenetic compensation and the animal is heterozygous with respect to epigenetically modified locus, negative phenotypic effects can be detectable. At least, we have seen many negative effects in the progeny, obviously heterozygous with respect to all epigenetic changes, obtained from drug-treated males and drug-naïve females. Thus, at least some effects of transgenerational epigenetic compensation are dominant.

The second reason of replacement of heritable epigenetic compensation by a set of mutations in regulatory sites of

different genes (each mutation with relatively small effect on phenotype) is also known from the experiments with paternal drug treatment. As a result of paternal drug treatment, usually only a few components of an acquired adaptation become epigenetically heritable, whereas the majority of components are not linked to any heritable change. That is why in the untreated descendants it is possible to observe phenotypic inversion – some quantitative phenotypic traits are changed in the opposite direction in comparison with paternal ones. It means that under natural conditions the external influence is still necessary for formation of the adaptive phenotype and the partial heritability of epigenetic compensation is not sufficient to form a complete acquired character in the progeny.

We guess that only the most critical components of an acquired compensation become epigenetically heritable (as a result of transgenerational epigenetic compensation of disturbed functionality), whereas for formation of other components (probably less critical) in the progeny, an appropriate external influence should be applied during ontogenesis of these descendants. As a final result of evolutionary episode we expect the genetic fixation of all components of useful phenotype. And this genetic fixation will be achieved by means of the genetic assimilation of the above-mentioned complex, consisted of two parts: 1) the heritable components of the epigenetic compensation and 2) the acquired components, produced by the direct influence of the external factors.

### "Ideal final result" and "ideal functional system"

In many places of the text we use the term "partial compensation" or "incomplete compensation". These terms force us to assume that in some other situation the "complete compensation" or "absolutely adapted state" can be achieved. This is a wrong presupposition concerning real living nature. Each organism consists of a set of functional systems, systems which are trying to reach or organize some positive results with a help of feedbacks (an important part) and with a help of other means. Each functional system is considered by us strictly as it was proposed by Peter Anokhin[28] (so, it is a physiological, but not a speculative philosophical category). Each functional system has positive and negative effects (cost factors). An ideal adaptation or ideal final result can be achieved only if we have functional system without any negative effect or cost factors. Real functional systems can evolve in this direction, but ideal final results usually will not be achieved (however some funny examples when positive effects are "free" can be collected).

Thus, there is always some internal force, which can promote evolution even under stable external conditions. The idea about "ideal final result" and "ideal system" was taken by us from the Theory of Inventive Problem Solving, developed by Genrich Altshuller[46-48]. For technical systems it is rather clear that they can be improved without dramatic increase of environmental pressure. Evolution of any well-known technical system, like bicycle, can serve as an example. The increased "requirements", which are usually applied by humans to technical systems, are more close to psycho-Lamarckian "wish" than to "environmental pressure".

### Nomogenesis

If we have steady increase of environmental pressure, applied to biological system and to particular functional system, all its components will become insufficient for desired function not simultaneously, but, first will be discovered the weakest component, which should be improved or replaced, then – the next one, and so on. Evolution of given functional system under condition of steady increase of environmental pressure is determined by internal features of particular functional system, its internal regularities. It means that evolution of this functional system will be mainly nomogenetic, where nomogenesis is defined as evolution on the basis of regularities, evolution determined by law, as it was proposed by Leo Berg[31]. Nomogenesis must be a part of evolutionary theory and this part was not covered in our article.

The sequential improvement of components of functional system, proposed by Nomogenesis, is quite opposite to the stochastic improvement of randomly chosen components of functional system, proposed by the previous theory (neo-Darwinism). The stochastic improvement of randomly chosen components was supported by neo-Darwinism mainly due to the general lack of efficiency of evolutionary process, taking place in accordance with this theory: any improvement should be accumulated – otherwise the evolutionary process will be too slow. Now it is clear that the improvement of the most critical component, not anyone, composes evolution of functional system during each evolutionary episode. Sometimes it seems that "load" is equally distributed between different components of functional system (there is no "rate-limiting" stage or reaction). This observation is correct only for evolutionary standard load. If the load will be increased, a few components will become more critical than the majority of others.

