Technical comment on ‘Negative-assortative mating for color in wolves’

## Background

Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) reported on “negative-assortative mating for color in wolves” from Yellowstone National Park, the “first documented case of significant negative-assortative mating in mammals.” Based on the close correspondence of genotype and allele frequencies observed in the wild to that predicted by their population genetic model, they conclude that “negative-assortative mating could be entirely responsible for the maintenance of this well-known color polymorphism.” While researching examples of nonrandom mating in the wild to teach in class I discovered that the results of their population genetic model are inconsistent with their stated assumptions, as I understand them. In this paper, I revisit the model with the following two objectives:

1. Demonstrate that the frequency of negative-assortative mating between gray and black pelage color morphs in their model does not follow from their assumptions; and
2. Derive results that are consistent with their assumptions.

I am critiquing only their model, not the data analysis or conclusions. Both the original model and the new model analyzed here lead to similar inferences about the maintenance of the pelage color polymorphism because the equilibrium genotype and allele frequencies are nearly the same in both models. However, it is important that the mathematical biology literature provide logically consistent analysis so that future researchers may benefit most from its insights.

## The frequency of assortative mating is inconsistent with the assumptions

Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) assume that a proportion matings are assortative, but the proportion they derive is much less (see Fig. @ref(fig:sample-space) for a graphical derivation). For consistency, I use the same symbols as Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) (Table @ref(tab:symbols) lists all symbols and their definitions). I infer three key assumptions from the two statements on the bottom-left of pg. 758 of Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)):

“gray wolves have a genotype of and an assumed frequency of and black wolves have genotypes and with frequencies and , respectively .”

“Assume that and are the proportions of negative-assortative mating and random mating, respectively, in the population.”

From these statements, I infer that:

1. , , and are mutually exclusive genotypes with frequencies , , and
2. Negative-assortative mating and random mating are mutually exclusive mating types with frequencies and
3. Genotype and mating type are independent ()

Other model assumptions made by Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) are common in population genetics. The model is deterministic, individuals are monoecious, generations are discrete and nonoverlapping, and individuals can mate multiply. Some of these assumptions are not be valid for wolves and different assumptions could result in qualitatively different outcomes. For example, if individuals only mate in pairs, this would constrain the amount of negative assortative mating that is possible when either allele is rare. While alternative assumptions may help understand how assortative mating maintains genetic variation, my purpose here is not to critique the assumptions of Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)), but rather to show their results are not logically consistent with their stated assumptions.

Glossary of mathematical symbols, variable string used in source code, and description.

| Symbol | Variable string | Description |
| --- | --- | --- |
|  |  | recessive beta-defensin variant |
|  |  | dominant beta-defensin variant |
|  |  | frequency of allele |
|  |  | frequency of allele |
|  |  | frequency of genotype |
|  |  | frequency of genotype |
|  |  | frequency of genotype |
|  |  | proportion negative-assortatively mating |

Based on these assumptions, I deduce that:

1. The probability of all genotype-mating type combinations must sum to 1
2. The probability of all genotypes in the assortative-mating subspace must sum to
3. The probability of all genotypes in the random-mating subspace must sum to

There are six mutually exclusive genotype-mating type combinations in the population sample space (Fig. a). Since , , and genotype is independent of mating type, the probability of all genotype-mating type combinations must sum to 1.

Furthermore, we know that within the negative-assortative and random mating subspaces, the total probability must sum to and , respectively:

The model in Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) is internally inconsistent because the proportion of negative-assortative matings does not equal as defined (Fig. @ref(fig:sample-space)b).

Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) state that the frequency of assortative matings is (*cf* top of pg. 759) and the frequency of random matings is . Applying these frequencies reveals that the proportion of assortative matings is not equal to as assumed:

There are no solutions to the expression above where the proportion of negative-assortative mating would equal when genotype and mating proportions are between 0 and 1. With their model, the actual proportion of negative-assortative would vary from 0 when or and when .

To summarize, a proportion should mate assortatively given the assumptions of their model, but only actually mate assortatively according to their results. In essence, they assign a proportion to mate assortatively, but then a proportion do not mate assortatively (the area of the gray regions in Fig. @ref(fig:sample-space)b), and are therefore not counted among the total number of matings. This is why the probabilities of all matings do not sum to 1. The logically consistent solution is to condition on the fact that if a mating is negative-assortative it must by definition have one gray and one black parent (Fig. @ref(fig:sample-space)b). In the next section, I use this approach to derive different results. In contrast, Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) effectively impose selection without ever stating that assumption. To deal with the fact that resulting genotype frequencies do not sum to 1, they regularize the frequencies (*cf* equation 1a-b), as normally done in models of selection. Regularization is appropriate with selection because selection shrinks or expands the sample space as long as average fitness does not equal 1. Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) impose selection because some of the assortative-mating individuals do not mate assortatively and are therefore not counted among the matings that result in offspring.

## Revised solutions consistent with model assumptions

The previous section showed that the frequency of assortative gray black matings was not derived in a manner logically consistent with the model’s assumptions. Here I derive new mating frequencies, genotype frequencies, and equilibria. This model was previously analyzed by Li ([1976](#ref-li_first_1976))] where in his notation , , and (*cf* pg. 367-368). I used Sympy version 1.8 ([Meurer et al. 2017](#ref-meurer_sympy:_2017)) for symbolic derivations through Python version 3.6 and the *R* package **reticulate** version 1.25 ([Ushey et al. 2022](#ref-ushey_reticulate_2022)). All other computations were performed in *R* version 4.2.0 ([R Core Team 2022](#ref-r_core_team_r_2022)). The source code is available in a public GitHub repository and will be archived on Zenodo upon publication.

