Impact of Transgenerational Epigenetic Inheritance on Evolution

A Theoretical Perspective

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In recent years, calls have been made to revise the Modern Evolutionary Synthesis (MS) to include insights about the contributions of developmental selection, niche construction and nongenetic transgenerational inheritance to the process of evolution (Jablonka 2017). The MS view in its typical interpretation describes evolution as the change in heritable genotypic frequency within and between populations as caused by the processes of natural selection, genetic drift and population division (Ashe et al. 2021). This definition of evolution excludes important transgenerational heritable characteristics and the mechanisms that impact them, such as parental effects, environmental modification, and epigenetic change. However, there is growing evidence that epigenetic markers exist that are both transgenerationally inheritable and induce meaningful change in an organism's characteristics (Ashe et al. 2021, Jablonka and Raz 2009). This suggests that mechanisms of transgenerational epigenetic inheritance should be included in a comprehensive definition of evolution. This aligns with previous critiques of the MS that have suggested broader definitions of evolution, such as the "changes in the nature and frequency of heritable types in a population" as suggested by Jablonka and Lamb (2007).

Considering this broader definition, it becomes important to understand the nature of the impact transgenerational epigenetics has on evolution. This question is complicated due to three axes of investigation: there are a variety of epigenetic mechanisms, a variety of ways in which transgenerational epigenetic inheritance can impact evolution and a variety of methodologies to explore the question. In this paper I focus on one of these methodologies, deriving insight from population genetic modelling techniques. Experimental or observational studies are limited by the resource demands of long-term analysis and isolating and measuring epigenetic change (Jablonka and Raz 2009). Modelling approaches can sidestep these challenges and possibly better inform future empirical studies. Geoghegan and Spencer (2012) initially developed the population epigenetic modelling approach and in two further papers expanded it, examining cases with partial modification and paramutation (Geoghegan and Spencer 2013a, 2013b). Subsequent entries in the field of population epigenetic modelling consider these three initial models foundational (Klironomos et al. 2013, Geoghegan and Spencer 2013c, Furrow 2014, Furrow and Feldman 2014, Greenspoon and Spencer 2018). For this reason, I will summarize and compare the results of these papers in light of the impact of transgenerational inheritance on evolution and then extend this analysis by introducing intuition from neutral formulations of the models and comparing phenotypic and genotypic variation at equilibrium with population genetic models without transgenerational epigenetics.

A series of mechanisms fall under the heading of epigenetic inheritance, which can be defined as the transmission of nongenetic gene-regulation-impacting information through cellular division (Ashe et al. 2021). ‘Transgenerational epigenetic inheritance’ narrows this

definition to the transmission of this information across organismal generations which requires both that the information survive the meiotic process and the epigenetic modification exists in the germline for multicellular organisms. Three major, but non-exhaustive, mechanics of epigenetic marking are highlighted in the literature: DNA methylation, chromatin structure, and regulatory RNA (Ashe et al. 2021, Jablonka 2017). Methyl groups attach principally to cytosine nucleotides and are concentrated in CG rich sites. These groups have been found to inhibit transcription of the surrounding sequence, but the exact effect is poorly understood and is likely to be regulated by chromatin structure and other characteristics of the sequences (Ashe et al. 2021). Further, methylation has been found to be conserved through mitosis and meiosis in some instances due to the symmetrical structure of CG-regions (Jablonka and Raz 2009). Chromatin structure impacts transcription by limiting transcriptase access to sections of the chromosome that are tightly wrapped in the structural histones (Jablonka and Raz 2009). The conservation of this structure through mitosis is well-known due to its contributions to differentiating tissues between separate cell-lines, but there is also evidence that this structure is maintained between generations (Ashe et al. 2021). A suite of small, persistent and poorly understood RNA sequences play a role in transcription and translation regulation, with the most notable family being 'small interfering RNA' (siRNA) (Ashe et al. 2021). These short RNA sequences attach to enzyme complexes that attach and inhibit homologous DNA sequences or mRNA sequences and persist within the cell body through meiosis and mitosis (Jablonka and Raz 2009).

Before I describe the metrics we will use to identify the relationship between transgenerational epigenetic inheritance and evolution, I first introduce terminology to

differentiate units of genetic and epigenetic variation. I will consider the typical definition of a **genetic allele**, as a sequence of nucleotides found at a specific locus. For example, I might name two alleles *a* and *A*. I will conceptualize each of the epigenetic modifications mentioned above as placing an **epigenetic marker** on a gene, indicating a functionally different version of that gene due to epigenetic modification. For example, there may be two different epigenetic markers for a gene, named 1 and 2. Note, that one 'epigenetic marker' might be an 'unmarked' state, such as no methyl groups present at the site but, as it differentiates from the alternative, we will consider both states as different markers. Then, an **epiallele** is a unique combination of genetic allele and epigenetic marker at a locus, so in a model with two genetic alleles (*a* and A) and two epigenetic markers (*1* and *2*) there would be four possible epialleles: a_1, a_2, A_1, A_2.

Consider again the definition of evolution from Jablonka and Lamb (2007) "changes in the nature and frequency of heritable types in a population". In the one-locus diploid models I will be reviewing the heritable types of interest are the unique pairs of epialleles. So, to assess their impact on evolution, I will measure epiallele frequency under a variety of epigenetic modification mechanisms. As epiallelic variation, by way of epigenotypic variation, serves as the fuel for evolutionary change, I will focus on metrics of variation specifically, not just epiallelic frequency. Further, as genetic variation is required for future genetic evolution, I will also focus on the impact of transgenerational epigenetic inheritance on the underlying genetic variation. I will also measure mean population fitness as an outcome of epiallelic frequency because it captures the important impact of evolution on population dynamics.

We will look at epiallelic variation through three metrics. The first, Metric A, is *the number of stable equilibria* for a given model and set of parameters. Geoghegan and Spencer (2012, 2013a, 2013b) identify the relationship between the number of stable equilibria and various parameters in the model which allows the identification of the conditions that can lead to states of high and low numbers of stable equilibria. A stable equilibrium can occur at a state with many or few epialleles present, so Metric A says nothing about the amount of within-population epiallelic variation. Its value instead comes from indicating between-population epiallelic variation. If a given parameter set, interpreted as a specific environment and set of species characteristics, has a high number of stable equilibria, then, all else held equal, separate populations are more likely to have distinct equilibrium states and thus varying epiallelic frequencies between populations. This may correspond with species-wide epigenotypic variation.

Metric B provides information about within-population variation by measuring the *proportion of the stable equilibria identified in Metric A that are internal*, meaning that all **epialleles** are present with a frequency greater than zero. Extinction of an epiallele, and most extremely, fixation of an epiallele, leads to low variation. Metric B, then, is correlated with how commonly a population at equilibrium experiences the likely-high-variation-state of internality. Though, two caveats should be made: first, the link between Metric B and internal outcomes for a population is mediated by the size of the basin of attraction for internal equilibria; secondly not all internally stable equilibria have high epiallelic variationas some epialleles may be present at very low, but non-zero frequencies.

Metric C is the *effective number of epialleles in the population at a stable equilibrium,* providing a finer metric of within-population variation. Using a diversity index, specifically the inverse Simpson index, Geoghegan and Spencer (2012, 2013a, 2013b) identify a metric that can be interpreted as the number of epialleles a population would have if they had the same diversity as the population in question but perfectly even epiallelic frequencies. The effective number of epialleles (n_e) is computed in a model with three epialleles using this equation:

$$\mathrm{n_e} = \frac{1}{p_1^2 + p_2^2 + \cdots + p_n \quad ^2}$$

Where p_i is the frequency of the i^{th} epiallele.

The previous three metrics are used by Geoghegan and Spencer (2012, 2013a, 2013b) in their analyses; to these I introduce Metric D to measure the impact of transgenerational epigenetic inheritance on genetic variation. Metric D identifies *the difference in genetic allele variation between the epigenetic model and a corresponding genetic model*. While the proper choice of corresponding genetic model depends upon application, I will often use the 'base genetic model' which is the same model with no epigenetic markers, leaving only the genetic component of differentiation between alleles. This model will include no mutation as the epigenetic model does not include genetic mutation. This comparison isolates the excess genetic variation introduced by the epigenetic dynamics. Alternatively, it captures the discrepancy in genetic variation predicted when a genetic-only model is misspecified for a population experiencing transgenerational epigenetic inheritance. Metric D may also entail a comparison with the 'equivalent genetic model' which is a genetic-only

model with the same number of alleles as epialleles in the model and a similar structure of differentiation. For example, the equivalent genetic model of the four epiallele model, a_1, a_2, A_1, A_2, described above would be a two-locus model with two genetic alleles at each locus: a, A, b, B.

Finally, I am interested in mean population fitness, which I denote as Metric E and can be calculated as the weighted average of epigenotypic fitnesses by the frequency of epigenotypes. Fitness is a typical measure of interest in the study of evolution as it determines population and species survival. Knowing how it is impacted by changing characteristics of the species and environment may identify in what situations epigenetics offers a survival advantage or disadvantage.

I will now review the model specifications and findings for each metric of four diploid population epigenetic models from three articles by Geoghegan and Spencer (2012, 2013a, 2013b). In the first three models they consider situations where an epigenetic modification is induced by a specific deme, or environment. An environment need not be interpreted as a physical region only, but also possibly as a condition such as drought, starvation, or exposure to a pathogen. Model One considers the simplest two epiallele model with no genetic variation, Model Two introduces genetic variation and Model Three introduces intermediary epigenetic states. Model Four is a paramutation model in which epigenetic modification is not induced on an allele by its environment but by its paired allele at the same locus.

For each model I will first describe the specification and set up of the model, then I will describe the behaviour of the neutral version of the model in which the fitnesses of all epigenotypes are equivalent, and finally I will discuss the results for each relevant metric.

Model One: Environmental epimodification model with two environments, one genetic allele and two epigenetic markers

Model One, as formulated in Geoghegan and Spencer (2012), describes a diploid population with no genetic variation, but two epigenetic markers, 1 and 2. Each individual in the population lives its life in Environment 1 or Environment 2, each of which corresponds to one of the two epigenetic markers. The conditions in Environment 1 induce the marking of an allele with epigenetic marker 1. A_2 epialleles present in individuals in Environment 1 are converted to A_1 epialleles with probability m_1, similarly A_1 epialleles are modified to A_2 at rate m_2 in Environment 2. We call these probabilities, m_1, m_2, the epigenetic modification rates and they have theoretical parallels with mutation rates in traditional population genetic models.