Evolution through sequential improvement of critical components was completely missed previously (was not discussed seriously) due to the extremely low general efficiency of evolutionary process, proposed by neo-Darwinism. Efficiency of a biological process (including evolutionary one) is defined as a ratio of its positive effect to cost factors (including negative or bad effects of the same process). This ratio for a particular functional system usually becomes better and better during phylogenesis and during some stages of ontogenesis. This principle was introduced as "The Principle of Efficiency" by Alexander Ugolev and it was developed using his own as well as other available physiological material[49,50]. The books of Alexander Ugolev (1985, 1987)[49,50] are not translated from Russian into English yet, including the last one: "Natural Technologies of Biological Systems" (1987)[50].

### Leo Berg and precession of characters

Transgenerational epigenetic compensation and its further genetic assimilation can explain (in some cases, at least) the phylogenetic acceleration, or the precession of phylogeny by ontogeny, discovered by Leo Berg in 1922[31].

By the term "precession of characters" Leo Berg understands the following series of phenomena (pp. 73-74[31]):

(1) *Paleontology* teaches us that in young forms characters not infrequently occur which, while disappearing with advancing age, reappear in more recent geological deposits both in the young and in the adult. In their development the young seem to be pushing ahead of their time.

(2) From the study of *embryology* we may gather that the larvae not infrequently possess morphological and physiological characters of a higher organization, which vanish in the adult

state. The adult thus seem to lag behind the young stages of their development.

(3) *Comparative anatomy* reveals the occurrence, in the more lowly organized groups, of characters which are peculiar to groups standing higher in the system. It often happens that in tracing the paleontological evolution of a group we observe that characters belonging to it already occurred in a lower group before the higher one came into existence: as, for instance, in the case of some Paleozoic organism which is beginning to shadow forth what will in time be fully developed in those of the Mesozoic era.

The precession of characters may thus be observed to occur in the development of both the individual (ontogeny) and of entire groups (phylogeny)[31].

In the course of evolution of a given species, when an acquired character has became inherited as a transgenerational epigenetic compensation, and further evolution proceeds through genetic assimilation of this transgenerational epigenetic compensation, it is possible the appearance and selection of a mutation with useful (in general), but rather strong influence on phenotype with multiple consequences (not all mutations, selected in the course of genetic assimilation, are the ones with subtle effect on phenotype). For example, in the experiment of Waddington with genetic assimilation of *bithorax* phenotype, such mutation with strong effect was found[51].

If any mutation with strong effect is found by genetic assimilation, this mutation will immediately induce the next wave of transgenerational epigenetic compensation.

If we compare the final result of genetic assimilation with the one of transgenerational epigenetic compensation, without any time limitation, we know that the genetic assimilation can achieve better optimization of phenotype, *i.e*. many single-nucleotide substitutions in many regulatory sites of several genes can form more precise phenotype, than the phenotype which can be formed by transgenerational epigenetic compensation, the compensation which is typically doing its job just "fast and dirty", with a crude and rather rough result.

In the instance of a new mutation with strong effect, it will be compensated initially by transgenerational epigenetic compensation. And this compensation, being unable to provide the precise adjustment of the whole ontogenesis, will try just to diminish all effects of mutation at late stages of ontogenesis. The effects of this mutation will be however visible at early ontogenetic stages, but will be completely disappeared later, at the adult stages of ontogenesis. However when transgenerational epigenetic compensation will be slowly (sometimes – very slowly) replaced by subtle-effect mutations in the course of genetic assimilation, the mutation will become visible in the adult phenotype, but without its previous negative physiological effects. The replacement of transgenerational epigenetic compensation by subtle-effect mutations in the course of genetic assimilation can form the "precession of characters" in evolution.

## Evolution of evolutionary theories

Evolutionary theories evolve about the same way as real biological objects. Each new evolutionary theory predicts the existence of more efficient evolutionary process than the evolutionary process, proposed by the previous evolutionary theory. For example, Lamarckian theory was replaced by Darwinian one, because Lamarckian theory predicted the existence of high cost factors – complex physiological mechanisms, mainly unknown, with unknown reliability and other unknown features, including possible disruption of descendant's ontogenesis due to simultaneous change of many hereditary factors; whereas clear mechanism was offered by the theory of Wallace-Darwin, which predicted slightly lower positive effects, but dramatically lower cost factors.