Table @ref(tab:probabilities) derives the probabilities of all possible outcomes and Table @ref(tab:genotypes) summarizes the frequency each mating combination. This is the exact same process used to derive frequency of mating combinations in positive-assortative mating models (e.g. [Hedrick and Ritland 2012](#ref-hedrick_population_2012)). Since I model random mating identically to the previous model, the frequencies of gray gray and black black matings are identical; only the frequency of gray black matings differs between models (Table @ref(tab:genotypes); Fig. @ref(fig:sample-space)c).

Code in the Supporting Information derives the expressions in (Table @ref(tab:genotypes)) analytically using a computer algebra system, but one can also use the Law of Total Probability to prove it. The Law of Total Probability for discrete probability distributions states that where is the probability of outcome conditional on outcome . The total probability of is the sum of conditional probabilities across all outcomes for event . Using the Law of Total Probability, the probability of a gray black mating is:

We already assume that and . With random mating, I arrive at the same expression as Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)), (*cf* top of pg. 759). If the mating is negative-assortative, then it *must* be a gray black mating. Therefore, . Putting these together, I obtain:

This result diverges from that given in Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)), where they report the frequency of gray black matings is (*cf* Table 1). One advantage of their result is that the frequency of assortative matings is 0 when the population is fixed at this locus ( or ). In contrast, Eq. conjures assortative matings from a population of wolves with identical coat color. This is a logical consequence of assuming that a proportion mate assortatively irrespective of the allele frequency. An alternative approach is to set bounds on as a function color morph frequency. For example, if we assume that wolves mate in monogamous pairs, then cannot exceed twice the frequency of the rarer color morph. Consider a single black wolf in pack of 100 (). The maximum value of is if there is one gray black mating and 49 gray gray matings. More generally, the maximum value of in a population of monogamous pairs can be found by solving Equation for when the maximum proportion of assortative matings is the lesser of and . When grey morphs are rarer than black morphs (), the maximum value of is ; when black morphs are rarer (), the maximum value of is . Different constraints on apply to a population where individuals can self-fertilize and/or mate with multiple individuals. Incorporating constraints on as a function of phenotypic frequency may be important, but the assumptions stated by Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) do not imply constraint on when one color morph is rare.

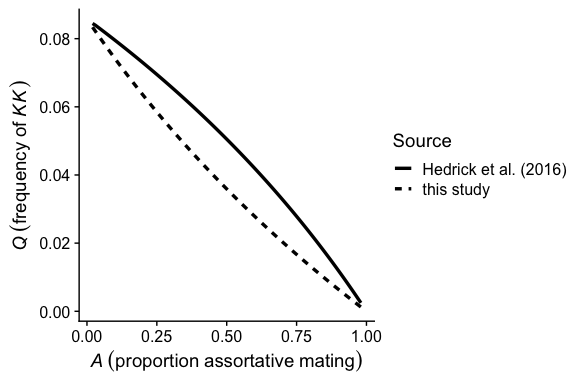
The probability of every mating outcome in the negative-assortative mating model analyzed by Hedrick *et al.* (2016). For the notation, the probability of event *X* is Pr[*X*]. The total probabilities for each row are derived from the product of all probabilities in the same row, Pr[Total] = Pr[Parent 1] Pr[Mating] Pr[Parent 2].

| Parent 1 | Pr[Parent 1] | Mating | Pr[Mating] | Parent 2 | Pr[Parent 2] | Pr[Total] | Color |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | assortative |  |  |  |  | Gray gray |
|  |  | random |  |  |  |  | Gray gray |
|  |  | assortative |  |  |  |  | Gray black |
|  |  | random |  |  |  |  | Gray black |
|  |  | assortative |  |  |  |  | Gray black |
|  |  | random |  |  |  |  | Gray black |
|  |  | assortative |  |  |  |  | Black black |
|  |  | random |  |  |  |  | Black black |

Hedrick *et al.* (2016) incorrectly derive the frequency of gray black. The corrected expressions are provided here.

| Color | Mating Genotypes | Frequency (Hedrick *et al.* 2016) | Frequency (this paper) |
| --- | --- | --- | --- |
| Gray gray |  |  |  |
| Gray black |  |  |  |
| Black black |  |  |  |

Despite the different frequency of gray black matings resulting from each model, the equilibrium genotype frequencies are very similar. In both models, , implying . I find that , which is close to the equilibrium values obtained in Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) through recursion (Fig. @ref(fig:fig2)). Next, I compared allele frequency change depicted in Figs. 3-4 of Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) to that predicted with the new model. The effect of on change in the frequency of the allele is qualitatively similar, but much faster with the new model (Fig. @ref(fig:fig3)). This is because the magnitude of allele frequency change far from the equilibrium is much greater with the new model (Fig. @ref(fig:fig4)). As a result, Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) overestimate how long it would take for to reach equilibrium given observed levels of assortative mating (). They conclude that would reach equilibrium at in 25 generations with . In the revised model, would reach equilibrium at in only 15 generations with and a starting allele frequency . Hence, the revised model actually lends credence to their conclusion that negative-assortative mating may be a better explanation than heterozygote advantage for variation at the beta definsin locus.



The equilibirum frequency of , the homozygote in this study (dashed line) and Hedrick (2016) (solid line) for possible values of , the proportion of wolves mating assortatively by color.

In conclusion, the logical inconsistency in Hedrick et al. ([2016](#ref-hedrick_negative-assortative_2016)) does not undermine their primary conclusion that negative-assortative mating by color may explain the distribution of genotype frequencies at the beta definsin locus in the Yellowstone population of wolves (*Canis lupus*). The new derivation here may prove useful to future research on negative-assortative mating.

# Literature cited

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