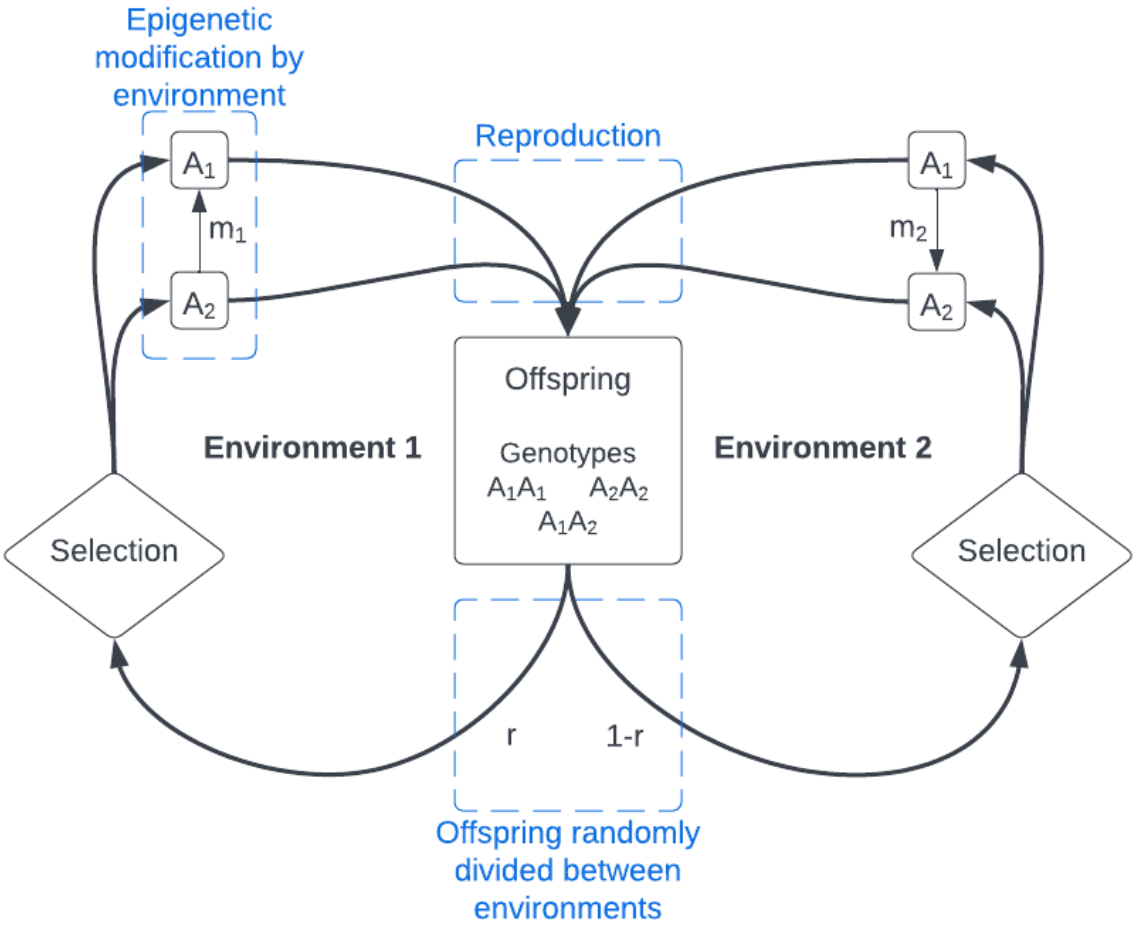

Fig. 1. Lifecycle diagram for Model One with two environments and a corresponding epigenetic marker for each environment and only one genetic allele. Individuals are randomly sorted into Environment 1 with probability r, with the rest going to Environment 2. There, they undergo selection according to environment-specific fitness for their epigenotype, then A_2 alleles in Environment 1 are converted to A_2 alleles with probability m_1 with the symmetrical process occurring in Environment 2. Gametes are then created and randomly paired across the whole population into new offspring that restart the cycle.

Fig. 1 illustrates the lifecycle of organisms in Model One. Individuals are randomly sorted into Environment 1 with probability r, with the rest going to Environment 2. The parameter r is interpreted as the frequency of Environment 1 in the meta-environment. Once sorted into the environment, organisms undergo selection, surviving the environment at a rate equal to their genotype's fitness in that environment then alleles undergo possible

epigenetic modification, as previously described. Gametes are then created and randomly paired across the whole population, forming new offspring that restart the cycle.

Individuals in this population have one of three epigenotypes: A_1A_1, A_1A_2, A_2A_2. Each epigenotype has two fitness values, one for each environment, resulting in six fitnesses within the model: $v_{11}, v_{21}, v_{31}, v_{12}, v_{22}, v_{32}$, where the first subscript denotes the genotype with 1 being A_1A_1, 2 indicating the epiheterozygote A_1A_2, 3 indicating A_2A_2. The second subscript indicates the environment. Using this lifecycle and notation, Geoghegan and Spencer (2012) derive the following recursion equations for the unnormalized proportions of A_1 and A_2 in the next generation, p_1^s and p_2^s:

$$p_1^s = rp_1(p_1v_{11} + p_2v_{21}) + m_1rp_2(p_1v_{21} + p_2v_{31}) + (1 - m_2)(1 - r)p_1(p_1v_{12} + p_2v_{22})$$

and

$$p_2^s = (1 - r)p_2(p_1v_{22} + p_2v_{32}) + m_2(1 - r)p_1(p_1v_{12} + p_2v_{22}) + (1 - m_1)rp_2(p_1v_{21} + p_2v_{31})$$

Briefly, to understand the first equation, $rp_1(p_1v_{11} + p_2v_{21})$ represents the unnormalized proportion of A_1 epialleles that are sorted into Environment 1 and survive, and $m_1rp_2(p_1v_{21} + p_2v_{31})$ represents the unnormalized proportion of A_2 epialleles that are sorted into Environment 1, survive, and are modified into A_1 epialleles. Finally, the last term, $(1 - m_2)(1 - r)p_1(p_1v_{12} + p_2v_{22})$, describes A_1 epialleles in Environment 2 that survive and are not modified to A_2. The terms in p_2^s are analogous.

Mean fitness of the population is expressed as $\overline{w} = p_1^s + p_2^s$. The normalized proportion or epiallele i in the next generation is expressed as $p_i' = \frac{p_i^s}{\overline{w}}$.

Neutral Model for Model One

To understand the behaviour of this model we will first look at its behaviour under neutral assumptions, where all fitness values in the model are equal. By also assuming the modification rates are equal in both environments, $m_1 = m_2 = m$ the expression at equilibrium simplifies a great deal:

$$p_1' = \frac{\mathrm{r}p_1(p_1 v_{11} + p_2 v_{21}) + m_1 \mathrm{r} p_2(p_1 v_{21} + p_2 v_{31}) + (1 - m_2)(1 - r)p_1(p_1 v_{12} + p_2 v_{22})}{\bar{w}}$$

$$p_1' = \frac{\mathrm{v}_{11}(\mathrm{p}_1 + \mathrm{p}_2)(\mathrm{r}p_1 + m_1 \mathrm{r} p_2 + (1 - m_2)(1 - r)p_1)}{\mathrm{v}_{11}(\mathrm{p}_1 + \mathrm{p}_2)(\mathrm{r}\mathrm{p}_1 + \mathrm{m}_1 \mathrm{r}\mathrm{p}_2 + (1 - \mathrm{m}_2)(1 - \mathrm{r})\mathrm{p}_1 + (1 - \mathrm{r})\mathrm{p}_2 + \mathrm{m}_2(1 - \mathrm{r})\mathrm{p}_1 + (1 - \mathrm{m}_1)\mathrm{r}\mathrm{p}_2)}$$

$$p_1' = \frac{(\mathrm{r}p_1 + m_1 \mathrm{r}(1 - p_1) + (1 - m_2)(1 - r)p_1)}{\mathrm{p}_1 + \mathrm{p}_2}$$

$$p_1' = \mathrm{r}p_1 + \mathrm{mr} - mrp_1 + (1 - \mathrm{r})\mathrm{p}_1 - \mathrm{m}(1 - \mathrm{r})\mathrm{p}_1$$

$$p_1' = \mathrm{mr} + (1 - \mathrm{m})\mathrm{p}_1$$

At equilibrium:

$$\mathrm{p}_1^* = \mathrm{mr} + (1 - m)\mathrm{p}_1^*$$

$$mp_1^* = mr$$

$$p_1^* = r \qquad \text{for } m \neq 0$$

In the neutral case, when the modification rate is constant across environments, the equilibrium frequency does not depend on the modification rate. The frequency of an allele tends towards the frequency of its corresponding environment. The speed of this convergence is linear in the modification rate and decreases with the distance from the equilibrium frequency:

$$\Delta p = p_1' - p_1 = rm + (1 - m)p_1 - p_1 = m(r - p_1)$$

If the constant rate of modification between environments assumption is relaxed, the expression for equilibrium is slightly more complicated:

$$p_1^* = m_1 r + p_1^*\left(1 - m_1 r - m_2(1-r)\right)$$

$$p_1^* = \frac{m_1 r}{m_1 r + m_2(1-r)}$$

The numerator of this equilibrium epiallele frequency is the product of the frequency of the corresponding environment and the modification rate in that environment. Essentially this tells us the proportion of other alleles that get modified into the allele of interest in each generation. In this case, it is the input of new A_1 alleles as a proportion of A_2 alleles in each generation. The denominator is the weighted average of modification rates by the frequency of their environments. The entire term can be thought of as the proportion of all epialleles subject to modification in any given time step that are in the corresponding environment (Environment 1 in this case). When the modification rates are equal, it is obvious that this value is just *r,* the frequency of the corresponding environment. To summarize, with neutral fitnesses the equilibrium frequency of an allele is positively associated with the frequency of its corresponding environment and modification rate in that environment and negatively correlated with the modification rate of other environments (and their frequencies, but this is just the inverse of the frequency of the alleles corresponding environment.

Simulation Methods in Geoghegan and Spencer (2012, 2013a, 2013b)

Equilibrium frequencies for the full Model One can be determined analytically, but the closed-form solutions are very complex. This complexity makes analytical stability analysis

unfruitful. To determine the stability of equilibria in Model One, the authors employ a simulation analysis. For each combination of the following set of r, m_1, m_2 values (0.1, 0.25, 0.5, 0.75, 0.9), 1000 sets of random fitnesses are generated from a uniform distribution. For each of these fitness sets, 1000 random starting frequencies are generated using the 'broken stick' approach employed in Marks and Spencer (1991) and the simulation is run using the above recursion equations. A stable equilibrium is identified when the absolute change in epiallele frequencies declines beneath 10^{-12}. These methods are employed in all future models.

Findings for Model One

The relationship between parameters and **Metric A**, the number of stable equilibria, resembles in some ways the patterns for a simple two allele, one locus genetic model. Either one or two stable equilibria is possible for each set of parameters. In the case of heterozygote advantage, where the mean fitness of the epiheterozygote, A_1A_2, weighted by the frequency of the environments is greater than the weighted mean fitness of either epihomozygote, then there will only be one stable equilibrium at intermediate values of p_1. The exact equilibrium frequency for A_1 will be positively correlated with the frequency of Environment 1 and the modification rate in Environment 1, and will be determined byt the fitness values of all epigenotypes in the population. The equilibrium frequency will be positively correlated with relatively high fitness values for epigenotypes containing A_1, and with relatively high fitness values for epigenotypes in Environment 1.

When there is epiheterozygote disadvantage, then two stable equilibriums are possible, equivalent in many ways to the fixation equilibria expected under the one-locus two-allele

genetic without mutation. However, these are never fixation equilibria as when $m_1, m_2 > 0$ and $0 < r < 1$ there is constant addition of both alleles due to epigenetic modification. In the case of $m_1 = m_2 = m$, as m increases these two stable equilibria are brought closer to moderate values, until at high levels of m only one stable equilibria is present, as pictured in Fig. 2. High values of m lead to more conversions of the epialleles, overwhelming the force of selection.

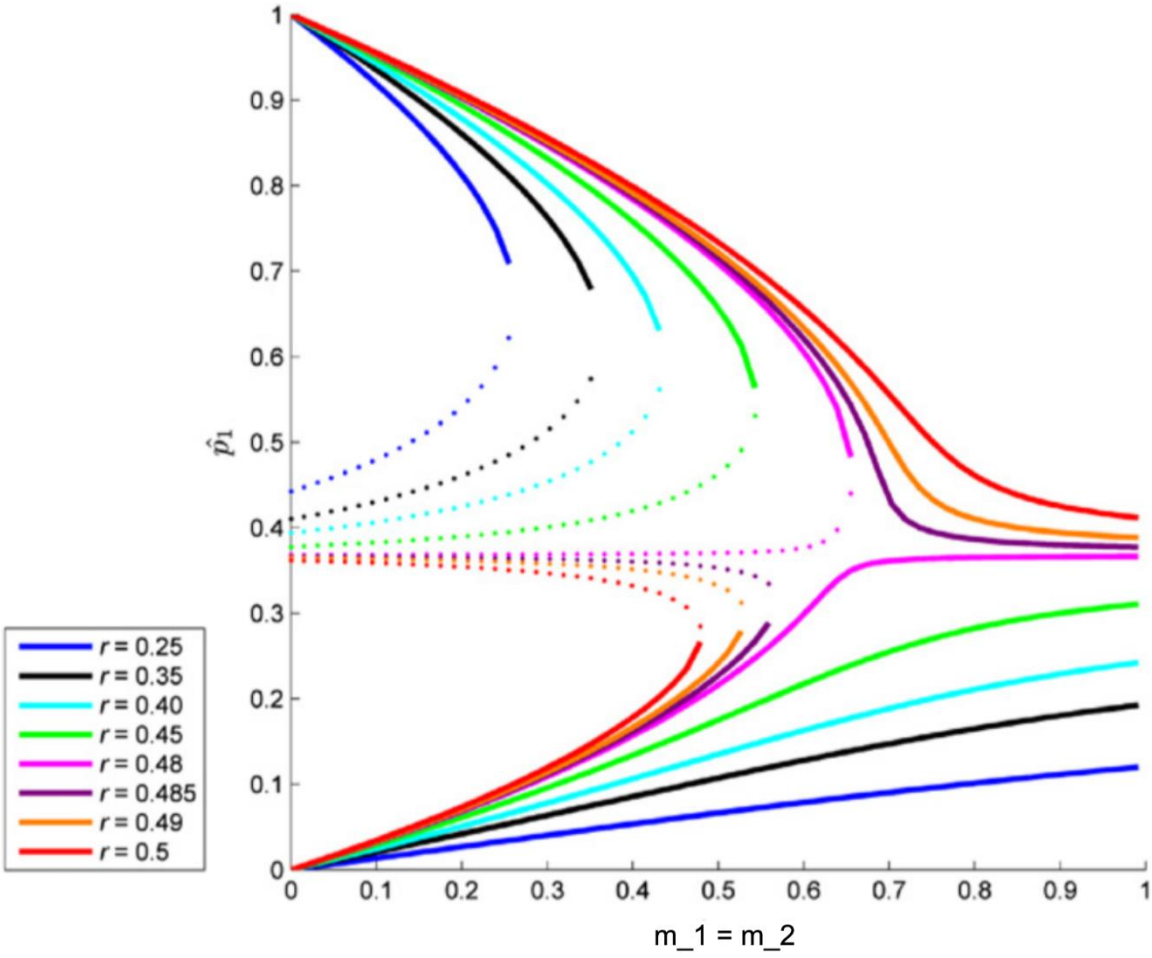

Fig. 2. Bifurcation diagram of Model One with heterozygote disadvantage for varying values of r. The equilibrium frequency of epiallele A_1 is represented on the y-axis while modification rate, assuming $m_1 = m_2$, is represented on the x-axis. Frequency of two stable equilibrium states decreases as modification rate increases. From Geoghegan and Spencer (2012).