We believe that the principle of efficiency is important for development of any biological theory, not only evolutionary one: new biological theory should predict the existence of more efficient biological process than it was previously assumed[7].

The second important feature of any significant biological theory, – not only evolutionary theory, – the introduction of a new distinction into particular field of science, the distinction between real biological objects, which was previously missing or supposed to be of no importance. The distinction should not be absolutely new, but it should be new for given branch of science.

For example, the theory of natural selection has introduced distinction between well fit and not-so-well fit animals of the same population. Before Wallace and Darwin all animals in one population (of the same gender) were considered more or less equal from the standpoint of evolution. Or another example: Gregor Mendel has introduced distinction between dominant and recessive alleles. More recent example: in 1965 the differences between males and females in variability and canalization of their ontogenesis were recognized as an enhancer of the efficiency of natural selection by Vigen Geodakian[52].

Better canalization of ontogenesis in females can be illustrated by many experiments, including recent experiment with $p66^{Shc}$ mutant mice and chronic cold-exposure[53]. In this experiment adult wild-type (+/+) and mutant (-/-) mice initially had the following body weight (g). Females: 26.0 (+/+), 25.6 (-/-); $\Delta$ = 0.4 g. Males: 30.4 (+/+), 29.6 (-/-); $\Delta$ = 0.8 g. After chronic (52-day) cold-exposure (3 h *per* day at +4 °C) their body weight (g) was as follows. Females: 26.1 (+/+), 26.1 (-/-); $\Delta$ = 0.0 g. Males: 30.0 (+/+), 28.1 (-/-); $\Delta$ = 1.9 g. In males, the $p66^{Shc}$ mutation not only induced greater initial drop in body weight in comparison with females (0.8 g *vs*. 0.4 g), but chronic cold-exposure made this drop even more pronounced (1.9 g), whereas body weight of mutant females was somehow normalized.

We have shown that mutant and wild-type animals behave very differently with respect to transgenerational epigenetic compensation. Transgenerational epigenetic compensation starts with mutants, if they are present in population, and convert their previously unfair phenotypes into phenotypes of hopeful monsters – into the animals with better overall fitness than wild-types of the same population. The ontogenesis of wild-types initially remains canalized, epigenetic compensation is not working in wild-types. But afterwards, if the population remains a random breeding one, their fitness will be decreased, because the combination of wild-type genotype with transgenerational epigenetic compensation, developed for mutants, has decreased fitness with respect to previously naive wild-types (*i.e.* wild-types without any compensation).

Thus, we have introduced the distinction between mutants and wild-types from the standpoint of transgenerational epigenetic compensation. Different animals in one population (namely: mutants and wild-types) are not equal with respect to transgenerational epigenetic compensation. Their differences produce the basis for speciation. Previously the distinction

between mutants and wild-types was completely ignored in the field of evolutionary epigenetic inheritance[18] (as well as by Lamarckian theory[32]), despite its postulated importance[25].

Another distinction, widely used by us, is that an acquired compensation is divided into hereditary and non-hereditary components during each evolutionary episode[6,7]. Only one or several (probably the most important) components are inherited. This allows to keep main positive effects and to decrease disruptive collateral factors. The hereditary components of an acquired compensation form "transgenerational epigenetic compensation".

### *P.S.*

Transgenerational epigenetic compensation extends the class of mutations those can be used as a basis for hopeful monsters formation. This class includes now many mutations with initially negative impact on fitness and survival. However not all mutations in a given species can be successfully compensated or compensated with an overshoot by means of hereditary epigenetic changes. For example, *Per2*$^{Brdm1}$ mutants were successful[22], but *p66*$^{Shc}$ mutants, being placed in very similar semi-natural experimental setup, were not[53].

With respect to semi-natural populations it should be noted, first of all, that the positive results of a transgenerational epigenetic compensation can be present in a semi-natural population only starting from the $F_2$-$F_3$ generation. In a mouse or rat population these positive results can be detected, if present, only during the second year of experiment or later. Many mutations can be wiped out before this time period, and, of course, they can not be used as a basis for hopeful monsters.

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