The frequency of fitness sets with two stable equilibria decreases as m increases. The proportion of fitness sets with two stable equilibria reaches 0 when m reaches a moderate value between 0.5 and 0.75, undetermined by Geoghegan and Spencer (2012). These findings for m can be generalized to the weighted average of m_1 and m_2 in cases where $m_1 \neq m_2$.

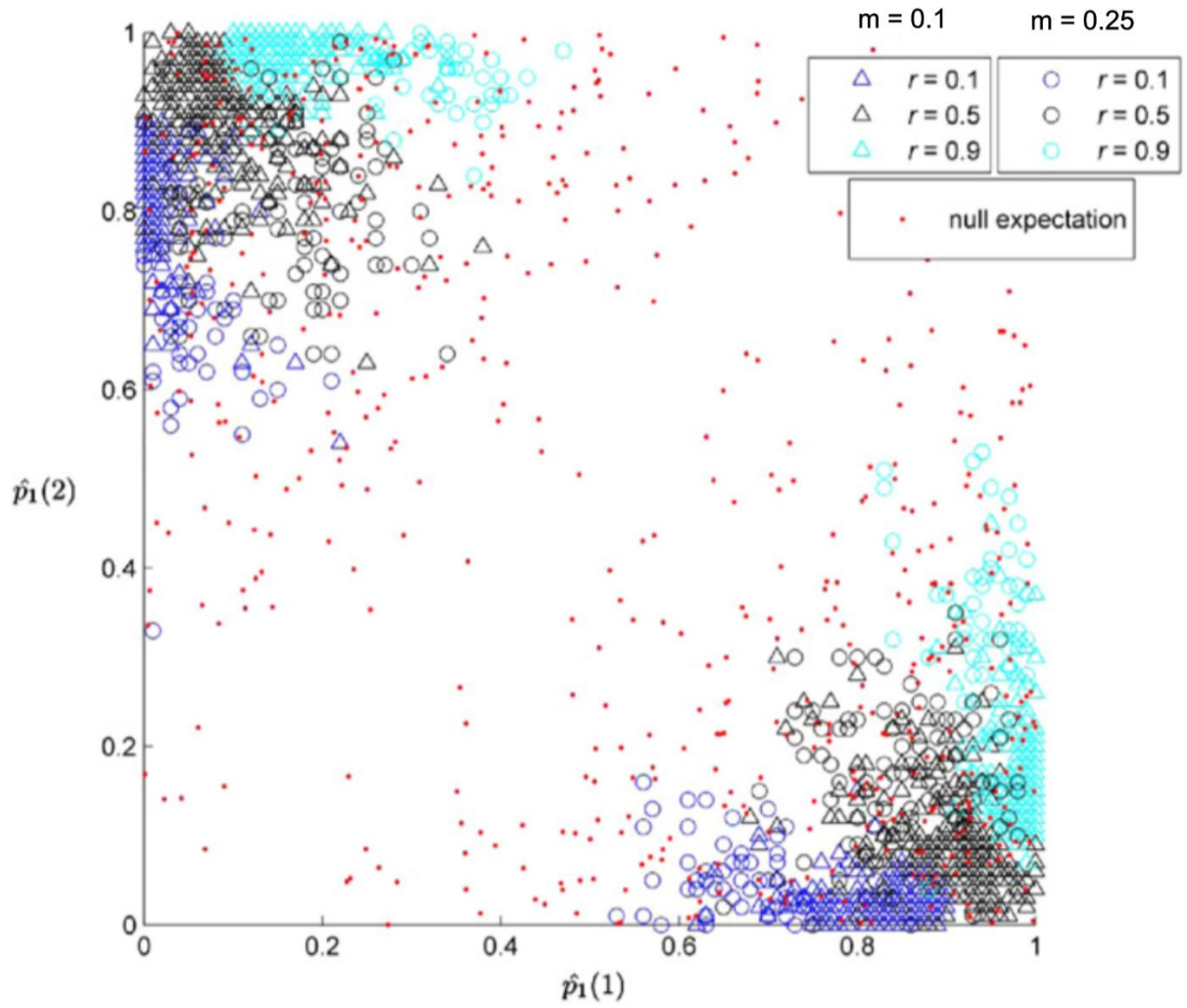

Fig. 3. Equilibrium frequencies of A_1 for cases with two stable equilibriums, represented by hollow circle and triangle markers. Equilibrium frequency for one equilibrium is represented on each axis, colours represent varying values of r, and shapes denote varying values of $m = m_1 = m_2$. From Geoghegan and Spencer (2012).

Besides epiheterozygote disadvantage and low mean modification rate, the third criterion required for the existence of two stable equilibria in a fitness set is a small

difference between modification rates, m_1 and m_2. When the difference increases, probability of two stable equilibria decreases as the modification rate advantage given to one epiallele over the other overwhelms any effects from fitness.

In this case **Metric B**, the proportion of internal stable equilibria, is always 1 when epigenetic dynamics are active, or $m_1, m_2 > 0$ and $0 < r < 1$, due to the constant addition of both epialleles in each time step from epimodification. Comparing to the base genetic case is meaningless, as the one allele genetic 'model' can also be seen as always internal as the only allele is always present at equilibrium. If we compare to an equivalent one-locus two-allele genetic model without mutation, Model One represents an increase in the proportion of internal stable equilibria. To the extent that Metric B provides meaningful information about epiallelic variation, this indicates that the existence of any epigenetic dynamic increases variation.

Metric C, the effective number of epialleles at equilibrium, is often low in cases with multiple stable equilibria, as these equilibria are typically near fixation. Geoghegan and Spencer (2012) find that the effective number of epialleles is inversely related with many of the conditions that promote two stable equilibrium states. This pattern can be observed in Fig. 3 by the clustering in the corners indicating values of p_1 near 1 or 0 for at least one of the two equilibria. The red dots in the figure show the null expectation for these equilibria, the highest and lowest values of three random numbers (representing the three possible equilibria for the parameter set, the middle of which is unstable). The actual equilibria frequencies for equilibria from two equilibria fitness sets is much more extreme than the

null expectation. Thus, epigenotypic variation, as measured by Metric C, is maximized under heterozygote dominance and high mean modification rates.

As there is no genetic variation in Model One, or the base genetic model, **Metric D** cannot be assessed.

For **Metric E**, Geoghegan and Spencer (2012) find that modification rate plays the role that mutation rate does in the equivalent genetic model in terms of mean population fitness. Due to epigenetic modification, maximum mean population fitness is not reached at equilibrium as alleles with lower marginal fitness are constantly introduced to the population due to modification.

Model Two: Environmental epimodification model with two environments, two genetic alleles and two epigenetic markers.

Model Two expands Model One with genetic variation by introducing a second genetic allele, represented by a. This results in four epialleles, A_1, A_2, a_1, a_2, ten possible epigenotypes and twenty fitness values in the model. Modification rates, m_1, m_2, are identical for a and A alleles, and all other conditions are identical. Better comparisons of genetic and epiallelic variation can be made with this formulation between the epigenetic and base genetic model.

Neutral Model for Model Two

All the insights from Model One can be extended to this model. Consider that a neutral version of the base two-allele genetic model with no mutation, in which all three genotype fitnesses are identical, will experience no change in allele frequency and starting

frequencies will always persist. Then the only axis of functional differentiation is epigenetic markers and the frequencies of epigenetic markers will follow the same dynamics as described in the neutral version of Model One, while the frequencies of genetic alleles will remain constant. This means that genetic variation, Metric D, is not impacted by the introduction of epigenetic dynamics under the neutral model, but this pattern does not hold when we assume there is variation among the epigenotype fitness values.

Findings for Model Two

The maximum value of **Metric A** is four stable equilibria for a given fitness set, but these conditions are rare, with only four examples found in all simulations. Many of the dynamics from Model One are maintained here. Like Model One, as $m = m_1 = m_2$ increases, the mean number of stable equilibria decreases as can be seen in Table 1.

(1)	(2)	(3)	(4)	(5)	(6)	(7)
m	No. of stable equilibria	Proportion of fitness sets in each category	Proportion of internal equilibria in each category	Normalized no. of total equilibria that are internal	Normalized total no. of equilibria in each category	Total proportion of internal equilibria
0.1	1	0.452	0.506	0.229	0.452	-
	2	0.480	0.315	0.302	0.960	-
	3	0.066	0.303	0.060	0.198	-
	4	0.002	0.000	0.000	0.008	-
	Total:	**-**	**-**	**0.591**	**1.618**	**0.365**
0.25	1	0.596	0.419	0.250	0.596	-
	2	0.392	0.071	0.056	0.784	-
	3	0.012	0.000	0.000	0.036	-
	Total:	**-**	**-**	**0.306**	**1.416**	**0.216**
0.5	1	0.660	0.410	0.271	0.660	-
	2	0.340	0.052	0.035	0.680	-
	Total:	**-**	**-**	**0.306**	**1.340**	**0.228**

Table 1. Stable equilibria and internal equilibria in Model 2 when r = 0.5. The first four columns are taken from Table 5 of Geoghegan and Spencer (2012), the final three are computed from that data. Column 3 represents the proportion of fitness sets with a particular number of stable equilibria. Column 4 reports the proportion of stable equilibria from fitness sets with a particular number of stable equilibria that are internal. Column 5 is the product of columns 2, 3 and 4, which represents the total number of internal stable equilibria in in fitness sets with a particular number of stable equilibria divided by the number of

fitness sets. Column 6 is the product of columns 2 and 3 which is the total number of stable equilibria in each category. Column 7 is the ratio of the totals in column 5 divided by column 6 and represents the proportion of stable equilibria that are internal for each value of m.

Table 1 also shows the proportion of stable equilibria that are internal, **Metric B**. Column 4, taken directly from Geoghegan and Spencer (2012), shows the proportion of stable equilibria that are internal for fitness sets with a particular number of stable equilibria. Internal equilibria are rare among fitness sets with high numbers of stable equilibria, as these stable equilibria are often genetic fixation equilibria, where one or the other genetic allele is extinct. I compute the total proportion of stable equilibria that are internal in Column 7, for three values of m. While the proportion of stable equilibria that are internal in each category declines as the modification rate increases (Column 4), the proportion of fitness sets with fewer stable equilibria increases as m increases (Column 3). This leads to a u-shaped relationship between the modification rate and the proportion of stable equilibria that are internal (column 7), initially declining as m increases and then increasing at some point near $m = 0.25$.

No data is provided in Geoghegan and Spencer (2012) about effective number of epialleles at equilibrium, **Metric C,** for Model Two. Data on epiallele frequencies at equilibrium across varying parameter values would be required to comment on this metric.

Information about **Metric D**, genetic variation, can be derived from the information provided in Table 1. Note that at equilibrium, if $m > 0$ and $0 < r < 1$, a_1 is present if and only if a_2 is present because an epigenetic marker cannot go to fixation due to constant introduction of both markers by epigenetic modification. The same is true for A_1 and A_2. Then, if both genetic alleles are present all four epialleles are present at equilibrium. Conversely, if all four epialleles are present (an internal equilibrium) then both genetic

alleles must be present. Therefore, all internal epigenetic equilibria are genetic polymorphic equilibria and vice versa. Now we can interpret the results in Column 7 of Table 1 as the proportion of stable equilibria that are genetic polymorphic equilibria and compare that to the base genetic model.

For a one-locus, two-allele, two-environment genetic model we can expect 0.25 of stable equilibria produced by simulation to be internal. This result is shown in Appendix 1. This expectation is constant across distributions of fitness values used in the model and confirmed (in a one-environment model) by simulations in Lewontin et al. (1977). . We can compare this base genetic model to the results from Model 2: for $m = 0.1$, 0.365 of stable equilibria are internal, for $m = 0.25$ it's 0.216 and $m = 0.5$ it's 0.228 (from Table 1, Column 7). For low modification rates, epigenetic dynamics increase genetic variation over the base model. At higher modification rates, the epigenetic mechanisms described in Model 2 slightly decrease genetic variation over the base genetic model. The mechanism behind this phenomenon is not clear. The random selection of more fitness sets does not seem to, by itself account for this change from the base genetic model. I suspect that it is a factor of the epiallele frequency-dependent nature of the effective genotypic fitnesses in the epigenetic model. These will influence the trajectory to equilibrium and may nudge the model towards heterozygote advantage or disadvantage states depending on the epimodification rate. But more work is required to understand this phenomena.

Model Three: Partial environmental epimodification model with two environments, one genetic allele and three epigenetic markers

Geoghegan and Spencer (2013a) introduce an amendment to Model One in which epigenetic modification induced by the environment can be incomplete. Environments 1 and 2 are renamed to α and β for clarity. The epigenetic marker associated with Environment α is 1 and marker 3 is associated with Environment β. Marker 2 represents a moderate epigenetic state between 1 and 3. This can be conceptually considered as a gene that has a moderate rate of methylation, histone wrapping or siRNA inhibition, while 1 and 3 represent the extremes. In an environment, alleles are partially modified at rate m_1 and fully modified at rate m_2. The authors often assume, for the sake of simplicity, that $m_1 = 2m_2$ and it is natural to assume that the rate of full modification m_2 is less than the rate of partial modification m_1. This means that A_3 alleles in Environment α are converted to A_1 alleles with probability m_2 and converted to the moderate epigenetic marker A_2 at rate m_1 with the symmetrical process occurring in Environment 2. Otherwise, the lifecycle continues as in Model One and is depicted completely in Fig. 4. For the three epialleles present, $A_1, A_2. A_3$, there are six unique epigenotypes with two fitness values each, one for each environment.

Neutral Model of Model Three

Assuming that all fitnesses are equal, the following relationships of frequencies at equilibrium can be derived from the recursion equations 1-7 in Geoghan and Spencer (2013a):

$$\mathrm{p}*_1 = \frac{r(m_1 p*_2 + m_2 p*_3)}{(1-r)(m_1+m_2)} \quad \mathrm{p}*_2 = \mathrm{rp}*_3 + (1-r)\mathrm{p}*_1 \qquad \mathrm{p}*_3 = \frac{(1-r)(m_1 p*_2 + m_2 p*_1)}{r(m_1+m_2)}$$

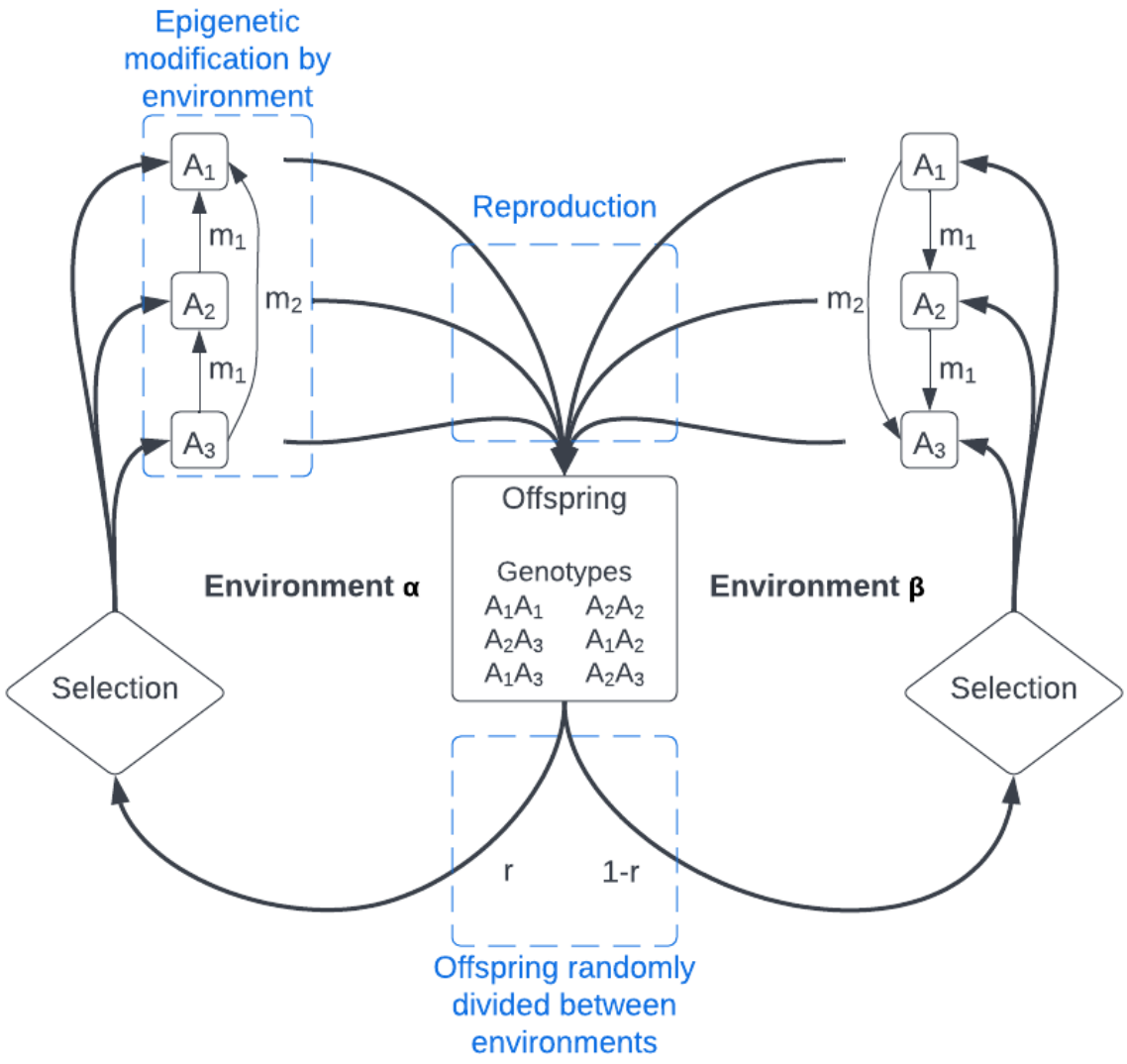

Fig. 4. Lifecycle diagram for Model Three with two environments and partial epigenetic modification in each environment and only one genetic allele. Individuals are randomly sorted into Environment α with probability r, with the rest going to Environment β. There, they undergo selection according to environment-specific fitness for their epigenotype, then A_3 alleles in Environment α are converted to A_1 alleles with probability m_2 and converted to the moderate epigenetic marker A_2 at rate m_1 with the symmetrical process occurring in Environment 2. Gametes are then created and randomly mated across the whole population into new offspring that restart the cycle.

The numerators of $p *_1$ and p $*_3$ are the rates at which new epialleles of A_1 and A_3 respectively are introduced to the population through epimodification, while the denominator is the proportion of that type of epiallele that are lost in each time step. This relationship between numerator and denominator is also true in the expression for $p *_2$ if

you multiply the whole expression by $\frac{m_1}{m_1}$: the numerator is the proportion of A_1 and A_3 epialleles that find themselves in the opposing environment and partially modified, while all A_2 epialleles face modification in each time step at rate m_1, the denominator.

This system of equations can be further solved if an assumption is made about the relationship between full and partial modification rates. Here, I imitate the assumption in Geoghegan and Spencer (2013) by taking $m_1 = 2m_2$. With that assumption, equilibrium frequencies of epialleles in the model can be written as functions of only r (see Sympy code in Appendix 3 for derivation):

$$p*_1 = \frac{r(2r+1)}{3} \qquad p*_2 = \frac{4r(1-r)}{3} \qquad p*_3 = \frac{(1-r)(3-2r)}{3}$$

These relationships are best expressed in Fig. 5 which shows that the extreme epigenetic states (A_1 and A_3) respond similarly to changes in environmental frequency as epialleles did in Model One, decreasing their equilibrium frequencies as their environmental frequency decreases. But the addition of the moderate epigenetic state, A_2, changes their shape into convex curves. The equilibrium frequency of A_2 is maximized at $r = 0.5$. Effective number of epialleles at equilibrium is maximized when environmental variation is maximized (moderate levels of r) in the neutral model. As epimodification is the determining factor of equilibrium frequencies, diverse environments promote balanced equilibrium frequencies by balancing the rates of epimodification to A_1, A_2, A_3 epialleles.

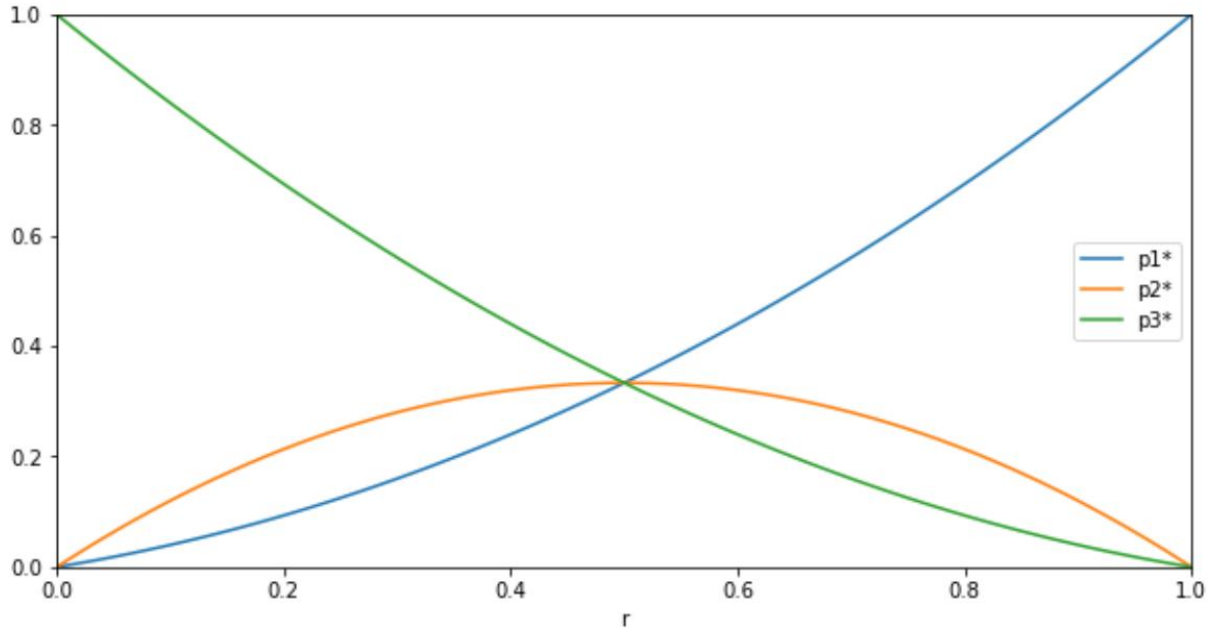

Fig. 5. Equilibrium frequencies in the neutral case for Model Three. Effective number of epialleles at equilibrium is maximized when environmental variation is maximized.

Findings for Model Three

In Models One and Two, changing the environmental frequency, r, seemed to have little to no impact on **Metric A**, the mean number of stable equilibria for fitness sets. However, in Model Three simulation analysis showed the mean number of stable equilibria is much higher at moderate levels of r. The mechanism of this change in behaviour is not explored in Geoghegan and Spencer (2013), but a hypothesis can be made based on the results of the neutral model. Moderate levels of r also promote the rate of production and equilibrium frequency of A_2 epialleles. As the existence of this moderate epigenetic marker is what differentiates this model from Model One, it would be fitting if the existence of this epiallele encouraged multiple stable equilibriums when its rate of production and equilibrium frequency in the model is maximized.

A maximum of three stable equilibria are possible for a fitness set. Two of those equilibria are familiar from Model One: one A_1 or A_3 epiallele dominates while the other and A_2 are rare. The third equilibrium is dominated by A_2 epialleles. Based on the results of the neutral model, I hypothesize that moderate values of r allow for greater A_2 frequencies at equilibrium and make this third equilibria possible, increasing the mean number of stable equilibria.

However, this phenomena, of more stable equilibria at moderate r values, is also observed when restrictions are imposed that make three equilibria impossible. Geoghegan and Spencer simulate the model making what I call the Adaptive Epiallele Assumption: that epigenotypes with A_1 epialleles have a fitness advantage over other equivalent epigenotyes in Environment α and symmetrically A_3 epialleles are favoured in Environment β. For example, $epigenotype\ A_1A_2$ has greater fitness than epigenotype A_2A_2 in Environment α. Under these conditions, three stable equilibria are impossible and the A_2 dominated equilibrium is not observed. However, the trend in more mean stable equilibria at moderate *r* persists. I hypothesize that the moderate epigenetic marker increases fitness sets that exhibit effective heterozygote disadvantage between A_1 and A_3; and that moderate values of r promote A_2 abundance and thus this effective heterozygote disadvantage in a way that is not captured in Model One.

Between population variation, we can conclude, is maximized in the partial modification model when environmental variation is high.

Similar to Model One, because there is no genetic variation in the model, all equilibria are internal, including all epigenetic markers, making **Metric B** irrelevant here.

If we make the Adaptive Epiallele Assumption, then only moderate levels of r show an increase in **Metric C**, mean effective alleles at equilibrium, over the base genetic model, which always has one allele present at equilibrium (see the first graph in Fig. 6). When we relax this assumption, this pattern remains for most levels of m_1 (still assuming that $m_1 = 2m_2$); moderate levels of r have the highest effective number of epialleles at equilibrium, but n_e, even in cases with extreme r values, is greater than one (see bottom two graphs in Fig. 6). However, at low values of m_1, effective number of epialleles is elevated for all values of r.

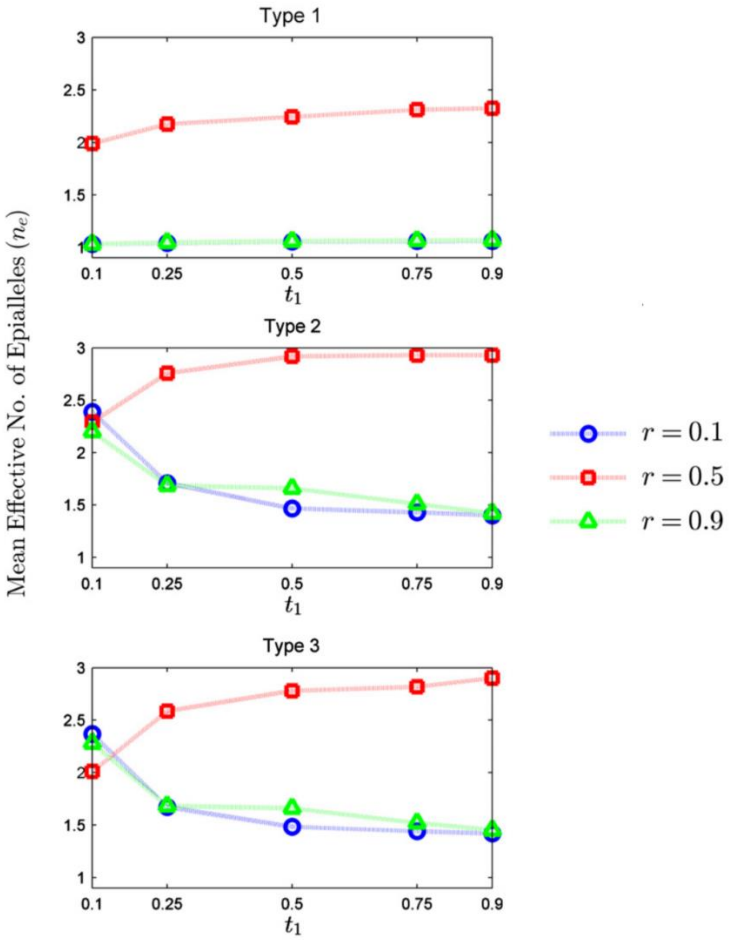

Fig. 6. Mean Effective Number of Epialleles for different restrictions on the model and varying values or r and m_1 (here expressed as t_1). The first graph, Type 1, indicates the case where the Adaptive Epiallele Assumption has been made. In Type 2 fitnesses are symmetric between environments (A_1A_1 epigenotypes have the same fitness in Environment α as A_3A_3 does in Environment β). In all simulations $m_1 = 2m_2$. Moderate values of r always produce greater effective number of alleles than

extremes except when m_1 is low and the Adaptive Epiallele Assumption is not being made. From Geoghegan and Spencer (2013a).

We can conclude then that if epigenetic modifications are partial and not adaptive to their inducing environments, they will greatly increase epigenotypic variation. Nonadpative modification avoids the compounding effect from adaptive modification of the modified alleles in one environment also having high fitness in that environment and strengthening fitness differences. This allows equilibria to exist with more epialleles present. But adaptive modifications only increase epigenetic variation in diverse environments. Consider that alleles in a model with adaptive modification and one environment that dominates face extreme selection pressures which is compounded by epimodification to the dominating environment. This results in one frequent epiallele. Only in diverse environments does the compounding effect of adaptive modification in one environment not overwhelm other forces.

No genetic variation is present in this model so comparisons of **Metric D** are irrelevant.

When we make the Adaptive Epiallele Assumption, mean population fitness, **Metric E**, is correlated with Metric C: balanced fitness values across epigenotypes leads to more epigenotypic variation and better mean population fitness. But by relaxing this assumption, this relationship reverses: the greater the number of effective epialleles at equilibrium, the lower the mean population fitness. Like Model One, higher modification rates introduce unfit alleles at equilibrium that decrease mean population fitness. This effect is mostly observed at extreme r values, but for moderate r values there is very little correlation between effective number of epialleles and mean population fitness. When one

environment dominates, the difference between marginal fitnesses for each allele is likely to be higher, because just the few fitness values in the frequent environment dominate. Moderate values of r have a moderating effect on marginal fitness values.

Model Four: Paramutation Model with Three Epialleles

Geoghegan and Spencer (2013b) introduce and analyze the behaviour of a simple paramutation model. In a paramutation model, epigenetic modification is not induced by the environment of the allele but by its paired allele in the genotype. There are some paramutagenic alleles, which we will call *a*, which have the ability to induce epigenetic modification in their partner allele in a diploid model. This may be due to a structure that allows a great number of bound methyl groups which can be easily transferred to the paired allele at the locus, modifying its function. Or it may be due to ability to encode siRNA that target that locus. In any case, when a genetically distinct paramutable allele, called A, is paired with a paramutagenic allele, *a,* there is a chance, *m*, that *A* will be epigenetically modified into the epigenetic allele *B.* A and B are genetically identical alleles (as indicated by both being capitalized in this nomenclature) but have two different epigenetic markers. In this model, B not only functions differently than A in terms of fitness but also is itself paramutagenic and is able to induce epigenetic modification in other paired alleles in the future.

The final characteristic that differentiates this model is the introduction of a chance of epigenetic reset from B to A in all time steps. The epigenetic modification is considered impermanent and B epialleles can revert to A alleles at a standard rate *t* in each generation.

The full lifecycle diagram is depicted in Fig. 7 which highlights that in this model epigenetic modification and reset happen concurrently.

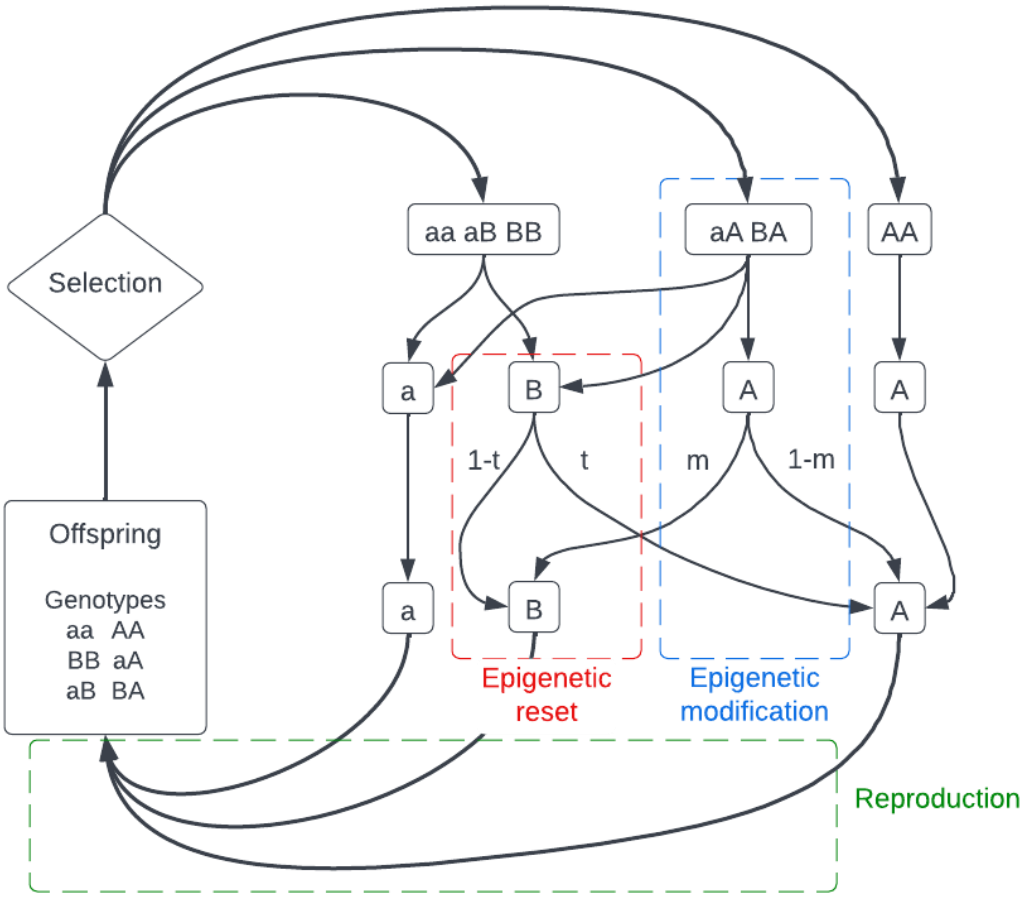

Fig. 7. Lifecycle Diagram for Model Four, paramutation with three epialleles. Offspring are produced and go through selection based on the fitness values of their epigenotypes. Then epigenetic modification of A, induced by partner alleles B or a happens concurrently with epigenetic reset and gametes are produced and mixed.

Neutral Model for Model Four

If we assume that the modification rate and the reset rate are identical, $m = t$, then a clear relationship between the equilibrium frequencies and the starting frequency of the

paramutagenic allele *a* can be derived under the neutral fitness assumptions. First note, similarly to the genetic components of Model Two, that under neutral assumptions there are no pressures acting on the frequencies of the genetic alleles *a* and *A* (remembering that epiallele B is merely an epigenetically marked form of A). For this reason, the frequency of epiallele *a* will remain constant in the neutral model and the equilibrium frequency will equal the initial frequency. As this is a fixed value, we will describe the equilibrium frequencies of the other epialleles based on this initial frequency of *a*. The following relationships are found in the neutral case:

$$p*_A = 1 - \sqrt{p*_a} \quad p*_B = \sqrt{p*_a}\left(1 - \sqrt{p*_a}\right)$$

These relationships are displayed in Fig. 6. Two major effects are at play here: as $p*_a$ increases, the frequency of the genetic allele A, and thus the combined frequency of the epialleles A and B must decreases. The frequency of B at equilibrium both depends on having a large enough stock of *a* to induce the epigenetic modification in B and enough A to be converted into B. Thus, increases in $p*_a$ at low overall levels of $p*_a$ are beneficial to $p*_B$ as *a* serves as a catalyst for paramutation, but after a certain point this beneficial effect of $p*_a$ is overwhelmed by the negative effect of the decrease in initial A that serves as fuel. This creates the hump-shaped relationship between $p*_a$ and $p*_B$ observed in Fig. 8.

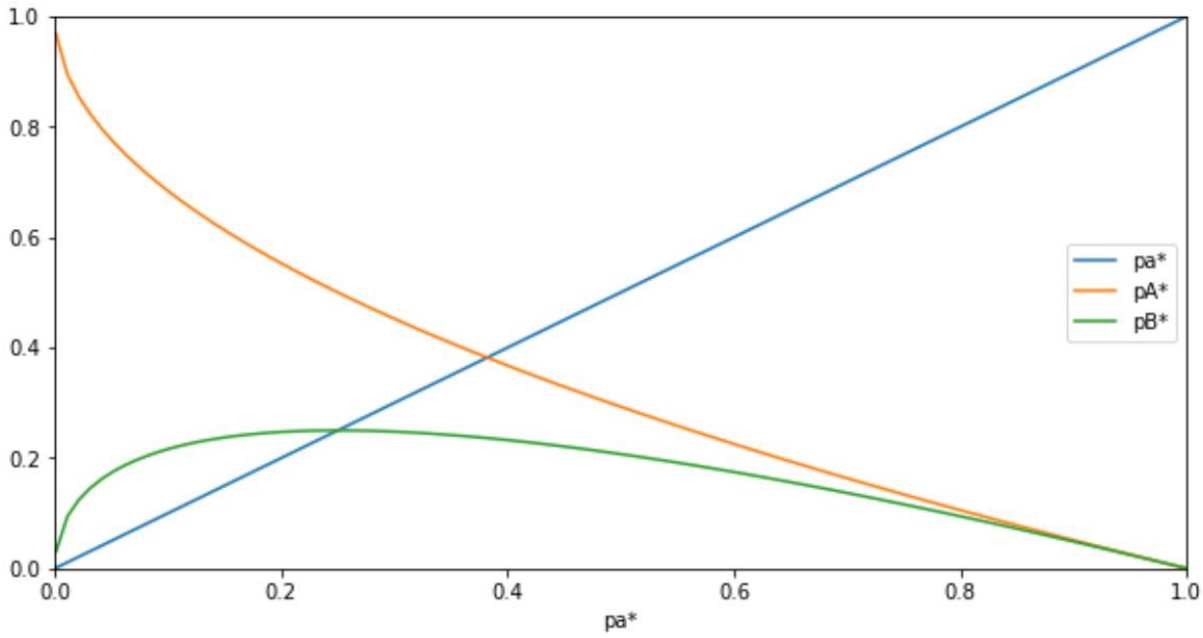

Fig. 8. Equilibrium frequencies across initial frequencies of epiallele *a* in the neutral case for Model Four.

Findings for Model Four

Four types of stable equilibria are possible when $m, t > 0$: fixation of a, fixation of A, elimination of a leading to a balance of A and B, and a fully internal equilibrium. Note that equilibria where just a and A, a and B, $or\ just\ B$ are present are impossible when $m, t > 0$, due to the constant introduction of A and B epialleles due to paramutation and reset A maximum of three stable equilibrium were found by simulation analysis for a single fitness set (Geoghegan and Spencer 2013b). Increasing the reset rate, t, decreased the mean number of stable equilibria for fitness sets. When t = 1, B epialleles are immediately reset to A in the generation following their paramutation, this approximates a model with only A and a epialleles present, which is just the underlying base genetic model with two alleles. Thus, as t increases, the model approaches this state and loses the chance of resulting in the extra stable equilibrium made possible by the third epiallele *B.* The relationship between

the modification rate, *m,* and **Metric A** is complex in this model and appears to change significantly if you relax the assumption that *a* and *B* induce epigenetic modification in *A* at the same rate. In general, the relationship is strongest at low levels of reset, *t*, where mean number of stable equilibria decreases as *m* increases, matching the behaviour of other models.

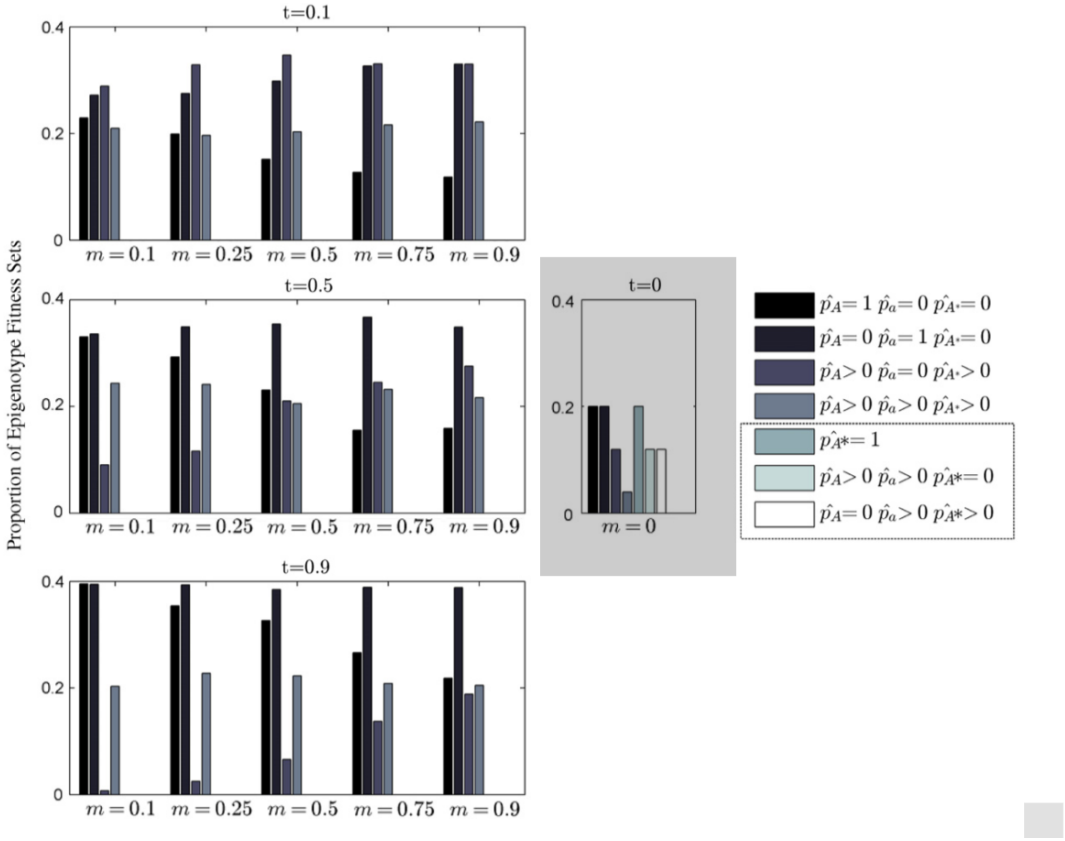

Fig. 9. Proportion of fitness sets that result in the four types of stable equilibria in Model Four across varying levels of *m* and *t*. Here epiallele B is denoted by A*. The proportion of fitness sets resulting in internal stable equilibria (denoted by the lightest blue-grey colour bar in the main diagram) seems to be consistent across values of m and t. The inset figure with the grey background shows the proportion of fitness sets resulting in 6 different equilibrium types when m, t = 0; which is equivalent to the three-allele, one-locus genetic model. From Geoghegan and Spencer (2013b).

The proportion of fitness sets with an internal stable equilibrium is consistent across values of t and m, with about 20% of fitness sets demonstrating this trait in the simulation analysis employed by Geoghegan and Spencer (2013b). This is demonstrated by the fourth,

lightest blue-grey bar in the main charts in Fig. 9. This is not quite the same metric as employed in the previous discussion of **Metric B** for Model Two where I examined the proportion of equilibria that were internal, making comparison difficult. However, we know that at low reset rates, *t*, mean stable equilibria decrease as m increases. Geoghegan and Spencer (2103b) also demonstrate that for most fitness sets, only one stable equilibria is internal. For low t and increasing m then, each fitness set has fewer equilibria, but the same proportion of fitness sets have internal equilibria, making internal equilibria a larger share of stable equilibria as m increases. Making conclusions about the rest of parameter space is unfortunately more difficult.

Fig. 10 shows that tor all values of m and t, mean alleles at equilibrium, **Metric C**, increases over the base 2-allele genetic model (which has effective alleles at equilibrium of 1.33). Except when t is very near to 1 and m is very near to 0 (approximating the 2-allele genetic model case) the mean number of epialleles at equilibrium is greater than the value for the equivalent three-allele genetic model (denoted by the grey line on Fig. 10). We can conclude that in comparison to the base genetic model epigenotypic variation always increases with the addition of paramutation dynamics and that the epigenetic variation from a paramutation model is almost always greater than a genetic model with an equivalent number of alleles as epialleles.

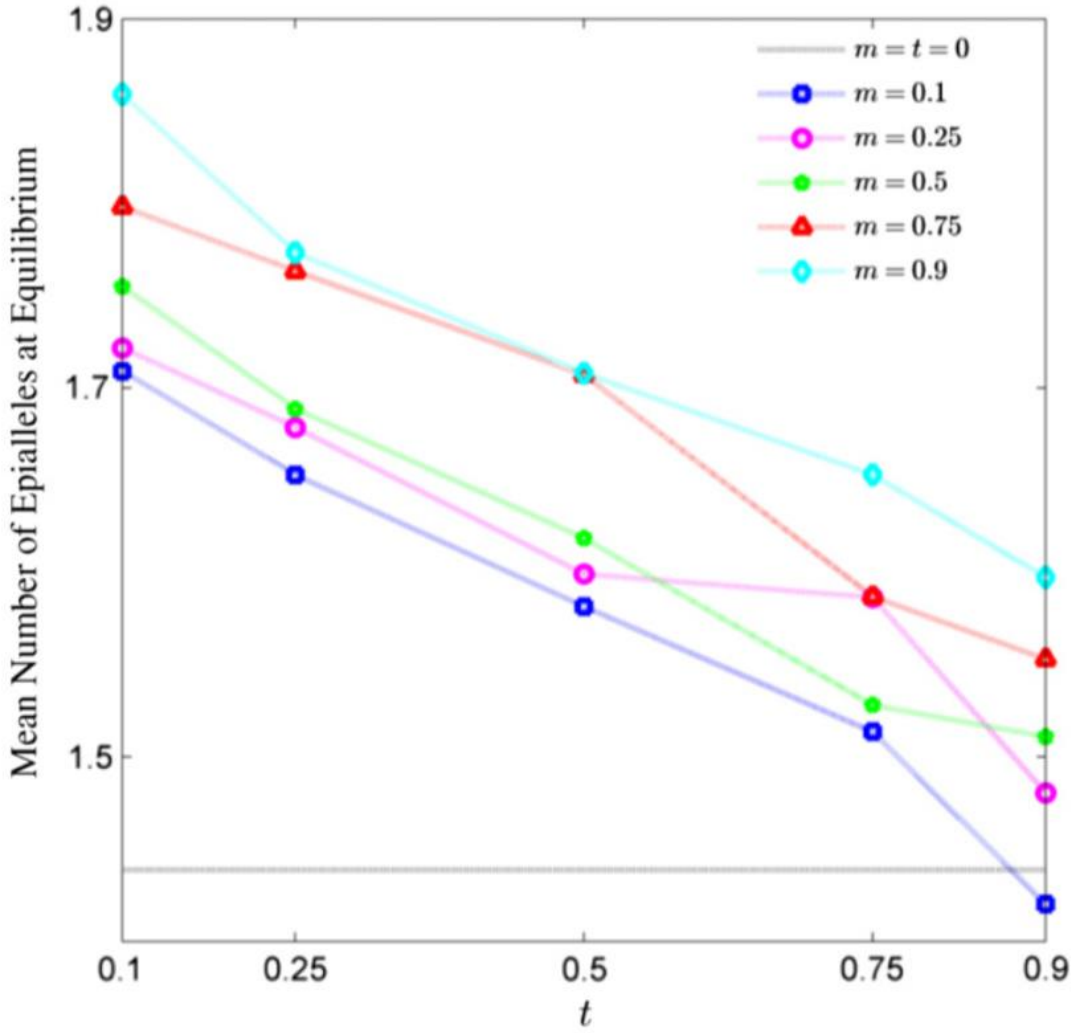

Fig. 10. Mean number of epialleles at equilibrium, Metric C, across levels of m and t. n_e decreases consistently with increasing reset, *t,* and decreasing modification rate, *m*. From Geoghegan and Spencer (2013b).

Effective alleles at equilibrium decreases with increasing t (reset rate), as B alleles are increasingly reset to A, lowering variation. This same metric increases with increasing modification rate, m.

It is not at first obvious why a condition with high modification rate, *m*, and low reset rate, *t*, does not decrease effective alleles at equilibrium as B would dominate A. But this intuition is gained when you realise that changes in t have a much higher impact on the model than changes in m. All B alleles are undergoing reset, no matter their allelic partners.

Where A alleles must be matched with B or a to be affected by modification. This means that the effect of t dominates m, and even high values of m cannot overwhelm it.

I turn my attention to evaluating **Metric D**, genetic variation, for this model. Considering the four types of equilibria that are possible in this model, one notices that there is no equilibrium where only *a* and *A* exist, or only *a* and *B*. This means that the only equilibria which are genetic polymorphisms are the fully internal equilibria. With this knowledge, we can identify the proportion of genetic polymorphic equilibria from the data presented in Fig. 9. As mentioned in the previous paragraph about Metric B, proportion of fitness sets with internal equilibria is constant at ~0.2 across all values of m and t. In comparison, 0.33 of fitness sets randomly generated for the base genetic 2-allele model have a genetic polymorphic equilibrium (Lewontin et al. 1978). Consistently then, modelling predicts that two allele systems where one allele is paramutagenic will have lower genetic variation at equilibrium on average then two allele systems with no epigenetic dynamics.

Geoghegan and Spencer (2013b) find that increasing the paramutation rate *m*, decreases mean population fitness, **Metric E**, across all simulation results and especially when the reset rate, *t*, is low. This effect is analogous to "mutation load" in genetic models with mutation.

Discussion

Summation and Comparison of Findings

Table 2 summarizes the findings and comparisons between Models One through Four for all Metrics A through E. The major findings are as follows:

	Model One – environmental modification without genetic variation.	**Model Two – environmental modification with genetic variation.**	**Model Three – partial environmental modification**	**Model Four - paramutation**
Metric A - # of stable eq.	Decreases with increasing m. Maximized under heterozygote disadvantage. Maximized when m_1 close to m_2. No relationship with r.	Decreases with increasing m. No relationship with r.	Maximized at moderate values of r.	Decreases with increasing m for low values of t. Decreases with increasing t.
Metric B – prop. of internal stable eq.	All equilibria internal.	Decreases as m increases to ~0.25 and then increases after.	All equilibria internal.	For low t values, increases as m increases.*
Metric C – effective # of epialleles at eq.	Increases with increasing m. Maximized under heterozygote advantage.	No data.	Maximized for moderate values of r. Increased by balanced fitness values. Increased when epialleles have no selective advantage in inducing environment.	Increases with increasing m. Decreases with increasing t.
Metric D – genetic variation at eq.	No genetic variation.	Measure proportion of stable equilibria that are genetic polymorphisms. Higher than base model for low values of m. Lower than base model for high values of m.	No genetic variation.	Measure proportion of fitness sets with genetic polymorphic equilibriums. Constant across all values of m and t at 0.2. Lower than base model in all cases.
Metric E – mean population fitness at eq.	Decreases with increasing m.	No data.	Under AEA: positive relationship with Metric C. Otherwise: negative relationship with Metric C and decreases with increasing m.	Decreases with increasing m; effect heightened under low reset rate.

Table 2. Summary and comparison of findings in Models One through Four for all five metrics. m = epimodification rate, t = reset rate, r = frequency of environment 1 or α *Note that the *proportion of fitness sets* with internal stable equilibria is constant across m, t at 0.2.

(1) In nearly all cases epigenotypic variation increases in the epigenotypic model over the base genetic model. The addition of epigenetic markers increases the number of epialleles and thus total variation. Also, epimodification, analogous to mutation, increases diversity by continually introducing less fit alleles at equilibrium. The major exception is when measuring epigenotypic variation with Metric B, but here comparisons with a base genetic model should be discarded because the number of epialleles decreases in the base genetic model making internal equilibria easier to attain.

(2) The mean number of stable equilibria (Metric A) decreases with increasing m in almost all models. Varying fitness among genotypes, specifically epiheterozygote disadvantage, tends to give rise to more co-existing stable equilibria in a fitness set. High rates of epimodification overwhelm the effect of these differences, pulling the system towards a single equilibria. There is no conclusive evidence of this relationship in Model Three.

(3) In the two models with multiple genetic alleles (Two and Four), genetic variation decreases when epigenetic modification is introduced to the model in almost all cases. The only exception is when modification rate is low in Model Two, then genetic variation is increased over the base genetic model.

(4) For the three models where mean population fitness was investigated, as m increases, mean population fitness at equilibrium decreases. This relationship can be understood as 'epimodification load' an analog to "mutation load" in genetic models, the

decreases in mean population fitness when mutation increases in genetic population models.

Suggestions for Models to be Studied

A major finding from research into epigenetic mechanisms is the relatively high rate of reset that epigenetic markers undergo int the processes of mitosis and meiosis. The chromosome restructuring in these periods leads to a high rate of disruption for epigenetic structures (Ashe et al. 2021). This sort of reset is analogous to the reset employed in the paramutation model in this paper. Intuitively, reset is the removal of an epigenetic marker to some 'unmarked' or default epigenetic state. This dynamic is not included in Models One through Three, and instead if an 'unmarked' epigenetic state is included it takes the form of one of the epialleles and 'reset' to this state only occurs as modification in one environment. Conceptually reset should be occurring in all environments whenever gametes are formed through meiosis.

To confuse matters, in the original Geoghegan and Spencer papers (2012 and 2013a) the term 'reset' is used to refer to the process I call modification and the parameter that I call 'm'. The action associated with this parameter I think is better conceptualized as a rate of modification and if reset were to be considered in models of this type it should be done in a way resembling reset in the paramutation model, Model Four. In that spirit, I outline two models, one simple and one more complicated, that introduce this process of reset to environmentally-induced epigenetic population models.

Model Five: Two Environment Model with Two Epigenetic Alleles: One 'Unmodified', One Induced by Environment

There are two environments in this model, one 'Ancestral' that does not induce any epigenetic modification and one 'Novel' environment that does. There are two epigenetic markers, one indicating the unmarked state, the other being marked. There is only one genetic allele. A is the unmarked epiallele, A_1 is the marked epiallele. As an A allele leaves the 'Novel' environment it is modified to A_1 with probability m. After, all A_1 alleles in both environments are reset to A with probability t.

I have performed preliminary analysis on this model (the code is provided in Appendix 2), running simulations for several fitness sets and across parameter space and found that it seems to generate very simple dynamics with a maximum of one stable equilibrium for all fitness sets examined. I have observed that the model is very sensitive to increasing reset rate and even moderate values overwhelm any variation in the modification rate.

Under neutral fitness conditions, the equilibrium frequencies of this model are:

$$p*_A = \frac{t}{(1-r)m(1-t)+t} \qquad p*_{A1} = \frac{(1-r)m(1-t)}{(1-r)m(1-t)+t}$$

These relationships are very similar to those observed in Model One: t is the rate at which new A epialleles are being introduced to the population as a proportion of p_{A1}, and $(1-r)m(1-t)$ is the rate at which new A_1 epialleles are introduced to the population as a proportion of p_A . Then, just like in Model One, the equilibrium frequency of an epiallele is the rate at which it is introduced, divided by the total rate that new epialleles are introduced.

The behaviour in this model is likely highly simplistic, but it is chosen as the simplest model available from which to build upon. To observe more characteristic epigenetic phenomena I suggest that a model with more than one 'modified' epigenetic marker is required, bringing us to Model Six.

Model Six: Two Environment Model with an 'Unmodified' Epigenetic Allele and Two Modified Epigenetic Alleles with Early and Limited Modification

There are two environments in this model, 1 and 2, both of which induce epigenetic modification of alleles to their corresponding epigenetic markers, 1 and 2. There is a third 'unmarked' epigenetic marker and only one genetic allele, leading to three epialleles: A, A_1, A_2.

Modification in this model is 'limited' meaning that modification only occurs for the 'unmarked' A epialleles. Modification in this model is also 'early' meaning that it occurs as an individual is sorted into an environment and before selection. Note that if we assume that A_1 and A_2 have higher fitnesses in their associated environments, high modification rates and high reset rates allow perfect adaptability and high mean population fitness in this model. High reset rates dictate that most epialleles are type A when entering an environment and high modification rates mean that most will be modified into, in this case, the preferred epiallele in the environment, which will increase fitness. This model then captures some of the more beneficial effects of epigenetic response that is observed in nature. Decreasing reset and modification rates captures some of the detrimental side effects of these transgenerational epigenetic mechanisms when organisms are left epigenetically mismatched to their environment.

The Unique Nature of Evolutionary Processes under Epigenetic Conditions

What makes analysis of evolution in the epigenetic context unique, especially when looking at genotypic frequencies, is the fact that fitness of genotypes is non-constant in epigenetic models. The marginal fitness of any genotype is frequency dependent on underlying epigenotypes. In this way, the genetic evolution of the system cannot be separated from its epigenetic components, making analysis that much more complicated and necessitating the simulation methods employed here. This fact is also a useful insight into how the overlaying of epigenotypic processes on top of a two-allele genetic model in Models Two and Four can impact the distribution of genetic polymorphisms at equilibrium. The introduction of epigenetic markers does not only change the fitness of a genotype (in an environment) but also makes it dynamic as a model iterates.

Best Practices for Observing Evolutionary Outcomes from Population Epigenetic Models

Geoghegan and Spencer (2012, 2013a, 2013b) did not approach their studies with the explicit goal in mind of evaluating the impact on evolution from epigenetic processes. As foundational papers in these modelling techniques, their findings focus on broad understandings of the behaviours of these models across parameter space and the limitations and interpretations of the numerical simulation techniques. Some of my conclusions in this study have been limited by a lack of reporting on important metrics in some studies and inconsistency in the characteristics of the models being measured. If one were to freshly approach the question of evaluating these models for their impact on

epigenotypic and genotypic frequencies and comparing these results with base and equivalent genetic models, a more comprehensive understanding could be achieved.

I see two major strategies for analysis: the remove-and-compare approach undertaken here and the trajectory approach that I will describe.

The remove-and-compare approach involves running an epigenetic model to equilibrium and comparing with a base or equivalent genetic model at equilibrium. If one wishes to compare genetic variation, you can remove epigenetic markers and do so. This is the approach undertaken in this paper, using the data presented by Geoghegan and Spencer (2012, 20131, 2013b) and comparing to genetic models. Re-simulating the results would allow asking and answering a further set of questions. First the proportion of parameter space, including starting frequencies, could be evaluated. One could ask what proportion of parameter space leads to genetic polymorphisms or equilibria with high effective numbers of epialleles. Further, we could better evaluate which starting frequencies and which fitness sets lead to these high variation outcomes. As not all parameter values are relevant or realistic in the natural context, knowing under what conditions these results matter allows us to evaluate how these findings can be applied.

The trajectory-approach compares the trajectory that the full epigenetic and base genetic models take to equilibrium. As natural systems often face disturbances that preclude any approximation of an equilibrium, the trajectory could be more important to consider than the equilibrium. If an epigenetic model approaches equilibrium quickly or slowly or spends more time in high or low variation states this could have meaning for the impact of epigenetic dynamics on long-term evolution. The functional, curve-based data

that results from this analysis will be more difficult to synthesize, so focus will likely need to be placed on comparing specific parameter sets of interest, such as those that replicate some natural conditions or produce the largest difference between trajectories. To my knowledge, no studies of this type using population epigenetic modelling have been completed, but Day and Bonduriansky (2011) perform a similar analysis using a model based on the Price equation and could serve as a guide to future efforts in population epigenetics.

Conclusion

The results of this paper show both that transgenerational epigenetic inheritance has a meaningful impact on evolution and that population epigenetic modelling is an effective way of deriving insights about these effects. If we consider the heart of a new evolutionary synthesis to be redefining evolution to include non-genetic components, then these results support this redefinition Transgenerational epigenetic inheritance impacts evolution through two axes: demonstrating a positive effect on epigenotypic variation, and a variable but mostly negative effect on genetic variation. These effects need to be understood for a complete comprehension of evolutionary processes. However, this paper also shows that traditional methods, like population genetic modelling, can be employed effectively in investigating this broader definition of evolution. Traditional methods need not be discarded in a new evolutionary synthesis.

The potential of population epigenetic modelling, I believe, has not yet been fully tapped. Approaches focused on identifying the regions of parameter space that lead to high variation outcomes, and trajectory-approaches will enrich our understanding further.

Additionally, the formulation possibilities of these models are not exhausted and there is more room for exploration, especially when incorporating epigenetic reset into these models. The methods and approach pioneered by Geoghegan and Spencer (2012, 2013a, 2013b) form a broad launch pad for future research.

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Appendix 1 – Simulation probabilities of internal equilibria in a one-locus, two-allele, *two environment*, genetic model

In order to determine the expected proportion of stable equilibria found in a simulation of the type described in Geoghegan and Spencer (2012) and Lewontin et al. (1978) that are internal, we must determine the probability of fitness sets that express overdominance, underdominance or directional selection. This is because we know the nature of stable equilibria under each of these cases. Fitness sets demonstrating directional selection, where $v_{11} > v_{12} > v_{22}$ or $v_{11} < v_{12} < v_{22}$, have one stable, and non-internal equilibrium. Fitness sets with underdominance where the heterozygote is selected against, $v_{11} > v_{12} < v_{22}$, have two stable, non-internal equilibria. Fitness sets with overdominance, where the fitness of the heterozygote is highest, $v_{11} < v_{12} > v_{22}$, have one stable and internal equilibria.

If we were interested in a one-locus, two-allele model with only one environment, the results of Lewontin et al. (1978) could directly tell us that each of these fitness cases occurs with a probability of $1/3$ and because of this and the characteristics mentioned above, internal stable equilibria occur with a frequency of $1/4$. When a second environment is added to the model, little changes in the purely genetic case: members of each genotype are split into the two environments at the same rate (r and 1-r) before they experience selection according to their specific fitness value in that environment. Because of this, each genotype can be thought of as having an effective fitness which is a weighted average of their fitness values in each environment. If v_{xyz} represents the fitness of genotype A_xA_y in environment z, then the effective fitness of genotype A_xA_y is:

$$v'_{xy} = rv_{xy1} + (1-r)v_{xy2}$$

With this change, the rest of the model can be considered to mathematically function in the exact same way, with no more impact from the two environments. However, what is now important is that the distribution of the effective fitness of a genotype in the two-environment model is no longer the same as the distribution of the fitness of the genotype in the one-environment model. If we consider the fitnesses for each environment to be chosen from a standard uniform distribution, the distribution of the effective fitness v'_{xy} is not uniform but follows an Irwin-Hall distribution which is the distribution of a sum of uniform random variables.

The question becomes: can the results of Lewontin et al. (1978) be extended to this two-environment case? The following calculations of the expected frequencies of the different fitness types for a fitness set, overdominance and underdominance (the frequency of directional selection can be derived from these other two as the remaining fitness sets), demonstrate that these results can be extended. It turns out that as long as the distrubtion of each fitness is identical the frequency of these fitness set types do not depend on the distribution of each fitness value. As the distribution of each fitness value is identical in the two-environment model, we can conclude that in the two-environment model

overdominance occurs with a frequency of 1/3, overdominance with a frequency of 1/3 and directional selection with a frequency of 1/3 and that ¼ of simulated equilibria will be internal.

Overdominance

$$P(v_{11} < v_{12}, v_{22} < v_{12} | v_{12}) = F_{v_{11}}(v_{12}) F_{v_{22}}(v_{12})$$

$$P(v_{11} < v_{12}, v_{22} < v_{12}) = \int_0^1 F_{v_{11}}(x) F_{v_{22}}(x) f_{v_{12}}(x) dx$$

All v_{11}, v_{12}, v_{22} have identical distributions, being the weighted (by r, 1-r) averages of two standard uniform random variables. So we can express the above formula in terms of the variable v with the same distribution:

$$= \int_0^1 F_v(x) F_v(x) f_v(x) dx$$

$$= \int_0^1 F_v(x)^2 f_v(x) dx$$

Let $u = F_v(x)$, $du = f_v(x)dx$, then:

$$P(v_{11} < v_{12}, v_{22} < v_{12}) = \int_0^1 u^2 \, du$$

$$= \frac{u^3}{3} |_0^1$$

$$= \frac{F_v(1)^3}{3} - \frac{F_v(0)^3}{3}$$

$$= \frac{1^3}{3} - \frac{0^3}{3}$$

$$= \frac{1}{3}$$

Underdominance (Heterozygote Disadvantage)

A similar process shows that the expected frequency of underdominant fitness sets in a simulation with uniformly distributed fitnesses is 1/3 also:

$$P(v_{11} > v_{12}, v_{22} > v_{12} | v_{12}) = \left(1 - F_{v_{11}}(v_{12})\right)\left(1 - F_{v_{22}}(v_{12})\right)$$

$$P(v_{11} > v_{12}, v_{22} > v_{12}) = \int_0^1 \left(1 - F_{v_{11}}(x)\right)\left(1 - F_{v_{22}}(x)\right) f_{v_{12}}(x)dx$$

All v_{11}, v_{12}, v_{22} have identical distributions, being the weighted (by r, 1-r) averages of two standard uniform random variables. So we can express the above formula in terms of the variable v with the same distribution:

$$= \int_0^1 \big(1 - F_v(x)\big)\big(1 - F_v(x)\big) f_v(x)dx$$

$$= \int_0^1 \big(1 - F_v(x)\big)^2 f_v(x)dx$$

Let $u = F_v(x)$, $du = f_v(x)dx$, then:

$$P(v_{11} > v_{12}, v_{22} > v_{12}) = \int_0^1 (1-u)^2\, du$$

$$= -\frac{(1-u)^3}{3}\Big|_0^1$$

$$= -\frac{\big(1 - F_v(1)\big)^3}{3} + \frac{\big(1 - F_v(0)\big)^3}{3}$$

$$= -\frac{(1-1)^3}{3} + \frac{(1-0)^3}{3}$$

$$= \frac{1}{3}$$

Appendix 2 – Python code for simulation of Model 5

A Jupyter Notebook file is also attached to this submission with this code.

```
from sympy import *

init_printing()

from matplotlib import pyplot as plt

colormap = plt.get_cmap("viridis")

divisions = 5 + 1


fig, axs = plt.subplots(nrows=(divisions), ncols=(divisions),
figsize=(((divisions-1)*4),((divisions-1)*4)))


for k in range(0,divisions):

    for j in range(0,divisions):

        for h in [0.1, 0.5, 0.9]:

            for i in range(0, divisions):


                r = k/(divisions-1)

                t = i/(divisions-1)

                s = j/(divisions-1)

                p1 = h

                p2 = 1 - p1

                v11 = 0.8

                v12 = 0.4

                v21 = 0.2

                v22 = 0.1

                v31 = 0.8

                v32 = 0.8

                record =[p2]

                change = 1

                while abs(change) > 10**-3 and len(record) < 51:

                    p1_s = r*(p1*(p1*v11 + p2*v21) + p2*s*(p1*v21 + p2*v31))
+ (1-r)*(p1*((1-t)+t*s)*(p1*v12+p2*v22) + s*p2*(p1*v22 + p2*v32))
```

```
                    mean_fitness = r*(p1*p1*v11 + 2*p2*p1*v21 + p2*p2*v31) +
(1-r)*(p1*p1*v12 + 2*p1*p2*v22 + p2*p2*v32)
                    p1_next = p1_s/mean_fitness
                    if p1_next > 1:
                        p1_next = 1
                    elif p1_next < 0:
                        p1_next = 0
                    change = p1_next - p1
                    record.append(1 - p1_next)
                    p1 = p1_next
                    p2 = 1 - p1


                axs[j, k].plot(record, label='t = '+str(t), color =
colormap(float(t)))
                axs[j, k].set_ylim(0,1)


                if j==0 and k==0 and h==0.1 and i==divisions-1:
                    axs[j, k].legend()


        if j==0:
            axs[j, k].set_title("r = "+str(r))
        if k==0:
            axs[j,k].set_ylabel("s = "+str(s), rotation=0, size='large')


plt.ylim(0,1)
plt.tight_layout
plt.savefig("MRdiploidwithfitness-"+str(v11)+"-"+str(v12)+"-"+str(v21)+"-
"+str(v22)+"-"+str(v31)+"-"+str(v32)+".png")
```

Appendix 3

Code for derivation of neutral equilibriums in Model 3. Jupyter notebook with this code attached to this submission.

```
from sympy import *
from sympy.solvers.solveset import linsolve
init_printing()
p1, p2, p3, r, t1, t2 = symbols('p1 p2 p3 r t1 t2')

# MODEL THREE
t1=t2*2
p1s = r*(t1*p2+t2*p3)/((1-r)*(t1+t2))
p2s = r*p3 + (1-r)*p1
p3s = (1-r)*(t1*p2+t2*p1)/(r*(t1+t2))
eqs = linsolve([p1s-p1, p2s-p2, p3s-p3, p1+p2+p3-1], p1, p2, p3).args[0]
eqs
pa, pA, pB, m1, m2, t, m = symbols('pa pA pB m1 m2 t m')
m1=m
m2=m
t=m
pAs = pA**2 + (1-m1)*pA*pa + (1-m2)*pA*pB + t*pB
pBs = (1-t)*pB + m1*pA*pa + m2*pA*pB
sols1 = nonlinsolve([pAs-pA, pBs-pB, pa+pA+pB-1], pA, pB).args[0]
sols2 = nonlinsolve([pAs-pA, pBs-pB, pa+pA+pB-1], pA, pB).args[1]
print(sols1[0])
print(sols1[1])
```

References

Ashe, A., Colot, V. & Oldroyd, B. P. How does epigenetics influence the course of evolution? *Philosophical Transactions of the Royal Society B: Biological Sciences* **376**, 20200111 (2021).

Bonduriansky, R. & Day, T. Nongenetic Inheritance and Its Evolutionary Implications. *Annual Review of Ecology, Evolution, and Systematics* **40**, 103–125 (2009).

Day, T. & Bonduriansky, R. A Unified Approach to the Evolutionary Consequences of Genetic and Nongenetic Inheritance. *The American Naturalist* **178**, E18–E36 (2011).

Furrow, R. E. & Feldman, M. W. Genetic Variation and the Evolution of Epigenetic Regulation. Evolution **68**, 673–683 (2014).

Furrow, R. E. Epigenetic Inheritance, Epimutation, and the Response to Selection. PLOS ONE 9, e101559 (2014).

Geoghegan, J. L. & Spencer, H. G. Population-epigenetic models of selection. *Theoretical Population Biology* **81**, 232–242 (2012).

Geoghegan, J. L. & Spencer, H. G. Exploring epiallele stability in a population-epigenetic model. *Theoretical Population Biology* **83**, 136–144 (2013a).

Geoghegan, J. L. & Spencer, H. G. The evolutionary potential of paramutation: A population-epigenetic model. *Theoretical Population Biology* **88**, 9–19 (2013b).

Geoghegan, J. L. & Spencer, H. G. The adaptive invasion of epialleles in a heterogeneous environment. *Theoretical Population Biology* **88**, 1–8 (2013c).

Greenspoon, P. B. & Spencer, H. G. The evolution of epigenetically mediated adaptive transgenerational plasticity in a subdivided population. Evolution **72**, 2773–2780 (2018).

Jablonka, E. & Lamb, M. Precis of Evolution in Four Dimensions. The Behavioral and brain sciences **30**, 353–65 (2007).Geoghegan, J. L. & Spencer, H. G. Population-epigenetic models of selection. *Theoretical Population Biology* **81**, 232–242 (2012).

Jablonka, E. & Raz, G. Transgenerational Epigenetic Inheritance: Prevalence, Mechanisms, and Implications for the Study of Heredity and Evolution. *The Quarterly Review of Biology* **84**, 131–176 (2009).

Jablonka, E. The evolutionary implications of epigenetic inheritance. *Interface Focus* **7**, 20160135 (2017).

Klironomos, F. D., Berg, J. & Collins, S. How epigenetic mutations can affect genetic evolution: model and mechanism. Bioessays **35**, 571–578 (2013).

Lewontin, R. C., Ginzburg, L. R. & Tuljapurkar, S. D. Heterosis as an explanation for large amounts of genic polymorphism. *Genetics* **88**, 149–169 (1978).

Lind, M. I. & Spagopoulou, F. Evolutionary consequences of epigenetic inheritance. *Heredity* **121**, 205–209 (2018).

Marks, R. & Spencer, H. The Maintenance of Single-Locus Polymorphism. II. The Evolution of Fitnesses and Allele Frequencies. *American Naturalist - AMER NATURALIST* **138**, (1991).

van der Graaf, A. *et al.* Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *Proceedings of the National Academy of Sciences* **112**, 6676–6681 (2015).